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# A Review of the Preclinical and Clinical Efficacy of Remdesivir, Hydroxychloroquine, and Lopinavir-Ritonavir Treatments against COVID-19

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## Abstract

In December of 2019, an outbreak of a novel coronavirus flared in Wuhan, the capital city of the Hubei Province, China. The pathogen has been identified as a novel enveloped RNA beta-coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus SARS-CoV-2 is associated with a disease characterized by severe atypical pneumonia known as coronavirus 2019 (COVID-19). Typical symptoms of this disease include cough, fever, malaise, shortness of breath, gastrointestinal symptoms, anosmia, and, in severe cases, pneumonia.<sup>1</sup> The high-risk group of COVID-19 patients includes people over the age of 60 years as well as people with existing cardiovascular disease and/or diabetes mellitus. Epidemiological investigations have suggested that the outbreak was associated with a live animal market in Wuhan. Within the first few months of the outbreak, cases were growing exponentially all over the world. The unabated spread of this deadly and highly infectious virus is a health emergency for all nations in the world and has led to the World Health Organization (WHO) declaring a pandemic on March 11, 2020. In this report, we consolidate and review the available clinically and preclinically relevant results emanating from in vitro animal models and clinical studies of drugs approved for emergency use as a treatment for COVID-19, including remdesivir, hydroxychloroquine, and lopinavir-ritonavir combinations. These compounds have been frequently touted as top candidates to treat COVID-19, but recent clinical reports suggest mixed outcomes on their efficacies within the current clinical protocol frameworks.

## Keywords

COVID-19, SARS-CoV-2, remdesivir, hydroxychloroquine, lopinavir-ritonavir

## Introduction

SARS-CoV-2 is a novel beta-coronavirus that has spread to virtually every part of the world.<sup>1</sup> SARS-CoV-2 is defined as severe acute respiratory syndrome coronavirus 2. This virus is characterized by a spherical morphology with several projections represented by the spike (S) glycoprotein. Several studies have suggested that bats are a likely natural reservoir of SARS-CoV-2. This hypothesis has merit, as it is known that various other coronaviruses, including SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV), have bats as their natural reservoir.<sup>2</sup> SARS-CoV-2 shares ~80% genomic homology with SARS-CoV-1 and ~40% homology with MERS-CoV.<sup>3</sup> Proteomic sequencing and phylogenetic analyses showed that similar viral repositories exist in several animals, such as pangolins and turtles, which may serve as intermediate hosts.<sup>4</sup>

As this is a novel pathogen, there are no vaccines yet developed, nor are there specific antiviral drugs that have

been authorized for use against SARS-CoV-2. The development of novel small molecules to treat coronavirus 2019 (COVID-19) will require an appropriate period of clinical testing before they are adopted for treatment based on the

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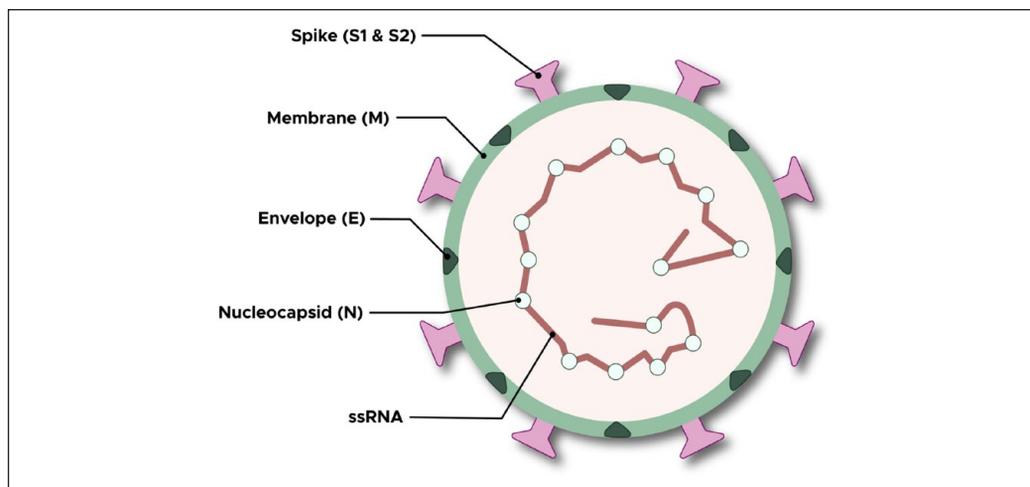
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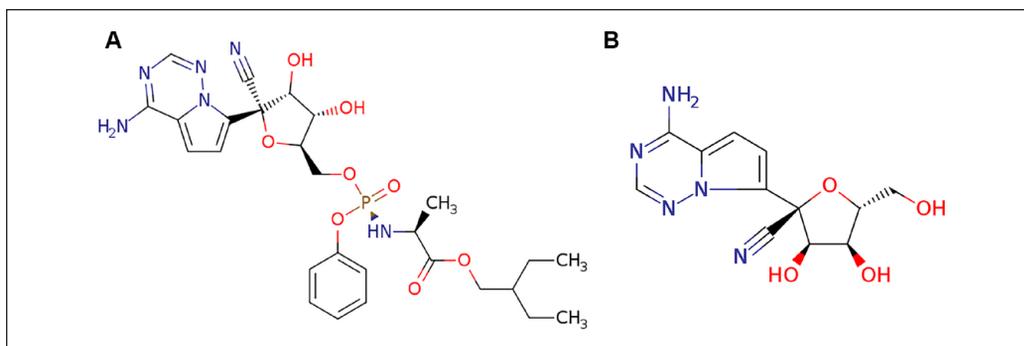
**Figure 1.** Schematic representation of a SARS-CoV-2 virion.

results of the controlled clinical trials. Thus, there is a critical need to rapidly identify safe and effective therapies. One of the most promising approaches to solve this problem is through screening of already approved drugs that can be repurposed for SARS-CoV-2. This methodology has identified drugs, including remdesivir (RDV), hydroxychloroquine (HCQ), and lopinavir-ritonavir (LPV/r), which all have primary indications as therapies against other pathogens but have been recently repurposed for COVID-19 due to lack of specific drugs. Although *in vitro* studies of these compounds have been promising, the clinical results that will be discussed later in this paper have been largely inconsistent. Because of this, on March 18, 2020, the World Health Organization (WHO) launched a multinational effort examining a number of drugs in clinical trials to evaluate their efficacy against COVID-19. The stand-alone drugs or combinations of drugs that are being tested include RDV; a combination of lopinavir and ritonavir; a combination of lopinavir, ritonavir, and interferon-beta (IFN $\beta$ ); and chloroquine (CQ) or HCQ. These treatment regimens will be evaluated relative to appropriate controls, with standard of care including respiratory support provided as required. It must be noted that even if these compounds exhibit suboptimal efficacy as stand-alone therapies, there are methods to increase treatment effectiveness. As our lab has recently proposed, we recommend a multifaceted viral target approach focusing on combinations of drugs, rather than monotherapy, using approved or experimental drugs.<sup>5</sup> We expect that this will not only enhance treatment efficacy but also hamper resistance and adverse effects through targeting multiple essential viral targets simultaneously. Further *in vivo* combinatorial testing must be done before using these as treatments on humans. This paper serves to consolidate the most prominent preclinical and clinical information currently available on these compounds.

### Viral Mechanism of Action

As with other coronaviruses, SARS-CoV-2 consists of four structural proteins that comprise a functional virion: the spike (S), envelope (E), membrane (M), and nucleocapsid (N) (**Fig. 1**). Similar to SARS-CoV-1, the SARS-CoV-2 S protein is a transmembrane glycoprotein consisting of two major exposed domains, where S1 is responsible for virus–host binding and S2 induces virus fusion within the endosome.<sup>6</sup> The S protein of SARS-CoV-2 uses the same entry receptor as the related SARS-CoV, human angiotensin-converting enzyme 2 (hACE2).<sup>7</sup>

Angiotensin-converting enzyme 2 (ACE2) is the primary host cell receptor responsible for SARS-CoV attachment and entry. Human ACE2 is present in a wide array of human tissues: lung epithelia, kidneys, testes, and small intestine.<sup>8</sup> Transmembrane serine protease 2 (TMPRSS2), also found in SARS-CoV, activates/cleaves S proteins to allow for the transmission of SARS-CoV through ACE2. The S protein consists of three sections: an ectodomain, a single-pass transmembrane anchor, and a short intracellular tail.<sup>9</sup> The ectodomain of the S protein consists of two subunits: S1 and S2. The S1 subunit contains a receptor binding domain (RBD) residing on its C terminus that is involved in binding to ACE2.<sup>10</sup> Like SARS-CoV, SARS-CoV-2 uses ACE2 receptor recognition but with key differences in the binding ridges of its S proteins. The presence of a unique four-residue motif (glycine–valine/glutamine–glutamate/threonine–glycine) with two flexible residues allows for a more compact folding of the ridge.<sup>11</sup> This results in closer contact between the S protein and ACE2. In addition, the RBD of the SARS-CoV-2 S protein is substantially more favorable for ACE2 due to its more hydrophilic environment.<sup>10</sup> Both of these differences cause stronger contact and a substantially higher binding affinity between the S protein and ACE2 in SARS-CoV-2



**Figure 2.** The chemical structure of **(A)** prodrug RDV and **(B)** its active form GS-441524.

compared with SARS-CoV. The S2 subunit mediates viral membrane fusion with the host cell.<sup>9</sup> It contains a fusion peptide and two heptad repeats: the HR1 and HR2 regions. These peptides are presumably responsible for fusion between viral and host cell membranes.

Coronaviruses are characterized by large (28–32 kb), highly conserved, nonsegmented, single-strand positive-sense RNA (+ssRNA) genomes.<sup>12</sup> The single-strand RNA genome of coronaviruses is readily translated by host cell machinery, as a 5' cap and a 3' poly-A tail flank on either side of the genome.<sup>13</sup> The SARS-CoV genome is translated into polyprotein products that undergo further processing by viral proteases in the formation of the replication–transcription complex.<sup>13</sup> The SARS-CoV-2 +ssRNA genome is composed of 29,903 nucleotides and its proteome consists of 29 proteins, several of which seem to be druggable.<sup>14</sup>

## Remdesivir

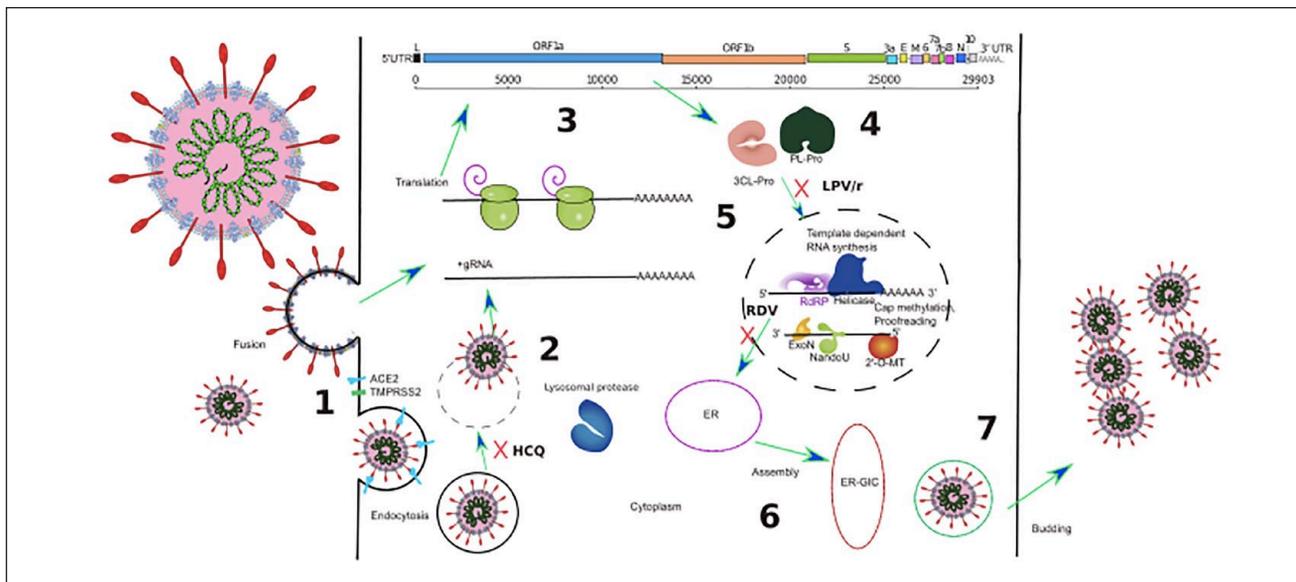
### Drug Background

RDV (**Fig. 2**) is a broad-spectrum antiviral agent, originally proposed for Ebola virus treatment, that has shown antiviral activity against SARS-CoV-1, MERS-CoV, and SARS-CoV-2 in a variety of in vivo and in vitro experiments.<sup>15–17</sup> The RDV prodrug is metabolized intracellularly to the active compound RDV (GS-441524), which is a triphosphoramidate adenosine nucleoside analog.<sup>15,18</sup> Prior in vitro and in vivo studies have identified RDV as having antiviral activity against SARS-CoV-1 and MERS-CoV. RDV exhibited dose-dependent reduction of SARS-CoV-1 replication in a human airway epithelial cell line ( $IC_{50}$ , 0.069  $\mu$ M).<sup>19</sup> Antiviral activity against MERS-CoV was also expressed by RDV in both human lung epithelial ( $IC_{50}$ , 0.025  $\mu$ M) and human airway epithelial cell lines ( $IC_{50}$ , 0.074  $\mu$ M). Further, the antiviral activity of RDV against SARS-CoV-1 was analyzed using an in vivo mouse model. RDV was administered to mice at a concentration of 50 mg/kg once a day or 25 mg/kg twice a day, and either 2 days or 5 days postinfection (dpi). Both RDV treatment concentrations resulted in a reduced viral load in the lungs of both the 2 and 5 dpi SARS-CoV-1-infected mice relative to vehicle-treated control mice.<sup>19</sup> In

vitro assessment was conducted on RDV-mediated inhibition of MERS-CoV in a Calu-3 human lung epithelial cell line. RDV displayed potent antiviral activity against MERS-CoV with an  $EC_{50}$  of 0.09  $\mu$ M. RDV's antiviral ability against MERS-CoV was also assessed via an in vivo mouse model. RDV (25 mg/kg twice a day) administered 24 h before MERS-CoV infection resulted in a significant decrease in viral load, lung hemorrhaging, and mortality relative to vehicle control.<sup>19</sup> The efficacy of prophylactic and therapeutic RDV treatment in combating MERS-CoV was also evaluated in a rhesus macaque animal model.<sup>20</sup> The MERS-CoV-infected rhesus macaques were divided into four groups, a prophylactic experimental group ( $n = 6$ ) that was administered RDV (5 mg/kg once a day until 6 dpi) 24 h before MERS-CoV inoculation, a treatment experimental group ( $n = 6$ ) that was administered RDV (5 mg/kg once a day until 6 dpi) 12 h after MERS-CoV inoculation, a prophylactic control group ( $n = 3$ ) that was administered the vehicle (1 mL/kg) 24 h before MERS-CoV inoculation, and a treatment control group that was administered the vehicle (1 mL/kg) 12 h after MERS-CoV inoculation. Prophylactic RDV administration resulted in significant positive clinical outcomes with virtually no gross or histological lung lesions relative to the control group. Therapeutic RDV administration resulted in better clinical outcomes and reduced gross and histological lung lesions relative to the control. Further prophylactic RDV treatment resulted in a significant reduction in viral load in the lungs relative to the control, and a less significant reduction of viral load in the lungs was also displayed in the therapeutic treatment of RDV relative to the control.<sup>21</sup> The antiviral activity of RDV against SARS-CoV-1 and MERS-CoV justified investigation of its efficacy as a possible treatment for COVID-19. Apparently, as of yet, there have not been clinical trials testing the antiviral activity of RDV against SARS-CoV-1 or MERS-CoV.

### Mechanism of Action against Coronaviruses

In RDV's active form, GS-441524 is a competitive inhibitor of RNA-dependent RNA polymerase (RdRp) by acting as an RNA-chain terminator, leading to the premature termination of viral RNA transcription<sup>15</sup> (**Fig. 3**). RDV incorporation



**Figure 3.** Illustration of the SARS-CoV-2 life cycle along with RDV-HCQ-LPV/r interaction and known mode of action. The infection cycle starts when the SARS-CoV-2 S protein binds to the hACE2 receptor. An S1-induced poststable S2 conformation allows either viral–host cell fusion or receptor-mediated endocytosis (1). Fusion directly allows the viral RNA to enter the host cell, but endocytosis requires lysosomal degradation of the coat and envelope for release of viral nucleocapsid in cytoplasm. HCQ is able to increase the endosomal and lysosomal pH, inhibiting complete viral endocytosis (2). The SARS-CoV-2 RNA genome is known to encode 29 viral proteins (3). A replicase is used to translate most of the viral genomic RNA to synthesize two replicase polyproteins, pp1a and pp1ab. The two major polyproteins are processed by two proteases, PLpro and 3CLpro, generating 16 nonstructural proteins (4). LPV/r is thought to inhibit both of these essential proteases. One of the nonstructural proteins produced by 3CLpro is RdRp. RdRp is involved in viral–host cell replication through catalyzing template synthesis of polynucleotides in the 5' to 3' direction (5). The active form of RDV (GS-441524) inhibits RdRp, consequently inhibiting new virion formation. The viral constituents that are created in the host cell are assembled to form a virion in the endoplasmic reticulum–Golgi apparatus compartment (6). Newly formed virions are then released from the cell through exocytosis within the smooth vesicles (7).

results in termination of RNA transcription three nucleotides from its incorporation and by escaping proofreading exonuclease activity.<sup>15</sup> RdRp has a critical role in RNA virus replication by catalyzing the template synthesis of polynucleotides in the 5' to 3' direction. RdRp is also essential for the initiation of RNA replication in the host cell, a key step in the RNA virus cycle of infection.<sup>22</sup> RdRp functionality requires SARS-CoV-2 accessory proteins, including nonstructural proteins (NSPs) 7 and 8, which increase template binding.<sup>23</sup> In SARS-CoV-1, without RdRp, there is a complete disruption of viral replication, which suggests its importance to the functionality of the virion.<sup>24</sup> A recent study has determined the cryoelectron microscopy structures of the RdRp complex in both the apo form and the other in a complex with the RDV.<sup>25</sup> This structural analysis further confirms that RDV is a strong inhibitor of RdRp.

### In Vitro Testing against SARS-CoV-2

RDV was first confirmed to have antiviral activity against SARS-CoV-2 from its inhibition of SARS-CoV-2 replication in vitro with Vero E6 cells with an  $EC_{50}$  of 0.77  $\mu\text{M}$ .<sup>17</sup> Further in vitro studies analyzing RDV's ability to inhibit

SARS-CoV-2 were performed in Vero E6 cells.<sup>16</sup> These in vitro experiments demonstrated reduction in the viral load of SARS-CoV-2-infected Vero E6 cells with an  $EC_{50}$  of 26.9  $\mu\text{M}$ .<sup>16</sup> Though wide variation between experiments is expected, there is an abnormally large 30-fold variation between these two reports. This can come from sourcing of the drug, improper titration, or other sources of error. More experimental work must be performed to get a clearer understanding of RDV's  $EC_{50}$ .

### Clinical Trials and Human Data

In the first case of a patient presenting with COVID-19 (a 35-year-old male) in the United States, RDV was administered as a compassionate-use antiviral treatment.<sup>26</sup> The SARS-CoV-2-infected patient was a relatively healthy nonsmoker who was admitted to the hospital on day 5 of illness. By day 10, the patient was given supplemental oxygen due to a decrease in oxygen saturation levels (90%), and by day 11 of illness, compassionate use of RDV was administered via infusion. On illness day 12, the clinical outcome measurements improved in the patient, with an increase in oxygen saturation and a discontinuation of

supplemental oxygen. This case report was published prior to the patient's discharge.<sup>26</sup> Clinical findings were also collected in patients ( $N = 53$ ) with severe COVID-19 who were administered compassionate use of RDV.<sup>27</sup> SARS-CoV-2-infected patients who were included in the study had oxygen saturation levels of 94% or lower, with 64% of patients receiving invasive ventilation. Patients were treated with RDV (200 mg on day 1 and 100 mg on days 2–10) for up to 10 days via infusion. Upon a median follow-up of 18 days after the first day of RDV treatment (interquartile range [IQR], 13–23), improvement in oxygen support was displayed in 68% of patients and a 13% mortality. Patients receiving invasive ventilation prior to initiation of treatment had a mortality rate of 18%, while patients not receiving invasive ventilation prior to initiation of treatment had a mortality rate of 5%.<sup>27</sup> This work is promising; however, these results are impossible to properly evaluate as they lack a proper control group. In a randomized, double-blinded, placebo-controlled, multicenter clinical trial conducted in 10 hospitals in Hubei, China, RDV efficacy was analyzed in patients with severe COVID-19 ( $N = 237$ ).<sup>28</sup> Patients enrolled in the study had oxygen saturation levels of 94% or less and had displayed symptoms 12 days or fewer prior to treatment. It is noteworthy that of the COVID-19 patients enrolled in this study, only 0.4% were on invasive ventilation prior to treatment. SARS-CoV-2-infected patients were randomly assigned to either an RDV treatment group ( $n = 158$ ) (200 mg on day 1 and 100 mg on days 1–10) or a placebo control group ( $n = 78$ ). The time to clinical improvement was not significantly different between the RDV treatment group and the placebo control group (IQR, 13–28 vs 15–28). Further, no significant difference was observed in the 28-day mortality rate between the RDV treatment group and the placebo control group (14% vs 13%). Analysis of the 28-day clinical improvement rate found no significant difference between the two groups; however, mortality was higher in the RDV treatment group (65% vs 58%). Examination of viral load in the upper and lower respiratory tract also revealed no major difference between the RDV treatment and placebo-dosed control groups.<sup>28</sup> An ongoing randomized, double-blinded, placebo-controlled clinical trial analyzing the effects of RDV treatment in patients with severe COVID-19 ( $N = 1063$ ) is currently being conducted by the U.S. National Institute of Allergy and Infectious Diseases (NIAID). Patients were randomly assigned into either an RDV treatment group (200 mg on day 1 and 100 mg on days 2–10) or a placebo control group. According to preliminary data from the trial, RDV treatment results in improved time of clinical improvement in comparison with the placebo control (11 vs 15 days). RDV treatment has also been shown in this ongoing study to have resulted in a decreased mortality rate relative to the placebo control group (8.0% vs 11.6%) (**Table 1**).

## Adverse Effects

As RDV is now authorized for emergency use for COVID-19 in several countries, any possible adverse effects must be noted. This is especially important in consideration of RDV relative to the other drugs noted in this paper, because its evaluation remains in the early stages, and therefore there is limited information available regarding the adverse effects of RDV, which has only been used to treat viral pathogens such as Ebola. Some notable side effects include, but are not limited to, elevation in hepatic enzymes, diarrhea, and renal impairment.<sup>27</sup> The lack of available information constricts our understanding of any possible adverse effects in the treatment of COVID-19 using RDV. RDV treatment has sometimes been shown to increase the levels of liver enzymes, which may be a consequence of inflammation or damage to hepatocytes.<sup>29</sup> Thus, it is of great importance that before prescribing RDV to a COVID-19 patient, a proper hematologic/organ-specific panel workup must be performed to test for any preexisting hepatic damage, as well as clinical monitoring during and after completion of RDV therapy. We are expecting that we will soon have a clearer understanding of the possible adverse effects on RDV in COVID-19 patients.

## Hydroxychloroquine

### Drug Background

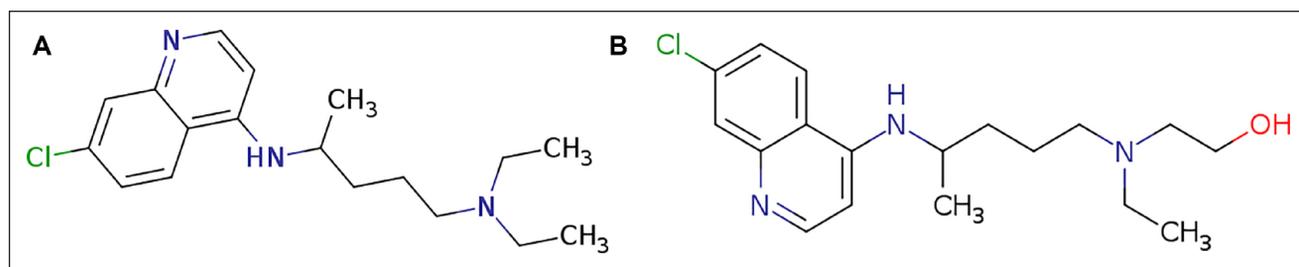
CQ is a 9-aminoquinoline that has been routinely used for the treatment of malaria and also as an anti-inflammatory drug for systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). HCQ is an analog of CQ in which one of the *N*-ethyl substituents of CQ is  $\beta$ -hydroxylated (**Fig. 4**). The activity of HCQ against malaria is equivalent to that of CQ, and HCQ is preferred over CQ when high doses are required because of the lower level of ocular toxicity of HCQ.<sup>30</sup> The use of HCQ or CQ as an anti-inflammatory stems from the compounds' ability to accumulate in the macrophages and lymphocytes. Studies in cell lines have shown that the use of HCQ or CQ reduces the secretion of proinflammatory cytokines, thereby suppressing an excessive host immune reaction.<sup>31</sup>

### Mechanism of Action against Coronaviruses

Although CQ and HCQ are widely used antimalarials, the *in vitro* antiviral activity of CQ has been known since 1969, although through an unknown mechanism.<sup>32</sup> Both CQ and HCQ are weak bases that affect vesicles leading to the dysfunction of several enzymes. The nonprotonated conjugated bases of these compounds are able to enter the host intracellular compartment, where they become protonated and are then trapped as cationic species unable to pass back across the cell membrane. These compounds are

**Table 1.** COVID-19 and Remdesivir Treatment Summary.

Study Type	Patients	Administration	Outcomes	Important Note
Observational	N = 53; severe COVID-19 (all ventilation)	Patients were treated with RDV (200 mg on day 1 and 100 mg on days 2–10) for up to 10 days via infusion	Improvement in oxygen support was displayed in 68% of patients and a 13% mortality was noted relative to the 18% in patients not receiving invasive ventilation prior to initiation of treatment	Impossible to evaluate; no control <sup>27</sup>
Randomized, double-blinded, placebo-controlled, multicenter clinical trial	N = 237; mild COVID-19 (no ventilation)	Randomly assigned to either an RDV treatment group (n = 158) (200 mg on day 1 and 100 mg on days 1–10) or a placebo control group (n = 78)	No significant difference	Slightly increased mortality in the RDV group <sup>28</sup>
Ongoing randomized, double-blinded, placebo-controlled clinical trial	N = 1063; severe COVID-19	Patients were randomly assigned into either an RDV treatment group (200 mg on day 1 and 100 mg on days 2–10) or a placebo control group	RDV treatment resulted in clinical improvement in comparison with the placebo control (11 vs 15 days); RDV treatment has also been shown in this ongoing study to have a decreased mortality rate relative to the placebo (8.0% vs 11.6%)	Still ongoing; United States ( <a href="https://www.niaid.nih.gov/news-events/nih-clinical-trial-shows-remdesivir-accelerates-recovery-advanced-covid-19">https://www.niaid.nih.gov/news-events/nih-clinical-trial-shows-remdesivir-accelerates-recovery-advanced-covid-19</a> )

**Figure 4.** The chemical structures of (A) CQ and (B) HCQ.

thus concentrated within acidic organelles such as endosomes and lysosomes, where the pH is low<sup>33</sup> (Fig. 3). CQ and HCQ are cellular autophagy inhibitors that are thought to interact with enveloped viruses at the late stages of replication.<sup>34</sup> As these compounds are bases, they increase the pH of lysosomal and trans-Golgi network vesicles, which consequently disrupt several enzymes, including acid hydrolases, and inhibit the posttranslational modification of newly synthesized proteins<sup>34</sup> (Fig. 3). In the case of SARS-CoV-1, HCQ has also been shown to interfere with the glycosylation of cellular receptors,<sup>35</sup> though the exact mechanism and consequence is not fully understood. CQ

and HCQ antiviral activity has been most noted as viruses enter their target cells through endosome-mediated endocytosis. As a virus is endocytosed within the host cell, it is within the lysosomal compartment where lysosomal enzymes (cathepsin CSTL) and a low pH unmask the heptad repeat subdomains of the S2 domain of the S glycoprotein. The trimer-of-hairpins structure acts as a class 1 viral fusion protein delivering nucleocapsid to the cytoplasm. HCQ is known to increase the pH of these lysosomes, which then effectively traps the virion within the vesicle, and it is hypothesized that virions can then be degraded by lytic enzymes and thus inactivated. Other mechanisms

have been proposed for how HCQ combats viruses. An increase in intracellular  $Zn^{2+}$  saturation and zinc ionophores in the host cell has been found to inhibit SARS-CoV-1 RNA replication.<sup>36</sup> HCQ is a zinc ionophore and induces an increase in intracellular  $Zn^{2+}$  concentration. CQ has been shown to bind to sialic acid residues, inhibiting the S protein from binding to sialic acid-containing gangliosides.<sup>37</sup>

### *In Vitro Testing against SARS-CoV-2*

In early in vitro studies, CQ was found to inhibit SARS-CoV-2 infection at a micromolar concentration with an  $EC_{50}$  of 1.13  $\mu$ M and a half-cytotoxic concentration ( $CC_{50}$ ) greater than 100  $\mu$ M.<sup>17</sup> Shortly after, another group found that HCQ was even more potent in inhibiting SARS-CoV-2 with an  $EC_{50}$  of 0.72  $\mu$ M.<sup>38</sup> HCQ antiviral activity against SARS-CoV-2 as well as cytotoxicity was measured in an in vitro Vero E6 cell line in comparison with CQ.<sup>30</sup> HCQ was found to be more cytotoxic than CQ ( $CC_{50}$ , 249.50 vs  $CC_{50}$ , 273.20  $\mu$ M), albeit a more potent antiviral against SARS-CoV-2 relative to CQ ( $EC_{50}$ , 4.51 vs 2.71  $\mu$ M). In a time-of-addition assay, HCQ and CQ treatment resulted in the blockage of viral transport from early endosomes to lysosomes, which is essential for SARS-CoV-2 release. The antiviral efficacy of HCQ in combination with azithromycin was analyzed in SARS-CoV-2-infected Vero E6 cells.<sup>39</sup> The HCQ–azithromycin combination was observed to have a significant inhibition of viral replication (5  $\mu$ M/5  $\mu$ M, 99.1% viral inhibition; 5  $\mu$ M/10  $\mu$ M, 97.5% viral inhibition).

### *Clinical Trials and Human Data*

In the case of COVID-19, CQ and HCQ are expected to show promising results in view of the antiviral effects seen in in vitro testing with these two compounds and their anti-inflammatory effects. There have been several studies that have demonstrated the potential efficacy for HCQ as an anti-COVID-19 therapeutic.<sup>40,41</sup>

In a case study, the clinical outcomes of a SARS-CoV-2-infected patient (39-year-old female), who, due to her RA medical history, was already on an oral HCQ treatment regimen (200 mg once a day), were measured.<sup>42</sup> Upon hospitalization, no treatments specifically targeting SARS-CoV-2 or inflammatory cascades were administered to the patient other than the continued use of HCQ. The patient was observed to have mild COVID-19 symptoms and was discharged from the hospital after 2 days.<sup>42</sup> In an uncontrolled, noncomparative clinical observational study, mild COVID-19 patients ( $n = 80$ ) were administered an HCQ–azithromycin combination (200 mg oral for three times a day for 10 days/500 mg on day 1 and 250 mg on days 2–4).<sup>43</sup> Patients received HCQ–azithromycin treatment for a mean of 4.9 days after onset of illness. HCQ–azithromycin

administration resulted in a promising clinical outcome (81.2% discharge rate) and low mortality rate (1.2%), but with no control group to compare these results to. Further, the HCQ–azithromycin combination resulted in a decrease in viral load (93% negative at day 8), but once again, there was no control for comparison. In a controlled clinical observational study, HCQ's antiviral ability in treating COVID-19 patients ( $N = 1376$ ) at a medical facility in New York City was analyzed.<sup>44</sup> SARS-CoV-2-infected patients enrolled in the study had oxygen saturation levels of 94% or less. Patients ( $n = 811$ ) given an HCQ regimen (600 mg on day 1 and 400 mg on days 2–4) were compared with patients who were given no HCQ ( $n = 565$ ). Patients in the HCQ treatment group were administered the drug within 48 h of presentation to the medical facility. It is essential to note that the HCQ-treated patients also differed by baseline characteristics with patients who did not receive HCQ, including with more severe acute respiratory distress syndrome (ARDS) (223 vs 360 PaO<sub>2</sub>/FIO<sub>2</sub>). A time-to-event analysis was conducted comparing the HCQ treatment group and the no-HCQ group with the primary endpoint, defined as either intubation or mortality. Administration of HCQ was suggested to be associated with a significant increase in serious complications in comparison with patients given no HCQ (32.3% vs 14.9%), granting a hazard ratio of 2.37 (95% confidence interval [CI], 1.94–3.02).<sup>44</sup> However, propensity score analyses granted a hazard ratio of 1.04 (95% CI, 0.82–1.32), and no major difference was found between HCQ-treated patients in comparison with patients given no HCQ.

In a New York-based retrospective, multicenter clinical observation, the antiviral ability of HCQ as well as HCQ–azithromycin was analyzed in COVID-19 patients ( $N = 1438$ ) (varied baseline characteristics).<sup>45</sup> The SARS-CoV-2-infected patients examined in the study were classified according to four different treatment groups: HCQ–azithromycin combination therapy ( $n = 735$ ), HCQ monotherapy ( $n = 271$ ), azithromycin monotherapy ( $n = 211$ ), and neither drug ( $n = 221$ ). HCQ was administered at a median of 1 day, and azithromycin was administered at a median of 0 days after admission. A primary outcome of mortality was analyzed and compared between the four treatment groups. Treatment of HCQ was suggested to be associated with a higher mortality rate among COVID-19 patients (HCQ–azithromycin, 25.7%; HCQ, 19.9%; azithromycin, 10.0%; neither drug, 12.7%). However, based on a Cox proportional hazards model, no notable difference was present in the mortality rate between the four treatment groups. HCQ–azithromycin combination therapy was granted a hazard ratio of 1.35 (95% CI, 0.76–2.40), HCQ monotherapy was granted a hazard ratio of 1.08 (95% CI, 0.63–1.85), and azithromycin monotherapy was granted a hazard ratio of 0.56 (95% CI, 0.26–1.21), in comparison with neither drug.<sup>45</sup> In a clinical observation study, HCQ's antiviral ability in treating COVID-19 patients requiring

supplemental oxygen ( $N = 173$ ) was examined.<sup>46</sup> Patients ( $n = 84$ ) administered an HCQ regimen within 48 h of admission to the hospital (600 mg once a day) were compared with a control group of patients ( $n = 89$ ) who were administered no HCQ. The overall survival rate by day 21 was analyzed as well as the survival rate without transfer to the ICU and the survival rate without ARDS. The overall survival rate by day 21 of HCQ-treated patients exhibited no significant difference in comparison with the control group that received no HCQ (89% vs 91%). Further, treatment with HCQ was suggested to have no significant difference in the survival rate without transfer to the ICU by day 21 in comparison with the control group (80% vs 75%). Similarly, no major difference was found in the survival rate without ARDS between the HCQ treatment group and the no-HCQ control group (70% vs 74%).<sup>46</sup> In an open-label, multicenter, randomized, controlled clinical trial, HCQ efficacy in COVID-19 patients was analyzed.<sup>47</sup> It is notable that of the SARS-CoV-2-infected patients ( $N = 150$ ) enrolled in the study, 99% had mild to moderate COVID-19. SARS-CoV-2-infected patients were randomly assigned to either an HCQ plus standard care treatment group ( $n = 75$ ) (1200 mg once a day on days 1–3 and 800 mg once a day for up to 14 days) or a standard care control group ( $n = 75$ ). The negative conversion rate of SARS-CoV-2 was measured in the COVID-19 patients. Analysis of the 28-day negative conversion rate of SARS-CoV-2 found no significant difference between patients given HCQ plus standard care and patients given only standard care (85.4% vs 81.3%). Likewise, there was no significant difference found in the median time to negative conversion between the HCQ plus standard care treatment group and the standard care control group (8 vs 7 days) (Table 2).<sup>48</sup>

### Adverse Effects

The use of CQ or HCQ has been common practice, especially in India and other malaria endemic countries, for several decades. These drugs have also been used in rheumatic and prophylactic conditions that have established a promising safety profile, where CQ and HCQ treatment showed little or no adverse conditions even during chronic administration.<sup>48</sup> However, in the case of use for COVID-19, there have been significant adverse effects associated with CQ and HCQ usage. On April 24, 2020, the U.S. Food and Drug Administration (FDA) issued a safety concern regarding the use of CQ and HCQ in COVID-19 patients. This was because of an increased number of reports showing serious heart rhythm complications in patients treated for COVID-19. This statement came at the moment when prescriptions for CQ and HCQ for the treatment of COVID-19 were increasing significantly. These serious cardiovascular complications include QT interval prolongation and ventricular tachycardia.<sup>49</sup> QT indicates the time during which ventricular

contraction and subsequent relaxation occurs. A recent clinical observation revealed that a significant number of patients (23%) treated with HCQ or HCQ–azithromycin ( $n = 90$ ) suffered prolonged QTc (corrected QT) intervals.<sup>50</sup> Further, HCQ–azithromycin was associated with a greater change of prolonged QTc intervals in comparison with HCQ monotherapy (median, 23 vs 5.5 QTc interval ms).<sup>50</sup> Another clinical observation analyzed the safety profile, in regard to prolonged QTc intervals, of HCQ and HCQ–azithromycin administration in COVID-19 patients ( $n = 40$ ).<sup>51</sup> SARS-CoV-2-infected patients were administered either HCQ monotherapy ( $n = 18$ ) or HCQ–azithromycin combination therapy ( $n = 22$ ). HCQ administration, with or without azithromycin, was associated with an increase in QTc intervals (93%), and prolonged QTc intervals were displayed in a significant portion of treated patients (36%). In the New York-based retrospective, multicenter clinical observation, HCQ–azithromycin administration in COVID-19 patients was associated with cardiac arrest in comparison with patients given neither drug.<sup>45</sup>

## Lopinavir-Ritonavir

### Drug Background

Prior in vitro and clinical studies have shown LPV/r therapeutic regimens to be effective antivirals in combating SARS-CoV-1. In vitro analysis of the antiviral ability of LPV/r indicated successful SARS-CoV-1 inhibition.<sup>52</sup> Lopinavir (4 µg/mL) and ribavirin (50 µg/mL) attained successful inhibition of SARS-CoV-1 in a fetal rhesus kidney-4 cell line, after 48 h of incubation.<sup>52</sup> The clinical effectiveness of LPV/r in treating SARS was tested in SARS-CoV-1-infected patients.<sup>52,53</sup> LPV/r (400 mg/100 mg twice a day) was administered to SARS-CoV-1 patients ( $n = 41$ ) alongside ribavirin and corticosteroids and compared with a matched historical control group ( $n = 111$ ), which was administered ribavirin alongside a corticosteroid.<sup>52</sup> The development of ARDS and mortality was measured in the patients at 21 days. The treatment group was found to have a drastic decrease in ARDS compared with the control group (2.4% vs 22.5%). Furthermore, the treatment group was found to have a decrease in mortality relative to the control group (0% vs 6.3%).<sup>52</sup> In another clinical study, LPV/r (400 mg/100 mg twice a day) was administered to two treatment groups, an initial treatment group ( $n = 44$ ) and a rescue treatment group ( $n = 31$ ), which were compared with corresponding matched historical control groups ( $n = 634$  and  $n = 343$ , respectively).<sup>53</sup> The rescue group is composed of COVID-19 patients that have already been administered some other therapy, but the treatment was ineffective. In the initial treatment of LPV/r in SARS-CoV-1-infected patients, a decrease in the intubation rate (0% vs 11.0%) and mortality (2.3% vs 15.6%) was found relative to the control group.

**Table 2.** COVID-19 and Hydroxychloroquine Treatment Summary.

Study Type	Patients	Administration	Outcomes	Important Note
Controlled clinical observational study	N = 1376; severe COVID-19 (all ventilation)	Patients (n = 811) given an HCQ regimen (600 mg on day 1 and 400 mg on days 2–4) were compared with patients who were given no HCQ (n = 565)	No significant difference	Patients differed by baseline characteristics; HCQ was associated with a significant increase in serious complication <sup>44</sup>
Retrospective, multicenter clinical observation	N = 1438; varied baseline characteristics	Four different treatment groups: HCQ–azithromycin combination therapy (n = 735), HCQ monotherapy (n = 271), azithromycin monotherapy (n = 211), and neither (n = 221)	No notable difference in the mortality rate between the four treatment groups	Rosenberg et al. <sup>45</sup>
Open-label, multicenter, randomized, controlled clinical trial	N = 55; mild/moderate COVID-19	Randomly assigned to either an HCQ plus standard care treatment group (n = 75) (1200 mg once a day on days 1–3 and 800 mg once a day for up to 14 days) or a standard care control group (n = 75)	No significant difference	99% of the patients enrolled in the study had mild to moderate COVID-19 <sup>47</sup>
Controlled clinical observational study	N = 173; severe COVID-19 (all ventilation)	Patients (n = 84) administered an HCQ regimen within 48 h of admission (600 mg once a day) were compared with a control group of patients (n = 89) administered no HCQ	No significant difference	Mahévas et al. <sup>46</sup>

However, in the rescue treatment group no major difference was observed in the intubation rate (9.7% vs 18.1%) or in mortality (12.9% vs 14.9%) in SARS-CoV-1 patients in comparison with the control group.<sup>53</sup> These findings demonstrated that LPV/r treatment performance in inhibiting SARS-CoV-1 is diminished in rescue therapy. Mixed success has been found in the LPV/r inhibition of MERS-CoV. In a Vero cell line, LPV/r was unable to generate a significant EC<sub>50</sub> in inhibiting MERS-CoV.<sup>54</sup> However, in the Huh7 cell line LPV/r was able to demonstrate anti-MERS-CoV activity with an EC<sub>50</sub> of 8 μM. In vitro assessment was conducted on the ability of LPV/r and IFNβ to inhibit MERS-CoV in a Calu-3 human lung cell line.<sup>19</sup> The LPV/r-IFNβ combination proved to be an inefficient combination, with the addition of LPV/r having no clear improvement in antiviral activity compared with IFNβ alone (EC<sub>50</sub>, 160 vs 175 IU/mL).<sup>19</sup> The ability of LPV/r to combat MERS-CoV

in vivo has been ambiguous. In a MERS-CoV-infected marmoset animal model, LPV/r administration diminished pathological features and improved clinical outcomes.<sup>55</sup> In another in vivo analysis, the LPV/r-IFNβ combination was administered in a mouse animal model.<sup>19</sup> A therapeutic dose of LPV/r-IFNβ was able to improve pulmonary function; however, the combination was not effective in reducing acute lung injury or viral load.<sup>19</sup> The relatively potent efficacy demonstrated by LPV/r against SARS-CoV-1 and MERS-CoV led to the investigation of repurposing LPV/r for SARS-CoV-2 treatment.

### Mechanism of Action against Coronaviruses

The SARS-CoV-1 papain-like cysteine protease is key in the processing of 16 viral proteins associated with RNA synthesis and proper replication of the SARS-CoV genome.<sup>56,57</sup>

Since the papain-like protease is critical in SARS-CoV-1 replication, it has been a target of interest in SARS-CoV-1 therapies. Lopinavir is a retroviral protease inhibitor commonly administered in coformulation with the structurally related ritonavir (LPV/r), a mutagenic guanosine analog that inhibits cytochrome P450 metabolism of lopinavir, in treatments for HIV-1<sup>18,58</sup> (Fig. 5). It has been demonstrated that lopinavir is a noncovalent competitive inhibitor of the SARS-CoV-1 papain-like protease<sup>58</sup> (Fig. 3). Further, computational work from our lab predicts that lopinavir is also able to inhibit the SARS-CoV-2 main protease.<sup>5</sup>

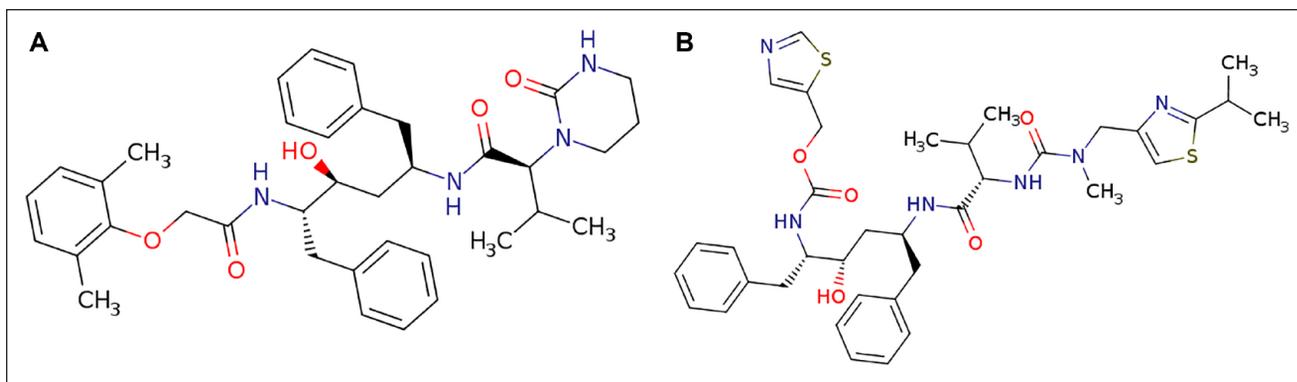
### *In Vitro Testing against SARS-CoV-2*

In vitro findings of the antiviral activity of lopinavir and ritonavir against SARS-CoV-2-infected Vero E6 cells have been encouraging. Lopinavir showed antiviral activity against SARS-CoV-2 in Vero E6 cells with an EC<sub>50</sub> of 26.1 μM.<sup>16</sup> However, ritonavir demonstrated optimal antiviral activity against SARS-CoV-2 in Vero E6 cells at a much higher EC<sub>50</sub> of >100 μM.<sup>16</sup>

### *Clinical Trials and Human Data*

A randomized controlled, open-label clinical trial was conducted in Wuhan, China, during the height of the epidemic.<sup>59</sup> Patients ( $n = 99$ ) infected with SARS-CoV-2 were randomly assigned into LPV/r treatment (400 mg/100 mg twice a day) or standard care ( $n = 100$ ) over the course of 14 days. Relatively no difference was found with the time of clinical improvement between patients administered LPV/r and patients administered standard care (16 vs 16 days). No significant difference was found in the 28-day mortality rate between patients administered LPV/r and patients administered standard care (19.2% vs 25.0%). Additionally, no major difference was found in the time from randomization to discharge between patients administered LPV/r and patients administered standard care (12 vs 14 days). Further, in the measurement of SARS-CoV-2 throat viral RNA quantification over the course of the study, LPV/r treatment did not reduce viral RNA loads in comparison with the standard care group (day 5: 34.5% vs 32.9%; day 10: 50.0% vs 48.6%; day 14: 55.2% vs 57.1%; day 21: 58.6% vs 58.6%; day 28: 60.3% vs 58.6%).<sup>59</sup> In a recent but limited study, the first set of patients infected with SARS-CoV-2 ( $n = 18$ ) in Singapore was analyzed.<sup>60</sup> Among the patients enrolled in the study, five patients were on an LPV/r treatment regimen (200 mg/100 mg twice a day for up to 14 days). Within 3 days of initiation of LPV/r treatment, there was a reduced need for supplemental oxygen in three of those patients. Additionally, within 2 days of initiation of LPV/r treatment, viral shedding was cleared in two of those patients. However, two patients who were administered LPV/r treatment developed respiratory failure within 3 days

of initiation of LPV/r treatment, with one patient being admitted to the ICU for assisted ventilation. Therefore, in this study, LPV/r treatment had no clear effect on decreasing viral load in comparison with patients who were not treated with LPV/r.<sup>60</sup> A case study of an index COVID-19 patient in Korea (54-year-old male) assessed the antiviral effectiveness of LPV/r treatment.<sup>61</sup> Over the course of hospitalization, the patient experienced mild symptoms of fever and dry cough. The patient began an LPV/r treatment regimen (two 200 or 50 mg pills twice a day) beginning on the eighth day of hospitalization and 10 days after onset of illness. Starting on the second day of LPV/r treatment, the SARS-CoV-2 viral load decreased, and there were no detectable virus titers by day 11 of hospitalization.<sup>61</sup> However, clinical improvement in the patient could have been the result of a natural immune response. In a case report, a COVID-19-infected patient (61-year-old female) with a history of RA was administered LPV/r therapy along with a continuation of HCQ treatment.<sup>62</sup> The SARS-CoV-2-infected patient was admitted to the hospital 4 days after symptom onset. On day 3 of admission, the patient developed an atypical pneumonia. Beginning on day 3 of admission, the patient was administered LPV/r (200 or 500 mg twice a day) alongside the continuation of select RA medications, including HCQ (200 mg once per day). The COVID-19 patient witnessed an improvement in symptoms and inflammatory markers over the course of 10 days after initiation of LPV/r treatment. On day 24 of admission, the viral load was diminished and the patient was discharged 2 days later.<sup>62</sup> Another small clinical study in Taiwan analyzed SARS-CoV-2-infected patients ( $n = 5$ ), two of which were administered an LPV/r treatment regimen (two 200 or 50 mg pills twice a day).<sup>63</sup> One patient who received LPV/r treatment was a 56-year-old woman who was administered the treatment on days 5–8 of illness. The patient underwent adverse gastrointestinal effects, a common side effect of LPV/r treatment, and was taken off LPV/r treatment by day 8 of illness. The other patient who received LPV/r treatment was a 53-year-old man who was administered the treatment on days 2–14 of illness. Cycle threshold (Ct) values were measured and no differences in viral shedding were found as detected by quantitative reverse transcriptase PCR (qRT-PCR). It was concluded that LPV/r treatment did not have an effect on shortening SARS-CoV-2 viral shedding, as there was no apparent differences in the Ct values compared with patients not administered LPV/r (0.9 per day vs 1.0 per day).<sup>63</sup> In contrast, a clinical trial comparing LPV/r-mediated and arbidol-mediated inhibition of COVID-19 was conducted in Wuhu, China.<sup>64</sup> SARS-CoV-2-infected patients ( $n = 34$ ) were given LPV/r treatment (400 or 100 mg twice a day) or arbidol (broad-spectrum antiviral) (0.2 g twice a day) ( $n = 16$ ). Patients treated with arbidol showed a drastic decrease in their viral loads by day 14 in comparison with patients treated with LPV/r



**Figure 5.** The chemical structure of (A) lopinavir and (B) ritonavir.

(0% vs 44.1%). Patients treated with arbidol also displayed a reduced duration of positive RNA test days in comparison with patients treated with LPV/r (9.5 vs 11.5 days).<sup>64</sup>

In a multicenter, open-label, randomized controlled clinical trial, LPV/r combination therapy with IFN $\beta$  and ribavirin was compared with LPV/r monotherapy in COVID-19 patients ( $N = 127$ ).<sup>65</sup> SARS-CoV-2-infected patients with mild to moderate COVID-19 symptoms were randomly assigned to either a triple-combination treatment group ( $n = 86$ ) (LPV/r–IFN $\beta$ –ribavirin) or a monotherapy control group ( $n = 41$ ) (LPV/r). COVID-19 patients in the treatment group were administered LPV/r (400 mg/100 mg twice a day), IFN $\beta$  (three doses of 8 million IU), and ribavirin (400 mg twice a day) for 14 days. COVID-19 patients in the control group were administered LPV/r (400 or 100 mg twice a day) for 14 days. The triple-combination treatment group (LPV/r–IFN $\beta$ –ribavirin) had a decreased time to negative viral load in comparison with the monotherapy control group (LPV/r) (7 vs 12 days). Further, improved clinical outcomes were increased in the triple-combination treatment group (LPV/r–IFN $\beta$ –ribavirin) in comparison with the monotherapy control group (LPV/r), in both the alleviation of symptoms (4 vs 8 days) and time to discharge (9.0 vs 14.5 days).<sup>65</sup> In a retrospective, single-center study, discharged COVID-19 patients ( $n = 94$ ) were analyzed.<sup>66</sup> A select portion of the SARS-CoV-2-infected patients in the retrospective study were on a combination therapy ( $n = 67$ ) (unspecified concentrations) of either interferon-alpha (IFN $\alpha$ ), LPV/r, and ribavirin ( $n = 21$ ) or IFN $\alpha$  and LPV/r ( $n = 46$ ). Time to discharge was correlated with SARS-CoV-2 mRNA conversion time in the IFN $\alpha$ , LPV/r, and ribavirin treatment group ( $p = 0.0215$ ) as well as the IFN $\alpha$  and LPV/r treatment group ( $p = 0.012$ ). Additionally, no significant difference was found between the two treatment groups in the time to discharge or the SARS-CoV-2 mRNA conversion times.<sup>66</sup> In a retrospective, single-center study, the antiviral ability of LPV/r in combination with arbidol was compared with the antiviral ability of LPV/r monotherapy in SARS-CoV-2-infected patients.<sup>67</sup> Patients,

without invasive ventilation, were enrolled in the study ( $N = 33$ ) and assigned to either an LPV/r (400 or 100 mg twice a day) and arbidol (200 mg every 8 h) combination treatment group ( $n = 16$ ) or an LPV/r (400 or 100 mg twice a day) monotherapy treatment group ( $n = 17$ ). In both the combination and monotherapy treatment groups, viral load analysis was conducted 7 and 14 days after initiation of treatment and chest CT scans were analyzed 7 days after initiation of treatment. An increase in negative SARS-CoV-2 tests was displayed in the LPV/r–arbidol combination treatment group in comparison with the LPV/r monotherapy treatment group (day 7: 75% vs 35% negative; day 14: 94% vs 53% negative). Further, the LPV/r–arbidol combination treatment group was associated with significant improvement in chest CT scans in comparison with the LPV/r monotherapy group (69% vs 29% improved).<sup>67</sup>

A clinical study conducted in Wenzhou, China, examined the effectiveness of LPV/r in combination with pneumonia-associated adjuvant therapy compared with only pneumonia-associated adjuvant therapy in COVID-19 patients ( $N = 47$ ).<sup>68</sup> SARS-CoV-2-infected patients were assigned to either a treatment group ( $n = 42$ ), administered LPV/r (400 or 100 mg twice a day or 800 or 200 mg once a day) alongside pneumonia-associated adjuvant therapy, or a control group (small,  $n = 5$ ), treated only with pneumonia-associated adjuvant therapy. Daily body temperatures were monitored and viral load analyses of the COVID-19 patients were analyzed over the course of 10 days after the initiation of treatment. In the patients whose body temperature was higher than 37.5 °C upon admission, LPV/r treatment in combination with pneumonia-associated adjuvant therapy was associated with a more rapid return to normal body temperature in comparison with the control (4.8 vs 7.3 days). Further, patients treated with LPV/r alongside pneumonia-associated adjuvant therapy were associated with a shorter time to testing negative for SARS-CoV-2 RNA in comparison with the control (7.8 vs 12.0 days) (Table 3).<sup>68</sup>

**Table 3.** COVID-19 and Lopinavir/Ritonavir Treatment Summary.

Study Type	Patients	Administration	Outcomes	Important Note
Randomized controlled, open-label clinical trial	<i>N</i> = 199; various baselines	Patients ( <i>n</i> = 99) infected with SARS-CoV-2 were randomly assigned into LPV/r treatment (400 mg/100 mg twice a day) or standard care ( <i>n</i> = 100) over the course of 14 days	No difference	Wuhan, China <sup>59</sup>
Multicenter, open-label, randomized controlled clinical trial	<i>N</i> = 127; mild to moderate COVID-19	Randomly assigned to either a triple-combination treatment group ( <i>n</i> = 86) (LPV/r–INFb–ribavirin) or a monotherapy control group ( <i>n</i> = 41) (LPV/r)	Improved clinical outcomes in (LPV/r–INFb–ribavirin) in both the alleviation of symptoms and time to discharge	No standard treatment control <sup>65</sup>
Retrospective, single-center study	<i>N</i> = 33; mild to moderate COVID-19	LPV/r (400 or 100 mg twice a day) and arbidol (200 mg every 8 h) combination treatment group ( <i>n</i> = 16) or an LPV/r (400 or 100 mg twice a day) monotherapy treatment group ( <i>n</i> = 17)	LPV/r–arbidol combination treatment group was associated with significant improvement in chest CT scans in comparison with the LPV/r monotherapy group (69% vs 29% improved)	Small sample size <sup>67</sup>
Controlled clinical observational study	<i>N</i> = 50	Patients were administered LPV/r treatment (400 or 100 mg twice a day) ( <i>n</i> = 34) or arbidol (broad-spectrum antiviral) (0.2 g twice a day) ( <i>n</i> = 16)	Patients treated with arbidol showed a drastic decrease in their viral loads by day 14 in comparison with patients treated with LPV/r (0% vs 44.1%); patients treated with arbidol also displayed a reduced duration of positive RNA test days in comparison with patients treated with LPV/r (9.5 vs 11.5 days)	China <sup>64</sup>

### Adverse Effects

In HIV trials, some of the most common adverse effects of LPV/r included diarrhea, nausea, vomiting, and headaches. There were instances of adverse side effects, such as myocardial infarction, pancreatitis, and hepatic failure, that were infrequent (less than 1%).<sup>69</sup> The adverse effects of LPV/r treatment in COVID-19 patients are less understood. The most common adverse symptoms of LPV/r were altered liver function and gastrointestinal problems, with varied severity.<sup>61,70</sup> LPV/r has the potential to interact with a variety of other drugs through several enzymes.<sup>69</sup> Some of these drug contradictions include propafenone, astemizole, flecainide, and pimozide, among others.<sup>69</sup> All of

these compounds are highly dependent on CYP3A or CYP2D6 for clearance, and for which elevated drug plasma concentrations can be lethal.

### Discussion

The rampant pace of SARS-CoV-2 transmission continues to drastically affect economies and health systems throughout the world. As this is a novel pathogen, there are no vaccines yet available, though several are in development and in the trial phase. Also, due to SARS-CoV-2's newness and novelty, there are no approved specific antiviral drugs to treat COVID-19. Furthermore, the discovery and development of novel compounds that specifically target SARS-CoV-2 will require

a sufficient period of preclinical testing predicting their efficacy and safety before they can enter clinical trials. Thus, the COVID-19 pandemic is a large-scale emergency that warrants the rapid evaluation and use of already approved drugs that can be repurposed for COVID-19. This methodology recommends the use of RDV, CQ or HCQ, and LPV/r to treat COVID-19 in emergency situations. The use of these drugs is in line with the WHO's guidance to further repurpose approved drugs that have demonstrated acceptable safety profiles. There has been widespread international promotion of drugs with unproven efficacies in treating COVID-19 without proper clinical evaluation. Our study has extensively searched available studies to compile this review to benefit physicians in making decisions in treating COVID-19 patients during the pandemic. Although there are promising outcomes with statistical significance in some of these clinical trials, many of these trials suggest that treatment with these drugs is not completely effective in improving recoveries in COVID-19 patients. There are several points that are of utmost importance, as summarized below:

1. RDV offers promise as a monotherapy against COVID-19, but the infancy of the drug makes it impossible to fully understand the adverse effects of this drug in humans.
2. Further, the prodrug of RDV, GS-441524, relies on cellular metabolic processes for activation, which makes it possible that there are variable activating processes in various cell types. Because of this, and the fact that we do not have a complete list of all of the cells and tissues that are infected by SARS-CoV-2, there may be physiological reservoirs that are effectively untreatable by RDV.
3. HCQ and CQ have been the most widely used treatments for COVID-19. These compounds are effective in blocking SARS-CoV-2 reinfection, but once there is active viral infection within the body, the risks of these drugs and lack of significant positive clinical impact make them a less desirable treatment option.
4. As of now, there is no strong evidence for the efficacy of LPV/r treatment against COVID-19, although, there is increasing evidence that an LPV/r-IFN $\beta$ -ribavirin combination does show promising results for the treatment of COVID-19.
5. Further robust, double-blinded, large sampled clinical trials are needed to comprehensively evaluate the suitability of these possible treatments.
6. Additionally, it is of great importance to understand the complete mechanism of action for each of these compounds to determine the suitability for combination therapy to increase the likelihood of success given the deficit of specific anti-COVID-19 therapies.
7. We recommend inclusion of more world-approved as well as experimental drugs to assess the possibility of repurposing. Through this, clinicians will be able to identify the best combinations of compounds that may be of greater efficacy against SARS-CoV-2, compared with monotherapies.

There is a possibility that these previously mentioned compounds may earn their place in the clinical realm as treatments of COVID-19. They may prove to be components of combination therapy rather than continue to be used in the manner in which they are currently utilized. Until a SARS-CoV-2-specific compound is developed and clinically approved, the most direct way to find a treatment is through a multifaceted drug-repurposed approach.

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