The Innovation

Review

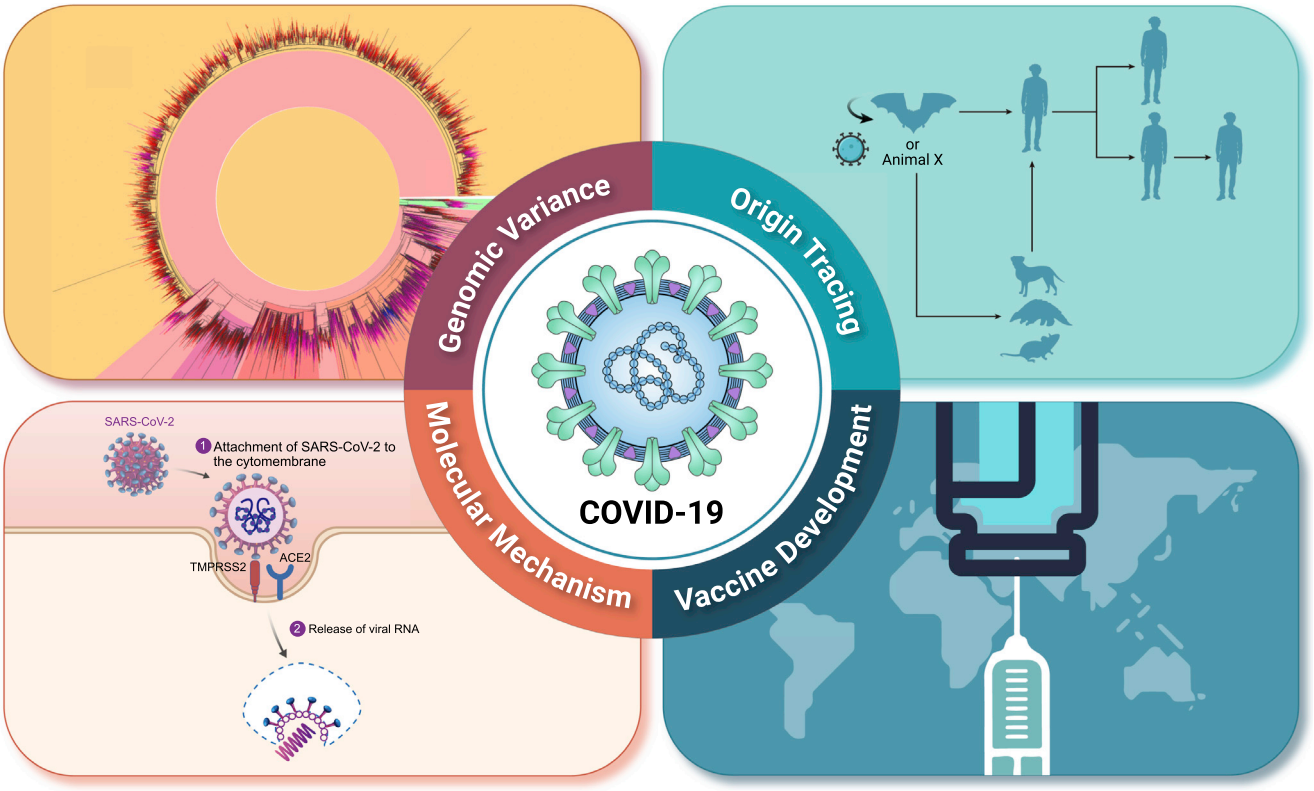
Genomic variation, origin tracing, and vaccine development of SARS-CoV-2: A systematic review

Tianbao Li,[1,2,3,8] Tao Huang,[4,8] Cheng Guo,[5] Ailan Wang,[2,3] Xiaoli Shi,[2,3] Xiaofei Mo,[2,3] Qingqing Lu,[2,3] Jing Sun,[6] Tingting Hui,[2] Geng Tian,[2,3] Leyi Wang,[7,*] and Jialiang Yang[1,2,3,*]

*Correspondence: leyiwang@illinois.edu (L.W.); yangjl@geneis.cn (J.Y.)

Received: October 31, 2020; Accepted: April 30, 2021; Published Online: May 10, 2021; https://doi.org/10.1016/j.xinn.2021.100116

Graphical abstract



Public summary

- Clinical manifestations and epidemiology of COVID-19
- The efficacy of the developed vaccine against SARS-CoV-2
- Phylogenetic tree of evolutionary relationships in current SARS-CoV-2 strains
- Structural analysis and origin tracking of SARS-CoV-2
- Mechanism involved in infection and immunological pathogenesis of SARS-CoV-2

Review

Genomic variation, origin tracing, and vaccine development of SARS-CoV-2: A systematic review

Tianbao Li,[1,2,3,8] Tao Huang,[4,8] Cheng Guo,[5] Ailan Wang,[2,3] Xiaoli Shi,[2,3] Xiaofei Mo,[2,3] Qingqing Lu,[2,3] Jing Sun,[6] Tingting Hui,[2] Geng Tian,[2,3] Leyi Wang,[7,*] and Jialiang Yang[1,2,3,*]

[1]Genetic Testing Center, Academician Workstation, Changsha Medical University, Changsha 410219, China

[2]Geneis (Beijing) Co., Ltd, Beijing 100102, China

[3]Qingdao Geneis Institute of Big Data Mining and Precision Medicine, Qingdao 266000, China

[4]Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, Shanghai 200031, China

[5]Center for Infection and Immunity, School of Public Health, Columbia University, New York, NY 10032, USA

[6]Department of Pathology, The George Washington University School of Medicine and Health Sciences, Washington, DC 20052, USA

[7]Veterinary Diagnostic Laboratory and Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL 61802, USA

[8]These authors contributed equally

*Correspondence: leyiwang@illinois.edu (L.W.); yangjl@geneis.cn (J.Y.)

Received: October 31, 2020; Accepted: April 30, 2021; Published Online: May 10, 2021; https://doi.org/10.1016/j.xinn.2021.100116

Citation: Li T., Huang T., Guo C., et al., (2021). Genomic variation, origin tracing, and vaccine development of SARS-CoV-2: A systematic review. The Innovation **2(2)**, 100116.

COVID-19 has spread globally to over 200 countries with more than 40 million confirmed cases and one million deaths as of November 1, 2020. The SARS-CoV-2 virus, leading to COVID-19, shows extremely high rates of infectivity and replication, and can result in pneumonia, acute respiratory distress, or even mortality. SARS-CoV-2 has been found to continue to rapidly evolve, with several genomic variants emerging in different regions throughout the world. In addition, despite intensive study of the spike protein, its origin, and molecular mechanisms in mediating host invasion are still only partially resolved. Finally, the repertoire of drugs for COVID-19 treatment is still limited, with several candidates still under clinical trial and no effective therapeutic yet reported. Although vaccines based on either DNA/mRNA or protein have been deployed, their efficacy against emerging variants requires ongoing study, with multivalent vaccines supplanting the first-generation vaccines due to their low efficacy against new strains. Here, we provide a systematic review of studies on the epidemiology, immunological pathogenesis, molecular mechanisms, and structural biology, as well as approaches for drug or vaccine development for SARS-CoV-2.

Key words: COVID-19; SARS-CoV-2; origin tracing; infection mechanism; SARS-CoV-2 vaccine

INTRODUCTION

Since early 2020, a novel highly infectious disease, coronavirus disease 2019 (COVID-19), emerged as a worldwide epidemic.[1,2] COVID-19 was declared to be a "pandemic" by WHO on March 11, 2020, and more than 40 million cases were confirmed and one million deaths recorded across more than 200 countries by November 1, 2020.[3,4] COVID-19 is a pulmonary disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the *Coronaviridae* family.[5,6] Coronaviruses contain a single-stranded RNA genome ranging from 25 to 32 kilobases and are generally categorized into four major genera: alpha-, beta-, gamma-, and delta-coronavirus.[7,8] SARS coronavirus caused an outbreak in 2002, resulting in more than 8,000 infections and 774 deaths across 37 countries.[9,10] Another coronavirus discovered in Saudi Arabia, MERS (Middle East respiratory syndrome), is reportedly responsible for 2,494 confirmed cases and 858 deaths since September 2012.[11] Several coronaviruses that cause mild to moderate symptoms in humans have not gained worldwide attention, whereas the HKU2 coronavirus, a newly reported mammalian coronavirus, was found to be responsible for fetal acute diarrhea syndrome in pigs in 2017.[12]

Coronaviruses have also been identified in several other mammalian hosts, such as birds, bats, civets, and mice, among other species.[13,14] Similarly, SARS-CoV-2 has raised major concerns globally, with clinical features ranging from mild or moderate upper respiratory symptoms to severe cases involving respiratory, gastrointestinal, hepatic, renal, and neurological system failure.[15–17] In the worst case scenarios, SARS-CoV-2 infects the lower respiratory tract, and can quickly develop into acute respiratory distress syndrome (ARDS), which requires mechanical oxygen support. The basic reproductive rate (R_0) is an epidemiological metric to estimate the extent of epidemic transmission without control measures, as well as to evaluate the efficiency of reducing the transmission of disease by human intervention. Initial evaluation of trends of COVID-19 transmission showed that R_0 is close to 2.5, which appeared similar to the spread of SARS-CoV in 2003, which had an R_0 of 2.90 (range 2.3–3.7). However, the basic reproduction number of SARS-CoV-2 dropped markedly to lower than 1 after implementing strict physical distancing and preventive hygiene measures.[18–20] Globally, the R_0 of SARS-CoV-2 ranges between 2.5 and 7.1 when in the absence of human intervention (Figure 1A).

To date, no effective methods have been found that can prevent the spread of the epidemic, apart from emphasizing the need for extended social distancing, molecular/antibody testing for infections, and contact tracing to identify and isolate infected patients.[5,21] In addition, antibody testing can be applied to identify individuals with previous SARS-CoV-2 infection, and those former patients with sufficient convalescent plasma may opt to donate blood to benefit the therapy of current patients. At the community level, antibody surveillance programs can be implemented to locally monitor public serological data to better inform public health policy, while increased molecular testing can be used to evaluate the efficacy of vaccination efforts.[22,23] Given these current strategies for epidemic control, development of affordable and accurate non-invasive nucleic acid and antibody tests is therefore urgently needed by community health services.

In this study, we conducted a systematic review focusing on genomic variation, origin tracking, epidemiological characteristics, transmission mode, and differences between virus strains, as well as pathological mechanisms, potential treatments, and recent advances in vaccine development.

Review

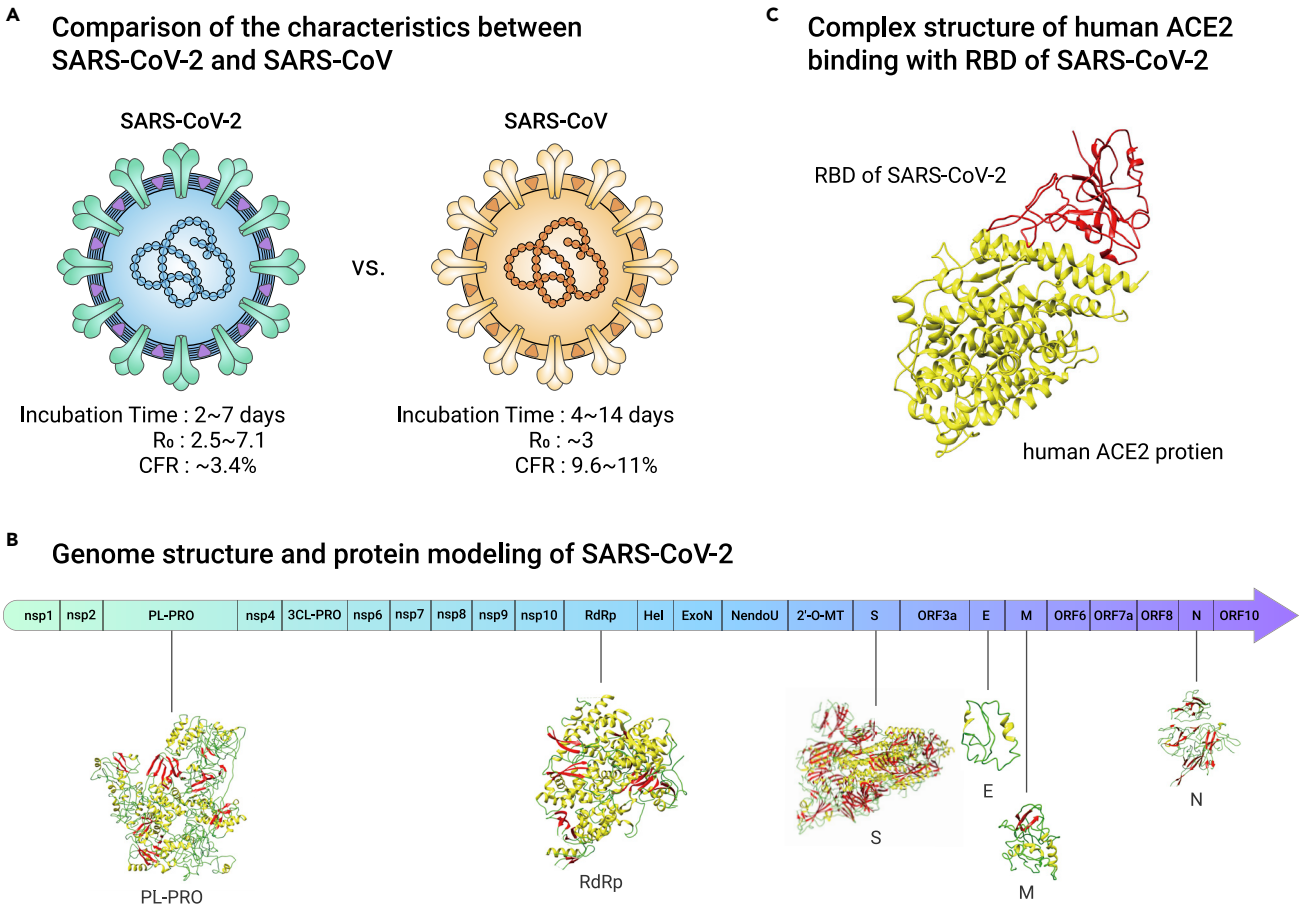


Figure 1. A summary of the epidemiology, molecular docking, genetic evolution, and genome structure of SARS-CoV-2 (A) Comparison of the characteristics between SARS-CoV-2 and SARS-CoV; (B) genome structure and protein modeling of SARS-CoV-2; and (C) complex structure of human ACE2 binding with RBD of SARS-CoV-2.

Clinical manifestations and epidemiology

COVID-19 was declared to be a "pandemic" by WHO on March 11, 2020, and more than 40 million cases were confirmed and one million deaths recorded across more than 200 countries by November 1, 2020. Similar to other coronaviruses, SARS-CoV-2 primarily spreads through respiratory droplets; although, this coronavirus was also potentially capable of transmission through the fecal-oral route. Recent reports have focused attention on shifts in the distribution of cases to an increasingly lower median age of occurrence, although the underlying reasons may be due to social practices rather than biological changes in infectivity (i.e., ignoring potential virus transmission conditions, relaxed social distancing, and less wearing of masks).[24,25] Multiple cluster cases have been reported as schools re-opened, especially in university dormitories, or at large-scale social events. Moreover, although the fecal-oral transmission route has not been verified, another study detected active virus in stools from SARS-CoV-2-positive patients, suggesting that the virus was able to invade through the gastrointestinal tract.[26,27]

Based on data from the Center for Disease Control and Prevention, the median age of library-confirmed cases (mostly through positive results of RT-PCR) is 51, 51% of whom are male.[28] Several studies have shown that the elderly and men have a relatively high susceptibility to SARS-CoV-2 infection, which aligned with previous studies that also reported similar patterns of slightly higher MERS-CoV and SARS-CoV infections among males than females.[29] Case fatality ratio (CFR) is an important factor for evaluating the severity of COVID-19.[30] Figure 1A presents comparative features between the CFRs of SARS-CoV-2 and SARS-CoV by the end of January 2020. These data show that the current international fatality ratio of SARS-CoV-2 is 0.7%, considerably lower than the previously reported CFR of 3.8%.

Global reports indicate that the clinical symptoms of SARS-CoV-2 patients are variable and relatively non-specific, with approximately 50% of infected patients appearing asymptomatic, exhibiting no obvious symptoms. Moreover, the early diagnostic window is short and may result in misdiagnosis since it cannot be easily differentiated from a common cold based purely on symptoms. In addition to respiratory symptoms, some patients report digestive symptoms, such as loss of appetite, stomach discomfort or nausea, and vomiting.[6] The viral load reaches its peak in the respiratory tract and it remains unclear if virus persistence in the gut is a driver of aggressive damage during COVID-19 progression.[31] Since the viral load increases relatively linearly, even after the active phase, viral RNA are still detectable after a patient has died.[32] A persistent infectious environment can potentially lead to dendritic cell damage or incomplete maturation and thereby impair T cell activation, even with a sufficient viral load to activate immune response. This process consequently results in a chronic infective status, and several studies have reported poor outcomes of SARS-CoV-2 infection due to chronic inflammation in patients.[33,34]

Some COVID-19 cases may progress quickly from a dry cough to ARDS or even to multi-organ failure, which then requires extracorporeal membrane oxygenation support. Laboratory examinations show several abnormalities associated with COVID-19: slightly increased blood cell counts, lymphopenia, increased C-reactive protein in most patients, as well as erythrocyte sedimentation.[35,36] Recent studies have shown increased lactose dehydrogenase in severely affected COVID-19 patients. Most infected patients with mild symptoms typically have a good prognosis with an approximately 14-day hospital stay. However, elderly patients or patients with an underlying disease frequently have worse prognoses and require an oxygen supply. Some severe cases even need intensive care unit treatment and the fatality rate may reach

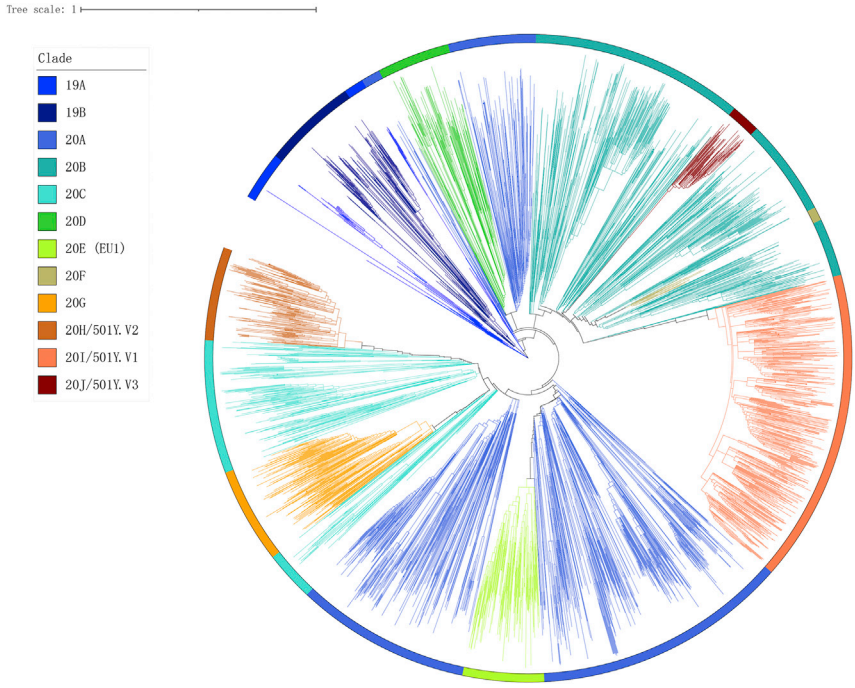


Figure 2. The phylogenetic history of the SARS-CoV-2 strains based on 3,991 genomes Colors in the legend represent each particular strain of SARS-CoV-2. The genomes of 3,991 SARS-CoV-2 strains were clustered into 12 classes, each of which showed similar genomic variation pattern.

17% among those patients.[37,38] One retrospective study revealed that a decrease in eosinophil counts was significantly associated with poor prognosis in patients.[39,40] To avoid the spread of COVID-19, clinical professionals and policymakers generally pay attention to pathogenesis and the overall infection process to establish effective measures for control of the disease. Previous studies have identified common patterns in chest CT imaging results, even before nucleic acid testing showed positive results.[41] The results from Santamarina et al. illustrated that over 95% of COVID-19 patients present bilateral lung opacities during CT scan, while lobular and sub-segmental areas of consolidation were unique features compared with that of the common cold or other types of pneumonias.[42] Romero et al. and Kamani et al. examined chest CTs of infected patients in different cohorts and found multiple regions of ground-glass opacities presenting with consolidation.[43,44] Moreover, evaluation by thoracic radiology has been considered an informative factor for discriminating between patients with or without COVID-19 infection. From a public health perspective, the rapid isolation of infected patients is crucial to minimize disease spread. Moreover, other CT features, including lymphadenopathy, pleural effusions, pulmonary nodules, or even white lung, can indicate patients with respiratory failure.[45,46]

The genomic variation of SARS-CoV-2 and origin tracking of COVID-19

SARS-CoV-2 is a beta-coronavirus that belongs to the same family as the highly pathogenic SARS-CoV and MERS-CoV, and contains the largest genome of all known RNA viruses.[47,48] Both SARS-CoV and MERS-CoV were first documented in bats or dromedary camels and then later transmitted to humans. However, possible intermediate hosts of the newly identified SARS-CoV-2 virus remain unknown.[49] Coronaviruses can infect humans primarily due to the unique structure of their spike protein and variable numbers of open reading frames (ORFs). Sequence analysis has shown that the SARS-CoV-2 genome could be divided into several ORFs, including ORF1a, ORF1b, ORF3a, ORF6, ORF7a, ORF7b, and ORF8, as well as the spike structural protein (S) viral envelope (E), membrane protein (M), and nucleoprotein (N) protein coding regions (Figure 1B). ORF1a/ORF1b cover approximately 67% of the SARS-CoV-2 genome. The RNA genome is wrapped by the N protein, which thus forms a coiled tubular structure. This helical nucleocapsid is surrounded by the viral E protein, which is associated with other structural proteins, such as the M and S proteins. Previous studies have reported that surface glycoproteins on the SARS-CoV spike protein play an important role in binding to the host receptor via their receptor-binding domains (RBDs).[50] The SARS-CoV infection process is reportedly mediated by a receptor for an angiotensin-converting enzyme (ACE2), whereas MERS-CoV primarily utilizes a dipeptidylpeptidase 4 receptor.[51,52] Alignment of the whole genomes of these 3 coronaviruses indicated a high degree of conservation in ORF1a and ORF1b, with only 5 nucleotides out of a total of 29,800 differing among them. SARS-CoV-2 is relatively similar to SARS-CoV, with some notable changes in amino acid sequence that current studies are aiming at characterize for their influence on the functionality or pathogenesis of SARS-CoV-2.

The phylogenetic history of the SARS-CoV-2 was reconstructed using a maximum likelihood approach[53] by Nextstrain[54] based on genomes of 3,991 strains[55], and the tree was shown and manipulated in itol.[56] The phylogenetic tree showed 12 different classes of genomic variations, including types 19A, 19B, 20A, 20B, 20C, 20D, 20E (EU1), 20F, 20G, 20H/501Y.V2, 20I/501Y.V1, and 20J/501Y.V3 (Figure 2). The major shared and other mutations within the spike protein of different strains are shown in Table 1. The clustered cases were shown to be relatively related to the geographical location and formed a mosaic pattern of phylogenetic placement in those countries. Types 19A and 20C are primarily found in the United States and Europe, 20E is the major type of SARS-Cov-2 strain in South America, type 20B is mainly present in Asia, while type 20H is now the major type in Africa. Among these, type A has been proposed as the ancestral variant of these three types of SARS-CoV-2.[57,58] Although the intermediate host in SARS-CoV-2 transmission to humans still remains unclear, several studies have suggested a warm-blooded vertebrate likely served as an intermediate host. The coronavirus genome displays an inherently high recombination frequency, as well as high mutation rates, which together facilitate their transmission among different species.[59] Korber et al. found that a mutated variant has arisen as the most prevalent strain in the worldwide epidemic.[60] This SARS-CoV-2 variant has a single amino acid conversion at residue D614G, resulting from a single, non-synonymous A-to-G nucleotide switch from that of the first reported SARS-CoV-2 reference genome. The proportion of newly infected patients infected by this SARS-CoV-2 variant has rapidly increased. The cryoelectron microscopy (cryo-EM) structure of the spike protein illustrated that

Table 1. Amino acid mutations emerged in spike proteins of six SARS-CoV-2 strains

	20A	20B	20C	20H	20I	20J
Clade	**B.1.525**	**P.2**	**B.1.526**	**B.1.351**	**B.1.1.7**	**P.1**
Shared mutations	S: D614G	S: D614G	S: D614G	S: D614G	S: D614G	S: D614G
	S: E484K	S: E484K	S: E484K	S: E484K	S: E484K	S: E484K
				S: N501Y		S: N501Y
				S: L18F		S: L18F
				S: K417N		S: K417N
			S: A701V	S: A701V		
		S: V1176F				S: V1176F
Other mutations	S: H69-		S: L5F	S: D80A	S: A570D	S: T20N
	S: V70-		S: T95I	S:D215J	S: P681H	S: P26S
	S: Y144-		S: D253G	S: L241-	S: T716I	S: D138Y
	S: Q52R			S: L242-	S: S982A	S: R190S
	S: A67V			S: A243-	S: D1118H	S: H655Y
	S: Q677H					S: T1027I
	S: F888L					

the D614 residue offers a hydrogen bond to link S1 and S2 subunits. Also, the mutational residue G614 could remove the hydrogen bond and increase main-chain flexibility. Residue 614 of the spike protein is close to the TMPRSS2 cleavage site. The titers in infectious virus experiments indicate that the entry of D614 into 293T cells was enhanced by TMPRSS2. However, G614 does not rely on TMPRSS2 to enhance 293T cell invasion. Fortunately, G614 was just increasing the viral load in patients and there is no evidence that this mutational residue will cause an increase in patient mortality.

Previous studies showed that SARS-CoV-2 is probably descended from a SARS-like bat CoV, with spike and accessory proteins showing lower similarity to MERS-CoV.[61] Moreover, SARS-CoV-2 is a novel beta-coronavirus, and its genome sequence shares only 79.0% and 51.8% identity with those of SARS-CoV and MERS, respectively, whereas its identity with the SARS-like coronavirus, RaTG13, originating in bats, reached 96% identity.[11] Bats can serve as a major reservoir for multiple zoonotic pathogens because of its living environment, thermoregulation, and self-elimination of many toxins and waste products. Thus, bats are more likely to be the reservoir of SARS-CoV-2 than other candidate species, although the chances of direct interactions between humans and bats is relatively low. Due to the low probability of direct species jumping due to infrequent encounters, many studies have investigated possible intermediate hosts between the reservoir (bat) and final host (human), with civet cats proposed as a strong candidate for the intermediate host.[62,63] A longitudinal study revealed the coexistence of highly diverse SARS-CoV strains in a single, specific cave containing almost all of the genetically different virus strains. One characteristic specific to coronaviruses is their high frequency of RNA recombination, which provoked us to speculate that newly emerged SARS-CoVs may arise through recombination between SARS-CoV strains in bats from other caves. Recent studies conducted by the Guo group indicated that the virus in the intermediate host was likely closer to snow mink coronavirus than bat coronavirus,[64] while another study reported that snakes most likely served as intermediate infection hosts.[65–67]

Infection mechanism and immunological pathogenesis of COVID-19

The spike (S) glycoprotein was reported as a key factor in facilitating SARS-CoV-2 to enter host cells (Figure 3). The S protein, a component of the membrane comprised of the S1 and S2 subunits, gives coronaviruses their characteristic "crown" appearance. The RBD and N-terminal domain (NTD) are found in the S1 subunit, while the S2 subunit contains the fusion peptide and heptad repeat regions, responsible for membrane fusion between virus and host cells.[68,69] Furthermore, a furan cleavage site for TMPRSS2 was reported at the boundary between S1 and S2, resulting in S protein cleavage during the progression of viral infection. This TMPRSS2 proteolytic cleavage site can also be used to distinguish SARS-CoV-2 from SARS-CoV. In addition, the SARS-CoV-2 RBD has a relatively higher binding affinity than that of SARS-CoV, which can at least partially explain why SARS-CoV-2 is highly contagious and exhibits increased infectivity.[70] A recent study showed that SARS-CoV-2 manipulates host splicing machinery during infection to affect viral replication. Moreover, other studies have suggested that the notch signaling pathway is involved in SARS-CoV-2 infection via the host protease, furin, which can interfere with viral entry into host cells.[71,72] The nucleocapsid (N) protein is located within virions and reportedly participates in packaging the RNA genome. The membrane (M) and envelope (E) structural proteins are essential for viral assembly and pathogenesis. M protein interactions lead to downregulation of mitochondrial fusion-mediated interferon-gamma responses by host cells.[73] By contrast, the E protein can interact with protein bromodomains BRD2 and BRD4 to regulate gene transcription.

Other work has shown that S protein binding affinity with ACE2 is 20-folder greater than that of SARS-CoV, and that high levels of ACE2 receptor have been associated with elevated risk for infection as well as poor prognosis during disease development.[74] Unfortunately, SARS-CoV-2 infection can result in hypoxic conditions which may ultimately lead to the onset of ARDS and/or toxic encephalopathy in its later stage.[75,76] Conti et al. revealed that SARS-CoV-2 could consistently induce an aggressive inflammatory response resulting in damage to airways, while severely affected patients died primarily due to the effects of ARDS.[77,78] In general, patients with ARDS present symptoms, such as difficulty breathing and low blood oxygen levels, causing them to succumb to secondary bacterial and fungal infections. Among these symptoms, ARDS is the major cause in 70% of COVID-19-related patient deaths.[79,80] Cell infiltration mediates pulmonary damage through excessive secretion of proteases and reactive oxygen species, and subsequently those inflammatory cells can directly damage lung structure, hinder macrophage infiltration, and induce diffuse alveolar damage and pulmonary edema.

Structural analysis of the virus, potential drug therapy, and vaccine development

Molecular docking analysis, followed by chemical stability studies, with subsequent target point determination, is the standard bioinformatics workflow that contributes to innovation in drug design, overcoming drawbacks of the traditional, more time-consuming, less predictable, drug development process. Among these processes, molecular docking can provide advantages in drug design, comparison, and evaluation of their efficacy. The COVID-19 pandemic is largely characterized by a lack of effective therapeutics, and with only a few candidates under clinical trial. Using the crystal structure of SARS-CoV-2 proteinase in conjunction with traditional herbal medicines in docking analyses yielded some promising terpenoid natural products that could inhibit the viral protease activity.[73,81] Another docking study that screened clinically approved medicines with the structure of SARS-CoV-2 M^{pro} suggested that lopinavir, ritonavir, and nelfinavir, and other drugs that were shown to be successful as antiviral treatments for HIV, can act as potential candidates for drug therapy of COVID-19.[82,83]

Protein-protein binding assays showed that ACE2 serves as the cellular binding receptor, of which a 17 amino acid of N-terminal signal sequence and 22 hydrophobic transmembrane sequence near the C terminus were found to be essential for the interaction. Moreover, ACE2 also contains a cytoplasmic domain with potential glycosylation sites that could mediate the initial host cell binding interaction.[84] To date, seven types of animal- and human-infecting coronaviruses have been reported, four of which only infect the upper respiratory tract and produce mild symptoms. However, three coronaviruses have been shown to infect and replicate in the lower respiratory tract of humans, causing pneumonia, ARD, and death.[85] Compared with SARS-CoV, SARS-CoV-2 can progress to critical or ARD within a relatively short period, i.e., consistently less than 10 days after symptom onset. In addition,

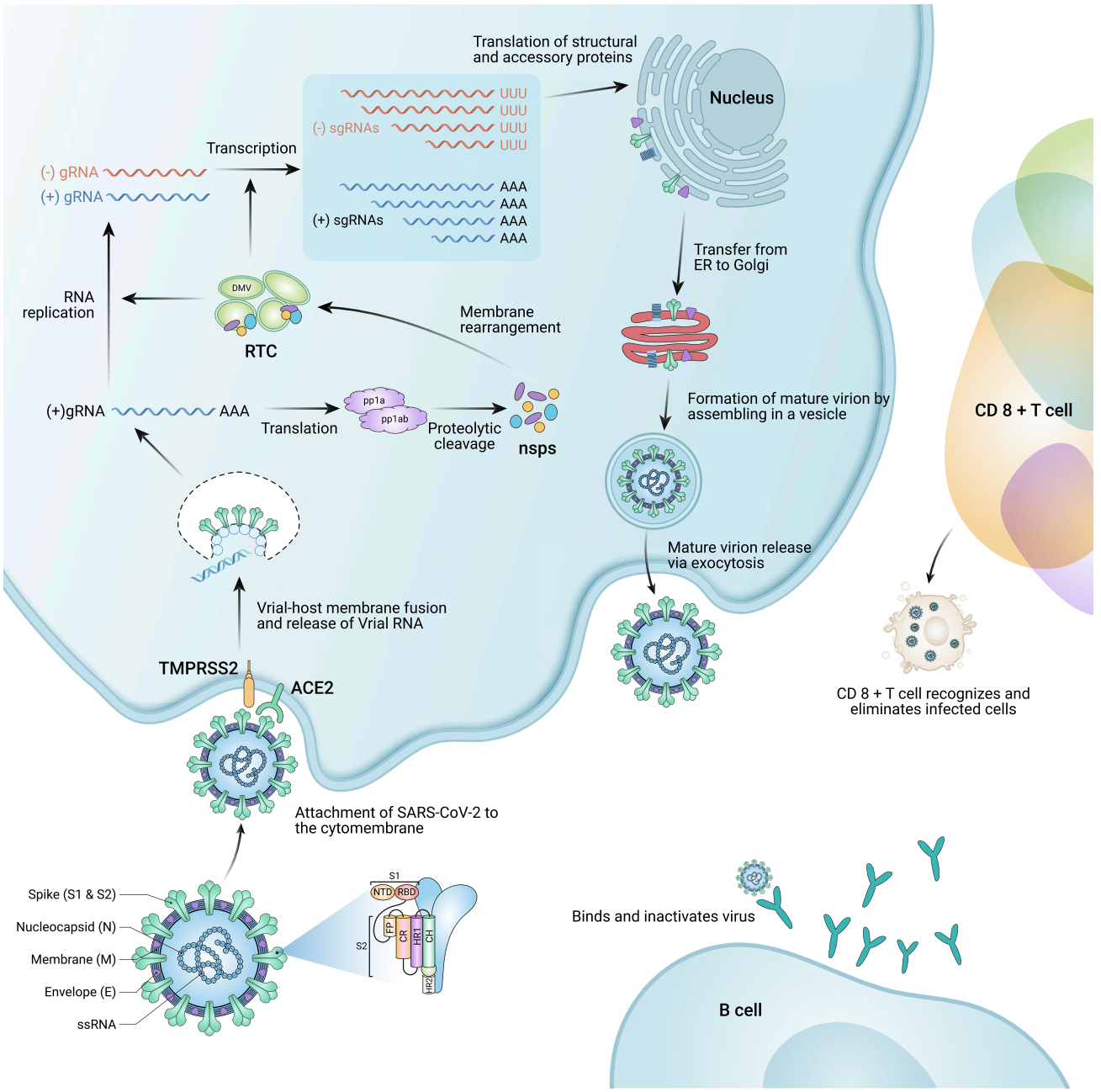


Figure 3. The mechanism of COVID-19 infection and the amplification process of SARS-CoV-2 as well as the response of the human immune system to the virus

SARS-CoV-2 exhibits similar characteristics in its host cell infection process as that of SARS-CoV in that they can both rely on the spike surface protein, a multifunctional molecule comprised of S1 and S2 subunits. The S1 subunit mediates host cell receptor binding, while the S2 subunit subsequently mediates viral fusion with the host cell. The structural conformation of the spike protein differs between the prefusion and post-fusion states, with membrane fusion serving as a key process in transitioning between the pre- and post-fusion conformations.

The S1 subunits are comprised of an NTD and RBD, through binding, which forms a complex of spike protein and human ACE2 (Figure 1C) for recognition of distinct host receptors before viral attachment. These two domains are connected by disulfide bonds at the beta-c2 and -c4 sites between five strands that form the first part, while the other one stabilizes another small flexible loop. The second S1 sub-domain is distributed on the protein surface and functions in the prefusion recognition process. Formation of the prefusion complex between SARS-CoV-2 S1 and human ACE2 NTD is initially driven by van der Waals forces, while H bond/salt bridge interactions drive further interactions. The SARS-CoV-2-CTD spans 195 residues, from T333 to P527, within which residues G466 to G502 form H bonds with amino acids of hACE2.[86,87] The interface was shown to contain three interaction regions, with a bridge forming between the alpha-1 helix and sites between the alpha-2 helix and loop 3–4. High-resolution imaging by cryo-EM further supported the formation of a stable prefusion complex.[88,89]

By contrast, the S2 subunit contains five functional domains, including a fusion peptide, heptad repeat N- and C-terminal regions (HR-N and HR-C), a transmembrane domain (TM), and a cytoplasmic domain.[90] The S2 subunit facilitates the viral fusion process via interaction with TMPRSS2 protein on the host cell surface. X-ray crystallography of the S2 subunit revealed a rod-like 6-HB fusion core that forms a deep hydrophobic groove adjacent to the HR1 domain, allowing HR2 binding to connect them.[91] Structural

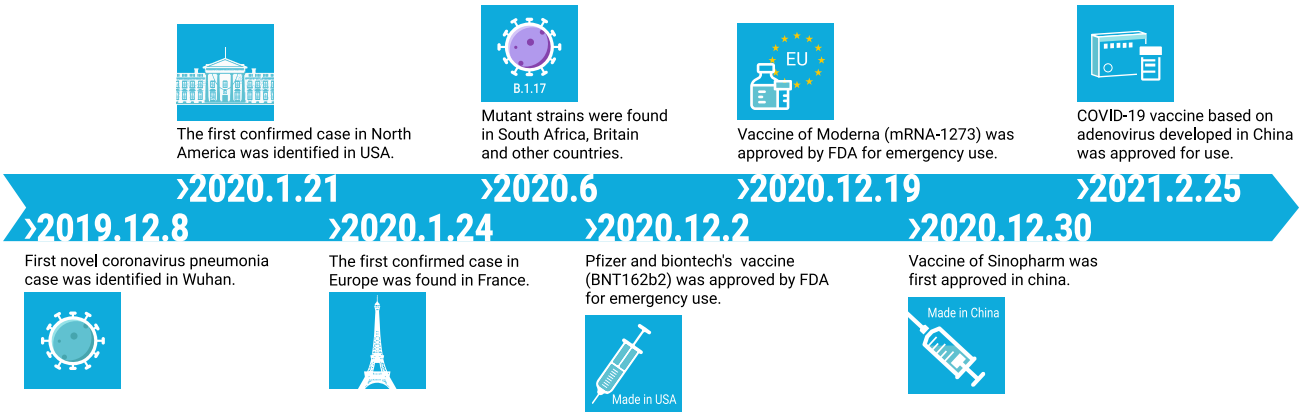


Figure 4. Timeline of COVID-19 disease and the progress of vaccine development

and biophysical evidence has together illustrated that the considerably higher infectivity of SARS-CoV-2 is due to the 20-fold higher affinity of S protein binding to human ACE2 than that of the SARS-CoV spike protein.

Vaccine development has been widely regarded as the most effective approach for prevention and management of COVID-19. The past year has seen substantial progress in the research and development of new coronavirus vaccines through the joint global efforts of medical and scientific research institutions and businesses, resulting in vaccine deployment in many countries and regions. The timeline of progression of SARS-CoV-2 vaccine development is shown in Figure 4. The WHO COVID-19 vaccine progress draft shows that 173 prospective vaccines for COVID-19 are currently in the preclinical stage as of January 2021,[92,93] while 64 candidate vaccines have entered clinical trials, 22 of which are in phase II/III or phase III clinical trials. The traditional strategy for development of coronavirus vaccines relies on virus inactivation, virus attenuation, and recombinant protein methods. In recent years, new technology platforms, such as viral vector vaccines and nucleic acid vaccines (mRNA vaccines and DNA vaccines), have opened up new avenues for vaccine development.[94]

Although nucleic acid vaccines can be produced quickly, this new type of vaccine requires relatively stringent transportation and storage conditions to ensure vaccine stability. In contrast, the preparation method for inactivated vaccines is well established and reliable, but the resultant vaccines provide relatively weak immunity. The use of recombinant protein technology is mature and considered safer than other vaccines, with low possibility of causing adverse reactions, but the immune response may also be insufficiently strong to control the virus. To date, three vaccines have been authorized in the United States, two of which are mRNA vaccines. On November 10, 2020, Pfizer and its partner BioNTech announced that their COVID-10 vaccine candidate BNT162b2 exhibited greater than 90% effectiveness among study participants.[95] The phase III clinical trial of BNT162b2 included a cohort of 43,538 participants, approximately 42% of which were recruited internationally, while 30% were US participants. Another American pharmaceutical company, Moderna, also successfully deployed an mRNA vaccine against SARS-CoV-2. Clinical safety trials showed that vaccinated people maintained high levels of antibodies for as long as 119 days.[96]

Other countries have focused on development of different types of vaccines. The Sinopharm Group announced approval of clinical trials for its vaccine based on inactivated virus. Both Sinopharm Group overseas phase III clinical studies were conducted in cohorts of more than 60,000 global volunteers and included greater than 6 months of observation data, which showed that antibodies are maintained at high levels, resulting in a 79.34% protection rate.[97,98] On November 23, 2020, AstraZeneca announced that its AZD1222 vaccine, produced in collaboration with Oxford University, could provide an average effectiveness of 70% against SARS-CoV-2.[99] On January 29, 2021, Johnson & Johnson announced that its single-dose new coronavirus vaccine (JNJ-78436735) in the phase III clinical study had reached all primary clinical endpoints and planned to submit an emergency use authorization application to the FDA. At 28 days following a single-dose vaccination, this vaccine had an overall effective rate of 66% in the prevention of moderate and severe COVID-19. Overall, enough vaccines for most populations would be necessary to decrease infection cases and stop the outbreak of COVID-19.[100]

Unfortunately, vaccine efficacy can be limited by the emergence of novel strains in human populations. In particular, the 20I (B.1.1.7) lineage from the United Kingdom, the South African 20H (B.1.351) lineage, and the Brazilian 20J (P.1) lineage (Figure 2) have spread globally due to their higher infectivity. Whether the existing vaccines remain effective against the newer virus variants has become a problem of great concern to both scientists and the general public, and several studies have (and continue to) explored the threat to vaccine protection posed by these variants. For example, the Pfizer BNT162b2 vaccine exhibited 95% efficacy against the original SARS-CoV-2 strain.[101] However, recent *in vitro* studies investigating the efficacy of serum of BNT162b2-vaccinated volunteers showed roughly equivalent neutralization of recombinant viruses expressing the P.1- and B.1.1.7 spikes, and a two-thirds reduction in efficacy for neutralization of those bearing the B.1.351 spike.[102] Similar results were obtained in an *in vitro* study of the Moderna mRNA-1273 vaccine in which researchers constructed a pseudovirus expressing complete spike proteins of the B.1.1.7 or B.1.351 variants. Immune serum was obtained from eight subjects from a phase I clinical trial and the degree of viral neutralization by these sera was determined. While the immune sera showed no significant neutralization of the B.1.1.7 variant, the neutralized B.1.351 mutant titer was reduced by 6.4-fold, but remained at a high level (1/290).

The Novavax NVX-CoV2733 vaccine is a recombinant protein vaccine produced using proprietary recombinant nanoparticles. Interim results of a phase III trial carried out in the UK with more than 15,000 volunteers aged between 18 and 84 years, including 27% over 65 years old, revealed an 85.6% efficacy of the vaccine against the B.1.1.7 variant, compared with 95.6% against the original strain. However, a phase II trial of NVX-CoV2733 in South Africa with more than 4,400 participants showed an overall efficacy of 49.4% (95% confidence interval [CI]: 6.1–72.8). The significant drop in the efficacy compared with the UK trial was due to the B.1.351 variant, carrying an E484K conversion, which is now predominant in South Africa. Sequencing of virus isolated from 27 South African SARS-CoV-2 cases indicated that 93% involved the B.1.351 variant.[103] It warrants mention that this study included 240 volunteers who were HIV-positive, and when this group was excluded from the analysis the protective efficacy was 60% (95% CI: 19.9–80.1).[104] Recently, a study was conducted to investigate neutralizing activity against the B.1.1.7 and B.1.351 variants by the inactivated virus vaccines BBIBP-CorV (Sinopharm) and CoronaVac (Sinovac) by comparing the serum neutralization titer of 50 patients with two doses of either BBIBP-CorV or CoronaVac vaccine with that of sera from 34 convalescents collected at 5 months after COVID-19 infection with COVID-19. The results suggested

Table 2. Current progress in vaccine development

Strategy	Developer	Protective effect	Cross-protection effect
Inactivated virus (PiCoVacc)	Sinovac, with National Institute for Communicable Disease Control and Prevention	a protective effect of 67% in Chile (real-world data)	slightly reduced effectiveness against B.1.1.7 spike-expressing recombinant virus and 3.3-fold reduction against B.1.351 spike-expressing recombinant virus (*in vitro*)
Inactivated virus	Wuhan Institute of Biological Products, Sinopharm, with Wuhan Institute of Virology, Chinese Academy of Sciences	the positive conversion rate of neutralizing antibody was 99.06%, and the protective effect was 72.51%	–
Inactivated virus (BBIBP-CorV)	Beijing Institute of Biological Products, Sinopharm, with Institute of Viral Disease Control and Prevention	the positive conversion rate of neutralizing antibody was 99.52%, and the protective effect was 79.34%	roughly equivalent against B.1.1.7 spike-expressing recombinant virus, but 2.5-fold lower efficacy against virus expressing the B.1.351 spike (*in vitro*)
Virus vector (Ad5)	CanSino Biological Inc. with Beijing Institute of Biotechnology	a protective efficacy against all symptoms of 68.83% (phase III clinical trial)	–
Virus vector (ChAdOx1)	University of Oxford, with AstraZeneca	the protective effect was 76% (phase III clinical trial), although some thrombotic events occurred; no definitive causal relationship between vaccine and thrombosis was found	–
LNP-mRNA (mRNA-1273)	Moderna, with National Institute of Allergy and Infectious Diseases	94.1% effective in phase III trial (95% CI: 89.3–96.8)	roughly equivalent against B.1.1.7 spike-expressing recombinant viruses, but 6.4-fold lower against B.1.351 spike-expressing virus
LNP-mRNA (BNT162b2)	BioNTech, with Fosun Pharma and Pfizer	95% in a clinical trial involving ~44,000 participants; the effective rate for preventing severe illness is 100%	roughly equivalent against P.1 spike- and B.1.1.7 spike-expressing recombinant viruses, but ~2/3 reduction in efficacy against B.1.351 spike-expressing virus (*in vitro* study)
Protein subunit (NVX-CoV2733)	Novavax	95.6% against the original strain (phase III trial in the UK)	85.6% effective against the B.1.1.7 variant (phase III trial in the UK); the protective efficacy was 60% (95% CI: 19.9–80.1) with 93% of cases involving the B.1.351 variant (phase II trial in South Africa)
Virus-vectored (Ad26)	Janssen Pharmaceutical Company	66.9% effective (95% CI: 59.0–73.4)	–
Protein subunit (ZF2001)	Anhui Zhifei Longcom Biopharmaceutical, with Institute of Microbiology, Chinese Academy of Sciences	the positive conversion rate was 96.6% in the phase II clinical trial, and the neutralizing antibody titer was 102.5	geometric mean titer was 106.1 (95% CI: 75.0–150.1) against the original virus strain and 66.6 (95% CI: 51.0–86.9) against the B.1.351 variant

that the B.1.1.7 variant showed little resistance to neutralization by either the convalescent or vaccinated sera, whereas B.1.351 showed 2-fold higher resistance to convalescent serum and 2.5- to 3.3-fold higher resistance to vaccinated serum than the original strains.[105] The current progress in main vaccine development is summarized in Table 2. Comparison details of the vaccines are provided in Table S1.

To better control the epidemic and reduce immune escape caused by virus mutations, Moderna developed the mRNA-1273.351 vaccine targeting the B.1.351 S protein. Moderna then developed a multivalent vaccine, mRNA-1273.211, which combined mRNA-1273 targeting the original strain and mRNA-1273.351 targeting B.1.351, to thus provide a broader range of protection. *In vivo* studies in a murine model indicated that vaccination with mRNA-1273.351 could increase the neutralizing antibody against the B.1.351 lineage, and that this vaccine thus far showed the highest efficacy for broad, cross-variant neutralization.

DISCUSSION

COVID-19 has spread globally to over 200 countries with more than 40 million confirmed cases and one million deaths as of November 1, 2020.[5] The total cases of COVID-19 are expected to be higher than reported due to the difficulty in identifying false-negative mild and asymptomatic cases. Moreover, accurate and reliable clinical diagnosis requires experienced professionals, while the use of symptom-suppressive medications before examination can also confound diagnosis. In most countries, an increasing trend in confirmed cases during the early stages of the outbreak is followed by an exponential growth trajectory before the epidemic peaks. Therefore, it is difficult to compare the rates of infection and fatality between countries due to differences in the stages of outbreak, highly variable scopes of population testing, differences in the burden on their respective health care systems, as well as the general health status of the population, and differences in average population demographics.[106] Moreover, the elderly and people with underlying chronic disease were more susceptible to infection by SARS-CoV-2 at the beginning of the outbreak.

The most common symptoms of early infection include mild fever, dry cough, and fatigue, while nasal congestion, diarrhea, and sore throat are rarely reported. However, some people close to COVID-19 patients, or in family cluster cases, initially showed no fever or respiratory symptoms but exhibited non-respiratory or cardiac-associated symptoms,

such as palpitation, arrhythmia, and cardiac shock accompanied by respiratory symptoms, dyspnea, or, occasionally, in the worst cases, ARD.[107,108] Although asymptomatic infection can be difficult to define in the early stages of infection, many patients eventually develop pneumonia; therefore, the clinical presentations range from asymptomatic to fatal multiple organ failures.

Binding of SARS-CoV-2 to the host ACE2 receptor activates fusion with the host cell and subsequent viral replication, leading to pyroptosis in the host cell and release of the virus. This process causes damage-associated molecular patterns, which are recognized by neighboring epithelial cells, alveolar epithelial cells, and vascular endothelial cells, consequently triggering a pro-inflammatory response. The inflammatory signal cascade entails cytokine release, which in turn recruits monocytes, macrophages, and T cells to those infection sites. Furthermore, these cytokine bursts can generate a pro-inflammatory feedback loop, resulting in a cytokine storm, eventually damaging pulmonary structure and function.[109] In addition, ACE2 regulates the renin-angiotensin system, which could be downregulated as viral load increases, thereby influencing fluid and electrolyte levels, and enhancing inflammatory response, vascular permeability, and infiltration of lymphocytes into the airway. Pulmonary recruitment of circulating immune cells and lymphocyte infiltration ultimately results in peripheral lymphopenia.

In summary, COVID-19 represents an urgent, worldwide health crisis. Systematic and rigorous review of genomic variations in SARS-CoV-2 and origin tracing of COVID-19 will benefit the prevention, diagnosis, and therapeutic strategies for patients suffering from COVID-19.

REFERENCES

1. Zon, L., Gomes, A.P., Cance, W.G., et al. (2020). Impact of COVID-19 pandemic on cancer research. Cancer Cell ***38***, 591–593.
2. Thompson, K.M., Kalkowska, D.A., and Badizadegan, K. (2020). A health economic analysis for oral poliovirus vaccine to prevent COVID-19 in the United States. Risk Anal. ***41***, 376–386.
3. De Filippo, O., D'Ascenzo, F., and Deferrari, G.M. (2020). COVID-19 pandemic and infarctions: another call to reorganise our healthcare systems. Heart ***106***, 1786–1787.
4. Khurana, D.K., Raheja, S.G., Dayal, M., et al. (2020). Covid 19: the new normal in the clinic: overcoming challenges in palliative care. Indian J. Palliat. Care ***26***, S81–S85.
5. Wong, R.S.Y. (2020). The SARS-CoV-2 outbreak: an epidemiological and clinical perspective. SN Compr. Clin. Med. 1–9.
6. Zampieri, N., Cinquetti, M., Murri, V., et al. (2020). Incidence of appendicitis during SARS-CoV-2 pandemic quarantine: report of a single area experience. Minerva Pediatr. https://doi.org/10.23736/S0026-4946.20.05901-0.
7. Parlakpinar, H., and Gunata, M. (2020). SARS-COV-2 (COVID-19): cellular and biochemical properties and pharmacological insights into new therapeutic developments. Cell Biochem. Funct. ***39***, 10–28.
8. Wu, N.C., Yuan, M., Bangaru, S., et al. (2020). A natural mutation between SARS-CoV-2 and SARS-CoV determines neutralization by a cross-reactive antibody. bioRxiv, 2020.09.21.305441.
9. Chu, H., Fuk-Woo Chan, J., Wang, Y., et al. (2020). SARS-CoV-2 induces a more robust innate immune response and replicates less efficiently than SARS-CoV in the human intestines: an ex vivo study with implications on pathogenesis of COVID-19. Cell Mol. Gastroenterol. Hepatol. ***11***, 771–781.
10. Sano, F., Yagasaki, H., Kojika, S., et al. (2020). Severe apparent life-threatening event (ALTE) in an infant with SARS-CoV 2 infection. Jpn. J. Infect Dis. https://doi.org/10.7883/yoken.JJID.2020.572.
11. Abdelrahman, Z., Li, M., and Wang, X. (2020). Comparative review of SARS-CoV-2, SARS-CoV, MERS-CoV, and influenza A respiratory viruses. Front Immunol. ***11***, 552909.
12. Gong, L., Li, J., Zhou, Q., et al. (2017). A new bat-HKU2-like coronavirus in swine, China, 2017. Emerg. Infect Dis. ***23***, 1607–1609.
13. Moses, M.O., Emikpe, A.O., Moses, M.K., et al. (2020). Combating COVID-19 lockdown inactivity in the African population: use of cultural practices and one health approach. Niger. J. Physiol. Sci. ***35***, 4–9.
14. Kitayama, T., Kitamura, H., Hagiwara, E., et al. (2020). COVID-19 pneumonia resembling an acute exacerbation of interstitial pneumonia. Intern. Med. ***59***, 3207–3211.
15. Lee, C.K., Leung, J.N.S., Cheng, P., et al. (2020). Absence of SARS-CoV-2 viraemia in a blood donor with COVID-19 post-donation. Transfus. Med. https://doi.org/10.1111/tme.12724.
16. Sinard, J.H. (2020). An analysis of the effect of the COVID-19 pandemic on case volumes in an academic subspecialty-based anatomic pathology practice. Acad. Pathol. ***7***, 2374289520959788.
17. Natarajan, H., Crowley, A.R., Butler, S.E., et al. (2020). SARS-CoV-2 antibody signatures robustly predict diverse antiviral functions relevant for convalescent plasma therapy. medRxiv, 2020.09.16.20196154.
18. Lyons, J., Akbari, A., Torabi, F., et al. (2020). Understanding and responding to COVID-19 in Wales: protocol for a privacy-protecting data platform for enhanced epidemiology and evaluation of interventions. BMJ Open ***10***, e043010.
19. Xu, W., Wang, M., Yu, D., et al. (2020). Variations in SARS-CoV-2 spike protein cell epitopes and glycosylation profiles during global transmission course of COVID-19. Front Immunol. ***11***, 565278.
20. Petersen, E., Koopmans, M., Go, U., et al. (2020). Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. Lancet Infect Dis. ***20***, e238–e244.
21. Al-Khani, A.M., Khalifa, M.A., Almazrou, A., et al. (2020). The SARS-CoV-2 pandemic course in Saudi Arabia: a dynamic epidemiological model. Infect Dis. Model. ***5***, 766–771.
22. Strassle, C., Jardas, E., Ochoa, J., et al. (2020). Covid-19 vaccine trials and incarcerated people—the ethics of inclusion. N. Engl. J. Med. ***383***, 1897–1899.
23. Poland, G.A., Ovsyannikova, I.G., Crooke, S.N., et al. (2020). SARS-CoV-2 vaccine development: current status. Mayo Clin. Proc. ***95***, 2172–2188.
24. Wise, J. (2020). Covid-19: suicidal thoughts increased in young adults during lockdown, UK study finds. BMJ ***371***, m4095.
25. Simonsen, A.B., Ruge, I.F., Quaade, A.S., et al. (2020). Increased occurrence of hand eczema in young children following the Danish hand hygiene recommendations during the COVID-19 pandemic. Contact Dermatitis ***84***, 144–152.
26. Weiner, L., Berna, F., Nourry, N., et al. (2020). Efficacy of an online cognitive behavioral therapy program developed for healthcare workers during the COVID-19 pandemic: the REduction of STress (REST) study protocol for a randomized controlled trial. Trials ***21***, 870.
27. Lesho, E., Reno, L., Newhart, D., et al. (2020). Temporal, spatial, and epidemiologic relationships of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) gene cycle thresholds: a pragmatic ambi-directional observation. Clin. Infect Dis. https://doi.org/10.1093/cid/ciaa1248.
28. Coiffard, B., Lepper, P.M., Prud'Homme, E., et al. (2020). Management of lung transplantation in the COVID-19 era—an international survey. Am. J. Transplant. ***21***, 1586–1596.
29. Ahmad, M.F., Mahakkanukrauh, P., and Das, S. (2020). The detection of SARS-CoV-2 virus in the vaginal fluid of females with severe COVID-19 infection: scientific facts. Clin. Infect Dis. ciaa1608.
30. Green, M.S., Peer, V., Schwartz, N., et al. (2020). The confounded crude case-fatality rates (CFR) for COVID-19 hide more than they reveal—a comparison of age-specific and age-adjusted CFRs between seven countries. PLoS One ***15***, e0241031.
31. Jha, A.K. (2020). Pulmonary vascular changes in acute respiratory distress syndrome due to COVID-19. Am. J. Respir. Crit. Care Med. ***203***, 259–260.
32. Allegra, A., Tonacci, A., Pioggia, G., et al. (2020). Vitamin deficiency as risk factor for SARS-CoV-2 infection: correlation with susceptibility and prognosis. Eur. Rev. Med. Pharmacol. Sci. ***24***, 9721–9738.
33. Nelde, A., Bilich, T., Heitmann, J.S., et al. (2020). SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. Nat. Immunol. ***22***, 74–85.
34. Caldas, L.A., Carneiro, F.A., Higa, L.M., et al. (2020). Ultrastructural analysis of SARS-CoV-2 interactions with the host cell via high resolution scanning electron microscopy. Sci. Rep. ***10***, 16099.
35. Singh, K., Mittal, S., Gollapudi, S., et al. (2020). A meta-analysis of SARS-CoV-2 patients identifies the combinatorial significance of D-dimer, C-reactive protein, lymphocyte, and neutrophil values as a predictor of disease severity. Int. J. Lab Hematol. ***43***, 324–328.
36. Cilloniz, C., Torres, A., Garcia-Vidal, C., et al. (2020). The value of C-reactive protein-to-lymphocyte ratio in predicting the severity of SARS-CoV-2 pneumonia. Arch. Bronconeumol. ***57 Suppl 1***, 79–82.
37. Finch, C.L., Crozier, I., Lee, J.H., et al. (2020). Characteristic and quantifiable COVID-19-like abnormalities in CT- and PET/CT-imaged lungs of SARS-CoV-2-infected crab-eating macaques (*Macaca fascicularis*). bioRxiv, 2020.05.14.096727.
38. Pautrat, K., and Chergui, N. (2020). SARS-CoV-2 infection may result in appendicular syndrome: chest CT scan before appendectomy. J. Visc. Surg. ***157***, S63–S64.
39. Sonnweber, T., Boehm, A., Sahanic, S., et al. (2020). Persisting alterations of iron homeostasis in COVID-19 are associated with non-resolving lung pathologies and poor patients' performance: a prospective observational cohort study. Respir. Res. ***21***, 276.
40. Chiumello, D., Busana, M., Coppola, S., et al. (2020). Physiological and quantitative CT-scan characterization of COVID-19 and typical ARDS: a matched cohort study. Intensive Care Med. ***46***, 2187–2196.
41. Yang, H., Lan, Y., Yao, X., et al. (2020). The chest CT features of coronavirus disease 2019 (COVID-19) in China: a meta-analysis of 19 retrospective studies. Virol. J. ***17***, 159.
42. Santamarina, M.G., Boisier Riscal, D., Beddings, I., et al. (2020). COVID-19: what iodine maps from perfusion CT can reveal—a prospective cohort study. Crit. Care ***24***, 619.

43. Romero, J., Valencia, S., and Guerrero, A. (2020). Acute appendicitis during coronavirus disease 2019 (COVID-19): changes in clinical presentation and CT findings. J. Am. Coll. Radiol. ***17***, 1011–1013.
44. Kamani, C.H., Jreige, M., Pappon, M., et al. (2020). Added value of (18)F-FDG PET/CT in a SARS-CoV-2-infected complex case with persistent fever. Eur. J. Nucl. Med. Mol. Imaging ***47***, 2036–2037.
45. Belfiore, M.P., Urraro, F., Grassi, R., et al. (2020). Artificial intelligence to codify lung CT in Covid-19 patients. Radiol. Med. ***125***, 500–504.
46. Bosso, G., Allegorico, E., Pagano, A., et al. (2020). Lung ultrasound as diagnostic tool for SARS-CoV-2 infection. Intern. Emerg. Med. ***16***, 471–476.
47. Daghir Janabi, A.H. (2020). Effective anti-SARS-CoV-2 RNA dependent RNA polymerase drugs based on docking methods: the case of Milbemycin, Ivermectin, and Baloxavir Marboxil. Avicenna J. Med. Biotechnol. ***12***, 246–250.
48. Srivastava, R., Daulatabad, S.V., Srivastava, M., et al. (2020). Role of SARS-CoV-2 in altering the RNA-binding protein and miRNA-directed post-transcriptional regulatory networks in humans. Int. J. Mol. Sci. ***21***, 7090.
49. Baronio, M., Freni-Sterrantino, A., Pinelli, M., et al. (2020). Italian SARS-CoV-2 patients in intensive care: towards an identikit for subjects at risk? Eur. Rev. Med. Pharmacol. Sci. ***24***, 9698–9704.
50. Perreault, J., Tremblay, T., Fournier, M.J., et al. (2020). Waning of SARS-CoV-2 RBD antibodies in longitudinal convalescent plasma samples within four months after symptom onset. Blood ***136***, 2588–2591.
51. Major, J., and Wack, A. (2020). An ace model for SARS-CoV-2 infection. J. Exp. Med. ***217***, e20201748.
52. Anand, S.P., Chen, Y., Prevost, J., et al. (2020). Interaction of human ACE2 to membrane-bound SARS-CoV-1 and SARS-CoV-2 S glycoproteins. Viruses ***12***, 1104.
53. Sagulenko, P., Puller, V., and Neher, R.A. (2018). TreeTime: maximum-likelihood phylodynamic analysis. Virus Evol. ***4***, vex042.
54. Hadfield, J., Megill, C., Bell, S.M., et al. (2018). Nextstrain: real-time tracking of pathogen evolution. Bioinformatics ***34***, 4121–4123.
55. Shu, Y., and McCauley, J. (2017). GISAID: global initiative on sharing all influenza data—from vision to reality. Eurosurveillance ***22***, 30494.
56. Letunic, I., and Bork, P. (2019). Interactive Tree of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. ***47***, W256–W259.
57. Shen, L., Dien Bard, J., Biegel, J.A., et al. (2020). Comprehensive genome analysis of 6,000 USA SARS-CoV-2 isolates reveals haplotype signatures and localized transmission patterns by state and by country. Front. Microbiol. ***11***, 573430.
58. Zheng, Y., Larragoite, E.T., Lama, J., et al. (2020). Neutralization assay with SARS-CoV-1 and SARS-CoV-2 spike pseudotyped murine leukemia virions. bioRxiv, 2020.07.17.207563.
59. Alexander, M.R., Schoeder, C.T., Brown, J.A., et al. (2020). Predicting susceptibility to SARS-CoV-2 infection based on structural differences in ACE2 across species. FASEB J. ***34***, 15946–15960.
60. Korber, B., Fischer, W.M., Gnanakaran, S., et al. (2020). Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell ***182***, 812–827.e9.
61. Ramlall, V., Zucker, J., and Tatonetti, N. (2020). Melatonin is significantly associated with survival of intubated COVID-19 patients. medRxiv, 2020.10.15.20213546.
62. Simonis, A., Theobald, S.J., Fatkenheuer, G., et al. (2020). A comparative analysis of remdesivir and other repurposed antivirals against SARS-CoV-2. EMBO Mol. Med. ***13***, e13105.
63. Li, T., Liu, Q., Garza, N., et al. (2018). Integrative analysis reveals functional and regulatory roles of H3K79me2 in mediating alternative splicing. Genome Med. ***10***, 30.
64. Guo, X., Li, Y., Li, H., et al. (2020). An improved multivariate model that distinguishes COVID-19 from seasonal flu and other respiratory diseases. Aging ***12***, 19938–19944.
65. Bhanu, P., Kumar, N.H., Kumar, S.H., et al. (2020). Comparative molecular docking analysis of the SARS CoV-2 spike glycoprotein with the human ACE-2 receptors and thrombin. Bioinformation ***16***, 532–538.
66. Qiao, J., Li, W., Bao, J., et al. (2020). The expression of SARS-CoV-2 receptor ACE2 and CD147, and protease TMPRSS2 in human and mouse brain cells and mouse brain tissues. Biochem. Biophys. Res. Commun. ***533***, 867–871.
67. Mukhopadhyay, D., and Mussa, B.M. (2020). Identification of novel hypothalamic microRNAs as promising therapeutics for SARS-CoV-2 by regulating ACE2 and TMPRSS2 expression: an in silico analysis. Brain Sci. ***10***, 666.
68. Maremanda, K.P., Sundar, I.K., Li, D., et al. (2020). Age-dependent assessment of genes involved in cellular senescence, telomere, and mitochondrial pathways in human lung tissue of smokers, COPD, and IPF: associations with SARS-CoV-2 COVID-19 ACE2-TMPRSS2-furin-DPP4 Axis. Front Pharmacol. ***11***, 584637.
69. Sarver, D.C., and Wong, G.W. (2020). Obesity alters Ace2 and Tmprss2 expression in lung, trachea, and esophagus in a sex-dependent manner: implications for COVID-19. bioRxiv, 2020.10.13.337907.
70. Chow, K.W., Kelly, D.J., Gupta, R., et al. (2020). Use of continuous glucose monitoring to assess TPN-induced hyperglycemia in an adult patient with severe COVID-19. JPEN J. Parenter. Enteral Nutr. ***45***, 208–211.
71. Wang, R., Simoneau, C.R., Kulsuptrakul, J., et al. (2020). Functional genomic screens identify human host factors for SARS-CoV-2 and common cold coronaviruses. bioRxiv, 2020.09.24.312298.
72. Wang, R., Hozumi, Y., Zheng, Y.H., et al. (2020). Host immune response driving SARS-CoV-2 evolution. Viruses ***12***, 1095.
73. Hong, M., Mandala, V., McKay, M., et al. (2020). Structure and drug binding of the SARS-CoV-2 envelope protein in phospholipid bilayers. Res. Sq. rs.3.rs-77124. https://doi.org/10.21203/rs.3.rs-77124/v1.
74. Liu, A., Zhang, X., Li, R., et al. (2020). Overexpression of the SARS-CoV-2 receptor ACE2 is induced by cigarette smoke in bronchial and alveolar epithelia. J. Pathol. ***253***, 17–30.
75. Bagnato, S., Boccagni, C., Marino, G., et al. (2020). Reply to "SARS-CoV-2-associated critical ill myopathy or pure toxic myopathy?". Int. J. Infect Dis. ***101***, 57.
76. Finsterer, J., and Scorza, F.A. (2020). SARS-CoV-2-associated critical ill myopathy or pure toxic myopathy? Int. J. Infect Dis. ***101***, 56.
77. Conti, P., Caraffa, A., Gallenga, C.E., et al. (2020). Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy. J. Biol. Regul. Homeost Agents ***34***, 1971–1975.
78. Bellgrau, D., and Modiano, J.F. (2020). The cytokine storm—an appropriate, overreactive response to SARS-CoV-2 or the wrong immune pathway? Scand. J. Immunol. ***93***, e12979.
79. Li, A., Garcia-Bengochea, Y., Stechel, R., et al. (2020). Management of COVID-19 myopericarditis with reversal of cardiac dysfunction after blunting of cytokine storm: a case report. Eur. Heart J. Case Rep. ***4***, 1–6.
80. Chitturi, K.R., Thacker, S., Al-Saadi, M.A., et al. (2020). Successful treatment of acute heart failure in COVID-19-induced cytokine storm with tocilizumab: a case report. Eur. Heart J. Case Rep. ***4***, 1–6.
81. Piccoli, L., Park, Y.J., Tortorici, M.A., et al. (2020). Mapping neutralizing and immunodominant sites on the SARS-CoV-2 spike receptor-binding domain by structure-guided high-resolution serology. Cell ***183***, 1024–1042.e21.
82. Rzepinski, L., Wawrzyniak, S., and Maciejek, Z. (2020). Immunocompromised myasthenia gravis patient not infected with SARS-CoV-2 after close exposure—what is the risk of COVID-19? Neurol. Neurochir Pol. ***54***, 481–482.
83. Pathak, S.S., Liu, D., Li, T., et al. (2019). The eIF2alpha kinase GCN2 modulates period and rhythmicity of the circadian clock by translational control of Atf4. Neuron ***104***, 724–735.e6.
84. Shi, M., Wang, L., Fontana, P., et al. (2020). SARS-CoV-2 Nsp1 suppresses host but not viral translation through a bipartite mechanism. bioRxiv, 2020.09.18.302901.
85. Liu, Q., Bonneville, R., Li, T., et al. (2017). Transcription factor-associated combinatorial epigenetic pattern reveals higher transcriptional activity of TCF7L2-regulated intragenic enhancers. BMC Genomics ***18***, 375.
86. Wang, Q., Zhang, Y., Wu, L., et al. (2020). Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell ***181***, 894–904.e9.
87. Wang, J., Li, T., Zan, H., et al. (2021). LUBAC suppresses IL-21-induced apoptosis in CD40-activated murine b cells and promotes germinal center B cell survival and the T-dependent antibody response. Front. Immunol. ***12***, 658048.
88. Yan, R., Zhang, Y., Li, Y., et al. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science ***367***, 1444–1448.
89. Goodman, M.L., Trinca, G.M., Walter, K.R., et al. (2019). Progesterone receptor attenuates STAT1-mediated IFN signaling in breast cancer. J. Immunol. ***202***, 3076–3086.
90. Xia, S., Liu, M., Wang, C., et al. (2020). Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res. ***30***, 343–355.
91. Yuan, M., Wu, N.C., Zhu, X., et al. (2020). A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science ***368***, 630–633.
92. Gee, J., Marquez, P., Su, J., et al. (2021). First month of COVID-19 vaccine safety monitoring—United States, December 14, 2020–January 13, 2021. MMWR Morb. Mortal. Wkly. Rep. ***70***, 283–288.
93. Yuan, B., Clark, C.A., Wu, B., et al. (2021). Estrogen receptor beta signaling in CD8(+) T cells boosts T cell receptor activation and antitumor immunity through a phosphotyrosine switch. J. Immunother. Cancer ***9***, e001932.
94. Pandey, A., Belbase, P., and Parajuli, A. (2021). COVID-19 vaccine development to vaccination. J. Nepal Health Res. Counc. ***18***, 807–809.
95. Dagan, N., Barda, N., Kepten, E., et al. (2021). BNT162b2 mRNA covid-19 vaccine in a nationwide mass vaccination setting. N. Engl. J. Med. ***384***, 10.1056/NEJMc2104281#sa1.
96. Mahase, E. (2021). Covid-19: UK approves Moderna vaccine to be given as two doses 28 days apart. BMJ ***372***, n74.
97. Ophinni, Y., Hasibuan, A.S., Widhani, A., et al. (2020). COVID-19 vaccines: current status and implication for use in Indonesia. Acta Med. Indones ***52***, 388–412.
98. Gao, Y., Liu, S., Guo, Q., et al. (2019). Increased expression of TRIP13 drives the tumorigenesis of bladder cancer in association with the EGFR signaling pathway. Int. J. Biol. Sci. ***15***, 1488–1499.

99. Wise, J. (2021). Covid-19: new data on Oxford AstraZeneca vaccine backs 12 week dosing interval. BMJ ***372***, n326.
100. Zhao, T., Hu, C., Ayaz Ahmed, M., et al. (2021). Warnings to the potential COVID-19 transmission risk: vaccine is not enough. Infect Control Hosp. Epidemiol. 1–4.
101. Polack, F.P., Thomas, S.J., Kitchin, N., et al. (2020). Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. N. Engl. J. Med. ***383***, 2603–2615.
102. Liu, Y., Liu, J., Xia, H., et al. (2021). Neutralizing activity of BNT162b2-elicited serum. N. Engl. J. Med. ***384***, 1466–1468.
103. Mahase, E. (2021). Covid-19: Novavax vaccine efficacy is 86% against UK variant and 60% against South African variant. BMJ ***372***, n296.
104. Callaway, E., and Mallapaty, S. (2021). Novavax offers first evidence that COVID vaccines protect people against variants. Nature ***590***, 17.
105. Wang, G.L., Wang, Z.Y., Duan, L.J., et al. (2021). Susceptibility of circulating SARS-CoV-2 variants to neutralization. N. Engl. J. Med. NEJMc2103022. https://doi.org/10.1056/NEJMc2103022.
106. Gu, T., Mack, J.A., Salvatore, M., et al. (2020). Characteristics associated with racial/ethnic disparities in COVID-19 outcomes in an academic health care system. JAMA Netw. Open ***3***, e2025197.
107. Pereda, N., and Diaz-Faes, D.A. (2020). Family violence against children in the wake of COVID-19 pandemic: a review of current perspectives and risk factors. Child Adolesc. Psychiatry Ment. Health ***14***, 40.
108. Sun, J., Cai, X., Yung, M.M., et al. (2019). miR-137 mediates the functional link between c-Myc and EZH2 that regulates cisplatin resistance in ovarian cancer. Oncogene ***38***, 564–580.
109. Jacob, F., Pather, S.R., Huang, W.K., et al. (2020). Human pluripotent stem cell-derived neural cells and brain organoids reveal SARS-CoV-2 neurotropism predominates in choroid plexus epithelium. Cell Stem Cell ***27***, 937–950.e9.

ACKNOWLEDGMENTS

The authors sincerely appreciate the originating and submitting of genetic sequences of SARS-CoV-2 and metadata made available through GISAID (https://www.gisaid.org/). This research was funded by Hunan Provincial Innovation Platform and Talents Program (No. 2018RS3105), the Natural Science Foundation of China (No. 61803151), the Natural Science Foundation of Hunan province (No. 2018JJ3570), the Project of Scientific Research Fund of Hunan Provincial Education Department (No 19A060 and 19C0185), and the project to introduce intelligence from oversea experts to the Changsha City (Grant No. 2089901).

AUTHOR CONTRIBUTIONS

L.W. and J.Y. conceived and coordinated the research. T.L., T.H., C.G., and A.W. searched literature and wrote the manuscript. X.S., T.H., G.T., Q.L., J.S. and X.M. reviewed the manuscript.

DECLARATION OF INTERESTS

T.L., A.W., X.S., X.M., Q.L., T.H., G.T., and J.Y. are currently employed by Geneis Beijing Co., Ltd. All other authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xinn.2021.100116.

LEAD CONTACT WEBSITE

Jialiang Yang: https://www.researchgate.net/profile/Jialiang-Yang
Leyi Wang: https://www.researchgate.net/profile/Leyi-Wang-2