



REVIEW

Drugs against SARS-CoV-2: What do we know about their mode of action?

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Summary

The health emergency caused by the recent Covid-19 pandemic highlights the need to identify effective treatments against the virus causing this disease (SARS-CoV-2). The first clinical trials have been testing repurposed drugs that show promising anti-SARS-CoV-2 effects in cultured cells. Although more than 2400 clinical trials are already under way, the actual number of tested compounds is still limited to approximately 20, alone or in combination. In addition, knowledge on their mode of action (MoA) is currently insufficient. Their first results reveal some inconsistencies and contradictory results and suggest that cohort size and quality of the control arm are two key issues for obtaining rigorous and conclusive results. Moreover, the observed discrepancies might also result from differences in the clinical inclusion criteria, including the possibility of early treatment that may be essential for therapy efficacy in patients with Covid-19. Importantly, efforts should also be made to test new compounds with a documented MoA against SARS-CoV-2 in clinical trials. Successful treatment will probably be based on multitherapies with antiviral compounds that target different steps of the virus life cycle. Moreover, a multidisciplinary approach that combines artificial intelligence, compound docking, and robust in vitro and in vivo assays will accelerate the development of new antiviral molecules. Finally, large retrospective studies on hospitalized patients are needed to evaluate the different treatments with robust statistical tools and to identify the best treatment for each Covid-19 stage. This review describes different candidate antiviral strategies for Covid-19, by focusing on their mechanism of action.

Highlights

- SARS-CoV-2 is a major threat to public health in the absence of drugs and vaccines.
- The development of antiviral agents is urgently needed to treat patients with Covid-19 and limit SARS-CoV-2 dissemination.

Abbreviations: 3CLpro, chymotrypsin-like 3; Abs, antibodies; ACE2, angiotensin-converting enzyme 2; CoV, coronaviruses; E, envelope protein; ExoN, exonuclease; FDA, Food and Drug Administration; HCQ, hydroxychloroquine; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; M, membrane protein; mAbs, monoclonal Abs; MERS, Middle East respiratory syndrome; MoA, mode of action; N, nucleoprotein; nsp, nonstructural protein; PLpro, papain-like; RdRp, RNA-dependent RNA polymerase; S, spike protein; SARS, severe acute respiratory syndrome; TMPRSS2, transmembrane protease serine 2.

- Several studies identified compounds that limit SARS-CoV-2 replication in vitro and determined the mode of action of some promising antiviral drugs.
- Drug repurposing for Covid-19 treatment is in progress, and the mode of action of these compounds needs to be clarified.

1 | INTRODUCTION TO SARS-CORONAVIRUS 2

Over the last 20 years, three coronaviruses (CoVs) that cause severe pulmonary infections in humans have crossed the species barrier.^{1,2} The last of these CoVs, named SARS-CoV-2, emerged in the Hubei province (China) in December 2019, and rapidly spread worldwide becoming a major public health threat.³ In approximately 20% of patients, the disease progresses to severe pneumonia, respiratory and multi-visceral failure, often leading to death of patients with

comorbidity.⁴ This worsening is associated with a deregulated immune response, including exacerbated production of pro-inflammatory cytokines.⁵ Therefore, specific and effective antiviral therapies are urgently needed, due to the absence of a vaccine.

Coronaviruses belong to the *Nidovirales* order, a group of enveloped viruses with genomic RNA of positive polarity. Their 27 to 34 kb genome encodes 16 nonstructural proteins (nsps) and 4 structural proteins (spike protein [S], envelope protein [E], membrane protein [M], and nucleoprotein [N]).⁶ The virus life cycle, described in Figure 1, begins by the attachment of the viral particle to the

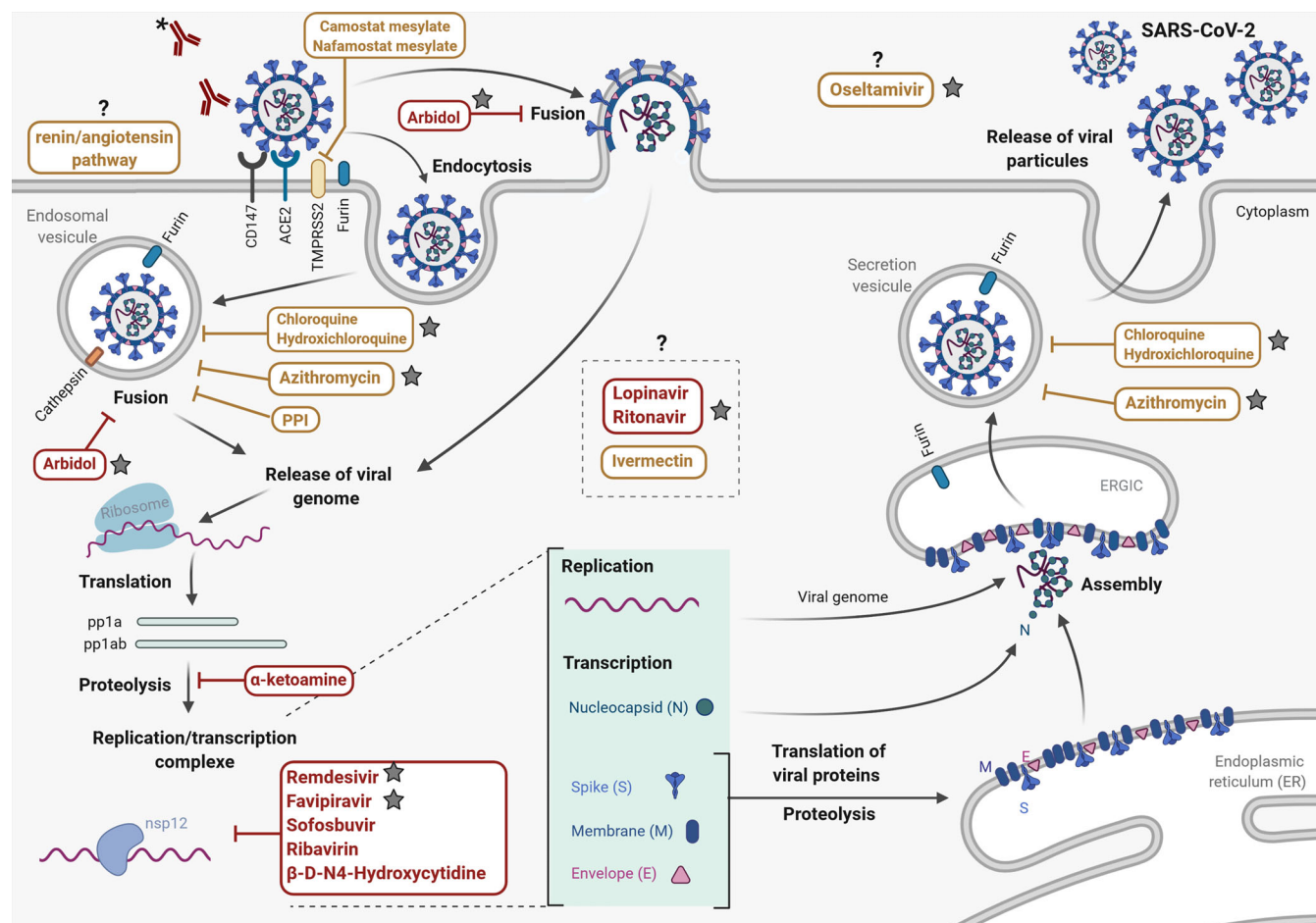


FIGURE 1 SARS-CoV-2 life cycle and potential mechanisms of action of currently evaluated therapeutics. SARS-CoV-2 life cycle and the presumed mechanisms of action (MoA) of the main SARS-CoV-2 replication inhibitors. Different compounds are assessed to find compounds targeting the different steps of SARS-CoV-2 life cycle. Molecules currently in clinical trials are indicated by a gray star. Therapeutic strategies based on antiviral compounds are indicated in red, and approved drugs used for other diseases or selected by virtual screening are indicated in yellow. The black question marks indicate unknown or elusive MoA in the context of CoV infection. *Corresponding to antibody (Ab) strategies including monoclonal antibodies and plasma from convalescent patient. The Biorender website was used to generate this figure

angiotensin-converting enzyme 2 (ACE2) cell surface receptor, mediated by the S protein.^{7,8} Virus entry is achieved by endocytosis and/or direct fusion of the cell and viral membranes. This step requires S protein activation. The S protein is synthesized as an inactive precursor that is inserted in the viral membrane, and requires two subsequent cleavages by cellular proteases to become functionally active. Different cellular proteases, such as furin-like enzymes and the transmembrane protease serine 2 (TMPRSS2), may cleave the S protein in two subunits, S1 and S2, in a process called "priming."^{9,10} A recent study showed that this furin-mediated cleavage is important for virus entry in lung cells.¹¹ In addition, for virus entry, a second proteolytic cleavage is required at the S2' site localized immediately upstream of the fusion peptide,^{12,13} and seems to involve at least TMPRSS2.¹⁴ The viral genome is then released into the cytoplasm of the infected cells. This allows the translation of the viral mRNA into two polyprotein precursors, pp1a and pp1ab, controlled by a -1 ribosomal frameshift. These polyprotein precursors are then cleaved by two viral proteases, chymotrypsin-like 3 (3CLpro) and papain-like (PLpro), to generate the 16 nsps (nsp1 to 16). Many of these proteins participate in the formation of the replication and transcription complex that orchestrates genome replication, mRNA synthesis and capping. At the final stage of viral infection, the N protein assembles with the neo-synthesized viral genome to form the nucleocapsid that associates with the viral structural proteins to generate new virions released by exocytosis.⁶

The ongoing Covid-19 pandemic has already caused at least 4 million confirmed cases and more than 250 000 deaths. In response to this global health emergency, public health measures to control the virus spread have been put in place and efforts to identify potential antiviral molecules and to develop a vaccine have been intensified. The explored antiviral strategies are mainly based on existing drugs that were developed to treat viral infection or other diseases. Moreover, the many different clinical trials currently in progress (more than 2400) are testing a limited set of drugs, alone or in combination.¹⁵ Although the drugs used in these therapeutic assays have shown some inhibitory effect in SARS-CoV-2-infected cells *in vitro*, the molecular bases of their antiviral activity are often poorly understood, except for nucleoside analogs (eg, favipiravir for influenza virus) and fusion inhibitors (eg, arbidol). In addition, while waiting for the final results of these clinical studies, important concerns have arisen, such as the risk of inappropriate self-treatment, the potential toxicity or adverse effects of some of these compounds, and the risk of depletion of pharmacy stocks needed for the treatment of other pathologies. On the other hand, the emergence of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) has led to a growing number of publications that describe new CoV inhibitors and precisely characterize their mode of action (MoA). In this review, we summarize promising candidate molecules that could be repurposed and anti-CoV-specific drugs derived from ongoing research on CoVs, for which we provide additional information on their potential MoA based on the current knowledge on SARS-CoV-2 life cycle. In this review, we do not describe vaccine strategies, and supporting treatments, although they are also important for Covid-19 management.

2 | CLASSICAL ANTIVIRAL APPROACHES

In the absence of specific therapeutics, supportive care (eg, oxygen therapy or mechanical ventilation) remains the only option for managing severe Covid-19 symptoms.¹⁶ To limit virus proliferation in the early disease stages and reduce its severity, one important strategy is to develop treatments based on existing antiviral compounds. Following the rapid emergence and spread of SARS-CoV in 2003 and MERS-CoV in 2012, important efforts were made to identify drugs that target specific steps of the coronavirus life cycle (Figure 1) including (a) the interaction with ACE2 receptors, (b) enzymes that catalyze S protein cleavage, (c) viral entry, (d) polyprotein processing, and (e) replication/transcription complex. These compounds have been rapidly screened in SARS-CoV-2-infected cells, and many of them are now assessed in small animal models and/or clinical trials.

2.1 | Antibody neutralization

A useful therapeutic strategy consists in interfering with the first step of the viral cycle by targeting the interaction of the viral S protein with the cell surface receptors. It was recently demonstrated that sera from patients with Covid-19 contain neutralizing antibodies (Abs) that can limit SARS-CoV-2 viral infection *in vitro*.¹⁷ Moreover, administration of plasma from convalescent patients has shown some efficacy in patients with respiratory distress, suggesting that passive immunity might help limit SARS-CoV-2 infection.¹⁸⁻²⁰ Clinical trials using plasmapheresis are under way to evaluate the efficacy of this strategy.^{21,22} Nevertheless, recent randomized clinical trial have not shown significant improve for patient under convalescent plasma therapy.²³ The development of monoclonal Abs (mAbs) against the viral S protein is also an option.²⁴ This approach was used in the latest Ebola outbreak and led to the development of ZMapp.²⁵ Neutralizing mAbs against the SARS- and MERS-CoV S protein have antiviral effect in cultured cells and animals infected with SARS-CoV or MERS-CoV.²⁶⁻³⁰ Thus, the development of mAbs targeting the S protein is currently in progress.³¹ The development of mAbs against ACE2 is also an interesting option to reduce viral entry,^{32,33} but could be more risky due to potential interference with the physiological function of the ACE2 receptor, which is involved in angiotensin maturation. Another possibility is to use human recombinant soluble ACE2 to block SARS-CoV-2 entry into the cell.³⁴ This strategy seems to limit SARS-CoV-2 infection in cell culture, but the stability of the soluble receptor in the serum of infected patients and the potential adverse effects in patients remain to be determined. Notably, it has also been shown that ACE2 therapy protects mice from lung injury that can be observed during SARS-CoV infection.³⁵ Interestingly, mAbs against other cellular receptors, such as CD147, also can block viral infection in cell culture, and the direct interaction between CD147 and the S protein has been demonstrated by coimmunoprecipitation.³⁶ These first results need be confirmed, but they suggest that SARS-CoV-2 could use an alternative entry

TABLE 1 Main antiviral molecules currently being tested against SARS-CoV-2 infection

Compounds	Target/MoA	Tests in vitro (CoV)	Clinical trials SARS-CoV-2
Arbidol hydrochloride	Endocytosis/inhibition membrane fusion	(38-40)	NCT04252885 (41,42)
Ribavirin	RNA polymerase/inhibition	(43,44)	No data
Remdesivir	RNA polymerase/inhibition	(45-48)	(49,50)
Favipiravir	RNA polymerase/inhibition	No data	ChiCTR2000030894, NCT04280705, NCT04292730, NCT04292899, NCT04315948, WHO Solidarity Trial (42,51)
β -d-N4-hydroxycytidine	RNA polymerase/inhibition	(52,53)	No data
Sofosbuvir	RNA polymerase/inhibition	(54)	No data
α -ketoamide inhibitor	Proteases/inhibition	(55)	No data
Ritonavir	Proteases/inhibition	(56)	NCT04252885 (41,49,57-59)
Lopinavir	Proteases/inhibition	(56,60)	NCT04252885 (41,49,57-59)
Nelfinavir	Proteases/inhibition	(61)	No data
Dolutegravir	Proteases/inhibition	(39)	No data

Abbreviations: CoV, coronaviruses; MoA, mode of action.

receptor in some cell types. A clinical trial using an anti-CD147 antibody (meplazumab) is currently ongoing in China³⁷; however, its antiviral effect is uncertain because the virus binds with higher affinity to the ACE2 receptor.³³

2.2 | Antiviral molecules

Although SARS-CoV-2 has spread very recently, several antiviral compounds previously tested against other pathogenic CoVs have been already tried patients with Covid-19 (Table 1). The first strategy is to interfere with virus entry using molecules that block virus-cell fusion. This includes umifenovir (Arbidol), an antiviral molecule initially developed for the treatment of influenza infection.⁶² Interestingly, umifenovir has a broad spectrum antiviral activity by inhibiting the entry of other viruses⁶³ and stimulating the immune response.³⁸ Moreover, umifenovir inhibits SARS-CoV-2 infection in vitro with an IC₅₀ of 10 μ M.³⁹ Although not yet approved by the FDA, this broad-spectrum antiviral molecule is used in China and Russia for influenza treatment, and therefore was rapidly included in several clinical trials alone or in combination with other compounds. The first comparative study performed in a small group of patients showed a significant decrease of the viral load in the arm treated with umifenovir in combination with the protease inhibitors lopinavir/ritonavir ($n = 16$) compared with the arm treated with lopinavir/ritonavir alone ($n = 17$).⁴¹ Nevertheless, one of the limitations of this study is the small-sample size and investigation including large cohort need to be performed.

After virus entry, the polyproteins pp1a and pp1ab are processed into 16 nsps by the viral proteases 3CLpro and PLpro. These cleavage events play a key role in the virus life cycle because they lead to the generation, among others, of the RNA-dependent RNA polymerase (RdRp) required for viral RNA replication and transcription. Due to the high conservation of the cleavage sites and of the protease structures, these proteases are an optimal antiviral target. A recent study described the X-ray structures of SARS-CoV-2 3CLpro alone and in

the presence of an α -ketoamide inhibitor.⁵⁵ Based on these findings, the α -ketoamide inhibitor was optimized and showed antiviral activity against SARS-CoV-2 at concentrations lower than 10 μ M in Calu3 lung cells. Protease inhibitors used in human immunodeficiency virus (HIV) therapy, such as lopinavir/ritonavir (a combination known as Kaletra), nelfinavir, also limit SARS-CoV-2 propagation in infected cells,^{39,57,64} and they are being tested in several ongoing clinical trials.^{56,60,61} However, the molecular basis of their inhibition mechanism has not been elucidated yet due to the fact that HIV and CoV proteases belong to different protease classes. Unfortunately, the first clinical trials reported that lopinavir, combined with the ritonavir that boosts lopinavir levels by interfering with cytochrome P450 metabolism, does not have any significant effect on SARS-CoV-2 infection.⁵⁸

Historically, the most attractive antiviral compounds are those that block virus replication by inhibiting RdRp activity. Nucleotide and nucleoside analogs are among the most promising groups of RdRp inhibitors. Their antiviral effect can be attributed to three nonmutually exclusive mechanisms. First, incorporation of nucleotide analogs (NAs) in the viral RNA by the error-prone polymerase can induce early chain termination in an obligate (immediate) or nonobligate fashion, resulting in incomplete, noninfectious viral RNA. The second mechanism, named error catastrophe, is associated with the insertion and extension of NAs throughout the viral RNA that result in many errors during RNA synthesis.^{65,66} Third, several NAs deplete the cytoplasmic levels of their equivalent native nucleotides, causing nucleotide pool imbalances that affect RdRp fidelity.⁶⁷ While nucleoside analogs have been successfully used for the treatment of other viral diseases, the situation is complicated in CoVs due to the presence of a viral exonuclease (nsp14 ExoN) with proof-reading activity that can reduce NA antiviral effect.^{68,69} Indeed, in vitro studies show that SARS-CoV polymerase can incorporate ribavirin triphosphate during replication, but this purine NA is detected by nsp14 ExoN and eliminated by the repair mechanism.⁶⁸ This might partly explain the poor antiviral effect of ribavirin in SARS- and MERS-CoV infection.^{43,70} On the other hand, molecules such as remdesivir, initially developed against Ebola virus,⁴⁵

appear to have an inhibitory effect against SARS-CoV and MERS-CoV in vitro at the submicro- to micromolar range, depending on the infected cell type.^{46,71,72} Remdesivir evaluation in a primate model infected by SARS-CoV showed that the antiviral activity is dependent on early treatment after infection.⁴⁷ Remdesivir is incorporated into nascent RNA by the SARS-CoV-2 RdRp complex.⁵⁴ Several clinical trials, such as the European DISCOVERY clinical program,⁴⁹ are assessing remdesivir safety and efficacy in hospitalized patients with Covid-19. The first results of these compassionate-use studies showed clinical improvement in 68% of patients treated with remdesivir in a small cohort ($n = 53$). Notably however, these studies were not randomized and furthermore did not report the change in viral load following treatment.⁵⁰ While some adverse effects were reported, including abnormal liver function, diarrhea, rashes, renal impairment, and hypotension, remdesivir was licensed in May 2020 as an emergence treatment against SARS-CoV-2.

The cytidine analog β -d-N4-hydroxycytidine (NHC) also is a potential anti-CoV compound. NHC inhibits MERS-CoV, SARS-CoV, and SARS-CoV-2 replication in vitro in the micromolar range (0.09–0.3 μ M), without apparent interference from the viral proofreading activity of nsp14.⁵² In infected cells, decreased viral replication was associated with increased mutation frequency, supporting a mechanism of lethal mutagenesis. Moreover, the orally bioavailable β -D-N4-hydroxycytidine-5'-isopropyl ester improved pulmonary function and reduced virus titer and body weight loss in mice infected with SARS- and MERS-CoV. These promising results suggest that NHC molecules might be considered for Covid-19 treatment,^{53,72} and should be assessed in clinical trials. Another potential treatment is the guanine analog favipiravir (T-705) that was initially developed for influenza virus,⁷³ but has a broad-spectrum activity, with antiviral effects against flaviviruses,^{74,75} alphaviruses,⁷⁶ noroviruses,⁷⁷ and Ebola virus.^{78–80} Favipiravir was approved for influenza treatment in Japan in 2014, and is currently assessed for Covid-19 treatment in several clinical trials.^{42,51} The preliminary results suggest a faster viral clearance time in the favipiravir arm compared with the control arm.⁵¹ More recently, in vitro studies demonstrate that favipiravir exerts an antiviral effect as a nucleotide analogue through a combination of chain termination, slowed RNA synthesis and lethal mutagenesis. The use of favipiravir in infected cells induces C-to-U and G-to-A changes.⁸¹ A last nucleoside analog potentially active against SARS-CoV-2 is sofosbuvir, a broad-acting antiviral approved for hepatitis C virus infection management. A recent study showed that sofosbuvir triphosphate is incorporated by recombinant SARS-CoV-2 RdRp during RNA elongation.⁵⁴ More work is needed to confirm its antiviral effect in infected cells and animal models before moving to clinical trials.

3 | DRUG REPOSITIONING STRATEGIES FOR COVID-19 TREATMENT

The fastest option for the treatment of Covid-19 is the identification of already approved drugs that were developed for other diseases, but that show inhibitory activity against SARS-CoV-2 in infected cells.

The main advantages of this strategy are that these drugs are available on the market, and their safety and toxicity profiles are already documented. However, their MoA against the virus is often speculative, and the efficacy and clinical doses required for treatment in patients with Covid-19 are unknown, due to the absence of large-scale clinical studies. Moreover, it is important to consider the risk of overdose and of unexpected increase of viral load or symptoms. Several teams have already set up platforms to screen FDA-approved libraries using SARS-CoV-2 infected cells.^{39,64,82} Although the chemical libraries and experimental conditions used in these studies are quite different, a comparative analysis of their results reveals that some compounds, such as chloroquine derivatives, have been identified in different experimental conditions. Moreover, several groups reported the antiviral activity of FDA-approved compounds, and some of them are already tested in clinical trials.¹⁵ In this review, we cannot discuss all drugs identified by this strategy. Therefore, we selected the key compounds that are already in clinical trials and the most interesting classes of compounds, based on their potential MoA on CoV life cycle inferred from their known activity.

3.1 | Immunomodulators

As some patients with Covid-19 show an inefficient antiviral response, it has been suggested that immunomodulators might restore the immune system homeostasis. For instance, interferon (IFN) could be used during the early stages of infection to boost the innate immune response and promote viral clearance. IFN treatments are being tested in several clinical trials, but results are not available yet. However, as SARS-CoV-2 infection may also be accompanied by a dysregulated immune response leading to a massive production of pro-inflammatory cytokines, IFN treatment could have unexpected effects.^{5,83–86} Indeed, IFN triggers overexpression of ACE2, even in cell lines with low basal expression level, leading to a larger dissemination of SARS-CoV-2.⁸⁷ Conversely, in the second phase of the disease, the use of molecules that limit the effect of the cytokine storm could be advantageous. For instance, the mAbs mepolizumab against interleukin (IL)-5, tocilizumab against IL-6 receptor,⁸⁸ as well as anakinra an interleukin 1 receptor antagonist⁸⁹ could control the immune response, and be of therapeutic interest for managing the production of pro-inflammatory cytokines in patients with severe infection.⁹⁰ Finally, Dexamethasone, used to reduce inflammation, has been tested in RECOVERY clinical trial and shown first promising results. Indeed, treatment with Dexamethasone reduces death by one-third to one-fifth.⁹¹

3.2 | Repositioned drugs that interfere with the viral life cycle

Several therapeutic molecules (Table 2), identified in antiviral screenings performed in infected cells, target the renin/angiotensin pathway.^{39,100,101} The MoA of these class of inhibitors is speculative.

TABLE 2 Main repositioning molecules currently being tested against SARS-CoV-2 infection

Compounds	Target/MoA	Tests in vitro (CoV)	Clinical trials SARS-CoV-2
<i>Antivirals</i>			
Nafamostat mesylate	TMPRSS2/inhibitor	(92)	No data
Camostat mesylate	TMPRSS2/inhibitor	(39,92)	No data
Chloroquine Hydroxychloroquine	pH increases in endosomal compartment	(39,48,93-96)	"Solidarity," "Discovery" NCT04358068 (49,97,98)
Azithromycin (antibiotic)	pH increases in endosomal compartment/ immunomodulator	(39)	NCT04358068 (98)
Omeprazole	PPI	(39)	No data
Vonoprazan	PPI	(39)	No data
Ivermectin	Limits viral infection	(99)	No data
Oseltamivir	Neuraminidase inhibitor	No data	No data

Note: Clinical trials web site: <https://clinicaltrials.gov/ct2/results?cond=SARS-CoV2&term=&cntry=&state=&city=&dist=>.

Abbreviations: CoV, coronaviruses; MoA, mode of action; PPI, proton pump inhibitor; TMPRSS2, transmembrane protease serine 2.

Interestingly, ACE2 belongs to this pathway. An *in vivo* study has shown that inhibition of the renin/angiotensin pathway upregulates ACE2 which can increase susceptibility to SARS-CoV-2 infection.¹⁰² More recently, it has been shown that treatment target this pathway is not associated with higher severity of COVID19¹⁰³ Additional work is needed to understand how these compounds inhibit the virus before planning clinical trials.

Once the virus binds to the target cell, the S protein is cleaved by cellular proteases into the S1 and S2 subunits (priming), and also upstream of the fusion peptide (S2' cleavage site). The serine protease TMPRSS2, which is strongly expressed in lung cells, can cleave a variety of CoV S proteins,^{12,13} and seems to play a key role in virus entry.¹⁴ Camostat mesylate, a TMPRSS2 inhibitor approved for clinical use, blocks S protein processing *in vitro*.⁹² Moreover, camostat mesylate and its derivative nafamostat mesylate can inhibit (in the nM to μ M range) the entry of vesicular stomatitis virus pseudotypes carrying the SARS-CoV-2 S protein in different cell types that overexpress TMPRSS2.^{92,104} This observation suggests that inhibition of S protein priming blocks ACE2-mediated entry. However, the inhibition observed with camostat mesylate and nafamostat mesylate in VeroE6 cells infected by SARS-CoV-2 is lower and probably not enough for a therapeutic utilization of camostat mesylate. The picture is further complicated because other cellular proteases could be implicated in S protein processing. Indeed, cathepsin L¹⁰⁵ and furin⁹ might be involved in S protein activation, depending on the infected cell type. Consequently, inhibitors of these different enzymes might limit virus propagation in some cell types, but their efficacy in the clinic could be limited. Perhaps, the best option is to combine furin and TMPRSS2 inhibitors to limit virus propagation.¹⁴

Interestingly, several compounds identified in different screens of infected cells are implicated in endosomal acidification.^{39,100,101} Increasing the endosomal pH might limit viral/endosomal membrane fusion, which is necessary for the release of the viral genome into the cytoplasm of the host cell. Two recent studies showed that proton pump inhibitors, such as omeprazole and vonoprazan, reduce the

infection of cells by SARS-CoV-2.^{39,64} In addition, chloroquine and hydroxychloroquine (HCQ), which have been extensively used for the treatment of malaria with known safety and efficacy, also limit acidification of endosomes, Golgi vesicles, and lysosomes.¹⁰⁶ These molecules significantly reduce SARS-CoV and SARS-CoV-2 infection *in vitro*.^{48,93-95,106,107} However, a recent study reported that HCQ does not have any effect on viral load levels and does not protect macaques against SARS-CoV infection.¹⁰⁸ Moreover, chloroquine derivatives have immunosuppressive effects and inhibit the activation of innate immune receptors, such as toll-like receptors¹⁰⁹ and have been associated with some adverse cardiac effects. Many clinical studies are currently testing these molecules,^{97,98} and their initial results are controversial.¹¹⁰ Randomized, double-blind studies with a larger number of patients should bring robust conclusions. Recent *in vitro* studies have also suggested the therapeutic effect of the HCQ-azithromycin combination.⁹⁸ Azithromycin is an antibiotic that has also antiviral activity in respiratory viral infections.¹¹¹ Interestingly, the MoA of azithromycin and HCQ may be related to the modulation of the endosomal and trans-Golgi network pH.^{112,113} In addition, azithromycin plays a role in regulating interleukin production that might help to control the immune response and prevent symptom worsening.¹¹⁴⁻¹¹⁶ Azithromycin may also inhibit viral invasion by interfering with the CD147-mediated recognition mechanism.¹¹⁷ Nevertheless, both the FDA and the World Health Organization (WHO) have removed their support for the use of HCQ and chloroquine for COVID-19, consequently the status of ongoing trials must be questioned.

More recently, it has been reported that ivermectin inhibits SARS-CoV-2 with an ~5000-fold reduction in viral RNA after 48 hours.⁹⁹ Ivermectin is a FDA-approved antiparasitic molecule, initially identified as an inhibitor of HIV protein nuclear import.¹¹⁸ Its broad-spectrum antiviral activity was documented *in vitro*.^{119,120} Ivermectin perturbs nuclear import through IMP α /β1; however, the role of this machinery during the CoV life cycle has not been elucidated yet. Nevertheless, an observational study reported survival benefits

during hospitalization,¹²¹ but no data on viral load, highlighting the necessity to validate this observation in clinical trials. Recent review presents the pharmacokinetic properties of ivermectin, and highlights that the dose currently used for parasitic disease do not effective against SARS infection.¹²²

Finally, oseltamivir, a neuraminidase inhibitor that prevents influenza viral particle release, is also investigated.¹²³ Like for ritonavir/lopinavir, there is no molecular basis to support these trials because to the best of our knowledge, CoVs (unlike influenza viruses) do not rely on neuraminidases during their life cycle. If antiviral effect are observed, the off-target mechanisms will have to be elucidated.

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CONFLICT OF INTEREST

The authors have no competing interest.

AUTHOR CONTRIBUTIONS

Coralie Valle, Baptiste Martin, Bruno Coutard, and Etienne Decroly were major contributors to writing the manuscript. Coralie Valle, Baptiste Martin, Franck Touret, Ashleigh Shannon, Bruno Canard, Jean Claude Guillemot, Bruno Coutard, and Etienne Decroly checked and revised the manuscript. All authors read and approved the final version.

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