Loyola University Chicago

Loyola eCommons

Chemistry: Faculty Publications and Other Works

Faculty Publications and Other Works by Department

9-17-2020

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

Dawid Maciorowski
Loyola University Chicago Stritch School of Medicine

Samir Z. El Idrissi
Loyola University Chicago

Yash Gupta
Loyola University Chicago Stritch School of Medicine

Brian J. Medernach
Loyola University Medical Center
Follow this and additional works at: https://ecommons.luc.edu/chemistry_facpubs

Michael B. Burns
Part of the Biochemistry Commons, Biology Commons, and the Chemistry Commons
Loyola University Chicago, MBURNS16@LUC.EDU
Author Manuscript
This is a pre-publication author manuscript of the final, published article.
See next page for additional authors

Recommended Citation

Maciorowski, Dawid; El Idrissi, Samir Z.; Gupta, Yash; Medernach, Brian J.; Burns, Michael B.; Becker, Daniel P. Ph.D.; Durvasula, Ravi; and Kempaiah, Prakasha. A Review of the Preclinical and Clinical Efficacy of Remdesivir, Hydroxychloroquine, and Lopinavir-Ritonavir Treatments against COVID-19. SLAS Discovery, 25, 10: , 2020. Retrieved from Loyola eCommons, Chemistry: Faculty Publications and Other Works, http://dx.doi.org/10.1177/2472555220958385

Authors

Dawid Maciorowski, Samir Z. El Idrissi, Yash Gupta, Brian J. Medernach, Michael B. Burns, Daniel P. Becker Ph.D., Ravi Durvasula, and Prakasha Kempaiah

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

Dawid Maciorowski[1,2*], Samir Z. El Idrissi[2], Yash Gupta[1,3], Brian J. Medernach[3], Michael B. Burns[2], Daniel P. Becker[2], Ravi Durvasula[1,3], Prakasha Kempaiah[1,3*]

[1]Loyola University Chicago Stritch School of Medicine, Chicago, IL, USA; [2]Loyola University Chicago, Chicago, IL, USA; [3]Department of Medicine, Loyola University Medical Center, Chicago, IL, USA

* Address correspondence to: Dawid Maciorowski and Prakasha Kempaiah, Ph.D, Loyola University Chicago Stritch School of Medicine, Chicago, IL-60153,USA, e-mails: dmaciorowski@luc.edu and Pkempaiah@luc.edu

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 SARS-CoV-2. The virus SARS-CoV-2 is associated with a disease

characterized by severe atypical pneumonia known as COVID-19. Typical symptoms of this disease include cough, fever, malaise, shortness of breath, GI symptoms, anosmia and in severe cases, pneumonia[1]. The high-risk group of COVID-19 patients includes people over the age of 60 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 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.

ABBREVIATIONS: COVID-19 (CoronaVirus Disease-2019); Middle Eastern Respiratory Syndrome (MERS); Severe Acute Respiratory Syndrome (SARS); Remdesivir (RDV); Hydroxychloroquine (HCQ); Chloroquine (CQ); Lopinavir-Ritonavir (LPV/r); Acute Respiratory Distress Syndrome (ARDS)

INTRODUCTION

SARS-CoV-2 is a novel beta-coronavirus that has spread to virtually every part of the world. 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 MERS-CoV have bats as their natural reservoir[2]. SARS-CoV-2 shares ~80% genomic homology with SAR-CoV-1, and ~40% homology with MERS-CoV[3]. 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[4].

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 COVID-19 will require an appropriate period of clinical testing before they are adopted for treatment based on the 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, hydroxychloroquine, and lopinavir/ritonavir, 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 WHO launched a multinational effort examining a number of drugs in clinical trials to evaluate their efficacy against COVID-19. The standalone drugs or combinations of drugs that are being tested

include remdesivir, a combination of lopinavir and ritonavir, a combination of lopinavir, ritonavir, and interferon beta, along with chloroquine or hydroxychloroquine. These treatments 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[5]. We expect that this will not only enhance treatment efficacy, but will 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 pre-clinical 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. These four proteins are the spike (S), envelope (E), membrane (M), and nucleocapsid (N) (**figure 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[6]. The S protein of SARS-CoV-2 uses the same entry receptor as the related SARS-CoV, human angiotensin-converting enzyme 2 (hACE2)[7].
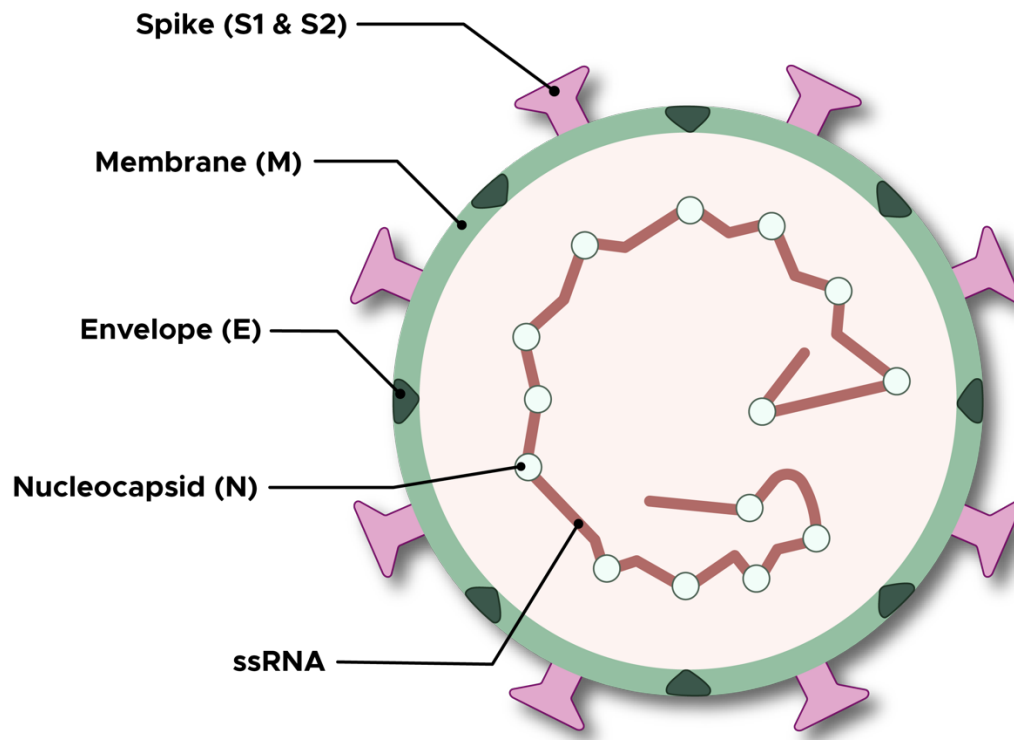
Figure 1. Schematic representation of a SARS-CoV-2 virion.

Angiotensin-converting enzyme 2 (ACE2) is the primary host cell receptor responsible for SARS-CoV attachment and entry. Human ACE2 (hACE2) is present in a wide array of human tissues: lung epithelia, kidneys, testis and small intestine[8]. 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[9]. 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[10]. 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[11]. This results

in closer contact between the S protein and ACE2. In addition, the RBD of the SAR-CoV-2 S protein is substantially more favorable for ACE2 due to its more hydrophilic environment[10]. Both of these differences cause stronger contact and a substantially higher binding affinity between the S protein and ACE2 in SARS-CoV-2 compared with SARS-CoV. The S2 subunit, mediates viral membrane fusion with the host cell[9]. 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, non-segmented, single stranded positive-sense RNA (+ssRNA) genomes[12]. The single strand RNA genome of coronaviruses is readily translated by host cell machinery, as a 5' cap as well as a 3' poly-A tail flank either side of the genome[13]. The SARS-CoV genome is translated into polyprotein products which undergo further processing by viral proteases in the formation of the replication-transcription complex[13]. 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[14].

REMDESIVIR

Drug Background. Remdesivir (RDV) (**figure 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[15–17]. The RDV prodrug is metabolized intracellularly to the active compound RDV (GS-441524), which is a triphophoramidate adenosine nucleoside analog[15,18]. 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 μM) [19]. Antiviral activity against MERS-CoV was also expressed by RDV in both human lung epithelial ($IC_{50=}$ 0.025 μM) and human airway epithelial cell lines (IC_{50} = 0.074 μM). Further, the antiviral activity of RDV against SARS-CoV-1 was analyzed using an in vivo mouse animal 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 post-infection (dpi). Both RDV treatment concentrations resulted in a reduced viral load in the lungs of both the 2 dpi and 5 dpi SARS-CoV-1 infected mice relative to vehicle treated control mice [19]. 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 μM. RDV antiviral ability against MERS-CoV was also assessed via an in vivo mouse model. RDV (25 mg/kg twice a day) administered 24 hours before MERS-CoV infection, resulted in a significant decrease in viral load, lung hemorrhaging, and mortality relative to vehicle control [19]. The efficacy of prophylactic and therapeutic RDV treatment in combating MERS-CoV was also evaluated in a rhesus macaque animal model[20]. The MERS-CoV infected rhesus macaques were divided into four groups, a prophylactic experimental group (n=6) that was administered with RDV (5 mg/kg once a day until 6 dpi) 24 hours before MERS-CoV inoculation, a treatment experimental group (n=6) that was administered with RDV (5 mg/kg once a day until 6 dpi) 12 hours after MERS-CoV inoculation, a prophylactic control group (n=3) that was administered with vehicle (1 mL/kg) 24 hours before MERS-CoV inoculation, and a treatment control group that was administered with vehicle (1 mL/kg) 12 hours 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 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[21]. 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.
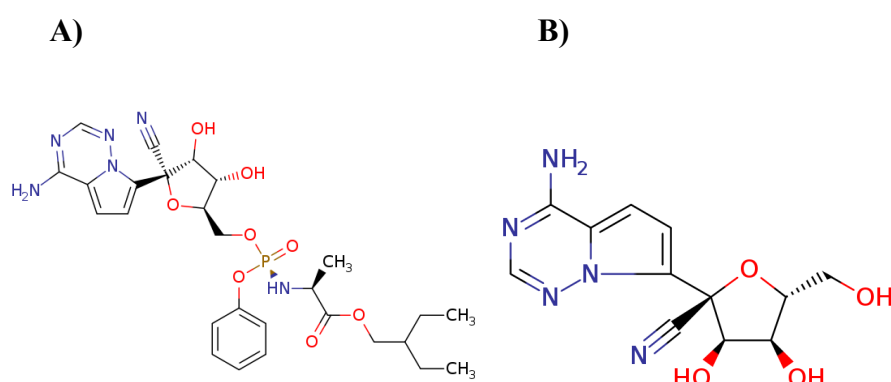
A)   B)

Figure 2. The chemical structure of (**A**) remdesivir (RDV) and (**B**) GS-441524

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[15] (**figure 3**). RDV incorporation results in termination of RNA transcription three nucleotides from its incorporation and by escaping proofreading exonuclease activity[15]. RdRp has a critical role in RNA virus replication by catalyzing the template synthesis of polynucleotides in the 5'-3' direction. RdRp is also essential for the initiation of RNA replication in the host cell, a key step in the RNA viruses cycle of infection[22]. RdRp functionality requires SARS-CoV-2 accessory proteins including

Non-Structural Protein (NSP) 7 and NSP 8, which increase template binding[23]. In SARS-CoV-1, without RdRp, there is a complete disruption of viral replication, which suggests it importance to the functionality of the virion[24]. A recent study has determined the cryo-electron microscopy structures of the RdRp complex in both, the apo form, and the other in a complex with the RDV[25]. This structural analysis further confirms that RDV is a strong inhibitor of RdRp.
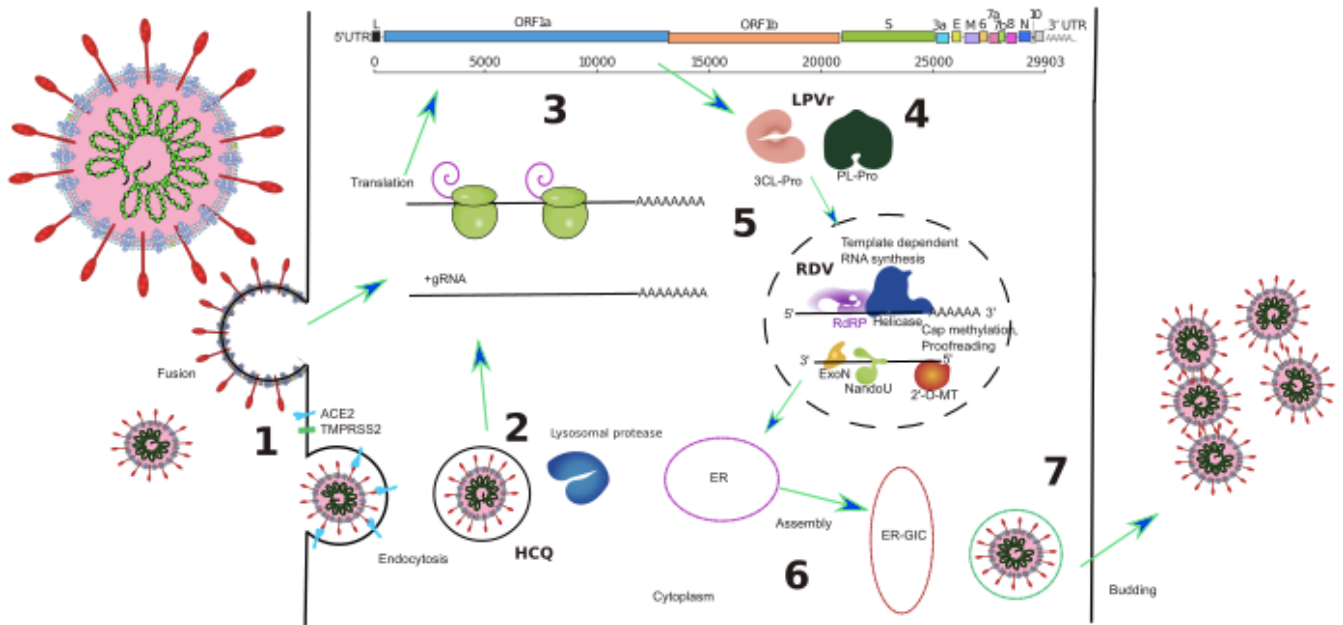

Figure 3. *Illustration of the SARS-CoV-2 life cycle along with RDV/HCQ/LPV interaction and known mode of action.* The infection cycle starts when SARS-CoV-2 spike protein binds to the human ACE2 receptor. An S1-induced post-stable S2 conformation allows either viral-host cell fusion (1). Fusion directly allows the viral RNA to enter the host cell, but endocytosis requires lysosomal degradation of 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 is thought to inhibit both of

these essential proteases. One of the nonstructural proteins produced by 3CLpro is RNA-dependent-RNA-polymerase (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).

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 with an EC_{50} of 0.77 μM[17]. Further in vitro studies analyzing RDV ability to inhibit SARS-CoV-2 were performed in Vero E6 cells[16]. 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 μM[16]. 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 U.S., RDV was administered as a compassionate-use antiviral treatment [26]. 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[26]. Clinical findings were also collected in patients (n=53) with severe COVID-19 who were administered compassionate use of RDV[27]. 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 day 2 to 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% [27]. 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) [28]. 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 day 1 to 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 to 28 vs IQR 15 to 28). Further, no significant difference was observed in the comparison of 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 RDV treatment and placebo-dosed control groups [28]. 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 United States 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 day 2-10) or into a placebo control group. According to preliminary data from the trial, RDV treatment results in improved time of clinical improvement in comparison to the placebo control (11 days 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%).

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 that 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[27]. The lack of available information constricts our understanding of any possible adverse effects in the treatment of COVID-19 using RDV. RDV treatment has been sometimes shown to increase the levels of liver enzymes, which may be a consequence of inflammation or damage to hepatocytes[29]. 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.

Study Type	Patients	Administration	Outcomes	Important Note
Observational	n=53; severe COVID-19 (all ventillation)	Patients were treated with RDV (200 mg on day 1 and 100 mg on day 2 to 10) for up to 10 days via infusion.	Improvement in oxygen support was displayed in 68% of patients and a 13% mortality noted relative to 18% in patients not receiving invasive ventilation prior to initiation of treatment	Impossible to evaluate, no control. [Grein, J. NEJM, 2020]
Randomized, double-blinded, placebo-	n=237; mild COVID-19 (no ventilation)	Randomly assigned to either an RDV treatment group	No significant difference	Slightly increased mortality in RDV group [Wang, Y, The Lancet, 2020]

controlled, multicenter clinical trial		(n=158) (200 mg on day 1 and 100 mg on day 1 to 10) or a placebo control group (n=78).		
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 day 2-10) or into a placebo control group.	RDV treatment resulted in improved time of clinical improvement in comparison to the placebo control (11 days vs 15 days). RDV treatment has also been shown in this ongoing study to have a decreased mortality rate relative to the	Still ongoing (https://www.niaid.nih.gov/news-events/nih-clinical-trial-shows-remdesivir-accelerates-recovery-advanced-covid-19)

			placebo (8.0% vs 11.6%).	

HYDROXYCHLOROQUINE

Drug Background. Chloroquine (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). Hydroxychloroquine (HCQ) is an analogue of CQ in which one of the N-ethyl substituents of CQ is β-hydroxylated (**figure 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[30]. The use of HCQ/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/CQ reduces the secretion of proinflammatory cytokines and thereby suppressing an excessive host immune reaction[31].
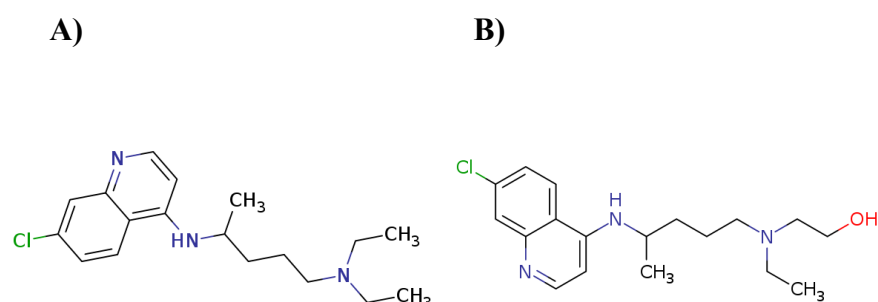
A)   B)

Figure 4. The chemical structure of (**A**) chloroquine (CQ) and (**B**) hydroxychloroquine (HCQ)

Mechanism of Action Against Coronaviruses. Although CQ and HCQ are widely used antimalarials, the in vitro antiviral activity of chloroquine has been known since 1969, although through an unknown mechanism[32]. Both CQ and HCQ are weak bases that affect vesicles leading to the dysfunction of several enzymes. The non-protonated 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 thus concentrated within acidic organelles such as endosomes and lysosomes where the pH is low [33] (**figure 3**). CQ and HCQ are cellular autophagy inhibitors that are thought to interact with enveloped viruses at the late stages of replication [34]. 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 post-translational modification of newly synthesized proteins[34] (**figure 3**). In the case of SARS-CoV-1, HCQ has also been shown to interfere with the glycosylation of cellular receptors[35], though the exact mechanism and consequence is not fully understood. CQ/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 unmasks the heptad repeats subdomains of the S2 domain of spike 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[36]. 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[37].

In vitro testing against SARS-CoV-2. In early in vitro studies, CQ was found to inhibit SARS-CoV-2 infection at micromolar concentration with an EC_{50} of 1.13 µM and a half-cytotoxic concentration (CC_{50}) greater than 100 µM[17]. Shortly after, another group found that HCQ was even more potent in inhibiting SARS-CoV-2 with an EC_{50} of 0.72 µM[38]. HCQ antiviral activity against SARS-CoV-2 as well as cytotoxicity was measured in an in vitro Vero E6 cell line in comparison to CQ[30]. HCQ was found to be more cytotoxic than CQ (CC_{50} 249.50 µM vs CC_{50} 273.20 µM), albeit a more potent antiviral against SARS-CoV-2 relative to CQ (EC_{50} 4.51 µM vs EC_{50} 2.71 µM). 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[39]. The combination of HCQ/azithromycin was observed to have a significant inhibition of viral replication (5 µM/5 µM 99.1% viral inhibition and 5 µM/10 µM 97.5% viral inhibition).

Clinical trials and human data. In the case of COVID-19, CQ/HCQ is expected to show promising results in view of the antiviral effects seen 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[40,41].

In a case study, the clinical outcomes of a SARS-CoV-2 infected patient (39 year old female), who, due to her rheumatoid arthritis (RA) medical history was already on an oral HCQ treatment regimen (200 mg a day), were measured[42]. 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 two days[42]. In an uncontrolled, non-comparative clinical observational study, mild COVID-19 patients (n=80) were administered a HCQ/azithromycin combination (200 mg oral for 3 times a day for 10 days/500 mg on day 1 and 250 mg on day 2-4)[43]. 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 this 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 to compare this to. In a controlled clinical observational study, HCQ antiviral ability in treating COVID-19 patients (n=1376) at a medical facility in New York City were analyzed[44]. 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 1400 mg on day 2-4) were compared to patients who were given no HCQ (n=565). Patients in the HCQ treatment group were administered the drug within 48 hours 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 PaO_2/FIO_2 vs 360 PaO_2/FIO_2). A time-to-event analysis was conducted comparing the HCQ treatment group and the no HCQ group with the primary end point defined as either intubation or mortality. Administration of HCQ was suggested to be associated with a

significant increase in serious complications in comparison to patients given no HCQ (32.3% vs 14.9%) granting a hazard ratio of 2.37 (1.94-3.02 with a 95% confidence interval)[44]. However, propensity-score analyses granted a hazard ratio of 1.04 (0.82-1.32 with a 95% confidence interval) and no major difference was found between HCQ treated patients in comparison to 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)[45]. 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%). Although, 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 (0.76-2.40 with a 95% confidence interval), HCQ monotherapy was granted a hazard ratio of 1.08 (0.63-1.85 with a confidence interval of 95%), Azithromycin monotherapy was granted a hazard ratio of 0.56 (0.26-1.21 with a confidence interval of 95%), in comparison to neither drug[45]. In a clinical observation study, HCQ antiviral ability in treating COVID-19 patients requiring supplemental oxygen (n=173) was examined[46]. Patients (n=84) administered an HCQ regimen within 48 hours

of admission to the hospital (600 mg once a day) were compared to 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 to 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 the ICU by day 21 in comparison to 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%)[46].In an open label, multicenter, randomized, controlled clinical trial, HCQ efficacy in COVID-19 patients was analyzed[47]. 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 day 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 days vs 7 days)[48].

Adverse Effects. The use of CQ/HCQ has been common practice especially in countries including India and other malaria endemic countries for several decades. These drugs have also been used in rheumatic and prophylactic conditions which have established a promising safety

profile, where CQ/HCQ treatment showed little or no adverse conditions even during chronic administration[49]. However, in case of use for COVID-19, there have been significant adverse effects associated with CQ/HCQ usage. On April 24, 2020, the United States Food and Drug Administration (FDA) issued a safety concern regarding the use of CQ/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/HCQ for the treatment of COVID-19 were increasing significantly. These serious cardiovascular complications include QT interval prolongation and ventricular tachycardia[50]. A recent clinical observation revealed that a significant number of patients treated with HCQ or HCQ/azithromycin (n=90) suffered prolonged QTc intervals (23%)[51]. Further, HCQ/azithromycin was associated with a greater change of prolonged QTc intervals in comparison to HCQ monotherapy (median 23 QTc interval milliseconds vs median 5.5 QTc interval milliseconds)[51]. 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)[52]. 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 was 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 cardiac arrest in comparison to patients given neither drug[45].

Study Type	Patients	Administration	Outcomes	Important Note
Controlled-	n=1376; severe	Patients (n=811)	No significant	Patients differed

clinical observational study	COVID-19 (all ventillation)	given an HCQ regimen (600 mg on day 1400 mg on day 2-4) were compared to patients who were given no HCQ (n=565).	difference	by baseline characteristics. HCQ was associated with a significant increase in serious complication. [Geleris, J, NJEM, 2020].
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, E. S, JAMA, 2020]
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	No significant difference	Enrolled in the study 99% had mild-to-moderate COVID-19. [Tang, N, JT and

		once a day on day 1-3 and 800 mg once a day for up to 14 days) or a standard care control group (n=75).		H, 2020]
Controlled-clinical observational study	n=173; severe COVID-19 (all ventilation)	Patients (n=84) administered an HCQ regimen within 48 hours of admission (600 mg once a day) were compared to a control group of patients (n=89) administered no HCQ.	**No significant difference**	[Mahévas, M, BMJ, 2020]

LOPINAVIR-RITONAVIR

Drug Background.

Prior in-vitro and clinical studies have shown LPV/r therapeutic regiments 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[53]. 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 hours of incubation[53]. The clinical effectiveness of LPV/r in treating SARS was tested in SARS-CoV-1 infected patients[53,54]. LPV/r (400 mg/100 mg twice a day) was administered to SARS-CoV-1 patients (n=41) alongside ribavirin and corticosteroids and compared to a matched historical control group (n=111) which had administered ribavirin alongside a corticosteroid[53]. 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 to 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%)[53]. 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 to corresponding matched historical control groups (n=634, n=343)[54]. 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. 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[54]. 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_{50} in inhibiting MERS-

CoV[55]. However, in the Huh7 cell line LPV/r was able to demonstrate anti-MERS-CoV activity with an EC_{50} of 8 μM. In vitro assessment was conducted on the ability of LPV/r and interferon beta (IFNb) to inhibit MERS-CoV in a Calu-3 human lung cell line[19]. The LPV/r-IFNb combination proved to be an inefficient combination, with the addition of LPV/r having no clear improvement in antiviral activity compared to IFNb alone (EC_{50} 160 IU/mL vs 175 IU/mL)[19]. 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[56]. In another in vivo analysis, LPV/r-IFNb combination was administered in a mouse animal model[19]. A therapeutic dose of LPV/r-IFNb was able to improve pulmonary function, however the combination was not effective in reducing acute lung injury or viral load[19]. 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[57,58]. 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 which inhibits cytochrome P450 metabolism of lopinavir, in treatment for human immunodeficiency virus (HIV)-1[18,59] (**figure 5**). It has been demonstrated that lopinavir is a noncovalent competitive inhibitor of the SARS-CoV-1 papain-like protease[59] (**figure 3**). Further, computational work from our lab predicts that lopinavir is also able to inhibit SARS-CoV-2 main protease[5].
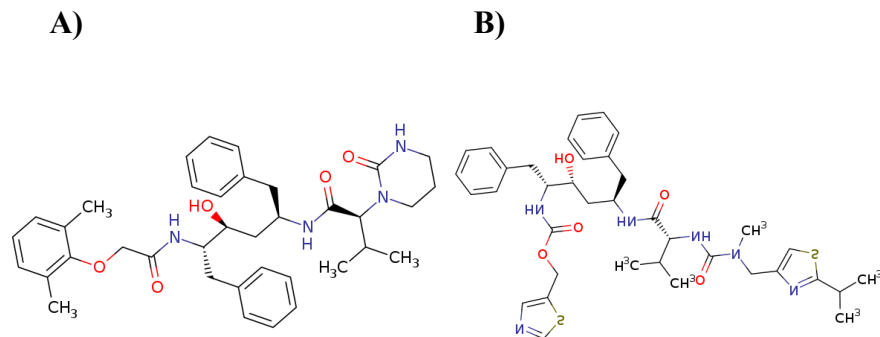
A)   B)

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

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 has been encouraging. Lopinavir showed antiviral activity against SARS-CoV-2 in Vero E6 cells with an EC_{50} of 26.1 μM[16]. However, ritonavir demonstrated optimal antiviral activity against SARS-CoV-2 in Vero E6 cells at a much higher EC_{50} of >100 μM[16].

Clinical trials and human data. A randomized controlled open-label clinical trial was conducted in Wuhan, China during the height of the epidemic[60]. 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 days 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 days 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 to 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%)[60]. In a recent but limited study, the first set of patients infected with SARS-CoV-2 (n=18) in Singapore was analyzed[61]. Among the patients enrolled in the study, 5 patients were on a 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 3 of those patients. Additionally, within 2 days of initiation of LPV/r treatment, viral shedding was cleared in 2 of those patients. However, 2 patients who were administered LPV/r treatment developed respiratory failure within 3 days of initiation of LPV/r treatment, with 1 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 to patients who were not treated with LPV/r[61]. A case study of an index COVID-19 patient in Korea (54-year old male) assessed the antiviral effectiveness of LPV/r treatment[62]. Over the course of hospitalization, the patient experienced mild symptoms of fever and dry cough. The patient began a LPV/r treatment regimen (two 200 mg or 50 mg pills twice a day) beginning on the 8th day of hospitalization and 10 days after onset of illness. Starting on the second day of LPV/r treatment SARS-CoV-2 viral load decreased as well as no detectable virus titers by day 11 of hospitalization[62]. 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[63]. 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 mg 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 viral load was diminished and the patient was discharged two days later[63]. Another small clinical study in Taiwan analyzed SARS-CoV-2 infected patients (n=5), two of which were administered a LPV/r treatment regimen (two 200 mg or 50 mg pills twice a day)[64]. One patient who received LPV/r treatment was a 56-year-old woman who was administered the treatment on day 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 to patients not administered LPV/r (0.9 per day vs 1.0 per day)[64]. In contrast, a clinical trial comparing LPV/r-mediated and arbidol-mediated inhibition of COVID-19 was conducted in Wuhu, China[65]. SARS-CoV-2 infected patients (n=34) were given LPV/r treatment (400 mg or 100 mg twice a day) or aribdol (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 to 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 to patients treated with LPV/r (9.5 days vs 11.5 days)[65].

In a multicenter, open-label, randomized control clinical trial LPV/r combination therapy with IFNb and ribavirin, was compared to LPV/r monotherapy in COVID-19 patients (n=127)[66]. 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-IFNb-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), IFNb (3 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 mg or 100 mg twice a day) for 14 days. The triple combination treatment group (LPV/r-IFNb-ribavirin) had a decreased time to negative viral load in comparison to the monotherapy control group (LPV/r) (7 days vs 12 days). Further, improved clinical outcomes were increased in the triple combination treatment group (LPV/r-IFNb-ribavirin) in comparison to the monotherapy control group (LPV/r), in both the alleviation of symptoms (4 days vs 8 days) and time to discharge (9.0 days vs 14.5 days)[66]. In a retrospective, single center study, discharged COVID-19 patients (n=94) were analyzed[67]. 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 IFNa, LPV/r and ribavirin (n=21) or IFNa and LPV/r (n=46). Time to discharge was correlated with SARS-CoV-2 mRNA conversion time in the IFNa, LPV/r and ribavirin treatment group (p=0.0215) as well as the IFNa, 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[67]. In a retrospective, single center

study, the antiviral ability of LPV/r in combination with aribidol was compared to the antiviral ability of LPV/r monotherapy in SARS-CoV-2 infected patients[68]. Patients, without invasive ventilation, were enrolled into the study (n=33) and assigned to either an LPV/r (400mg or 100 mg twice a day) and arbidol (200 mg every 8 hours) combination treatment group (n=16) or an LPV/r (400 mg 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 days and 14 days after initiation of treatment as well as chest CT scans analyzed 7 days after initiation of treatment. An increase in negative SARS-CoV-2 tests were displayed in the LPV/r-arbidol combination treatment group in comparison to the LPV/r monotherapy treatment group (day 7: 75% vs 35% negative and 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 to the LPV/r monotherapy group (69% vs 29% improved)[68].

A clinical study conducted in Wenzhou, China examined the effectiveness of LPV/r in combination with pneumonia-associated adjuvant therapy compared to only pneumonia-associated adjuvant therapy in COVID-19 patients (n=47)[69]. SARS-CoV-2 infected patients were assigned to either a treatment group (n=42), administered LPV/r (400 mg or 100 mg twice a day or 800 mg 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 to the control (4.8 days 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 to the control (7.8 days vs 12.0 days)[69].

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 including myocardial infarction, pancreatitis, and hepatic failure, which were infrequent (less than 1%)[70]. The adverse effects of LPV/r treatment in COVID-19 patients is less understood. The most common adverse symptoms of LPV/r were altered liver function and gastrointestinal problems, with varied severity[62,71]. LPV/r has the potential to interact with a variety of other drugs through several enzymes[70]. Some of these drug contradictions include propafenone, astemizole, flecainide, pimozide, among others[70]. All of these compounds are highly dependent on CYP3A or CYP2D6 for clearance, and for which elevated drug plasma concentrations can be lethal.

Study Type	Patients	Administration	Outcomes	Important Note
Randomized controlled open-label clinical trial	n=199; various baselines	Patients (n=99) infected with SARS-CoV-2 were randomly assigned into LPV/r treatment (400 mg/100 mg twice a day) or	No difference	Wuhan, China [Cao, B, NJEM, 2020].

		standard care (n=100) over the course of 14 days.		
Multicenter, open-label, randomized control clinical trial	n=127; mild to moderate COVID-19	Randomly assigned to either a triple combination treatment group (n=86) (LPV/r-INFb-ribavirin) or a monotherapy control group (n=41) (LPV/r).	Improved clinical outcomes in (LPV/r-IFNb-ribavirin) in both the alleviation of symptoms and time to discharge.	No standard treatment control [Hung, I. F.-N, The Lancet, 2020]
Retrospective, single center study	n=33; mild to moderate COVID-19	LPV/r (400mg or 100 mg twice a day) and arbidol (200 mg every 8 hours) combination treatment group (n=16) or an LPV/r (400 mg or 100 mg twice a day) monotherapy treatment group	LPV/r-arbidol combination treatment group was associated with significant improvement in chest CT scans in comparison to the LPV/r monotherapy group (69% vs 29% improved).	Small sample size [Deng, L. J of Infection, 2020].

		(n=17).		
Controlled-clinical observational study	n=50	Patients were administered LPV/r treatment (400 mg or 100 mg twice a day) (n=34) or aribdol (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 to 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 to patients treated with LPV/r (9.5 days vs 11.5 days).	[Zhu, Z., J of Infection, 2020].

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 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/HCQ, and LPV/r to treat COVID-19 in emergency situations. The use of these drugs is in line with the World Health Organization's (WHO) guidance to further repurpose approved drugs that have demonstrated acceptable safety profiles. There has been widespread international promotion of drugs with unproven in treating COVID-19 without proper clinical evaluation. Our study has extensively searched available studies to compile into this review to benefit physicians in making decisions in treating the COVD-19 patients during this 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 are not completely effective in improving recoveries in COVID-19 patients. There are several points that are of utmost importance, as summarized below:

1. Remdesivir (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. 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. Hydroxychloroquine (HCQ) and chloroquine (CQ) have been the most widely used treatments for COVID-19. These compounds are effective in blocking SARS-CoV-2 pre-infection, 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 lopinavir-ritonavir (LPV/r) treatment against COVID-19, although, there is increasing evidence that a LPV/r-IFNb-ribavirin combination does show promising results for the treatment of COVID-19.

5. Further robust, double-blind, large sampled clinical trials are needed to comprehensively evaluate 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 to monotherapies.

There is a possibility that these previously-mentioned compounds may earn their place in the clinical realm as treatments of COVID-19 and may prove to be components of combination therapy rather than the manner they are currently being 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.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

REFERENCES

(1)   Gandhi, R. T.; Lynch, J. B.; del Rio, C. Mild or Moderate Covid-19. *N Engl J Med* **2020**, NEJMcp2009249.

(2)   Calisher, C. H.; Childs, J. E.; Field, H. E. et al. Bats: Important Reservoir Hosts of Emerging Viruses. *Clin. Microbiol. Rev.* **2006**, *19* (3), 531–545.

(3)   Cao, Y.; Deng, Q.; Dai, S. Remdesivir for Severe Acute Respiratory Syndrome Coronavirus 2 Causing COVID-19: An Evaluation of the Evidence. *Travel Medicine and Infectious Disease* **2020**, 101647.

(4)   Lam, T. T.-Y.; Shum, M. H.-H.; Zhu, H.-C. et al. Identifying SARS-CoV-2 Related Coronaviruses in Malayan Pangolins. *Nature* **2020**.

(5) Gupta, Y.; Maciorowski, D.; Mathur, R.; et al. *Revealing SARS-CoV-2 Functional Druggability Through Multi-Target Cadd Screening of Repurposable Drugs*; preprint; other, 2020.

(6) Wrapp, D.; Wang, N.; Corbett, K. S.; et al. Cryo-EM Structure of the 2019-NCoV Spike in the Prefusion Conformation. *Science* **2020**, *367* (6483), 1260–1263.

(7) Hoffmann, M.; Kleine-Weber, H.; Schroeder, S. et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**.

(8) Li, M.-Y.; Li, L.; Zhang, Y.; Wang, X.-S. Expression of the SARS-CoV-2 Cell Receptor Gene ACE2 in a Wide Variety of Human Tissues. *Infect Dis Poverty* **2020**, *9* (1), 45.

(9) Glowacka, I.; Bertram, S.; Muller, M. A.; et al. Evidence That TMPRSS2 Activates the Severe Acute Respiratory Syndrome Coronavirus Spike Protein for Membrane Fusion and Reduces Viral Control by the Humoral Immune Response. *Journal of Virology* **2011**, *85* (9), 4122–4134.

(10) Lu, G.; Wang, Q.; Gao, G. F. Bat-to-Human: Spike Features Determining 'Host Jump' of Coronaviruses SARS-CoV, MERS-CoV, and Beyond. *Trends in Microbiology* **2015**, *23* (8), 468–478.

(11) Simmons, G.; Gosalia, D. N.; Rennekamp, A. J.; et al. Inhibitors of Cathepsin L Prevent Severe Acute Respiratory Syndrome Coronavirus Entry. *Proceedings of the National Academy of Sciences* **2005**, *102* (33), 11876–11881.

(12) Qian, Z.; Dominguez, S. R.; Holmes, K. V. Role of the Spike Glycoprotein of Human Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Virus Entry and Syncytia Formation. *PLoS ONE* **2013**, *8* (10), e76469.

(13) Chen, Y.; Liu, Q.; Guo, D. Emerging Coronaviruses: Genome Structure, Replication, and Pathogenesis. *J Med Virol* **2020**, *92* (4), 418–423.

(14) Helmy, Y. A.; Fawzy, M.; Elaswad, A.; et al. The COVID-19 Pandemic: A Comprehensive Review of Taxonomy, Genetics, Epidemiology, Diagnosis, Treatment, and Control. *JCM* **2020**, *9* (4), 1225.

(15) Gordon, C. J.; Tchesnokov, E. P.; Feng, J. Y. et al. The Antiviral Compound Remdesivir Potently Inhibits RNA-Dependent RNA Polymerase from Middle East Respiratory

Syndrome Coronavirus. *J. Biol. Chem.* **2020**, *295* (15), 4773–4779. https://doi.org/10.1074/jbc.AC120.013056.

(16) Choy, K.-T.; Wong, A. Y.-L.; Kaewpreedee, P. et al. Remdesivir, Lopinavir, Emetine, and Homoharringtonine Inhibit SARS-CoV-2 Replication in Vitro. *Antiviral Research* **2020**, *178*.

(17) Wang, M.; Cao, R.; Zhang, L. et al. Remdesivir and Chloroquine Effectively Inhibit the Recently Emerged Novel Coronavirus (2019-NCoV) in Vitro. *Cell Res* **2020**, *30* (3), 269–271.

(18) McCreary, E. K.; Pogue, J. M. Coronavirus Disease 2019 Treatment: A Review of Early and Emerging Options. *Open Forum Infectious Diseases* **2020**, *7* (4), ofaa105.

(19) Sheahan, T. P.; Sims, A. C.; Graham, R. L.; et al. Broad-Spectrum Antiviral GS-5734 Inhibits Both Epidemic and Zoonotic Coronaviruses. *Sci. Transl. Med.* **2017**, *9* (396), eaal3653.

(20) de Wit, E.; Feldmann, F.; Cronin, J.; et al. Prophylactic and Therapeutic Remdesivir (GS-5734) Treatment in the Rhesus Macaque Model of MERS-CoV Infection. *Proceedings of the National Academy of Sciences* **2020**, *117* (12), 6771–6776.

(21) de Wit, E.; van Doremalen, N.; Falzarano, D. et al. SARS and MERS: Recent Insights into Emerging Coronaviruses. *Nature Reviews Microbiology* **2016**, *14* (8), 523.

(22) Yap, Y.; Zhang, X.; Andonov, A.; He, R. Structural Analysis of Inhibition Mechanisms of Aurintricarboxylic Acid on SARS-CoV Polymerase and Other Proteins. *Comput Biol Chem* **2005**, *29* (3), 212–219.

(23) Subissi, L.; Posthuma, C. C.; Collet, A.; et al. One Severe Acute Respiratory Syndrome Coronavirus Protein Complex Integrates Processive RNA Polymerase and Exonuclease Activities. *Proc Natl Acad Sci USA* **2014**, *111* (37), E3900–E3909.

(24) Ju, J.; Li, X.; Kumar, S.; Jockusch, S.; et al. *Nucleotide Analogues as Inhibitors of SARS-CoV Polymerase*; preprint; Pharmacology and Toxicology, 2020.

(25) Yin, W.; Mao, C.; Luan, X.; et al. Structural Basis for Inhibition of the RNA-Dependent RNA Polymerase from SARS-CoV-2 by Remdesivir. *Science* **2020**, eabc1560.

(26) Holshue, M. L.; DeBolt, C.; Lindquist, S. et al. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med* **2020**, *382* (10), 929–936.

(27) Grein, J.; Ohmagari, N.; Shin, D. et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. *N Engl J Med* **2020**, NEJMoa2007016.

(28) Wang, Y.; Zhang, D.; Du, G. et al. Remdesivir in Adults with Severe COVID-19: A Randomised, Double-Blind, Placebo-Controlled, Multicentre Trial. *The Lancet* **2020**, S0140673620310229.

(29) Eastman, R. T.; Roth, J. S.; Brimacombe, K. R. et al. Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19. *ACS Cent. Sci.* **2020**, acscentsci.0c00489.

(30) Liu, J.; Cao, R.; Xu, M. et al. Hydroxychloroquine, a Less Toxic Derivative of Chloroquine, Is Effective in Inhibiting SARS-CoV-2 Infection in Vitro. *Cell Discov* **2020**, *6* (1), 16.

(31) Jeong, J. Y.; Jue, D. M. Chloroquine Inhibits Processing of Tumor Necrosis Factor in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages. *J. Immunol.* **1997**, *158* (10), 4901–4907.

(32) Inglot, A. D. Comparison of the Antiviral Activity in Vitro of Some Non-Steroidal Anti-Inflammatory Drugs. *Journal of General Virology* **1969**, *4* (2), 203–214.

(33) Goldman, S. D.; Funk, R. S.; Rajewski, R. A. et al. Mechanisms of Amine Accumulation in, and Egress from, Lysosomes. *Bioanalysis* **2009**, *1* (8), 1445–1459.

(34) Savarino, A.; Boelaert, J. R.; Cassone, A. et al. Effects of Chloroquine on Viral Infections: An Old Drug against Today's Diseases. *The Lancet Infectious Diseases* **2003**, *3* (11), 722–727.

(35) Vincent, M. J.; Bergeron, E.; Benjannet, S.; et al. Chloroquine Is a Potent Inhibitor of SARS Coronavirus Infection and Spread. *Virology journal* **2005**, *2* (1), 69.

(36) te Velthuis, A. J. W.; van den Worm, S. H. E.; Sims, A. C. et al. Zn2+ Inhibits Coronavirus and Arterivirus RNA Polymerase Activity In Vitro and Zinc Ionophores Block the Replication of These Viruses in Cell Culture. *PLoS Pathog* **2010**, *6* (11), e1001176.

(37) Fantini, J.; Di Scala, C.; Chahinian, H. et al. Structural and Molecular Modelling Studies Reveal a New Mechanism of Action of Chloroquine and Hydroxychloroquine against SARS-CoV-2 Infection. *International Journal of Antimicrobial Agents* **2020**, 105960.

(38) Yao, X.; Ye, F.; Zhang, M. et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clinical Infectious Diseases* **2020**, ciaa237.

(39) Andreani, J.; Le Bideau, M.; Duflot, I. et al. In Vitro Testing of Combined Hydroxychloroquine and Azithromycin on SARS-CoV-2 Shows Synergistic Effect. *Microbial Pathogenesis* **2020**, *145*, 104228.

(40) Chen, Z.; Hu, J.; Zhang, Z. et al. *Efficacy of Hydroxychloroquine in Patients with COVID-19: Results of a Randomized Clinical Trial*; preprint; Epidemiology, 2020.

(41) Gao, J.; Tian, Z.; Yang, X. Breakthrough: Chloroquine Phosphate Has Shown Apparent Efficacy in Treatment of COVID-19 Associated Pneumonia in Clinical Studies. *Biosci Trends* **2020**, *14* (1), 72–73.

(42) Dousa, K. M.; Malavade, S. S.; Furin, J. et al. A SARS-CoV-2 Infection in a Patient on Chronic Hydroxychloroquine Therapy: Implications for Prophylaxis. *IDCases* **2020**, *20*, e00778.

(43) Gautret, P.; Lagier, J.-C.; Parola, P. et al. Clinical and Microbiological Effect of a Combination of Hydroxychloroquine and Azithromycin in 80 COVID-19 Patients with at Least a Six-Day Follow up: A Pilot Observational Study. *Travel Medicine and Infectious Disease* **2020**, 101663.

(44) Geleris, J.; Sun, Y.; Platt, J. et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* **2020**, NEJMoa2012410.

(45) Rosenberg, E. S.; Dufort, E. M.; Udo, T. et al. Association of Treatment With Hydroxychloroquine or Azithromycin With In-Hospital Mortality in Patients With COVID-19 in New York State. *JAMA* **2020**.

(46) Mahévas, M.; Tran, V.-T.; Roumier, M. et al. Clinical Efficacy of Hydroxychloroquine in Patients with Covid-19 Pneumonia Who Require Oxygen: Observational Comparative Study Using Routine Care Data. *BMJ* **2020**, m1844.

(47) Tang, N.; Bai, H.; Chen, X. et al. Anticoagulant Treatment Is Associated with Decreased Mortality in Severe Coronavirus Disease 2019 Patients with Coagulopathy. *Journal of Thrombosis and Haemostasis* **2020**.

(49) Haładyj, E.; Sikora, M.; Felis-Giemza, A. et al. Antimalarials - Are They Effective and Safe in Rheumatic Diseases? *Reumatologia* **2018**, *56* (3), 164–173.

(50) Roden, D. M.; Harrington, R. A.; Poppas, A. et al. Considerations for Drug Interactions on QTc in Exploratory COVID-19 (Coronavirus Disease 2019) Treatment. *Circulation* **2020**, CIRCULATIONAHA.120.047521

(51) Mercuro, N. J.; Yen, C. F.; Shim, D. J. et al. S. Risk of QT Interval Prolongation Associated With Use of Hydroxychloroquine With or Without Concomitant Azithromycin Among Hospitalized Patients Testing Positive for Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol* **2020**.

(52) Bessière, F.; Roccia, H.; Delinière, A. etl. Assessment of QT Intervals in a Case Series of Patients With Coronavirus Disease 2019 (COVID-19) Infection Treated With Hydroxychloroquine Alone or in Combination With Azithromycin in an Intensive Care Unit. *JAMA Cardiol* **2020**.

(53) Chu, C. M. Role of Lopinavir/Ritonavir in the Treatment of SARS: Initial Virological and Clinical Findings. *Thorax* **2004**, *59* (3), 252–256.

(54) Chan, K. S.; Lai, S. T.; Chu, C. M. et al. Treatment of Severe Acute Respiratory Syndrome with Lopinavir/Ritonavir: A Multicentre Retrospective Matched Cohort Study. *Hong Kong Med J* **2003**, *9* (6), 399–406.

(55) Dyall, J.; Coleman, C. M.; Hart, B. J. et al. Repurposing of Clinically Developed Drugs for Treatment of Middle East Respiratory Syndrome Coronavirus Infection. *Antimicrob. Agents Chemother.* **2014**, *58* (8), 4885–4893.

(56) Chan, J. F.-W.; Yao, Y.; Yeung, M.-L. et al. Treatment With Lopinavir/Ritonavir or Interferon-B1b Improves Outcome of MERS-CoV Infection in a Nonhuman Primate Model of Common Marmoset. *J Infect Dis.* **2015**, *212* (12), 1904–1913.

(57) Wu, A.; Peng, Y.; Huang, B. et al. Genome Composition and Divergence of the Novel Coronavirus (2019-NCoV) Originating in China. *Cell host & microbe* **2020**.

(58) Báez-Santos, Y. M.; St. John, S. E.; Mesecar, A. D. The SARS-Coronavirus Papain-like Protease: Structure, Function and Inhibition by Designed Antiviral Compounds. *Antiviral Research* **2015**, *115*, 21–38.

(59) Ratia, K.; Pegan, S.; Takayama, J. et al. A Noncovalent Class of Papain-like Protease/Deubiquitinase Inhibitors Blocks SARS Virus Replication. *Proceedings of the National Academy of Sciences* **2008**, *105* (42), 16119–16124.

(60) Cao, B.; Wang, Y.; Wen, D. et al. A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. *New England Journal of Medicine* **2020**.

(61) Young, B. E.; Ong, S. W. X.; Kalimuddin, S. et al. for the Singapore 2019 Novel Coronavirus Outbreak Research Team. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *JAMA* **2020**, *323* (15), 1488.

(62) Lim, J.; Jeon, S.; Shin, H.-Y. et al. Case of the Index Patient Who Caused Tertiary Transmission of Coronavirus Disease 2019 in Korea: The Application of Lopinavir/Ritonavir for the Treatment of COVID-19 Pneumonia Monitored by Quantitative RT-PCR. *J Korean Med Sci* **2020**, *35* (6), e79.

(63) Song, J.; Kang, S.; Choi, S. W. et al. Coronavirus Disease 19 (COVID-19) Complicated with Pneumonia in a Patient with Rheumatoid Arthritis Receiving Conventional Disease-Modifying Antirheumatic Drugs. *Rheumatol Int* **2020**, *40* (6), 991–995.

(64) Cheng, C.-Y.; Lee, Y.-L.; Chen, C.-P. et al. Lopinavir/Ritonavir Did Not Shorten the Duration of SARS CoV-2 Shedding in Patients with Mild Pneumonia in Taiwan. *Journal of Microbiology, Immunology and Infection* **2020**, S168411822030092X.

(65) Zhu, Z.; Lu, Z.; Xu, T. et al. Arbidol Monotherapy Is Superior to Lopinavir/Ritonavir in Treating COVID-19. *Journal of Infection* **2020**, S0163445320301882.

(66) Hung, I. F.-N.; Lung, K.-C.; Tso, E. Y.-K. et al. Triple Combination of Interferon Beta-1b, Lopinavir–Ritonavir, and Ribavirin in the Treatment of Patients Admitted to Hospital with COVID-19: An Open-Label, Randomised, Phase 2 Trial. *The Lancet* **2020**, S0140673620310424.

(67) Yuan, J.; Zou, R.; Zeng, L. et al. The Correlation between Viral Clearance and Biochemical Outcomes of 94 COVID-19 Infected Discharged Patients. *Inflamm. Res.* **2020**, *69* (6), 599–606.

(68) Deng, L.; Li, C.; Zeng, Q. et al. Arbidol Combined with LPV/r versus LPV/r Alone against Corona Virus Disease 2019: A Retrospective Cohort Study. *Journal of Infection* **2020**, S0163445320301134.

(69) Ye, X.-T.; Luo, Y.-L.; Xia, S.-C. et al. Clinical Efficacy of Lopinavir/Ritonavir in the Treatment of Coronavirus Disease 2019. *European Review for Medical and Pharmacological Sciences* **2020**, *24* (6), 3390–3396.

(70)   Cvetkovic, R. S.; Goa, K. L. Lopinavir/Ritonavir: A Review of Its Use in the Management of HIV Infection. *Drugs* **2003**, *63* (8), 769–802.

(71)   Zhang, Y.; Xu, Q.; Sun, Z. et al. Current Targeted Therapeutics against COVID-19: Based on First-Line Experience in China. *Pharmacological Research* **2020**, 104854.