REVIEW ARTICLE

Current status of drug development against SARS CoV-2 infections

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Abstract

SARS CoV-2 and its associated disease Covid-19 first occurred in China at the end of 2019 and conquered the world in a storm. As of June 24, 2020, the World Health Organization listed 9,129,146 confirmed cases, accompanied by 473,797 deaths. An initial response by many countries was to lock down their economies, which helped flattening the curve at a high economic cost. The long-term solution will be vaccines to prevent infection and treatment drugs.

This minireview focuses on drugs against the virus itself. Among the drugs that interfere with the virus' ability to attach to and invade the human cell, camostat mesylate looks promising *in vitro*, but clinical trials have not been completed yet. A phase II trial has been completed for recombinant human angiotension converting enzyme 2 that blocks the spike protein from binding to cellular ACE-2. Hydroxychloroquine is probably the most controversial of all drugs; after initial excitement, the Federal Drug Administration revoked the emergency use of this drug against SARS CoV-2. Among the inhibitors of the RNA dependent RNA polymerase of the virus, remdesivir, faripiravir which is already in a phase IV trial, and tenofovir will be discussed. Additional drugs included in this study are lopinavir/ritonavir that have previously been used against HIV and the antiparasitic drug ivermectin. Many of the presented drugs have previously been used for a different disease and are currently being trialed against SARS CoV-2.

Keywords: SARS CoV-2, Covid-19, pandemic, drug development, clinical trials

Introduction

The virus SARS CoV-2 and its associated disease COVID-19 originated in China [1] and then spread across the world at an alarming speed. On March 11, 2020, COVID-19 was declared a pandemic by the World Health Organization (WHO; www.who.int/data). By June 24, the WHO listed a total of 9,129,146 cases and 473,797 deaths worldwide. Having learned lessons from the 2003 pandemic on SARS CoV, a team of Chinese researchers postulated a four step control path for future coronavirus epidemics; prevention, rapid response, reducing viral transmission, and treatment [2]. Within the US, the Centers for Disease Control and Prevention (CDC; www.cdc.gov) list 2,336,615 cases as of June 24 and 121,117 deaths. Testing can be done for both, the actual virus by means of reverse transcription PCR, as well as antibodies [3-5]. Test protocols are available through the CDC website (www.cdc.gov/coronavirus/2019-ncov/lab/). Contact tracing is also considered helpful. As one example, the State of North Dakota provides the new Care19 app to aid this purpose (www.health.nd.gov). To prevent economically costly lock-downs, preventative approaches include vaccine development (for a review, see [6]). Simultaneously, efforts are undertaken to develop a myriad of different treatment options.

This mini-review focuses on drugs against SARS CoV-2 infections. The emphasis is on drugs that inhibit the virus, as opposed to those that modulate the innate immune system. Drugs are included that fit into one of three categories; i) block attachment and entry of the virus into the human cell, ii) inhibit the RNA dependent RNA polymerase; iii) others. Information given about the selected drugs include basics of the molecular mechanism or signal transduction pathway if known, other diseases that these drugs were previously used for, and research efforts that demonstrate effectiveness against SARS CoV-2. Information about ongoing clinical trials will be included as this gives an indication to when the drug may become available.

1. SARS CoV-2

The family Coronaviridae includes seven viruses that can cause human disease, of which SARS-CoV caused outbreaks in 2003, 2012, and 2019, respectively (for recent reviews see [7, 8]). In this sense, the world is just seeing the third pandemic in two decades of a coronavirus [9]. The large degree of similarity between the SARS CoV-2 genome sequence and those of other bat coronaviruses implies that the original reservoir for this new virus may be the bat as well, with the possibility of intermediate mammals sold at the Wuhan food market [1].

A big distinction of SARS CoV-2 from the previous SARS CoV and MERS is the way the disease presents itself. Some of the are actually similar among the three pathogens, such as shortness of breath, muscle ache, and fever [10]. Early symptoms include sore throat and headache [10]. The progression of Covid-19 after the early onset is more difficult to predict or even understand. Entering the peripheral blood from the lungs, the virus is eventually capable of attacking just about any organ that

has the angiotensin converting enzyme-2 receptor (ACE-2), which includes lungs, heart, renal and gastrointestinal tract [11]. The ability to travel from the blood to the intestines explains why SARS CoV-2 can be detected in feces [12]. From the onset of the symptoms to acute respiratory distress syndrome (ARDS), it takes about 8 days [3].

In a review article, Lin and coworkers [13] hypothesize a pathogenesis pathway that divides the clinical phase of severe SARS CoV-2 infection into three phases: viremia (day 7 to 10 after infection), acute pneumonia (day 7 to 10 through day 14 to 21), recovery (after day 14-21). B cells and T cells in blood decrease in a linear manner throughout all three phases [13]. Inflammatory cytokines increase sharply at the onset of the acute phase [13]. These so called cytokine storms have been known to complicate other infectious diseases and were proposed as targets for immunomodulatory therapy previously [14]. Among the comorbidities that distinguish patients with a severe progression of SARS CoV-2 infection from non-severe ones are hypertension, respiratory system disease, and cardiovascular diseases [15]. A new study that associated variations in the genetic make up of patients with respiratory failure determined that people in the blood group A had a higher risk at developing severe disease with an odds ratio of 1.45, while patients in the blood group O had a lower risk with an odds ratio of 0.65 [16].

Symptoms of SARS CoV-2 infections that go beyond acute respiratory distress syndrome (ARDS) are for example neurological (systematically reviewed by [17]). Neurological symptoms include dizziness, stroke, seizures, encelopathy, and coma [17]; risk factors for cerebrovascular diseases as a consequence of SARS CoV-2 infection include advanced age, heart failure, coronary artery disease, diabetes, and obesity [17]. Cardiac injury and heart failure also fall into this category of non-ARDS symptoms of patients with a severe disease progression. It is believed that pericytes with high expression of ACE-2 may be the target for SARS CoV-2, as patients with basic heart failure showed increased expression of ACE-2 [18]. Altogether, the symptoms caused by SARS CoV-2 are diverse and not limited to respiratory distress.

The early intervention by means of economic lock down with the purpose to slow the spread of SARS CoV-2 and 'flatten the curve' was successful in many countries. A study from Italy and Spain analyzed data with quasi-Poisson regression and came to the conclusion that the first lockdown reduced slopes, but trends kept rising. In contrast, the second lockdown decreased trends and slopes for both countries[19]. A study published in Nature determined the effect of anti-contagion policies among China, South Korea, Italy, Iran, France, and the US and concluded that the collective lockdowns in those six countries prevented 62 millions of confirmed SARS CoV-2 cases, with may translate to ~530 millions of total infections [20]. However, continued lockdowns come at an economic cost and it may be a better approach to use the lockdown and post-lockdown time to develop vaccines and treatment drugs against SARS CoV-2 [21].

Vaccine development has been reviewed by Chen and coworkers [6]. Vaccine development has been facilitated by

previous vaccine design concepts for SARS Co-V. Current vaccine development programs include but are not limited to an adenovirus-vectored vaccine by Johnson and Johnson, a live attenuated vaccine by Codagenix, a protein based vaccine by the University of Queensland, a recombinant nanoparticle vaccine by Novavax, an S-trimer recombinant protein vaccine by Clover Biopharmaceuticals, and a coronavirus RBD protein based vaccine by Baylor College of Medicine and Fudan University, New York.

2. Treatment drugs against SARS CoV-2

The drugs that are being developed to treat Covid-19 patients can be divided into two mechanistic categories, based upon their target; the virus itself, or the human immune system [22]. To provide focus, this article will be limited to those drugs that act against the virus itself. Many of the selected drugs were originally directed against a different disease and have recently been tested against SARS CoV-2. This strategy is believed to shorten the time until the drug will become available to patients. Differences between the discussed drugs include the target (*e.g.* spike protein, ACE-2 receptor, replication machinery) and the virus that the drug was previously used and approved for (*e.g.* HIV, Ebola, influenza, hepatitis B). Information on the drug targets, aspects of the molecular mechanisms or signal transduction pathway if known, current state of research, and examples of clinical trials are summarized in Table 1.

Table 1. Summary of drugs to treat infection with SARS CoV-2

Name of drug	Drug target	Mechanism	Current state	Trial/Reference[1]
Camostat mesylate	TMPRSS22 (serine protease)	Block cell entry	Clinical trial ongoing in US	NCT04353284 (phase II) NCT04321096 (phase I and II)
rhACE-2	ACE-2 (receptor)	Block cell entry	Phase II trial completed	NCT01597635 (Phase II) [23][2]
Hydroxychloroquine	pH ACE-2 glycosylation	Block cell entry	Clinical trials ongoing and completed	NCT04351620 (Phase I) NCT04345692 (Phase III) NCT04308668 (Phase III) [24][2]
Remdesivir	RdRP	Adenosine analog	Clinical trials ongoing in the US and France	NCT04323761 (expanded access) NCT04365725
Favipiravir	RdRP	Guanine analog	Clinical trials ongoing and completed world wide	ChiCTR2000029600 (Phase II) [25][2] NCT04359615 (Phase IV) NCT04387760 (Phase II and III) NCT04411433 (Phase III)
Tenofovir	RdRP	Acyclic analog of d-AMP	One clinical trial, US and Spain	NCT04334928
Lopinavir/Ritonavir	Cysteine protease	Replication complex	Clinical trials ongoing and completed in China	NCT04252885 (Phase IV) ChiCTR2000002930 8 [26][2]
Ivermectin		Reduces viral RNA	Clinical trials ongoing world wide	NCT04343092 (Phase I, completed) NCT04425707 NCT04374279 (Phase II)
Simeprevir	Mpro protease		Research only [27]	Not yet

[1]The selected clinical trials were obtained from https://clinicaltrials.gov and the Chinese Clinical Trial Registry (www.chictr.org).
[2]For completed trials, the reference for the publication is indicated (if available).

2.1 Blocking the entry path that requires ACE-2 and TMPRS22

The first step in the replication cycle of SARS CoV-2 is attachment to the angiotensin converting enzyme 2 (ACE-2) on the human host cell by means of a specific recognition sequence in the spike protein on the virus [28]. One process that leads to the formation of a virus-human cell fusion is proteolytic cleavage of the spike protein by a transmembrane serine protease designated TMPRS22 [28]. Both, ACE-2 and TMPRS22 have important functions in the human body and have been proposed as drug targets for diseases that are unrelated to SARS CoV-2. ACE-2 was proposed as a target for cardiovascular therapies [29] with the ultimate goal to reduce blood pressure through inhibition of ACE-2 [30]. TMPRSS22 is the target for camostat mesylate that inhibits trypsin, plasmin, kallikrein, and thrombin and is prescribed to reduce pancreatitis associated pain [31].

Camostat mesylate was the most effective and recommended for animal studies in a study that tested the efficacy at inhibiting viral entry into host cells of several commercially available inhibitors [28]. A mouse model that was previously done to test numerous inhibitors against SARS CoV-1 and MERS demonstrated that camostat might be an excellent lead candidate for the development of novel treatment against viruses that use serine proteases for cell entry, such as coronaviruses and filoviruses [32]. A clinical trial (NCT04353284) by Yale University (United States) to test camostat mesylate in COVID-19 outpatients is not yet recruiting. The purpose of this phase 2 trial is to test whether orally given camostat mesylate might reduce complications of SARS CoV-2 infections, as well as reducing transmission of the virus. A second clinical trial (NCT04321096) by the University of Aarhus (Denmark) includes two cohorts of patients, hospitalized ones and outpatients.

An intriguing thought was to block ACE-2 by providing the soluble recombinant human angiotension converting enzyme 2 (rhACE-2) to block the spike protein from binding to cellular ACE-2. A completed phase II clinical trial (NCT01597635) by GlaxoSmithKline showed that angiotensin II levels decreased rapidly following infusion of the rhACE-2 drug GSK2586881, while being tolerated well by patients with ARDS [23]. It was believed that GSK2586881 decreased serum levels of angiotensin II by directing the substrate away from ACE-2, thus preventing activation of ACE-2 and preventing ARDS. Initial research with SARS CoV-2 demonstrated that the rhACE-2 drug APN01 inhibited SARS CoV-2 replication in tissue cultures of engineered human cells by 1,000 to 5,000 times [33].

An especially controversial drug is hydroxychloroquine that inhibits pH dependent steps of the life cycle of coronaviruses [34]. Chloroquine and hydroxychloroquine have been used to treat malaria for a long time. Lately, effectiveness has been reduced by the increasing development of resistance in the *Plasmodium falciparum* parasite [35], a long list of moderate to severe side effects including nausea, vomiting, and diarrhea [36], as well as unsuitabiliby for certain categories of patients including those that are in high risk categories for SARS CoV-2 (*e.g.* elderly) [37]. Additionally, chloroquine and

hydroxychloroquine have been used to treat autoimmune diseases such as rheumatoid arthritis, where drug treatment during the onset of rheumatoid arthritis reduces the incidence of chronic kidney disease [38].

Hydroxychloroquine increases the endosomal pH that is required for the membrane fusion between coronaviruses and the human host cell [34]. In addition, it inhibits the replication of SARS CoV by interfering with the glycosylation of ACE-2 [39]. Shortly after the onset of the COVID-19 pandemic, there were several Chinese publications on the effectiveness of hydroxychloroquine treatment of COVID-19 patients and one clinical study showed promising early results [40]. A small non-randomized clinical trial from France demonstrated that hydroxychloroquine treatment was significantly associated with a reduction in SARS CoV-2 load, an effect that was reinforced by additionally administering azithromycin [41]. It did not take long for another group of researchers from France to directly refute the previous paper by stating that there was no evidence of benefit of the combined hydroxychloroquine/azithromycin treatment [42]. Based on 'limited *in-vitro* and anecdotal clinical data', the US Food and Drug Administration (FDA) authorized an emergency approval to prescribe hydroxychloroquine to COVID-19 patients prior to the completion of clinical trials [43].

Currently ongoing clinical trials on the effectiveness of hydroxychloroquine for COVID-19 patients include NCT04351620 by the University of Chicago (United States), a phase I trial on the effects of high doses of hydroxychloroquine for ambulatory patients with mild COVID-19 symptoms and NCT04345692 by Queen's Medical Center (United States), a phase III clinical trial to study hydroxychloroquine in hospitalized COVID-19 patients. Both these trials are in the recruitment stage. Most importantly, in the phase III trial NCT04308668 hydroxychloroquine failed to prevent illness or confirmed infection when administered within four days of exposure to the virus [24]. The FDA used the outcome of this trial to revoke the emergency use authorization for hydroxychloroquine on June 15, 2020 (www.fda.gov).

2.2 Inhibiting the RNA dependent RNA polymerase of the virus

RNA dependent RNA polymerase (RdRP) is a key protein in the replication cycle of RNA viruses. It catalyzes RNA strands that are complementary to the original RNA (without a DNA template), starting out at the 3' end in a primer-independent of primer-dependent manner [44, 45]. Numerous inhibitors of the SARS CoV-2 RdRP have been described. An *in silico* study modeled the binding affinity of nucleotide analogs to RdRP and hypothesized the potential effectiveness of Remdesivir (adenosine analog), Favipiravir (guanine analog), Tenofovir (acyclic analog of d-AMP), several other nucleotide inhibitors, and interestingly also hydroxychloroquine [46]. Analysis of the cryo-EM structure of the SARS CoV-2 RdRP demonstrated that the RNA template is inserted into a central channel of the enzyme. Remdesivir is covalently bound to the first replicated base pair and consequently terminates chain elongation and thus prevents viral replication [47].

Remdesivir has undergone clinical trial as a therapeutic against the Ebola virus in the Democratic Republic of Congo, which was unable to confirm effectiveness against the virus, but proved that it is safe to treat patients [48]. Several studies demonstrated antiviral activity of remdesivir against SARS CoV, MERS, and SARS CoV-2 *in vitro* [49-51]. Gilead Sciences has a patent on remdesivir as treatment for arenaviruses and coronaviruses [52]. Clinical trials in the US and other countries are numerous. NCT04323761 by Gilead Sciences tests remdesivir for the treatment of SARS CoV-2 infection. NCT04365725 by Assistance Publique - Hôpitaux de Paris (France) is recruiting volunteers for a retrospective study, where the effect of remdesivir on hospitalized adult Covid-19 patients will be determined. A small cohort study with 61 patients yielded clinical improvement in 26 out of 53 patients (68%), while data from 8 patients could not be analyzed [53]. The FDA granted an emergence use authorization to treat the most severely ill patients with remdesivir on May 1, 2020.

Faripiravir has a similar mode of action as remdesivir, but has been less investigated at the clinical level and needs further investigation [54]. It structurally resembles guanine and also inhibits RdRP by competing with purine nucleosides [55]. It has been developed by Toyoma Chemicals and is approved for the treatment of influenza in Japan after a phase III clinical trial was completed in Japan[56], in addition to two phase II studies in the US. Faripiravir was on trial in China as a treatment of SARS CoV-2 infections (ChiCTR2000029600). Comparing 35 patients that were treated with favipiravir with 45 patients in the untreated control group demonstrated a shorter viral clearance time among the drug treated patients (median 4 days vs 11 days) [25]. A number of clinical trials across the world are currently in the recruitment or not yet recruitment phase. The phase IV trial NCT04359615 from Shahid Beheshti University of Medical Sciences (Iran) studies the treatment of hospitalized patients with faripiravir. NCT04387760 by Royal College of Surgeons in Ireland (Ireland) compares faripiravir and hydroxychloroquine, with the hydroxychloroquine trial being in phase II and the faripiravir trial in phase III. NCT04411433 from Hacettepe University (Turkey) is a phase III trail for the use of favipiravir to treat SARS CoV-2 patients.

Tenofovir is used to treat hepatitis B, especially to prevent transmission of the virus from pregnant mothers to their unborn babies [57]. It is also commonly used to treat HIV patients [58] and is considered safer than other antiretroviral drugs [59, 60]. A molecular docking study using RdRP as a target determined that Tenofovir among other RdRP inhibitors might be potent drugs against SARS CoV-2 [61]. A single clinical trial involving tenofovir (NCT04334928) from Plan Nacional sobre el Sida (Spain) and Harvard School of Public Health (United States) seeks to study an assortment of drugs including tenofovir (and hydroxychloroquine) for health care workers. This is a phase III trial and they are currently in the recruitment phase.

2.3 Other SARS CoV-2 drugs

Lopinavir and Ritonavir are inhibitors of an aspartyl protease of HIV and have been used

as a combination drug for many years [62], though cardiac abnormalities were observed in HIV infected children on antiretroviral therapy [63]. They also reduce viral activity against SARS CoV [64] and MERS [65] through the coronaviral nsp5 protease (3CLpro), a cysteine protease that controls the replication complex and is essential for virus replication. Clinical studies demonstrated effectiveness against SARS CoV and MERS as well. A phase IV clinical study (NCT04252885) by Guangzhou 8th People's Hospital (China) is currently recruiting and seeks to study the efficacy of lopinavir/ritonavir/arbidol against SARS CoV-2. A completed randomized and controlled open-label train from China (ChiCTR20000029308) tested lopinavir/ritonavir on hospitalized adult patients with severe SARS CoV-2 infections. Comparing 99 patients that received lopinavir/ritonavir treatment with 100 patients of the control group, the researchers could not observe any benefit of the treatment. Gastrointestinal events were more common in the treatment group, while other adverse events occurred more often in the control group [26].

Ivermectin has been discovered as drug against parasitic nematode infections in humans and animals some 35 years ago [66] and it was the joint focus of the 2015 Nobel Prize in Physiology or Medicine. In the live stock industry, ivermectin and other macrocyclic lactones are commonly used against anthelmints in the US cattle industry [67]. For the human health market, ivermectin has been used for the control and elimination of lymphatic filariasis [68]. The FDA has approved ivermectin as a broad spectrum anti-parasitic agent. In recent years, activity against a number of viruses has been demonstrated as well [69-72]. In the case of HIV and dengue virus, ivermectin dissociates the IMPα/β heterodimer which is involved in the transport of viral proteins within the human cell [72]. Lately, it has been shown that ivermectin acts against SARS CoV-2 by reducing viral RNA [73]. Side effects can include severe headaches, myalgia, and motor deficit [74].

The list of clinical trials to test the effect of ivermectin on SARS CoV-2 is long. A completed phase I trial (NCT04343092) by the University of Baghdad (Iraq) tested ivermectin as an add on therapy against SARS CoV-2. NCT04425707 by the Ministry of Health and Population (Egypt) is recruiting for treating SARS CoV-2 infections with ivermectin. John's Hopkins University is not yet recruiting for a phase II trial (NCT04374279) that is looking to demonstrate recovery from SARS CoV-2 infection by ivermectin.

An entire group of inhibitors was proposed by Bafna and coworkers, who investigated the structural similarity of SARS-CoV2 Mpro and hepatitis C virus (HCV) NS3/4A Proteases [27]. The structural similarity of these two proteases appears striking and includes the binding pocket. Computational docking of existing HCV protease inhibitors into the active site of the M^{pro} protease of SARS-CoV2 gave rise to the idea that these drugs that are currently used against HCV may also be effective against SARS CoV-2 [27]. The list of drugs includes Glecaprevir, Boceprevir, Sovaprevir, Vaniprevir, and Simeprevir, of which the latter had the highest scoring

complex. Experimental research, followed potentially by clinical trials will have to confirm whether any of these drugs can be used against SARS CoV-2.

Conclusion

Altogether, half a year into the Covid-19 pandemic, a tremendous amount of research has been done on a myriad of different treatment options. A popular and possibly successful approach is to use drugs against previous diseases, including HIV, Ebola, influenza, and hepatitis B or even non-infectious diseases such as cardiovascular disease or pancreatitis. The approach is to test these already approved drugs against SARS CoV-2, particularly if previous research already indicated effectiveness against SARS CoV and/or established safe use for at least some categories of patients. In the case of successful *in vitro* experiments, clinical trials have been initiated, some have been completed. The drugs discussed in this mini-review are in different stages of this progression, up to completed phase II and phase III trials and ongoing phase IV trials. Some drugs, such as hydroxychloroquine raised excitement after *in vitro* experiments, but failed to deliver promising results in clinical trials. Among the many different drugs, this author hopes that one or several will be successful in clinical trials up to phase III or phase IV and will become available to the world population within a year or two. Seeing as i) the many risk factors that patients may have seriously impact the severeness of the SARS CoV-2 infection, and ii) each of the discussed drugs has different already established side effects that may exclude certain patients from being treated with the drug, this author hopes that the large diversity of the proposed drugs may enable us to use different drugs for different categories of Covid-19 patients.

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Conflict of Interest

The author declares no conflict of interest.

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