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Review

COVID-19 Transmission, Current Treatment, and Future Therapeutic Strategies

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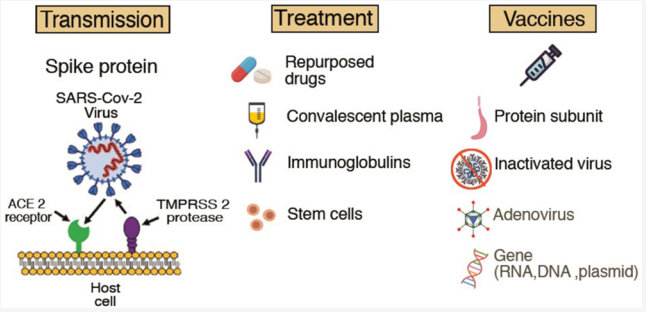
ABSTRACT: At the stroke of the New Year 2020, COVID-19, a zoonotic disease that would turn into a global pandemic, was identified in the Chinese city of Wuhan. Although unique in its transmission and virulence, COVID-19 is similar to zoonotic diseases, including other SARS variants (e.g., SARS-CoV) and MERS, in exhibiting severe flu-like symptoms and acute respiratory distress. Even at the molecular level, many parallels have been identified between SARS and COVID-19 so much so that the COVID-19 virus has been named SARS-CoV-2. These similarities have provided several opportunities to treat COVID-19 patients using clinical approaches that were proven to be effective against SARS. Importantly, the identification of similarities in how SARS-CoV and SARS-CoV-2 access the host, replicate, and trigger life-threatening pathological conditions have revealed opportunities to repurpose drugs that were proven to be effective against SARS. In this article, we first provided an overview of COVID-19 etiology vis-à-vis other zoonotic diseases, particularly SARS and MERS. Then, we summarized the characteristics of droplets/aerosols emitted by COVID-19 patients and how they aid in the transmission of the virus among people. Moreover, we discussed the molecular mechanisms that enable SARS-CoV-2 to access the host and become more contagious than other betacoronaviruses such as SARS-CoV. Further, we outlined various approaches that are currently being employed to diagnose and symptomatically treat COVID-19 in the clinic. Finally, we reviewed various approaches and technologies employed to develop vaccines against COVID-19 and summarized the attempts to repurpose various classes of drugs and novel therapeutic approaches.



KEYWORDS: *COVID-19, SARS-CoV-2, vaccines, therapeutic strategies, transmission*

INTRODUCTION

In this article, we reviewed the current state of knowledge on the transmission of SARS-CoV-2 virus from patient to host, assessed mathematical models employed to evaluate the risk of viral aerosol/droplet tra*y*nsmission, and discussed potential routes of SARS-CoV-2 viral entry into the human host and the underlying cellular mechanisms. In addition, we outlined the clinical manifestations of COVID-19 and commented on the capabilities of existing diagnostic methods to detect the virus in humans. Further, we discussed novel therapeutic strategies to curb the virus, specifically focusing on the current efforts employed for developing an effective vaccine and drug repurposing strategies to combat the virus.

As published research on COVID-19 is extensive and continues to grow in volume, a complete review of the existing knowledge is not practically feasible. Hence, we direct the readers' attention to excellent articles that provided informative reviews on various topics that are not adequately covered in this review. Those include the following:

i. Effective methods for prevention of person-to-person transmission of SARS-CoV-2.[1]
ii. Clinical features of COVID-19 in symptomatic[2−4] and asymptomatic patients.[5]
iii. Review on the estimates of incubation[6] and infectious[7] periods of COVID-19.
iv. Human immune responses to SARS-CoV-2.[8]
v. Association between COVID-19 mortality and pre-existing comorbidities.[9−12]
vi. Various types of vaccines in clinical development.[13]
vii. Human immune response against COVID-19 vaccines.[8]
viii. Monoclonal antibody therapy for SARS-CoV-2 versus SARS-CoV and MERS-CoV.[14]
ix. Effect of experimental treatments, which are currently in clinical trials, on mortality, and length of hospital stay.[15]

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Table 1. Similarities and Differences among Betacoronaviruses[a]

	SARS-CoV	SARS-CoV-2	Pangolin-CoV	Bat CoV RaTG13
Genus	Betacoronavirus (+ssRNA enclosed in capsid)			
Genomic Sequence Similarity	0.797		0.9102	0.96
Genome Structure		Novel putative short proteins potentiate replication and transmission		
Spike Protein Functional Similarity	Binds human ACE2 receptor			
Spike Sequence Similarity	>0.90			0.975
Spike RBD Sequence Similarity	<0.75			
Spike Protein Sequence		4-amino acid insertion at the S1/S2 site compared to Pangolin-CoV		0.93
Spike Protein Structure		Adds furin cleavage site at the S1/S2 site compared to Pangolin-CoV		
Mpro (Main Protease) Sequence Similarity	0.96			
Mpro (Main Protease) Active Site Features		Different size and shape compared to SARS-CoV and flexible loop blocks active site.		
Sequence similarity at 7 conserved ORF1ab replicase domains	0.944			
Papain-like Protease Sequence Similarity	> 0.9			
Helicase Sequence Similarity	> 0.9			
RNA-dependent RNA polymerase Sequence Similarity	> 0.9			

[a]All sequence similarities are with respect to SARS-CoV-2. Purple-shaded items indicate similarity or, where relevant, degree of similarity. Blue-shaded items are unique to SARS-CoV-2.

COVID-19 OVERVIEW

Pandemics: A Historical Perspective. Disease outbreaks appear as a sudden spike of illness in a particular area or community. When the outbreak is not contained, it spreads over a large population and affects an entire region or community of people, causing an epidemic. As infected people and/or objects contaminated with infectious material spread across the globe, an epidemic turns into a pandemic.[16] Through the 16th and 19th centuries, pandemics such as smallpox, plagues, and cholera destroyed many cities throughout Europe and Asia. The 20th century witnessed the spread of the Spanish Flu (1918−1919), a pandemic caused by the H1N1 strain of influenza that is most likely spread by soldiers returning home from World War I.[17,18] Influenza recurred in the human population with a variety of mutations causing pandemics such as Asian flu (1957−1958) and swine flu in 2009 that together killed more than a million people.[19,20] Some of the influenza strains persisted with humanity, causing seasonal influenza, leading to thousands of deaths every year. Concerted efforts of the scientific community to tackle such pandemics have eventually led to research advances, which ultimately helped to develop vaccines for combating the seasonal flu.

Zoonotic Origins of Pandemics. Most pandemics encountered by humanity in recent times are zoonoses that are generally transmitted to humans via direct contact with animal body fluids or via vectors that carry zoonotic pathogens.[21] For example, the HIV/AIDS pandemic is believed to have originated in chimpanzees.[22] Another example is Ebola (2014−2016) that spread from bats to humans.[23] Influenza often originates in avian or swine hosts before being transmitted to humans. Severe acute respiratory syndrome (SARS), in 2002−2003, and Middle-East respiratory syndrome (MERS), which has continued to spread in the Arabian peninsula since 2012, were believed to have been transmitted to humans via palm civet cats[24] and dromedary camels,[25] respectively. Zoonoses amplify in the bodies of animals (animal reservoirs) without being fatal to the host. Bats, for example, serve as perfect reservoirs as they have adequate interferons to protect themselves from the actual disease while still amplifying the pathogen load. As zoonoses migrate across species, the pathogens carve out robust evolutionary routes and transform themselves into the most virulent and contagious strains.[21]

Origins of Coronavirus Disease-19 (COVID-19). The current pandemic, COVID-19, is believed to have emerged from an animal host. The COVID-19 is caused by the SARS-CoV-2 virus, whose genome shares 96% similarity with betacoronavirus isolated from a bat in 2013 (RaTG13). The sequence of the receptor-binding motif (RBM) of SARS-CoV-2, which is critical for host infection, also shares a high-sequence similarity with the betacoronavirus isolated from a Malayan pangolin.[24] In fact, the percentage of bases identical to the SARS-CoV-2 RBM sequence is higher for pangolin-CoV (75/76 = 98.7%) than for RaTG13 (59/76 = 77.6%). Wong et al. (2020) observed that 5 of the amino acids shared uniquely between SARS-CoV-2 and pangolin-CoV occur at the key sites engaged in host binding. Therefore, it has been speculated that SARS-CoV-2 originated in bats and went through multiple recombination events as it migrated through other mammals.[24]

The Similarity and Differences among SARS-CoV-2 and Other Coronaviruses. Coronaviruses contain a positive-sense single-stranded RNA (+ssRNA) enclosed in a capsid with spikes, which resemble solar corona. Relative to other positive RNA viruses, coronaviruses have a large genome and possess sophisticated machinery to overtake host cells. They are known to cross species barriers, infect humans, and hijack the host cells to replicate further and spread. As of now, there are no effective means of prevention or treatments against coronaviruses, which have become a significant source of respiratory disease outbreaks. Four of the six coronaviruses that were previously known to infect humans cause common colds, upper respiratory, and intestinal illnesses. Of these, betacoronaviruses like SARS-CoV and Middle-East respiratory syndrome coronavirus (MERS-CoV) cause severe and often fatal lower respiratory tract infections.[26] Viral RNA isolated

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Table 2. Similarities and Differences between SARS and COVID-19[a]

	SARS	COVID-19
Pre- Transmissibility	NO	YES
Mild Case Transmissibility	NO	YES
Reproduction Number (R_0)	1.7-1.9 (WHO)	2.0-2.5 (WHO); 5.7 with 95% CI: 3.8-8.9 (CDC)
Number of Reported Cases	More than 8000	31.44 million through September 22, 2020
Number of Reported Deaths	774	967,197 through September 22, 2020
Mortality Rate	About 9%	3.1%
Primary Mode of Transmission	Infectious respiratory droplets dispersed from mucous membranes	
Ability to Survive on Surfaces	YES	
Median Incubation Period	4-7 days	
Maximum Incubation Period	14 days	
Potential to cause severe respiratory infection	YES	
Potential to infect CNS and brain	YES	

[a]Purple-shaded items indicate similarity; yellow-shaded represent relative levels, and blue-shaded items are unique to COVID-19.

from COVID-19 patients in Wuhan was sequenced and a betacoronavirus with unique genomic features, including a couple of novel putative short proteins that potentiate the replication and transmission of the viral proteins was identified.[27] Multiple independent genomic sequencing studies conducted on SARS-CoV-2 viral RNA isolated from several COVID-19 patients have demonstrated a phylogenetic relationship to a bat coronavirus (bat-CoV-RATG13) and a pangolin coronavirus (pangolin-CoV) at the whole-genome level and a very close association to SARS−Co-V at the molecular level (Table 1).[27−30] In particular, these two coronaviruses exhibited similarities in the coding region of the spike protein (S-protein), which enables the virus to bind to the cell surface receptors and facilitate its entry into the human host.[30] Zhou et al. (2020) conducted a series of in vitro experiments to show that SARS-CoV-2 infected cells that express angiotensin-converting enzyme 2 (ACE2) receptors, thus providing strong evidence that the virus enters cells by binding to the ACE2 receptor, which was also shown to mediate SARS−Co-V internalization.[31] Comparative information on these and other essential features between SARS−Co-V and SARS-CoV-2 are provided in Table 1.

Similarities and Differences between SARS and COVID-19. Similarities between SARS and COVID-19. Like the more advanced cases of COVID-19, SARS manifested as a rapidly progressing viral pneumonia. The primary mode of transmission of SARS and COVID-19 appears to be via infectious respiratory droplets dispersed from the mucous membranes (Table 2). SARS-CoV and SARS-CoV-2 are reported to have similar stability and decay rate in aerosols and on several surfaces.[32,33] It has been demonstrated that both can survive for up to 3 days on plastic and up to 2 days on stainless steel, with similar decay profiles of the virus titer on each surface.[32] The median incubation period, which is the time from the initial exposure until the onset of symptoms, appears to be around 4−7 days,[33] and the maximum incubation period could be up to 14 days for both SARS and COVID-19.[33,34]

Differences between SARS and COVID-19. SARS had a mortality rate of about 9%, which is 4−10 times higher than that of COVID-19. Unlike SARS-CoV-2, there were no reports of SARS-CoV transmission before symptoms appeared, and mild SARS-CoV infections were believed to be not transmittable (Table 2).[33] The basic reproduction number, R_0, defined as the average number of secondary infections produced by an infected person, is used to describe the transmission potential of infectious diseases. Using the World Health Organization (WHO) estimates, Petrosillo predicted that the R_0 for SARS is in the range of 1.7−1.9, whereas, for COVID-19, it was predicted to range between 2.0 and 2.5.[35] However, the Centers for Disease Control and Prevention (CDC) estimated that the R_0 for COVID-19 is much higher (5.7; 95% CI: 3.8−8.9).[36] A larger difference in the frequency of COVID-19 cases compared to SARS cases and its ability to spread rapidly across the globe indicates that the R_0 value for COVID-19 is most likely closer to the CDC estimate (Table 2).

SARS-COV-2 TRANSMISSION

Transmission of SARS-CoV-2 from the Patients to the Host. Frequent sneezing and dry coughing exhibited by the COVID-19 patient generate viral plumes of thousands of droplets per cubic centimeter. Since SARS-CoV-2 infection is believed to be transmitted by aerosols and/or droplets, it is imperative to assess their particle characteristics, aerodynamic behavior, and their propensity to bypass various physiological barriers to enter the host body.[37]

Characteristics of Droplets/Aerosols Emitted by COVID-19 Patients. It was initially thought that the pathogens are carried from the patient via larger droplets, which settle on the surfaces and are then carried to the host by the dust rising from the dried droplets. It has recently been identified that sneezing and dry cough suffered by COVID-19 patients generate droplet sizes ranging between 0.6 and 100 μm, and the number of droplets increases proportionately with coughing rate.[38] More than 97% of these droplets tend to be lower than 50 μm, and a majority of them are smaller than 10 μm.[39−41] Pre- or asymptomatic patients can also generate and emit large quantities of droplets, smaller than 1 μm, through normal breathing and speech.[42]

The particle size distribution may shift even lower when the airborne droplets are evaporated to form droplet nuclei. The droplet nuclei formation is dependent on the ambient temperature and humidity, as well as on the particle size of the droplet. The droplets less than 10 μm have a greater potential to turn into droplet nuclei before settling. These droplets remain suspended in the cloud of air emitted by the cough or due to the ambient airflow. The droplets with a diameter less than 50 μm survive longer in the plume without any significant evaporation[43,44] and contaminate distant

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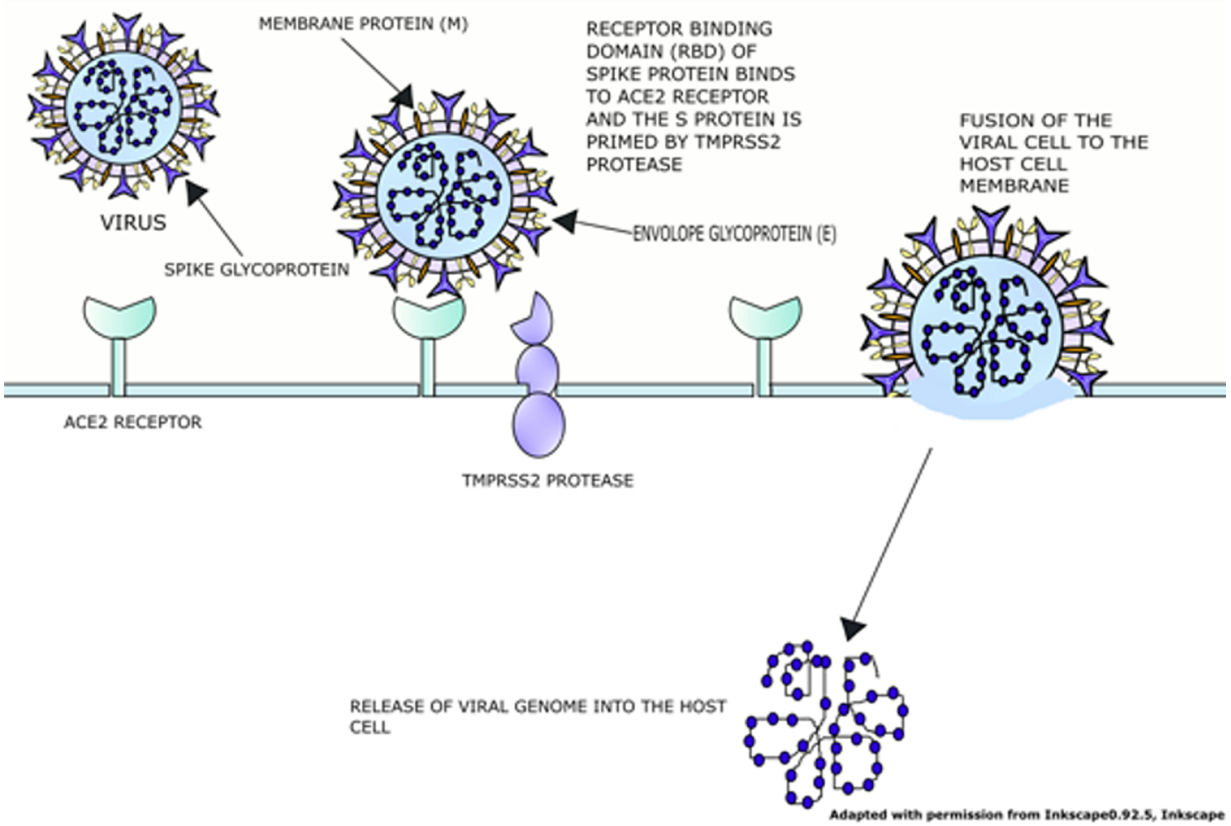

Figure 1. Host receptor interaction with the SARS-CoV-2 spike protein and subsequent viral cell fusion with the host cell membrane.

surfaces as well as ventilation systems.[41] Like most viruses, the average size of SARS-CoV-2 is around 0.1 μm.[37] Therefore, even 1−10 μm aerosol particles are sufficiently large to carry a viable viral particle load.[45,46]

Transmission of Airborne Viral Particles. Expulsion of air due to exhalation, sneezing, and coughing results in the release of multiphase turbulent flow, which is generally composed of hot moist air. The locally moist and warm atmosphere within the turbulent air helps the droplets escape evaporation much longer; this considerably extends the lifetime of the droplet from a fraction of a second to minutes.[47] Additionally, coughing and sneezing also generate the aerosol plumes at a high enough velocity to infect someone who is standing in proximity to the patient. Under optimal conditions of humidity and temperature, the aerosol droplets of all sizes can travel up to 7−8 m.[47,48]

Risk of Infection. A recent report has shown that SARS-CoV-2 aerosols remain viable in the air for a duration of at least 3 h with a half-life of about 1 h and is contagious to infect the human host.[32] The risk of infection when in close proximity with a COVID-19 patient was assessed by several mathematical models, of which the Wells and Riley model is the most widely used. Wells and Riley have conducted seminal research in quantifying airborne infection rates in confined spaces.[49,50] Their work has culminated in the Wells−Riley eq (eq 1), which computes the number of new cases infected (N_c) over time (t) based on the infective (I) and susceptible (S) people in a space with ventilation rate, Q, typically expressed in m^3/S, and quantity of infectious material in the air, q where the pulmonary ventilation rate of susceptible individuals is p m^3/S.

$$N_c = S(1 - e^{-Iqpt/Q}) \quad (1)$$

The Wells−Riley equation has been successfully employed to predict the measles outbreak in schools and has also been employed to evaluate the impact of airflow and ventilation on infection rates.[51] One of the limitations of this model is that it assumes a well-mixed room with uniform distribution of the aerosol particles throughout the space, which is not always possible even with a well-designed ventilation system. Therefore, the model may fall short of predicting the risk of COVID-19 infection in workspaces with several ventilation zones and in public spaces with activities, such as loud speaking and signing, that generate large droplets. Another major limitation of the Wells−Riley model is the representation of the infectious dose as the "quantum" of infection (q), which is defined as the number of infectious droplet nuclei required to infect 1−1/e (about 63.2%) susceptible people.[52] While this is a simple approach that is analogous to the quantity and virulence of infectious material in the air, it cannot fully capture the complex interaction between various physicochemical and biological factors that drive the infection. This limitation is being addressed by the development of detailed dose−response models and stochastic modeling approaches.[53−56]

Cellular Mechanisms Underlying SARS-CoV-2 Entry into the Human Host. Viruses have been known to enter the host cells via receptor-mediated endocytosis,[57] which is triggered when the receptor-binding domain of the virus binds to the corresponding receptor on the host cell.

Role of Angiotensin-Converting Enzyme-2 Receptor. It has been previously reported that the S-protein of SARS-CoV demonstrated an affinity for the ACE-2 receptor,[58] which served as an entry point for SARS-CoV viral RNA into the host cells.[59] Due to the high structural homology between the S-

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

protein of SARS-CoV and SARS-CoV-2, the ACE-2 receptor also facilitates the cellular entry of SARS-CoV-2[60] (Figure 1).

Studies conducted on human HeLa cells and in mice with and without ACE-2 expression have provided experimental evidence that the ACE-2 receptors are most likely involved in the cellular entry of the SARS-CoV-2 virus (Wuhan strain).[31] Similarly, the SARS-CoV-2 infection of BHK21 cells that were transfected with human and bat ACE-2 receptors was higher compared to the BHK21 cells that do not express ACE-2 receptors.[61] Biophysical and structural evidence demonstrates that the ACE-2 binding affinity of the SARS-CoV-2 S-protein ectodomain is 10−20-fold higher than that of SARS-CoV S-protein,[62] which is claimed to be responsible for differences in the contagious nature of SARS-CoV-2 and SARS-CoV.[63]

Although the ACE-2 receptor shares a considerable homology with the ACE-1 receptor, due to the smaller size of the active site on ACE-2 receptors and amino acid differences in the binding pocket, ACE-2 cannot be inhibited by the conventional ACE inhibitors like lisinopril, enalapril, and ramipril.[64] Additionally, there is no evidence that angiotensin receptor blockers (ARBs) such as losartan block ACE-2. Further discussion regarding ARBs is provided in the later sections.

The Link between the SARS-CoV-2 and TMPRSS2. Uptake of SARS-CoV-2 by the host cells is not only dependent on the binding of S-protein of the virus to the ACE-2 receptor but also requires the S-protein priming by transmembrane protease serine-2 (TMPRSS2),[30,31] which is critical for the fusion of the virus with the host cell membrane and its subsequent entry into the host cell (Figure 1). Thus, the synergistic activity of the ACE-2 receptor and TMPRSS2 is needed for SARS-CoV-2 entry into the host. It was further noted that TMPRSS2 is highly expressed and widely distributed compared to the ACE2 receptors, suggesting that the ACE2 receptor might be the rate-limiting factor for SARS-CoV-2 entry during the initial stage of infection.[30] Although TMPRSS2 is a key component for the viral infection, other proteases such as cathepsin B/L could act as a substitute for TMPRSS2. Hence, it may be important to inhibit both these proteases to prevent the cellular entry of SARS-CoV-2. However, when it comes to the transmission and pathogenesis of the virus, TMPRSS2 is believed to play a more prominent role compared to the cathepsin B/L.[61]

The Link between the SARS-CoV-2 and Furin. Extensive bioinformatics analyses identified the presence of a unique amino acid (PRRA) sequence between the S1 and S2 subunits in the S-protein of SARS-CoV-2. This amino acid sequence could be cleaved by furin, a type-1 membrane-bound protease,[65] expressed in various organs such as the brain, lungs, gastrointestinal (GI) tract, liver, and pancreas that are vulnerable to viral entry. The action of furin has been documented in other coronaviruses as well as in HIV, where it acts on the viral envelope protein.[66] The action of furin on the S-protein of SARS-CoV-2 could enhance its cellular entry by exposing the binding and fusion domains and enhance the viral transmissibility and pathogenesis.[65] Furin has also been detected in T cells, which circulate throughout the body. The circulating cells could form a feed-forward loop, which could facilitate the furin-dependent viral replication and contribute to the cytokine storm in some patients.[66] Therefore, furin presents an additional pathway that could be pharmacologically targeted to curb the spread of SARS-CoV-2.[67]

Potential Routes of SARS-CoV-2 Entry in the Human Host. Given the significance of cell surface proteins ACE2, TMPRSS2, and furin in enabling the entry of SARS-CoV-2 into the human host,[68] we assessed the expression levels of the genes that code for these proteins in several human bulk tissue and cell types. Transcriptomic gene expression was assessed in 54 healthy tissue types from the Genotype-Tissue Expression (GTEx) project.[69] According to the GTEx data, ACE2 is highly expressed in the testis and ileum (>10 transcripts per million, or TPM) as well as in adipose tissue, kidney, heart, and thyroid (>5 TPM). Further, there are 19 GTEx tissue types, including lung, with reasonable ACE2 expression (1 TPM). On the other hand, TMPRSS2 expression is at least 1 TPM in about 18 GTEx tissue types, and the highest levels were found in the prostate, stomach, colon, pancreas, lung, small intestine, and salivary glands (>40 TPM). While furin is ubiquitously expressed in all 54 GTEx tissue types, the highest furin expression is observed in the liver, lung, thyroid, whole blood, and skin (>100 TPM) (Figure S1 and Table S1). Zou et al. (2020) reported a single-cell (RNA-Seq) study measuring relative transcription levels of ACE2 in various tissues.[70] In the lung, ACE2 was most highly expressed in alveolar type 2 (AT2) epithelial cells and respiratory epithelial cells. In the heart, ACE2 was highly expressed in myocardial cells; in the digestive system, ACE2 transcription was the most prominent in ileal and esophageal epithelial cells. ACE2 transcription within the urinary system was the most prolific in proximal tubule cells of the kidney and urothelial cells of the bladder (Table S2). Since SARS-CoV-2 enters the host through ACE2 receptor-mediated uptake, higher expression of ACE2 in the cells that form physiological barriers protecting various organs from viral entry could make the human host susceptible to SARS-CoV-2 infection.

Oral Mucosa. H. Xu et al. (2020) investigated ACE2 expression in the oral cavity using 13 normal-adjacent tissues from The Cancer Genome Atlas (TCGA) and 14 tissue types from Functional Annotation of The Mammalian Genome Cap Analysis of Gene Expression (FANTOM5 CAGE) data sets.[71] The results demonstrated that the ACE2 is expressed on the oral mucosa and is highly enriched in the tongue epithelial cells. These findings emphasize the susceptibility to the oral cavity for SARS-CoV-2 infection.

Upper Respiratory Tract. The SARS-CoV-2 viral RNA was detected in the upper respiratory tract in both symptomatic and asymptomatic patients, highlighting the possible role of the nasal epithelium as a viral reservoir and in spreading the virus across the respiratory mucosa.[30] The presence of high viral titer in the nasal epithelium could be due to the high expression of ACE2 receptors in the respiratory mucosa.[30,72]

Lower Respiratory Tract. Since lungs are one of the first organs affected in COVID-19,[73,74] it is crucial to recognize the mechanisms of viral entry into the lower respiratory tract and identify cellular targets in the lung susceptible to SARS-CoV-2 infection. The majority of SARS-CoV-2-laden droplets/aerosols generated by the patient are in the ideal size range (1−10 μm) to gain access to the deeper quarters of the lung. Single-cell transcriptomic study of healthy lung tissue samples obtained from multiple donors have shown that the AT2 epithelial cells expressed the highest levels of ACE2 compared to other lung cell types (Table S3).[75] Despite relatively modest expression levels of ACE2 in bulk lung tissue, its higher expression in the AT2 cells may provide SARS-CoV-2 access to the lung tissue. Moreover, AT2 cells provide platforms for

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

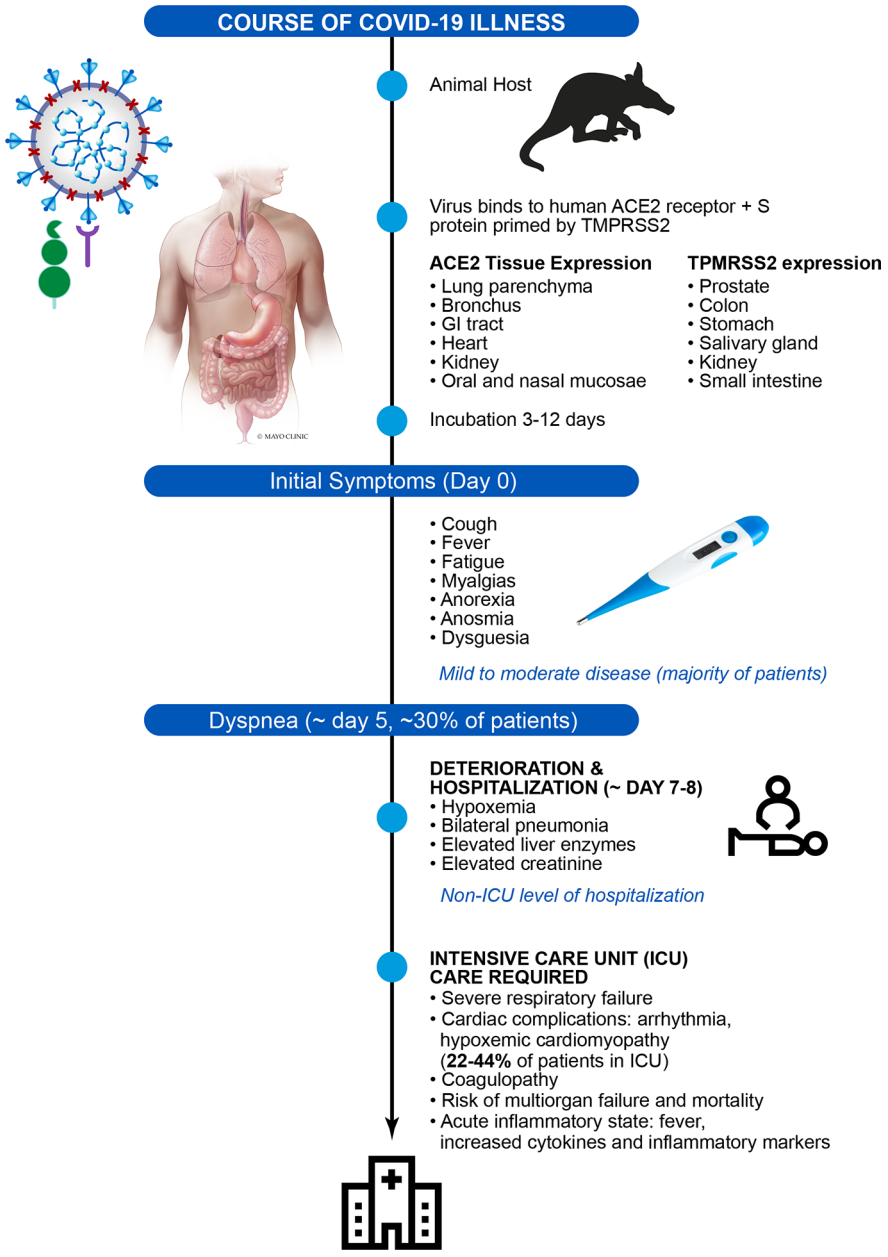

Figure 2. A visual representation of the course of COVID-19 illness.

SARS-CoV-2 viral replication and also manifest increased levels of inflammatory cytokines.[76]

Entry into the Central Nervous System and Brain. In a recent study, neurological symptoms were evident in 36% out of 214 COVID-19 patients tested, and 46% of those patients exhibited severe neurological deficits.[77] Several other studies have also reported the prevalence of neurological symptoms in COVID-19 patients.[78] In another large prospective study conducted among hospitalized COVID-19 patients in New York, neurologic disorders were detected in 13.5% of the patients. The occurrence of neurologic disorders was found to confer a higher risk of in-hospital mortality.[79] This clinical evidence may fuel speculation about the propensity of SARS-CoV-2 to permeate the brain barriers and trigger neurological damage. Once in the nasal cavity, the virus could infect the olfactory sensory neurons in the olfactory epithelium, the only part of the CNS exposed to the external environment, as well as the trigeminal nerve to gain access to the CNS.[80] The SARS-CoV-2 virus could also penetrate the olfactory mucosa lining the cribriform plate and traverse along the perineuronal space to access the cerebrospinal fluid (CSF) in the subarachnoid space.[81] Although the New York study reported no evidence of meningitis, encephalitis, and myelitis, which indicate SARS-CoV-2 invasion of the CNS,[79] researchers from Beijing Ditan hospital have demonstrated the presence of SARS-CoV-2 in the CSF of patients by genome sequencing. Moreover, the drainage of the virus-laden CSF to the cervical lymph nodes may lead to the activation of immune response[82] and most likely triggers SARS-CoV-2-associated encephalitis in COVID-19 patients.[83] The SARS-CoV-2 virus could also enter the brain from the systemic circulation via the blood−brain barrier.[84] Cytokine syndrome leads to the blood−brain barrier breakdown, which has been associated with the development of acute necrotizing encephalopathy in COVID-19 patients.[85] This could increase viral transmission via the paracellular

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

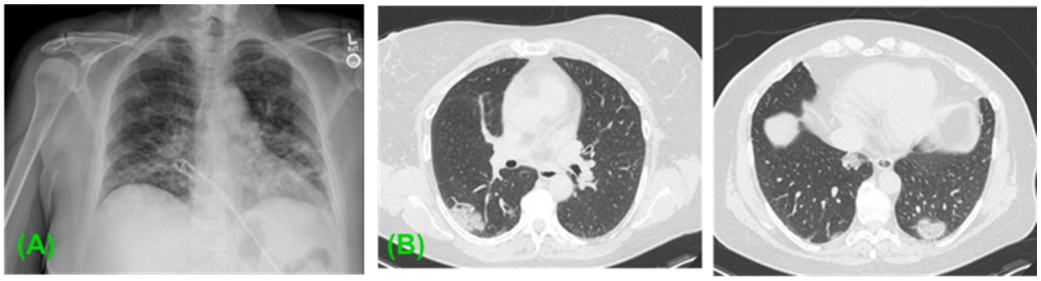

Figure 3. Radiographic findings of COVID-19 pneumonia. (A) A 72-year-old female with a past medical history significant for type 2 diabetes, hypertension, hyperlipidemia, and hypothyroidism present with fevers and shortness of breath that developed 8 days prior to admission to the hospital for acute respiratory failure. History of contact with a COVID-19 positive individual. Portable chest X-ray shows scattered bilateral pulmonary infiltrates most prominent in the left lower lung. (B) A 56-year-old female with 5 days history of intermittent fever and dyspnea. PCR was positive for COVID-19. HRCT shows patchy bilateral, peripherally, and lower lobe predominant subsolid nodules.

spaces between the endothelial cells of the BBB, in addition to their usual transendothelial route.

Breaching GI Epithelium. The virus present in the nasal cavity is cleared into the GI tract by the mucociliary system. Moreover, large droplets/droplet nuclei that are unable to enter the deeper lungs tend to be cleared into the GI tract. Although viruses cannot survive the strongly acidic environment of the stomach, there is substantial evidence that the GI tract may be a potential transmission route and target organ of the SARS-CoV-2 virus.[86]

A recent study by Lin et. al showed that 58 out of 95 patients exhibited GI symptoms. Gastroscopy examination of these patients showed SARS-CoV-2 infection in the esophagus, stomach, duodenum, and rectum. Another study conducted in 138 hospitalized COVID-19 patients has shown that a significant portion of patients (10.1%) initially presented diarrhea and nausea prior to the development of fever and dyspnea, thus suggesting the possibility of virus infection via the GI tract.[87] The expression of ACE2 and TMPRSS2 is high in absorptive enterocytes and in ileal epithelial cell subclusters, respectively. Moreover, ACE2 and TMPRSS2 were found to be highly coexpressed in colon enterocytes.[86] Since the coexpression of ACE2 and TMPRSS2 is essential for SARS-CoV-2 viral entry, the enteric symptoms observed in COVID-19 patients might be due to the invasion of SARS-CoV-2 across the gut epithelial barrier. In comparison to COVID-19 patients without diarrhea, COVID-19 patients with ceased diarrhea or with ongoing diarrhea displayed elevated fecal calprotectin concentrations,[88] which are significantly correlated with serum interleukin-6 (IL-6) concentrations. Clinical evidence of diarrhea as well as elevated levels of fecal calprotectin and serum interleukin-6 in COVID-19 patients suggest that SARS-CoV-2 most likely generates an acute intestinal inflammatory response.[89,90]

COVID-19 CLINICAL MANIFESTATIONS, DIAGNOSIS, AND TREATMENT

Clinical Manifestations. The clinical manifestations of COVID-19 are not specific but somewhat similar to many viral illnesses. A high-level description and visualization of the disease and its symptoms are presented in Figure 2. After an incubation period of about 4−14 days, most individuals develop symptoms that can range from mild to very severe and even fulminant disease.[91−93] The most common manifestations are cough (46−82%), fever (77−98%), fatigue, anorexia, and myalgias (muscle pain),[77] although anosmia (loss of sense of smell) and dysgeusia (loss of sense of taste) are frequently seen and are believed to be characteristic, but not exclusive, to COVID-19.[94] Sore throat, headache, and rhinorrhea (runny nose) are also reported. Gastrointestinal symptoms such as nausea and diarrhea and accompanying abdominal pain may precede the respiratory symptoms in up to 10% of patients.[95] Asymptomatic individuals can test positive for COVID-19 (30%). However, the majority of individuals will present mild to moderate disease (55%). About 30% of patients may develop dyspnea (shortness of breath) around day 5 after the disease onset. Deterioration in the second week of illness is typical in patients with a more severe form of the disease. These patients commonly require hospitalization by day 7 or 8[28,96] and manifest hypoxemia (low blood oxygen) as well as bilateral pneumonia (75%).[97] Elevation of the liver enzymes and creatinine are also common. Most hospitalized patients require a standard level of care, although about 20% may deteriorate quickly after the onset of dyspnea and develop severe respiratory failure.[95]

Complications. Acute respiratory distress syndrome (ARDS) is one of the most severe complications of patients with COVID-19. It is associated with prolonged hospitalization and high mortality, especially if patients develop multiorgan system failure.[77] Respiratory support is crucial and ranges from high flow oxygen to providing noninvasive as well as invasive mechanical ventilation. Prone positioning has been noted to be beneficial in improving the oxygenation.[98] A subset of patients may develop an acute inflammatory state with fevers and increased expression of inflammatory markers as well as cytokines, similar to that observed in cytokine release syndrome.[95]

Higher incidence of cardiovascular complications such as arrhythmias, hypoxemic cardiomyopathy, and acute cardiac injury are frequently seen (22−44%) in the intensive care unit (ICU) patients compared to non-ICU patients (2%). These could precede or develop during the multiorgan system failure of ARDS. Healthcare providers caring for these patients should be aware of these complications;[77,99] however, this is not an indication for routine telemetry monitoring or the need to screen for biomarkers for myocardial injury (troponins or BMP) unless there is evidence of myocardial ischemia or worsening heart failure. Additionally, QT should be documented on every patient as some of the drugs used in these patients may cause QT-interval prolongation.

A coagulopathic stage resulting in microvascular thrombosis or disseminated intravascular coagulation has been associated with COVID-19 infection.[100−102] Interestingly, while thrombotic complications are common, bleeding is a rare

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

complication even in the presence of abnormal coagulation laboratory studies. Coinfections with another virus, especially seasonal influenza and other bacterial microorganisms, have been described in about 10% of the patients.

Risk Factors. Several factors have been associated with poor disease prognosis, among which increasing age (>65 years old), cigarette smoking, diabetes, hypertension, cardiovascular disease, chronic lung disease, malignancy, and immunosuppressed status are the most common. Other poor indicators are lymphopenia, thrombocytopenia, and elevated inflammatory markers (IL-6, ferritin, ESR).[99,103]

COVID-19 Diagnosis. *Laboratory Findings.* The complete blood cell count can be normal, but the most common abnormal laboratory findings are lymphopenia (63%), leukopenia (9−25%), leukocytosis (24−30%), and thrombocytopenia (36%). Liver enzymes are elevated in about 37% of the patients. Other inflammatory markers [erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)], D-dimer, ferritin, and IL-6, are also commonly elevated. Procalcitonin is usually normal but can be high, especially if there is a superimposed bacterial infection.[77,95,104]

Radiographic Findings and Other Imaging Studies. It is important to be aware that up to 50% of the patients may have a normal chest X-ray (CXR), especially in the early stages of the disease. However, for those that develop pneumonia, typical CXR findings reveal bilateral peripheral patchy opacities (Figure 3A). A high-resolution CT of the chest (HRCT) is more sensitive, especially in the early stages. Common findings include patchy areas of ground-glass opacities, mostly peripheral and with lower lobe predominance. Areas of consolidation may be present, especially as the disease progresses (Figure 3B). Some features resemble those of organizing pneumonia. These findings are not specific to COVID-19 and can be seen in other viral pneumonia. Therefore, HRCT of the chest should not be used as a screening test for patients with suspected COVID-19, but rather be employed to evaluate clinical deterioration.[97,105]

Point of Care Ultrasound. It is especially helpful for those patients in the ICU setting to assess the lung (presence of B-lines, consolidations with air bronchogram, pleural effusions) and heart function without the risk of having to transport critically ill patients for radiologic procedures and to avoid unnecessary exposure.[106]

Diagnosis and PCR Testing. Clinical presentation, laboratory, radiological features, and exposure history (travel, positive contact, etc.) should raise the suspicion for COVID-19 infection. However, a definitive diagnosis should be made with microbiologic testing by the confirmation of the presence of SARS-CoV-2 RNA in clinical specimens. Initially, a reverse-transcription polymerase chain reaction (RT-PCR)-based test was only performed by the CDC. However, similar tests are now also available at several hospitals and commercial laboratories. The sensitivity of the test is about 70−75%. Common factors that can affect the positivity of the test are the type and quality of the specimen (nasopharyngeal has better sensitivity than oropharyngeal), stage and severity of the disease (in the early stages, viral concentrations are higher in the oropharynx, while sputum and bronchoalveolar lavage tend to have higher sensitivity as the disease progresses), and the characteristics of the specific test.[107] A negative test does not rule out COVID-19. Therefore, if there is a high clinical suspicion for COVID-19 infection, PCR should be repeated in about 24−48 h; meanwhile, the patient should remain in isolation.[108]

Current Treatment Strategies for COVID-19. Due to the unknown efficacy of the available antiviral drugs, the standard of care, especially for those patients with mild disease, should center on the prevention of transmission. Close monitoring is important for patients that are being managed at home, and prompt escalation of care is required if deterioration occurs.[107] Data regarding the risk of increasing viral replication versus anti-inflammatory benefits of corticosteroids is inconclusive.[109] However, they can be considered in the presence of other indications such as severe COPD.[110] The use of inhalers is preferred over nebulized therapies to avoid aerosol-generating procedures that could potentially increase airborne viral spread.[111] Nonsteroidal anti-inflammatory drugs (NSAIDs) have been deemed to affect the levels of ACE2 receptors in epithelial cells and potentially increase viral infection. However, this is debatable. It is uncertain if all NSAIDs have the same potential for adverse reactions in COVID-19. NSAIDs are proposed to lead to a theoretical increased risk of ARDS via leukotriene release and, subsequently, bronchoconstriction.[112,113]

Therefore, the use of NSAIDs for symptom relief should be individualized. The European Medicines Agency (EMA) and the World Health Organization (WHO) do not recommend that NSAIDs be avoided. The use of acetaminophen is generally preferred in hospital settings due to the increased risk of bleeding and renal injury associated with NSAIDs. The use of ACE inhibitors and angiotensin receptor blockers has also been controversial. However, the American Society of Cardiology and the European Society of Cardiology currently do not recommend initiation or discontinuation of these agents.[35,114] The decision to administer antiviral and other anti-inflammatory therapies to COVID-19 patients should be made on a case-by-case basis, if possible, in consultation with infectious disease specialists, and preferably as part of a clinical trial or registry.

Patients with moderate to severe illness frequently benefit from oxygen supplementation (nasal cannula and high flow oxygen), and for those with acute respiratory failure, noninvasive and invasive mechanical ventilation is frequently needed. Positive airway pressure (PAP) should be used with the recognition that it is an aerosol-generating procedure and requires a higher level of personal protection equipment (PPE) used by healthcare providers.[35,107] Pharmacological prevention of venous thromboembolism should be offered to all hospitalized patients unless there are specific contraindications due to the increased risk of venous thromboembolism in these patients.

FUTURE THERAPEUTIC STRATEGIES

COVID-19 Prevention: Vaccine Development. More than 200 clinical trials are currently underway to test various novel and repurposed compounds against COVID-19 (Table S4). However, the promise of vaccines is alluring as they have the potential to prevent disease transmission in a larger population. Before deploying these vaccines in a larger population, their safety and efficacy should be thoroughly established; ineffective vaccines may not only fail to protect the individual from the virus but could also cause disease through antibody-dependent enhancement or other mechanisms.[115,116]

Technologies Employed in COVID-19 Vaccine Development. To create a safe and effective vaccine against SARS-

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

CoV-2, researchers around the world are harnessing various technologies that were previously attempted against other viral infections, particularly against SARS-CoV. The most promising of these technologies could be broadly classified as protein subunit vaccines, inactivated vaccines, viral vector vaccines, and gene vaccines.[115]

Protein Subunit Vaccines. A protein subunit vaccine formulation often incorporates components of the pathogen that activate the host immune system[117] in a novel delivery vehicle such as liposome, virosomes, or polymeric nanoparticles.[118] Of these, liposomes and virosomes are being widely employed in vaccine development against SARS-CoV-2 because they not only function as delivery systems for subunit antigens but also as highly versatile adjuvants.[119] Liu et al. have developed a cationic liposome protein subunit vaccine, which contains the S1 subunit of the SARS-CoV-2 virus and two types of adjuvants, monophosphoryl lipid A (MPLA), a toll-like receptor 4 (TLR4) agonist, and CpG ODN, a toll-like receptor 9 (TLR9) agonist.[120] In addition, the incorporation of cationic ingredients like 1,2-dioleoy-l-3-trimethylammonium-propane (DOTAP) was shown to improve the liposome's interaction with antigen-presenting cells.[121] Compared to the traditional S1 subunit vaccine with alum adjuvant, the liposome vaccine produced a stronger T cell immunity in mice by enabling both CD4+ and CD8+ cells. The liposome can also induce the production of IgA, which will provide the host with possible mucosal defense.[120] Virosomes are lipid vesicles around 150 nm in size and contain viral proteins. Virosomes are biologically degradable, nontoxic, and do not form antiphospholipid antibodies. As an adjuvant, virosomes are superior to liposomes because they can protect pharmaceutically active substances within endosomes from proteolytic degradation until they enter the cytoplasm.[122] Virosomes were previously used to deliver vaccines against SARS-CoV and MERS-CoV.[123]

Building on their previous efforts to develop the SARS-CoV vaccine, Texas Children's Hospital Center for Vaccine Development at the Baylor College of Medicine is developing a subunit vaccine against SARS-COV-2 that consists of the S-protein receptor-binding domain (RBD). Like their previous vaccine against SARS-CoV, the current vaccine formulation is most likely composed of a recombinant RBD polypeptide formulated with alum or synthetic TLR4 agonist known as glucopyranosyl lipid A (GLA).[124] The University of Queensland and Novavax have been developing an immunogenic virus-like nanoparticle vaccine using a recombinant S-protein.[125] The Novavax vaccine was currently in phase 3 trial at the time of writing,[126] NVX-CoV2373 demonstrated mild or no reactogenicity in a majority of patients; the adjuvanted regimen induced a T helper 1 response without causing serious adverse effects.[127] In addition, Clover Biopharmaceuticals has been developing a highly purified S-trimer vaccine using their patented Trimer-Tag technology,[125] which was previously employed to develop subunit vaccines against HIV, RSV, and Influenza.[128] Clover Biopharmaceuticals completed enrollment of subjects in a phase 1 trial for dose escalation on their subunit vaccine with CpG 1018 adjuvants developed by GlaxoSmithKline (GSK) and Dynavax Technologies (Table S5).[129,130] Dynavax's CpG 1018 adjuvant is a TLR9 agonist, which was shown to stimulate the CD4+ and CD8+ T cells, and has a good safety profile.[131]

Inactivated Virus Vaccines. Inactivated vaccine consists of attenuated viral particles or bacterial pathogens that evoke an immune response but not the infection. Since these vaccines do not provide long-lasting immunity, booster doses are often required. Inactivated viral vaccines are formulated by propagating and concentrating large quantities of viral particles and inactivating them via chemical and/or physical methods. Often, ascorbic acid,[132] binary ethylenimine,[133] gamma irradiation,[134] or relatively high-temperature treatments[135] are employed to inactivate the viral particles. Analysis confirming virus inactivation needs to be carefully performed to achieve a high degree of safety.[136]

The first inactivated COVID-19 vaccine is being developed by the Wuhan Institute of Biological Products affiliated with the China National Pharmaceutical Group (Sinopharm). This vaccine is produced by propagating the virus in the Vero cell line, employing β-propiolactone as the inactivating agent, and incorporating alum as the adjuvant. In phase 1/2 clinical trials, the vaccine generated minimal adverse reactions and has been reported to produce antibodies in all participants.[137] The most common adverse events associated with the vaccine include injection site pain and fever, both of which were self-limited and mild. Its efficacy and long term adverse reactions are currently being assessed in phase 3 trials.[137,138] Another inactivated vaccine, CoronaVac (formerly PiCoVacc), is being developed by China's Sinovac Biotech Ltd. The vaccine consists of the CN2 strain of the SARS-CoV-2 virus isolated from bronchoalveolar lavage fluid samples of hospitalized patients. A comparison of the whole genome from different passages of the viral stock suggested that the CN2 strain had optimum genetic stability. The safety and immunogenicity of CoronaVac were tested in a rhesus macaques model[139] and were further established in phase 2 clinical trials. Currently, phase 3 clinical trials are underway in 8870 participants.[140]

The University of Wisconsin, Madison, in collaboration with vaccine companies FluGen and Bharat Biotech, has developed an inactivated vaccine against SARS-CoV-2 called CoroFlu for intranasal administration.[141] The vaccine is based on FluGen's influenza vaccine, M2SR, which induces the immune response against influenza. Now, FluGen has inserted SARS-CoV-2 S-protein gene sequences into M2SR to induce an immune response against SARS-CoV-2.[141] Although vaccines are primarily administered via the invasive parenteral route,[142] a noninvasive nasal immunization has the potential to induce robust mucosal and systemic immune responses against respiratory viral infections.[143,144]

Adenovirus Vaccines. Adenoviruses have double-stranded linear DNA enveloped in an icosahedral capsid.[145] Adenoviruses activate the innate as well as adaptive immunity in mammalian hosts and trigger the release of pro-inflammatory cytokines. These inflammatory cytokines further elevate the immune response by stimulating immune cells such as cytotoxic T lymphocytes, which recognize and kill virus-infected cells.[146] Adenoviral vectors have been previously employed against diseases like influenza, Ebola, SARS, influenza, and HIV. Oxford University's, Jenner Institute, CanSino biologics, and Johnson and Johnson have been testing this vector to develop vaccines against COVID-19 (Table S5). Cansino's vaccine, Ad5-nCoV is in phase 2 clinical trials.[147] In addition, Oxford University researchers employ a chimpanzee adenoviral vaccine vector, AZD1222, which is a nonreplicating virus. The genetic sequence encoding the S-protein of SARS-CoV-2 is encapsulated in the AZD1222 construct.[148] A single dose of this vaccine has been claimed to generate a strong immune response without causing infection in the vaccinated

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

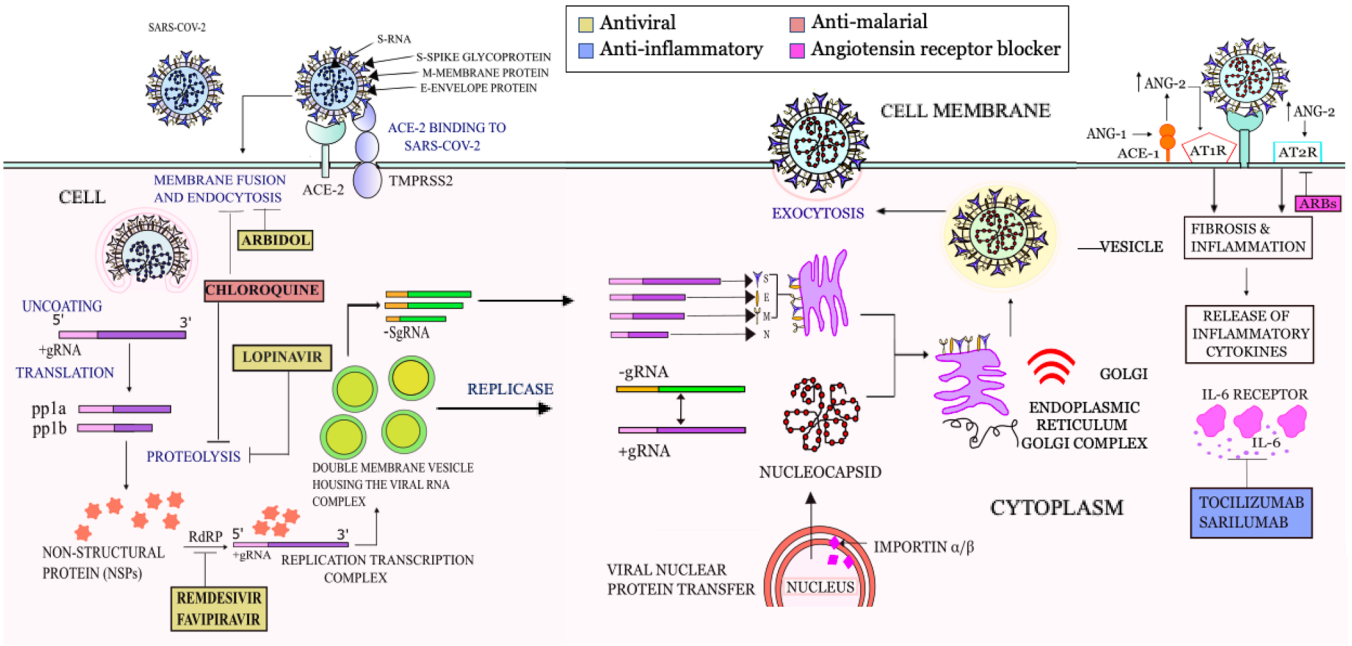

Figure 4. Cellular and molecular mechanisms underlying viral cell fusion and replication of SARS-CoV-2 in human cells. Impact of various drugs/pharmacological agents on critical processes involved.

patient. Hence, this vaccine is believed to be safer for children, the elderly, and individuals with pre-existing conditions such as diabetes. AstraZeneca and the University of Oxford have been collaborating in developing this vaccine; phase 1 and 2 trials demonstrated an acceptable safety profile and confirmed neutralizing antibody response against SARS-CoV-2.[148]

Further, a recombinant novel coronavirus vaccine, which incorporates a replication-defective adenovirus type-5 as the vector to express the SARS-CoV-2 S-protein, has been developed in China by CanSino Biologics and Institute of Biotechnology of the Academy of Military Medical Sciences (Table S5); a randomized, double-blind, placebo-controlled, phase 2 trial is ongoing since April 2020. This vaccine has demonstrated a significant neutralizing antibody response against the SARS-CoV-2 spike protein in healthy adults of age 18 years or older.[149]

Although adenoviral vectors are being widely considered to deliver COVID-19 vaccines, they are currently in the experimental phases, and no vaccine using this platform has been approved for human use against infectious diseases. In addition to the serious risk of inflammatory reactions posed by adenoviral vectors, like those observed in AstraZeneca trials, immunity against adenoviral vectors is also possible since humans are commonly exposed to adenoviruses.

Gene Vaccines. Gene vaccines involve direct administration of a DNA plasmid, which codes for the particular target antigen. This type of vaccine has many potential advantages over conventional vaccines in terms of stimulating both B and T cell responses and has a better safety profile. Since they are devoid of any infectious agents, they could be administered to immunocompromised patients.[150] The DNA plasmid incorporated in the vaccine can be accurately designed using the knowledge of the viral genome. Synthetic DNA vaccines accelerate the developmental process of a vaccine as they allow for the quick design of multiple vaccine candidates for preclinical testing, facilitate scalable manufacturing of large quantities of vaccine products, and encounter less regulatory hurdles for clinical translation. Moreover, the synthetic DNA is temperature-stable and has a longer shelf life.[151]

Gene-based vaccines against SARS-CoV-2 are mostly being developed against the S-protein. The vaccine is expected to trigger the expression of spike antigens in the host, which then induce antibodies capable of inhibiting S-protein recognition by the host receptors. Inovio Pharmaceuticals employs DNA-plasmid pGX9501 in their vaccine candidate, which encodes for the S-protein of SARS-CoV-2. Their previous studies have shown that the immunization of animal models with DNA vaccines encoding MERS-CoV S-protein could protect the animal from the disease. Given the shared global protein fold architecture between SARS-CoV-2 and MERS-CoV S-proteins, a synthetic DNA vaccine, IN0−4800 (Inovio Pharmaceuticals), was formulated based on their prior vaccine constructs.[152] Currently, INO-4800 is in phase 2 clinical trials, where the vaccine is administered to healthy adults by an intradermal route followed by electroporation.[153]

In addition to DNA, mRNA could also be used in gene vaccines. While DNA vaccines act on the nucleus, mRNA vaccines act in the cytosol; hence, they are not required to cross the nuclear membrane. Additionally, RNA vaccines are known to induce a more potent memory in the immune system and therefore require lower doses than the DNA vaccines. However, RNA vaccines are not as stable as DNA vaccines; they are heat-labile and are prone to hydrolysis by ribonucleases present in circulation.[154] To improve the stability and deliverability to the host, mRNA vaccines are formulated as lipid nanoparticles using cationic lipids and lipopolymers that can be electrostatically complexed with the negatively charged RNA.[155] Moderna (Cambridge, MA) uses this approach for its SARS-CoV-2 (Table S5) vaccine. Their lipid nanoparticle formulation consists of mRNA-1273, which encodes a stabilized prefusion spike trimer, S-2P, and is currently in phase 3 clinical trials.[156] In addition, Pfizer's nucleoside-modified mRNA (modRNA) candidate, BNT162b1 (encodes an optimized SARS-CoV-2 full-length

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

S-protein), is also formulated as a lipid nanoparticle. The phase 1/2 studies[157] have shown that BNT162b1 induced a stronger CD8-T cell response, which could promote the generation of CD4 T cells and neutralizing antibodies,[156,157] in comparison to Moderna's vaccine candidate.[156,158] In phase 3 interim analysis, the mRNA-based vaccine candidate BNT162b2 demonstrated a 90% efficacy rate over the placebo, 7 days after the second dose.[159,160]

Even after the development of a safe and effective COVID-19 vaccine, the challenges encountered in manufacturing, distributing, and administering the vaccine to the vulnerable population throughout the world is fraught with many challenges. Specifically, distributing the vaccine in developing countries could be very challenging as the cold chain required for the stability and activity of the vaccine is not adequately established.[161]

Repurposing Approved Drugs to Treat COVID-19 Patients. There are several investigational drugs currently in clinical trials (Table S4) to treat COVID-19 patients. These drugs were selected based on their putative mechanisms inhibiting viral entry into the host and subsequent viral replication and are currently being investigated or were already investigated in human clinical trials (Figure 4). While some of these drugs have been used previously to treat SAR-CoV infections, a few of them are being used for the first time to target SAR-CoV-2 infection.

Antiviral Drugs. The FDA approved remdesivir (Gilead Sciences, Inc.) for the treatment of COVID-19 requiring hospitalization in patients 12 years of age and older.[162] Remdesivir is an RNA-dependent RNA polymerase inhibitor, which is believed to incorporate itself into SARS-CoV-2 RNA and inhibit its further replication (Figure 4). Remdesivir was also administered to one of the first COVID-19 patients in the United States via intravenous (IV) administration. No adverse effects were reported, and the patient's clinical condition improved even after the supplemental oxygen was discontinued. After the IV administration of 200 mg of remdesivir on the first day and 100 mg on each subsequent day for 9 days, the overall clinical improvement was observed in 36 of 53 COVID-19 patients.[163]

Lopinavir and ritonavir in an antiretroviral therapy has also has also been studied in treating patients with COVID-19 but failed to show benefit over standard care.[164] Umifenovir inhibits the S-protein/ACE2 interaction and is approved in Russia and China for influenza prophylaxis. In a study conducted in Russia during the 2004 SARS outbreak, the activity of umifenovir against SARS-CoV has been established using in vitro models[165,166] and is currently being investigated for COVID-19 treatment.[167] Favipiravir, which is approved in Japan for influenza, inhibits RNA polymerase and blocks viral replication (Figure 4). When compared with umifenovir, treatment with favipiravir resulted in the better clinical recovery of moderate COVID-19 infections in a prospective randomized study.[167]

Antimalarial Drugs. Chloroquine and hydroxychloroquine have been approved by the Food and Drug Administration (FDA) for the treatment of malaria and inflammatory disorders such as rheumatoid arthritis. Being weak bases, chloroquine and hydroxychloroquine are believed to inhibit viral replication by increasing the endosomal vesicular pH and engendering the inactivation of proteases such as acid hydrolases, which are pH-dependent (Figure 4). Consequently, post-translational modifications of newly synthesized proteins,[168] including the formation of the S-protein envelope, which allows for SARS-CoV-2 binding to ACE2 receptors and subsequent endocytosis,[31] are inhibited. Clinical trials conducted in China demonstrated that chloroquine may reduce the progression of pneumonia and replication of the SARS-CoV-2 virus in a small group of 100 patients.[169]

Despite these putative therapeutic benefits of chloroquine, hydroxychloroquine did not appear to have the same positive clinical effects. A randomized, double-blind, placebo-controlled clinical trial conducted in subjects that had high-risk or moderate-risk exposure to COVID-19 showed that hydroxychloroquine provided no significant benefits in preventing illness when used within 4 days of the exposure.[170] Hydroxychloroquine is a cautionary tale: despite promising in vitro data and its inhibition of viral entry mechanisms, patient outcome data did not necessarily show a benefit.

Angiotensin Receptor Blockers and Statins. Therapeutic drug combinations such as statins and angiotensin receptor blockers (ARBs) are promising therapies for potentially averting ARDS in COVID-19 patients. Statins and ARBs could alleviate the host response to the infection through their immunomodulatory properties that reduce the release of inflammatory cytokines (Figure 4).[171] Pro-inflammatory cytokines, in due course, cause endothelial barrier leakage by disrupting the integrity of endothelial tight junctions and trigger pneumonia.[172] The leaky barrier allows for the accumulation of fluid from the blood in the interstitial lung tissue, presumably due to an increase in the expression of angiopoietin-2.[172] Statins and ARBs decrease the production of angiopoietin-2 and help restore the endothelial barrier integrity.[172] Although ARBs and ACE inhibitors appear to upregulate ACE2 expression and increase the potential of SARS-CoV-2 binding,[173] some argue that ACE inhibitors and ARBs may have a beneficial effect by decreasing overall inflammation.[174] Several cardiology associations strongly recommend continuing treatment with ACE inhibitors/ARB in patients who were previously taking these drugs as the benefit outweighed the risk. Khera and colleagues found that patients with both hypertension and COVID-19 treated with ACE inhibitors were 40% less likely to be hospitalized than those not treated with ACE inhibitors. Interestingly, this effect was not seen in patients treated with ARBs.[175] More information is needed to understand the clinical impact of ACE or ARBs on COVID-19 treatment.

Impact of Formulation and Route of Administration on the Efficacy of Antivirals. As described earlier, pneumonia is one of the severe symptoms observed in COVID-19 patients. Administering therapeutic concentrations of remdesivir to the lungs is pivotal to avoid further spread of the virus into the lungs. Sun et al. (2020) have demonstrated that remdesivir IV administration alone cannot achieve clinical efficacy due to low lung distribution and poor cellular permeability of remdesivir as well as its active nucleoside triphosphate metabolite.[176] However, a combination drug delivery approach involving IV administration along with pulmonary nebulization of remdesivir was deemed effective in achieving therapeutic drug concentrations in the lungs to reduce SARS-CoV-2 viral replication.[176] The IV administration of remdesivir is only possible in a healthcare setting, whereas patients could self-administer remdesivir via a nebulizer. However, further studies are needed to determine the role of nebulized remdesivir in early COVID-19.

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Novel Therapies. *Convalescent Plasma Treatment.* Convalescent plasma treatment is being developed at a remarkable speed. In August 2020, the FDA authorized the use of high titer and low titer convalescent plasma for the treatment of patients hospitalized with COVID-19.[177] The convalescent patient plasma may contain antibodies that not only block viral infection but also improve the clearance of cells infected with the virus.[178,179] Thus, convalescent plasma from patients recovered from COVID-19 might be useful to alleviate symptoms in critically ill patients.[180] When five COVID-19 patients suffering from ARDS were infused with 400 mL of convalescent plasma (with SARS-CoV-2-specific antibody binding titer greater than 1:1000 and neutralization titer greater than 40), viral load decreased within 3−4 days after the infusion; moreover, the majority of the patients did not need mechanical ventilation following 12 days of plasma infusion.[180] In this small sample study, patients have also received steroids and other antiviral agents. In a larger study of 5000 patients, convalescent plasma appeared to be safe in hospitalized patients with COVID-19.[181] Recent reports from the follow-up study conducted on 35,322 transfused COVID-19 patients have shown that the patients receiving high IgG plasma (>18.45 signal-to-cutoff ratio (S/Co)) had lower mortality (8.9%) than those receiving medium (4.62−18.45 S/Co) or low IgG plasma (<4.62 S/Co).[182] Although data support the safety and potential efficacy of convalescent plasma, rigorous randomized clinical trials are needed to determine which subset of patients and to what extent they are most likely to benefit.

Immunoglobulins. Monoclonal antibody therapy could potentially be an effective clinical treatment against COVID-19.[183] It targets a single epitope, which allows for a higher specificity on a predetermined target. Monoclonal antibodies that are produced on a large scale have reduced batch variations in comparison to polyclonal antibodies.[184] Lilly has developed a neutralizing monoclonal antibody drug candidate, bamlanivimab (LY-CoV555), from the convalescent plasma of COVID-19 patients. Bamlanivimab has an activity against the SARS-CoV2 receptor-binding domain.[185] It blocks the attachment of the virus to the host cell and prevents its entry into human cells. In the phase 2 clinical trial for bamlanivimab in outpatients, a reduction in viral load was observed at day 11 when compared to the placebo group. The symptom severity and COVID-19 hospitalization rate were also reduced in patients who received bamlanivimab.[185] It currently has received emergency use authorization by the FDA for the treatment of COVID-19 in recently diagnosed patients.[186] Regeneron has developed a novel two monoclonal antibody cocktail, REGN-COV2, from humanized VI mice and blood from recovered COVID-19 patients. It is known to reduce SARS-CoV-2 viral infectivity by binding to the RBD of the spike protein at two distinct, nonoverlapping locations. This interaction hinders the binding of the virus to the host cell and is able to neutralize the virus and prevent infection.[187] Moreover, the two-antibody cocktail combination prevents mutant viral escape, which is usually seen with single antibody therapies. REGN-COV2 prevents the escape of viral mutants by simultaneously binding to two distinct regions of the virus.[188] REGN-COV2 is being evaluated in four late-stage clinical trials. Two phase 2/3 clinical trials conducted on hospitalized and nonhospitalized patients have shown a reduction of viral symptoms in nonhospitalized patients.[189,190] Two phase 3 recovery and prevention trials of hospitalized COVID-19 patients are currently underway.[191] Antibody immunotherapy is therapeutically promising against SARS-COV2, but still, there are certain challenges. Patients who have previously died of SARS-CoV infection have exhibited a strong neutralizing antibody response in addition to pulmonary inflammation. Due to a pathological link between the neutralizing antibody response and pulmonary inflammation, it is necessary to consider the patients' adaptive immune responses when administering an antiviral immunotherapy.[192] Additionally, patients with severe COVID-19 may not be responsive to antibody therapy because they would have developed several underlying conditions such as acute inflammation and coagulopathy during the course of infection. In such cases, the patient's condition deteriorates so drastically that decreasing viral load in these patients may not be helpful.[193]

Despite being promising therapeutic agents to treat COVID-19, immunoglobulins pose several challenges for large scale manufacturing and quality control. Moreover, immunoglobulins have to be given intravenously, at doses as high as 8 g for REGN-COV2, which requires hospitalization of the patient. These constraints may increase the expense and limit the widespread use of immunoglobulins for COVID-19 treatment.[194]

Inflammatory Modulators. A multicenter randomized controlled trial investigating the efficacy and safety of tocilizumab in COVID-19 treatment has been conducted in Wuhan, China. Tocilizumab is a monoclonal antibody which blocks IL-6 receptors.[195] Initial studies concluded that tocilizumab was associated with reduced mortality and clinical improvement in patients with severe COVID-19.[196] Further studies are needed to confirm the efficacy and safety of tocilizumab prior to routine use in clinical practice.

Interferons are a family of proteins produced by the host cells in response to a viral infection. Interferon-β is known to increase the production of anti-inflammatory cytokines and downregulate the production of pro-inflammatory cytokines.[197] Interferon-α is known to extend the activated T cell response, increase humoral immunity, and antigen-presenting cell response.[198] Interferon-α has been used in combination with ribavirin to treat MERS-CoV.[199] Inhalation formulation of interferon-β-1a, which is expected to decrease symptoms of respiratory illness and pneumonia in COVID-19 patients, is currently in phase 2 clinical trials (Synairgen, England). Eculizumab, a monoclonal antibody that binds to complement component 5, is currently being evaluated to treat COVID-19. Preliminary evidence demonstrated that 4 patients with severe ARDS or pneumonia in the intensive care unit recovered after treatment with eculizumab.[200]

Stem Cell Therapies. Expanded umbilical cord mesenchymal stem cells (UC-MSCs) have been regarded as a possible treatment for SARS-CoV-2. The MSCs have powerful immunomodulatory properties and can secrete anti-inflammatory factors. Theoretically, the accumulation of MSCs in the lung could protect alveolar epithelial cells and improve lung function. Promising preclinical and preliminary clinical data have demonstrated the feasibility of stem cell therapy to enhance the recovery of COVID-19 patients.[201] Among the types of stem cells that are available for clinical use, UC-MSCs appear to be the best candidates to treat coronavirus.[201] The UC-MSCs derived from umbilical cords have a rapid doubling time, which makes it easy to scale up in the lab. Moreover, they can be harvested noninvasively, unlike bone marrow stem cells.

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Chinese investigators have reported IV infusion to be the ideal route of administration for stem cells in COVID-19 patients as the stem cells are mainly confined to the lungs following IV administration. This approach may be particularly beneficial since the lungs are the most affected organs in COVID-19 patients.[201] Overall, using UC-MSCs is relatively inexpensive and presents a potential treatment option for COVID-19.[201]

SUMMARY

Despite similarities in the clinical manifestations and molecular mechanisms with other diseases caused by betacoronaviruses, COVID-19 turned out to be so contagious and deadly that it triggered commitment and collaboration among scientists across the globe to protect humanity against this juggernaut. This coordinated effort has not only been accelerating our understanding of COVID-19 pathophysiology and its clinical manifestations but also contributing to the better prognosis of hospitalized patients. Vaccine development against COVID-19 is in full swing, and several vaccine candidates are in phase 3 trials. On the other hand, high-throughput drug discovery platforms are being harnessed to repurpose existing drugs and develop formulation strategies for COVID-19 treatment. As of now, there are more than 300 clinical trials underway to test the safety and efficacy of various drug candidates in COVID-19 patients. A recent flurry of articles in various scientific journals on COVID-19 etiology, pathophysiology, clinical treatments, and drug discovery, as well as on repurposing efforts, are serving as beacons of optimism that a vaccine and/or effective treatment may soon become available for this devastating disease. The current review article is intended to summarize COVID-19 pathophysiology from the perspective of pharmacological interventions that are being investigated and provide a snapshot of this rapidly moving frontier in the fight against COVID-19.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.0c00608.

Transcriptomic gene expression of ACE2, TMPRSS2, and furin in various organs from the genotype-tissue expression (GTEx project); (PDF)

Relative transcription levels of ACE2 in various tissues from a single-cell (RNA-Seq) study; ACE2, TMPRSS2, and furin expression levels in various cell types from a single-cell transcriptomic study; repurposed drugs in clinical trials for COVID-19; vaccines in clinical trials for COVID-19 (XLSX)

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V.S.S. and J.A.W. are co-first authors. K.R.K. and K.K.K. are co-senior authors.

Notes

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Mol. Pharmaceutics XXXX, XXX, XXX−XXX

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