

COVID-19: Search for Therapeutics

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Abstract

Human coronaviruses (HCoVs) cause respiratory diseases infecting the upper and/or lower respiratory tract. The six human coronaviruses so far identified are HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU-1, SARS-CoV, and MERS-CoV. Four of these coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU-1) are known as circulating common coronavirus found continuously in the human population causing mostly common cold, with few cases of severe diseases. In late December 2019, a novel human coronavirus, now called SARS-CoV-2, was identified during an outbreak in Wuhan, China. The disease spectrum caused by this virus is now called COVID-19 (Coronavirus Infectious disease 2019). This novel coronavirus has spread globally resulting in a world-wide pandemic that continues to rage as of now. SARS-CoV-2 has a high case morbidity and mortality rate and is high risk to the elderly populations, immune-compromised populations, and to those who have other critical issues like heart disease, diabetes, etc.

In this review, we summarize the latest information of the epidemiology, pathogenesis, and clinical aspects of SARS-CoV-2, and discuss the current scientific and therapeutic advancements for clinical treatment of this pandemic novel coronavirus.

Introduction

In November 2002, a novel coronavirus, SARS-CoV, was first detected in China, while the larger outbreak was not confirmed until 2003. This virus caused pneumonia and other respiratory complications, referred to as Severe Acute Respiratory Syndrome (SARS). This epidemic spread to 29 countries, and infected about 9000 people with a more than 10% mortality rate [1-5]. SARS was identified as a human coronavirus, adding to four known coronaviruses that primarily cause common cold in humans (HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43) [6-8]. In 2012, MERS-CoV (Middle East Respiratory Syndrome coronavirus) was first identified in Saudi Arabia, isolated from a human with pneumonia [9]. As of March 12, 2019, a total of 2,494 cases with MERS were found in 27 countries with a mortality rate of more than 30% [10,11]. In December 2019, a new severe acute respiratory syndrome (SARS)-associated coronavirus was identified during an outbreak in Wuhan, China, originally called 2019-nCoV [12]. This new virus primarily causes pneumonia, among other syndromes, and the spectrum of diseases caused by it is called COVID-19 (Coronavirus Infectious Disease 2019). This virus was later named SARS-CoV-2. In March 2020, WHO declared COVID-19 as a global pandemic disease [13]. As of August 10, 2020, 89,270 people in China had been infected with SARS-CoV-2 with more than 4,693 fatalities. In the USA, there have been over 4.9 million confirmed cases with 160K deaths, and worldwide there have been more than 19.7M cases with 728K fatalities [14]. In this review, we will summarize the current knowledge of human coronavirus infection, with emphasis on therapeutic strategies.

A: Human Coronavirus and its Different Types:

Coronaviruses (CoVs) are single-stranded positive-sense RNA viruses whose genome (>27kb) is encapsulated within a lipid membrane envelope carrying spike protein [15]. This envelope is studded with glycoprotein spikes that give coronaviruses their crown-

like appearance [16,17]. Table 1 displays the sources of Coronavirus and their classifications.

A-1. Zoonosis: SARS-CoV is known to spread from bats to civets and then to humans [18], while MERS-CoV spreads from bats to dromedary camels and then to humans [19]. Genome sequencing of SARS-CoV-2 showed that about 96% overall genome sequence were identical with Bat CoV-RaTG13 [20], suggesting that human-SARS-CoV-2 and Bat coronavirus might share a common ancestor [21,22]. However, protein sequence alignment and phylogenetic analysis showed similar residues of receptors in many species, such as turtles, pangolins and snakes [23].

A-2. Classifications: Coronaviruses are divided into four genera: alpha, beta, gamma, and delta [24]. α - and β -CoV are able to infect mammals, while γ - and δ -CoV tend to infect birds. Among the four common human coronaviruses HCoV-229E and HCoV-NL63 are of alpha-type, while HCoV-OC43 and HCoV-HKU1 are in the group of beta-type coronaviruses. These four coronaviruses cause mild to moderate upper-respiratory tract illnesses, including the common cold [25,26]. SARS-CoV, MERS-CoV, and SARS-CoV-2 are all beta-coronaviruses and cause a severe lower respiratory tract infection with higher rates of morbidity and mortality than the α - human coronaviruses [12].

Based on current epidemiological investigation, the incubation period of SARS-CoV-2 ranges from 1–14 days while most commonly it is 3–7 days, similar to other coronaviruses. However, SARS-CoV-2 is

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Table 1. Human Coronavirus and its Different Types [21]

Cartoon of Human Coronavirus					Hemagglutinin (HE)		
					Lipid Bilayer		
					Envelope Glycoprotein (E)		
					Membrane Protein (M)		
					Nucleoprotein (N)+ RNA		
					Spike Protein (S)		
Common Human Coronaviruses					Other Human Coronaviruses (Severe Disease Causing Pathogens)		
	HCoV-229E	HCoV-NL63	HCoV-OC43	HCoV-HKU1	HCoV-SARS	HCoV-MERS	HCoV-SARS-2
Zoonosis	Bats → Camels (?) → Human	Bats → (?) → Human	Rodents → Bovines → Human	Rodents → (?) → Human	Bats → Palm Civets → Human	Bats → Dromedary Camels → Human	Bats → Pangulins (?) → Human
Classifications	α-Type	α-Type	β-Type	β-Type	β-Type	β-Type	β-Type
Incubation Period	2-5 days	2-5 days	2-5 days	2-5 days	2-11 days	2-13 days	3-6 days
Clinical Symptoms	Malaise, Headache, Sneezing, Nasal discharge, Sore throat, Fever, and Cough	Cough, Fever, Hypoxia, Croup, Rhinorrhea, Tachypna	Malaise, Headache, Sneezing, Nasal discharge, Sore throat, Fever, and Cough	Fever, Running nose, Cough, Dyspnea	Fever, Myalgia, Headache, Dry cough, Respiratory Distress, Diarrhea, Dyspnea	Fever, Myalgia, Headache, Cough, Sore throat, Respiratory Distress, Diarrhea, Dyspnea, Pneumonia	Fever, Myalgia, Headache, Dry cough, Respiratory Distress, Diarrhea, Dyspnea, Pneumonia
Fatality Rates	None	None	None	None	~10%	~35%	~4%

known to be contagious during this latency period prior to symptoms appearance [27,28].

A-3. Transmissibility and Pathogenicity: Coronaviruses express several structural proteins including the glycosylated spike (S) protein that mediates the host cell invasion via binding to a cellular receptor protein. Cell tropism is defined by the entry cellular receptor employed by the coronavirus, via its S glycoprotein. SARS-CoV, SARS-CoV-2, and H-CoV-NL63 utilize Angiotensin-Converting Enzyme-2 (ACE2) as the cellular receptor, whereas MERS-CoV uses Dipeptidyl-Peptidase-IV (DPP-IV), H-CoV-229E uses Amino-Peptidase N (APN), HCoV-HKU1 and HCoV-OC43 employ Sialic Acids as receptors [28-33]. Additionally, all of the α-CoV S-protein contain a sialic-acid binding domain [34,35].

The coronavirus infection process requires S-protein priming, which is facilitated usually by host serine proteases, whether membrane-associated, or extra-cellular. SARS-CoV-2 appears to use cell-bound transmembrane serine protease 2 (TMPRSS2-11?) [11]? from cleavage of S into S1 and S2 upon binding to ACE2 [28]. A portion of the S proteins on the coronavirus virion, may also be cleaved already prior to export of virion outside the cell by proteases in the Golgi apparatus. The viral S glycoprotein thereby consists of two subunits, S1 and S2 [36]. While the receptor-binding-domain (RBD) of S1 protein determines the virus-host range and cellular tropism, the S2 protein with its two tandem domains, heptad repeats 1 (HR1) and heptad repeats 2 (HR2) mediates virus-cell membrane fusion [37,38]. Following fusion, the viral genome is released into the cytoplasm in a Cathepsin L and pH dependent manner, similar to Influenza or Ebola virus (Figure 1) [39-43]. Virion fusion is followed by endocytosis, and uncoating of virion in the cytosol with release of the genomic RNA and virion proteins. These processes are not well studied.

Like many other RNA viruses, coronavirus replicates in the cytoplasm of the infected cells. [39-43]. The viral genomic RNA

is released into the cytoplasm forming replication-transcription complexes (RTC) in double-membrane vesicles [44-46]. The RTC replicates continuously and synthesize a nested set of subgenomic RNAs [47,48], which encode accessory proteins and structural proteins. The viral genome encodes several nonstructural proteins including RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro), and papain-like protease (PLpro) [49,50]. The viral RdRp synthesizes a full-length negative-strand RNA template for making more viral genomic RNA. The RdRp also contains a nuclease function with limited proof-reading ability, which is why coronaviruses do not mutate as rapidly as many other RNA viruses such as influenza [51].

Translation of the positive strand viral RNA into two viral polyproteins, pp1a and pp1ab occurs utilizing cellular machinery. This is followed by cleavage into individual effector proteins carried out by viral proteinases, 3CLpro and PLpro [49,50]. PLpro further may deubiquitinate certain host cell proteins, including interferon factor 3 and NF-κB, resulting in immune suppression [50,52].

In the endoplasmic reticulum (ER) and Golgi the newly formed genomic +ve RNA, nucleocapsid proteins and envelope glycoproteins assemble and form lipid-coated viral particles [53,54]. Mature coronavirus particles are subsequently transported to the cell surface [46]. Coronaviruses appear to exit cells via cell lysis rather than export/expulsion, although these processes are not well studied. [55].

Cryo-EM structure analysis has revealed that the binding affinity of SARS-CoV-2 S-protein to ACE2 is about 10–20 times higher than that of SARS-CoV S-protein [56,57]. It is speculated that this may contribute to the reported higher transmissibility and contagiousness of SARS-CoV-2 as compared to SARS-CoV [58]. SARS-CoV-2 progeny viruses are released into the luminal side of the human airway, facilitating spread and transmission through droplets and aerosols from coughing and sneezing [10,58,59]. Importantly, sustained human-to-human transmission, along with many exported cases across the globe

have led to a world-wide pandemic. However, the disease severity is not necessarily linked to transmission efficiency but may be due to other factors such as age, immunosuppression, or coexisting conditions (such as diabetes or heart disease) [60]. Prior exposure to common cold coronaviruses is thought to provide protection from SARS-CoV-2 resulting in milder disease [61]. Such protection can possibly result from HCoV-NL63 infection, as this virus also uses ACE2 receptor, and its S-protein bears significant similarity with the SARS-CoV-2 S-protein [62].

Zhou et al. have confirmed that the SARS-CoV-2 uses the same cellular entry receptor, ACE2, as SARS-CoV [20]. Yet SARS-CoV-2 is substantially less virulent (~3% mortality) compared to SARS-CoV (~10% mortality) and MERS-CoV (~35% mortality) [10, 63]. HCoV-NL63 also binds to the ACE2 receptor, has become endemic in the human population, but usually causes only a mild upper respiratory tract disease, except in sporadic cases wherein the same clinical syndrome as COVID-19 is reported but with reduced morbidity [23,64]. Thus, the mechanisms of human-to-human transmission and pathogenicity

of SARS-CoV-2 reflecting its now-proven pandemic potential are still unknown.

Further, Zhang et al. (2020) recently have found the presence of SARS-CoV-2 in fecal swabs and blood, indicating the possibility of multiple routes of transmission [65]. It is highly transmissible in humans, especially in the elderly and people with underlying diseases [66-69].

A-4. Symptoms of COVID-19: The main clinical symptoms of COVID-19 patients include fever, cough, and fatigue [70]. In addition to the Acute Respiratory Disease Syndrome (ARDS), or Severe Acute Respiratory Syndrome (SARS), the SARS-CoV-2 virus can cause adverse effects in the digestive system, urogenital system, central nervous system, heart, and circulatory system as well [71,72]. Its pathogenicity reflects the broad distribution of ACE2 in human cell lines and tissues, thus causing disease in multiple organs. Additionally, multiple inflammatory syndromes (especially in children, dubbed "MIS-C"), and multi-organ failures can result from the dysregulated

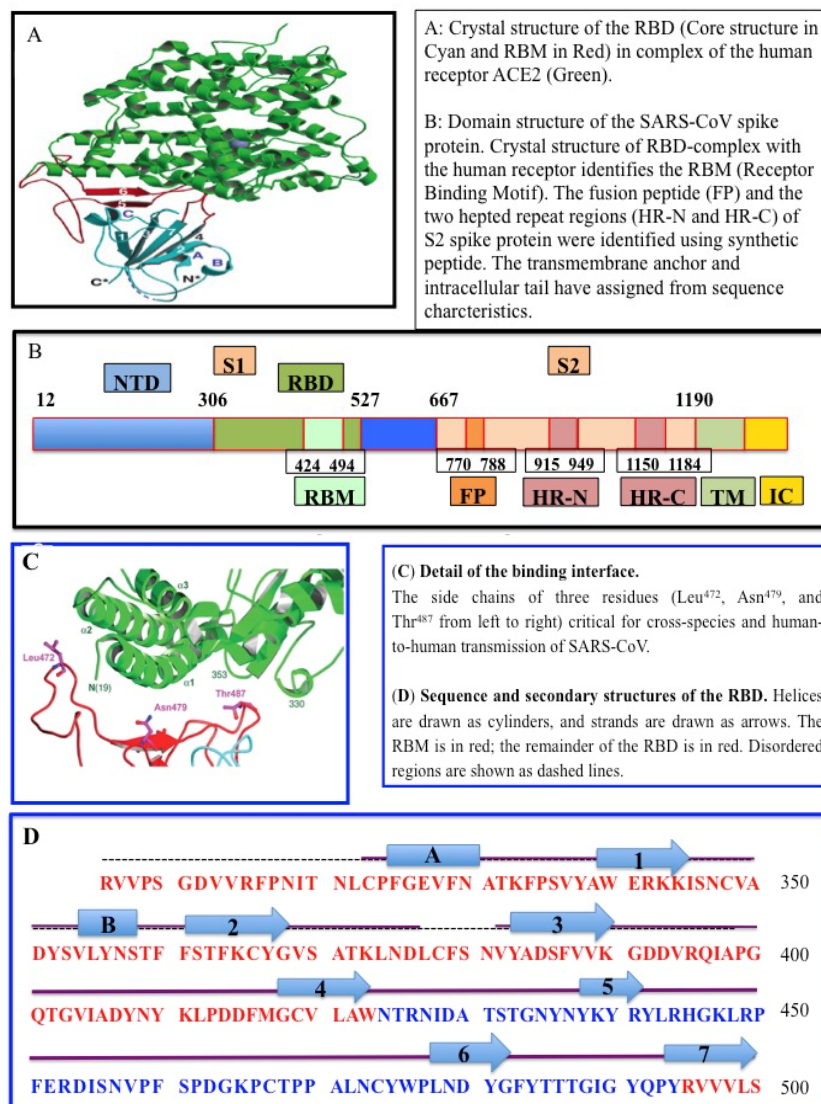


Figure 1. SARS Coronavirus spike receptor-binding domain (RBD) complexed with ACE2 receptor (A, B), structure of SARS EBD complexed with the receptor (C, D) [108]

attack by the host immune system against the virus directed at clearing out infected cells. People with underlying diseases, like cancer, heart problems, diabetes, and obesity, are susceptible to infection and prone to serious outcomes, which include ARDS, cytokine storm, and death [73, 74].

B. Diagnosis of COVID-19 in Human:

Chest radiography can reveal a typical feature of bronchiolitis with specific features, enabling reliable diagnostics especially in an emergency setting. The most common Chest X-ray pattern was multifocal and peripheral, associated with interstitial and alveolar opacities. In contrast CT exhibited ground glass opacities and was less diagnostic for differentiating the morbidity level of the syndrome [75].

Any unknown pathogen identification is possible using molecular biology tools but may be difficult and time-consuming; however, genome-specific PCR primers can be designed for RT-PCR analysis to specifically detect the virus. FDA has approved several tests on an emergency approval basis for the detection of COVID-19 [76].

Antigen Tests: Detection of one or more SARS-CoV-2 protein antigens in the patient blood. These are quick but not very sensitive nor specific. A positive antigen test means current infection by SARS-CoV-2 or antigen from a different virus that is cross-reactive to the antibodies used in the detection kit. A positive test should be confirmed by a viral RNA test (see below). A negative test means the antigen level in the sample is below detection limit, but does not rule out current infection, so it must be repeated later as the circumstances require.

Nucleic Acid Amplification Tests (Molecular tests): Detection of viral RNA (vRNA) in the human specimen by RT-PCR suggests that the virus might be present. However, if the amount of viral RNA in a particular sample is too low or contaminated with other kind of virus, molecular tests can yield a false negative result. The American Society for Microbiology has developed step-by-step verification procedures for commercial use of COVID-19 diagnostic tests. Both qualitative and quantitative tests are available now.

Antibody Tests (Serology): Generally blood samples are used as a specimen for the presence of antibodies produced in response to SARS-CoV-2 infection, however it does not test for the presence of the virus itself. Therefore, it does not indicate whether a person is currently infected or not, but whether the person was subjected to an infection in the past.

An antibody test can yield a false negative result if the test is conducted too early before the antibodies have developed, or too late, after the antibodies have subsided. It can yield a false positive result if patient antibodies are from a different infection but cross-reactive to SARS-CoV-2 antigen.

The Centers for Disease Control and Prevention (US CDC) has developed a test for SARS-CoV-2 in respiratory and serum samples from infected persons [77]. NIAID (National Institute of Allergy and Infectious Diseases) is also trying to develop additional diagnostic tests for COVID-19. These tests are expected to facilitate preclinical studies and the development of therapeutics [78].

C. Genome Analysis of Coronavirus and its implications to COVID-19 (Figure 1):

All coronaviruses express a set of structural proteins in the order of 5' - spike (S) - envelope (E) - membrane (M) and nucleocapsid (N) - 3', as well as some additional nonstructural proteins. The structural

gene region also harbors several ORFs that are interspersed along the structural protein coding genes. The location and number of these accessory ORFs vary between the CoV species [79]. The replicase gene is comprised of two overlapping ORFs (open reading frames) that encode up to 16 non-structural proteins required for viral replication and the modulation of any antiviral responses.

Alignment of the HCoV-NL63 and HCoV229E genomes, both alpha-coronaviruses, show the highest sequence similarity, except within the M gene [48]. A careful analysis has shown a large insert of 537 nucleotides are present in the 5' portion of the S gene of HCoV-NL63 but not in HCoV-229E. A BLAST search, further did not find any similarity between the additional 179-amino acid domain of the S protein of HCoV-NL63 with any other coronavirus sequence deposited in the GeneBank [57,80].

HCoV-NL63 uses ACE2 receptor to bind for invasion and replicates efficiently in monkey kidney cells, as do SARS-CoV, and SARS-CoV-2, though the S proteins of HCoV-NL63 do not match with the two SARS-CoVs sequences [54]. It appears that sequence homology is not the determining factor in receptor binding, and that the RBD quaternary structural features and specific interactions govern binding affinities.

D. Cell Culture Models for COVID-19 Infection:

Different cell culture models are there which can mimic different epithelial tissues [81]. Tracheo-bronchial cells which are the first targets of human respiratory viruses can be cultured in air-liquid interface (ALI). Apical side of the cell layer while exposed to the air, the basolateral side is submerged in the medium. The expression pattern of both primary human bronchial ALI and epithelium *in vivo* are comparable. Therefore, it appears that these culture methods can be used for virus replication as well as interactions with the host cells [82]. HCoV-HKU1 can propagate in alveolar HAE cultures (Human airway epithelial cell culture), and shows a better tropism for alveolar type II cells [83].

To date, most inhibitors are studied using HCoV-susceptible cell lines. However, an organotypic human airway culture system, since they contain many different cellular sub-populations, may represent an improved system for the testing of new antiviral compounds [84]. Such tissue culture models would require significant efforts for validation of assays and for interpretation of datasets due to heterogeneity of cell populations with variable content.

E. Therapeutics (Table 2): As of June 1, 2020, no specific approved treatment for COVID-19 is currently available. Remdesivir and favipravir, both small chemical antivirals that affect replication cycle, have been approved in different countries under emergency use guidelines. With the knowledge of the genome as well as protein structural information from previously discovered six human coronaviruses, several investigators are examining the development of therapeutics. So far 71 treatments and 47 vaccines are in development. *Treatments* includes various antibodies, antivirals, corticosteroids, immune suppressants, cell-based therapies, and most commonly, re-purposed drugs [85]. Some potential *treatments* are listed in the Table 2, whose targets are known and also preapproved as a medicine for other disease [23]. Candidate vaccines for COVID-19 that are in clinical phase are listed in the Table 3 [86-88].

E-1. Antiviral Compounds: Inhibition of viral attachment to the host cells and entry should be the prime target of several antiviral compounds [28]. Chloroquine, generally known as an anti-malarial drug, interferes with the terminal glycosylation of ACE2, reducing

Table 2. Some Possible Therapeutics of COVID-19 [23]

Some Potential Medicines	Target(s)	Approved Medicine for	Mechanism of action on SARS-CoV-2
1) Lopinavir 2) Rupintrivir 3) Small molecule Inhibitor : (3-1): 4-methyl-N-[(1S)-2-oxo-2-[[[(1S,2E)-1-(2-phenylethyl)-3-(phenylsulfonyl)-2-propen-1-yl-amino]-1-(phenylmethyl)ethyl]-1-piperazinecarboxamide. (3-2): (αR)-α-[[3-(4-chloro-2-fluorophenyl)-1-oxo-2-propen-1-yl]amino]-N-[(1R)-1-methyl-2-(2-oxo-3-pyrrolidinyl)ethyl]-benzenepropanamide	An inhibitor that may disrupt the function of 3CLpro and PLpro, which was tested against SARS-CoV.	<i>Lopinavir</i> : Approved for HIV infection	An inhibitor that may disrupt the function of 3CLpro and PLpro, which was tested against SARS-CoV
1) Remdesivir 2) Ribavirin 3) Aurine tricarboxylic acid 4) Favipiravir (Favilavir)	RNA-dependent RNA polymerase (RdRp) for replicating viral genome	<i>Remdesivir</i> : Ebola virus infection <i>Favipiravir</i> : Viral infections	An inhibitor of viral RdRp, as tested against SARS-CoV in cell Culture
1) Arbidol 2) Camostat mesylate	<i>Arbidol</i> : A viral surface protein (S) for binding to host cell receptor ACE2/. <i>Camostat mesylate</i> : Inhibitor of TMPRSS2	<i>Arbidol</i> : Influenza antiviral Drugs <i>Camostat mesylate</i> : Antiviral Dug	<i>Arbidol</i> : Inhibits Viral protein binding to ACE2 <i>Camostat mesylate</i> : Prevents cleavage of the spike protein in SARS-CoV-2, which is necessary for the virus to infect cells.
Benzopurpurin B	NSP15 (poly(U)-specific endo-ribonuclease		Suppress viral infectivity by inhibiting endo-ribonuclease NSP15
Chloroquine	Endosome/ACE2	Malaria	Elevates endosomal pH and interferes with ACE2 glycosylation
Antiviral Antibodies	S-Protein and other antigens		Neutralizing Antibodies
Nanoviricides: Venus Fly-Trap	S-Protein		Multipoint binding sites on Virus particles; and destruction

Table 3. COVID-19 candidate vaccines in clinical phase [83, 113, 114]

Candidate	Vaccine characteristics	Developer	Status
DNA Based (INO-4800)	DNA plasmid vaccine with electroporation	Inovio Pharmaceuticals	Phase I NCT04336410
Non-replicating viral vector (Ad5-nCoV)	Non-replicating viral vector; Adenovirus Type 5 vector	CanSino Biologics	Phase II NCT04313127
Protein subunit (NVXCoV-2373)	Full length recombinant SARS-CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix.	Novavax/Emergent Biosolutions/ Praha Vaccines/ Serum Institute of India/ AGC Biologics	Phase I/II NCT04368898
Inactivated virus (PiCoVacc)	Inactivated virus, plus Adjuvant	Sinovac	Phase II
Inactivated virus (Unnamed)	Inactivated virus	Beijing Institute of Biological Products and Sinopharm	Phase II ChiCTR2000032459
mRNA vaccine (mRNA 1273)	LNP-encapsulated mRNA vaccine encoding S protein	Moderna and NIAID	Phase I (NCT04283461)
Inactivated virus (Unnamed)	Inactivated virus	Wuhan Institute of Biological products and Sinopharm	Phase I/II
Adenovirus Vaccine (AZD 1222)	Adenovirus Vaccine	University of Oxford and AstraZeneca	Phase IIb/III
Inactivated virus (Unnamed)	Inactivated virus	Institute of Medical Biology and Chinese Academy of Medical Sciences.	Phase I
RNA (3LNP-mRNAs)	RNA	BioNTech/ Fosun Pharma/Pfizer	Phase I/II (NCT04368728)
LV-SMENP-DC	Lentiviral-modified DC's expressing minigene of selected viral proteins; administered with antigen-specific CTLs	Shenzhen Geno-Immune Medical Institute	Phase I (NCT04276896)
Pathogen-specific aAPC	Lentiviral-modified aAPCs expressing synthetic minigene of selected viral proteins	Shenzhen Geno-Immune Medical Institute	Phase I (NCT04299724)

ACE2 levels, and thus limits virus-receptor binding and thereby entry into the host cell [88]. Furthermore, endosomal acidification which is needed for viral genome release into the host-cell cytoplasm may be inhibited by Chloroquine [88], or its more potent chemical derivative, Hydroxychloroquine [20,23], that have shown promise in testing with SARS-CoV-2 *in vitro*. Recently, Wang et al. have shown that chloroquine effectively inhibits SARS-CoV-2 *in vitro* [89], however controversies regarding the efficacy vs. toxicity of these therapeutics remain.

Camostat and Nafamostat, inhibitors of TMPRSS2, have shown effectiveness against coronaviruses entry into the host cells (e.g. MERS-CoV) [28, 90]. Further, endocytic entry of β -coronaviruses can be blocked by Imatinib, an Abelson (Abl) kinase inhibitor [91]. All of these potential therapeutics inhibited the replication of MERS-CoV, SARS-CoV, and HCoV-229E in the low-micromolar range, which is indicative of their potential utility as a broad-spectrum antiviral remedies [83].

E-2. Protease Inhibitors to Block Viral Evasion: The viral evasion needs proteases like 3CLpro and PLpro, which help the translation of essential viral polyproteins (pp1a and pp1ab) and then further proteolysis to form effector proteins [92]. Kaletra, a combination of Lopinavir & Ritonavir, inhibits the activity of 3CLpro and is approved for the treatment of HIV/AIDS [93]. Preliminary clinical trials using Kaletra for the treatment of SARS-CoV-2, however were disappointing [94]. Efforts are still going on to test in combination with other antiviral drugs.

Disulfiram, an FDA-approved alcohol-aversion drug, was found to inhibit papain-like protease in MERS-CoV [95]. Numerous other inhibitors are being examined, with promising results against SARS viruses [83].

E-3. Inhibition of Viral replication: A number of components including the viral RNA-Dependent RNA polymerase (RdRp) and Helicase (nsP13) protein are required for the virus replication [92]. The small molecule 1,2,4-triazole is a derivative that inhibited the viral NTPase/helicase of SARS- and MERS-CoVs with low cytotoxicity [96,97], and could be considered for use against SARS-CoV-2.

Remdesivir (GS-5374), an adenosine analog and RdRp inhibitor, developed to combat other viruses (e.g. Ebola virus) has shown strong activity against SARS-CoV-2 *in vitro* [68,98,99], but only limited effectiveness in some of its clinical trials [100]. While it shows strong activity in cell culture studies, its clinical activity is limited by its rapid metabolic conversion to nearly inactive form, and its maximum dosage is limited by organ toxicities including liver and kidney. Favipiravir, another RdRp inhibitor approved for emergency use in novel Influenza in Japan, was approved in Japan and in China for emergency use for COVID-19. However, it has very limited clinical effectiveness, which is comparable to or less than that of remdesivir.

E-4. Immune therapy: The first line of defense against any pathogens is the innate immune system of the host. The receptor determinant N-acetyl-9-O-acetylneuraminic acid or O-Acetylated Sialic acid Interferon (IFN) System coordinates with various cellular effector proteins and impair virus propagation and transmission. In general, early infection with HCoV-229E, MERS- or SARS-CoVs do not express any innate immunity in the primary target cells of the human airway [101]. This is most likely due to the fact that 5' terminal of the viral mRNA being capped is indistinguishable from the host cellular mRNAs, and therefore not detectable by cellular immune sensors [89,102-104]. In addition, various other CoV accessory proteins inhibit interferon signaling at different stages of the host-innate-immune response [105,106].

Sarilumab and Tocilizumab, the monoclonal antibodies (mAbs) against the IL-6 receptor, could be possible therapeutics for COVID-19 patients, but may develop a risky cytokine storms [107]. Recent *in vitro* experimental data find more susceptibility of SARS-CoV-2 to type-1 interferon (e.g. Interferon- β) [108].

A combination of treatments such as steroids, interferon, and the antiviral drug Ribavirin has shown an effective antiviral effect [109-112]. Ribavirin is highly toxic to red blood cells.

E-5. Vaccines: Vaccines against the spike proteins (S) of both SARS-CoVs and MERS-CoVs pathogens are promising in animal models [113,114]. A selection of COVID-19 candidate vaccines that are in clinical phase are shown in Table 3. [115-117].

E-6. Animal Model for Vaccine Development: Traditionally, studies with respiratory viruses use mice and ferrets as an animal model. However, no animal model could represent human infection or disease correctly. Currently, there is no validated animal model for SARS-CoV-2 infection, and therefore there is no animal model for testing efficacy of either therapeutics or vaccines for COVID-19.

Further, adapted cell-cultured viruses may not show the same properties as wild-type viruses. SARS-CoV was able to replicate in different strains of inbred mice but to enhance their clinical signs, human ACE2 has been introduced. Transgenic animal models (mouse) expressing human ACE2 have become available. This provides for emulation of virus entry in human cells, but does not mimic the human response to the virus. These models are not yet validated for clinical pathology to the best of our knowledge. However, cells isolated from these transgenic h-ACE2 animals could be used for infection inhibition studies *in vitro* [118,119].

Considering the emergence of SARS-CoV-2, along with SARS- and MERS-CoVs, the pandemic potential of coronaviruses is now well established, and animal model development is necessary. Currently, a few animal models for MERS-CoV have been established by introducing the human variant of the viral receptor DDP4 (Dipeptidyl Peptidase 4) in place of the mice variant. However, the method of insertion of human Dipeptidyl Peptidase 4 (hDPP4) negatively affects the degree of pathogenesis [120,121]. Various non-human primates (NHPs) can be naturally infected with both MERS-, and SARS-CoVs, but the disease presentation and pathogenesis differs among the subspecies. Furthermore, NHP models are expensive and come with ethical obstacles [122].

E-7. Novel nanomedicine approaches: U.C. San Diego engineers coated a polymer using an extract of lung cell membranes and demonstrated that this so called “nanosponge” indeed acted as a decoy for the SARS-CoV-2 virus *in vitro* [123]. Such an undefined materials cannot be advanced into regulatory frameworks.

Considering the merits and demerits of the above approaches, Nanoviricides, Inc., a Shelton (CT) based biotech company dedicated to antiviral research, has developed a platform technology that goes beyond what antibodies can do for attacking a virus. We have developed a “Venus-Fly-Trap” for virus particles. The antiviral drug platform that we call “nanoviricide” provides multiple binding sites that mimic the cellular receptor on a polymeric micelle in high density. The binding sites are made up of small chemical virus-binding ligands designed using molecular modeling. Virion binding to the nanoviricide would lead to a multi-point attack on the virus particle, with the metastable polymeric micelle inverting and fusing into the lipid membrane of the virus envelop, thereby destabilizing the virus particle. Antibodies,

in contrast, only bind to the virus via two points, requiring multiple antibodies to tag the virus, and further require the patient's immune system to destroy the virion. In addition antibodies are highly specific and single point mutations can allow virus escape. In contrast, the ligands are designed to be broad-spectrum, and to mimic the conserved features of the binding of virion to its cognate receptor, thereby minimizing the possibility of viral escape (Table 2). In order to assess how broad-spectrum effect our drug candidates may possess we are testing our compounds now against two different coronaviruses, 229E and NL63 (Work is in Progress). ([https://www.marketwatch.com/press-release/nanoviricidesdevelops-highly-effective-broad-spectrum-drug-](https://www.marketwatch.com/press-release/nanoviricidesdevelops-highly-effective-broad-spectrum-drug-candidates-against-coronaviruses-2020-05-12)

[candidates-against-coronaviruses-2020-05-12](https://www.marketwatch.com/press-release/nanoviricidesdevelops-highly-effective-broad-spectrum-drug-candidates-against-coronaviruses-2020-05-12). *NanoViricides Develops Highly Effective Broad-Spectrum Drug Candidates Against Coronaviruses*. Published: May 12, 2020).

Conclusions

Since no vaccines are currently available for any of the respiratory coronaviruses (SARS, MERS and SARS-CoV-2), it is necessary to investigate the spread of infections and to follow the protection guidelines. In accordance with other recent reviews on this area [74], we agree that more detailed epidemiological studies are required to determine the pathological effects on other body/organ systems. Finally, a more detailed knowledge of the genomes and proteomes of previously described coronaviruses HCoV-NL63, HCoV-229E, SARS-CoV, MERS-CoV, and their role in the pathogenesis, is needed to better understand infection as well as for improved drug design. An appropriate animal model also needs to be developed that mimics the human clinical pathology. Further, the development of an accurate diagnosis of COVID-19 will shed light on the true incidence of this virus infection.

Conflict of interest

The authors declare no competing interests.

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