ACS Infectious Diseases

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Review

Recent Insights into Emerging Coronavirus: SARS-CoV-2

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Cite This: https://dx.doi.org/10.1021/acsinfecdis.0c00646

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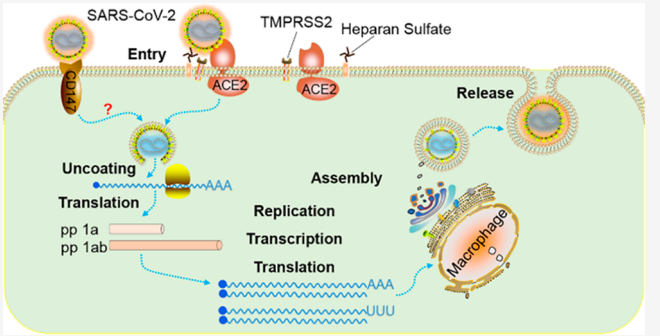
ABSTRACT: The SARS-CoV-2 outbreak that emerged at the end of 2019 has affected more than 58 million people with more than 1.38 million deaths and has had an incalculable impact on the world . Extensive prevention and treatment measures have been implemented since the pandemic. In this Review, we summarize current understanding on the source, transmission characteristics, and pathogenic mechanism of SARS-CoV-2. We also detail the recent development of diagnostic methods and potential treatment strategies of COVID-19 with focus on the ongoing clinical trials of antibodies, vaccines, and inhibitors for combating the emerging coronavirus.



KEYWORDS: *SARS-CoV-2, COVID-19, pathogenesis, diagnosis, therapy, antibody, vaccine, inhibitor, transmission, pandemic*

Tracing back to the emergence of COVID-19, 27 cases of acute respiratory pneumonia of unknown etiology were confirmed in Wuhan, China by December 2019. Common symptoms of the infection include loss of taste and smell, dry cough, dyspnea, fever, and radiological manifestations of bilateral lung infiltration. On January 7, 2020, viral strains were successfully isolated from clinical samples. Through genome-wide sequencing and phylogenetic analysis, researchers discovered that the viral genome is 89.1% similar to that of a coronavirus found in bats.[1] Wu et al. then published the genome information on the novel coronavirus immediately on virological.org and the GenBank database.[1,2] These platforms facilitate rapid information sharing, allowing scientists around the world to keep tracing the emergence of 2019-nCoV, which is now known as SARS-CoV-2. The epidemic has spread rapidly across the world with the first case reported internationally in Thailand on January 13, followed by Japan,[3] South Korea,[4] the United States,[5] European countries,[6] Australia,[7] and other countries. To date, COVID-19 (official name for the novel coronavirus infection) has affected 213 countries with the United States leading the number of confirmed cases, and most recently, Africa is facing an acceleration in the rate of infection. On March 12, COVID-19 was listed as a pandemic by the World Health Organization (WHO), the first since the H1N1 pandemic in 2009. It was the first time that a coronavirus infection has been assessed as a pandemic, surpassing the total count of the SARS outbreak in 2003.

As of November 22, the number of confirmed cases has reached >58 million, including >1.38 million deaths. It is estimated that about 250 million people around the world will be infected by SARS-CoV-2 by June 2021, with 1.75 million deaths.[8] Many countries have paid huge economic prices by taking unprecedented measures to curb the spread of the virus, such as imposing large-scale isolation and quarantine, closure of borders, restrictions on public gatherings, and nationwide blockades.[9] The pandemic has had an inestimable impact on and will lead to an inevitable decline in the global economy.[10,11] Clinical trials in other areas have been halted, as governments have stepped up efforts and adopted social quarantine measures to slow attacks on COVID-19.[12] Figure 1 depicts a series of important events and scientific discoveries since the advent of SARS-CoV-2. Herein, we summarize the latest understanding on the origin, transmission, and pathogenic mechanism of SARS-CoV-2. Current methods and progress for the diagnosis and treatment of COVID-19 are reviewed. Based on these run-throughs, we provide insights on the development in combating COVID-19.

VIRUS, RESERVOIRS, AND ITS TRANSMISSION

It was demonstrated that 2019-nCoV shares <90% homology of its conserved replicase domain with other betacoronaviruses. It was identified as a novel virus belonging to the genus Betacoronavirus, subgenus Sarbecovirus of the Coronaviridae family, and has been identified as SARS-CoV-2 (Figure 2).[13] Similar viral sequences have been isolated from animals,

Special Issue: Antiviral Therapeutics

Received: September 11, 2020

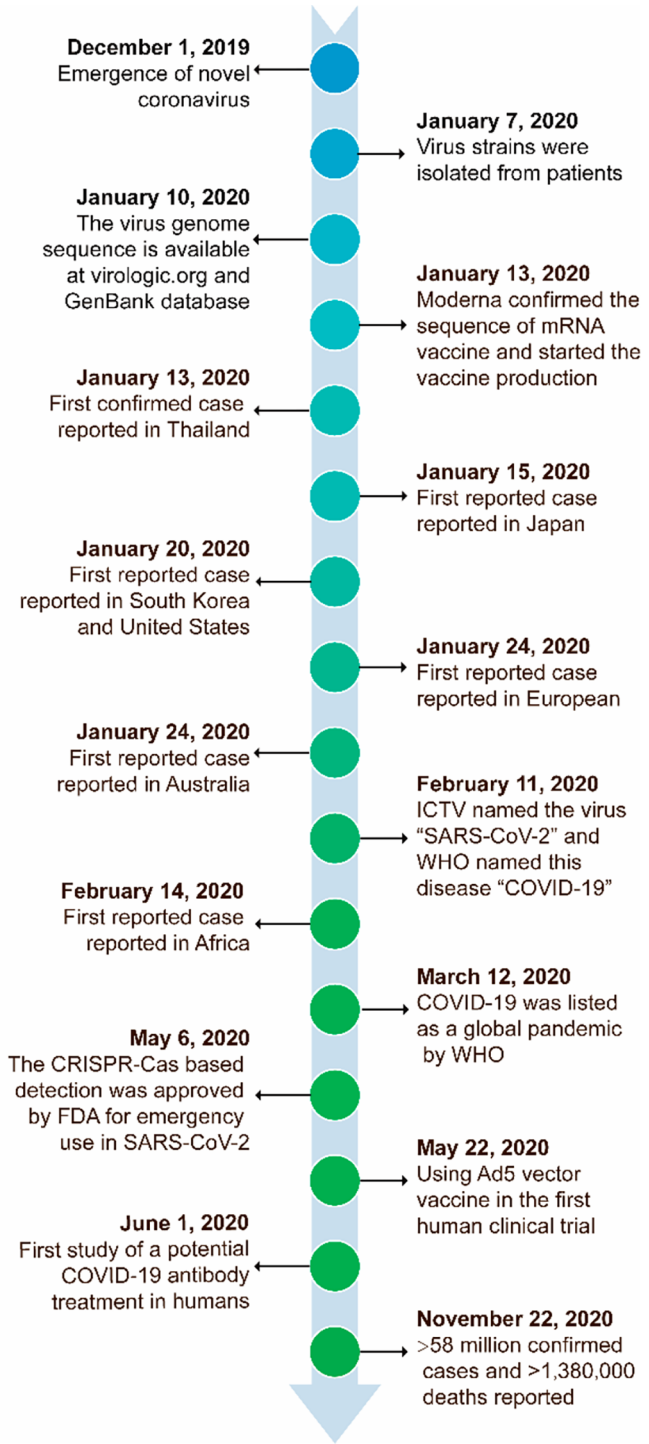


Figure 1. Key dates of the emergence of the SARS-CoV-2 pandemic and relevant important findings.

revealing the potential source of SARS-CoV-2.[14] The genomic sequences of SARS-CoV-2 isolated from patients were compared with viral libraries and were discovered to be closely related to two species of bat-derived SARS-CoV-like coronaviruses: bat-SL-CoVZC45 and bat-SL-CoVZXC21, with a similarity of 88% in genomic sequence.[15] In a genome-wide association study, SARS-CoV-2 was found to have a similarity of 79.5% in genomic sequence to the SARS coronavirus and to be 96% homologous to that of a bat-derived coronavirus, suggesting that bats are the main source of transmission of SARS-CoV-2.[16] Aside from bats, pangolins have been regarded as a possible source of SARS-CoV-2 transmission as well. Lam et al. discovered that pangolin-derived coronaviruses were closely associated with two sublineages of SARS-CoV-2-related coronaviruses through metagenomic sequencing.[17] The pangolin-derived coronavirus showed high similarity to SARS-CoV-2 in the receptor binding domain, which shares 97.4% homology in their amino acid sequences, having a higher homology compared to that between a bat-derived coronavirus and SARS-CoV-2 (89.2%). However, another study deduced that the pangolin may not be the intermediate host of the COVID-19 outbreak, although the pangolin-derived coronavirus showed a high similarity with the SARS-CoV-2 genetic sequence.[18] Noteworthily, the comparative analysis of genomic sequences between SARS-CoV-2 and MERS-CoV revealed that there were three homologous genomic regions in their ORF1ab genes that could promote recombination. Once the two viruses recombine to form a new chimeric virus, it may pose a greater threat. On March 24, 2019, the Guangdong Wildlife Rescue Center received 21 Malay pangolins from the Guangdong customs, of which 16 dead pangolins were observed to have lung swelling, containing foamy fluid, accompanied by symptoms of pulmonary fibrosis. Hepatomegaly and splenomegaly were also observed in some of the dead pangolins.[19] The viral metagenomic study speculated the deaths of these pangolins might be associated with coronaviruses.[19] Although the exact origin of SARS-CoV-2 remains unspecified, great attention is required to monitor pangolin-associated activities in reducing the risk for COVID-19.

SARS-CoV-2 is pleomorphic or generally spherical with a diameter range of 80−160 nm.[20] The virion contains a positive-sense single-stranded ribonucleic acid (RNA) genome of about 30 kb with a 5′ cap structure and a 3′ poly(A) tail (Figure 3). It generally consists of 15 open reading frames (ORFs) that encode 29 proteins. It can be divided into two parts, namely, the region encoding nonstructural proteins (ORF1a and ORF1ab) and the conserved structural protein region. The former accounts for about two-thirds of the total genome length, while the latter occupies the remainder. The ORF1a/ORF1ab are translated into polyprotein 1a (pp1a) and polyprotein 1ab (pp1ab), respectively. Polyprotein 1a (pp1a, 440−500 kDa) is cleaved into 11 nonstructural proteins (nsps) by papain-like protease (PLpro) and 3C-like protease (3CLpro, M^{pro}), while pp1ab (740−810 kDa) is translated after a ribosomal frameshift occurs in the upstream-1 position of the ORF1a stop codon and is then cleaved into 16 nsps. The conserved structural protein region of 3′ end contains 13 ORFs, encoding four main structural proteins, namely, spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein, as well as 9 hypothetical cofactors. Among these nine cofactors encoded by structural proteins, it is worth noting that ORF3b and ORF10 of SARS-CoV-2 show low homology to SARS-CoV.[21,22]

At present, COVID-19 can be identified mainly through direct transmission, aerosol transmission, and contact transmission (i.e., infections caused by droplets of sneezing, coughing, talking, and exhaled gas inhaled in close proximity (within about 6 feet); infections caused by inhaled aerosols in the air; and also infections caused by indirectly contacting contaminated droplets deposited on the surface of the objects via the mouth, nose, eyes, and other mucous membranes).[23,24] Among these routes of transmission, the first two ways via air

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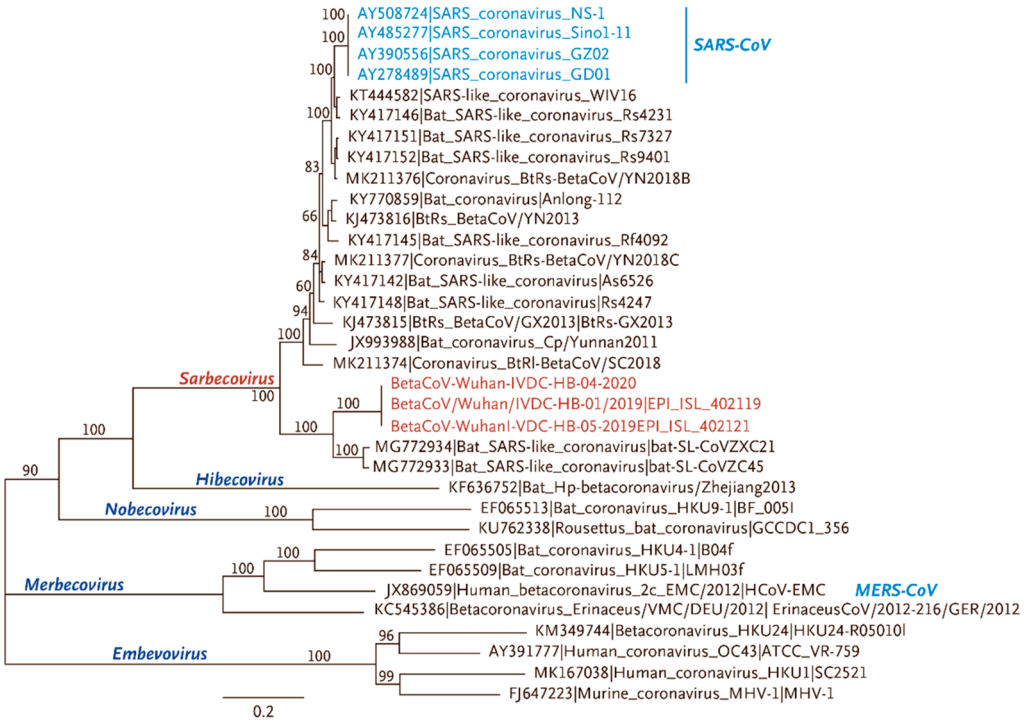

Figure 2. Full-length phylogenetic analysis of SARS-CoV-2 and other betacoronavirus genomes in the orthocoronavirinae subfamily.[13] Reproduced with permission from ref 13. Copyright 2020, Massachusetts Medical Society.

transmission are the main ways for COVID-19.[25] RT-PCR tests showed that SARS-CoV-2 genomes were detected on the surfaces of the workplace in COVID-19 quarantine centers as well as in the air particles of the intensive care units (ICUs).[26] In an outbreak area of northern Italy, the viral RNA of SARS-CoV-2 was also detected in the air particles, indicating SARS-CoV-2 could be transmitted via airborne transmission.[27] It was deduced that SARS-CoV-2 forms clusters with suspended particles in the air, reducing the diffusion coefficient while enhancing survival and persistence in the atmosphere. Speculation that cats could be the intermediate hosts of SARS-CoV-2 has been made as the virion was demonstrated to replicate effectively in cats and can be transmitted among their species through respiratory droplets.[28,29] However, there is no clear correlation if SARS-CoV-2 can be transmitted between the animals or from infected animals to humans, e.g., dogs.[30] SARS-CoV-2 was shown to have poor replicability in dogs, pigs, chickens, and ducks.[29] Aside from direct transmission, there is a concern that fetuses may be at risk of congenital COVID-19. However, there is no evidence that COVID-19 in pregnancy may lead to transplacental transmission of SARS-CoV-2 infection or serious adverse consequences in newborns.[31] It is difficult to trace the modes of transmission for SARS-CoV-2 and can only be disclosed over time and experience.

■ PATHOGENESIS OF SARS-COV-2 INFECTION

The virus must first enter the cells of host organisms and establish infection by reproducing in the cells using the host cells' resources. SARS-CoV-2 infects a host when its S protein binds to the host cell's surface receptor, angiotensin-converting enzyme 2 (ACE2) (Figure 4), which was previously known as the interferon-stimulated gene (ISG) in the epithelial cells of the barrier tissue.[32] A recent study suggested that glycosaminoglycans (GAGs) on the surfaces of host cells might interact with the S1/S2 proteolytic cleavage site (681−686 (PRRARS) and another site (453−459 (YRLFRKS)) of the SARS-CoV-2 S protein to assist viral particles' entry.[33] The binding of S protein with ACE2 was facilitated through heparan-sulfate-dependent behavior.[34] The S protein is a highly glycosylated type I membrane protein that can be digested by furin-like protease into a trimer composed of two domains: receptor binding domain (RBD) S1 and fusion domain S2.[35−37] Type II transmembrane serine protease (TMPRSS2) as well as cathepsin B and L (CatB/L) hydrolyze the S2′ site of the fusion domain S2,[38−40] triggering the dissociation of S1 and the irreversible refolding of S2, resulting in a conformational change of the S protein and subsequently the fusion of the viral envelope and endosome. Alternatively, the S protein of SARS-CoV-2 binds to the surface receptor CD147 on the host cell, which is considered to be a new way to mediate the invasion of the virus.[41] The N protein then releases the viral RNA through the process of capsid dissociation.[42,43] About two-thirds of the viral RNA is translated into two large polyproteins (pp1a and pp1ab). Hydrolysis of these polyproteins by PLpro and 3CLpro leads to the production of 16 kinds of mature nonstructural proteins (nsp1 nsp16) and the formation of a viral replicase−transcriptase complex. This complex uses positive-strand RNA as a template to produce a negative-strand RNA and a subgenome (−) RNA (through a discontinuous extension mechanism).[44] The former RNA is used as a template for generating new positive-strand RNA, and the latter is used to create subgenomic (+) mRNAs for translating structural proteins.[45] The S protein, E protein, and M protein then enter the endoplasmic reticulum, while the N protein binds to the positive-strand RNA to form a nucleoprotein complex. The assembly of virion is completed in a Golgi apparatus and ready to be released from the infected cells via a vesicle to complete its life cycle.[21]

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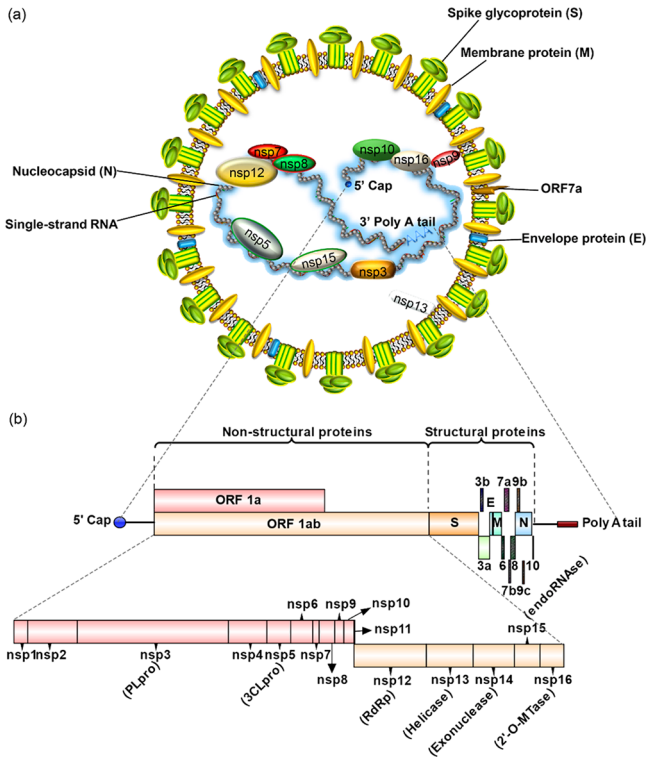

Figure 3. Structure of the SARS-CoV-2 virion and its genomic organization. (a) The general structure of the SARS-CoV-2 virion and its encoded proteins. (b) The genome organization of SARS-CoV-2 consists of 14 ORFs, including 2 ORFs (ORF1a and ORF1ab) adjacent to the 5′ cap region that encodes for nonstructural proteins required for viral replication and ORFs that encode structural proteins: spike (S) protein, membrane (M) protein, envelope (E) protein, and nucleocapsid (N) protein along with their accessory proteins.

SARS-CoV-2 infected humans by binding to ACE2 in the nasal epithelial mucosal cells and entering the lungs through respiratory tracts.[46] Type III interferon (IFN-γ) produced by dendritic cells in the lungs in response to viral pathogen-associated molecular pattern (PAMP) destroys the barrier and inhibits tissue repair and the proliferation of pulmonary epithelial cells, causing the lungs to be vulnerable to infections of deadly bacteria.[47,48] At the same time, IFN-γ can also upregulate the expression of ACE2 in respiratory epithelial cells,[32] steering a higher risk for COVID-19. SARS-CoV-2 releases IL-6 and tumor necrosis factor-α (TNF-α) through infecting type II alveolar epithelial cells and alveolar macrophages, accompanied by an increase in chemokines.[32,49] The virus further induces the recruitment of circulating mononuclear macrophages and neutrophils to aggravate the inflammation of the lungs through the production of proinflammatory factors, reactive oxygen species, and other active substances. The formation of a "cytokine storm" increases capillary permeability and causes a leakage of blood and inflammatory factors. When protein-rich tissue fluid leaks into the alveoli and interstitium, it will cause alveolar and pulmonary interstitial edema. In addition, the decrease of alveolar surfactant causes pulmonary atrophy, which leads to the decrease of oxygen diffusion capacity. The imbalance of ventilation/the blood flow ratio aggravates lung ischemia and hypoxia. It eventually leads to intractable hypoxemia and respiratory failure.[50] IL-6 and TNF-α, especially the former, are important endogenous sources of fever. In response to these factors, hepatocytes release acute phase proteins, i.e., serum amyloid protein A-1 (SAA1), serum amyloid protein A-2 (SAA2), and C-reactive protein (CRP). Other acute phase proteins released, e.g., complement 6 (C6) and complement factor B (CFB), are also involved in the complement activation pathway.[32] It was observed that COVID-19 patients have high levels of IL-1b, chemokine (C−X−C motif) ligand 10 (CXCL10), and monocyte chemoattractant protein-1 (MCP-1), which may activate the T helper cell 1 (Th1) cellular

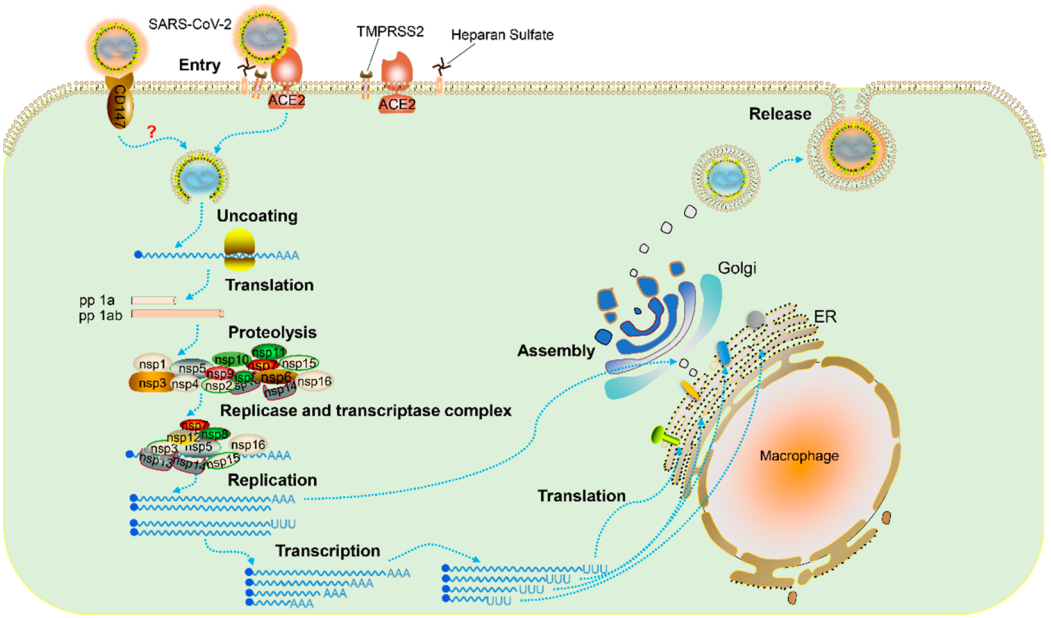

Figure 4. Life cycle of SARS-CoV-2. SARS-CoV-2 first enters the host cell by binding to the host cell receptor ACE2 or CD147 (which requires further study). Viral RNA is released into the host cell through uncoating of N protein and is then translated into a polymerase complex needed for viral replication. The viral life cycle ends with the release of an assembled virion from the host cell.

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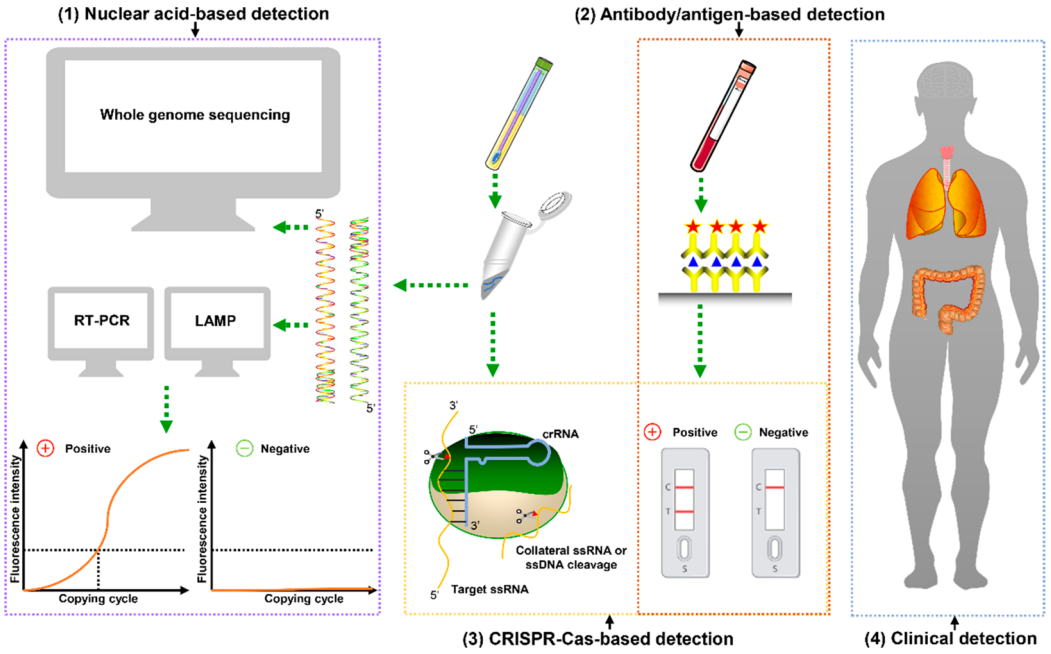

Figure 5. Types of methods for COVID-19 detection. The diagram of four main methods for COVID-19 detection. The nucleic-acid-based and CRISPR-Cas-based detections are mainly based on the amplification of viral RNA in samples and further realized by genome-wide sequencing, RT-PCR, LAMP, and CRISPR-Cas; the antibody/antigen-based detection works by screening for the presence of SARS-CoV-2-specific antibodies in blood, serum, or plasma samples. The clinical detection of COVID-19 is mainly based on specific clinical manifestations of patients to assist in the diagnosis of COVID-19.

response.[51] SARS-CoV-2 was detected in stool samples,[52] indicating it may enter the peripheral blood from the lungs and attack absorptive intestinal epithelial cells in the intestine, where the virus productively infects.[32,53] Unlike individuals with influenza viral infection, only a very small amount of interferon was detected in peripheral blood or lungs of COVID-19 patients.[54,55] The differential response of interferon induced by systemically and locally induced dendritic cells and their respective roles in the pathogenesis and severity of COVID-19 remain unclear and require further investigation.

The incubation period of SARS-CoV-2 takes about 1−14 days. It varies from person to person, depending mainly on the age and physical condition of an individual.[56] Common symptoms of COVID-19 include fever, dry cough, loss of taste and smell, and tiredness.[51] According to the reported confirmed cases, approximately 5% of COVID-19 patients were classified as acute. COVID-19 is generally diagnosed based on chest computed tomography (CT) with bilateral lung with ground-glass shadow. This symptom may eventually develop into acute respiratory distress syndrome (ARDS), which could lead to death.[57] The severity of COVID-19 depends mainly on the host factors, i.e., age and health conditions (e.g., lymphocytopenia and hypercytokinemia). Viral mutation generally does not affect clinical outcomes significantly.[58] It takes about 1−3 weeks on average to recover from COVID-19.[57]

■ DETECTION OF SARS-COV-2

Rapid, accurate, and precise SARS-CoV-2 diagnostic testing is vital in suppressing the COVID-19 pandemic. Current diagnostic testing of SARS-CoV-2 includes detecting through nucleic acids, antigens/antibodies, CRISPR-Cas and clinical symptoms (Figure 5). Table 1 compares the advantages and disadvantages of different detection methods for SARS-COV-2. Detection, sampling, and processing methods of samples are directly affecting the accuracy of diagnostic results. To date, false-positive or false-negative results of nucleic acid tests cannot be avoided completely.

Nucleic-Acid-Based Detection. In the early stage of the SARS-CoV-2 outbreak, it was necessary to use whole-genome sequencing when the SARS-CoV-2 genome sequence was unknown. By studying the viral genome isolated from COVID-19 patients, scientists can quickly recognize the evolutionary path of the virus, its mode of transmission, and the presence of mutants. St. Hilaire et al. have developed pathogen-oriented low-cost assembly and resequencing (POLAR) detection methods based on whole-genome sequencing technology.[59] POLAR has enhanced sensitivity by amplifying the entire SARS-CoV-2 genome. It detects more than 95% of SARS-CoV-2 at concentrations of 84 genome equivalents per mL, which is higher than most detection limits of the current available diagnostic methods.

RT-PCR detection is based on defining the gene sequence of SARS-CoV-2 using specific primers, which is the gold standard for the SARS-CoV-2 infection. The target nucleic acid is amplified exponentially by reverse transcription and polymerase chain reaction and subsequently verified by agarose gel electrophoresis. Hebert et al. developed a cheap, high-throughput SARS-CoV-2 detection method based on RT-PCR to analyze 10 000 samples in 1 operation at a price of $1 per sample.[60] However, the operation of RT-PCR is tedious. Aerosols produced can contaminate the amplification products while opening the lid of the instrument. The more common method for SARS-CoV-2 detection is reverse transcription quantitative real-time PCR (RT-qPCR). It can realize real-time fluorescence monitoring by adding a fluorescence reagent to the reaction system. This not only facilitates the process of reading the results but also prevents possible contamination of the sample after opening the instrument's lid. However, it

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ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 1. Comparison of Different Detection Methods for SARS-CoV-2

Detection methods	Testing material	Benefits	Challenges
Nucleic-acid-based detection	Nasopharyngeal/oropharyngeal swab, saliva, bronchial lavage fluid, bronchoalveolar lavage fluid, etc.	Good accuracy, high sensitivity, high specificity	Highly dependent on handling skills, time-consuming
Antibody/antigen-based detection	Serum (antibody); nasopharyngeal swab, saliva, other lower respiratory tract secretions, blood, feces (antigen)	Fast, easy in handling, inexpensive	Antibody-based detection (excluding antigen-based) is not suitable for preliminary diagnosis, low-throughput analysis, accuracy depending on the specificity of antibody/antigen
CRISPR-Cas-based detection	Nasal swabs, nasopharyngeal/oropharyngeal swabs, etc., and samples of bronchoalveolar lavage fluid	Easy in handling, high sensitivity, fast, good accuracy, inexpensive	The detection system needs to be further optimized (such as CRISPR-Cas enzymes to tolerate different sample conditions), achieving automation and industrialization from testing technology
Clinical detection	CT image and blood	Convenient and intuitive, can be used for preliminary diagnosis	Requires professional personnel, highly dependent on handling skills, requires access to selected equipment, can only be used as auxiliary means of diagnosis

requires RNA extraction from nasopharyngeal (NP) swabs, which depends on the supply of RNA extraction kits and is relatively cumbersome. Lately, Bruce et al. has successfully detected SARS-CoV-2 RNA from NP samples by RT-qPCR without RNA extraction with an accurate recognition rate of 92%.[61] The results of thermal inactivation or lysis of NP swab samples with an increasing number of amplification cycles were also consistent with the standard detection methods.[62,63] Digital PCR (dPCR) is a breakthrough quantitative analysis technology developed in recent years. It divides targeted nucleic acids into many separate and parallel PCR reactions to achieve PCR amplification of a single-molecule template. During amplification, chemical reagents and dye-labeled probes can be used to detect the amplification of specific sequences. The sensitivity of reverse transcription digital PCR (RT-dPCR) for SARS-CoV-2 is 3 times higher than that of RT-qPCR in a comparative analysis of 194 clinical pharyngeal swab specimens.[64] RT-dPCR is a powerful supplement to the current standard diagnosis method RT-PCR, as RT-dPCR has a better detection limit, sensitivity, and accuracy, reducing the rate of false detection.[65,66]

Loop-mediated isothermal amplification (LAMP) is a single-tube technique for amplifying DNA at a constant temperature. Compared with conventional PCR, it does not require a thermal cycler, which greatly reduces the complexity of the instrument. It is a more convenient and quicker method for SARS-CoV-2 nucleic acid detection with a detection limit of about 10^2 RNA copies per reaction (close to the detection limit of RT-qPCR).[67,68] It can detect the ORF1ab gene, E gene, and N gene, simultaneously within 30 min.[69] Compared with NP swab sampling, it was more sensitive in detecting SARS-CoV-2 in the saliva of COVID-19 patients.[70] Saliva sampling does not require any RNA extraction steps, reducing the risks of medical staff and the pain of individuals in the sampling process.[71] LAMP technology shows a good prospect in large-scale screening.

Antibody/Antigen-Based Detection. SARS-CoV-2 contains a variety of structural proteins with different antigenic epitopes. Using the principle of specific binding between an antigen and antibody, the presence of SARS-CoV-2 in the sample can be detected by an antibody. Seo et al. constructed a sensor using a graphene sheet coated with specific antibodies of SARS-CoV-2 S protein for detecting SARS-CoV-2 in clinical samples.[72] The detection limits of this sensor for SARS-CoV-2 in culture medium and clinical samples were 1.6×10^1 pfu/mL and 2.42×10^2 copies/mL, respectively. Sofia 2 SARS Antigen FIA (Quidel Corporation) was the first test for SARS-CoV-2 antigen. It uses a fluorescence-based sandwich immunoassay to detect the presence of the viral N protein. Alternatively, the detection of antibodies within blood can be used for COVID-19 diagnosis. When infected by SARS-CoV-2, humans produce specific antibodies in fighting against COVID-19 along with immune cells. By detecting these identified antibodies, we can indirectly judge if a person is infected with SARS-CoV-2 via the principle of specific binding of antigen and antibody. Antibodies for detection are mainly divided into two categories. IgM mainly occurs in the early stage of COVID-19, while IgG occurs at a later stage. At present, there are at least 23 commercial kits based on antibody/antigen available for testing. Although serological tests are rapid and require no special equipment, their usefulness may be limited in the diagnosis in the early stage of COVID-19, as it takes days to

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ACS Infect. Dis. XXXX, XXX, XXX−XXX

weeks after the onset of symptoms to develop a detectable antibody response.

CRISPR-Cas-Based Detection. The use of a qPCR detection method for COVID-19 diagnosis is inconvenient, as samples are required to be sent to the laboratory for testing, and this prolongs the time of diagnosis and treatment. Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) and DNA endonuclease-targeted CRISPR trans reporter (DETECTR) have made real-time (point-of-care) diagnosis of COVID-19 possible. SHERLOCK and DETECTR are simple, rapid, and economical detection platforms based on Cas13 and Cas12a, respectively. Both can reliably detect incredibly low concentrations of SARS-CoV-2 nucleic acids. The Cas13 and Cas12a target the adjacent ssRNA and ssDNA by cutting and degrading the genes of interest, respectively. These subsequently activate the connected report groups to generate signals on test papers. SHERLOCK technology can be completed in an hour and detects only 10^2 copies/mL of SARS-CoV-2.[73] The upgraded detection method, SHERLOCK testing in one pot (STOP), does not require purification of RNA. It can start directly from a patient's sample, and all the chemical reactions involved for detection can be completed within a test tube.[74] STOP demonstrated 100% specificity and 97% sensitivity in a controlled experiment of 12 positive and 5 negative SARS-CoV-2 samples. Broughton et al. developed a CRISPR-Cas12-based lateral flow (DETECTR) detection technique that allows rapid detection of SARS-CoV-2 RNA from a respiratory swab extract (<40 min).[75] In the clinical samples of 36 COVID-19 patients and 42 patients with other respiratory diseases, the positive and negative prediction of DETECTR were 95 and 100%, respectively, indicating a low false-positive rate.

At present, the Sherlock CRISPR SARS-CoV-2 kit has been granted FDA emergency authorization to detect SARS-CoV-2. This was the first time the FDA has authorized the use of CRISPR for infectious disease testing. These CRISPR-Cas-based detection methods described above present diagnosis results by visualizing the capillary action of analytes through a solid supporting material made of antibodies. There is also an alternative CRSPR-Cas-based detection method for consideration, Cas13-based, rugged, equitable, scalable testing (CREST). CREST uses a fluorescent LED imaging device to display data, offering great convenience for the diagnosis of COVID-19.[76] In addition, a scalable multipathogen detection platform based on CRISPR-Cas detection developed by Ackerman et al. can reliably test more than 4500 target samples on a single array, representing high-throughput CRISPR-Cas-based detection.[77] In brief, CRISPR-Cas-based detection methods are still in their introductory phase and need to be further optimized.

Clinical Detection. In the early stage of patients with SARS-CoV-2 infection, it was observed that the total number of white blood cells and lymphocytes generally decreased, while liver enzymes, lactate dehydrogenase (LDH), myoglobin and muscle enzymes, CRP, and the erythrocyte sedimentation rate increased in most patients. Chest CT scans usually showed multiple small plaques and interstitial changes. In severe patients, D-dimer increased, and peripheral blood lymphocytes decreased progressively with CT imaging of multiple ground-glass opacities and infiltration shadows in both lungs. ICU patients often have increased inflammatory factors with CT imaging of lung consolidation and rare pleural effusion. Elecsys IL-6 is an inflammatory response diagnosis kit that has gained emergency authorization from the FDA to help in diagnosing patients with SARS-CoV-2 infection if they have severe inflammatory responses. It helps doctors to decide if patients require the use of mechanical ventilation, which helps keep patients alive but does not promote recovery. Mei et al. used an artificial intelligence (AI) algorithm to combine chest CT scans with clinical symptoms, exposure history, and laboratory tests to quickly diagnose patients infected with SARS-CoV-2.[78] This AI model demonstrated better sensitivity than senior radiologists in an analysis of 279 patients. The AI system performed better for diagnosing infected patients who tested positive for RT-PCR but showed normal CT scans. Radiologists, on the other hand, identified all patients as negative SARS-CoV-2 infected cases. This case study has shown the potential value of AI systems in diagnosing SARS-CoV-2 infected patients.

POTENTIAL THERAPEUTIC STRATEGIES

In view of the rapidly spreading and relatively high mortality of COVID-19, it is urgent to fill in the gap of effective and specific treatment of SARS-CoV-2.[57] At present, there are >6000 clinical trials ongoing in searching for potential therapeutic agents for treating COVID-19.

Antibodies. Neutralizing antibodies have great potential in curbing COVID-19, as they are highly specific. In general, antibodies prevent viruses from entering host cells by selectively binding to the surface epitopes of the viral particles. Recombinant neutralizing antibodies isolated from COVID-19 patients are the most direct and fastest possible intervention for the treatment or prevention of COVID-19.[79] It was demonstrated that recovered COVID-19 patients generally possess immunoglobulin G (IgG) antibodies.[80] Ju et al. obtained 206 specific antibodies against SARS-CoV-2 S protein RBD from the plasma of 8 patients by single-B-cell sequencing.[81] All of these antibodies had strong affinity and neutralizing activity to RBD of SARS-CoV-2. There was no cross-recognition to RBD of S protein of SARS-CoV or MERS-CoV. Two of the antibodies, P2C-1F11 and P2B-2F6, can competitively bind to ACE2 and block its fusion with S protein RBD. Crystal structure analysis showed that the binding targets of P2C-1F11 and P2C-1C10 were different, suggesting that the two antibodies may act synergistically if combined. Using the same technique, Shi et al. isolated 11 SARS-CoV-2 neutralizing monoclonal antibodies (mAbs) from peripheral blood mononuclear cells of convalescent patients.[82] Two of the monoclonal antibodies, CA1 and CB6, specifically bound to the SARS-CoV-2 S protein on the surfaces of transfected HEK293T cells and showed good neutralizing activity. The therapeutic and preventive effects of CA1 and CB6 antibodies were tested in rhesus monkeys infected with SARS-CoV-2 and showed promising results in reducing viral load within 4 days. In terms of the prevention effect, administration of the CB6 managed to keep the viral load at its lowest number ($<10^3$ RNA copies/mL) after the monkeys were infected with SARS-CoV-2. At present, the company Junshi Bio has developed an injectable monoclonal antibody (JS016), which is now in a Phase I clinical trial approved by the State Drug Administration of China. Other reported monoclonal antibodies including B38 and H4 also showed neutralization ability to SARS-CoV-2 *in vitro* and effectively blocked the binding of S protein RBD to cellular receptor ACE2.[83] Cao et al. screened 8558 viral protein binding antibody sequences from 60 convalescent patients and successfully identified 14 highly

https://dx.doi.org/10.1021/acsinfecdis.0c00646
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 2. Selected Trials of Monoclonal Antibody/Convalescent Plasma for COVID-19

Types	Sponsor/Country	Description	Phase study	Clinical trial number
Monoclonal antibody	Xijing Hospital/China	Preliminary efficacy of tocilizumab treatment in the patients with COVID-19	Phase IV	ChiCTR2000033705
	Junshi Biosciences/China	JS016: human monoclonal antibody that targets the SARS-CoV-2 S protein	Phase II	NCT04441918
	Vir Biotechnology, Inc./United States	VIR-7831 and VIR-7832: modified antibodies isolated from a patient who recovered from SARS	Phase II/III	NCT04545060
	Eli Lilly/United States	LY3819253 (LY-CoV555): a specific monoclonal antibody against the SARS-CoV-2 S protein	Phase III	NCT04501978
	Tychan Pte Ltd./Singapore	TY027: a SARS-COV-2 specific monoclonal antibody	Phase I	NCT04429529
	Regeneron/United States	REGN-CoV2: a cocktail of the human antibodies REGN10933 and REGN10987	Phase III	NCT04425629
	AstraZeneca, Parexel/United Kingdom	AZD7442: a combination of two mAbs (AZD8895 & AZD1061) against the SARS-CoV-2 S protein	Phase I	NCT04507256
	Sorrento Therapeutics, Inc./United States	STI-1499: a monoclonal antibody which targets the COVID-19 S protein	Phase I	NCT04454398
	Mabwell (Shanghai) Bioscience Co., Ltd./China	MW33: a recombinant fully human antibody to coronavirus	Phase I	NCT04533048
	Stanford University/United States	Anti-SARS-CoV-2 IgY: an anti-SARS-CoV-2 chicken egg antibody	Phase I	NCT04567810
	HiFiBiO Therapeutics/United States	HFB30132A: a SARS-CoV-2 neutralizing antibody engineered with specific sequences	Phase I	NCT04590430
	Celltrion/South Korea	CT-P59: an anti-SARS-CoV-2 monoclonal antibody	Phase II/III	NCT04602000
Convalescent plasma	DRK-Bluspendedienst Baden-Württemberg-Hessen gGmbH/Germany	A randomized, prospective, open label clinical trial on the use of convalescent plasma compared to best supportive care in patients with severe COVID-19	Phase II	2020-001310-38
	Fundació Clínic per a la recerca Biomèdica/Spain	Plasma turnover in patients with COVID-19 disease and invasive mechanical ventilation: a randomized study	Phase II	2020-001722-66
	Ruprecht-Karls-Universität Heidelberg/Germany	A Randomized Open Label Phase-II Clinical Trial with or without Infusion of Plasma from Subjects after Convalescence of SARS-CoV-2 Infection in High-Risk Patients with Confirmed Severe SARS-CoV-2 Disease	Phase II	2020-001632-10
	Institute of Blood Transfusion, Chinese Academy of Medical Sciences/China	Convalescent plasma for the treatment of severe novel coronavirus pneumonia (COVID-19): a prospective randomized controlled trial	N/A	ChiCTR2000029757
	Renmin Hospital of Wuhan University/China	A randomized, double-blind, parallel-controlled trial to evaluate the efficacy and safety of anti-SARS-CoV-2 virus-inactivated plasma in the treatment of severe novel coronavirus pneumonia (COVID-19)	N/A	ChiCTR2000030929
	Joakim Dillner, Karolinska University Hospital/Sweden	Convalescent Plasma as Treatment for Acute Coronavirus Disease (COVID-19)	Phase I/II	NCT04390178
	Gailen D. Marshall Jr., MD PhD/United States	COVID-19 Convalescent Plasma (CCP) Transfusion	Phase I	NCT04412486
	Direction Centrale du Service de Santé des Armées/France	Efficacy of Convalescent Plasma Therapy in the Early Care of COVID-19 Patients	Phase III	NCT04372979
	University of Pennsylvania/United States	COVID-19 Convalescent Plasma for the Treatment of Hospitalized Patients with Pneumonia Caused by SARS-CoV-2	Phase I	NCT04397757
	University of Sao Paulo General Hospital/Brazil	Treatment of Patients With COVID-19 With Convalescent Plasma	Phase II	NCT04415086
	Federal Research Clinical Center of Federal Medical & Biological Agency/Russia	Hyperimmune Convalescent Plasma in Moderate and Severe COVID-19 Disease	Phase II	NCT04392414
	Biofarma/Indonesia	Convalescent Plasma Therapy in Patients With COVID-19	Phase I	NCT04407208
	University of Oxford/United Kingdom	A randomized trial of treatments to prevent death in patients hospitalised with COVID-19 (coronavirus)	Phase II/III	ISRCTN50189673

[a]ARDS, acute respiratory distress syndrome; CP, convalescent plasma; IgM and IgG, immunoglobulin M and G; IVIG, intravenous immunoglobulin; SOC, standard of care; N/A, not available. Sources: https://apps.who.int/trialsearch/.

https://dx.doi.org/10.1021/acsinfecdis.0c00646
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 3. Overview of Vaccine Production Platforms and Technologies for SARS-CoV-2

Vaccine name/clinical trial number		Organizations	Description	Phase Study
Inactivated vaccines	ChiCTR2000031809	Wuhan Institute of Biological Products	Propagating the virus in the cell and then using chemical reagents to inactivate the virus	Phase I/II/III
	CoronaVac	Sinovac Biotech Co.	Propagating the virus in the cell and then using chemical reagents to inactivate the virus	Phase I/II/III
	BBIBP-CorV	Beijing Institute of Biological Products	Propagating the virus in the cell and then using chemical reagents to inactivate the virus	Phase I/II/III
	V-SARS	Immunitor LLC	Heat-inactivated plasma of donors with COVID-19	Phase I/II
	BBV152A, B, C	Bharat Biotech International Limited, Indian Council of Medical Research	Vaccines consisting of whole, inactive SARS-CoV-2 virus particles	Phase I/II
	NCT04412538	Chinese Academy of Medical Sciences	Propagating the virus in the cell and then using chemical reagents to inactivate the virus	Phase I/II
Live attenuated vaccines	BCG vaccine	Multiple organizations	A live attenuated vaccine consisting of the bacteria that causes bovine tuberculosis	Phase III/IV
	MMR vaccine	Kasr El Aini Hospital	Live-attenuated strains of the viruses caused by these	Phase III/IV
	Oral poliovirus vaccine	Multiple organizations	Consisting of live attenuated polioviruses of the three serotypes	Phase III/IV
	CDX-CoV	Codagenix/Serum Institute of India	Deoptimized live attenuated vaccines	Preclinical
Recombinant protein vaccines	AV-COVID-19	Aivita Biomedical, Inc.	Individual DC loaded with antigens from the SARS-CoV-2 coronavirus to prevent COVID-19	Phase I/II
	NVX-CoV2373	Novavax	An intramuscularly delivered nanoparticle vaccine created by infecting Sf9 insect cells with baculoviruses, viral vectors that express the SARS-CoV-2 S protein	Phase I/II/III
	KBP-COVID-19	Kentucky BioProcessing, Inc.	Generating SARS-CoV-2 antigens by transferred virus into the tobacco plants	Phase I/II
	SCB-2019	Clover Biopharmaceuticals	A recombinant subunit vaccine candidate for COVID-19	Phase I
	MVC-COV1901	Medigen Vaccine Biologics Corp.	Vaccine consisting of the SARS-CoV-2 S protein with adjuvants of CpG 1018 and aluminum	Phase I
	COVAX-19	GeneCure Biotechnologies	A protein subunit vaccine that combines recombinant SARS-CoV-2 S protein with Advax adjuvant	Phase I
	PittCoVacc	University of Pittsburgh Medical Center	Microneedle array delivered SARS-CoV-2 subunit vaccines	Preclinical
	DPX-COVID-19	IMV Inc.	Peptide epitopes from the SARS-CoV-2 S proteins	Preclinical
Viral-vector-based vaccines	AZD1222 (ChAdOx1 nCoV-19)	University of Oxford	Attenuated adenovirus that displays the SARS-CoV-2 S protein on its surface	Phase III
	Ad26.COV2.S	Janssen Vaccines & Prevention B.V.	An adenovirus serotype-26-vector-based vaccines expressing the SARS-CoV-2 S protein	Phase III
	Gam-COVID-Vac	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation	An adenoviral-based vaccine that displays the SARS-CoV-2 S protein on its surface	Phase I/II/III
	TMV-083	Institut Pasteur	Live attenuated recombinant measle vaccine virus vector expressing a modified glycoprotein of SARS-CoV-2	Phase I
	V591	Merck Sharp & Dohme Corp.	A SARS-CoV-2 vaccine candidate using an attenuated measles virus as a vector	Phase I
	Ad5-nCoV	CanSino Biologics Inc.	Incorporating a full-length SARS-CoV-2 S protein into a replication-defective adenovirus type 5 vector	Phase I/II/III
	LV-SMENP-DC	Shenzhen Geno-Immune Medical Institute	Modifying DC with lentivirus vectors expressing COVID-19 minigene SMENP and immune-modulatory genes	Phase I/II
	Covid-19/aAPC Vaccine	Shenzhen Geno-Immune Medical Institute	The aAPC transformed with lentivirus vector to present SARS-CoV-2 antigen	Phase I
	AdCOVID	Altimmune Inc.	Single-dose, intranasal vaccine based on RD-Ad5 vector technology	Preclinical
	T-COVIDTM	Altimmune Inc.	An investigational intranasal immune modulator vaccine base on RD-Ad5 vector technology	Preclinical
Nucleic acid vaccine	mRNA-1273	Moderna	Lipid nanoparticle dispersion containing an mRNA that encodes for the prefusion stabilized S protein of SARS-CoV-2	Phase I/II
	AG0301-COVID19	AnGes, Inc.	A DNA vaccine encoding antigens from SARS-CoV-2	Phase I/II
	ZyCoV-D	Zydus Cadila, Cadila Healthcare Limited	A DNA-plasmid-based vaccine against SARS-CoV-2	Phase I/II
	GX-19	Genexine, Inc.	A DNA vaccine expressing SARS-CoV-2 S protein antigen	Phase I/II
	BNT162	Biontech RNA Pharmaceuticals GmbH, Pfizer	Four individual lipid nanoparticle-encapsulated mRNA vaccines (2 modRNA, 1 uRNA, and 1 saRNA)	Phase I/II/III
	INO-4800	Inovio Pharmaceuticals	A double-stranded DNA plasmid that encodes antigens found in SARS-CoV-2	Phase I/II
	bacTRL-Spike	Symvivo Corporation	The live bacterium *Bifidobacterium longum* containing DNA plasmids encoding SARS-CoV-2 S protein	Phase I
	LUNAR-COV19	Arcturus Therapeutics, Inc.	Self-replicating mRNA vaccine that is devoid of any viral material or coadjuvants	Phase I/II
	CVnCoV	CureVac AG	Optimized mRNA vaccine	Phase I
	ChiCTR2000034112	Yunnan Walvax Biotechnology Co., Ltd.	mRNA-based vaccines	Phase I

https://dx.doi.org/10.1021/acsinfecdis.0c00646
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 3. continued

[a]BCG, bacille Calmette-Guerin; MMR, measles-mumps-rubella; DC, dendritic cells; S, spike; Advax, a polysaccharide adjuvant derived from delta inulin; aAPC, artificial antigen presenting cells; RD-Ad5, replication-deficient adenovirus 5; modRNA, modified mRNA; uRNA, uridine containing mRNA; saRNA, self-amplifying mRNA. Sources: ClinicalTrials.gov, chictr.org.cn, clinicaltrialsregister.eu, and ctri.nic.in.

active neutralizing antibodies.[84] Among them, the most active BD-368-2 antibody was neutralized by pseudovirus and native virus, and the half-maximal inhibitory concentrations (IC50) were 1.2 and 15 ng/mL, respectively. BD-368-2 showed effectively preventive and therapeutic effects in hACE2 transgenic mice. When BD-368-2 was injected 24 h before SARS-CoV-2 infection, the viral infection in mice was completely inhibited, and the preventive effect was demonstrated by decreasing the viral load by ∼2400 times.

It was demonstrated that convalescent serum from horses and SARS-CoV patients can cross-neutralize COVID-19.[16,39,85] Pinto et al. identified antibody S309 from individuals infected with SARS-CoV.[86] The antibody can effectively neutralize SARS pseudovirus and SARS-CoV-2. Vir Biotechnology has cooperated with GlaxoSmithKline in modifying the S309 antibody and developing two new antibodies VIR-7831 and VIR-7832, which both have potential therapeutic effects on COVID-19 with an extended half-life and are now in the preclinical trial stage. Alternatively, Wang et al. identified antibody 47D11 as able to neutralize SARS2-S pseudotype VSV by targeting S1B (residues 338-506) RBD of SARS-CoV-2.[87] Humanized anti-CD147 monoclonal antibody Meplazumab (maprozumab) can also effectively treat COVID-19 patients safely,[88] as demonstrated by clinical trials.

Plasma therapy has previously been used to treat SARS-CoV and Ebola.[89,90] Clinical trials in China have shown that convalescent plasma therapy effectively treated COVID-19 in high-risk patients.[91] As of November 22, 2020, there have been more than 200 projects of plasma therapy for clinical trial research (Table 2), based on data from the International Clinical Trials Registry Platform (ICTRP).

The use of antibodies is promising in combating the COVID-19 pandemic. However, mutation of the SARS-CoV-2 surface antigen is one of the concerns if antibodies are used as a strategy to combat COVID-19 pandemics. It was demonstrated that antibody cocktail therapy is effective against the S protein mutation of SARS-CoV-2 in humans.[92] More importantly, it should be noted that the antibodies induced by a primary infection can bridge the secondary infected viral strain with the Fc receptor in the constant region of IgG antibodies on immune cells, thus increasing the probability of the virus to enter immune cells and cause more serious disease recurrence.[93] This effect of antibody-dependent enhancement (ADE) should be carefully detected by monoclonal antibodies and convalescent sera in the treatment of COVID-19. Therefore, it is necessary to take immediate action to monitor the Fc-binding domain of mutant monoclonal antibodies for maintaining the neutralization potential of the antibody and also preventing the uptake of immune cells at the same time. The design of Fc-optimized antibodies[94] and nanoantibodies[95−98] for the treatment and prevention of therapeutic antibodies against COVID-19 represents a new effective approach in fighting against the virus.

Vaccines. There are currently no data available on the protective duration of immunity against SARS-CoV-2. The protective duration of immunity against SARS-CoV in recovered individuals was shown to be last up to 10 years, suggesting the potential importance of research and development of vaccines for the novel coronavirus.[99] Developing vaccines for SARS-CoV-2 can protect humanity from the persistent threat of COVID-19.[100] Research in genomic and structural biology has provided a strong basis for the development of vaccines.[101] Vaccines can be developed in various forms: an inactivated vaccine, a live attenuated vaccine, a recombinant protein vaccine, a viral vector vaccine, and a nucleic acid vaccine (mRNA/DNA vaccine). More than 170 vaccine candidates for SARS-CoV-2 are under development across the world, of which at least 50 of them are being assessed in clinical trials at present (Table 3). An ideal vaccine should show consistent immune responses at all stages of clinical trials. Nucleic acid (DNA and RNA) vaccines have the greatest potential in the speed of research and development, because they can be produced synthetically and do not need to be cultured or fermented.[102] Nucleic acid vaccines, e.g., mRNA-1273, BNT162INO-4800, bacTRL-Spike, LUNAR-COV19, AG0301-COVID19, ZyCoV-D, GX-19, and CVnCoV, are studied in clinical trials. To date, there is no approved nucleotide vaccine for SARS-CoV-2 by any regulatory agencies in the US or EU. In particular, ShaCoVacc (BD131), an mRNA vaccine based on a virus-like particle (VLP) delivery system, demonstrated great potential in combating COVID-19.[103] Most recently, Zhang et al. developed a candidate vaccine, which utilized mRNA encapsulated by lipid nanoparticles encoding SARS-CoV-2 RBD, to induce strong anti-SARS-CoV-2 neutralizing antibodies and Th1-biased cellular responses in mice and nonhuman primates. When the mice were inoculated with two doses of ARCoV, it could completely protect the mice from the attack of SARS-CoV-2 mouse adaptive strain.[104]

Focusing on the epitopes associated with strong neutralization activity produced by a B cell response is conducive in the development of vaccines with long-term protective effects. This strategy of developing a recombinant subunit vaccine can minimize the production of non-neutralizing or weakly neutralizing antibodies in the immune body, thus weakening ADE.[105] An extensively used approach is to design antigenic epitopes based on the three-dimensional structure of S proteins and effective neutralizing antibodies at the molecular level. As the antibody binds to the RBD of S protein and the RBD immunogen can induce antibodies with higher affinity than the naive S protein, the vaccine sequence is usually designed to select an appropriate sequence of RBD-related regions rather than the whole S protein.[106] More recently, the constructed MERS-CoV dimerized RBD antigen successfully induced a high concentration of neutralizing antibody in a mouse model to protect mice from MERS-CoV infection.[107] This design strategy provides a new idea for the research and development of SARS-CoV-2 vaccines. Current ongoing clinical trials on recombinant subunit vaccines for SARS-CoV-2 include NVX-CoV2373, SCB-2019, COVAX-19, PittCoVacc, KBP-COVID-19, MVC-COV1901, and DPX-COVID-19. Viral vector vaccines are formed by inserting selected gene fragments encoding antigens into highly safe viral vectors. When the recombinant viral vector vaccine is injected into the body, the

https://dx.doi.org/10.1021/acsinfecdis.0c00646
ACS Infect. Dis. XXXX, XXX, XXX−XXX

specific antigens are produced, and cellular and humoral immune responses are induced. There are replicable and nonreplicable types of viral vectors. Current ongoing clinical trials on viral vectors for SARS-CoV-2 vaccines include AZD1222 (formerly known as ChAdOx1nCoV-19), Ad5-nCoV, LV-SMENP-DC, Covid-19/aAPC Vaccine, AdCOVID, Ad26.COV2.S, Gam-COVID-Vac, TMV-083 V591, and T-COVIDTM. Inactivated vaccine is the utilization of chemically treated, proliferated live viral strains that lose the ability to replicate but retain the ability of inducing humoral immune responses. This method is risky if the "inactivation" process is not handled properly, as the virus may reinfect the host. CoronaVac, BBIBP-CorV, V-SARS, and inactivated vaccines from the Wuhan Institute of Biological Products and Beijing Institute of Biological Products have been developed on the basis of this technology and are currently being assessed in clinical studies. In addition, repurposed attenuated vaccines, such as bacille Calmette-Guerinhave (BCG) and measles-mumps-rubella (MMR) vaccines, have shown protective effects against respiratory infections.[108,109] The antigen of an attenuated vaccine has low pathogenicity. However, it should be noted that attenuated vaccines still have the ability to replicate. At present, there are more than 100 institutions conducting related clinical trials on attenuated vaccines. CDX-CoV, developed by Codagenix and the Serum Institute of India, represents one of the potential candidates of attenuated vaccines for COVID-19.

Geographical diversity often requires more than one effective vaccine approach. Cooperation between the government, academia, and industry in leveraging their respective strengths is important for developing effective vaccines against SARS-CoV-2.

Inhibitors. Research and development of drugs for viral infections are generally divided into two categories: pathogen-directed and host-directed inhibitors. Although clinical trials of more than 250 drugs are ongoing, no specific treatment for COVID-19 has been approved. Figure 6 shows the development of COVID-19 inhibitors in clinical investigations (dated November 22th). In Table 4, selected inhibitors for clinical evaluation of COVID-19 are listed.

Pathogen-Directed Inhibitor. Developing antivirals for SARS-CoV-2 from scratch could take years. The repurposing of the existing FDA-approved drugs is the most feasible way to fight COVID-19. Viral RNA/DNA polymerase plays a key role in the viral replication cycle.[110] Remdesivir, developed by Gilead Sciences, is a nucleotide analogue with broad-spectrum antiviral activity that inhibits RNA-dependent RNA polymerase. It showed good *in vitro* and *in vivo* performance against coronaviruses that cause Middle East respiratory syndrome (MERS) and SARS. It has been granted "Emergency Use Authorization" and has been officially approved as the first treatment for COVID-19 by the FDA in the US. When clinicians injected the drug intravenously into severe patients with COVID-19 in the hospital, it shortened the recovery time to an average of 11 days, compared with placebo treatment that required 15 days.[111] Favipiravir, an oral antiviral drug developed by the Japanese Toyoda Akio Chemical Company, is mainly used to treat influenza by selectively inhibiting the RNA polymerase necessary for viral replication. The drug used the same mechanism to combat SARS-CoV-2.[112] It is currently assessed in more than 26 clinical trials ranging from phase II to IV, showing positive effects in shortening the treatment time and improving the lung condition of COVID-19 patients.[113]

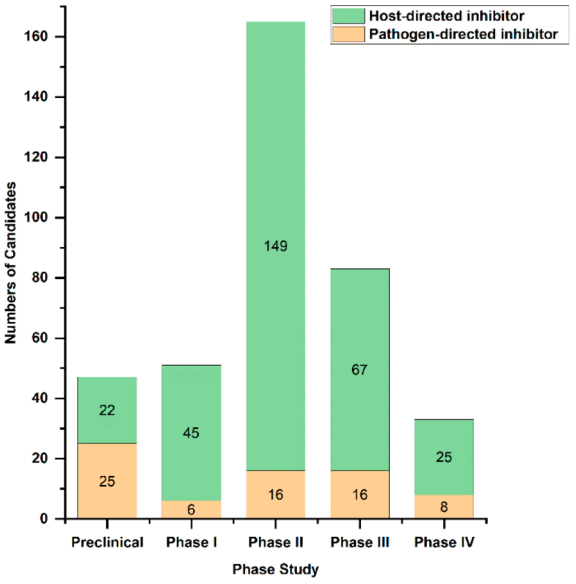

Figure 6. Clinical pipeline for COVID-19 candidate drugs. Data sources: ClinicalTrials.gov; clinicaltrialsregister.eu; www.chictr.org.cn.

However, the Italian Pharmaceutical Agency has noted that it is too early for the available evidence to support this drug for COVID-19 treatment.[114] Danoprevir is an effective inhibitor of HCV protease (NS3/4A). The structure of 3CLpro of SARS-CoV-2 is similar to that of HCV and HIV protease. A clinical study on 11 danoprevir-treated COVID-19 patients showed that the respiratory symptoms were significantly improved, and pulmonary imaging showed that acute exudative lesions recovered significantly. It was proven safe and well-tolerated in all patients, representing a potential drug for effective COVID-19 treatment.[115] Other RNA polymerase inhibitors, such as ribavirin (NCT04605588), molnupiravir (NCT04575597), and clevudine (NCT04347915), have also been assessed with clinical studies for combating COVID-19. Proteases of SARS-CoV-2 have also become important research targets for the development of COVID-19 inhibitors. Lopinavir−ritonavir is a combination of HIV protease inhibitors that slow down AIDS by inhibiting HIV replication or growth. The combination has been used to treat HIV infections in adults and children for 14 days or more. Lopinavir alone showed inhibitory activity on SARS-CoV *in vitro*.[116] Administration of lopinavir−ritonavir for COVID-19 treatment was found to be beneficial to some secondary end points, but no clear clinical benefits were observed.[117] However, clinical trials of 127 patients showed that combination therapy of interferon-β-1b, lopinavir−ritonavir, and ribavirin was superior to lopinavir−ritonavir alone in relieving symptoms as well as shortening virus shedding time and hospitalization times for COVID-19 patients with mild/moderate symptoms.[118] The safety and efficacy of HIV protease inhibitors (e.g., darunavir/cobicistat and ASC09/ritonavir) for COVID-19 treatment are still under evaluation. The anticancer drug selinexor blocks nuclear and cytoplasmic transport, isolates key viral accessory proteins and genomic materials in the host nucleus, and reduces viral replication and immune pathogenicity. It is potentially useful in COVID-19 treatment. A clinical study on selinexor in the treatment of COVID-19 infection is still ongoing.[119]

Dai et al. designed and synthesized two peptides 11a and 11b based on the three-dimensional structure of coronavirus

https://dx.doi.org/10.1021/acsinfecdis.0c00646
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 4. Some Clinically Evaluated Inhibitors for the Treatment of COVID-19

Inhibitors		Organizations	Description	Phase Study	Clinical Trial Number
Pathogen-directed inhibitor	Remdesivir	Gilead	Viral RdRp inhibitor	Phase III (FDA approved)	NCT04280705
	Favipiravir	Promomed, LLC, Shahid Beheshti University of Medical Sciences		Phase III/IV	NCT04542694, NCT04359615
	Danoprevir	Ascletis Pharmaceuticals Co., Ltd.	Inhibitor of the hepatitis C NS3/4A protease	Phase IV	NCT04291729
	Ribavirin	Bausch Health Americas, Inc., SynaVir	Adenosine and guanosine analogue	Phase I/II/III	NCT04605588
	Molnupiravir	Merck Sharp & Dohme Corp.	Nucleoside analogue	Phase II/III	NCT04575597
	Clevudine	Bukwang Pharmaceutical	Viral protease inhibitor	Phase II	NCT04347915
	Lopinavir	Beijing YouAn Hospital, Tongji Hospital		Phase I/II/III/IV	NCT04286503, NCT04255017
	Ritonavir	Darrell Tan, Ascletis Pharmaceuticals Co., Ltd.		Phase III/IV	NCT04321174, NCT04345276
	Darunavir	Shanghai Public Health Clinical Center		Phase III	NCT04252274
	ASC09	Tongji Hospital		Phase III	NCT04261270,
	Selinexor	Karyopharm Therapeutics, Peter MacCallum Cancer Centre	An inhibitor of chromosome region maintenance 1	Phase II/III	NCT04355676, NCT04534725
Host-directed inhibitor	APN01	Apeiron Biologics	A competitive ACE2 inhibitor	Phase II	NCT04335136
	Nafamostat	Latus Therapeutics	A serine protease inhibitor	Phase II/III	NCT04473053
	Sarilumab	Sanofi/Regeneron, MJM Bonten	IL-6/IL-6R inhibitor	Phase III/IV	NCT04327388, NCT02735707
	Siltuximab	University Hospital, Ghent,		Phase III	NCT04330638
	Tocilizumab	Queen's Medical Centre, Hadassah Medical Organization		Phase III/IV	NCT04412772, NCT04377750
	Anakinra	Fundacion Miguel Servet, MJM Bonten	IL- 1 blocker	Phase II/III/IV	NCT04443881, NCT02735707
	Canakinumab	Novartis		Phase II/III	NCT04510493
	Infliximab	Tufts Medical Center, Daniel Benjamin	TNF-α inhibitor	Phase II/III	NCT04425538, NCT04593940
	Adalimumab	Shanghai Changzheng Hospital		Phase IV	ChiCTR2000030089
	Emapalumab	Swedish Orphan Biovitrum	IFN-γ inhibitor	Phase II	NCT04324021
	Baricitinib	Eli Lilly, Cambridge University Hospitals NHS Foundation Trust	JAK inhibitor	Phase III/IV	NCT04421027, NCT04390464
	Ruxolitinib	Novartis, Incyte Corporation		Phase III	NCT04334044, NCT04362137
	AMY-101	Amyndas Pharmaceuticals S.A.	Inhibitor of complement C3	Phase II	NCT04395456
	Dexamethasone	Chattogram General Hospital, ClinAmygate	A steroid drug	Phase III/IV	NCT04499313, NCT04530409
	Nitric oxide	Massachusetts General Hospital, Bellerophon Pulse Technologies	An endogenous biomolecule	Phase II/III	NCT04305457, NCT04421508

[a]RdRp: RNA-dependent RNA polymerase; ACE2: angiotensin-converting enzyme 2; IL-6/IL-6R: interleukin-6/interleukin 6 receptor; IL-1: interleukin-1; TNF: tumor necrosis factor; JAK: Janus kinase.

main protease.[120] The two compounds exhibited excellent antiviral activity *in vitro* against SARS-CoV-2 M^{pro} with IC50 values of approximately 0.053 and 0.040 μM, respectively. At the same time, the antiviral activity test results showed that the compounds 11a and 11b had good antiviral activity with half-maximal effective concentration (EC50) values of 0.53 and 0.72 μM, respectively. More importantly, these two compounds show good pharmacokinetic properties and safety profiles *in vivo* and have great potential to be developed into new anti-SARS-CoV-2 drugs.

Host-Directed Inhibitor. Developing host-directed inhibitors is also an extensive research strategy for mitigating or treating diseases. Host-directed inhibitors block targeted key regulatory factors in the host immune response system during viral infection. Compared with drugs targeting dozens of proteins encoded by SARS-CoV-2, there are more drugs that have been developed to target proteins in host cells. Also, host proteins are highly conserved in evolutionary pathways, and drugs targeting them are less likely to develop drug resistance than antiviral drugs that target viruses directly.[121] APN01 is one of the host-directed inhibitors that competitively inhibit the binding of virus to endogenous ACE2 on the cell membrane.[121] It was proven that APN01 endows anti-inflammatory properties against ARDS. It is being evaluated in a clinical trial (NCT04335136) for COVID-19 treatment. Nafamostat mesylate can effectively block the fusion of MERS-CoV with host cells by targeting binding to TMPRSS2.[122] It effectively inhibited the fusion of SARS-CoV-2 with human lung epithelial cell-derived Calu-3 cells, and its EC50 value was about 10 nM,[123] showing good potential for COVID-19 treatment. At present, clinical trials on nafamostat (such as NCT04473053) are being evaluated. Chloroquine/hydroxychloroquine (CQ/HCQ) belong to the quinolone family with similarity in clinical indications and retinal toxicity. They can accumulate in the lysosome and increase pH to block the activity of lysosomal enzymes, thus inhibiting the fusion of virus endocytosis in lysosomes. CQ showed the ability to inhibit the proliferation of SARS-CoV-2 *in vitro*.[124] A clinical study (NCT04328272) suggested that COVID-19 patients treated with CQ/HCQ showed improvement in clinical

https://dx.doi.org/10.1021/acsinfecdis.0c00646
ACS Infect. Dis. XXXX, XXX, XXX−XXX

symptoms, while others showed that the administration of HCQ has no beneficial clinical benefits.[125−127] Another clinical study demonstrated administration with CQ shortens the median time for obtaining undetectable viral RNA, and no serious adverse reactions were observed.[128] However, the death rate of the administration with HCQ (25.7%) was barely higher than that of standard treatment (23.5%).[125] There is no evidence that HCQ is effective as a preventive drug for COVID-19 infection. In view of these conflicting findings of CQ/HCQ treatment, the US FDA has agreed to withdraw the authorization for emergency use of this drug.

Cytokine storm is a severe immune overreaction, which can lead to respiratory damage and life-threatening respiratory complications in COVID-19 patients. Blocking cytokine storms such as interleukin-6 (IL-6), IL-1, TNF-α, and IFN-γ help to fight against COVID-19 patients. There are >60 and >25 clinical trials of specific monoclonal antibodies that target IL-6/IL-6R (e.g., sarilumab, siltuximab, and tocilizumab) and IL- 1 blockers (e.g., anakinra[129] and canakinumab[130]), respectively. TNF-α inhibitors (e.g., infliximab, adalimumab, and etanercept) have been proven to reduce inflammation in diseases such as rheumatoid arthritis and inflammatory bowel disease. A clinical study demonstrated that the TNF-α inhibitor showed comparable efficacy in the treatment of COVID-19 patients with that of a pre-existing condition of inflammatory bowel disease patients.[131] The efficacy and safety on reducing inflammation and respiratory distress using the IFN-γ inhibitor emapalumab were evaluated in clinical trial (NCT04324021) for COVID-19 treatment.[132] Janus kinase (JAK) inhibitors may reduce the ability of infected host cells to produce more viruses and cytokine storms by inhibiting JAK1 and JAK2. There are more than 30 registered randomized controlled trials evaluating the efficacy of JAK inhibitors baricitinib and ruxolitinib for COVID-19 treatment (e.g., NCT04421027, NCT04390464, NCT04334044, NCT04362137).[132]

The complement system plays important roles via an innate immune response against viral infection. Activation of complement C3 had been shown to aggravate SARS-CoV-related ARDS.[133] In C3-deficient mice infected with SARS-CoV, infiltration of neutrophils and inflammatory monocytes in the lungs decreased significantly, suggesting that inhibition of C3 helps in alleviating lung injury. AMY-101 (peptide inhibitor of central complement C3) had been shown to interfere with the releasing of IL-6 in a whole blood infection model. It is currently being assessed in clinical trials for COVID-19 treatment (NCT04395456).[134] IL-17 gene-deficient mice and mice receiving the anti-IL-17 antibody showed an increased survival rate of acute lung injury with reduced lung infiltration and an increased lung pathology score after a lipopolysaccharide (LPS) challenge.[135] In contrast, the administration of exogenous IL-17 intensifies the production of TNF, IL-1β, IL-6, and CXCL2 induced by LPS, revealing the role of IL-17 as a key upstream regulator of the inflammatory pathway. It may be a potential therapeutic target for COVID-19 treatment.

The immune system plays a vital role in combating COVID-19.[136] Dexamethasone is a steroid drug that suppresses the immune system and reduces inflammation. A clinical trial showed that the mortality rate of severe patients (28%) was lower with treatment with dexamethasone compared to those that received conventional treatment (41%).[137] However, for patients with mild symptoms, the therapeutic effect of the drug is not significant.

Nitric oxide is an endogenous biomolecule produced by vascular endothelial cells and nerve tissue cells. Its main functions include regulating blood pressure, phagocytizing foreign toxic substances, and inhibiting platelet thrombosis. During the establishment of inflammation, emphysema, or cystic fibrosis, blood vessels and capillaries carrying oxygen will contract. Inhaling nitric oxide helps dilate the contracted blood vessels and improve oxygen transport capacity. There are more than 15 clinical trials ongoing assessing the safety and efficacy of nitric oxide, and it seems that nitric oxide is effective in improving blood oxygen saturation.[138]

Gordon et al. studied the interaction between SARS-CoV-2 protein and human protein by affinity-purification mass spectrometry and found that the proteins involved in mRNA translation and sigma receptors in the endoplasmic reticulum can be used as promising drug targets for COVID-19.[22] Two inhibitors that hinder the translation of mRNA have been found to have strong antiviral effects and have been studied in clinical trials for COVID-19 treatment: zotatifin and plitidepsin. Zotatifin and plitidepsin were originally used in advanced solid tumors and myeloma treatments, respectively. There are a variety of drugs that were demonstrated to effectively inhibit SARS-CoV-2 *in vitro* via hindering sigma receptors in the endoplasmic reticulum, i.e., antihistamines (cloperastine and clemastine), antipsychotics (haloperidol and melperone), antimalarials (hydroxychloroquine), hormones (progesterone), and antianxiety drugs (siramesine). Bojkova et al. used proteomic methods to systematically analyze the effects of cellular pathways (translation, splicing, carbon metabolism and nucleic acid metabolism) involved in SARS-CoV-2 infection.[139] Experimental data suggested that some small molecular inhibitors can be used to target these pathways for COVID-19 treatment, i.e., translation inhibitors (cycloheximide and amitine), a splice inhibitor (pladienolide B), a hexokinase inhibitor (2-deoxy-D-glucose), and inosine monophosphate dehydrogenase (ribavirin). The aforementioned small molecules can inhibit viral replication at nontoxic concentrations *in vitro*. All of the inhibitors mentioned show promising potential for COVID-19 treatment, but more research is required to ensure the safety and efficacy before they can be widely utilized.

Other Therapies. Other potential treatments such as traditional Chinese medicine and cell-based therapy have been utilized in combating COVID-19, and further details can be found in a review published by Luo et al.[140] These treatments have also alleviated pulmonary lesions caused by SARS-CoV-2. Remarkably, Case13d, a recently discovered RNA-directed RNA endonuclease derived from *Ruminococcus flavefaciens* XPD3002, demonstrated target RNA degradation with the help of CRISPR RNAs (CrRNAs).[141] Abbott et al. adopted PAC-MAN (prophylactic antiviral CRISPR in human cells) based on a CRISPR-Cas13d system to degrade viral sequences in many highly conserved regions of the SARS-CoV-2 genome and specifically cut SARS-CoV-2 sequences.[142] It had been proven to effectively degrade RNA of live influenza A virus (IAV) in human lung epithelia l cells. This strategy shows promise for inhibiting SARS-CoV-2. mRNA allows rapid translation of required proteins and undergoes self-degradation *in vivo*. Blanchard et al. transcribed mRNA *in vitro* to express Cas13a, which can effectively degrade IAV RNA in infected

https://dx.doi.org/10.1021/acsinfecdis.0c00646
ACS Infect. Dis. XXXX, XXX, XXX−XXX

mice and reduce SARS-CoV-2 replication *in vitro*, paving the way for COVID-19 treatment.

CONCLUSIONS AND FUTURE PERSPECTIVES

SARS-CoV-2 is a new betacoronavirus causing pandemics all over the world. It is vital to understand the origin and transmission characteristics of SARS-CoV-2 and diagnostic methods and effective treatment strategies of COVID-19 to halt the pandemic. Previous epidemics have taught us that stigmatizing the spread of the virus only puts people at greater risk and reduces access to care.[143] Airborne transmission is the main route of COVID-19. Social distancing has proved effective in halting the epidemic, as demonstrated in China and many other countries that later followed the path.[10] It is very likely that the epidemic will rebound if a movement control order is loosened to avoid an economic downturn. Despite the fact that the number of reported new infections has stabilized in some countries, we need to keep close surveillance of this viral infection. The asymptomatic characteristic of the infection has promoted the spread of COVID-19 rapidly.[144] Nucleic-acid-based detection remains the most important detection method at present, but there are still drawbacks associated with the current methods. More efforts need to be made in acquiring detection methods that save time with better portability while maintaining good sensitivity, accuracy, and precision.

Development of new drugs or treatments for COVID-19 takes time and money. In dealing with the emergency, known clinical drugs have been assessed and repurposed to treat COVID-19. There are a lot of clinical trials being conducted worldwide in assessing drugs and/or treatments for COVID-19. Research and development based on antibodies, vaccines, and inhibitors is advancing at an unprecedented rate. With at least 600 potential treatments for COVID-19 and thousands of clinical trials being conducted worldwide, standardized approaches should be established and followed in clinical evaluation of drugs or treatments instead of cutting corners and resorting to risky and unproven solutions. ADE is one of the biggest concerns for COVID-19. Mutation of a viral surface antigen, such as the D614G mutation of SARS-CoV-2 S protein,[145] is one of the potential targets in developing antibodies and vaccines for COVID-19. Drugs targeting host intracellular proteins may be less specific and cause cytotoxic side effects. It may even suppress the host's immune system and hinder the body's ability to manage viral infection. In addition, the lungs of COVID-19 patients are vulnerable to recurrent infection of deadly bacteria. During COVID-19 treatment, suboptimal or inappropriate use of antimicrobials may lead to long-term transmission of antimicrobial resistance in an acute nursing environment. It is important for physicians to prescribe antibiotics at the right dose and for the correct time frame. Specific inhibitors targeting the three-dimensional structure of SARS-CoV-2 M^{pro} have been developed and demonstrated good antiviral activity with good safety profile.[120]

Undoubtedly, tremendous progress has been made in discovering the origin, transmission, pathogenesis, diagnosis, and treatment of the SARS-CoV-2 infection. However, more research is required to continue our fight with SARS-CoV-2, and cooperation between governments, academia, and industries are crucial to make this happen. In facing more and more of these outbreaks (e.g., SARS, MERS, Zika, Ebola), should human beings reflect on their relationship with nature and recognize that health mainly depends on the continuous stability and operation of the biosphere life support system?

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the National Key R&D Program of China (2018YFC1105402), the National Natural Science Foundation of China (21875189 and 61935017), the National Science Basic Research Plan in Shaanxi Province of China (2020JQ-148, 2020GXLH-Z-013, and 2019KJXX-064), and the Fundamental Research Funds for the Central Universities.

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