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Viewpoint

COVID-19: Living through Another Pandemic

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ABSTRACT: Novel beta-coronavirus SARS-CoV-2 is the pathogenic agent responsible for coronavirus disease-2019 (COVID-19), a globally pandemic infectious disease. Due to its high virulence and the absence of immunity among the general population, SARS-CoV-2 has quickly spread to all countries. This pandemic highlights the urgent unmet need to expand and focus our research tools on what are considered "neglected infectious diseases" and to prepare for future inevitable pandemics. This global emergency has generated unprecedented momentum and scientific efforts around the globe unifying scientists from academia, government and the pharmaceutical industry to accelerate the discovery of vaccines and treatments. Herein, we shed light on the virus structure and life cycle and the potential therapeutic targets in SARS-CoV-2 and briefly refer to both active and passive immunization modalities, drug repurposing focused on speed to market, and novel agents against specific viral targets as therapeutic interventions for COVID-19.

As first reported in December 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused an outbreak of atypical pneumonia in Wuhan, China, that has since spread globally.[1] The disease caused by this new virus has been named coronavirus disease-2019 (COVID-19) and on March 11, 2020 was declared a global pandemic by the World Health Organization (WHO).[1] Currently, there are seven known human coronaviruses classified into two broad genera of alpha- and beta-coronaviruses. The alpha-coronaviruses comprise HCoV-NL63 and HCoV-229E, while the beta-coronaviruses comprise HCoV-OC43, HCoV-HKU1, SARS, Middle East Respiratory Syndrome virus (MERS), and SARS-CoV-2.[2] The alpha-coronaviruses and HCoV-OC43 and HCoV-HKU1 are among the causes of the common cold and have been circulating in human and animal populations for many years.[2] All these viruses originate from a common ancestor and enter the human population through zoonotic transfer or species jumping.[3] Although the first four known human coronaviruses originated from birds, SARS, MERS, and SARS-CoV-2 appear, on the basis of gene sequence analysis, to have originated from bats.[4] However, in each case, these more recent viruses appear to have been transmitted through an intermediate host such as a civet, a small nocturnal mammal native to tropical Asia and Africa (SARS), a camel (MERS), or a pangolin (SARS-CoV-2) after acquiring additional mutations.[2] Bats harbor more strains of coronavirus than other mammals, estimated to range from 5000 to 10,000 distinct subtypes.[5] Therefore, additional epidemics are highly likely to occur in the future due to the abundant number of coronaviruses present in the bat population.

As of May 6th, 2020, more than 3.7 million cases of SARS-CoV-2 positive patients have been reported worldwide with over 260,000 deaths, reflecting a ~6.8% case fatality rate. While the infection fatality rate is currently unknown, and likely to be lower than the current case fatality rate, estimates suggest it is close to 1%, or approximately 10 times the infection fatality rate of seasonal influenza (flu), which is fatal in only ~0.1% of infected patients.[6] In contrast to previous coronavirus epidemics (Table S1), COVID-19 is indiscriminately wreaking havoc globally with no apparent end in sight due to its high virulence and the absence of resistance among the general population.

In general, all pandemics pass through three phases until they become endemic. The first phase of "seeding" or slow spread is often not noticed early enough, leading to dissemination of the disease before effective countermeasures can be initiated. During the second phase, there is a rapid increase in cases until a peak occurs in the number of infected individuals; parallel efforts to control and contain the virus can mitigate this phase. In the third phase, the infection rate curve will start to decrease until the disease becomes extinct or endemic. The kinetics of increase and decrease in the rate of infections can vary significantly between populations depending on the use of preventive measures and the availability of effective treatments. Previous coronavirus outbreaks and the current pandemic highlight the urgent unmet medical need to expand and focus our research tools on these long neglected infectious diseases and to prepare for future inevitable pandemics. Herein, we briefly recap the current and potential future therapeutic interventions for SARS-CoV-2 and highlight the recently published crystal structures of the SARS-CoV-2 main protease and its inhibitors as novel agents against SARS-CoV-2.

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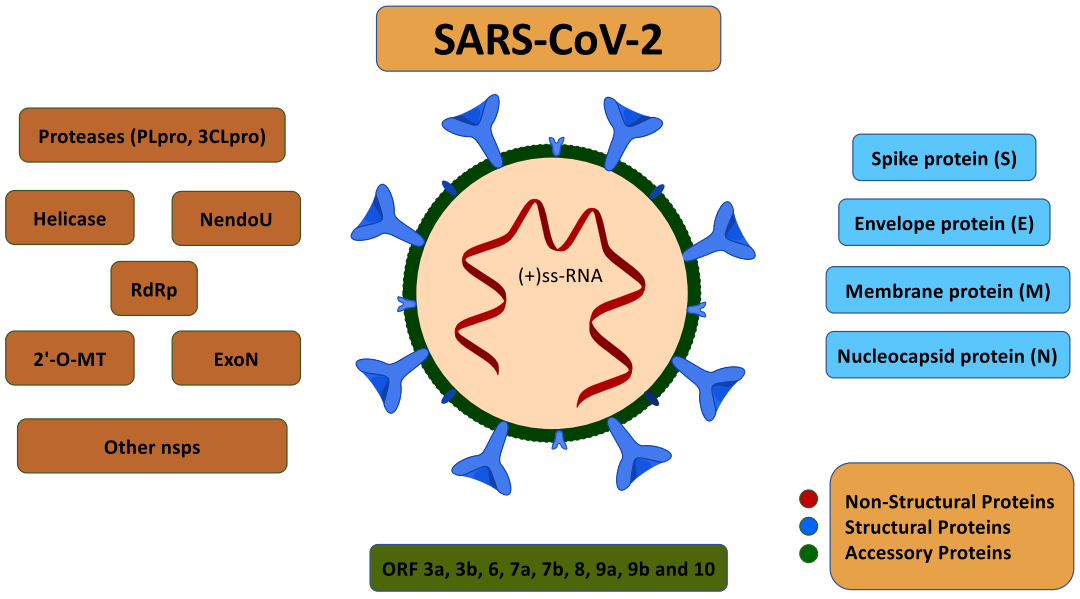

Figure 1. SARS-CoV-2 encoded proteins.

VIRUS STRUCTURE AND LIFE CYCLE

SARS-CoV-2 is an enveloped, nonsegmented single stranded, positive sense RNA virus. It has one of the largest genomes among all RNA viruses, comprising approximately 30 kilobases (kb) (NC_045512.2). SARS-CoV and SARS-CoV-2 belong to the same *Coronaviridae* family that also includes the highly fatal Middle East Respiratory Syndrome virus (MERS) that appeared in 2012.[7] SARS-CoV-2 enters cells through the interaction of its surface Spike protein with the host receptor, angiotensin-converting enzyme 2 (ACE2).[8] Subsequent proteolytic cleavage by the host serine protease TMPRSS2 or perhaps other proteases allows subsequent cell entry by endocytosis.[8] Upon membrane fusion and endocytosis, the viral nucleocapsid with its genome payload is released into the cytoplasm of the infected cell. Following its release into the host cell, the virus usurps portions of the endoplasmic reticulum to form numerous double membrane vesicles.[9] These vesicles are perfect sanctuaries to protect the viral genome and allow an efficient replication process to occur through a macromolecule complex called the replication–transcription complex (RTC).[10] The viral genome is subsequently translated into viral polyproteins using the host cell protein translation machinery, which are then cleaved into structural and nonstructural viral proteins by two viral proteases, M^{pro} and PL^{pro}.[11] This step is followed by the assembly of viral particles (virions) in the endoplasmic reticulum/golgi compartment.[12] The packaged virions are then transported to the cell surface, are released from the cells through exocytosis, and proceed to infect other cells.

CLINICAL COURSE AND OUTCOMES OF COVID-19

SARS-CoV-2 likely binds to epithelial cells in the nasal cavity during the asymptomatic state of the disease (initial 1−2 days of infection) where there might be some local propagation of the virus but with a limited innate immune response. Within the next few days the infection starts in the upper airway and during this stage the infection can be detected by nasal swabs or sputum as well as early markers of the innate immune response. About 80% of infected patients show mild symptoms that are mostly restricted to the upper and conducting airways.[13] However, about 20% of infected patients will progress to develop a lower respiratory tract infection leading to hypoxia, and lung damage. These patients are liable to succumb to acute respiratory distress syndrome (ARDS), which is frequently fatal. Patients with comorbidities such as cardiovascular disease, diabetes mellitus, hypertension, chronic lung disease, cancer, chronic kidney disease, and obesity (body mass index ≥30) and ARDS are at increased risk of death.[14] The available data indicate that the viral infection can produce an excessive immune reaction known as cytokine release syndrome (CRS) or "cytokine storm" associated with elevated levels of interleukin-6 (IL-6).[15] Laboratory findings associated with worse clinical outcomes include lymphopenia, and elevations in liver enzymes, lactate dehydrogenase (LDH), inflammatory markers (e.g., C-reactive protein [CRP], ferritin), D-dimer, prothrombin time (PT), troponin and creatine phosphokinase (CPK), and acute kidney injury.[15] Chest CT scans in patients with COVID-19 commonly show ground-glass opacification, consistent with viral pneumonia with abnormalities more likely to be bilateral with a peripheral distribution involving the lower lobes.[16] While SARS-CoV-2 entry is dependent on ACE2 in lung cells,[8] ACE2 expression is not exclusive to the lungs, and higher relative ACE2 expression is observed in heart, kidney, GIT, and testes (Gene ID: 59272). The organ- and cell-specific expression of ACE2 suggests that it may play a role in the regulation of cardiovascular and renal function as well as fertility. Surprisingly, the 2003 SARS-CoV infection was shown to downregulate the expression of ACE2 in lung tissue reducing transmissibility but increasing virulence.[17] ACE2, a component of the renin-angiotensin system (RAS), catalyzes the cleavage of angiotensin I into angiotensin 1−9 and angiotensin II into the vasodilator angiotensin 1−7.[18] The balance between angiotensin II and angiotensin (1−7) is critical since angiotensin II elicits vasoconstriction via the angiotensin AT1 receptor, whereas angiotensin (1−7) exerts a vasodilatory effect mediated by AT2 with multiple beneficial effects on the cardiovascular and respiratory system.[18] While ACE2 appears to be involved in the hypertension and respiratory manifestations of severely ill patients, the benefits and merits of ACE

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inhibitors (ACEI) or angiotensin receptor 1 blockers (ARBs) in the SARS-CoV-2 patient is still controversial.[18] The priming of the S viral protein by TMPRSS2 is crucial for viral entry where TMPRSS2 expression is very high in the lungs, kidneys, and prostate tissue (Gene ID: 7113).[8] TMPRSS2 is predominantly expressed in the luminal cells of the prostate epithelium, where its expression is regulated positively by androgens and negatively regulated by estrogens. The hormonal regulation of TMPRSS2 has been suggested to be linked to the fact that men are at higher risk than women to become seriously ill with COVID-19.[19] TMPRSS2 knockout in mice is not lethal. In contrast, humans are intolerant to the loss of function of ACE2.[19] Concerns about the effect of ACEI and AT receptor blockers in COVID-19 patients are actively being investigated in the clinic, with the most recent reports offering encouragement that they may have a beneficial effect.

■ POTENTIAL DRUG TARGETS

SARS-CoV-2 viral RNA encodes several proteins that are potentially druggable targets (Figure 1 and Table S2), including four structural proteins: the Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N).[1] The 1273 amino acid, 141 kDa, Spike protein is heavily N-glycosylated and is a major inducer of host immune responses. The 222 amino acid M protein has three transmembrane domains and is the most abundant structural protein in the virion. The 75 amino acid E protein is important for assembly and release of the virus. The 419 amino acid nucleocapsid protein forms a protective protein shell around the virus genetic material and is encased in a lipid envelope that is usurped from the host cell. Matrix protein connects the membrane to the nucleocapsid protein. There are also 16 nonstructural proteins (nsp1−16)[1] including several for which there are X-ray crystallography-derived structural data: RNA dependent RNA polymerase (nsp12, RdRp), a papain-like protease (nsp3, PLpro), the main protease (nsp5, $3CL^{pro}$, or M^{pro}), and exonuclease/N7-methyltransferase (nsp14, ExoN). RdRp catalyzes synthesis of the full length negative-strand RNA template used by RdRp to make more viral genomic RNA. The SARS-CoV-2 genome also contains a number of open reading frames (ORFs): namely, ORF 1a proposed to encode nsp1 to nsp11; ORF1b, is proposed to encode nsp12 to nsp16, essential for viral replication, and ORFs 3a, 3b, 6, 7a, 7b, 8, 9a, 9b, and 10, which encode for accessory proteins[1] (Table S2).

■ TREATING VIRAL INFECTION

Immunization against SARS-CoV-2. The development of a manufacturable, safe, and effective vaccine may take 12−18 months. Several phase I clinical trials are currently recruiting participants to test the safety, reactogenicity, and immunogenicity of several investigational SARS-CoV-2 vaccines (Tables S3 and S4). An mRNA vaccine based on the Spike protein began human clinical trials within a record 63 days from first publication of the SARS-CoV-2 sequence (NCT04283461). It has been suggested that the mutation rate for SARS-CoV-2 is expected to be low, raising hope that a successful vaccine will provide life-long immunity.[20] Hyper-immune globulin isolated from the sera of convalescent patients having high titers of antibodies against SARS-CoV-2 or even their whole blood may provide instant "passive" short-lived immunity mainly via viral neutralization. Antibody dependent therapy, for example targeting the Spike protein might represent the most efficient, near-term therapeutic intervention if regulatory and safety requirements can be addressed. With over 1.3 million positive cases of COVID-19 in the US based solely on the results of RNA molecular tests, large-scale antibody testing should be expedited to identify individuals who have been exposed to the virus but were never officially confirmed to have COVID-19. An understanding what levels of antibody confer immunity postinfection could be used to determine who may be less likely to transmit the virus and thus may be able to go back safely to work.

Drug Repurposing. Developing highly selective SARS-CoV-2 specific new drugs will take many years. Alternatively, repurposing of existing, approved drugs can present a more rapid strategy to identifying drugs effective in treating COVID-19 (Tables S3 and S4 and Figure S1).[11] Repurposing of drugs that would block SARS-CoV-2 entry and/or replication are urgently needed to mitigate the symptomatic burden of the disease. Unfortunately, the HIV protease inhibitors ritonavir/liponavir failed to show efficacy in SARS-CoV-2 infected patients.[21] Hydroxychloroquine, which may act by increasing the pH within lysosomes, was granted FDA authorization for use in emergency cases. Several antiviral agents are being tested such as the RdRp inhibitor remdesivir and the approved anti-influenza drug faviprivir.[8] Remdesivir was previously tested in humans with Ebola virus disease and also in animal models of MERS and SARS-CoV.[11] At least six clinical trials are evaluating remdesivir in SARS-CoV-2 patients. Other drugs that might inhibit RdRp include the broad-spectrum antiviral drug ribavirin. RdRp conservation among RNA virus families makes it an exciting target for the discovery of newer agents. The Spike protein, ACE2, and TMPRSS2 may also represent interesting therapeutic targets for current drug repurposing efforts. Camostat mesylate, approved in Japan for treatment of pancreatic inflammation, has been shown to block TMPRSS2 activity.[22] Arbidol, which is hypothesized to block Spike/ACE2 binding, is being investigated clinically, and a clinical trial was recently launched to study the effect of thiazide, thiazide-like diuretics, calcium channel blockers, ACE inhibitors, and angiotensin receptor blockers in COVID-19 (NCT04330300). The availability of soluble recombinant hACE2 encouraged its testing in two clinical trials; although one was terminated (NCT04287686) the other is currently active (NCT04335136). Monoclonal antibodies, especially for interleukin-6 (IL-6) or its receptor, are also being considered for the control of SARS-CoV-2 associated respiratory exacerbations.[11] Interestingly, several Janus Kinase (JAK) inhibitors such as baricitinib and ruxolitinib are currently being evaluated given their involvement in interleukin signaling pathways. Another currently recruiting clinical trial is testing quinolone, macrolide, and β-lactam antibiotics against COVID-19 (NCT02735707). Multiple groups have tested FDA approved drugs in various *in vitro* assays as well as in computational screens. Many of these drugs show inhibitory activities, although not always at a concentration that may be safely achieved in patients.[23] Controlled clinical trials of these agents are mandatory to assess their efficacy and safety without creating false positive hope or depleting the supplies of drugs needed to treat the diseases for which they were initially approved.

Novel Agents. M^{pro} and PL^{pro} are cysteine proteases responsible for the cleavage of viral polypeptides into functional proteins for virus replication and packaging within host cells.[24] These enzymes represent the best characterized

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drug targets among coronaviruses and are currently the focus of attention among scientists seeking novel coronavirus small molecule therapeutics.[25] M^{pro} is shared by all coronavirus genera and has similarity to the $3C^{pro}$ of the *Enterovirus* genus in the picornavirus family.[24] M^{pro} contains a Cys···His catalytic dyad with an additional α-helical domain involved in the dimerization of the protease, which is essential for its catalytic activity.[25] The enteroviral $3C^{pro}$ functions as a monomer featuring a classical Cys···His···Glu/Asp catalytic triad.[24] Yet, they share the almost absolute requirement for Gln in the P1 position of the substrate and space for only small residues such as Gly, Ala, or Ser in the P1′ position. Since no human proteases with a similar cleavage specificity are known, it may be possible to identify highly selective M^{pro}/$3C^{pro}$ inhibitors, which display minimal inhibition of host proteases.[26] The 3-D structures of unliganded SARS-CoV-2 M^{pro} and of its complex with a peptidomimetic α-ketoamide inhibitor (**11r**) have been solved[26] and were used to support the design of an optimized derivative (**13b**) through docking studies (Figure S2). α-Ketoamides can interact with the catalytic center of M^{pro} through two hydrogen bonding interactions rather than only one as with other warheads such as aldehydes or Michael acceptors.[24] Nucleophilic attack of the α-keto group by the catalytic Cys residue results in reversible formation of a thiohemiketal. These α-ketoamides feature a 5-membered rigid γ-lactam as a mimic of the P1 residue, glutamine, required for M^{pro} specificity, with the advantage of reducing the loss of entropy upon binding.[24] Follow up optimization efforts guided by docking to the SARS-CoV-2 M^{pro} co-crystal structure with **11r**, included incorporation of the P3-P2 amide bond into a pyridone ring as in **13a**. The resulting half-life of **13a** in plasma was enhanced by 3 fold relative to **11r**, *in vitro* kinetic plasma solubility improved by a factor of ∼19 and thermodynamic solubility by a factor of ∼13. **13a** inhibited purified recombinant SARS-CoV-2 M^{pro}, SARS-CoV M^{pro}, and MERS-CoV M^{pro} in the submicromolar range.[26] Modification of the P1′ and P3 moieties of **13a** afforded an optimized derivative **13b** which was crystallized with SARS-CoV-2 M^{pro} (PDB: 6Y2G and 6Y2F). Both **13a** and **13b** displayed good stability in mouse and human microsomes. **13b** (3 mg/kg) showed longer $t_{1/2}$, t_{max}, and residence time compared to **13a** (20 mg/kg) in CD-1 mice. Both compounds showed lung tropism which is thought to be beneficial. While the development of these ketoamides into clinical candidates requires additional safety studies, the availability of their crystal structures is of great importance in facilitating the discovery and development of other M^{pro} inhibitors. One of the suggested agents for testing is the previously reported Rhinovirus and SARS-CoV M^{pro} inhibitor clinical candidate rupintrivir (AG-7088) (Figure S2). In addition, other groups recently reported M^{pro} crystal structures with inhibitors such as the peptidomimetic Michael acceptor **N3** (PDB: 6LU7) and the reversible inhibitor **X77** (PDB: 6W63) (Figure S2).[27] A large array of M^{pro} crystal structures with multiple covalent and noncovalent fragments were solved through an exceptionally large screen with vast opportunities for fragment growing and merging. The cryo EM structure of SARS-CoV-2 RdRp was recently solved, showing nearly an identical sequence to its SARS-CoV homologue.[28] RdRp should be another high priority target for therapeutic intervention given that lead inhibitors such as remdesivir already exist.

CONCLUSIONS

Since 2003, the three pandemics caused by SARS, MERS, and SARS-CoV-2 are believed to have initiated as a result of bat coronaviruses crossing the species barrier. Therefore, other epidemics are likely to occur in the future due to the effectively unlimited supply of coronaviruses present in the bat population. Equally concerning is the fact that RNA viruses lack a genomic proof-reading mechanism and therefore are prone to mutation, raising the specter that once a new coronavirus begins circulating in the human population it will be extremely difficult to eliminate. Finally, for a drug to be most effective, it must reach its site of action (e.g., lung tissues) in sufficient quantity to clear the virus. With nearly three million reported cases, >260,000 deaths, millions out of work, and billions of dollars in lost revenue, it appears that SARS-CoV-2 has altered daily life for a sustained period of time. This cascading effect has generated unprecedented momentum and scientific effort around the globe to fight this virus. Success will require creative thinking, new technologies, innovative approaches, particularly focused on speed to market, and combinations of different modalities to effectively combat not just the current foe but also future coronaviruses. We remain hopeful that we will be more prepared for the emergence of a potential "SARS-CoV-3".

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.0c00224.

Tables showing viral outbreaks since year 2000, different targets expressed in SARS-CoV-2 proteome, unique therapeutic interventions in recruiting and nonrecruiting clinical studies for COVID-19, and structures of select approved or investigational drugs tested for drug repurposing against SARS-CoV-2; structures of the novel inhibitors obtained from the PDB for SARS-CoV-2 M^{pro} (PDF)

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Notes

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