

## Treatments for COVID-19: emerging drugs against the coronavirus

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**Summary.** The Coronavirus disease 19 (COVID-19) outbreak has been recognized as a global threat to public health. It is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and no effective therapies currently exist against this novel viral agent. Along with extensive public health measures, an unprecedented global effort in identifying effective drugs for the treatment is being implemented. Potential drug targets are emerging as the result of a fast-evolving understanding of SARS-CoV-2 virology, host response to the infection, and clinical course of the disease. This brief review focuses on the latest and most promising pharmacological treatments against COVID-19 currently under investigation and discuss their potential use based on either documented efficacy in similar viral infections, or their activity against inflammatory syndromes. Ongoing clinical trials are also emphasized. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** COVID-19, SARS-CoV-2, drugs, therapy, antivirals, inflammation

### Introduction and aim

Human coronaviruses (HCoVs) have traditionally received relatively little attention due to their mild phenotypes in humans, and are considered inconsequential pathogens causing approximately 15% of “common cold” in healthy people (1,2). However, in less than two decades, we have already encountered two highly pathogenic and large-scale epidemic HCoVs, namely SARS-CoV in 2003 and MERS-CoV in 2012 (3), with a case fatality rate of 9.5 and 35%, respectively (4). In late December of 2019, the scientific community has been informed of an emerging outbreak caused by a novel pathogen from a viral family, formerly thought to be relatively benign, but later recognized as global threat to public health and named COVID-19 by World Health Organization (WHO). In less than two months we rapidly learned that COVID-19 infection is not like the seasonal flu, with one in five patients presenting with a severe infection and requiring oxygen or even mechanical ventilation (5).

As of April 25<sup>th</sup>, 2.686.785 laboratory-confirmed cases of COVID-19 were reported involving 213 countries and resulting in more than 184.681 confirmed deaths worldwide (6). As pauci-symptomatic (and even asymptomatic) people may be fueling the coronavirus spread, COVID-19 burden is likely underestimated, thus further complicating a careful monitoring of the natural course of the disease, the rate of its spreading, as well as the development of effective epidemic prevention strategies (7).

While witnessing a rapidly increasing number of new cases, a greater understanding of the etiology and pathogenesis of this previously unknown illness has been progressively gained, from viral gene sequencing to elucidation of transmission route, from virulence mechanisms to the investigation of the wide variability of clinical spectrum observed in routine practice. Along with extensive public health measures promoting social distancing and reducing the massive strain on the healthcare infrastructure, global efforts are currently directed to the development of a vaccine

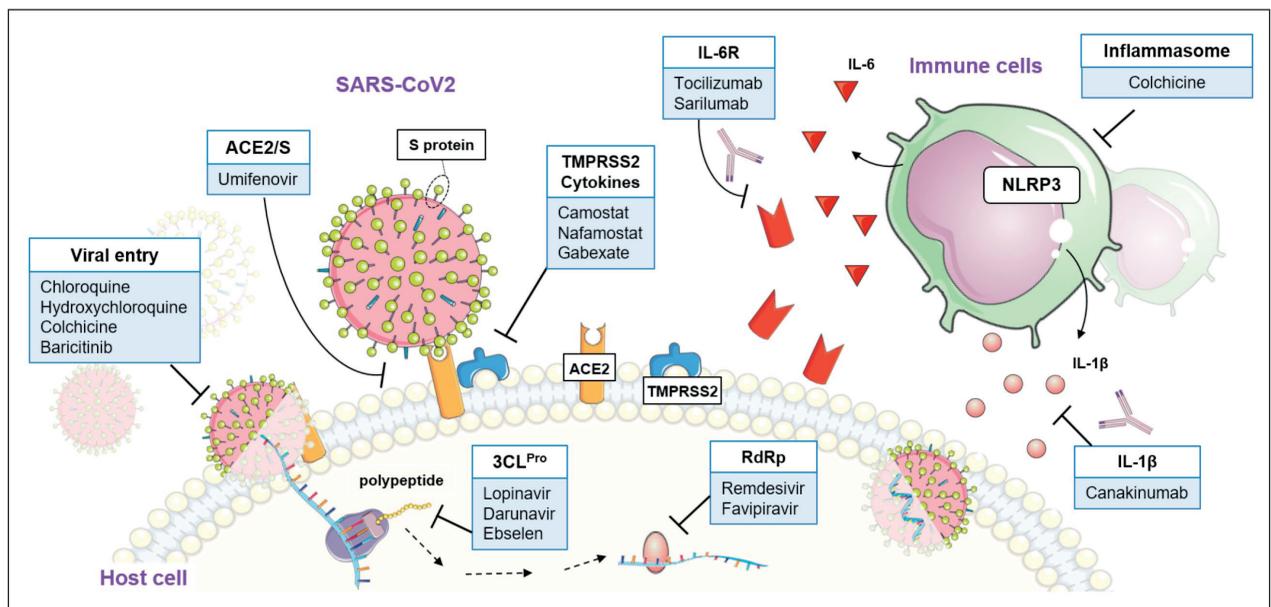
and therapeutic agents while optimizing marketed medications, mainly employed in severe respiratory viral infections. Nevertheless, no effective prophylactic or post-exposure therapies are currently available. Here, we briefly review the latest and most promising pharmacological treatments against COVID-19 currently under investigation and discuss their potential use by virtue of either documented efficacy in similar viral infections, or their activity against the so-called “cytokine storm” or the newly defined sepsis-induced-coagulopathy (8). Figure 1 illustrates the main pharmacological strategies proposed so far.

### COVID-19 disease and therapeutic strategies

A fast-evolving understanding of how the SARS-CoV-2 targets cells around the body and a mounting clinical evidence of the COVID-19 disease course in hospitalized patients have been providing potential targets for drug therapy (9-11). While we are just beginning to probe the degree of lung damage, clinical reports suggest that other organs can also be injured, including heart, liver, kidney and the lower gastroin-

testinal tract. Emerging evidence suggests that COVID-19 may demand a multi-target approach thus involving viral entry and lifecycle, host response or even both.

SARS-CoV-2 enters host cells by taking advantage from the binding of the viral spike (S) protein to host cell angiotensin-converting enzyme 2 (ACE2) receptor and the type 2 transmembrane serine protease (TMPRSS2). Overall, coronavirus entry into susceptible cells requires the concerted action of receptor-binding and proteolytic processing of the S protein to promote virus-cell fusion (12-14). Of note, this route of entry was found being 100- to 1000-fold more efficient than the endosomal pathway and the availability of proteases in the extracellular milieu being a key factor of tropism (15). Drugs preventing viral entry by inhibiting TMPRSS2 (i.e. camostat mesylate), targeting S protein/ACE2 interaction (i.e. umifenovir), inhibiting viral entry and endocytosis (i.e. chloroquine/hydroxychloroquine) may all stand as potential therapies along with drugs targeting viral polyprotein synthesis (i.e. lopinavir, remdesivir, etc). To date, such strategies seem to hold the greatest promise when applied early in the course of the illness, but their usefulness in ad-



**Figure 1.** Graphic summary of the emerging drug targets for the treatment of COVID-19. The pharmacological targets (white text boxes) and the relative proposed drugs (light blue text boxes) are noted. Abbreviations: ACE2, angiotensin converting enzyme 2; TMPRSS2, type 2 transmembrane serine protease; S protein, spike protein; 3CLPro, 3-chymotripsin like protease; RdRp, RNA dependent RNA polymerase; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; IL-1β, interleukin 1 beta; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3. Images from Smart Servier Medical Art (<https://smart.servier.com/>) have been combined for creating the illustration. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (<https://creativecommons.org/licenses/by/3.0/>).

vanced stages is still quite uncertain (16, 17) (Fig.1).

The effectual host immune response, including innate and adaptive immunity against SARS-CoV-2, may also serve as a therapeutic avenue with inhibition of IL-6 signaling playing a protective role if given at the time of overly elevated immune response to the virus. In this context, it has been speculated that drugs targeting immune regulation pathway (i.e. tocilizumab, sarilumab) may control the extreme cytokine reaction (termed “cytokine storm”) that is accompanied by infiltration of inflammatory monocytes/macrophages into the lung without deleterious effects on virus replication (5,18). A stage classification system has been recently proposed to improve the therapeutic strategies employed in COVID-19 patients so far, by distinguishing the phase where the viral pathogenicity dominates versus when the host inflammatory response overtakes the pathology (18). Such approach may be helpful to better correlate stage of illness severity with response to therapy and clinical outcomes, and to weight the benefit/risk ratio for the currently investigated pharmacological treatments.

The clinical spectrum of COVID-19 is very wide, ranging from unspecific symptoms (i.e. fever, dry cough), sometimes combined with mild pneumonia and dyspnea and very often associated with olfactory and gustative disorders, to severe pneumonia and altered gas exchange, resulting in approximately 5% of infected patients with severe lung dysfunction, or multiple (extra pulmonary) organ failure (19,20). While we are gaining better knowledge of the clinical features of the disease, a mounting evidence is disclosing risk factors and biochemical parameters associated with poor prognosis that could serve as therapeutic targets. Haemostatic abnormalities, including disseminated intravascular coagulation (DIC), occur in patients affected by COVID-19 along with increased levels of D-dimer. Additionally, the severe inflammatory response, critical illness, and underlying traditional risk factors may all predispose to thrombotic events (21). As a result, one of the most significant poor prognostic features in those patients is the development of coagulopathy. Of note, platelet activation is also likely to be contributing to the hypercoagulability, but the extent to which it does, and which signaling pathways are involved are a matter of debate. In this still evolving clinical scenario,

the use of heparins has been investigated by virtue of their anticoagulant activity, protective action in the vascular endothelium and pleiotropic properties (8).

Overall, based on the aforementioned premises, a rising number of trials are currently authorized worldwide (902 as of April 26<sup>th</sup>) (22), thirteen of which are in Italy (23).

## Antiviral drugs

### *Lopinavir/Ritonavir*

Lopinavir/ritonavir is an orally administered, fixed dose combination approved by EMA and FDA for the treatment of HIV infections. Both the antiretrovirals are protease inhibitors, although lopinavir is the active antiviral moiety. Lopinavir inhibits the proteases of the human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2), thus preventing the cleavage of the gag-pol polyprotein, and producing an immature virus, which is not infectious. Therefore, the antiviral effect mediated by lopinavir is mainly attributable to its ability to prevent the infection of susceptible cells. However, lopinavir has low bioavailability due to a high first-pass effect mediated by the cytochrome P450 3A4 (CYP3A4) in the liver. By inhibiting the CYP3A4 isoenzyme, ritonavir acts as a pharmacokinetic “booster”, reducing the metabolism of lopinavir and increasing its plasma half-life (24). Currently, the lopinavir/ritonavir combination is used for the treatment of adult patients, adolescents and children (aged from 14 days and older) with HIV-1 infection, often associated to other antiviral drugs (25).

Several scientific evidences argued for the off-label use of lopinavir/ritonavir in patients with SARS-CoV-2 infection. In particular, *in vitro* and *in vivo* studies have shown that lopinavir may inactivate 3-chymotrypsin-like protease (3CL<sup>pro</sup>, also known as Main protease, M<sup>pro</sup>), which represents a key target for human coronavirus replication (i.e. SARS-CoV and MERS-CoV) (26,27). Very recently, one *in vitro* study showed that lopinavir inhibits also SARS-CoV-2 replication, with a half-maximal effective concentration (EC<sub>50</sub>) at 26.63 μM (28).

Studies conducted both on patients and on animal models suggested that the lopinavir/ritonavir

combination was able to improve the clinical and symptomatic course of respiratory syndromes caused by SARS-CoV or MERS-CoV (29-31). A clinical trial evaluating lopinavir/ritonavir potential in SARS showed some benefits, such as reduced intubation rates and mortality, particularly if treatment was started early, i.e. within the first 7-10 days of exposure (32). However, the observational retrospective nature of the study prevents for conclusive recommendations.

In February 2020, short guidelines issued by the Zhongnan hospital of Wuhan University for the diagnosis and treatment of SARS-COV-2 pneumonia were published, which recommend the use of lopinavir/ritonavir in the initial stages of disease to reduce mortality and glucocorticoid consumption based on results from a brief systematic review of the literature (33). More recently, an open-label randomized controlled trial (LOTUS China - Lopinavir Trial for Suppression of SARS-CoV-2 in China) evaluated efficacy and safety of lopinavir/ritonavir versus standard care on 199 hospitalized adult subjects with COVID-19 pneumonia (17). In the study, 99 subjects were treated with lopinavir/ritonavir (400 mg/100 mg twice daily for up to 14 days) in addition to standard therapy and 100 patients with only standard therapy. The results showed no statistically significant difference in terms of clinical improvement (hazard ratio [HR] for symptom improvement, 1.31; 95% confidence interval [CI], 0.95-1.80), viral clearance and 28-day mortality (19.2 % vs. 25.0%; absolute difference -5.8% in favor of the antiviral combination did not reach statistical significance; 95% CI, -17.3%-5.7%). Although the delayed treatment start (median time from symptom onset to randomization, 13 days, interquartile range [IQR] 11-16) may partially accounts for the ineffectiveness of treatment, a post hoc analysis by subgroups showed no benefit in patients treated within 12 days from the onset of symptoms (HR, 1.25; 95% CI, 0.77-2.05). Moreover, 13 patients (~14%) discontinued therapy early because of gastrointestinal adverse events. The safety profile of lopinavir/ritonavir is of particular concern in SARS-CoV-2 infected patients. Common lopinavir/ritonavir adverse effects include nausea, diarrhea (up to ~30%), and hepatotoxicity (2%-10%), and these conditions may be exacerbated in COVID-19 patients, since 20% to 30% of them present with transaminases

elevation (34). Other detrimental factors may be drug-drug interactions (i.e. co-administration of CYP3A4 substrates/inducers) and potential severe adverse drug reactions (pancreatitis, liver damage, cardiac conduction abnormalities) (25).

Although several RCTs are ongoing, the current evidence suggests poor benefit from lopinavir/ritonavir therapy. Treatment should be limited to COVID-19 patients of lesser severity and applied in the initial stages of the disease.

In addition, the darunavir/cobicistat combination (i.e., protease inhibitor/CYP3A4 inhibitor) is being also used as an alternative to lopinavir/ritonavir because of its greater intestinal tolerability (35). An *in vitro* study showed also activity of darunavir against SARS-CoV-2 (36). In COVID-19 patients, it is administered as one tablet (darunavir 800 mg/cobicistat 150 mg) daily for 5 days in combination with standard treatments. A phase 3, randomized, open label clinical trial evaluating efficacy and safety of darunavir/cobicistat for the treatment of pneumonia caused by SARS-CoV-2 is ongoing, with an estimated primary completion date at 31 August 2020 (37).

#### *Remdesivir*

Remdesivir (GS-5734) is a monophosphoramidate pro-drug of the nucleoside analogue of adenine GS-441524. Inside the cells, GS-441524 is converted into the pharmacologically active form of triphosphate (GS-443902), which in turn is able to inhibit the viral RNA-dependent RNA polymerase (RdRp). Emerged during the screening of agents against RNA viruses, remdesivir proved promising against Ebola infections, inhibiting RdRp with a low  $EC_{50}$  and with a high selectivity towards the viral enzyme (38). Remdesivir has shown broad-spectrum antiviral activity with good *in vitro* and *in vivo* efficacy on animal models against various RNA viruses, such as SARS-CoV and MERS-CoV, including SARS-CoV-2 ( $EC_{50}$ : 0.77  $\mu$ M;  $EC_{90}$ : 1.76  $\mu$ M) (16,39,40). Hence, several *in vitro* studies point to remdesivir as a promising candidate for the treatment of respiratory disease COVID-19, particularly in association with chloroquine (16). Remdesivir is currently not approved for marketing in any country for any indication, and must be obtained via compassionate use or enrollment in a clinical trial. As recom-

mended for compassionate use, treatment may begin following a worsening of the patient's clinical status. A phase 1 clinical trial, evaluating safety and pharmacokinetics of single- and multiple-dose regimen, found that intravenous infusions of remdesivir between 3 and 250 mg were well tolerated, with no signs of liver or kidney damage (41). In this dose range, kinetics was linear, showing intracellular half-life of more than 35 hours. On a multiple-dosing regimen the treatment induces a reversible transaminase elevation.

Firstly used in clinic for the treatment of Ebola virus disease (42), remdesivir has been successfully used for treating COVID-19, as reported by two recent case reports (43,44).

Treatment schedule consists of a 200-mg loading dose on day 1, followed by 100-mg daily infusions (1 hour) for 5 or 10 days, depending on the active protocol. Several ongoing studies are evaluating remdesivir efficacy and safety in COVID-19, including two randomized open-label phase 3 trials promoted by Gilead Sciences, enrolling patients with moderate (NCT04292730, EudraCT 2020-000842-32) (45) or severe (NCT04292899, EudraCT 2020-000841-15) (46) disease. These trials are also active in Italy, where the Italian Medicines Agency (AIFA) cooperates in identifying centers with the largest number of hospitalized patients (note: "Azienda Ospedaliero-Universitaria di Parma", Italy, is actively contributing to both studies) (47). The potential of remdesivir in COVID-19 is tested in Italy also in the context of the WHO-promoted SOLIDARITY mega-trial (32 centers involved) (48) and several compassionate use programs (49). Other clinical trials are currently testing remdesivir worldwide, such as DisCoVeRy - an open-label randomized study based on a WHO adaptive protocol to compare various treatments in hospitalized patients, promoted by the French INSERM (NCT04315948 (50), EudraCT 2020-000936-23); an international double-blind placebo-controlled study sponsored by the U.S. National Institute of Allergic and Infectious Diseases (NIAID), conducted in USA, South Korea and Singapore (NCT04280705) (51). Two randomized double-blind placebo-controlled studies in China, on patients with mild/moderate (NCT04252664) (52) and severe (NCT04257656) (53) COVID-19, are currently suspended and termi-

nated, respectively.

Very recently, a study evaluated clinical outcomes in a cohort of 53 patients hospitalized for severe COVID-19 in USA, Europe, Canada and Japan, treated with intravenous remdesivir for compassionate use (54). Median symptom duration before receiving the treatment was 12 days (IQR, 9 to 15), 30 patients (57%) receiving mechanical ventilation and 4 (8%) being on extracorporeal membrane oxygenation (ECMO). During a median follow-up of 18 days, the need for oxygen support improved in 36 (68%) patients; 17 of 30 patient receiving mechanical ventilation were extubated; 26 patients (47%) were discharged; 7 patients died (overall mortality, 13%; 18% in those receiving mechanical ventilation; 5% in those receiving noninvasive ventilation or oxygen supplementation). The study suggests substantial benefit from remdesivir treatment in people with severe COVID-19. However, the study lacks a comparison group, thus preventing definitive conclusions and pointing to RCT results for further recommendations.

#### *Chloroquine and hydroxychloroquine*

The therapeutic use of chloroquine (CQ) and hydroxychloroquine (HCQ) dates back to the mid-twentieth century. These two drugs are usually indicated in the treatment of both malaria and some autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis (55,56). After oral intake, both drugs are well absorbed in the gastrointestinal tract. They are metabolized by hepatic deamination with elimination half-lives that can reach 45 days and are mainly excreted via the kidneys.

These two drugs have almost identical mechanisms of action. Their pharmacological effects include stabilization of lysosomal membranes, inhibition of polymorphonuclear chemotaxis and phagocytosis, interference with pro-inflammatory cytokine production (e.g., interleukin 1 beta) by monocytes and inhibition of the release of superoxide by neutrophils (56).

CQ and HCQ showed antiviral effects against SARS-CoV-2 infection models *in vitro*. These agents appear to block viral entry into the cells through endosomal acidification, which mediates the virus-host cell fusion, proteolytic processing and inhibition of host receptor glycosylation (57,58). In addition, CQ has been

reported to block SARS-CoV-2 replication at concentrations that can be achieved after a 600-mg oral loading dose in humans (16). *In vitro*, CQ and HCQ exert their antiviral activity against SARS-CoV-2 with  $EC_{50}$  in the low micromolar range, although HCQ shows higher potency ( $EC_{50}$ : 6.14  $\mu$ M [HCQ] vs 23.90  $\mu$ M [CQ]) (59). Moreover, the immunomodulating activity exerted by these drugs may synergistically enhance their antiviral action *in vivo*.

Optimal dosing for CQ and HCQ in COVID-19 has not been univocally defined. Some evidences reported 500 mg orally once or twice daily on 5-10 days for CQ (60). As for HCQ, pharmacokinetic simulations recommended an oral loading dose of 400 mg twice daily on day 1, then 200 mg twice daily in the next 2-5 days (59).

Noteworthy, the use of CQ or HCQ in COVID-19 patients is not yet supported by high-quality scientific evidences. A brief letter from China reported that CQ is superior to control in improving the disease course in more than 100 patients with COVID-19 pneumonia (61). Although this could be the first evidence of clinical benefits from CQ treatment in such context, trial design and outcome data are still lacking, thus preventing any further inference. Two small clinical trials studied the therapeutic potential of HCQ in COVID-19 patients. A Chinese study of 30 patients on standard care (supportive care, antivirals), randomly (1:1) assigned to HCQ 400 mg daily, for 5 days, or standard care only, reported no improvement in viral clearance (93.3% vs 86.7%, respectively;  $p > 0.05$ ) (62). On the other hand, a French study on 36 patients (20 on treatment, 16 on control arm) assigned to HCQ 200 mg every 8 hours or to standard supportive care reported higher viral clearance in HCQ group (14/20, 70%) compared to control group (2/16, 12.5%). Moreover, authors claimed further improvement by adding azithromycin to HCQ vs HCQ alone (6/6, 100% vs 8/14, 57%, respectively).

Data from these studies should be interpreted with care since they present major limitations, such as small sample size and poor study design. In particular, the French study did not control for patient dropout (early cessation due to critical illness and/or toxicity) and baseline viral load. Clinical or safety outcomes were also not reported. A recent retrospective

analysis of data from 368 patients hospitalized with confirmed SARS-CoV-2 infection in USA found no evidence that using HCQ in combination with or without azithromycin reduced the risk of mechanical ventilation COVID-19 patients (63). On the contrary, they found association of increased overall mortality in patients treated with HCQ alone.

These findings highlight the importance of awaiting the results of ongoing prospective RCTs (64,65) before recommending a widespread use of these drugs. Although CQ and HCQ are in general well tolerated, their use should be carefully monitored in some patient subsets. Serious adverse effects may occur, such as hemolysis (in patients with glucose 6-phosphate dehydrogenase deficiency), retinal toxicity, hearing reduction, neuropsychiatric symptoms, agranulocytosis, hypoglycaemia, hypotension and prolongation of the QT tract (66). For this reason, CQ or HCQ should not be administered in patients receiving concomitant QT-interval prolonging agents (e.g. amiodarone, azithromycin or other macrolides and fluoroquinolones). Compared to CQ, HCQ seems to determine a slightly milder toxicity profile in animal models and in humans (67,68).

#### *Other antivirals*

Several antivirals have been used since the COVID-19 outbreak in China and worldwide. However, most of them, including oseltamivir and ribavirin, have shown limited value or futility (10).

Umifenovir (also known by its trade name Arbidol) is a more promising antiviral agent, approved in Russia and China for the treatment and prophylaxis of influenza at the dose of 200 mg orally every 8 hours. In addition, umifenovir showed *in vitro* activity against SARS-CoV (69). Its mechanism of action is peculiar, since the drug seems to block the interaction between S protein and ACE2 receptor, thus preventing the fusion of viral envelope with cell membrane (70). Currently 8 clinical trials are studying umifenovir in COVID-19 patients (71), in combination with other therapeutics or in monotherapy (72). At moment, clinical evidence in COVID-19 is limited to observational data, though suggesting lower mortality and higher discharge rate in umifenovir-treated patients (73).

Favipiravir is an antiviral drug, approved in Japan (trade name, Avigan) for the treatment of influenza from

new or re-emerging viral strains which do not respond to conventional antiviral therapies (74). Many countries have used it off-label to treat new viral infections, including Ebola and Lassa. More recently, it was approved in China for the treatment of COVID-19. Favipiravir is a prodrug analogue of a purine nucleotide, favipiravir ribofuranosyl-5'-triphosphate (favipiravir-RTP). The active metabolite is recognized as a substrate, which selectively inhibits the RNA-dependent RNA polymerase (RdRp) of RNA viruses, halting viral replication. The catalytic domain of RdRp is preserved in various types of RNA viruses, which supports the wider antiviral spectrum of favipiravir. In addition, the drug has been shown inducing a high rate of RNA mutations, thus generating a non-viable viral phenotype (75,76). The *in vitro* EC<sub>50</sub> estimates of favipiravir showed much higher potency against influenza, compared to Ebola and SARS-CoV-2 (77,78). Accordingly, dosing regimens *in vivo* have been modified in COVID-19 studies, in which oral loading doses of 1800 mg twice daily on day 1, followed by 800 mg twice daily in the next 2-5 days have shown higher antiviral potency (79,80). After oral dosing, favipiravir showed almost complete bioavailability (97,6%) but complex nonlinear pharmacokinetics, which is also affected by differences in body weight and ethnicity (81). The elimination half-life is approximately 2-5 hours; however, lower than expected plasma levels have been observed in patients with Ebola or severe flu, raising concerns about bioavailability and/or metabolism alterations in seriously ill patients (82,83). The drug is overall well tolerated, with adverse reactions, such as hyperuricemia, diarrhea, elevated transaminases and neutropenia, occurring in a dose-dependent manner (81,84).

To date, limited clinical experience supports the use of favipiravir for COVID-19. A prospective, randomized, multicenter study compared favipiravir with umifenovir (120 patients in each arm) for treating moderate to severe COVID-19 infection (85). Favipiravir improved clinical recovery at day 7 in patients with moderate infections (71.4% favipiravir vs 55.9% umifenovir,  $p=0.0199$ ). No significant differences were observed by comparing treatments in severe or severe-moderate (combined analysis) arms.

Recently, preliminary results from an open-label, controlled, non-randomized clinical trial on 80 patients

with laboratory-confirmed COVID-19 and with the onset of symptoms in the 7 days prior to enrollment in the study have been published (86). Favipiravir showed a higher antiviral activity than lopinavir/ritonavir, in terms of virus elimination and rate of improvement of thoracic CT imaging. However, these results must be interpreted with caution considering the preliminary nature of the study, which has methodological limitations (open-label study, no randomization, in both groups patients took interferon alfa-1b by aerosol, exclusion of severely ill patients).

Several clinical studies testing efficacy and safety of favipiravir in COVID-19 are ongoing (87), including a multi-center, randomized, double-blind, placebo-controlled study coordinated in Italy at the "ASST Fatebenefratelli Sacco", Milan (NCT04336904) (88).

Other investigational compounds, such as DAS181 (Fludase), have been considered promising based on their mechanism of action and preclinical data, showing broad spectrum antiviral activity (89-91). DAS181 is a novel sialidase fusion protein which enzymatically cleaves viral receptors on the host epithelial cell, preventing the binding of influenza, parainfluenza, and other viruses (91). No clinical evidence has been reported so far, but at least four clinical trials testing its potential against COVID-19 are underway (92) (NCT04324489; NCT04298060; NCT03808922; NCT04354389 - this is a multicenter, randomized, placebo-controlled, double-blind study coordinated in Italy by "Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico Milano" and "Azienda Ospedaliero-Universitaria Policlinico di Modena" with estimated primary completion by June 2020 (93)).

Very recently, more than ten thousand pharmacologically active compounds, including many approved drugs, were screened as inhibitors of the SARS-CoV-2 main protease (3CL<sup>pro</sup> or M<sup>pro</sup>) (94). Out of six agents selected, ebselen (also known as PZ-51 or DR-3305) exhibited higher potency (half-maximal inhibitory concentration: 0.67  $\mu$ M) and promising antiviral activity in cell-based *in vitro* assays. This is of interest because ebselen has already been tested in clinical setting because of its antioxidant or lithium-mimetic properties (95). Further studies are needed to test its *in vivo* efficacy against SARS-CoV-2 infection.

## Drugs targeting inflammation and hypercoagulability

Great expectations are currently placed on the use of drugs selectively blocking the inflammatory response triggered by SARS-CoV-2 and the associated thromboembolism. Moreover, other agents affecting crucial signaling pathways in host cells have been proposed. Since these drugs could potentially modify the course of the disease even in patients with severe infections, the topic is of great interest. However, no high-quality evidence exists and no general recommendations for their use can be made yet.

Some repurposed and investigational drugs are reviewed here below.

### *Tocilizumab*

Interleukin 6 (IL-6) is a cytokine relevant to many inflammatory and metabolic processes in the body (96). High levels of IL-6 are implicated in the pathogenesis of various inflammatory and autoimmune disorders, including many forms of rheumatic diseases. IL-6 is also involved in the cytokine release syndrome (CRS), a systemic inflammatory response triggered by several factors such as infections and certain drugs, also known as “cytokine storm” (96,97). In CRS, plasma and tissue levels of various pro-inflammatory cytokines (e.g. TNF- $\alpha$ , IL-1, IL-6) and chemokines (e.g., IL-8) increase, producing long-term damage and fibrosis of the lung tissue, clotting disorders and multi-organ dysfunction.

Tocilizumab (trade name RoActemra) is a humanized monoclonal antibody that blocks the interleukin 6 receptor, thus inhibiting the downstream inflammatory cascade. Tocilizumab is indicated for the treatment of severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, juvenile idiopathic polyarthritis and for the treatment of chimeric antigen receptor T-cell (CAR-T) induced CRS (98) in adults and pediatric patients (aged 2 years or older).

The rationale for its off-label use in patients with severe COVID-19 is based on the evidence that SARS-CoV-2 infection induces an excessive and aberrant host immune response (99). This response may further evolve toward an acute respiratory distress syndrome and, in most critically ill patients, triggering a

“cytokine storm” (100). By analogy with the treatment of CAR-T associated CRS, tocilizumab has been used in small groups of severe COVID-19 cases, with early reports of success. A Chinese report of 21 patients with severe or critical COVID-19 pneumonia showed that treatment with tocilizumab 400 mg IV reduced oxygen demand in 15/20 (75%) patients, induced resolution of lung injury as reported by CT scan in 19/21 (91%) patients, normalization of lymphocyte count (10/19), reduction of PCR levels (16/19) and hospital discharge (19/21), with an average hospitalization of 13.5 days (101). However, the small sample size and the lack of a comparison group prevent any firm conclusion about the drug’s specific effects.

By the end of April 2020, more than twenty-five RCTs are testing the efficacy of tocilizumab, alone or in combination, in COVID-19 patients with severe disease are ongoing (102) (e.g., three studies authorized by AIFA in Italy (23): NCT04317092 – a study coordinated by “Istituto Nazionale Tumori, IRCCS, Fondazione G. Pascale”, Naples (103); NCT04346355 – another study involving also the “Azienda Ospedaliero-Universitaria di Parma” (104); NCT04320615 – a multicenter study promoted by F. Hoffmann-La Roche Ltd (105)). Treatment consists of one single intravenous infusion of tocilizumab, at the dose of 8 mg/kg, up to a maximum dose of 800 mg. Primary completion of these studies is expected between May and December of 2020.

The safety profile of tocilizumab should be monitored, since peculiar toxicity may occur. As an immunosuppressive drug, there is a risk of reactivating latent infections (i.e., mycobacteria). Other adverse reactions include neutropenia and thrombocytopenia, hepatotoxicity and severe hypersensitivity reactions. It is known that IL-6 reduces the expression and activity of specific cytochrome P450 (CYP) enzymes, particularly CYP3A4. Therefore, caution should be used for potential interactions, as tocilizumab can reverse the inhibitory effect of IL-6 and restore the CYP3A4 activity, modifying the exposure and activity of CYP3A4 substrate drugs (106,107).

### *Sarilumab*

Sarilumab (trade name Kevzara) is a fully human monoclonal antibody that binds specifically to

both soluble and membrane-bound IL-6 receptors, inhibiting IL-6 mediated signaling. Hence, it shares its mechanism of action, most of its therapeutic indications, and toxicity, with tocilizumab. Currently, high (400 mg IV) or low (200 mg IV) dosing protocols are under investigation. At least ten studies are evaluating efficacy and safety of sarilumab in monotherapy or in combination in COVID-19 patients (108) (e.g., NCT04327388 – a multicenter study promoted by Sanofi/Regeneron Pharmaceuticals, authorized in Italy by AIFA and involving the “Azienda Ospedaliero-Universitaria di Parma” (109)).

#### *Anakinra and Emapalumab*

Anakinra (trade name Kineret) is a biological, recombinant analogue of the human interleukin-1 type I receptor (IL-1RI) antagonist (IL-1RA). In particular, the drug inhibits the biological response to IL-1 $\alpha$  and IL-1 $\beta$ , two important pro-inflammatory cytokines (110). In European Union (EU) countries, it is authorized for the treatment of rheumatoid arthritis (RA), Cryopyrin-Associated Periodic Syndromes (CAPS) and Still's Disease, in monotherapy or in combination with other anti-inflammatory and disease-modifying drugs (111).

Emapalumab (trade name Gamifant) is a fully human monoclonal antibody, which binds and neutralizes interferon- $\gamma$  (IFN- $\gamma$ ) (112,113). This cytokine is critical for innate and adaptive immunity against viral and other infections, exerting important immunomodulatory effects. Moreover, aberrant IFN $\gamma$  expression has been found in some autoinflammatory syndromes and autoimmune diseases (114,115). In November 2018, emapalumab received the global approval for the treatment of pediatric and adult patients with primary hemophagocytic lymphohistiocytosis (HLH) with refractory, recurrent, or progressive disease or intolerance to HLH therapy (112,113). In EU, the drug received orphan designation and Priority Medicine (PRIME) status by the European Medicines Agency (EMA) (116).

The rationale for the off-label use of anakinra or emapalumab in the treatment of COVID-19 patients lies in their ability of counteracting the hyper-inflammatory response triggered at the lung level and the “cytokine storm” (see above). At the moment there

is no information on these alleged positive effects, but the great interest is attested by the clinical studies launched worldwide. By the end of April 2020, at least seven phase 2 or 3 RCTs have been registered for anakinra (117), and one study evaluating the efficacy and safety of both anakinra and emapalumab versus standard of care (118). The latter is an open label, controlled, parallel group, 3-arm, multicenter study coordinated in Italy and promoted by Swedish Orphan Biovitrum (SOBI) company (NCT04324021 – authorized by AIFA (23), it also involves “Azienda Ospedaliero-Universitaria di Parma” as a research center (118)). Anakinra and emapalumab are administered according to a complex dosing scheme, consisting of intravenous infusions over several days and at different dosages. Importantly, all patients being treated with emapalumab receive also methylprednisolone, as recommended. The primary completion date is estimated on July 2020.

#### *Colchicine*

Colchicine is an old drug, initially extracted from the autumn crocus (*Colchicum autumnale*) and used for centuries in counteracting various inflammatory diseases, though its mechanism of action became clearer in the last decades. Colchicine disrupts microtubule dynamics, both by inhibiting tubulin polymerization and by fostering its depolymerization (119,120). The block of microtubules affects many cell pathways involved in the inflammation machinery, thus impairing the expression of cell adhesion molecules and the secretion of several cytokines and chemokines (121,122). In particular, through this mechanism colchicine counteracts the assembly of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome, thereby reducing the intracellular processing and the release of IL-1 $\beta$  and other interleukins, including IL-6 (123). Moreover, it is known that colchicine concentrates in neutrophils and macrophages, prolonging its own action. Beyond its anti-inflammatory and immunomodulating activities, colchicine showed also some antiviral properties. By damaging microtubules, the drug reduced the cell entry and the intracellular trafficking of viral particles (124,125). Interestingly, microtubules seems to play a role also in the replication of SARS-CoV, by favouring

the formation of double-membrane vesicles in infected cells (126). Noteworthy, other studies showed that the envelope protein E (viroprotein E) of SARS-CoV, as well as the SARS-CoV accessory protein open reading frames (SARS 3a) alter ionic equilibrium in intracellular compartments and activate NLRP3 (127,128). However, whether SARS-CoV-2 may replicate the same mechanisms is still unknown.

Colchicine is currently used in the treatment of gout, pericarditis, Familial Mediterranean Fever and Behçet's disease (122). Its ability in counteracting the "cytokine storm" lays the ground for its use also in COVID-19 patients. The specific inhibition of NLRP3 exerted by colchicine further supports its use in this context. Colchicine is a well-known, inexpensive, well tolerated drug, with a long-standing use in clinic and this could be an advantage over newer immunomodulating drugs.

These premises prompted researchers and clinicians to test efficacy and safety of colchicine in SARS-CoV-2 infected patients. At least seven studies have been registered at the moment, including two Italian studies authorized by AIFA (EudraCT 2020-001475-33 (129) – an interventional, pilot, multicenter, randomized, open-label, phase 2 study, coordinated by the "Azienda Ospedaliero-Universitaria di Perugia"; EudraCT 2020-001258-23, NCT04322565 (130) – a multicenter, randomized, open-label, phase 2 study on COVID-19 patients at low or intermediate risk, coordinated by the "Azienda Ospedaliero-Universitaria di Parma"). Colchicine is orally dosed 1 mg daily, half dose (0.5 mg) daily in case of acute or chronic kidney failure, or 0.5 mg every other day in case of advanced liver impairment. Moreover, caution should be applied for the concomitant use of colchicine and strong inhibitors of P-glycoprotein or CYP3A4 (e.g., clarithromycin, erythromycin) for the risk of drug-drug interactions.

### *Heparins*

Since its discovery in 1916, the efficacy of heparin for the prevention and treatment of venous thrombosis and pulmonary embolism, the prevention of mural thrombosis after myocardial infarction (MI), and the treatment of patients with unstable angina and MI has been largely documented. However, unfractionated heparin (UFH) displays some limitations, based on its

pharmacokinetic, biophysical, and non-anticoagulant biological properties, that paved the way to the development of low-molecular-weight heparins (LMWHs). LMWHs, derived from heparin by chemical or enzymatic depolymerization, have shown a more predictable anticoagulant effect than UFH, are easier to administer, and may not require monitoring (131-133).

Preliminary laboratory information and earlier clinical observations indicated that the plasma of COVID-19 patients is hypercoagulable, thus suggesting that early anticoagulation may inhibit clotting formation and reduce micro-thrombi, thereby reducing the risk of major organ damage. The use of anticoagulants (such as heparin) for patients with severe COVID-19 was initially recommended by an expert consensus in China (134). Two subsequent studies evaluated the potential benefit of LMWHs in patients with COVID-19.

In a retrospective study of 449 patients with severe COVID-19, 7-day anticoagulant therapy mainly with LMWH appeared to be associated with lower mortality in the subpopulation meeting sepsis-induced coagulopathy criteria or with markedly elevated D-dimer (8). While the study suggests that there is a survival advantage for those patients with COVID-19 who receive administration of prophylactic dose of heparin agents, it did not describe to what extent heparin improves the abnormal coagulation. In the second study, which was not peer-reviewed before publication, LMWH was found improving the coagulation dysfunction of COVID-19 patients and exerting anti-inflammatory effects by reducing IL-6 and increasing lymphocyte percentages (135). Furthermore, it has been suggested that the non-anticoagulant properties of heparin, e.g. its anti-inflammatory function, may also be relevant in this setting as well as heparin ability to protect the endothelium may help impacting the microcirculatory dysfunction and possibly limiting organ damage. Finally, another interesting concept is the potential antiviral action of heparin by virtue of its poly-anionic nature that would make heparin a potential effective inhibitor of viral attachment as documented in herpes simplex virus or SARS-associated CoV infections (136-138).

Nevertheless, additional findings from larger and randomised studies are needed to determine whether

heparin may be beneficial in COVID-19 patients. In this context, matters worthy of investigation are the proper timing, dosages and administration scheme of heparins, as well as the evaluation whether *in vitro* studies of anti-inflammatory functions, endothelial protection and viral inhibition should be considered independently of anticoagulant properties (20,139).

Earlier case reports and later clinical observations suggested that the incidence of venous thromboembolism (VTE) could be higher among COVID-19 patients with increased D-dimer levels predicting VTE risk thus raising questions on the clinical significance of increased D-dimer in severe COVID-19 and the role of anticoagulant therapy in the prophylaxis and treatment of these patients. However, it is also possible that a pulmonary embolism could occur in more severely ill COVID-19 patients before hospitalizations thus clarifying the documented ineffectiveness of prophylactic doses of heparins during their hospital stay (20).

Recently, both the International Society on Thrombosis and Haemostasis (ISTH) and the American Society of Hematology (ASH) suggested that all hospitalized COVID-19 patients should receive thromboprophylaxis, or full therapeutic-intensity anticoagulation if such an indication is present (140). However, some argue that it would be necessary to recalibrate the recommended approach to the use of heparin by considering early initiation of therapeutic anticoagulation with UFH prior to significant clinical deterioration to avoid further decline in patients without significant bleeding risks (141). Furthermore, the use of therapeutic doses of UFH or LMWH is currently not supported by evidence outside of established diagnoses of VTE or as a bridging strategy in patients on vitamin K antagonists (VKA), and cannot be recommended as a standard treatment for all COVID-19 patients (20). Overall, the hypothesis of improving the clinical outcome of COVID-19 patients by simple and inexpensive antithrombotic drugs is very attractive, but more robust evidence from properly designed clinical trials with strong endpoints are necessary.

#### Miscellanea

The whole scientific community and health professionals are involved in an enormous effort aimed at evaluating as many therapeutic options as possible.

Thousands of compounds are indeed considered (142), and the number of those deemed promising grows on a daily basis. This brief overview of emerging drugs must be necessarily selective. Nevertheless, due to the peculiar mechanism of action proposed, the following therapeutic and investigational compounds deserve a brief mention.

Baricitinib is a small molecule inhibitor of Janus kinase subtype 1 and 2 (JAK1, JAK2), approved in EU and USA for the treatment of rheumatoid arthritis resistant to TNF $\alpha$ -antagonist therapy (143,144). Baricitinib has been selected as a molecule potentially useful in patients with COVID-19 because of its dual anti-inflammatory and antiviral activity *in vitro*. The latter has been recently reported by a study identifying baricitinib as an inhibitor of AP2-associated protein kinase 1 (AAK1), a protein playing a key role in endocytosis and viral entry in the alveolar type 2 epithelial cells (145,146). Clinical trials studying baricitinib in COVID-19 patients are ongoing (147).

In Italy, AIFA also authorized Ruxolitinib, another JAK1/2 inhibitor, and Canakinumab, a monoclonal antibody targeting IL-1 $\beta$  potentially counteracting the “cytokine storm”, for compassionate use in COVID-19 patients (49).

Camostat mesilate is a non peptidic, orally bioavailable serine protease inhibitor, approved in Japan for the treatment of pancreatitis. It can also inhibit TMPRSS2, the host cell serine protease which is important for viral entry (14,148), and the same mechanism has been recently shown for the mucolytic cough suppressant drug bromhexine (149). Both drugs are currently studied as a promising therapeutic agent in COVID-19 (150,151).

Gabexate mesilate is another synthetic serine protease inhibitor indicated for the anticoagulant treatment in disseminated intravascular coagulation (152). Gabexate is also able to reduce the production of inflammatory cytokines and its use has also been explored in cancer, ischemia/reperfusion injury and pancreatitis (153-155). The drug exerts potent anticoagulant actions as a direct inhibitor of kallikrein, plasmin and thrombin (156). Finally, gabexate could block the entry of the virus into lung cells by inhibiting TMPRSS2. Nafamostat mesilate, a molecule structurally related to gabexate, has been shown to inhibit TM-

PRSS2 (157,158) and is already under clinical testing in COVID-19 (159).

### Controversies

Since ACE2 has been discovered as the cell-entry receptor for SARS-CoV-2, scientists and clinicians questioned whether ACE inhibitors or angiotensin receptor blockers may have any therapeutic or harmful role in treating COVID-19 patients (160). Data reported so far remain contradictory and inconclusive, warranting the indication of continuing therapy for patients already treated with these drugs (161,162).

Another controversial issue concerns the use of corticosteroids. As effective anti-inflammatory drugs, they may reduce lung damage associated with SARS-CoV-2 infection, thwarting the onset of ARDS. However, the risk-benefit balance is unclear, since delayed viral clearance or secondary infections may arise due to their broad immunosuppressive activity (163). Unless otherwise indicated for concomitant use (e.g., methylprednisolone and emapalumab) or comorbidities, the current evidence warns about their use in this context.

### Conclusions and perspectives

The world is currently facing one of the largest public health emergencies since the 1918 influenza outbreak. From the beginning, the scientific community has devoted enormous efforts in organizing a rapid and effective response against this new viral disease. It is worth noting that the pathogenetic mechanisms of SARS-CoV-2 have been only partially unraveled. Of great practical relevance is the fact that, it is still not known if stable and effective immunological memory against SARS-CoV-2 may develop after the first infection. Data on recovered patients are still scarce and anecdotal, and the use of convalescent plasma, isolated from putatively immune subjects, as a post exposure prophylaxis led to conflicting results (164). Notwithstanding, over 45 studies using hyperimmune plasma have been launched worldwide (165), including two Italian studies coordinated in Pavia (166) and Bergamo (167), whose preliminary results seem very encouraging.

Pending further research, enormous resources have been also ploughed into vaccine development

programs. Vaccine is certainly the most effective resource for preventing new coronavirus epidemics in the long term. As of this writing, more than 120 vaccines are in the pipeline, 70 of which are in preclinical evaluation and 7 already in clinical development (mainly in phase 1) (168).

In contrast, no drug has been approved so far for the treatment of COVID-19. Though promising pharmacological strategies are emerging in parallel with the growing knowledge of the disease (Fig. 1), the availability of higher quality clinical evidence in the near future remains compelling, since most of the studies completed so far showed high risk of bias or did not undergo a rigorous peer-review process.

Hundreds of phase 2 or phase 3 clinical trials are currently ongoing and even more are on the starting blocks. Old and recent drugs are being repurposed while a number of investigational compounds showed promise in preclinical and clinical studies. Expensive biological drugs, such as monoclonal antibodies, are being employed for compassionate use or in clinical studies, generating wide interest. However, it is likely that their indications will eventually be restricted to the most severe forms of the disease.

The identification of drugs with a good efficacy and safety profile, allowing a satisfactory control of COVID-19 not only in hospitals, but also at home, is a rational goal to be achieved in the near future.

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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