Review

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An overview of potential therapeutic agents to treat COVID-19

Xueqin Dong[1], Zhenxue Tian[2], Chengwu Shen[3], Cuirong Zhao[3,*]

[1] Community Health Service Center of the Qianfo Mountain Office, the People's Hospital of Lixia District of Jinan, Ji'nan, Shandong, China;
[2] Department of Pharmacy, Qingdao Municipal Hospital, Qingdao, Shandong, China;
[3] Department of Pharmacy, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan, Shandong, China.

SUMMARY The emerging novel coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has swept across the world and become a global threat to public health. More than 200 countries and territories worldwide are suffering from this COVID-19 pandemic. Worryingly, no specific vaccines or drugs have been approved for the prevention or treatment of COVID-19. Under the pressure of a sustained rise in the incidence and mortality of COVID-19, an unprecedented global effort is being implemented to identify effective drugs to combat the current coronavirus. As the understanding of SARS-CoV-2 virology, the underlying mechanism by which it attacks host cells, and the host response to the infection rapidly evolves, drugs are being repurposed and novel drugs are being identified and designed to target the SARS-CoV-2 pathogenesis. Presented here is a brief overview of both virus-based and host-based potential therapeutic drugs that are currently being investigated.

Keywords coronavirus, COVID-19, severe acute respiratory syndrome coronavirus 2, potential therapeutic agents, virus-based, host-based

1. Introduction

Since China first reported an unusual type of pneumonia on December 31, 2019, the number of people identified with this pneumonia has been increasing at an alarming rate, leading to a worldwide public health emergency. This new infectious disease, officially named coronavirus disease (COVID-19) on February 11, 2020 by the World Health Organization (WHO), is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On March 11, 2020, the COVID-19 outbreak was officially declared a pandemic by the WHO. The disease is characterized by flu-like symptoms, such as cough, fever, myalgia, and fatigue. Although some infections are asymptomatic, many patients develop pneumonia, and some patients even develop severe and fatal respiratory diseases. As noted by the WHO, a total of 32,029,704 confirmed cases and 979,212 deaths worldwide were caused by COVID-19 as of September 25, 2020 (*1*). Nevertheless, there are no approved vaccines or specific drugs available for the prevention and treatment of COVID-19 at this moment. Given the threat of the pandemic and urgent need for effective vaccines and antivirals, vigorous efforts are being made globally to stop the COVID-19 epidemic. Compared to *de novo* drug development, drug repurposing offers advantages in taking less time and involving less cost, so it may be an ideal strategy for finding and identifying effective and safe potential therapeutic agents for the disease (*2*).

A better understanding of SARS-CoV-2 virology, the underlying mechanisms by which it attacks host cells, and the host response to the infection is crucial to drug discovery and repurposing. Like severe acute respiratory syndrome coronavirus (SARS-CoV) that emerged in 2002 and Middle East respiratory syndrome coronavirus (MERS-CoV) that was identified in 2012, SARS-CoV-2 is a lipid-enveloped, single-stranded, positive sense RNA virus that is a zoonotic β-coronavirus (*3*). The SARS-CoV-2 genome, first published on January 24, 2020 (*4*), shares a nucleotide identity of 82% with SARS-CoV (*5*). Studies have confirmed that SARS-CoV-2 and SARS-CoV bind to the same host cell surface receptor, angiotensin-converting enzyme 2 (ACE2), *via* their structural spike glycoprotein (S protein) (*6*). Host transmembrane serine protease 2 (TMPRSS2), along with ACE2 and virus S protein, is responsible for virus fusion and entry, and the three have been studied as potential targets for screening therapeutic compounds and repurposing drugs (*7,8*). In addition, many agents have been studied and identified based on virus-specific nucleic acids or proteins such as RNA-dependent RNA polymerase (RdRp), 3-chymotrypsin-like protease 3Clpro (also termed Mpro), and papain-like proteases (PLpro), which play an important role in virus replication

(*8,9*). The mechanism of host response to the infection also offers attractive targets for potential therapies (*8,10*). The possible life cycle of SARS-CoV-2 in host cells and host immune responses is shown in Figure 1. Presented here is a brief overview of both virus-based and host-based potential therapeutic drugs that are being investigated. Some examples are listed in Table 1 and their potential targets are shown in Figure 1.

2. Therapeutic agents targeting SARS-CoV-2 entry

The recognition and docking between virus S protein and host receptor ACE2 is the first step – and a critical one – for SARS-CoV-2 entry into susceptible cells (*11*). The binding process also requires the priming of the S protein by cleaving S protein by TMPRSS2 into two functional subunits, S1 and S2 (*7*). S1 is responsible for

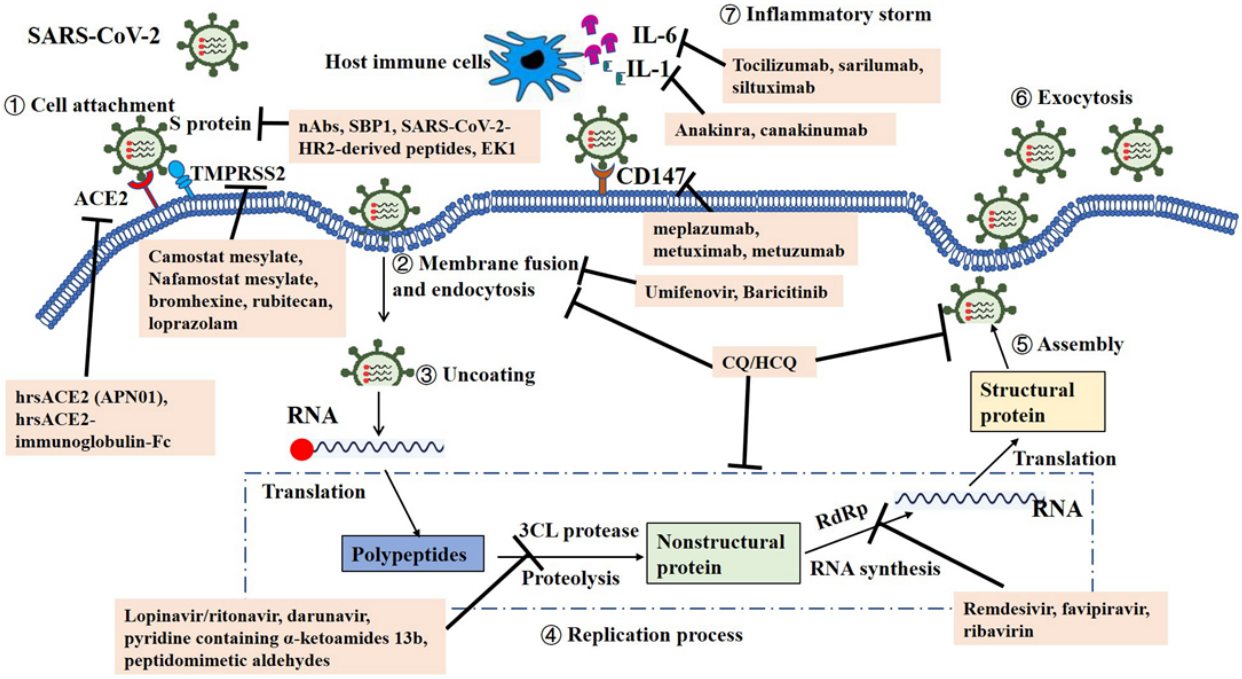

Figure 1. Possible life cycle of SARS-CoV-2 and potential agents. ① SARS-CoV-2 attaches to the host cell through the bind between the virus S protein and cellular receptors, such as angiotensin-converting enzyme 2 (ACE2) or transmembrane glycoprotein CD147. ② At this point, transmembrane protease serine 2 (TMPRSS2) cleaves and activates the S protein, leading to membrane fusion and virus entry *via* an endosomal pathway. ③ After entering the host cell, the viral RNA is introduced into the cytoplasm. ④ Then, with the help of encoded proteases including 3C-like protease (3CLpro) and RNA-dependent RNA polymerase (RdRP), SARS-CoV-2 produces new genomic RNA. ⑤ The assembled virion is formed and ⑥ released from the infected cells *via* exocytosis. ⑦ Uncontrolled replication promotes infection with SARS-CoV-2 and causes host immune responses and inflammatory cytokine storms. Proposed potential agents to treat SARS-CoV-2 and their possible targets are shown with bold lines. (CQ/HCQ: Chloroquine and hydroxychloroquine; nAbs: S protein-neutralizing antibodies; SBP1: peptide binder targeting the receptor-binding domain (RBD) of the S protein; EK1: a peptide fusion inhibitor; hrsACE2 (APN01): recombinant human soluble ACE2 protein)

Table 1. Potential agents for SARS-CoV-2

Mechanisms	Targets	Potential agents
Inhibiting SARS-CoV-2 entry	S protein	S protein-neutralizing antibodies (nAbs), peptide binder SBP1, SARS-CoV-2-HR2-derived peptides, peptide fusion inhibitor EK1
	ACE2	hrsACE2 (APN01), hrsACE2-immunoglobulin-Fc
	TMPRSS2	Camostat mesylate, nafamostat mesylate, bromhexine, rubitecan, loprazolam
	CD147	Meplazumab, metuximab, and metuzumab
	membrane lipids	Umifenovir
	AAK1	Baricitinib
Interrupting the process of SARS-CoV-2 replication	3CL protease	Lopinavir/ritonavir, darunavir, pyridine containing α-ketoamides 13b, peptidomimetic aldehydes
	RdRp	Remdesivir, favipiravir, ribavirin
Affecting host immune responses	IL-6 receptor	Tocilizumab, sarilumab, siltuximab
	IL-1 receptor/ IL-1β	Anakinra, canakinumab
Potential therapeutic agents with multiple mechanisms		Chloroquine and hydroxychloroquine, Interferons

viral attachment to the target host cells. The S2 subunit facilitates viral fusion with the cellular membrane, allowing virus entry *via* endocytosis (*7*). This complex process provides insights with which to screen potential drugs.

2.1. Screening for virus-based therapeutic agents targeting SARS-CoV-2 entry

The screening of virus-based therapeutic agents targeting SARS-CoV-2 entry is mostly focused on the virus' structural S protein. S protein-neutralizing antibodies (nAbs) could prevent SARS-CoV-2 infection *via* passive immunization and may become a better strategy for COVID-19 treatment (*12*). In molecular docking experiments, Pandey *et al.* found that 10 natural compounds (flavonoids/non-flavonoids) effectively bind to the C-terminal region of the SARS-CoV-2 S protein's two subunits, displaying a higher affinity than that of hydroxychloroquine (HCQ) (*13*). This binding likely interferes with interaction between the virus S protein and host ACE2 receptor or internalization during fusion (*14*). That study also suggested that fisetin, quercetin, and kamferol bind to the complex of hACE2-S with a low binding free energy and exhibit drug-like properties (*13*). A study has indicated that the S protein binds to the ACE2 receptor in its receptor-binding domain (RBD) (*15*). The peptide binder SBP1, which was synthesized to target the RBD of the S protein, could potentially keep SARS-CoV-2 from entering into host cells (*16*). A functional analysis of the S2 subunit revealed that the fusion of the viral and host cell membranes is mediated by the formation of a six-helical bundle (6-HB) from the interaction between heptad repeat 1 (HR1) and HR2 of S2 (*17*). The HR1 region is conserved among various human coronaviruses. Therefore, HR1 and HR2 may good targets for identifying fusion inhibitors against SARS-CoV-2. Based on experience with SARS-CoV (*18*), HR1- and HR2-derived peptides (named SARS-CoV-2-HR1P and SARS-CoV-2-HR2P, respectively) were designed to act as a SARS-CoV-2 membrane fusion inhibitor (*19*). Although their actual effect and safety need to be verified further, SARS-CoV-2-HR2P with potent fusion-inhibiting activity was found to be a promising therapeutic for treatment of SARS-CoV-2. In contrast, HR1P did not markedly inhibit virus fusion (*19*). The peptide fusion inhibitor EK1, which was designed based on the HR1 region, exhibited obvious fusion inhibitory activity with lower immunogenicity and better safety (*20*). Thus, EK1 may also be a potential treatment for COVID-19, although it needs to be verified.

2.2. Screening of host-based therapeutic agents targeting SARS-CoV-2 entry

The ACE2 receptor and TMPRSS2 are valuable targets for host-based therapeutic drug development involving the inhibition of SARS-CoV-2 entry. The recombinant human soluble ACE2 protein (hrsACE2, APN01) dose-dependently bound to cellular ACE2, suppressed SARS-CoV-2 replication, and significantly reduced viral loads in Vero cells (*21*). A point worth noting is that APN01 also inhibited SARS-CoV-2 infection in human kidney organoids and engineered blood vessels. The actual efficacy with which APN01 reduces the viral load and mitigates symptoms in patients with COVID-19 is being verified in a randomized, multicenter clinical trial (NCT04335136). In addition, the hrsACE2-immunoglobulin-Fc, formed by fusing hrsACE2 to an immunoadhesin, may be a good choice to suppress SARS-CoV-2 infection *in vitro* and potentially *in vivo* (*22*).

Given the key role of host cell serine protease TMPRSS2 in virus entry, protease inhibitors may be a treatment option for patients with COVID-19 (*7*). By inhibiting the activity of TMPRSS2, the clinically-proven serine protease inhibitors camostat mesylate and nafamostat mesylate have been found to drastically reduce SARS-CoV-2 infection in human lung Calu-3 cells (*7*) and simian Vero E6 cells (*9*), respectively. Both marketed drugs have already been approved to treat other diseases in Japan for many years and are clinically safe, suggesting that they should be considered as promising therapeutic drugs to treat SARS-CoV-2 without safety concerns. Moreover, the approved mucolytic cough suppressant bromhexine was also found to inhibit TMPRSS2 (*23*). These three marketed drugs are currently being tested in clinical trials as promising therapeutic agents against COVID-19 (*10*). In addition, the approaches of *in silico* structure-based virtual screening and molecular docking have identified the oral topoisomerase I inhibitor rubitecan and benzodiazepine loprazolam as potent candidates for combating SASRS-CoV-2 by inhibiting TMPRSS2 (*24*). Besides host ACE2 receptor and its partner protease TMPRSS2, transmembrane glycoprotein CD147 was found to mediate another novel route for SASRS-CoV-2 invasion of host cells (*25*). Thus, the humanized anti-CD147 antibodies meplazumab, metuximab, and metuzumab are considered promising host-based therapeutic agents for treating COVID-19, though they need to be verified and examined further (*26,27*).

The wide-spectrum antiviral drug umifenovir (arbidol) is another therapeutic agent targeting SARS-CoV-2 entry predominantly by intercalating into membrane lipids and inhibiting viral fusion with host cell membranes (*28*). Currently, several phase IV clinical trials have been completed or are underway in China to confirm the efficacy of arbidol in the treatment of COVID-19 (*29*). Arbidol was recommended to treat COVID-19 in the latest therapy guidelines issued by the National Health Commission of the People's Republic of China on August 8, 2020 (*30*). Baricitinib, the small

molecule inhibitor of Janus kinase subtype 1 and 2 (JAK1/2) approved for the treatment of rheumatoid arthritis, has been selected as a potential therapeutic agent in clinical trials involving patients with COVID-19 because of its interaction with endocytosis kinase regulator AP2-associated protein kinase 1 (AAK1), whereby it interrupts endocytosis and virus entry (*31*).

3. Potential therapeutic agents targeting SARS-CoV-2 replication

Following SARS-CoV-2 entry into the host cell, the viral RNA is introduced and the process of replication begins. Some functional proteins, such as RdRP, helicase 3CL protease, and PL protease, are vital for SARS-CoV-2 replication and have become potential targets for virus-based drug development to treat COVID-19 (*8*).

3.1. Potential therapeutic agents targeting proteases

The replicase complex, involving 3CLpro and the secondary papain-like protease 2 (PL2pro), facilitates viral replication (*32*). Since 96% of the SARS-CoV-2 3Clpro sequence is identical to that of SARS-CoV (*33*) and there is no human homolog of 3CLpro, 3CLpro has become an ideal target for drug discovery and repurposing (*34*). The combination of lopinavir/ritonavir (LPV/RTV), with LPV acting against 3CLpro and RTV increasing the LPV half-life by inhibiting cytochrome P450, was approved for treatment of human immunodeficiency virus (HIV) (*35*). Based on its efficacy against SARS-CoV and MERS-CoV (*36,37*), LPV/RTV was tested for the treatment of SARS-CoV-2. Although one *in vitro* study tested lopinavir against SARS-CoV-2 (*38*), only a few clinical trials have noted clinical improvement in patients with COVID-19 receiving lopinavir/ritonavir (*39,40*). Most clinical trials have found no clinical benefit from lopinavir/ritonavir in patients with mild, moderate, or severe COVID-19 (*41,42*). There may be benefits when lopinavir/ritonavir is combined with other drugs or used in the early stage of COVID-19. Currently, routine use of lopinavir/ritonavir is not recommended, and further studies are needed to confirm its efficacy. Despite the discouraging results with lopinavir and ritonavir, 3CLpro is still a potential therapeutic target for screening agents against SARS-CoV-2. Eleven approved or investigational drugs, such as the tyrosine kinase inhibitors poziotinib and fostamatinib, the antipsychotic drug ziprasidone, and the detoxification drug folinic acid, were identified as potential covalent inhibitors of SARS-CoV-2 3CLpro according to the steric-clashes alleviating receptors (SCAR) protocol (*43*). Other marketed drugs identified as SARS-CoV-2 3Clpro covalent inhibitors include lurasidone, talampicillin, ribavirin, and telbivudine (*24,44*). With the help of target-based virtual ligand screening, Wu *et al.* found that the anti-hypertensive drugs telmisartan and nicardipine, anti-bacterial agents including doxycycline, lymecycline, demeclocycline, and oxytetracycline, and conivaptan for treatment of hyponatremia displayed the highest binding affinity to 3CLpro (*45*). A growing number of marketed drugs, investigational compounds, and phytochemicals were identified as potent inhibitors of SARS-CoV-2 3CLpro according to computational methods (*46,47*), suggesting promising strategies for drug repurposing. The Michael acceptor inhibitor N3 and the organoselenium compound ebselen were found to inhibit SARS-CoV-2 in simian Vero E6 cells (*46*). A newly designed pyridine containing α-ketoamides (13b) displayed a strong inhibitory effect on purified recombinant SARS-CoV-2 3CLpro, favorable pharmacokinetic properties, and strong lung tropism in mice (*33*), suggesting a role for this specific type of 3CLpro inhibitor in COVID-19 therapy. Two newly designed and synthesized peptidomimetic aldehydes, termed 11a and 11b, displayed excellent inhibitory action against 3Clpro in simian Vero E6 cells infected with SARS-CoV-2 and SARS-CoV-2 (*34*). Because of its favorable pharmacokinetic properties and low toxicity in beagles and Sprague Dawley rats, 11a is considered a promising agent for COVID-19 therapy (*34*).

Another protease PLpro, which is crucial for correcting virus replication (*48*), is considered a potential target for developing therapeutic agents against SARS-CoV-2. Target-based virtual ligand screening has indicated that a series of marketed drugs, including the antibiotics cefamandole, chloramphenicol, and tigecycline, the anti-virus agents ribavirin, thymidine, and valganciclovir, and natural products such as platycodin D and catechin compounds, bind to PLpro with a high affinity, indicating their potential for SARS-CoV-2 treatment (*45*).

Darunavir, one of the second generation of HIV-1 protease inhibitors, drastically inhibited the replication of SAR-SCoV-2 *in vitro* (*49*). However, results from a randomized, open-labeled single-center, controlled phase III trial revealed that the combination of darunavir/cobicistat was not efficacious in reducing the duration of therapy or alleviating symptoms in patients with COVID-19 (NCT04252274). Further studied are need to evaluate the efficacy and safety of darunavir in the treatment of COVID-19.

3.2. Potential therapeutic agents targeting RdRp

RdRp is critical for the machinery of viral RNA transcription and replication. In addition to virus replication rates and fidelity, the virus' ability to mutate and adapt to new environments is determined by RdRp (*50*). At the protein level, the amino acid sequence of RdRp of SARS-CoV-2 is approximately 96% identical to that of SARS-CoV. Moreover, their protein structures are similar (*51*), indicating that potent inhibitors of the

RdRp of SARS-CoV are likely suppress SARS-CoV-2 RdRp (*52*). Thus, conserved RdRp has been recognized as a potential target for screening agents against SARS-CoV-2. The adenosine analogue remdesivir (GS-5734) was originally designed for the Ebola virus and exhibits broad-spectrum antiviral activity against several RNA viruses including SARS-CoV, MERS-CoV, and SARS-CoV-2 (*53,54*). Remdesivir can recognize the key component of RdRp nsp12 and join nascent viral RNA chains, leading to premature termination of RNA synthesis (*55*). Wang *et al.* found that micromolar concentrations of remdesivir can effectively block SARS-CoV-2 infection in Vero E6 cells, particularly in combination with chloroquine (*53*). A high intracellular concentration of remdesivir in its active form has been observed in rhesus monkeys and remdesivir retains its good pharmacokinetic properties, indicating its potential for clinical treatment of COVID-19. Since remdesivir has not been approved for treatment for any disease worldwide, it must be used compassionately or in enrolled clinical trials. One multicenter, double-blind, placebo-controlled, well-conducted RCT from China found that remdesivir reduced the time to clinical improvement in patients with COVID-19. However, the efficacy of remdesivir did not differ significantly from that of a placebo (*56*). The first American patient with COVID-19 received remdesivir and recovered in January 2020 (*57*). Remdesivir was also reported to reduce the time to recovery and tended to have a survival benefit for patients with COVID-19 in a clinical trial conducted by the Unites States National Institute of Health (*58*). Encouraged by these results, remdesivir was authorized by the United States Food and Drug Administration (FDA) for emergency use to treat inpatients with COVID-19 (*59*). Currently, a number of clinical trials to confirm the efficacy and safety of remdesivir for COVID-19 treatment are underway around the world (*10*).

Another nucleotide analogue suggested for COVID-19 treatment is favipiravir (Avigan, T-705), which has been approved for treatment of influenza in China and Japan (*49*). Favipiravir blocks the replication of RNA viruses by selectively inhibiting RdRp and is unlikely to generate resistant viruses (*60*). Since the RdRp gene of SARS-CoV-2 is similar to that of influenza virus, favipiravir may be a promising therapeutic for COVID-19 (*4*). The potential inhibitory effect of favipiravir on SARS-CoV-2 was demonstrated in Vero E6 cells *in vitro* (*53*). Preliminary results of clinical trials have indicated that favipiravir is effective at improving clinical outcomes for patients with COVID-19 (*61*). Compared to lopinavir/ritonavir, favipiravir resulted in faster improvement of chest images and faster viral clearance with fewer adverse effects during the treatment of patients with COVID-19 (*62*). Another randomized, multi-center, open labeled study revealed that favipiravir effectively decreased the incidence of cough and pyrexia and improved 7-day clinical recovery in inpatients with moderate-to-severe COVID-19 (*63*). However, there were no significant differences between groups receiving favipiravir and umifenovir. Although more clear evidence of the efficacy and safety of favipiravir is being assembled in multiple clinical trials (*10*), favipiravir may be one of the most promising anti-SARS-CoV-2 drugs and it has a relatively high level of patient compliance (*49*).

The guanosine analogue ribavirin has been proposed as a potent drug to treat SARS-CoV-2. Although there several RCTs to test its efficacy are underway, ribavirin is not recommended to treat patients with COVID-19 because of its apparent inactivity and hemolytic toxicity. In China, the latest treatment guidelines for COVID-19 (*30*), recommend a combination of ribavirin and lopinavir/ritonavir or interferon. However, use of ribavirin or lopinavir/ritonavir alone to treat COVID-19 is not recommended.

Computer-aided drug screening is being extensively used to discover new drugs and repurpose existing ones targeting RdRp in order to treat COVID-19. *in silico* virtual screening by Pokhrel *et al.* indicated that quinupristin bound across the conserved RNA tunnel of RdRp, possibly resulting in the arrest of viral replication (*64*). Elfiky cited ribavirin, sofosbuvir, remdesivir, tenofovir, galidesivir, and the guanosine derivative (IDX-184) as potent drugs for COVID-19 therapy since they tightly bind to SARS-CoV-2 RdRp in a model (*65*). In a target-based virtual ligand screening study, some marketed drugs such as the anti-bacterial agent novobiocin, the anti-fungal drug itraconazole, the muscle relaxant drug pancuronium bromide, and natural products or derivatives exhibited a high binding affinity to the RdRp of SARS-CoV-2 (*45*). The approved anti-HCV drug elbasvir was predicted to bind tightly and preferentially to RdRp, PLpro, and helicase of SARS-CoV-2, suggesting it could efficiently stop virus replication alone or in combination with other agents (*66*).

4. Discovery of potential therapeutic agents based on host immune responses

Pneumonia, lymphocyte exhaustion and peripheral lymphopenia, and a cytokine storm are the typical features of severe COVID-19 (*67*). When SARS-CoV-2 infects the host cell, the innate and adaptive immune responses are activated in the host's body. A variety of antibodies are produced to fight the virus. The uncontrolled inflammatory innate responses may induce a storm of pro-inflammatory cytokines including IL-1β, IL-6, and TNF, which cause tissue damage, leading to acute respiratory distress syndrome (ARDS). Thus, the host immune response provides a therapeutic avenue to treat COVID-19.

Based on experience with other viral diseases, the most direct but potentially effective treatment for

COVID-19 is using convalescent plasma (CP), which can be obtained from patients who have fully recovered from the SARS-CoV-2 infection (*68,69*). Patients who have recovered from SARS-CoV-2 have developed viral antibodies against SARS-CoV-2 at a high titre (*70*). Antibodies in the CP can neutralize the virus directly, suppress viremia, and boost the immunity of the patient. Studies in China have found that CP shows promising in improving the clinical condition of patients with severe COVID-19 (*69,71*). Hospitals in New York City are preparing to use CP as a promising treatment for COVID-19. Currently, a number of RCTs examining CP in the treatment of COVID-19 are underway in various countries. The human monoclonal neutralizing antibody CR3022, isolated from a convalescent patient with SARS-CoV, was reported to strongly bind to the conserved receptor-binding domain (RBD) in SARS-CoV-2 and SARS-CoV (*72*). CR3022 might be a potential therapeutic candidate to prevent and treat COVID-19, and especially for patients in life-threatening condition.

Neutralizing mAbs and small molecule inhibitors targeting pro-inflammatory cytokines and downstream signaling components may also useful in controlling the cytokine storm and alleviating immune injury (*29,67*). Tocilizumab, a recombinant human anti-IL-6 receptor antibody, has being investigated for off-label use in patients with severe COVID-19. Studies from China and Italy showed that tocilizumab potentially controlled fever and improved respiratory function in patients with severe COVID-19 (*73,74*). A number of RCTs are underway to evaluate the efficacy of tocilizumab, alone or in combination, in severely ill patients with COVID-19 (10). Other anti-IL-6 receptor antibodies studied for the treatment of COVID-19 include the humanized monoclonal antibody TZLS-501, sarilumab, and the recombinant human-mouse chimeric monoclonal antibody siltuximab (*75*). TZLS-501 has been shown to significantly reduce circulating levels of IL-6 in the blood (*75*). Clinical trials to test the safety and efficacy of siltuximab and sarilumab in patients with severe COVID-19 have begun (*10*). Several RCTs to study the efficacy of recombinant human IL-1 receptor antagonist anakinra in COVID-19 therapy are planned. The JAK1/2 inhibitor ruxolitinib and the anti-IL-1β monoclonal antibody canakinumab are compassionately used for COVID-19 treatment in Italy (*10*). Moreover, NLRP3 (NOD-, LRR-, and pyrin domain-containing 3) inflammasome, which plays an important role in inflammatory cytokine production, and its inhibitors have garnered attention as potential agents to treat SARS-CoV-2 (*76*).

In China, the latest treatment guidelines for COVID-19 recommend immunotherapies with CP and intravenous human immunoglobulin and tocilizumab to treat severe cases with rapid progression or a high level of IL-6 (*30*).

5. Other potential therapeutic agents with multiple mechanisms

Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ), used to treat malaria and autoimmune diseases, have gained greater attention as promising therapeutic agents for the treatment of COVID-19 (*77,78*). To date, these agents have shown therapeutic activity against several viruses such as SARS-CoV and MERS-COV, suggesting they could be effective in treating SARS-CoV-2 infection (*79*). Proposed mechanisms by which CQ/HCQ combats SARS-CoV-2 include blocking virus entry into host cells, arresting viral replication, assembly, and budding, attenuating the inflammatory reaction, and inhibiting autophagy (*80,81*).

CQ/HCQ are weak bases and can elevate endosomal pH, thereby interfering with virus-host cell fusion during SARS-CoV-2 endocytosis (*78*). Another mechanism by which CQ/HCQ blocks SARS-CoV-2 entry into host cells is by inhibiting glycosylation of the ACE2 receptor and viral envelope glycoproteins (*53,79*). CQ/HCQ may arrest SARS-CoV-2 replication and budding by inhibiting specific enzymes that are necessary for virions assembly and budding from the cell membrane (*81*).

Another reason why CQ/HCQ may be a potential therapeutic agent for COVID-19 is because of their profound anti-inflammatory action to reduce pro-inflammatory cytokine and superoxide release, presumably by inhibiting the Toll-like receptor (TLR) pathway and upregulating the cyclin dependent kinase inhibitor p21 (*82,83*). Studies with Vero E6 cells have shown that CQ/HCQ has a potent antiviral effect against SARS-CoV-2 (*53,77*), with HCQ having relatively higher potency (*84*). That said, results from clinical trials of CQ/HCQ to treat patients with COVID-19 are inconsistent. A Chinese study with more than 100 patients showed that CQ is superior to the control in improving lung imaging findings, increasing the negative conversion rate, and shortening the duration of treatment (*85*). Two clinical trials suggested that HCQ improved clinical outcomes for patients with COVID-19 (*86*). However, HCQ did not improve viral clearance in another study with 30 patients with COVID-19 (*87*). The macrolide antibiotic azithromycin was shown to significantly enhance HCQ efficacy by increasing the virologic cure rate and reducing the duration of therapy in one case series from France (*88*). In contrast, another case series in France reported that a combination of HCQ and azithromycin had disappointing results in critically-ill patients with COVID-19 (*89*). Moreover, a retrospective analysis study from the US (*90*) found that the risk of mechanical ventilation for inpatients with COVID-19 could not be reduced by HCQ alone or in combination with azithromycin. Furthermore, increased overall mortality was found to be related to HCQ treatment alone. Therefore, there is a dire need for quality scientific evidence to confirm the efficacy

and safety of CQ/HCQ alone or in combination with azithromycin for the treatment of COVID-19. A large number of RCTs are ongoing (*80*). Interestingly, CQ/HCQ can induce the uptake of zinc into the cell cytosol, which has been shown to halt coronavirus replication by targeting RdRp (*91*). Thus, synergistic zinc supplementation may be necessary to improve the therapeutic effects of CQ/HCQ in patients with COVID-19 (*92*). Currently, many protocols have approved HCQ for the treatment of COVID-19, and especially when combined with other antiviral drugs (*78,84*). Instead of HCQ, chloroquine phosphate is recommended for the treatment of COVID-19 in the latest Chinese treatment guidelines (*30*). Post-exposure prophylaxis against SARS-CoV-2 with CQ/HCQ is not recommended in light of safety concerns (such as worsening vision, QT prolongation, hypoglycemia, and development of a rash).

Interferons (IFN) have antiviral activity by inhibiting viral replication and immunomodulatory action by interacting with toll-like receptors (*93*). IFN-α and IFN-β have been found to have potent inhibitory activity against SARS-CoV and MERS-CoV (*94,95*). Compared to SARS-CoV, SARS-CoV-2 was found to be more susceptible to IFNs and inhalation of IFN-α 2b significantly reduced the infection rate. Thus, IFN-α 2b can be used for prophylaxis against the SARS-CoV-2 infection (*96*). In China, IFN-α is recommended for treatment of COVID-19 alone or in combination with ribavirin and the antiviral drugs lopinavir/ritonavir (*30*). The efficacy and safety of this COVID-19 treatment strategy is being evaluated in a trial in China (ChiCTR2000029387). Reduced INF-β was reported to be directly associated with increased susceptibility to developing severe respiratory diseases in patients with a viral infection (*97*). The SARS-CoV-2 infection was found to decrease INF-β production in body (*98*). One study has suggested that a combination of INF-β and ribavirin offers promise as a treatment for SARS-CoV-2 (*99*). Clinical trials using IFN-β or inhaled IFN-β (SNG001) to treat COVID-19 are ongoing in the UK (*100*).

6. Conclusion

Effective therapeutic agents are urgently needed to globally combat the ongoing COVID-19 pandemic. This review has summarized potential therapeutic drugs targeting SARS-CoV-2 entry and replication and the host response to the infection. Although some of the drugs mentioned have yielded promising results, no specific drug is capable of treating COVID-19 according to a substantial amount of quality scientific evidence. The combination of antiviral and anti-inflammatory drugs may be more effective. Drug safety, a high level of efficacy, and availability should be full considered in COVID-19 therapy. Findings from ongoing clinical trials and advances in vaccine research will be critical to defeating the SARS-CoV-2 infection.

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**Address correspondence to:*
Cuirong Zhao, Department of Pharmacy, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan, Shandong 250021, China.
E-mail: xiaozhao511@163.com

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