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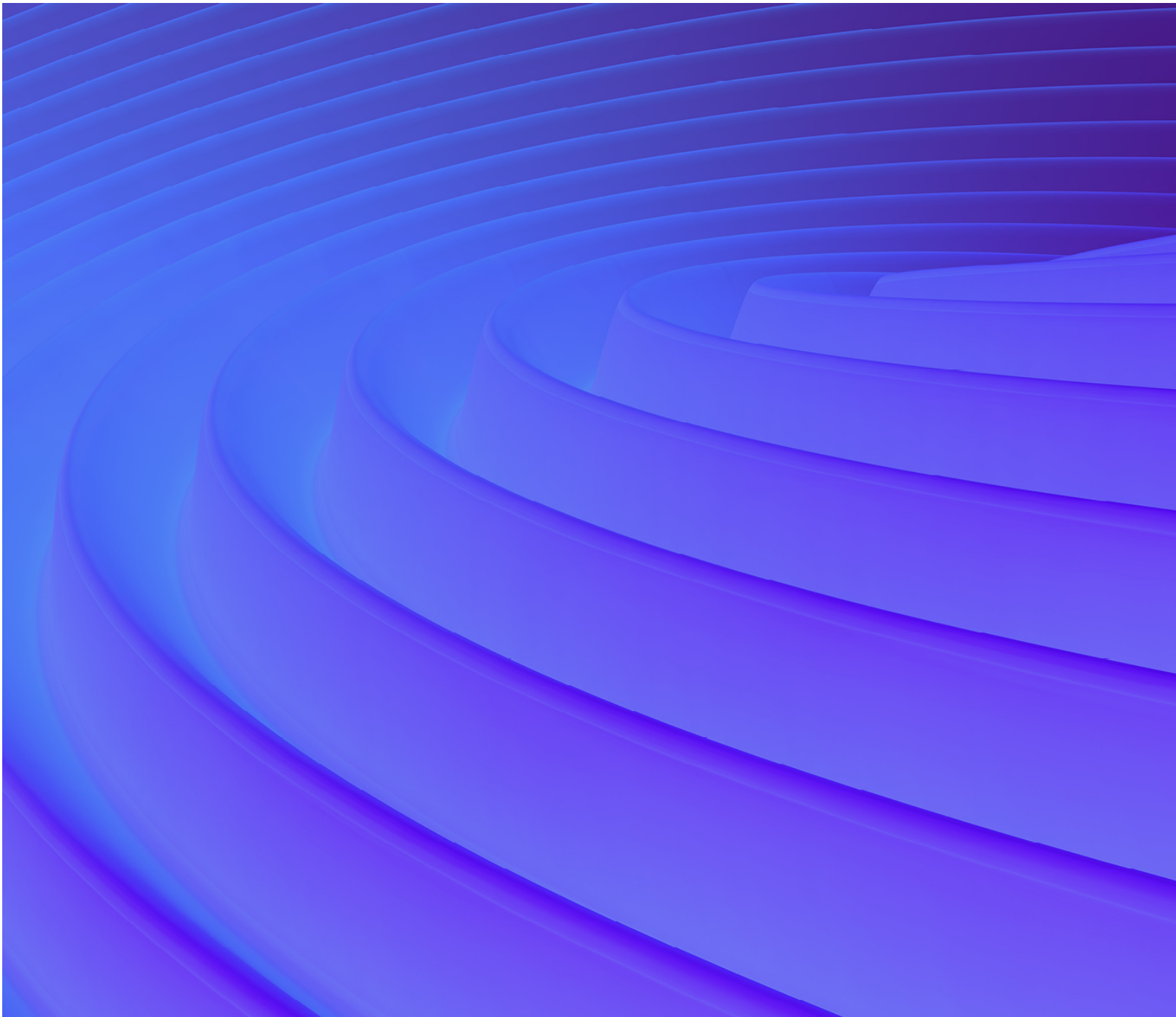
Disease Briefing: Coronaviruses



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Coronavirus: Disease Briefing

Facts about Coronaviruses

Coronaviruses are a group of large, enveloped, positive-sense, single-stranded RNA viruses belonging to the order Nidovirales, family Coronaviridae, subfamily Coronavirinae. More than two dozen different species are known and have been divided into four genera (alpha, beta, gamma and delta) characterized by different antigenic cross-reactivity and genetic makeup. Only the alpha- and betacoronavirus genera include strains pathogenic to humans and other mammals (Paules, C.I. et al (2020); Chen, Y. et al (2020)).

The first known coronavirus, the avian infectious bronchitis virus, was isolated in 1937 and was the cause of devastating infections in chickens. The first human coronavirus was isolated from the nasal cavity and propagated on human ciliated embryonic trachea cells in vitro by Tyrrell and Bynoe in 1965. However, coronaviruses have been present in humans for at least 500-800 years, and all originated in bats (Su, S. et al (2016); Yang, Y. et al (2020); Pooladanda, V. et al (2020)).

Coronaviruses have long been recognized as important veterinary pathogens, causing respiratory and enteric diseases in mammals as well as in birds. Until 2019, only six coronaviruses were known to cause disease in humans: HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory virus coronavirus (MERS-CoV) (Skariyachan, S. et al (2019); Bonilla-Aldana, D.K. et al (2020)). HCoV-229E and HCoV-NL63 are alphacoronaviruses; the rest are betacoronaviruses (Yang, Y. et al (2020)). The first four are endemic locally; they have been associated mainly with mild, self-limiting disease, although HCoV-HKU1 can cause pneumonia. SARS-CoV and MERS-CoV can cause severe illness (Song, Z. et al (2019); Paules, C.I. et al (2020)). SARS-CoV and MERS-CoV are among the pathogens included in the World Health Organization's Blueprint List of Priority Diseases (Bonilla-Aldana, D.K. et al (2020)).

Given the high prevalence and wide distribution of coronaviruses, their large genetic diversity as well as the frequent recombination of their genomes, and increasing activity at the human-animal interface, these viruses are recognized as an ongoing threat to human health (Hui, D.S. et al (2020); Zhu, N. et al (2020)). This fact again became strikingly evident in late 2019 and early 2020, when a novel coronavirus was discovered to be the cause of a large and rapidly spreading outbreak of lower respiratory tract infection and disease, including potentially fatal pneumonia, in Wuhan, China (WHO statement regarding cluster of pneumonia cases in Wuhan, China (World Health Organization, January 9, 2020); Coronavirus disease (Covid-19) pandemic (World Health Organization)). The virus--provisionally designated 2019-nCoV and later given the official name SARS-CoV-2, due to its similarity to SARS-CoV--was isolated and the viral genome sequenced. SARS-CoV-2 was characterized as a betacoronavirus and recognized as the seventh discrete coronavirus species capable of causing human disease (Zhu, N. et al (2020)). The disease caused by the virus was officially named Coronavirus Disease 2019 (Covid-19) by WHO.

Morphology, Structure and Replication

Coronaviruses are so named because of their characteristic solar corona (crown-like) appearance when observed under an electron microscope. This appearance is produced by the peplomers of the surface (or spike; designated S) glycoprotein radiating from the virus lipid envelope (Chen, Y. et al (2020); Yang, Y. et al (2020)).

Coronaviruses have four major structural proteins. The S glycoprotein is a major antigen responsible for both receptor binding and cell fusion (Song, Z. et al (2019)) and the membrane glycoprotein (M) is involved in budding and envelope formation; the M protein has also been found to play a pivotal role in virion assembly. The viral genome is associated with the basic phosphoprotein nucleocapside (N) within the capsid. The envelope (E) protein is a highly hydrophobic protein encasing the entire structure of the coronavirus. The genome is nonsegmented, positive single-stranded RNA of about 26-32 kb, making it the longest RNA viral

genome known, and contains at least six different open reading frames. The RNA molecule has a methylated cap in 5' and a poly-A tail in 3' (Schoeman, D. et al (2019); Chen, Y. et al (2020): Pillaiyar, T. et al (2020)).

Coronaviruses are capable of adapting quickly to new hosts through the processes of genetic recombination and mutation in vivo. As RNA viruses, coronaviruses rely on RNA-dependent RNA polymerase (RdRp) to replicate the virus genome. The intrinsic error rate of RdRp is approximately 1,000,000 mutation/site/replication, resulting in continuous point mutations. Point mutations alone are not sufficient to create a new virus, however; this can only occur when the same host is simultaneously infected with two coronavirus strains, enabling recombination. One coronavirus can gain a genomic fragment of hundreds or thousands base-pair long from another CoV strain when the two co-infect the same host, enabling the virus to increase its ecological niche or to make the leap to a new species (Raj, V.S. et al (2014); Gralinski, L.E. et al (2015)). This susceptibility enabled the emergence in approximately two decades of three new human coronavirus species with epidemic potential: SARS-CoV, MERS-CoV and SARS-CoV-2 (Chen, J. (2020)).

Epidemiology, Morbidity and Mortality

Coronaviruses, along with influenza, parainfluenza, RSV and rhinoviruses, cause mild, self-limited upper respiratory tract infections including the common cold (Pillaiyar, T. et al (2020)) and pneumonia. Coronaviruses are responsible for one-third of cold cases. Coronaviruses can also cause gastroenteritis in humans as well as a plethora of diseases in other animals (Berry, M. et al (2015); Su, S. et al (2016)). Unlike other coronaviruses pathogenic in humans, SARS-CoV, MERS-CoV and SARS-CoV-2 can cause severe acute respiratory disease, multi-organ failure and/or death.

In a comprehensive epidemiology study conducted over a nine-year period in Sao Paulo, Brazil, human coronaviruses were detected in 7.7% of respiratory samples analyzed. The researchers looked at 1,137 samples obtained from asymptomatic individuals, general community, patients with comorbidities and hospitalized patients. NL63 was the most frequently detected coronavirus overall (50.0%), followed by OC43 (27.3%), albeit with variations by year: in 2004, HCoV-229E was the predominant strain circulating (61.5%) (Cabeça, T.K. et al (2013)). 229E is distributed globally (Su, S. et al (2016)).

A study of 559 upper respiratory samples obtained from adults with acute respiratory infections in Beijing, China in 2014 showed that HCoV-OC43 was present in 12.5%, with prevalence peaking in autumn (Hu, Q. et al (2014)). OC43, which has diverged into five distinct genotypes, is distributed globally (Su, S. et al (2016)).

HCoV-NL63 was first isolated from a respiratory sample obtained from pediatric patients in different geographic areas in 2004. The virus, which is now known to be distributed globally, accounts for approximately 4.7% of common respiratory illness worldwide (Su, S. et al (2016)). HKU1 is less commonly isolated, causing a generally mild and self-limited infection that is indistinguishable from other respiratory viruses. It appears to be globally distributed (Su, S. et al (2016)).

An analysis of 686 adult patients presenting with acute respiratory infections in Mallorca, Spain (January 2013-February 2014) showed that 7% overall were caused by coronavirus, including 21.6% of patients in whom viral infection was implicated. The most prevalent strain identified was OC43 (50.0%), followed by NL63 (29%) and 229E (21%). Fifty-two percent of patients with CoV infections required hospitalization, and two patients required intensive care. No CoV infections were fatal in this study (Reina, J. et al (2014)).

A coronavirus that killed nearly 25,000 piglets in 2016-2017 in China emerged from horseshoe bats near the origin of the SARS-CoV, which emerged in 2002 in the same species of bats (Rhinolophus spp). The virus, named swine acute diarrhea syndrome coronavirus (SADS-CoV), has not been confirmed to infect humans (Zhou, P. et al (2018)).

Facts about SARS-CoV

Severe acute respiratory syndrome (SARS) was a viral illness caused by a novel coronavirus and affecting the respiratory system. It originated in the Chinese province of Guandong in November 2002 and soon spread throughout Asia, North America and Europe. Worldwide, 33 countries and regions on five continents reported SARS cases, although the most severely affected were mainland China and Hong Kong. By spring 2003, SARS became recognized as a global health threat. The rapid spread of the virus to different continents following the initial outbreak underscored the ease with which infectious diseases can be spread internationally within a highly mobile global population (Heymann, D.L. et al (2013); Yang, Y. et al (2020)).

Although the disease has been absent since 2003, the swift and extensive spread of SARS underlined the need for ongoing surveillance of this and related coronavirus, as well as the maintenance of capacity for rapid response should it reemerge. Equally important lessons of the SARS outbreak were the need for transparency in information sharing and the importance of international coordination of response (McCloskey, B. et al (2020)). In the post-SARS era, the government of mainland China has invested heavily in public health, infectious disease surveillance, response and reporting, enabling the country to respond more effectively to subsequent health threats such as H7N9 avian influenza (Zhang, Y. et al (2013)) and Covid-19 (Hui, D.S. et al (2020)).

The lessons learned from SARS have also been applied effectively on the international level in terms of response to the ongoing Middle East respiratory virus (MERS-CoV) outbreak, which emerged in 2012 and is caused by a different strain of coronavirus. These lessons were again put to test in 2020 with the emergence and explosive spread of Covid-19, initially in mainland China and later globally (Perlman, S. (2020)).

SARS-CoV Morphology, Structure and Replication

On March 24, 2003, scientists in Hong Kong and at the U.S. Centers for Disease Control and Prevention (CDC) reported the first preliminary evidence that a new coronavirus was the causative agent of SARS. On April 17, 2003, the WHO formally announced that the causative agent of SARS was a newly discovered member of the coronavirus family, which was not known to exist in humans before the disease was recognized. The new coronavirus was only distantly related to previously known and characterized coronaviruses (Berry, M. et al (2015)). Its origin was eventually traced to bats, with the masked palm civet (Civettictis civetta), a tree-dwelling cat, serving as a possible intermediary host that enabled the jump to humans (Song, Z. et al (2019)).

The SARS-CoV virion is spherical with an average diameter of 78 nm. The helical nucleocapsid is enclosed by an envelope (Goldsmith, C.S. et al (2004)) that is covered with club-shaped, long peplomers about 20 nm long, giving it the typical crown-like appearance.

The organization of SARS-CoV is similar to that of other coronaviruses, with the gene order being 5', replicase [rep], spike [S], envelope [E], membrane [M], nucleocapsid [N], 3', flanked by short untranslated regions (Du, L. et al (2009); Song, Z. et al (2019)). Sequences potentially coding for five more nonstructural proteins are interspersed between the ORF S and N.

The genome contains a total of 11 predicted open reading frames that potentially encode as many as 23 mature proteins (Ruan, Y.J. et al (2003)). The two principal ORFs, occupying about two-thirds of the genome, code for two major polyproteins, ORF1a and ORF1b. The polyproteins are cleaved by proteolysis to produce nonstructural proteins, the most important of which are the RNA-dependent RNA polymerase (Rep) and an ATPase helicase (Hel). The SARS-CoV has some genetic characteristics that are slightly different from other coronaviruses. There is a short anchor in the S protein, the number and location of the small ORFs are different, there is only one PLP-protease, and a unique, short lysine-rich region exists in the nucleocapsid protein. The biologic significance of these variations is unknown (Rota, P.A. et al (2003); Marra, M.A. et al (2003)).

Coronaviruses enter cells via binding to a host receptor followed by membrane fusion. ACE2 was identified as the cell receptor for SARS-CoV (Wan, Y. et al (2020)). The SARS-CoV virus

acidification of endosomes for a productive infection, suggesting a pH-dependent mechanism (Simmons, G. et al (2004)). Coronaviruses replicate in the cytoplasm, where viral RNA is synthesized in a specific, flask-shaped compartment surrounded by a double membrane (Gosert, R. et al (2002)). The SARS-CoV infection is associated with ultrastructural changes both in vivo and in cultured cells. These changes include formation of double-membrane vesicles, presence of nucleocapsid inclusions and granulations in the cytoplasm (Goldsmith, C.S. et al (2004)).

The first gene to be translated is a viral RNA polymerase, called replicase, which initially transcribes full-length, negative strand (or antisense) copies of the genome. These negative strands are then used as templates to produce mRNAs that transcribe viral genes. Those subgenomic transcripts are nested, and have identical 5' regions, non-translated, and a poly-A tail in 3'. The different, nested transcripts are not produced by splicing, but by the activity of the viral RNA polymerase. The viral RNA polymerase interacts with a repeated intergenic sequence (TRS, transcription regulating sequence) located between the viral genes and allows the link between the 5' leader sequence and the start of each gene. The replication mechanism has not been completely described, but it is likely to proceed through subgenomic-size, minus-strand RNAs containing the anti-leader sequence. Large granular areas containing viral RNA and proteins that are not seen in cells infected by other coronaviruses may be observed in cells infected by the SARS-CoV. These regions may be viral translation centers (Goldsmith, C.S. et al (2004); Song, Z. et al (2019)).

The viral particles assemble in the Golgi, accumulate in dilated vesicles that are then transported and secreted to the cell surface, where they are released by exocytosis.

The SARS-CoV has biological characteristics that differ from previously known coronaviruses. SARS-CoV is tropic for Vero cells (a cell line derived from the African green monkey kidney epithelial cells), it grows at 37°C in contrast to other coronaviruses that grow at lower temperature, and can infect the lower respiratory tract (Vicenzi, E. et al (2004)). The SARS coronavirus genome is between 29705 and 29751 nucleotides (NCBI Sequence Viewer: SARS coronavirus). The SARS virus genome did not match any of the three previously known groups of coronaviruses, and had only a weak antigenic relationship to coronaviruses 229E and OC43. The polymerase gene is closely related to the bovine and murine coronaviruses in group 2, but also has some characteristics of avian coronaviruses in group 3. The SARS-CoV does not have a hemagglutinin-esterase present in group 2 and some group 3 coronaviruses, but it has a single papain-like proteinase that is present in group 3 coronaviruses (Holmes, K.V. et al (2003)). The differences between SARS-CoV and other coronaviruses pointed to a new group (Marra, M.A. et al (2003); Rota, P.A. et al (2003)) that was phylogenetically equidistant from the three known groups at that time. A new coronaviruses group 4 was proposed, of which the SARS-CoV is the only member. The discovery of SARS-CoV drove the search for other, previously unknown, human coronaviruses. Two such viruses were identified shortly thereafter: HCoV-NL63 (2004) and HCoV-HKU1 (2005). Both appear to be distributed worldwide, and at least the former has been circulating in human populations for centuries (Berry, M. et al (2015); Abdel-Moneim, A.S. (2014)).

Transmission

The SARS coronavirus was transmitted through large droplets and via direct contact (Wong, S.S. et al (2008)). The virus can reach a concentration of about 100 million particles per mL in sputum (Drosten, C. et al (2003)) and can survive on contaminated surfaces and objects at room temperature for up to six days (Cleri, D.J. et al (2010)).

Two major factors contributed to the rapid spread of SARS: a highly mobile international population and high urban population densities (Arita, I. et al (2003)).

Attack rates were higher than 50% in the healthcare setting during the outbreak, while household transmission was less efficient (6-8%) (Goh, D.L. et al (2004); Lau, J.T. et al (2004)). Simulation studies performed after the outbreak suggested that physicians and other health care workers were the principal vectors of SARS transmission in the hospital setting (Cleri, D.J. et al (2010)). Practices such as use of ventilators and nebulized bronchodilators may cause aerosols

and spread of droplets containing virus. The risk of spreading the virus may also be increased by cardiopulmonary resuscitation, bronchoscopy, endotracheal intubation, airway and sputum suction (Cleri, D.J. et al (2010); Chen, W.Q. et al (2009)).

Virus load and shedding peaked at approximately 10 days from the appearance of clinical symptoms, when the patient's status worsened and required medical attention. Thus patients were most infectious at the time of seeking health care. Viral shedding continued for at least 13 more days (range 2-60 days) (Cleri, D.J. et al (2010)). Patients were not infectious during the incubation period (Zeng, G. et al (2009)).

A few patients were identified as SARS "superspreaders" who spread the virus efficiently because they harbored above-normal levels of virus (Yang, Y. et al (2020)). Superspreading seems to be associated with high virus titer, aerosol generation, contamination of the environment, and close contact with others in a healthcare setting (Cleri, D.J. et al (2010)).

Symptoms and Disease

The SARS-CoV preferentially infects the lower respiratory tract, resulting in a severe, acute viral pneumonia. The WHO case definition for probable SARS included high fever (>38°C) or history of fever in the previous 48 hours; new infiltrates on chest x-ray suggestive of pneumonia; flu-like symptoms (chills, cough, malaise, myalgia) or history of exposure to SARS-CoV; and one or more positive diagnostic tests for SARS (Cleri, D.J. et al (2010)). Unfortunately, the initial symptoms and clinical appearance were not easily distinguishable from other common respiratory infections, and fever was sometimes absent in older adults.

Analysis of both autopsy samples and experimentally infected animals indicates that the SARS-CoV infection in the lung affects the pneumonic areas and is detected in type 2 pneumocytes (Gralinski, L.E. et al (2015)). Morphological changes in tissues included diffuse alveolar damage, denudation of the bronchial epithelium, loss of cilia, and squamous metaplasia. Giant-cell infiltration, hemophagocytosis and cytomegalic alveolar pneumocytes were also observed in some cases (Liu, J. et al (2020)). The infection progresses through an inflammatory or exudative phase (characterized by hyaline-membrane formation, pneumocyte proliferation and edema), a proliferative phase and a fibrotic phase (Gralinski, L.E. et al (2015)).

The respiratory tract was the main target of the SARS-CoV, although the gastrointestinal tract could also be involved (Paules, C.I. et al (2020)). Infection of the central nervous system was also reported (Zhang, D.M. et al (2008)). Symptomatically, SARS generally followed a triphasic pattern. In the first week after infection, symptoms usually consisted of fever and myalgia. These early symptoms may have been related to direct viral cytopathic effects, since increases in viral load could be detected by PCR during this phase of the disease. Seroconversion was detected during the second week and was followed by a reduction of viral load. The innate immune response was insufficient to control the SARS-CoV infection because decreases in viral load are coincident with the specific antibody response (Peiris, J.S. et al (2003)). A third phase occurred in 20% of infected patients and was characterized clinically by disease progression that could not be explained by uncontrolled viral replication. This phase could be the result of an excessive and aberrant albeit ineffective host immune response, ultimately leading to SARS-associated lung damage and, potentially, death (Gralinski, L.E. et al (2015); Zumla, A. et al (2020)).

Symptoms of SARS during the 2003 outbreak were not identical in all patients. Nearly 100% of adults and children presented with fever, and approximately half with cough and/or myalgia. Only a few patients had upper respiratory symptoms. Diarrhea was reported in 11-15% of patients at presentation (Cleri, D.J. et al (2010)) and in up to 40-70% of hospitalized patients (Hui, D.S. (2005)). Lymphopenia, leukopenia, thrombocytopenia were detected in some patients. Elevation of enzymes such as lactate dehydrogenase, aspartate aminotransferase and creatinine kinase levels indicated an effect of SARS on the liver in some patients (Drosten, C. et al (2003): Cleri, D.J. et al (2010)). Others presented with symptoms unexpected in a respiratory infection, such as acute abdominal pain (Poutanen, S.M. et al (2003)). Pulmonary infiltrates were present on chest radiography. The changes in lung tissue pointed to damage inflicted by cytokines and chemokines (Gralinski, L.E. et al (2015)).

During the outbreak, about 40% of infected patients developed respiratory failure requiring assisted ventilation, however 90% of patients recovered within a week after the first appearance of symptoms. Smokers required mechanical ventilation more frequently than nonsmokers (Poutanen, S.M. et al (2003)). Older patients had greater morbidity and mortality, the result of an aging-related attenuation in the adaptive immune response (Frieman, M. et al (2008); Schäfer, A. et al (2014)).

Fatal SARS was the result of progressive respiratory impairment caused by damage to the lung alveoli. While the mortality rate during the SARS outbreak was <1% for patients under age 24 (Hui, D.S. et al (2010)), it increased to about 13% in patients under age 60, and was much higher (approximately 50%) in those over 60 and in those developing acute respiratory distress syndrome (approximately 50%) (Cleri, D.J. et al (2010); Schäfer, A. et al (2014)). The overall mortality rate during the outbreak was approximately 10%. Fatal cases of SARS-CoV infection were characterized by aberrant interferon signaling and a dysregulated adaptive immune response, or "cytokine storm" (Liu, J. et al (2020)).

Independent correlates of adverse clinical outcome included known history of diabetes/hyperglycemia, advanced age, male gender, comorbid hepatitis, high neutrophil counts at admission and high levels of lactate dehydrogenase, reflecting tissue necrosis related to the immune hyperactivity (Cleri, D.J. et al (2010); Hui, D.S. et al (2010)). A positive association was reported between air pollution and higher case-fatality rates (Cleri, D.J. et al (2010)). Host genetic variants may have also influenced variations in disease response (Schäfer, A. et al (2014)).

SARS infection was less prevalent as well as less aggressive in young children (Berry, M. et al (2015)). The highest rates of infection occurred in people of 20-39 years of age, whereas only 1% of cases occurred in children under age 10 years (Liang, W. et al (2004)). High rates among young adults may reflect cases among healthcare workers, while similar high rates in older people may be the consequence of nosocomial infections.

A prospective, observational study reported in 2007 was the first to provide comprehensive information regarding the long-term outcomes of SARS survivors. The 117 SARS survivors from Toronto, Ontario, underwent physical examination, pulmonary function testing, chest radiography and the six-minute walk test, filled out quality-of-life surveys and provided information regarding healthcare utilization at three different points (3, 6 and 12 months) following hospital discharge. The results showed that most SARS survivors had recovered fully from the physical illness by one year. However, general health, vitality and social functioning were below normal in many SARS survivors one year after illness, and many patients reported being unable to return to their pre-SARS level of work. Health care utilization, especially with respect to psychiatric care, was significantly higher than normal during the period of evaluation, and patients reported important decrements in mental health. Family caregivers of SARS survivors also reported suffering psychological consequences (Tansey, C.M. et al (2007)). A later study of 22 long-term survivors in Toronto established that chronic post-SARS morbidity persisted for up to 20 months after onset of illness. Symptoms included chronic widespread musculoskeletal pain, fatigue, depression and disordered sleep (Moldofsky, H. et al (2011)). A long-term follow-up study reported by Hong Kong researchers also found significant psychiatric morbidities and persistent fatigue in 233 SARS survivors at the fourth year of follow-up (Lam, M.H. et al (2009)); another Hong Kong follow-up study suggested that long-term impairment was more pronounced in health care workers (Ngai, J.C. et al (2010)).

Epidemiology and Cost of the SARS Epidemic

A total of 8,422 cases and 919 resulting deaths resulted worldwide during the SARS outbreak. Mainland China was hardest hit, with 5,328 cases and 349 deaths (Yang, Y. et al (2020)). Epidemiologic studies estimated that the average incubation time was 6.4 days. Mortality was 6.8% in younger patients and was as high as 43% in patients over the age of 60 years (Cleri, D.J. et al (2010)). The global case-fatality rate was 11% (Wong, S.S. et al (2008)), albeit with significant variation between regions (Lau, E.H. et al (2010)).

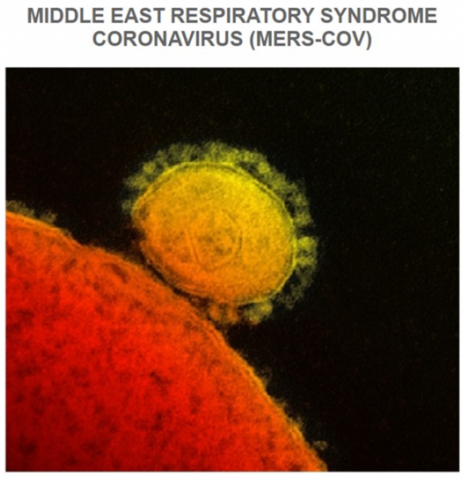
The SARS epidemic had important economic implications, with a global economic impact over two years estimated at between USD 40 billion (Ayittey, F.K. et al (2020)) and up to USD 100 billion (Paules, C.I. et al (2020)). The total economic impact of SARS in mainland China in 2003 has been estimated at USD 25.3 billion (Zhang, Y. et al (2013)), including losses to the tourism sector in Beijing alone estimated at USD 1.4 billion (Beutels, P. et al (2009)).

The rapid and effective containment of SARS just months after its international recognition was achieved thanks to an unprecedented international collaboration between researchers, healthcare providers and health authorities (Braden, C.R. et al (2013)). However, factors and circumstances that caused the emergence of SARS are not understood and a reemergence of the disease remains possible, particularly in light of the fact that animal reservoirs of this and other coronaviruses still exist (Berry, M. et al (2015); Yang, Y. et al (2020)).

For more epidemiology information, consult the Incidence and Prevalence Database (IPD): **IPD: Severe acute respiratory syndrome (SARS).**

Facts about MERS-CoV

In September 2012, WHO reported two cases of acute respiratory illness, ultimately fatal, accompanied by renal failure and caused by a previously unknown human coronavirus (Milne-Price, S. et al (2014); Chan, J.F. et al (2015)). The earliest known case has been traced to April 2012 (Chan, P.K. et al (2013)). The novel betacoronavirus responsible for the disease, formally named Middle East respiratory syndrome coronavirus (MERS-CoV), appears to have originated in bats (Zumla, A. et al (2015)) and uses dromedary camels as intermediate hosts (Cho, H. et al (2018)). Although it also pertains to the Coronavirinae family, the new virus was shown to be genetically different from the SARS coronavirus and to use a different host-cell receptor, identified as dipeptidyl peptidase 4 (DPP4, also known as CD26) (Li, F. et al (2019)). In a human lung epithelial cell assay, MERS-CoV was shown to elicit a distinct pattern of host gene expression responses. The virus is a cause for concern due to its zoonotic potential and the high case fatality rate (approximately 35%) (Li, F. et al (2019)).



Transmission electron micrograph of a single Middle East Respiratory Syndrome Coronavirus (MERS-CoV) virion. Credit: NIAID/RML

WHO has released interim guidelines for the appropriate care of patients in whom this infection is suspected (see **Clinical management of severe acute respiratory infection when Middle East respiratory syndrome coronavirus (MERS-CoV) infection is suspected - Interim guidance (World Health Organization, 2019)**). See **WHO Global Alert and Response (GAR): Coronavirus infections** and **CDC - Coronavirus home page** for up-to-date information from WHO and CDC.

MERS-CoV Morphology, Structure and Replication

MERS-CoV is a positive-sense, enveloped, single-stranded RNA virus with a genome size of 29.9 kB. It is classified as a betacoronavirus, and is more closely related to bat coronaviruses such as HKU4 and HKU5 than it is to SARS-CoV. Seroepidemiology studies have failed to uncover evidence of past infections with MERS-CoV in the general population of the affected geographic region, supporting the affirmation that this was a new virus (Chan, J.F. et al (2015)).

The genome arrangement of MERS-CoV is 5' - replicase - structural proteins (spike - envelope - membrane - nucleocapsid) - poly(A) - 3', similar to other coronaviruses. The virus has 10 open reading frames (ORFs) and 16 putative nonstructural proteins that are involved in the processes of viral transcription and replication (Chan, J.F. et al (2015); Skariyachan, S. et al (2019)).

The virus gains entry into the host cell by binding to DPP4 receptors expressed in the lower airway as well as in the kidney and other organs (Paules, C.I. et al (2020)). It uses host proteases to gain entry into lung cells. The protease furin activates the S protein on the viral envelope, mediating membrane fusion and enabling virus entry into the host cell (Banik, G.R. et al (2015); Tang, T. et al (2020)). Like the SARS-CoV, the Middle East respiratory virus is able to overcome the host innate immune response until high virus titres have been achieved, and induces cytokine dysregulation (Gralinski, L.E. et al (2015); Skariyachan, S. et al (2019)).

Transmission

The MERS-CoV virus presumably originated in bats, although it was initially unclear how it made the leap from bats to humans (Abdel-Moneim, A.S. (2014)). CDC investigators were first to identify dromedary camels as an intermediate or amplifying host and the most likely source of zoonotic transmission in the Middle East (Arabi, Y.M. et al (2017); Killerby, M.E. et al (2020)). Several possible routes of spread exist, including direct contact with the animals--particularly juvenile camels--and their bodily fluids, as well as meat handling and/or consumption of unpasteurized camels' milk (Widagdo, W. et al (2019); Killerby, M.E. et al (2020)).

Although it is primarily a zoonotic virus, nonsustained human-to-human transmission has been confirmed in 53-60% of all cases, albeit predominantly in health care settings and family clusters. Humans are considered terminal or transient hosts, however, with an R0 of <1 (Killerby, M.E. et al (2020)). Patients with severe to fatal infection are more likely to transmit the virus, since they shed a higher amount of virus progeny in comparison to those with asymptomatic or mild infection (Widagdo, W. et al (2019)). Like SARS-CoV, droplets are believed to constitute the principal mode of transmission of MERS-CoV (Cho, H. et al (2018)). Nosocomial spread, i.e. contamination via contact with virus on environmental surfaces, was also confirmed during the Korean outbreak in 2015 (Bin, S.Y. et al (2016); Cho, H. et al (2018)).

Symptoms and Disease

The incubation period is approximately 5 days (range 2-15 days), with 94% of patients showing signs of disease by day 12 (Chan, J.F. et al (2015)). Typical presenting symptoms are nonspecific and include fever, chills, nonproductive cough, dyspnea, rigor, headache, myalgia and malaise. Some patients present with gastrointestinal symptoms, including diarrhea, nausea and vomiting, and abdominal pain. Acute renal impairment is a unique feature of MERS and occurs with significantly greater frequency than was seen in patients with SARS (Song, Z. et al (2019); Paules, C.I. et al (2020)).

Pathological features of MERS-CoV infection include exudative pulmonary edema, diffuse alveolar damage with hyaline membranes, type II pneumocyte hyperplasia, interstitial pneumonia, and necrosis of the bronchial submucosal glands (Liu, J. et al (2020)).

Symptoms and manifestations of Middle East respiratory syndrome range from mild or asymptomatic infection to severe pneumonia, acute respiratory distress, septic shock and multiorgan failure resulting in death (Zumla, A. et al (2015); Zumla, A. et al (2016)). Respiratory failure with ARDS and multiorgan dysfunction syndrome are not uncommon, and the majority of patients with these complications will require admission to the intensive care unit within 2-5 days

of symptom onset. The median time from symptom onset to invasive ventilation and/or extracorporeal membrane oxygenation in these patients is 4.5 to 7 days (Chan, J.F. et al (2015)). Risk of severe disease is higher in men over age 45, people with preexisting medical conditions including diabetes, obesity chronic kidney disease, chronic cardiac disease and COPD (Chan, J.F. et al (2015); Zumla, A. et al (2016)), and in health care workers.

While the early case-fatality rate was close to 60%, this has decreased with improved awareness and surveillance; however, mortality remains above 35% (Al-Tawfiq, J.A. et al (2014); Chafekar, A. et al (2018)). The probability of a fatal outcome is much greater among patients aged 50 years and older as compared to younger patients (77% vs. 22%, respectively) (Cauchemez, S. et al (2014)). Mortality is also higher in men and in patients with multiple comorbidities (Banik, G.R. et al (2015); Chan, J.F. et al (2015)).

Epidemiology of MERS

Since September 2012, cases of MERS-CoV have been reported in 27 countries including Italy, the Netherlands, France, Germany, Italy, Tunisia, Malaysia, United Kingdom, United States, Iran, Egypt, Lebanon and Turkey (Chafekar, A. et al (2018); Middle East respiratory syndrome coronavirus (MERS-CoV) (World Health Organization), consulted March 19, 2020). Initial cases were restricted to the Middle East as well as two cases in the U.K. among family members of an infected individual who had recently traveled from Saudi Arabia. Several cases later occurred in clusters, including a hospital outbreak in Saudia Arabia, and confirmed that the virus can be transmitted between humans during close contact (Assiri, A. et al (2013); Zumla, A. et al (2015)). As of January 2020, the World Health Organization had been notified of 2,519 laboratory-confirmed human cases of infection with the virus and 866 resulting deaths (Middle East respiratory syndrome coronavirus (MERS-CoV) (World Health Organization), consulted March 19, 2020). The case-fatality rate remains extremely high: in excess of 30% (Salamatbakhsh, M. et al (2019)).

Published epidemiology figures reflect only the number of patients with clinical manifestations of MERS. However, a study of the general population of Saudi Arabia suggests that the rate of asymptomatic disease is much higher. Based on a serosurvey of individuals aged 15 and older who were seen by a health care professional or participated in a national burden-of-disease study between December 2012 and December 2013, nearly 45,000 people in that country were estimated to be seroprevalent for MERS-CoV, and may constitute a source of infection for individuals who do not come into contact with camels (Müller, M.A. et al (2015)). Moreover, a study of travelers to countries affected by MERS between September 2012-2016 has enabled a more precise estimate of the number of severe MERS cases in those countries (Saudi Arabia, United Arab Emirates, Jordan and Qatar). The researchers estimated that approximately 3,300 cases of severe disease occurred in that span of time, a number that is 2.3 times greater than the total number of laboratory-confirmed infections (O'Hagan, J.J. et al (2016)).On May 20, 2015, the index case in what became the largest outbreak of MERS-CoV outside the the kingdom of Saudi Arabia was reported in the Republic of Korea. The index patient had recently traveled to four countries in the Middle East, and returned to Korea while still asymptomatic. Between May 2015 and June 2016, there were 185 laboratory-confirmed cases, including 38 fatalities, in Korea, as well as an additional case in China. The outbreak cost the central government of the Republic of Korea USD 860 million in concept of quarantine system reform, emergency support for hospitals and other MERS response activities,and loans for affected medical institutions. Direct medical costs of the outbreak were approximately USD 12 million (Joo, H. et al (2019)).

The epidemiology of new MERS infections appears to follow a seasonal pattern, with outbreaks in the spring of 2013, 2014 and 2015 coinciding with the months when camels give birth (Al-Tawfiq, J.A. et al (2014)).

Although the data is still evolving, the basic reproduction number (R0) for the MERS-CoV is generally considered to be less than 1, indicating low pandemic potential unless the virus mutates (Killerby, M.E. et al (2020)).

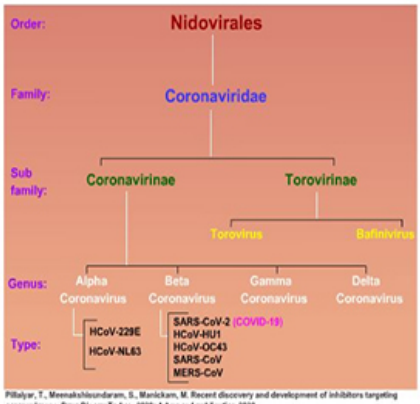
For more epidemiology information, consult the Incidence and Prevalence Database (IPD): **IPD: Middle East respiratory syndrome coronavirus (MERS-CoV)**.

Facts about COVID-19

In December 2019, a new coronavirus began causing febrile respiratory illness in mainland China; two months later, the rapidly spreading disease was officially named Coronavirus Disease 2019 (Covid-19) by WHO (Lai, C.C. et al (2020)). Earliest reports of the illness were issued by doctors in the densely populated city of Wuhan, Hubei province. Index cases were linked to the Huanan wholesale seafood market, which was immediately closed. Although the initial cases were traced to zoonotic transmission, human-to-human transmission was soon documented, both in healthcare settings and in familial clusters (Chan, J.F. et al (2020); Li, Q. et al (2020)). In fact, following the initial leap across the species barrier, human-to-human transmission quickly became responsible for widespread and rapid dissemination of the virus across populations with no preexisting immunity (Chen, J. (2020)); the disease spread from a single focal point across the entire country of China in just 30 days (Wu, Z. et al (2020)). By the end of January 2020, the new coronavirus had already established a foothold on four continents (Asia, Australia, Europe and North America) (Triggle, C.R. et al (2020)).

The pathogen--originally termed 2019-nCoV and later designated SARS-CoV-2--was sequenced and identified as a betacoronavirus belonging to the sarbecovirus subgenus, with approximately 80% similarity in genetic sequence to SARS-CoV (Zhu, N. et al (2020); Perlman, S. (2020)) overall, and more than 90% sequence identity with respect to various essential enzymes (Morse, J.S. et al (2020)). The new virus is even more closely related (more than 90% sequence homology) to Bat-CoV-RaTG13, which was previously identified in Rhinolophus affinis (intermediate horseshoe bat) from Yunnan Province (Yang, Y. et al (2020); He, F. et al (2020)).

Coronavirus Taxonomy

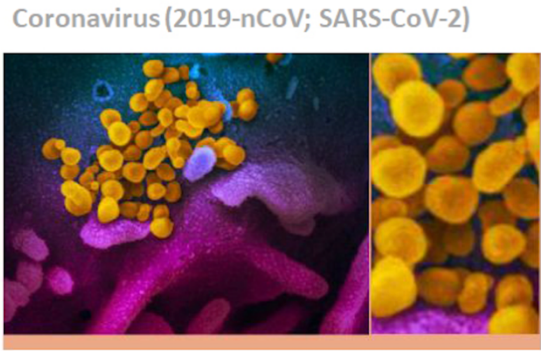


SARS-CoV-2 Morphology, Structure and Replication

The SARS-CoV-2 viral genome is a single-stranded, positive-sense RNA with 10 open reading frames (ORFs) encoding for four structural (S, E, M and N), 16 nonstructural (including 3-chymotrypsin-like protease, papain-like protease, helicase, and RNA-dependent RNA polymerase) and several accessory proteins (Li, G. et al (2020); Tang, T. et al (2020); Pooladanda, V. et al (2020)).Like SARS-CoV, the new coronavirus deploys the densely glycosylated S protein (which consists of S1 and S2 subunits) for virus-host cell receptor interaction and viral entry via the membrane pathway (Walls, A.C. et al (2020)), with ACE2 serving as its binding receptor on the host cell (Wan, Y. et al (2020)). The S protein is also the major antigenic determinant, and is targeted by the host antibody response (Tang, T. et al (2020)). The S1 subunit, as the receptor

binding domain, engages with the ACE2 receptor on the host cell surface (Kumar, G.V. et al (2020); Walls, A.C. et al (2020)). The virus uses the host cellular serine protease TMPRSS2 for S protein priming (also known as cleavage). During this step, which is essential for entry into the host cell, the S protein is cleaved and its subunits are separated, exposing the S2 subunit, which mediates cell membrane fusion (Hoffmann, M. et al (2020); Profile of a killer: The complex biology powering the coronavirus pandemic (Nature News, May 4, 2020)). ACE2 receptors are expressed on a variety of cells, including arterial and venous endothelial cells, arterial smooth muscle, upper and lower respiratory tract epithelial cells, small intestinal epithelial, renal and immune cells (Madjid, M. et al (2020)). However, TMPRSS2 must be expressed on the same cell at the same time in order for the virus to successfully enter. ACE2/TMPRSS2 co-expressing cell subsets--and hence, those at the greatest risk of infection--include mucus-secreting goblet cells in the nose, type II pneumocytes in the alveoli of the lung, and absorptive enterocytes in the intestines. In the absence of host proteases, however, coronaviruses are alternatively able to penetrate the host cell via clathrin- and non-clathrin-mediated endocytosis (Tang, T. et al (2020)).

Due to its similarities to SARS virus, the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV) named the new virus SARS-CoV-2. The native animal host of SARS-CoV-2 is presumed to be a bat; a wild animal--most likely, a pangolin--is believed to have served as an amplifying intermediate host (Lu, R. et al (2020); Yang, Y. et al (2020); Zhang, T. et al (2020)), as bat-derived coronaviruses cannot directly infect humans (Wang, R. et al (2020)). The binding affinity of the SARS-CoV-2 S protein for ACE2 is 10- to 20-fold greater than that of SARS-CoV, which may help to explain its more rapid spread through human populations (He, F. et al (2020); Wrapp, D. et al (2020)).



Source: NIAID-RML NIAID had producted images of the novel coronavirus (SARS-CoV-2, previously known as 2019-nCoV) on its scanning and transmission eletron microscopes on Tuesday Feb 11, 2020. SARS-CoV-2 causes COVID-19 disease, which has grown to be a global public health emergency since cases were first detected in Wuhan, China in December 2019.

Transmission

Person-to-person transmission of the virus is primarily via inhalation of suspended respiratory secretions, i.e., droplets generated when an infected individual coughs, sneezes or speaks, or through direct contact with an infected patient (Lai, C.C. et al (2020); Guo, Z.-D. et al (2020)). There is increasing evidence that the virus can also be transmitted in aerosolized microparticles of saliva, e.g., those produced when speaking, and that in a confined environment, such particles can remain suspended in the air for up to 14 minutes (Stadnytskyi, V. et al (2020)). Viral load in saliva peaks at presentation and remains high for at least the first week of symptomatic illness, gradually declining thereafter but remaining detectable for 20 days or more (Kai-Wang, K. et al (2020)). The virus can also be transmitted via fomites. It remains viable for up to 24 hours on cardboard and for up to 72 hours on plastic and stainless steel (van Doremalen, N. et al (2020)). Infectious droplets and body fluids can also contaminate the human conjunctival epithelium, producing ocular complications that may then progress to respiratory infection; this route of transmission was reported in Wuhan, China (Lu, C.W. et al (2020)). At later stages of infection, viral persistence has been detected in anal swabs, blood and serum, suggesting additional shedding mechanisms and the potential for transmission via the oral-fecal or body fluid routes (Zhang, W. et al (2020)). The risk of vertical transmission remains unclear. No virus

was detected in amniotic fluid, cord blood, neonatal throat swab or breastmilk samples obtained from six women developing laboratory-confirmed Covid-19 in late pregnancy (Chen, H. et al (2020)). In another sample of 33 Covid-19-positive mothers, three infants presented with early-onset SARS-CoV-2 infection, despite strict infection control and prevention procedures during the delivery. Vertical maternal-fetal transmission could not be ruled out in this cohort (Zeng, L. et al (2020)).

A study of the transmission dynamics in the first 425 confirmed cases in Wuhan concluded that SARS-CoV-2 is extremely contagious, and estimated a basic reproduction number (R0) of 2.2 (Li, Q. et al (2020)); later studies with more data suggested a higher R0 of 2.24-3.58 (Lai, C.C. et al (2020)). An active monitoring study of U.S. patients infected with Covid-19 found that the symptomatic secondary attack rate was just 0.45% among all close contacts, but increased to 10.5% among household members (Burke, R.M. et al (2020)), while a contact tracing study in Taiwan found an overall secondary clinical attack rate of 0.7%, increasing to 4.6% in household family contacts and 5.3% in nonhousehold family contacts (Cheng, H.Y. et al (2020)). A retrospective cohort study analyzed 391 Covid-19 cases and 1,286 close contacts in the Chinese city of Shenzhen between January 14 and February 12, 2020. The household secondary attack rate in this cohort was 11.2%, with children as likely as adults to become infected but less likely to have severe symptoms. The R0 was 0.4 (Bi, Q. et al (2020)). Similar to SARS, superspreading events have been reported during the Covid-19 outbreak (Liu, Y. et al (2020)).

It soon became apparent that the infection could be transmitted by individuals during the prodromal period (Heymann, D.L. et al (2020)), as well as by those who remain asymptomatic throughout their infection (Yang, Y. et al (2020)). According to a study of 28 infector-infectee pairs, the serial interval--the time from symptom onset in a primary patient to the onset of symptoms in a secondary patient--of Covid-19 (4.0 to 4.6 days) is close to or shorter than its median incubation period (5.1 days). This finding is significant because it suggests a more important role of presymptomatic transmission, implying that the isolation of cases as a means of curtailing the outbreak might not be as effective as once believed (Lauer, S.A. et al (2020); Nishiura, H. et al (2020)). This finding was reinforced in a study of 77 transmission pairs, in which 44% of secondary cases were infected during the presymptomatic stage of the index case's illness. Assuming an incubation period of 5.2 days, viral shedding began 2.3 days before symptom onset, peaking at 0.7 days before onset of symptoms, and appeared to decline quickly over the next 7 days (He, X. et al (2020)).

Symptoms and Disease

The incubation period ranges from 2-14 days (median 5.1 days) (Lauer, S.A. et al (2020)), after which Covid-19 disease manifestation varies widely, from an asymptomatic carrier state to pneumonia or acute respiratory distress syndrome (ARDS). Up to 30% of infections are asymptomatic (Lai, C.C. et al (2020); Nishiura, H. et al (2020)). Symptomatic respiratory illness ranges from mild to severe, with symptoms that include (from most to least common) fever, cough, dyspnea, myalgia, headache and diarrhea. Chest CT scan reveals the presence of bilateral ground-glass opacities (Huang, C. et al (2020); Wu, Z. et al (2020); Lai, C.C. et al (2020)). First reported anecdotally, acute anosmia and/or ageusia (sudden loss of olfactory and gustatory function, respectively) is now recognized as a strong indicator of Covid-19, particularly of mild to moderate severity (Vaira, L.A. et al (2020)).

In an early description of 41 clinical cases in Wuhan, China, clinical presentations were very similar to those of SARS and included fever (98%), cough (76%) and myalgia or fatigue (44%). All patients had pneumonia with abnormal findings on chest CT; 32% had underlying diseases including diabetes, hypertension and cardiovascular disease. The most severely ill patients developed ARDS, a syndrome characterized by the acute onset of hypoxemic respiratory failure with bilateral infiltrates, requiring ICU admission and oxygen therapy. Critically ill patients showed elevated plasma levels of IL-2, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A and TNF-alpha--a so-called "cytokine storm"--, corresponding with disease severity. The mortality rate in this early patient set was approximately 15% (Huang, C. et al (2020); Zumla, A. et al (2020)), and primarily involved older patients with serious underlying diseases or conditions. A later analysis of a larger group of Chinese patients (N = 44,672) found an overall mortality rate of 2.3%, which increased

with age, from zero in children under 9 to 14.8% in those over 80 (Unknown Author (2020); Wu, Z. et al (2020)). The presence of comorbidities, including cardiovascular disease and diabetes, was shown in various patient series to be associated with a significantly higher risk of mortality (Madjid, M. et al (2020)). Other factors associated with poor prognosis include indicators of increased disease severity (oxygenation, respiratory rate, leukocyte/lymphocyte count, chest imaging findings), disseminated intravascular coagulation, older age and delay in diagnosis. Male gender and African American race have been linked to more severe disease and worse outcomes in several patient groups (Lai, C.C. et al (2020)).

As the numbers of infected and seriously ill patients grow worldwide, there is a growing understanding of the full scope and impact of SARS-CoV-2 infection on the body. In addition to its well-known respiratory effects, the viral infection--as well as the drugs used to treat it and the host inflammatory and immune response--are capable of potentially causing much broader devastation affecting organs ranging from the brain and eyes to the gastrointestinal tract, liver, kidneys, heart and circulatory system (How does coronavirus kill? Clinicians trace a ferocious rampage through the body, from brain to toes (Science news, April 17, 2020)) as well as peripheral and central nervous system and skeletal muscle (Mao, L. et al (2020)). An usually high incidence of disseminated intravascular coagulation, localized pulmonary thrombotic microangiopathy and venous thromboembolism has been reported in severely ill patients as well as those with only mild to moderate disease; coagulopathy in the Covid-19 patient population is driven by the immune response and is associated with increased risk of fatal outcome (Tang, N. et al (2020); Levi, M. et al (2020)). In a preliminary report of 184 Dutch ICU patients with laboratory-confirmed Covid-19 pneumonia, the incidence of thrombotic complications was remarkably high: 31% (Klok, F.A. et al (2020)); in a follow-up analysis of the same 184 critically ill patients, the investigators found that the incidence of a composite endpoint comprising symptomatic acute pulmonary embolism, deep vein thrombosis, ischemic stroke, myocardial infarction and/or systemic arterial embolism was 49%. Of special concern, large-vessel ischemic stroke and other severe consequences of thrombotic disease have been described as presenting symptoms of Covid-19 in young, otherwise healthy individuals (Oxley, T.J. et al (2020)).

A challenge for scientists studying Covid-19 is that those who are infected with subclinical or mild disease might not present to health care centers, impacting the accuracy of total case counts and calculation of case-fatality rates. Moreover, asymptomatic, seemingly healthy individuals can spread the virus to their contacts at home and at work as well as during travel (Munster, V.J. et al (2020); Bai, Y. et al (2020)).

Epidemiology

According to WHO, as of June 5, 2020, more than 6.5 million laboratory-confirmed cases of Covid-19 had been diagnosed and reported in 216 countries, areas or territories worldwide. Although early cases were concentrated in mainland China, the outbreak in that country began slowing in late February, at the same time that it began picking up in other countries. Beginning on February 25, more new cases of Covid-19 were reported each day from countries outside China than from mainland China itself; a week later, the number of daily deaths outside mainland China began to surpass those inside the country, and on March 16, the total number of cases outside mainland China overtook the total number of Chinese cases. As of June 5, WHO had confirmed 387,298 deaths from Covid-19 worldwide (Coronavirus disease (Covid-19) pandemic (World Health Organization), consulted June 5, 2020). For contrast, SARS-CoV caused more than 8,000 symptomatic infections resulting in 800 deaths, and MERS-CoV to date (as of February 4, 2020) has infected 2,494 individuals and caused 858 deaths (Wu, J.T. et al (2020)). On January 30, under recommendation from the International Health Regulations (2005) Emergency Committee, the Director-General of WHO declared the Covid-19 outbreak a Public Health Emergency of International Concern (PHEIC) (Unknown Author (2020)). On March 11, WHO characterized the outbreak a global pandemic (WHO Director-General's opening remarks at the media briefing on COVID-19 -- 11 March 2020 (World Health Organization press release)). To track the outbreak in real time, click here: Coronavirus

COVID-19 global cases dashboard (Johns Hopkins University Center for Systems Science and Engineering).

The rapid spread and ease of transmission of the virus are causing global alarm. Experts point out that although the virus poses a relatively low health threat at the individual level, it is easily transmissible and thus poses a significant risk at the population level. Careful surveillance of SARS-CoV-2 virus is critical to monitor its future host adaption, viral evolution, infectivity, transmissibility and pathogenicity (Huang, C. et al (2020)). Modeling studies suggest that the initial, most severe pandemic wave of SARS-CoV-2 will be followed by recurrent wintertime outbreaks until either herd immunity is achieved or a vaccine is widely available. If immunity is short-lasting, pandemic waves may recur every year or two. If immunity is permanent, however, the virus could largely disappear within five or more years of the first major outbreak (Kissler, S.M. et al (2020)).

For more epidemiology information, consult the Incidence and Prevalence Database (IPD): IPD: Covid-19 (2019 novel coronavirus).

Morbidity and Mortality

The case-fatality rate in a study of the first 44,000 cases of Covid-19 in mainland China was 2.3% (Unknown Author (2020)). Mortality among symptomatic patients is estimated to be in the range of 0.5% to 4%, while among patients who require hospitalization, the rate increases to 5% to 15%. In the early Hubei Province case series, mortality among critically ill patients ranged from 22% to 62%. These numbers will change as the outbreak evolves (Murthy, S. et al (2020)). As the pandemic continued to progress, and taking into account the total spectrum of disease, the overall case-fatality ratio in mainland China was estimated in a modeling study at 1.38%, ranging from 0.31% in those under 60 years of age to 6.38% in those 60 years and older. In an international sample of patients included in the same modeling study, the case-fatality ratio was estimated at 2.7% overall, ranging from 1.4% in patients aged < 60 years to 4.5% in those aged 60 years and older (Verity, R. et al (2020)).

In Italy, a much higher case-fatality rate of 7.2% (as of March 17, 2020) was reported. Three factors that potentially explain this difference were identified: the older age of the population (23% aged 65 years or older), testing strategies (only symptomatic individuals are tested) and the definition of Covid-19-related death applied in that country. Uniform testing and reporting guidelines are needed in order to generate standard epidemiology data across all affected countries (Onder, G. et al (2020)). Throughout the entire EU/European Economic Area (EEA) plus U.K., the Covid-19 mortality rate among hospitalized patients was estimated to be 22% (Covid-19 surveillance report - Week 21 (European Centre for Disease Prevention and Control), consulted June 4, 2020).

Early in the U.S. outbreak, the mortality rate among residents of a long-term care facility in Washington state was 33.7% (McMichael, T.M. et al (2020)).As the pandemic progresses, racial differences in outcomes have increasingly been reported, particularly in the U.S. A retrospective cohort study explored racial and ethnic differences in the clinical course and outcomes of U.S. Covid-19 patients. The study was conducted in Louisiana and included patients from an integrated-delivery health system from March 1 and April 11, 2020. A total of 3,481 Covid-19 patients were included in the study, of whom 1,382 were hospitalized (39.7%; 319 white non-Hispanics and 1,063 black non-Hispanics). Among hospitalized Covid-19 patients, 76.9% were black. The hospital course of Covid-19 patients who were hospitalized during the study period was reported as follows for white versus black non-Hispanics: coinfection with pneumonia, 36.4% vs 38.3%; acute renal failure, 10.7% vs 15.3%; acute hepatic injury, 0.6% vs 0.2%; cardiomyopathy or congestive heart failure, 0% vs 0.2%; hypoxic respiratory failure, 24.8% vs 25.4%. The clinical outcomes of Covid-19 patients who were hospitalized in the same period were reported as follows for white versus black non-Hispanics: still admitted, 4.1% vs 4.6%; discharged alive from the hospital, 65.8% vs 73.8%; died, 30.1% vs 21.6%. The median length of hospital stay in the two groups was 7.0 days and 6.0 days, respectively. Of 326 patients who died from Covid-19, 70.6% were black (Price-Haywood, E.G. et al (2020)).

The leading causes of death in patients with Covid-19 are respiratory failure subsequent to ARDS (70%) and sepsis (28%) (Tay, M.Z. et al (2020)). An autopsy study of 12 consecutive Covid-19 deaths in Hamburg, Germany, revealed deep venous thrombosis in 7 of 12 patients (58%); venous thromboembolism was not suspected before their deaths. In four patients, pulmonary embolism was the direct cause of death (Wichmann, D. et al (2020)).

Risk of death is highest among the elderly and patients with comorbidities, but the disease affects individuals of all ages. An analysis of data on 2,135 pediatric cases of Covid-19 reported to the Chinese Center for Disease Control and Prevention between January 16 and February 8, 2020 confirmed that the disease does not spare children, although manifestations may be more mild. Infants were especially vulnerable (Dong, Y. et al (2020)). More recently, reports from Europe and North America have described clusters of children and adolescents requiring admission to intensive care units with a multisystem inflammatory condition with some features similar to those of Kawasaki disease and toxic shock syndrome. Case reports and small series have described a presentation of acute illness accompanied by a hyperinflammatory syndrome, leading to multiorgan failure and shock. The WHO has developed a preliminary case definition and established a digital platform for standardized, anonymized clinical data collection in order to obtain greater understanding of this syndrome (Multisystem inflammatory syndrome in children and adolescents temporally related to COVID-19 -- Scientific brief (World Health Organization, May 15, 2020)).

U.K. researchers conducted a modeling study to estimate the overall impact of Covid-19 on mortality during government-mandated lockdowns. This includes not only deaths directly caused by the viral infection, but also excess deaths in individuals indirectly affected by the pandemic, e.g., by altered access to health services; the physical, psychological and social effects of distancing; or adverse economic changes. They estimated one-year mortality under various scenarios including full suppression, partial suppression, mitigation and doing nothing. Excess deaths in the U.K. under these scenarios ranged from 2-7 excess deaths with full suppression to between 18,374 and 73,498 in a mitigation scenario, and would be between 146,996 to 587,982 under a policy of "do nothing" (Banerjee, A. et al (2020)).

Economic Impact

In an intricately intertwined global economy, the costs of disruption due to the Covid-19 pandemic are becoming starkly apparent. This unprecedented event has interrupted global trade and supply chains, depressed asset prices, and forced multinational corporations to make difficult decisions based on limited and constantly evolving information (Ayittey, F.K. et al (2020)).

The eventual impact of the Covid-19 pandemic on industries including tourism, manufacturing, commerce and trade, as well as its impact on global supply chains, has been and will continue be vast, both within China and globally. According to one estimate, China's GDP year-on-year growth during Q1 2020 will be down at least 4.5%, while global GDP will be suppressed by approximately 0.42% during Q1. This is comparable to the World Bank's estimates of global GDP loss due to a severe influenza outbreak: 0.5%, equivalent to USD 300 billion, and dwarfs the losses attributed to the 2002-03 SARS outbreak, when China played a much smaller role in the global economy (Ayittey, F.K. et al (2020)).

The economic impact of the Covid-19 pandemic and resulting partial economic shutdown in the U.S. has been estimated at 5% of GDP per month, equivalent to USD two trillion if said shutdown lasts for two months (Walensky, R.P. et al (2020); Policy brief - The cost of COVID-19: A rough estimate of the 2020 US GDP impact (Mercatus Center at George Mason University, April 2020)).

Diagnosis

During the SARS epidemic, the FDA and CDC collaborated on the validation and licensing of SARS diagnostic tests. Approaches to diagnostic testing include serologic detection, virus isolation in cell culture, electron microscopy and detection of viral RNA by molecular methods.

Both ELISA and immunofluorescent serologic tests for detecting coronavirus antibodies were developed (Suresh, M.R. et al (2008)). The availability of RNA sequence information on a number of strains of SARS viruses facilitated the subsequent development of rapid diagnostic tests. Molecular tests based on reverse transcription polymerase chain reaction (RT-PCR) detect viral RNA. RT-PCR was an early detection test available for SARS-CoV, but its sensitivity was low, identifying only 37.5-50% of probable cases (Suresh, M.R. et al (2008)).

Two-step conventional and one-step quantitative RT-PCR techniques were routinely used during the SARS outbreak (Peiris, J.S. et al (2008)). A report from the CDC indicated that real-time RT-PCR was more sensitive than conventional RT-PCR, potentially providing a useful technique for detecting virus in the early phases of the diseases, when virus titer was low (Emery, S.L. et al (2004)). ELISA detection of anti-nucleocapsid protein (NP) antibodies, which peak early in infection, was identified by Canadian investigators as a more reliable and specific method of diagnosing SARS (Suresh, M.R. et al (2008)).

Various diagnostic tests have been used in the detection of MERS-CoV infection, including serological assays, immunofluorescence assays, ELISA, protein microarray, micro-neutralization assays and Western blot--all of which have limitations (Banik, G.R. et al (2015))--, as well as RT-PCR, which is most specific and sensitive (Skariyachan, S. et al (2019)). WHO recommends that screening RT-PCR target the upE gene, and that positive samples be retested targeting the ORF1a, ORF1b or N gene. Testing should use samples obtained from the lower respiratory tract, e.g., bronchoalveolar lavage or tracheal aspirate, where viral load is greatest (Banik, G.R. et al (2015); Zumla, A. et al (2015)). However as the procedure for collecting these specimens is invasive, upper respiratory specimens are sometimes used instead (Chan, J.F. et al (2015)).

Researchers at the University of Texas and NIH have developed asymmetric five-primer reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays for the detection of MERS-CoV. The RT-LAMP assays are designed to amplify MERS-CoV genomic loci located within the ORF1a and ORF1b genes and the upE gene, and will enable the development of portable point-of-care diagnostics (Bhadra, S. et al (2015)).

In December 2019, a novel coronavirus, later identified as SARS-CoV-2, was first identified in samples taken from three patients with acute respiratory disease in Wuhan, China. The genetic sequence of SARS-CoV-2 was made available to the WHO on January 12, 2020, facilitating the production of specific diagnostic PCR tests to detect the novel coronavirus infection (Hui, D.S. et al (2020); Zhu, N. et al (2020)). The virus was first isolated from bronchoalveolar lavage fluid; however, viral RNA has also been detected in blood and stool samples (Wang, W. et al (2020)). With increased experience, the most commonly used diagnostic samples are those taken from the upper (nasopharyngeal) or lower (induced sputum, endotracheal aspirates, bronchoalveolar lavage) respiratory tract (Murthy, S. et al (2020)). The Beijing Center for Disease Prevention and Control and the University of Hong Kong (Chu, D.K.W. et al (2020)) as well as several Chinese biotech companies have developed nucleic acid test kits. Aiming to shorten the diagnosis time, Jiangsu Qitian Gene Technology together with the National Institute for Viral Disease Control and Prevention, developed test kits with an isothermal amplification instrument that automatically interprets the results in minutes, with both sensitivity and specificity values of 100%.

On February 5, 2020, the U.S. FDA issued an emergency use authorization that would allow emergency use of the CDC's own 2019-nCoV Real-Time RT-PCR Diagnostic Panel (FDA takes significant step in coronavirus response efforts, issues emergency use authorization for the first 2019 novel coronavirus diagnostic (FDA news release, February 4, 2020)). The diagnostic is a RT-PCR test for the detection of SARS-CoV-2 from respiratory secretions (nasal or oral swabs), but was initially plagued with a high rate of inconclusive or invalid results (Sharfstein, J.M. et al (2020)). Novacyt has also launched a quantitative PCR assay, targeting the unique SARS-CoV-2 genome sequences without the need for cold chain shipping. In addition, Co-Diagnostics is using the Coprimer multiplexing technology to differentiate between similar genetic sequences, thereby reducing false positive diagnosis. Also, at Meridian Bioscience, the molecular diagnostic test (Meridian Lyo-Ready 1-Step RT-qPCR Mix) can be prepared and freeze-dried, making it highly stable and only requiring the addition of the patient sample to run the assay. In Europe, among others, Ares Genetics is collaborating with BGI Group to make real-

time fluorescence PCR tests for the new coronavirus, producing results in several hours. On March 21, the FDA granted emergency use authorization of the first rapid, point-of-care diagnostic for the U.S. market: Cepheid's Xpert Xpress SARS-CoV-2. The test has been designed to operate on any of Cepheid's more than 23,000 automated GeneXpert Systems worldwide, with a detection time of approximately 45 minutes.

Hong Kong researchers developed three different PCR assays targeting the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp)/helicase (Hel), spike (S) and nucleocapsid (N) genes, and compared each of them with the RdRp-P2 assay used in many European laboratories. They found that the COVID-19-RdRp/Hel assay did not cross-react with other human-pathogenic coronaviruses and respiratory pathogens in cell culture and clinical specimens; in contrast, the RdRp-P2 assay did cross-react with SARS-CoV in cell culture (Chan, J.F. et al (2020)).

Patients testing positive for Covid-19 on PCR should undergo imaging studies in order to detect lung damage at the early stages. Non-contrast-enhanced chest computed tomography (CT) plays an important role at this stage of diagnosis, and enables the detection of bilateral, multifocal patchy ground glass opacities, which are characteristic chest CT imaging features of Covid-19 pneumonia (Xu, X. et al (2020); Li, Y. et al (2020)). Patients should also be tested for influenza and other viruses, as coinfection is associated with worse outcomes (Yang, Y. et al (2020)).

In the U.S., Mammoth Biosciences and Sherlock Biosciences are using CRISPR (clustered regularly interspaced short palindromic repeats)-Cas12 to develop rapid point-of-care (POC) SARS-CoV-2 tests (Fast, portable tests come online to curb coronavirus pandemic (Nature Biotechnology News, March 23, 2020)). Mammoth scientists have created the SARS-CoV-2 DETECTR, a CRISPR-Cas12 based lateral flow assay that detects SARS-CoV-2 from extracted patient sample RNA and takes 30 minutes to perform. The assay involves simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT-LAMP) from RNA extracted from nasopharyngeal or oropharyngeal swabs in universal transport media. This is followed by Cas12 detection of predefined coronavirus sequences and then cleavage of a reporter molecule confirms detection of the virus. Sherlock Biosciences developed the SHERLOCK CRISPR SARS-CoV-2 test, which works by programming a CRISPR molecule to detect the specific genetic signature for SARS-CoV-2 in a nasal, nasopharyngeal or oropharyngeal swab or specimen of bronchoalveolar lavage (BAL). When the signature is found, the CRISPR enzyme is activated and releases a detectable signal. In May 2020, Sherlock received Emergency Use Authorization (EUA) for the test kit, which provides results in less than one hour (First CRISPR test for the coronavirus approved in the United States (Nature News, May 8, 2020)).

Quantitative RT-PCR based assays need expensive lab instrumentation and are usually conducted in public health laboratories. The SARS-CoV-2 DETECTR assays have comparable accuracy to qRT-PCR, employ isothermal signal amplification for rapid target detection without the need for thermocycling, show single nucleotide target specificity, are integrated with portable, low-cost lateral flow strips, and follow a quick development cycle.

RT-PCR, RT-LAMP, RT-insulated isothermal PCR (RT-iiPCR) and one-step rRT-PCR assays diagnose SARS-CoV-2 genetic material (Pang, J. et al (2020)). Rapid diagnostics now include antibody tests that screen for immunoglobulins generated soon after a primary infection. An antibody test for SARS-CoV-2 has been given emergency use authorization by the FDA (qSARS-CoV-2 IgG/IgM Rapid Test - Letter of Authorization (FDA news release, April 1, 2020)). The NIH (NIH begins study to quantify undetected cases of coronavirus infection (National Institutes of Health news release, April 10, 2020)) and other laboratories such as Stanford Medicine are also studying similar tests.

Given that the SARS-CoV-2's RNA genome is stable, antibody tests indicate immunity to the virus. Separate research groups are using the virus's spike protein to detect circulating antibodies 2-15 days (severe cases) or 10-15 days (mild cases) after symptoms emerge. Neutralizing antibodies may signify protective capacity in vivo or indicate the presence of protective helper or cytotoxic T cells. Together, they may confer lifetime immunity or for a maximum period of three years, as noted for SARS and MERS.

Yet, several limiting factors regulate immune levels. All patients (mild/asymptomatic/severe infection status) may not bear similar potential to generate high antibody titers. Even if protective antibodies are triggered, it is unclear if they will also protect against future (secondary) infections. Furthermore, if antibody titers weaken with age that could explain geriatric case pathology. Antibody detection can lead the way to two therapies: herd immunity, where up to 75% of antibody-positive (immune) cases can mediate protection for the remaining population; and plasma therapy for severe cases (What Do Antibody Tests For SARS-CoV-2 Tell Us About Immunity? (The Scientist, April 15, 2020)).

The generation of an antibody response during natural infections has led to the introduction of "immunity passports" in the context of COVID-19 (WHO scientific brief, April 24, 2020) in Chile. An immunity passport declares that the holder has recovered from Covid-19 and allows travelers to clear airport security. France, the U.K. and some U.S. sectors are interested in incorporating such passports in their reopening strategy. The detection of antibodies to SARS-COV-2 form the basis for this "risk-free certificate", claiming that the holder is protected against re-infection. This privilege is based on the assumption that a single Covid-19 infection triggers necessary protective antibodies, moreover that immunity both at the individual and collective level (herd immunity) will slow down transmission and protect the uninfected cases. However, the WHO has cautioned that this assumption--that one-time infection leads to protective immunity--needs to be proven (). In South Korea, 2% of recovered cases have again tested positive. The corresponding rates in mainland China are 5-10%. Recovered patients can suffer relapses, either due to a second infection or reactivation of the virus reservoir in the body.

Differential Diagnosis

Pneumonia of other viral or bacterial origin --especially Streptococcus pneumonia, Haemophilus influenzae, Moraxella catarrhalis, methicillin-resistant Staphylococcus aureus and Legionella spp.-- were included in the differential diagnosis of SARS. Other febrile viral diseases included in the differential diagnosis were seasonal and avian influenza, respiratory syncytial virus, varicella zoster virus, human metapneumovirus and hantavirus. When appropriate, other epidemic or population-wide diseases were taken into consideration, e.g. smallpox, tularemia, anthrax, viral hemorrhagic fever or plague (Cleri, D.J. et al (2010)).

In the case of Covid-19, the differential diagnosis includes most of the aforementioned infections as well as noninfectious diffuse pulmonary diseases, e.g., dermatomyositis or vasculitis. Travel history and contact tracing will help to inform the diagnosis (Tian, X.L. et al (2020); Yang, Y. et al (2020)).

Prevention

Without effective drugs or vaccines against the infectious agents (Li, G. et al (2020)), society-level interventions such as isolation/social distancing, quarantine and community containment are the most effective means of controlling a coronavirus outbreak with epidemic potential (Wilder-Smith, A. et al (2020)). Although authorities may be reluctant to impose these measures due to their economic and social impact, the success of these strategies was demonstrated during the SARS outbreak in Singapore, where application of infection control measures resulted in a decrease in R0 (secondary infection rate) from 7 at week 1 to <1 after week 2 (Cleri, D.J. et al (2010)). Soon after the Covid-19 outbreak began to expand, Chinese authorities imposed restrictions on movement in and around Wuhan, the major air and train transportation hub of central mainland China. Transportation and activities throughout the country were subsequently limited (Wu, Z. et al (2020)). Based on assumptions of exponential growth of the outbreak (estimated R0 = 2.68), WHO-linked epidemiology experts recommend stringent controls in order to prevent independent, self-sustaining outbreaks in countries around the world (Wu, J.T. et al (2020)). This is especially important given the increasingly clear role of asymptomatic individuals, including children, in spreading Covid-19.

On the personal level, hygiene measures are recommended to prevent the spread of disease in situations where individuals are in contact with patients or contaminated fomites (Chen, Y. et al

(2020)). Washing hands with soap and water or with alcohol-based handrubs is effective for interrupting virus transmission. In general, coronaviruses are able to survive on metal, glass and plastic surfaces at room temperature for up to nine days, but can be inactivated by disinfection with ethanol (62-71%), hydrogen peroxide (0.5%) or sodium hypochlorite (0.1%) (Kampf, G. et al (2020)). The MERS virus is capable of surviving in the environment for up to 48 hours at 20°C and for 24 hours at 30°C (Chan, J.F. et al (2015)). The SARS-CoV-2 virus is stable and viable on surfaces made of plastic or stainless steel for up to 72 hours, on cardboard for up to 24 hours, and on copper for up to four hours. The virus is viable in aerosols for at least three hours (van Doremalen, N. et al (2020)). Personal protective equipment, including eye protection, is thus recommended for health care personnel, as well as surgical masks or N95 disposable filtering respirators (Huang, C. et al (2020)). Airborne precautions should be applied especially when performing aerosol-generating procedures such as intubation (Paules, C.I. et al (2020)). All potentially infectious specimens should be handled and transported with caution, and must be tested in laboratories meeting WHO BSL3 standards (Chan, J.F. et al (2015)).

As a result of the SARS outbreak, WHO revised the rules for reporting infectious diseases by its member states. The previous reporting requirements, formulated in 1951, required reporting for plague, cholera and yellow fever only, and the resulting delay in reporting cases early in the outbreak was likely to have contributed to its rapid spread (Wu, Z. et al (2020)). The efficient and collaborative international response to the MERS outbreak beginning in 2012, and again to the Covid-19 outbreak in late 2019, testifies to the improvements made (Chan, J.F. et al (2015); Paules, C.I. et al (2020)). In 2017, WHO placed SARS-CoV and MERS-CoV on its Priority Pathogen list, with the goal of galvanizing research and development into countermeasures against CoVs (Paules, C.I. et al (2020)).

As the three major coronavirus outbreaks have clearly demonstrated, increasing overlap between human and animal ecosystems provides greater opportunities for viruses to cross the species barrier. Prevention of future outbreaks of zoonotic disease requires improved coordination between experts in human and veterinary medicine as well as stricter laws governing the raising, transportation, slaughter and sale of wild animals (Wang, R. et al (2020); Yang, Y. et al (2020)).

Chemoprophylaxis

Studies such as Mexico's PHYDRA trial (NCT04318015) and the U.K.'s COPCOV trial (NCT04303507) are evaluating the potential efficacy of the antimalarial drugs chloroquine/hydroxychloroquine for chemoprophylaxis in health care personnel who are in contact with Covid-19 patients. The Indian Council of Medical Research recommends the prophylactic use of hydroxychloroquine by all health care professionals in that country who are in contact with patients known or suspected to be infected with SARS-CoV-2, as well as for asymptomatic household contacts of confirmed cases (Agrawal, S. et al (2020); Rathi, S. et al (2020)). In June, U.S. and Canadian researchers published results of a randomized, double-blind, placebo-controlled trial evaluating hydroxychloroquine for postexposure prophylaxis. They enrolled 821 asymptomatic adults who had a high- or moderate-risk household or occupational exposure to someone with confirmed Covid-19, i.e., at a close distance for a period of >10 minutes while wearing insufficient personal protective equipment. Subjects were treated with placebo or active drug within four days of exposure. The incidence at 14 days of new illness compatible with Covid-19 did not differ significantly between subjects receiving hydroxychloroquine (11.8%) versus placebo (14.3%) (Boulware, D.R. et al (2020)).

Health Canada has provided regulatory clearance for a phase II study evaluating the broad-spectrum antiviral agent favipiravir as a preventative measure against Covid-19 outbreaks. Approximately 760 participants, both residents and staff, at 16 long-term care homes across Ontario, are expected to enroll in the partially blinded, cluster-randomized, placebo-controlled trial, the primary objective of which will be to evaluate the efficacy of favipiravir for 25 days compared with placebo as a prophylaxis to prevent Covid-19 outbreaks in these high-risk settings. The primary endpoint will be outbreak control, defined as no new cases of Covid-19 in residents for 24 consecutive days up to day 40 after the start of prophylaxis, with secondary objectives including measures of safety, rates of infection, disease progression and fatality rates.

Vitamin D is known to enhance the production of antimicrobial peptides in the respiratory epithelium, and may help tamper the inflammatory response to infection by modulating immune cell function (Laird, E. et al (2020)). In a systematic review and meta-analysis conducted prior to the Covid-19 pandemic, vitamin D supplementation was shown to be safe and provide some protection against acute respiratory tract infections. The reviewers analyzed individual participant data (N=10,933) obtained in 25 randomized controlled trials. Protective effects were observed with daily or weekly doses of vitamin D, albeit not with bolus dosing, and were strongest in individuals with the most severe vitamin D deficiency at baseline (hydroxyvitamin D < 25 nmol/L) (Martineau, A.R. et al (2017)). A growing body of circumstantial evidence links vitamin D status with outcomes in Covid-19 patients. The administration of vitamin D supplements to vulnerable individuals, such as nursing home residents, may be warranted during the current pandemic (Mitchell, F. (2020)). The COVIDENCE UK study, now recruiting volunteers, will assess how diet and lifestyle factors--including vitamin D status--affect the transmission of SARS-CoV-2, severity of Covid-19 symptoms, speed of recovery and long-term effects.

Vaccines

The successful containment of coronavirus epidemics in farm animals by vaccines, including those based on either killed or attenuated virus, supports the initiation of vaccine development. However, it remains unknown whether coronavirus infections in humans produce a lasting immune response that could be replicated with a vaccine. Nonetheless, given the rapid propagation of SARS-CoV-2 throughout the global population and the complete lack of preexisting immunity in humans to this highly contageous virus, a vaccine is widely accepted as the only tool available to enable an eventual return to normalcy. The pandemic has created an urgent need to develop and test one or more safe and effective vaccines, and then to manufacture and quickly distribute them in quantities sufficient to immunize a sufficiently large number of individuals to provide herd immunity, which would protect the entire global community. This challenge requires full and unprecedented collaboration from industries, governments and academia (Corey, L. et al (2020)).

The S protein is currently considered to be one of the most promising targets for coronavirus vaccine development (Li, F. et al (2019); Song, Z. et al (2019)), due to its ability to induce neutralizing antibodies, and hence is being targeted for the development of anti-MERS-CoV (Yong, C.Y. et al (2019)) as well as anti-SARS-CoV-2 vaccines. Upon emergence of SARS-CoV-2, both the S and N proteins were identified as potential vaccine antigens, based on their previously demonstrated ability to induce potent and long-lived immune responses in SARS-CoV (Ahmed, S.F. et al (2020)).

Vaccine platforms with potential application in human coronavirus vaccine development include gene-based vaccines (e.g., nucleic acid DNA and mRNA, replicating and non-replicating viral vectors), and protein-based vaccines (e.g., whole inactivated virus or recombinant protein subunits). DNA- and mRNA-based platforms have the greatest potential for rapid development in an outbreak situation. They can be made quickly because they require no culture or fermentation, being based instead on a viral sequence (Lurie, N. et al (2020); Corey, L. et al (2020); Graham, B.S. (2020)). The two main types of RNA vaccines are non-replicating mRNA and self-amplifying (also known as replicon) RNA vaccines. mRNA vaccines are designed to induce the cytoplasmic expression of chimeric mRNAs containing curated ORF viral sequences. Once injected, the delivered mRNA is processed by immune cells and begins to produce the targeted functional protein (generally S) directly via translation, at the same time activating other immune cells (B cells and T cells) to recognize the newly produced viral protein and to generate antibodies (Wang, F. et al (2020)). Adjuvants can be added to these vaccines to boost and prolong the immune response (Graham, B.S. (2020)).

Several companies are focusing on nucleic acid vaccine platforms for rapid development of a Covid-19 vaccine (Corey, L. et al (2020)). One such vaccine is mRNA-1273, an mRNA vaccine against SARS-CoV-2 encoding for a pre-fusion, stabilized form of the S protein. The vaccine candidate was selected by Moderna in collaboration with investigators from Vaccine Research Center at NIAID and is undergoing an extremely accelerated development timeline. The first

clinical batch of the vaccine was completed on February 7, 2020 and underwent analytical testing; it was shipped to NIH on February 24, just 42 days from sequence selection. The first participant in the NIAID-led phase I study of mRNA-1273 was dosed on March 16. The vaccine has received fast-track designation from FDA. On May 6, the FDA completed its review of the company's IND application for a phase II study in 600 healthy subjects, which is expected to begin shortly. The protocol is being finalized for a phase III study, which could begin in early summer of 2020.

Coalition for Epidemic Preparedness Innovation (CEPI) provided early funding for advancement of Oxford University's vaccine candidate against COVID-19 into clinical safety testing. In March of 2020, CEPI expanded a previous collaboration with Oxford to use their ChAdOx1 viral vector technology to develop a vaccine candidate against the novel coronavirus. ChAdOx1 is a replication-deficient simian adenoviral vaccine vector, and this vaccine platform has been used to produce vaccine candidates against multiple pathogens, including influenza, chikungunya and zika. CEPI provided initial funding for the Oxford project to support the manufacture of COVID-19 vaccine materials required for preclinical and phase I development. Only six weeks later, Oxford initiated phase I/II studies of their ChAdOx1 nCoV-19 vaccine candidate. In May, the vaccine was licensed to AstraZeneca for global development and distribution.

CanSino and the Beijing Institute of Biotechnology are also developing an adenoviral vector-based Covid-19 vaccine. The vaccine, a replication-defective Ad5-vectored vaccine expressing the spike glycoprotein of SARS-CoV-2, was evaluated in a phase I safety study enrolling 108 healthy adults. The vaccine was tolerable and immunogenic at 28 days post-vaccination, inducing rapid and specific T-cell responses beginning 14 days post-injection; SARS-CoV-2-specific antibody and T-cell responses were lowest in those subjects with high preexisting Ad5 neutralizing antibody titers. The incidence of adverse effects was high and included injection-site pain, fever, fatigue and headache, although these were generally transient and self-limiting. The investigators concluded that further evaluation of the vaccine is warranted (Zhu, F.C. et al (2020)). A phase II trial is ongoing in China.

Given the rapid increase in and accelerated pace of vaccine development during the Covid-19 pandemic, the WHO designed the SOLIDARITY vaccine trial. This large, international, multisite trial has an adaptive design and will enable the concurrent evaluation of the benefits and risks of various candidate vaccines that are deemed promising (WHO R&D blueprint: Novel coronavirus - An international randomised trial of candidate vaccines against COVID-19 (World Health Organization, April 9, 2020)). In the U.S., the National Institutes of Health has established the Accelerating Covid-19 Therapeutic Interventions and Vaccines (ACTIV) partnership, which will develop a collaborative framework for prioritizing vaccines as well as drug candidates, including streamlining clinical trials, coordinating regulatory processes and/or leveraging assets among all partners to rapidly respond to the Covid-19 and future pandemics (NIH to launch public-private partnership to speed COVID-19 vaccine and treatment options (National Institutes of Health news release, April 17, 2020)).

Albeit controversial, human challenge studies have been proposed as a method of accelerating the testing of vaccines under conditions of urgency, such as a pandemic. These would be conducted later in the testing process, following the successful completion of safety, dose finding and immunogenicity studies. Challenge studies involve the deliberate infection of human volunteers with a viral pathogen in order to evaluate efficacy of a candidate vaccine(s), and would entail a significantly shorter timeframe than that needed for standard phase III field trials. Study participation would be limited to previously uninfected individuals deemed to be at low risk of complications or mortality, who would be randomized to receive either an investigational vaccine or placebo in order to detect differences in response to the viral challenge (Eyal, N. et al (2020)). The WHO has published a policy brief outlining key criteria that would need to be satisfied in order for such unconventional studies to be ethically acceptable (Key criteria for the ethical acceptability of COVID-19 human challenge studies - WHO Working Group for Guidance on Human Challenge Studies in COVID-19 (World Health Organization, May 6, 2020)).

A potential risk of any antiviral vaccine, and one that also exists with coronavirus vaccines, is that of vaccine-enhanced disease. One such syndrome, known as antibody-dependent enhancement (ADE), occurs when exposure to the virus upregulates the expression of both neutralizing and non-neutralizing antibodies, rendering the individual's immune system more, rather than less, reactive to a secondary infection. ADE has been observed in cats vaccinated against feline infectious peritonitis virus, a member of the coronavirus family. A related syndrome, known as vaccine-associated enhanced respiratory disease (VAERD), may emerge in individuals vaccinated with conformationally incorrect antigens. This results in two major types of immunological phenomena that correlate with VAERD: one is a relatively high ratio of binding antibody to neutralizing antibody. The other is induction of a Th2-driven allergic immune response, which may paradoxically potentiate airway dysfunction and delay viral clearance. Prior to widespread vaccination of citizens, rigorous testing in appropriate animal models and early-stage clinical studies must be conducted to detect the risk of both ADE and VAERD, as well as longer-term studies to determine the risk of enhancement following reexposure to a virus that continues to circulate in the population (Lurie, N. et al (2020); Corey, L. et al (2020); Graham, B.S. (2020)).

Development of a MERS vaccine has been facilitated by the recent development of small animal models that effectively replicate MERS-CoV transmission and symptomatic human disease (Schindewolf, C. et al (2019)). In contrast, vaccine research for Covid-19 has been hindered by the lack of a suitable animal model for testing. Transgenic mice expressing the human ACE2 receptor, first developed during the SARS outbreak, are again being bred (Labs rush to study coronavirus in transgenic animals -- some are in short supply (Nature News, March 9, 2020)).

The following tables present an up-to-date overview of the development of potential coronavirus vaccines against Covid-19 and MERS.

Drug name	Organizations	Description	Phase
VPM-1002	Vakzine Projekt Management	Tuberculosis vaccine consisting of a live attenuated recombinant Mycobacterium bovis BCG strain with deleted urease C gene (UreC), expressing fusion protein comprising Mycobacterium bovis Ag85B antigen fused to listeriolysin (Hly) from Listeria monocytogenes	Phase III
AZD-1222	University of Oxford	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of replication - deficient chimpanzee adenovirus Oxford 1(ChAdOx1) vector encoding SARS - CoV - 2 spike (S) gene	Phase II/III
Ad5-nCoV	Beijing Institute of Biotechnology; CanSino Biologics	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of a replication - defective adenovirus type 5 vector encoding SARS - CoV - 2 spike (S) gene	Phase II
BNT-162a1	Pfizer; BioNTech	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine	Phase I/II
BNT-162b1	Pfizer; BioNTech	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine	Phase I/II

BNT-162b2	Pfizer; BioNTech	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine	Phase I/II
BNT-162c2	Pfizer; BioNTech	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine	Phase I/II
LV-SMENP-DC	Shenzhen Genoimmune Medical Institute	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of dendritic cells modified with lentiviral vectors (NHP/TYF) expressing a SARS - CoV - 2 SMENP minigene encoding multiple viral genes (spike (S), membrane (M), envelope (E), nucleocapsid (N) and protease (P)) and immune - stimulating regulatory genes (CNX, GM - CSF and IL - 15); administered together with SARS - CoV - 2 antigens - specific peripheral blood mononuclear cells (PBMC) - derived cytotoxic T lymphocytes (CTLs)	Phase I/II
NVX-CoV2373	Novavax	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising recombinant COVID - 19 spike glycoprotein; encapsulated in nanoparticles	Phase I/II
PiCoVacc	Sinovac	Human SARS - CoV - 2 (Covid - 19 coronavirus) inactivated vaccine; expressed in Vero cells	Phase I/II
1088181	Wuhan Institute of Biological Products	Human SARS - CoV - 2 (Covid - 19 coronavirus) inactivated vaccine	Phase I/II
INO-4800	Inovio Pharmaceuticals	Human SARS - CoV - 2 (Covid - 19 coronavirus) DNA vaccine	Phase I
mRNA-1273	National Institutes of Health (NIH); Moderna	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine encapsulated in lipid nanoparticles	Phase I
1084319	Shenzhen Genoimmune Medical Institute	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of artificial antigen presenting cells (aAPC) carrying SARS - CoV - 2 antigens	Phase I

Immuvac	Cadila Pharmaceuticals	Poly - TLR agonist polyantigenic vaccine containing heat - killed Mycobacterium W	Clinical
CORVax12	OncoSec Medical	Composition comprising human SARS - CoV - 2 (Covid - 19 coronavirus) DNA vaccine and a plasmid encoding human interleukin - 12 (IL - 12) (TAVO(TM))	IND Filed
T-COVID	Altimmune	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine based on adenovirus type 5 vector	IND Filed
1092642	ImmunityBio	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consiting of E1, E2b, E3 - deleted adenovirus type 5 vector encoding SARS - CoV - 2 spike (S) and nucleocapsid (N) gene	IND Filed
AAVCOVID	General Hospital Corp.	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of adeno - associated viral (AAV) vector encoding Spike glycoprotein (S) of SARS - CoV - 2	Preclinical
AVI-205	Abvision (AVI)	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine based on the Spike protein, produced on the ImmunoBuster - II(TM) platform	Preclinical
Ad26 SARS-CoV-2	Johnson & Johnson	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of replication - incompetent adenovirus vector serotype 26 (Ad26) encoding SARS - CoV - 2 proteins; produced based on AdVac(R) technology and expressed in human PER.C6® cell line	Preclinical
COVID-19 S-Trimer	Clover Biopharmaceuticals	Human SARS - CoV - 2 (Covid - 19 coronavirus) subunit trimer vaccine comprising recombinant COVID - 19 spike glycoprotein; produced using Trimer - Tag(R) technology	Preclinical
DPX-COVID-19	IMV Inc.	Human SARS - CoV - 2 (Covid - 19 coronavirus) peptide vaccine formulated with the DepoVax(TM) vaccine delivery technology	Preclinical
EXG-5003	Elixirgen Therapeutics	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising self - replicating RNA (srRNA) expressing the receptor binding domain (RBD) of the SARS - CoV - 2 spike protein	Preclinical

IBIO-200	iBio (US)	Human SARS - CoV - 2 (Covid - 19 coronavirus) virus - like particle vaccine; generated by FastPharming System(TM)	Preclinical
IPT-001	INTELLiSTEM Technologies	Human SARS - CoV - 2 (Covid - 19 coronavirus) peptide vaccine based on the Spike (S) and Nucleocapsid (N) proteins, developed using the Intellipeptidome(TM) platform	Preclinical
LUNAR-COV19	Duke University; Arcturus Therapeutics	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine comprising a self - replicating RNA based on STARR technology platform delivered using Lipid - enabled and Unlocked Nucleomonomer Agent modified RNA (LUNAR(R)) platform	Preclinical
MV-SARS-CoV-2	Themis Bioscience	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising measles vector encoding SARS - CoV - 2 antigens	Preclinical
PittCoVacc	University of Pittsburgh	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of microneedle (MNA) patch comprising recombinant spike glycoprotein (S) of SARS - CoV - 2 expressed in 293HEK cells	Preclinical
STI-6991	Sorrento Therapeutics	Human SARS - CoV - 2 (Covid - 19 coronavirus) decoy cellular vaccine consisting of irradiated replication - deficient K562 human myelogenous leukemia cells expressing spike protein of SARS - CoV - 2 virus	Preclinical
TNX-1800	Tonix Pharmaceuticals	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising an engineered, live replicating strain of horsepox virus (HPXV) encoding the Spike glycoprotein (S) of SARS - CoV - 2	Preclinical
TNX-1810	Tonix Pharmaceuticals	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising an engineered, live replicating strain of horsepox virus (HPXV) encoding a protein of COVID - 19 virus	Preclinical
TNX-1820	Tonix Pharmaceuticals	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising an engineered, live replicating strain of horsepox virus (HPXV) encoding a protein of COVID - 19 virus	Preclinical

TNX-1830	Tonix Pharmaceuticals	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising an engineered, live replicating strain of horsepox virus (HPXV) encoding a protein of COVID - 19 virus	Preclinical
ZIP-1642	Ziphius Therapeutics	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine encoding different Covid - 19 antigens, including Spike (S) protein	Preclinical
bacTRL-Spike	Symvivo	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of probiotic composition comprising Bifidobacterium longum transduced with a DNA plasmid encoding spike protein from SARS - CoV - 2	Preclinical
1091995	Verndari	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine consisting of microneedle array dermal patch (VaxiPatch(TM)) comprising purified recombinant spike glycoprotein (S) of SARS - CoV - 2	Preclinical
1080103	CureVac	Human SARS - CoV - 2 (Covid - 19 coronavirus) mRNA vaccine	Preclinical
1091920	Vaxart	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising replication incompetent adenovirus 5 (rAd) vector encoding a SARS - COV - 2 antigen and a dsRNA TLR3 ligand as an adjuvant	Preclinical
1086209	Kentucky BioProcessing (KBP)	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine; expressed in tobacco plant cells	Preclinical
1086184	Osaka University; AnGes	Human SARS - CoV - 2 (Covid - 19 coronavirus) plasmid DNA vaccine	Preclinical
1092384	Abnova	Human SARS - CoV - 2 (Covid - 19 coronavirus) self - amplifying mRNA vaccine encapsulated in lipid nanoparticles	Preclinical
1083739	Mitsubishi Tanabe Pharma	Human SARS - CoV - 2 (Covid - 19 coronavirus) plant - derived virus - like particle (VLP) vaccine	Preclinical
1092152	Heat Biologics	Human SARS - CoV - 2 (Covid - 19 coronavirus) vaccine comprising engineered heat shock protein gp96 and SARS - CoV - 2 antigens	Preclinical

Experimental vaccines for prevention of MERS-CoV in active preclinical and clinical development

Drug name	Organizations	Description	Phase
BVRS-GamVac-Combi	Ministry Healthcare Russian Federation	Middle East respiratory syndrome coronavirus (MERS - CoV) vaccine comprising a combined heterolgous adenoviral vector	Phase I/II
GLS-5300	Inovio Pharmaceuticals; GeneOne Life Science	Middle East Respiratory Syndrome DNA vaccine using the SynCon (TM) technology, encoding MERS spike protein	Phase I/II
ChAdOx1 MERS	Vaccitech Ltd.; University of Oxford	Middle East respiratory syndrome recombinant (MERS) vaccine consisting of replication - deficient simian adenovirus vector ChAdOx1 carrying full - length spike gene of MERS - CoV camel isolate; under the control of human cytomegalovirus major immediate early promoter (IE CMV)	Phase I
MVA-MERS-S	Ludwig-Maximilians-Univ. Muenchen	Middle East respiratory syndrome coronavirus (MERS - CoV) vaccine comprising modified vaccinia virus encoding full - length S protein of MERS - CoV, under the control of early/late promoter PmH5	Phase I
GREVAX/MERS	Greffex	Recombinant adenoviral vector developed using GREVAX Universal Platform (GREVAX vector) encoding Middle East respiratory syndrome coronavirus (MERS - CoV) antigens	Preclinical
MVA-MERS-S_DF1	Universitaetsklinikum Hamburg-Eppendorf	Middle east respiratory syndrome (MERS) vaccine consisting of a modified vaccinia ankara (MVA) virus encoding MERS - CoV spike (S) protein antigens	Preclinical

Treatment

There is no approved drug therapy for SARS, MERS, Covid-19 or any other coronavirus infection at this time (Li, G. et al (2020)). Early diagnosis, isolation and supportive care, including supply of supplemental oxygen, are the mainstay of treatment for patients with severe disease (Murthy, S. et al (2020); Yang, Y. et al (2020)). Treatment approaches, all of which are investigational, target either the virus itself or the host response, although for patients with severe disease, a combination regimen targeting both of these aspects of the disease might be most effective.

When it emerged in 2003, SARS was an unknown disease and treatment was empirical. Initial efforts to treat the disease with broad-spectrum antibodies from human immune serum globulins were unsuccessful. Some nonspecific immunosuppressive treatments or broad-spectrum antiviral agents, such as ribavirin, were of limited success (Zumla, A. et al (2016)). Combination therapy with ribavirin and corticosteroids was frequently administered as first-line treatment for SARS, based on promising results observed in some of the earliest patients treated, although data obtained subsequently failed to confirm ribavirin's anticipated anti-SARS-CoV activity in vitro (Cleri, D.J. et al (2010)). Some physicians preferred to delay administration of corticosteroids until the second week of infection in order to reduce side effects. The HIV protease inhibitor combination lopinavir/ritonavir, which inhibits the major CoV protease 3CLpro, was the most effective treatment for SARS (Zumla, A. et al (2016)). Twenty-one day ARDS and death rates were lowest in subjects treated with a combination of ribavirin, lopinavir/ritonavir and a corticosteroid (Pillaiyar, T. et al (2020)).

At the outset of the MERS-CoV outbreak, NIH researchers screened a panel of 290 approved and investigational drugs with defined cellular targets in order to determine the potential for repurposing any of them to treat SARS and/or MERS. They found that 33 compounds were active against MERS-CoV, 6 against SARS-CoV and 27 against both coronaviruses. The active drugs were grouped into 13 therapeutic classes and included antibacterial and antiparasitic agents, neurotransmitter inhibitors, estrogen receptor antagonists, kinase signaling inhibitors, inhibitors of lipid or sterol metabolism, protein-processing inhibitors, and inhibitors of DNA synthesis/repair (Dyall, J. et al (2014)). In another repurposing study, Dutch investigators screened a library of 348 FDA-approved drugs for anti-MERS-CoV activity in cell culture and found four (chloroquine, chlorpromazine, loperamide, and lopinavir) that were capable of inhibiting MERS-CoV replication at low micromolar concentrations, and further evaluation of these compounds was recommended. In MERS-CoV-infected patients, administration of drugs such as these--even if not 100% effective in blocking viral replication--could provide a window of opportunity during which the patient's immune system might begin to respond to the infection (de Wilde, A.H. et al (2014)). A systematic review of drugs evaluated in preclinical and clinical studies against MERS-CoV found that the combination of lopinavir/ritonavir and interferon-beta-1b gave excellent results in common marmosets, and has progressed to testing in a randomized control trial setting. Ribavirin and interferon were the most widely used combination in observational studies, and may warrant further investigation (Momattin, H. et al (2019)).

In early 2020, as the number of people affected by the Covid-19 outbreak steadily multiplied and with a lack of virus-specific therapies, scientists began to investigate various host-directed therapies with demonstrated safety that could be repurposed to treat the most seriously ill patients. Candidate drugs included metformin, glitazones, fibrates, sartans and atorvastin for boosting the immune response; zinc and other metal-containing supplements with antiviral activity; cyclosporine, lopinavir/ritonavir, interferon beta-1b, ribavirin and remdesivir, also for their antiviral activity; various cellular therapies; and anti-IL-6 monoclonal antibodies (MAbs) such as tocilizumab (Zumla, A. et al (2020)). Other host-directed therapeutic options that could be repurposed, based on previous lines of investigation into SARS-CoV and MERS-CoV, include JAK-STAT kinase inhibitors, which have potent antiinflammatory effects (Stebbing, J. et al (2020)), nutritional interventions (vitamins A, C, D and E, B vitamins, omega-3 polyunsaturated fatty acids, selenium, zinc and iron), immuno-enhancing agents (interferons, IVIG, thymosin alpha-1, thymopentin, levamisole and ciclosporin), convalescent plasma and traditional Chinese

medicine. Suggested virus-directed approaches include the antimalarial agent chloroquine; flavonoids, for their antiviral and antioxidant activity; the virucidal anthraquinone emodin and the antipsychotic agent chlorpromazine, both of which block the interaction of the viral S glycoprotein with the ACE2 binding receptor; and MAbs directed against S glycoprotein (Zhang, L. et al (2020); Pillaiyar, T. et al (2020)).

In March, WHO announced the initiation of the SOLIDARITY trial, a large international study designed to test various treatment approaches. More than 70 countries have said they will join the study, which will initially evaluate four different drugs or combinations: remdesivir, lopinavir/ritonavir (Kaletra), Kaletra plus interferon-beta, and chloroquine. Also in March, SOLIDARITY's European counterpart, DISCOVERY, was launched in various European countries (Belgium, France, Germany Luxembourg, the Netherlands, Spain, Sweden and the U.K.). This study will enroll 3,200 patients who will be treated with remdesivir, Kaletra with or without IFN-beta, or hydroxychloroquine. Both SOLIDARITY and DISCOVERY are adaptive trials, meaning that ineffective experimental treatments can very quickly be dropped and replaced by other molecules that emerge from research efforts. Both studies will compare the active treatments to standard of care.

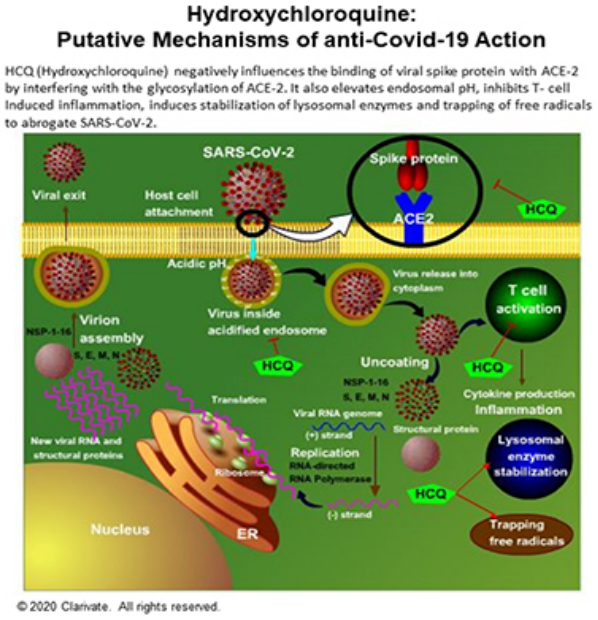
Broad-Spectrum Antiviral Agents

The aminoquinoline antimalarial agents chloroquine and its more soluble and better tolerated metabolite hydroxychloroquine have broad antiviral spectrums in vitro, with activity against DNA as well as RNA viruses including anti-SARS-CoV-2. They also exert antiinflammatory and immunomodulatory effects. Chloroquine has a variety of effects, although it is still unknown which (if any) of these are relevant to Covid-19. It acts by increasing endosomal pH required for fusion of a virus with the host cell, as well as by interfering with glycosylation of virus cell surface receptors. Chloroquine may also interfere with posttranslational modification of viral proteins, interrupting the process of viral replication and reducing infectivity (Colson, P. et al (2020); Devaux, C.A. et al (2020)).

Investigational use of chloroquine in Chinese patients with Covid-19 led to more rapid declines in fever and improvements in lung CT images, and was associated with a shorter recovery time as compared with control groups. Based on this promising profile, low cost, favorable safety profile and easy availability of the drug, approximately a dozen clinical trials were initiated to evaluate chloroquine for the treatment of Covid-19 pneumonia (Gao, J. et al (2020)). One small, open-label, nonrandomized French trial claimed significant reductions in viral load in patients treated with hydroxychloroquine plus azithromycin (Gautret, P. et al (2020)); however, the study had several methodological limitations, meaning that additional studies--including studies to determine the optimum dose and dosing regimen--are required before chloroquine/ hydroxychloroquine regimens can be widely recommended (Sanders, J.M. et al (2020)). The addition of azithromycin is based on its CYP450-inhibitory effects, which may reduce the metabolism of hydroxychloroquine. However, the American College of Cardiology notes that chloroquine, hydroxychloroquine and azithromycin all prolong QT interval, raising concerns about the risk of arrhythmic death from individual or concurrent use of these medications, and recommends close clinical monitoring (Ventricular arrhythmia risk due to hydroxychloroquine-azithromycin treatment for COVID-19 (American College of Cardiology, March 29, 2020)); this cardiotoxic effect was reported in a Brazilian study, and led to a recommendation against use of high-dose chloroquine in combination with azithromycin and oseltamivir in critically ill patients (Silva Borba, M.G. et al (2020)). Others have expressed concern that chloroquine/hydroxychloroquine may adversely affect the host adaptive immune response to the virus by down-regulating IL-2 and the favorable antiinflammatory Th2 response. This paradoxical effect was observed in patients administered the drugs to treat chikungunya virus infection (Guastalegname, M. et al (2020)). During the peak of the outbreak in that country, Chinese experts issued a consensus statement regarding the use and appropriate dosing of chloroquine (Unknown Author (2020)). In late March, in response to a request from the Biomedical Advanced Research and Development Authority (BARDA), the U.S. FDA issued an Emergency Use Authorization to allow the donation of hydroxychloroquine sulfate and

chloroquine phosphate to the Strategic National Stockpile. Under the EAU, the drugs could be distributed and used for hospitalized adult and adolescent patients with Covid-19, as appropriate, when a clinical trial is not available or feasible (Chloroquine phosphate and hydroxychloroquine sulfate for treatment of COVID-19 - Letter of authorization (Food and Drug Administration, March 28, 2020)). The EMA, in contrast, issued a statement just days later emphasizing that the antimalarials should be used to treat Covid-19 only in the context of a clinical trial or emergency use program (COVID-19: chloroquine and hydroxychloroquine only to be used in clinical trials or emergency use programmes (European Medicines Agency, April 1, 2020)).

In May 2020, researchers from Harvard Medical School and collaborators published a multinational registry analysis of real-world outcomes obtained in more than 96,000 Covid-19 patients, including those who were (n=14,888) treated with chloroquine or hydroxychloroquine, with or without a macrolide, and control patients (n=81,144) who did not receive any of these regimens. The article (now retracted) reported no evidence of benefit with any of these regimens; in fact, each of the antimalarial drug-containing regimens was associated with decreased in-hospital survival as well as an increased frequency of ventricular arrhythmias when used for treatment of Covid-19. On the basis of this report, on May 25 WHO temporarily paused the hydroxychloroquine arm of the Solidarity Trial in order for the Data Safety Monitoring Board (DSMB) to review safety and mortality data. Following this review, the DSMB found no reasons to modify the trial, and investigators involved in the study were notified by WHO to resume hydroxychloroquine treatment on June 3. That same day, the editors of the Lancet issued an 'Expression of Concern' to alert readers to the fact that serious scientific questions regarding the data analysis had been brought to their attention (see An open letter to Mehra et al and The Lancet (J. Watson et al., May 28, 2020)). On June 4, the authors of the Lancet study retracted their article, stating that third-party peer reviewers were unable to replicate the analyses presented in the paper.



Defensins, including alpha- and beta-defensins, are constitutively or inducibly expressed by humans and other organisms to protect against invading microorganisms. They have broad-spectrum antimicrobial activity, with potent killing effects against bacteria, fungi, mycoplasma, viruses and tumor cells (Park, M.S. et al (2018); Li, G. et al (2020)). Defensin-mimetic therapeutics are a novel class of antimicrobial peptide (AMPs) mimetics, also termed host defense protein (HDPs) mimetics, that are more stable and potent than natural defensins. These compounds show antibacterial, antiviral, antifungal, antiinflammatory and anticancer activities through their

effects on the innate and adaptive human immune system. Defensin mimetics may be useful in the treatment of coronavirus infections, including Covid-19.

Ribavirin is a ribonucleoside analogue that is active against some coronaviruses, as well as respiratory syncytial virus and metapneumoviruses. Because of its relatively broad spectrum of antiviral activity, ribavirin was one of the first compounds tested for its clinical efficacy against SARS. Early therapy with ribavirin, particularly when combined with corticosteroids, was associated with variable outcomes in SARS patients (Cleri, D.J. et al (2010); Sanders, J.M. et al (2020)). Ribavirin has also been tested in the rhesus macaque model of MERS-CoV, which is a model of mild to moderate human disease. The results obtained--IFN-a2b plus ribavirin reduced virus replication, moderated the host response and improved clinical outcome--support use of the combination to treat patients with MERS (Falzarano, D. et al (2013)). However, in an observational study of 349 critically ill MERS patients, of whom 144 received ribavirin/rIFN (ribavirin and/or rIFN-alfa2a, rIFN-alfa2b or rIFN-beta1a), the treatment was not associated with any reduction in 90-day mortality or in faster MERS-CoV RNA clearance (Arabi, Y.M. et al (2019)). Adverse events, including dose-dependent anemia, are a significant concern with ribavirin, and have been cited as one factor potentially limiting its utility in patients with Covid-19 (Li, G. et al (2020)). Nonetheless, in mid-April 2020, Bausch Health announced that it had initiated a clinical trial program in Canada evaluating ribavirin for inhalation in combination with standard-of-care therapy for the treatment of hospitalized adult patients with respiratory distress resulting from Covid-19 infection. The initial clinical study has been approved by Health Canada and was expected to be initiated within a few weeks. The company is also in discussions with health authorities in multiple countries regarding additional studies to evaluate ribavirin as a treatment for Covid-19 infection. Additionally, the Bausch Foundation is continuing to work directly with health authorities in Italy to make ribavirin for inhalation available free of charge in compassionate use in Italian hospitals.

Viral Entry Inhibitors

The process of coronavirus replication is well understood. Several unique steps have been identified as potential targets for antiviral drugs. The first step in the replication process--viral fusion with the host cell--could potentially be blocked by entry inhibitors or membrane fusion inhibitors, similar to antivirals used for HIV infection.

Angiotensin-converting enzyme 2 (ACE2) receptors are highly expressed on pulmonary cells, primarily in type II alveolar epithelial cells. Type II alveolar cells produce pulmonary surfactant, which maintains the stability of pulmonary tissue by reducing the surface tension of fluids that coat the lung. However, ACE2 also serves as the entry receptor for some coronaviruses, including SARS-CoV and SARS-CoV-2. The spike (S) protein of SARS engages ACE2 as the entry receptor and then uses the host cell-surface protein TMPRSS2 (transmembrane serine protease 2), which is co-expressed on bronchial epithelial cells, for S priming. The latter step enables fusion of viral and cellular membranes and viral entry into the cell (Stopsack, K.H. et al (2020)). The resulting injury to type II alveolar cells may help to explain the severe lung injury observed in Covid-19 patients.

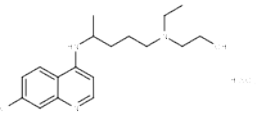
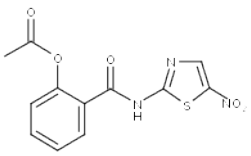
Umifenovir (also known as arbidol), marketed in some countries for the treatment of influenza, has long been studied as a potential treatment for other viral infections. It is an efficient inhibitor of SARS-CoV-2 virus infection in vitro (Wang, X. et al (2020)). Umifenovir has a unique mechanism of action targeting the S protein/ACE2 interaction, and thus is capable of inhibiting membrane fusion of the viral envelope (Sanders, J.M. et al (2020)). Chinese researchers evaluated the antiviral effects and safety of umifenovir in combination with lopinavir/ritonavir in patients with laboratory-confirmed Covid-19. Fifty patients were enrolled and were divided into two treatment groups, receiving either lopinavir/ritonavir (400 mg/100 mg) twice a day for a week (n = 34), or umifenovir (0.2 g) given three times a day (n = 16). None of the patients developed severe pneumonia or ARDS. Fever was the most common symptom at the onset of illness, with most patients having a short duration of fever (< 7 days); there was no difference in fever duration between the two groups. On day 7 after admission, the viral load was undetectable in half of the patients receiving umifenovir and in 23.5% of those treated with lopinavir/ritonavir. On day 14 after the admission, viral load was undetectable in all the patients

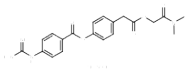
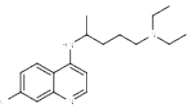
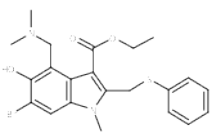
in the umifenovir group; however, viral load was detectable in 44.1% of patients who received lopinavir/ritonavir. Patients treated with umifenovir had a shorter duration of positive RNA test versus those treated with lopinavir/ritonavir. As for safety, no apparent side effects were found in either treatment group. Overall, these findings indicate that umifenovir monotherapy could be superior to lopinavir/ritonavir in treating Covid-19 (Zhu, Z. et al (2020)).

Administration of human recombinant soluble ACE2 has been explored as a method of preventing viral entry into host cells, i.e., as a neutralizing agent (Tay, M.Z. et al (2020)). This approach, known as ACE2 enhancement therapy, has been tested successfully in vitro and in human capillary and human kidney organoids. It should be noted that viral inhibition in these models, albeit dose-dependent, was not complete, suggesting that in addition to ACE2, SARS-CoV-2 may also use some other co-receptor. Alternatively, there may be other as-yet-unknown factors that mediate infection of ACE2-expressing cells in the upper respiratory tract (Monteil, V. et al (2020)). In late February, a pilot investigator-initiated clinical trial evaluating recombinant human ACE2 (rhACE2), APN-01, was launched to treat patients with severe Covid-19 infection in the People's Republic of China. A total of 24 patients were to be treated for 7 days in the randomized, unblinded study, with the aim of obtaining preliminary data on the impact of rhACE2 on biological, physiologic, and clinical outcomes, as well as safety in patients with severe SARS-CoV-2 infection. These data will be assessed to determine whether a phase IIb clinical trial in a larger number of patients is warranted.

As explained above (see SARS-CoV-2 Morphology, Structure and Replication), the S protein consists of S1 and S2 subunits, which are the receptor binding domain and membrane fusion domain, respectively. The S1 domain is poorly conserved across different members of the coronavirus family, which may explain why monoclonal antibodies developed for SARS--most of which were targeted at S1--have shown limited efficacy against SARS-CoV-2. The membrane fusion domain, on the other hand, is one of the best conserved regions of the S protein across all species. Drugs and biologics targeted to S2, therefore, may be more broadly applicable in the treatment of this and future CoV outbreaks (Tang, T. et al (2020)).

Viral entry inhibitors under active development for the treatment of coronavirus infection

Drug name	Organizations	Description	Phase	Structure
Hydroxychloroquine sulfate	Sanofi	Autophagy Inhibitors; Palmitoyl-Protein Thioesterase 1 (PPT1) Inhibitors; Spike Glycoprotein (S) (SARS-CoV-2; COVID-19 Virus)/ACE2 Interaction Inhibitors; Viral Attachment Inhibitors	Phase III	
Nitazoxanide	Romark	Hemagglutinin (HA) (Viral) Inhibitors; Myc Proto-Oncogene Protein (c-Myc) Inhibitors; Protein Disulfide-Isomerase A3 (PDIA3) Inhibitors; Pyruvate Synthase (Pyruvate-Ferredoxin Oxidoreductase; PFOR) (Bacterial) Inhibitors; Pyruvate Synthase (Pyruvate-Ferredoxin Oxidoreductase; PFOR) (Protozoal) Inhibitors; Signal Transduction Modulators; Viral Fusion Inhibitors; Viral Maturation Inhibitors	Phase III	

Leronlimab	CytoDyn	Anti-CD195 (CCR5); Signal Transduction Modulators; Viral Entry Inhibitors	Phase II/III	
Camostat mesylate	Aarhus University	Transmembrane Protease Serine 2 (TMPRSS2) Inhibitors; Trypsin Inhibitors; Viral Entry Inhibitors	Phase I/II	
1086612	Chongqing Sidemu Biotechnology	Anti-GM-CSF; Drugs Acting on NKG2D; Drugs Targeting Angiotensin-I Converting Enzyme-Related Carboxypeptidase (ACE2); Signal Transduction Modulators; Viral Fusion Inhibitors	Phase I/II	
LY-3819253	Lilly	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Phase I	
REGN-3048	Regeneron	Anti-Spike Glycoprotein (MERS-CoV); Viral Fusion Inhibitors	Phase I	
REGN-3051	Regeneron	Anti-Spike Glycoprotein (MERS-CoV); Viral Fusion Inhibitors	Phase I	
SAB-301	SAB Biotherapeutics	Anti-Spike Glycoprotein (MERS-CoV); Viral Fusion Inhibitors	Phase I	
Chloroquine phosphate	Guangdong Zhongsheng Pharmaceutical; University of Oxford	Apoptosis Inducers; Histamine N-methyltransferase (HNMT) Inhibitors; Spike Glycoprotein (S) (SARS-CoV-2; COVID-19 Virus)/ACE2 Interaction Inhibitors; Viral Attachment Inhibitors	Clinical	
Umifenovir hydrochloride	Wuhan Tongji Hospital	Capsid Assembly (Hepatitis B Virus) Modulators; Spike Glycoprotein (S) (SARS-CoV-2; COVID-19 Virus)/ACE2 Interaction Inhibitors; Viral Attachment Inhibitors	Clinical	
CMAB-020	Mabpharm	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Basigin (BSG; CD147)/ACE2 Interaction Modulators; Viral Fusion Inhibitors	Preclinical	

COVI-SHIELD	Sorrento Therapeutics	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical
JS-016	Shanghai Junshi Biosciences	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical
LCA-60	Vir Biotechnology	Anti-Spike Glycoprotein (MERS-CoV); Viral Fusion Inhibitors	Preclinical
REGN-COV2	Regeneron	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical
STI-1499	Sorrento Therapeutics	Angiotensin-I Converting Enzyme-Related Carboxypeptidase (ACE2) Inhibitors; Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Signal Transduction Modulators; Viral Fusion Inhibitors	Preclinical
VIR-7831	Vir Biotechnology; GlaxoSmithKline	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical
VIR-7832	Vir Biotechnology; GlaxoSmithKline	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical

Inhibitors of Host Proteases

A number of host proteases have been shown to proteolytically process the S protein, which determines coronaviral entry into the host cell. These include cathepsin, furin, trypsin (Millet, J.K. et al (2015); Kilianski, A. et al (2014)), and type II transmembrane serine protease (TMPRSS2) (Stopsack, K.H. et al (2020)).

Following attachment of the SARS-CoV-2 S protein to the ACE2 receptor on the host cell, the spike protein is cleaved by TMPRSS2, allowing the S2 subunit virus to drive fusion of the viral membrane with the host cell (Profile of a killer: The complex biology powering the coronavirus pandemic (Nature News, May 4, 2020)). TMPRSS2 has been identified as a promising anti-Covid-19 drug target, with the advantage that drugs acting on this target are already approved for marketing. The TMPRSS2 inhibitor camostat mesilate is marketed in Japan for the treatment of pancreatitis, and has been identified as a suitable candidate for repurposing in the treatment of Covid-19 (Hoffmann, M. et al (2020)). In April, Danish investigators began enrolling patients with PCR-confirmed SARS-CoV-2 in a placebo-controlled study (NCT04321096) that will attempt to determine whether camostat mesilate is able to act on the lung cells targeted by the virus and prevent it from infecting them.

In a comparative study, the related TMPRSS2 inhibitor nafamostat mesylate was found to be 15-fold more potent than camostat in blocking SARS-CoV-2 entry in vitro, with significantly higher antiviral efficiency. Nafamostat is already approved by the FDA and has a proven safety profile, supporting its evaluation in patients with Covid-19 infection (Hoffmann, M. et al (2020)).

The mucolytic agent bromhexine was found in a repurposing study to be a potent and specific TMPRSS2 inhibitor with a potentially superior safety profile (Maggio, R. et al (2020)), supporting its evaluation in patients with Covid-19.

Viral Enzyme Inhibitors

Viral enzymes involved in the process of replication within the host cell have also been identified as potential drug targets. Inhibitors of viral proteases may block cleavage of the polymerase protein to inhibit viral RNA synthesis. Nucleoside inhibitors might specifically inhibit viral replication without causing damage to the host cell. Targeted inhibitors of the serine proteases, which are required to activate the viral infectivity of some coronaviruses, may block the later stages of the viral life cycle (Kilianski, A. et al (2014); Zhou, Y. et al (2015)). The main protease (Mpro, also called 3CLpro) is one of the best characterized drug targets for coronaviruses. Mpro, together with the papain-like proteases, is required for processing polyproteins that are translated from the viral RNA. It has been identified as a promising target for anti-SARS-CoV-2 compounds (Zhang, L. et al (2020)).

The HIV protease inhibitor combination lopinavir/ritonavir has progressed furthest in development for treatment of MERS-CoV. Following successful preclinical evaluation of lopinavir/ritonavir plus interferon-beta1b, in which significant reductions in mortality were obtained in a marmoset model, clinical evaluation of the combination was recommended (Chan, J.F. et al (2015)). The ongoing MIRACLE trial is evaluating the efficacy and safety of lopinavir/ritonavir plus recombinant interferon-beta1b compared to placebo--both given in combination with optimal supportive care--in patients with laboratory-confirmed MERS-CoV infection requiring hospital admission (Arabi, Y.M. et al (2018)). The justification for using the two in combination is that ritonavir, in addition to inhibiting protease, is also an inhibitor of cytochrome P4503A4. It thereby reduces the metabolism and enhances and prolongs the action of the second protease inhibitor, lopinavir.

Since the combination of lopinavir and ritonavir was already available in the Wuhan, China hospital where early SARS-CoV-2-infected patients were treated, a trial was quickly initiated to assess the efficacy and safety of the combination to treat Covid-19 (Huang, C. et al (2020)). The randomized, controlled, open-label trial, designated LOTUS China, enrolled 199 patients who were SARS-CoV-2-positive on RT-PCR, had confirmed pneumonia on chest imaging and had oxygen saturation (Sao2) of less than or equal to 94% while breathing ambient air or Pao2:Fio2 ratio of less than or equal to 300 mgHg. Eligible patients were randomized to receive either lopinavir/ritonavir (400 mg/100 mg p.o.) twice daily in combination with standard care, or standard care alone, for 14 days; standard care included supplemental oxygen, noninvasive and invasive ventilation, antibiotic agents, vasopressor support, renal-replacement therapy and extracorporeal membrane oxygenation (ECMO), as required. The results showed no difference overall in time to clinical improvement or mortality between the lopinavir/ritonavir and standard care groups. In the intention-to-treat population, however, initiation of lopinavir/ritonavir therapy within 12 days after the onset of symptoms was associated with shorter time to clinical improvement, whereas initiation of treatment after this point was not. Twenty-eight-day mortality rates were lower in the active treatment vs. standard care groups (19.2% vs. 25.0%), and ICU stay was shorter (6 days vs. 11 days). The percentage of patients with clinical improvement at day 14 was also higher in the lopinavir/ritonavir group versus standard care (45.5% vs. 30.0%). Addition of lopinavir/ritonavir did not result in decreased viral RNA load in throat or duration of viral RNA detectability as compared with standard care alone. Of note, the overall mortality rate (22.1%) was substantially higher than that reported in initial descriptive studies (11% to 14.5%), indicating a high overall degree of severity in the study population (Cao, B. et al (2020)).

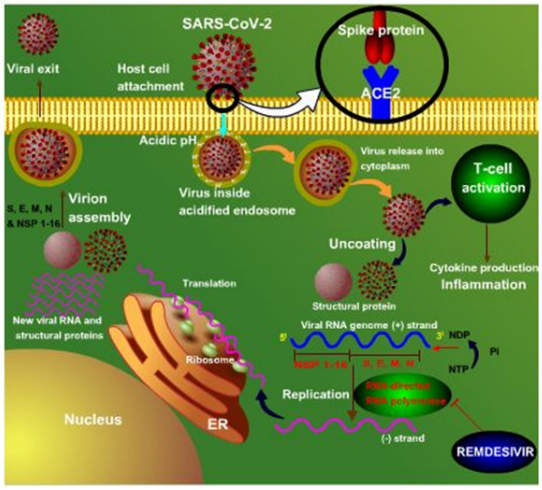
In March 2020, a clinical trial was initiated in Spain to evaluate a novel multidrug approach for reducing the duration of infection and transmission of the SARS-CoV-2 virus. A total of around

200 people testing positive for SARS-CoV-2 will receive the anti-HIV drug combination darunavir/cobicistat plus hydroxychloroquine to try to reduce the number of days they are contagious. In addition, about 3,000 of their direct contacts will receive hydroxychloroquine to see if it prevents infection. It is estimated that people with the virus are contagious for 14 days after first showing symptoms and that they can infect 5-15% of their contacts. The study will determine whether this regimen is able to reduce these numbers.

In the case of RNA viruses such as coronavirus, the most specific target is the RNA-directed RNA-polymerase (RdRp), which directs the processes of viral genome replication and transcription. This key enzyme shows significant differences between positive-sense viruses, such as SARS-CoV-2, and negative-sense RNA viruses (Buonaguro, L. et al (2020)). The RdRp inhibitor remdesivir showed broad-spectrum antiviral activity against coronaviruses in vitro and in vivo, inhibiting the replication of both endemic and zoonotic strains in cell culture. In a relevant murine model of SARS-CoV infection, prophylactic administration of remdesivir prevented development of symptomatic disease; postexposure administration was also effective in mitigating the immunopathological phase of disease, improving respiratory function and reducing viral load (Sheahan, T.P. et al (2017)). In 2020, based on these and other studies suggesting its anti-CoV activity (Sheahan, T.P. et al (2020); Wang, M. et al (2020)) and at the request of treating physicians, remdesivir was supplied by the manufacturer for experimental use in China, to treat hospitalized adult patients with Covid-19 illness. In January, in its R&D Blueprint report, WHO said it considered remdesivir to be the most promising candidate for treatment of Covid-19, based on its broad antiviral spectrum, available in vitro and in vivo data, and the extensive clinical safety database (WHO R&D blueprint report - Informal consultation on prioritization of candidate therapeutic agents for use in novel coronavirus 2019 infection (World Health Organization, January 24, 2020)). In April, results were announced from a compassionate-use study in 53 hospitalized patients (22 in the U.S., 22 in Europe or Canada, and 9 in Japan) who were treated with a 10-day course of remdesivir. At baseline, 30 patients (57%) were receiving mechanical ventilation and four (8%) were receiving ECMO. At a median follow-up of 18 days, 36 patients (68%) registered improvement in oxygen-support class, including 17 of 30 mechanically ventilated patients (57%) who were successfully extubated. Twenty-five patients (47%) were released from the hospital, while seven (13%) died (Grein, J. et al (2020)). Later that month, preliminary results of the NIAID-sponsored ACTT-1 trial, which enrolled 1,063 hospitalized adults with laboratory-confirmed Covid-19, were announced. The preliminary findings indicate that patients randomized to a 10-day course of treatment with remdesivir had a 31% faster time to recovery than those who received placebo (11 days vs. 15 days, respectively). Results also suggested a survival benefit, with a mortality rate of 8.0% for the group receiving remdesivir versus 11.6% for the placebo group (Beigel, J.H. et al (2020)). The same day, Gilead released topline results from the first open-label, phase III SIMPLE trial indicating patients who received a shorter, 5-day course of remdesivir experienced similar clinical improvement as patients who received a 10-day treatment course (Goldman, J.D. et al (2020)), which could significantly expand the number of patients who could be treated with the current supply of the investigational drug. On May 1, the FDA issued an emergency use authorization (EUA) for remdesivir in the U.S., allowing the drug to be distributed and used by licensed health care providers to treat adults and children hospitalized with severe Covid-19. Severe Covid-19 is defined as patients with an oxygen saturation of less than or equal to 94% on room air, or requiring supplemental oxygen, mechanical ventilation or ECMO (Coronavirus (COVID-19) update: FDA issues emergency use authorization for potential COVID-19 treatment (FDA news release, May 1, 2020)). The following week, the Japanese Ministry of Health, Labour and Welfare (MHLW) granted regulatory approval of remdesivir as a treatment for SARS-CoV-2 infection under an exceptional approval pathway. The exceptional approval was granted due to the Covid-19 pandemic and references the EUA of remdesivir in the United States. Of note, the Japanese regulatory process does not include an emergency use provision.

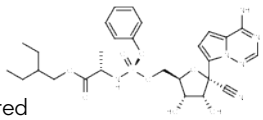
Remdesivir: Treatment for SARS-CoV-2 Infection (COVID-19)

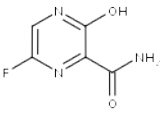
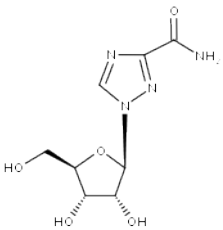
Remdesivir is an investigational antiviral drug that inhibits viral RNA-directed RNA polymerase to disrupt replication of a new viral genome. It functions by blocking addition of new nucleotides to the 3' OH group of template RNA.



Favipiravir, a nucleoside analogue that is marketed in Japan for the treatment of influenza A and B, is being evaluated as a potential broad-spectrum antiviral for use in the Covid-19 outbreak (Sanders, J.M. et al (2020)). Like remdesivir, favipiravir inhibits RNA-directed RNA polymerase of various RNA viruses; in addition to influenza, it has been found to inhibit the replication of yellow fever virus, Ebola virus, norovirus and chikungunya virus (Li, G. et al (2020)). Although favipiravir was not highly active against SARS-CoV-2 in vitro (Wang, M. et al (2020)), its commercial availability and favorable tolerability profile support clinical testing in Covid-19 patients, with studies in the planning stages or already underway in several countries.

Viral enzyme inhibitors under active development for coronavirus infection

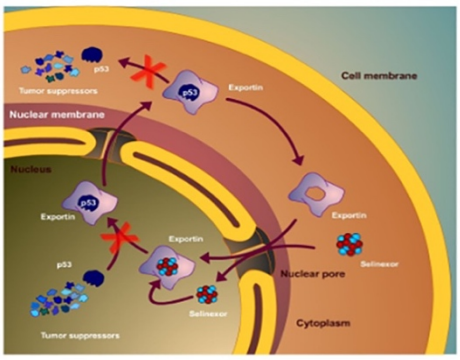
Drug name	Organizations	Description	Phase	Structure
Remdesivir	Gilead	RNA-Directed RNA Polymerase (RdRp) (Ebola Virus) Inhibitors; RNA-Directed RNA Polymerase (RdRp) (MERS-CoV) Inhibitors; RNA-Directed RNA Polymerase (RdRp) (SARS-CoV) Inhibitors; RNA-Directed RNA Polymerase (RdRp) (SARS-CoV-2; COVID-19 Virus) Inhibitors; Viral Replication Inhibitors	Registered	
ASC-09/ritonavir	Ascletis	HIV Protease Inhibitors; HIV-1 Protease Inhibitors; Tumor Necrosis Factor Receptor Superfamily Member 6 (CD95)/PLC-gamma-1 Interaction Inhibitors	Phase III	
Darunavir/cobicistat	Shanghai Public Health Clinical Center	Cytochrome P450 CYP3A4 Inhibitors; HIV Protease Inhibitors	Phase III	

Favipiravir	FUJIFILM Toyama Chemical	RNA-Directed RNA Polymerase (RdRp) (Influenza A Virus H1N1) Inhibitors; RNA-Directed RNA Polymerase (RdRp) (NS5B) (HCV) Inhibitors; RNA-Directed RNA Polymerase (RdRp) (SARS-CoV-2; COVID-19 Virus) Inhibitors; Viral Replication Inhibitors	Phase III	
Lopinavir/ritonavir	King Abdullah International Med Res Cent	HIV Protease Inhibitors; HIV-1 Protease Inhibitors; Tumor Necrosis Factor Receptor Superfamily Member 6 (CD95)/PLC-gamma-1 Interaction Inhibitors	Phase II/III	
Ribavirin	Bausch Health	Equilibrative Nucleoside Transporter ENT1 Inhibitors; Inosine 5'-Monophosphate Dehydrogenase (IMPDH) Inhibitors; RNA-Directed RNA Polymerase (RdRp) (SARS-CoV-2; COVID-19 Virus) Inhibitors; Viral Replication Inhibitors	IND Filed	

Other Viral Replication Inhibitors

In late April 2020, the first patient was treated in a randomized phase II study evaluating low-dose oral selinexor in hospitalized patients with severe COVID-19 (XPORT-CoV-1001; NCT04349098). Selinexor is a selective inhibitor of nuclear export (SINE) compound that blocks Exp1, a cellular protein encoded by the gene XPO1 which is involved in both the replication of SARS-CoV-2 and the host inflammatory response to the virus. SINE compounds have been identified as having the potential to interfere with key host protein interactions with influenza, RSV and other viruses including SARS-CoV-2, with Exp1 being one of the host proteins with the highest number of functional connections with SARS-CoV proteins. SINE compounds have also demonstrated potent antiinflammatory activity through the inhibition of nuclear factor kappaB (NF-kappaB), leading to reductions in cytokines such as IL-6, IL-1, IFN-gamma and others in a variety of models, which may be particularly beneficial to hospitalized patients with COVID-19 and other severe viral infections.

EXPORTIN-1 RECEPTOR ANTAGONISM - SELINEXOR



Interferons

The host immune response, including the innate interferon response, is crucial for controlling viral replication. This response involves stimulation by the virus of pattern recognition receptors, which triggers the transcriptional induction of type I and III interferons (IFN-I and IFN-III), activation of the JAK1/TYK2-STAT1/2 pathway and the subsequent upregulation of IFN-stimulated genes (ISGs). Coronaviruses are capable of suppressing this response in order to evade the host immune system and continue replicating unchecked; SARS-CoV-2 is particularly adept at such evasion. However, they may be responsive to treatment with interferons, particularly recombinant forms (Zumla, A. et al (2016); Blanco-Melo, D. et al (2020); Jamilloux, Y. et al (2020)).

The antiviral activity of interferon-beta, interferon-alfa and interferon-gamma was evaluated in SARS-CoV strains isolated from patients in Frankfurt and Hong Kong and replicated in Vero and Caco-2 cell lines (Hensley, L.E. et al (2004)). IFN-beta showed good antiviral activity, inhibiting SARS-CoV replication in both cell lines. IFN-alfa was also active, but with a sensitivity index 50-90 times lower than that for IFN-beta. IFN-gamma was slightly more active than IFN-alfa in one cell line but was completely inactive in the other (Cinatl, J. et al (2003)). MERS-CoV has been shown to be 50-100 times more susceptible in vitro than SARS-CoV to treatment with IFN-alfa (Abdel-Moneim, A.S. (2014)). In vitro in Vero cells, SARS-CoV-2 was more susceptible than SARS-CoV to both IFN-alfa and IFN-beta, the latter being slightly more effective in reducing viral titers (Mantlo, E. et al (2020)).

In early 2020, a phase II trial was launched in Hong Kong to test the efficacy of lopinavir/ritonavirin, with or without the addition of ribavirin and IFN-beta-1b, in 127 hospitalized patients with mild to moderate Covid-19. The combination regimen incorporating IFN-1beta and ribavirin was shown to be safe, and was superior to lopinavir/ritonavir alone in alleviating symptoms as well as shortening the duration of viral shedding and hospital stay. The investigators concluded that future placebo-controlled clinical studies evaluating IFN beta-1b as a backbone of antiviral therapy are warranted (Hung, I.F. et al (2020)). Chinese Covid-19 guidelines list interferons as an alternative for use in the setting of combination therapy (Sanders, J.M. et al (2020)).

Recombinant interferon therapy has long focused on type I IFNs (IFN-alpha and -beta) for potentiation of the innate antiviral response. During the Covid-19 pandemic, attention turned to the potential contribution by type III interferons, particularly IFN-lambda, in mediating antiviral resistance in cells. Type III IFNs have antiviral and tissue-protective activities; their expression is induced at a lower viral burden compared to type I IFNs. Also in contrast with type I IFNs, which signal through a receptor complex (IFNAR) present on a multitude of host cells, type III IFNs signal through a unique heterodimeric receptor complex (IFNLR), the expression of which is limited to epithelial cells and a subpopulation of immune cells, including neutrophils and B cells. Administration of recombinant or pegylated IFN-lambda, either as prophylactic therapy or at an early stage of Covid-19, could result in expression of ISGs and induce a localized antiviral response in respiratory epithelial cells, while reducing the systemic side effects and inflammation associated with type I IFNs (Prokunina-Olsson, L. et al (2020); Andreakos, E. et al (2020)).

Interferons under active development for treatment of coronavirus infection

Drug name	Organizations	Description	Phase
1086588	Shanghai Jiao Tong University (SJTU)	Recombinant human interferon alpha - 1b	Phase III
Interferon-beta	Synairgen	Interferon beta 1a (IFN - b1a)	Phase II

FP-1201	Faron	Recombinant human interferon beta - 1a	Clinical
Peginterferon lambda-1a	Eiger BioPharmaceuticals	Pegylated (20kD) recombinant human interferon lambda 1 (IFNL1/IL29)	Clinical
Human leukocyte interferon alpha	AIM ImmunoTech	Interferon alpha proteins comprising approximately 166 amino acids ranging in molecular weights from 16, 000 to 27, 000 daltons	Preclinical

Monoclonal Antibodies

Monoclonal antibodies (MAbs), including MAbs directed at neutralizing the virus or those designed to modulate the host response, often represent the first line of investigation and defense against emerging diseases. Murine, chimeric and fully human monoclonal antibodies have been tested; the latter are preferred due to their reduced immunogenicity (Jin, Y. et al (2017); Shanmugaraj, B. et al (2020)).

Various MAbs were evaluated during the SARS outbreak. Most of these were directed at the S1 fragment of the spike protein, with the aim of blocking its interaction with the cellular binding receptor ACE2 (Shanmugaraj, B. et al (2020)). Neutralization of Middle East respiratory syndrome coronavirus has been attempted using a related strategy targeting the receptor (CD26/DPP4) binding domain of the MERS-CoV spike glycoprotein. One such MAb designated m336 neutralized the virus with exceptional potency, and was reported to have great potential as a candidate therapeutic or as a reagent to facilitate the development of MERS-CoV vaccines (Ying, T. et al (2014)). Japanese researchers also investigated anti-CD26 MAb for MERS-CoV and have identified the humanized MAb YS110 as a promising candidate, with the advantage that this agent has already undergone clinical testing for other indications (Ohnuma, K. et al (2013)).

MAbs directed to the S protein of SARS-CoV-2 have also been described in the literature. Such neutralizing antibodies could be used to reduce the course of infection or in the setting of prevention (Tian, X. et al (2020); Kumar, G.V. et al (2020)). However, the vast majority of MAbs in development for Covid-19 target the host immune response and cytokine storm, as discussed in a later section of this report.

Monoclonal antibodies under active development for coronavirus infections

Drug name	Organizations	Description	Phase
Canakinumab	Novartis	Anti-IL-1beta; Signal Transduction Modulators	Phase III
Lenzilumab	Humanigen	Anti-GM-CSF; Signal Transduction Modulators	Phase III
Tocilizumab	Roche; Chugai Pharmaceutical	Anti-Interleukin-6 Receptor Subunit Alpha (CD126; IL-6R); Signal Transduction Modulators	Phase III
Emapalumab	Swedish Orphan Biovitrum	Anti-IFN-gamma; Signal Transduction Modulators	Phase II/III

IFX-1	InflaRx	Anti-C5 (Complement 5)	Phase II/III
Leronlimab	CytoDyn	Anti-CD195 (CCR5); Signal Transduction Modulators; Viral Entry Inhibitors	Phase II/III
Olokizumab	R-Pharm	Anti-IL-6; Signal Transduction Modulators	Phase II/III
Sarilumab	Sanofi; Regeneron	Anti-Interleukin-6 Receptor Subunit Alpha (CD126; IL-6R); Signal Transduction Modulators	Phase II/III
Astegolimab	Genentech	Anti-Interleukin-1 Receptor-Like 1 (IL1RL1; ST2)	Phase II
Avdoralimab	Innate Pharma	Anti-Anaphylatoxin Chemotactic Receptor 1 (C5aR; CD88); Signal Transduction Modulators	Phase II
Camrelizumab	Southeast University	Anti-PD-1; Immune Checkpoint Inhibitors	Phase II
Gimsilumab	Roivant Sciences	Anti-GM-CSF; Signal Transduction Modulators	Phase II
LY-3127804	Lilly	Angiogenesis Inhibitors; Anti-ANGPT2 (Angiopoietin 2)	Phase II
Sirukumab	Janssen	Anti-IL-6; Signal Transduction Modulators	Phase II
[131I]-Metuximab injection	Fourth Military Medical University	Anti-CD147 (Basigin (BSG; CD147); Signal Transduction Modulators	Phase I/II
LY-3819253	Lilly	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Phase I
Mavrilimumab	Kiniksa Pharmaceuticals	Anti-CSF2RA (Granulocyte-Macrophage Colony-Stimulating Factor Receptor Subunit alpha); Signal Transduction Modulators	Phase I
REGN-3048	Regeneron	Anti-Spike Glycoprotein (MERS-CoV); Viral Fusion Inhibitors	Phase I
REGN-3051	Regeneron	Anti-Spike Glycoprotein (MERS-CoV); Viral Fusion Inhibitors	Phase I
TJ-003234	I-Mab Biopharma	Anti-GM-CSF; Signal Transduction Modulators	Phase I

Eculizumab	Alexion Pharmaceuticals	Anti-C5 (Complement 5)	Clinical
IC-14	Implicit Bioscience	Anti-CD14	Clinical
Namilumab	Izana Bioscience	Anti-GM-CSF; Signal Transduction Modulators	Clinical
Siltuximab	EUSA Pharma	Anti-IL-6; Signal Transduction Modulators	Clinical
CERC-002	Cerecor	Anti-TNFSF14 (Tumor Necrosis Factor Ligand Superfamily Member 14; LIGHT)	IND Filed
Ravulizumab	Alexion Pharmaceuticals	Anti-C5 (Complement 5)	IND Filed
CMAB-020	Mabpharm	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Basigin (BSG; CD147)/ACE2 Interaction Modulators; Viral Fusion Inhibitors	Preclinical
COVI-SHIELD	Sorrento Therapeutics	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical
JS-016	Shanghai Junshi Biosciences	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical
LCA-60	Vir Biotechnology	Anti-Spike Glycoprotein (MERS-CoV); Viral Fusion Inhibitors	Preclinical
STI-1499	Sorrento Therapeutics	Angiotensin-I Converting Enzyme-Related Carboxypeptidase (ACE2) Inhibitors; Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Signal Transduction Modulators; Viral Fusion Inhibitors	Preclinical
VIR-7831	Vir Biotechnology; GlaxoSmithKline	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical
VIR-7832	Vir Biotechnology; GlaxoSmithKline	Anti-S (Spike Glycoprotein (SARS-CoV-2; COVID-19 Virus); Viral Fusion Inhibitors	Preclinical

Immunoglobulin Therapy

Experimental use of passive antibody therapy, including i.v. immunoglobulins or convalescent plasma, was described during the 2003 SARS epidemic (Mair-Jenkins, J. et al (2015); Roback, J.D. et al (2020)). The principal mechanism of action of convalescent plasma--i.e., blood plasma obtained from patients who have overcome a specific infection--is expected to be viral neutralization, although other mechanisms may also be involved, such as antibody-dependent cellular cytotoxicity and/or phagocytosis (Casadevall, A. et al (2020)).

Again during the MERS-CoV outbreak in 2015, some South Korean patients were treated with convalescent plasma. A systematic review and meta-analysis of healthcare databases and so-called grey literature describing the use of convalescent plasma, serum or hyperimmune immunoglobulin derived from convalescent plasma to treat severe acute respiratory infections of viral origin concluded that the approach was safe and may decrease the risk of mortality (Mair-Jenkins, J. et al (2015)). However, Saudi Arabian scientists reported that clinical trials evaluating this therapy would be challenging due to the limited availability of suitable donors, i.e., individuals with sufficiently high neutralizing antibody titers (Arabi, Y. et al (2016)).

Convalescent plasma was used to treat some patients in China with Covid-19, although not in the setting of controlled clinical trials (Roback, J.D. et al (2020)). The potential of the treatment to improve clinical outcomes in patients with laboratory-confirmed Covid-19 and acute respiratory distress syndrome was evaluated in 5 critically ill patients who had severe pneumonia with rapid progression and continuously high viral load despite antiviral treatment and who were receiving mechanical ventilation. The treatment consisted of convalescent plasma with a SARS-CoV-2-specific antibody (IgG) binding titer greater than 1:1000 and a neutralization titer greater than 40 that had been obtained from 5 patients who recovered from COVID-19; it was administered 10-22 days after admission. Following the transfusion, body temperature normalized within 3 days in 4 of 5 subjects, the Sequential Organ Failure Assessment (SOFA) score decreased from 2-20 before to 1-4 after and Pao2/Fio2 improved in 4 of 5 patients, increasing within 12 days from 172-276 to 284-366. Viral loads became negative within 12 days and SARS-CoV-2-specific ELISA and neutralizing antibody titers increased from 40-60 before to 80-320 on day 7. ARDS resolved in 4 patients at 12 days after transfusion and 3 patients were weaned from mechanical ventilation within 2 weeks. Three of 5 patients had been discharged from the hospital at the time of reporting and 2 were in stable condition 37 days after transfusion. Though the effects of treatment may have been influenced by the fact that other treatments such as antivirals were also given, further study in a larger number of patients appeared warranted, and perhaps different timings of administration after admission to hospital (Shen, C. et al (2020)). As stated above, this treatment is thought to work by suppressing viremia, which typically peaks in the first 10-14 days of illness; thus, convalescent plasma should be administered in the early stages of disease, or in the setting of prophylaxis, in order to be most effective (Chen, L. et al (2020); Casadevall, A. et al (2020)). In March 2020, the U.S. FDA issued a notice stating that investigators wishing to study the compassionate use of convalescent plasma to treat patients with Covid-19 are encouraged to submit requests to FDA for investigational use under the traditional IND regulatory pathway (Investigational COVID-19 convalescent plasma - Emergency INDs (Food and Drug Administration, March 25, 2020)). In April, the FDA issued guidance for health care providers and investigators regarding the administration and study of Covid-19 convalescent plasma during the public health emergency (Investigational Covid-19 convalescent plasma - Guidance for industry (Food and Drug Administration, April 2020)).

An alternative to convalescent plasma, which contains both IgG and IgM but varies in antibody specificity and titer depending upon donor characteristics, is hyperimmune globulin (H-Ig), which contains standardized antibody doses but is devoid of IgM due to fractionation (Roback, J.D. et al (2020)). In March 2020, Takeda announced that it was initiating the development of a highly purified anti-SARS-CoV-2 polyclonal H-Ig designated TAK-888 to treat high-risk individuals with COVID-19. Takeda is in discussions with national health and regulatory agencies and healthcare partners in the U.S., Asia and Europe to rapidly advance research into TAK-888. This requires access to source plasma from people who have successfully recovered from COVID-19 or who have been vaccinated, once a vaccine is developed.

Corticosteroids

Corticosteroids were widely used during the SARS epidemic, although there was little consensus at the time regarding optimal treatment regimens. A review published some years later by Chinese researchers concluded that corticosteroid therapy had a positive impact on oxygenation index (OI), used as a measure of efficacy. Among the 225 SARS patients treated at a single Chinese center in 2003, the use of corticosteroids increased OI from an average of 237 mmHg at baseline to 335 mmHg after steroid administration. The optimum dose was determined to be 1-3 mg/kg (or 160-240 mg/day) for a total accumulated dose of 1000-2000 mg. The optimum duration of treatment was 8-14 days (Jia, W.D. et al (2009)).

Data obtained in a Hong Kong hospital support use of pulsed methylprednisolone as rescue therapy only during the later stages of SARS; administration during the earlier phases of disease appeared to actually prolong viremia (Hui, D.S. et al (2010)). In fact, later analysis showed that prolonged methylprednisolone use was associated with worse outcomes, including disseminated fungal infection and avascular osteonecrosis, and increased 30-day mortality (Pillaiyar, T. et al (2020)); as such, corticosteroids should be used only with caution in the treatment of patients with MERS (Zumla, A. et al (2015)).

Based on previous experience with SARS and MERS, routine use of corticosteroids was not initially recommended in patients in Wuhan with Covid-19 (Huang, C. et al (2020); Lai, C.C. et al (2020)), although this guidance continues to evolve. Precise use of corticosteroids, adhering strictly to the most recent treatment guidelines (Treatment of patients with nonsevere and severe coronavirus disease 2019: An evidence-based guideline (April 2020)), may be warranted to treat appropriately selected patients with novel coronavirus pneumonia in the phase of ARDS, when they may inhibit the cytokine storm and prevent multiorgan damage and septic shock (Zhou, W. et al (2020)). Given potential harms and lack of proven benefit, however, this should always be in the setting of a randomized, controlled trial (Sanders, J.M. et al (2020)).

Targeting the Cytokine Storm

A model has been proposed of the pathogenesis of acute respiratory distress syndrome, such as that occurring in patients with advanced Covid-19. Lung vascular permeability increases in the early or exudative stage, causing the alveolar air space and interstitium to become flooded with protein-rich edema fluid and triggering an inflammatory response (Sapru, A. et al (2015); Tay, M.Z. et al (2020)). Pulmonary or systemic inflammation is both triggered by and prompts the further systemic release of proinflammatory cytokines, sometimes termed a "cytokine storm." Alveolar macrophages release cytokines (IL-6, IL-10 and TNF-alpha), which recruit and activating neutrophils in the lungs (Pedersen, S.F. et al (2020)), leading to further release of inflammatory mediators (leukotrienes, antioxidants, platelet-activating factor and neutrophil elastase). All of these substances have harmful effects on the capillary endothelium and alveolar epithelium, and hence disrupt the epithelial barrier between capillaries and airspaces. As a result, the airspaces and interstitium are flooded with edema fluid, protein and cellular debris. In the resulting cascade of events, surfactant is disrupted, airspaces collapse and there is an imbalance ("mismatch") between ventilation and perfusion, causing hypoxemia (Sweeney, R.M. et al (2016); Tay, M.Z. et al (2020)). In patients with severe Covid-19, the cytokine storm is manifested by an increase in white blood cell count but a simultaneous and significant decrease in CD4+ and CD8+ T cell and natural killer (NK) cell counts, indicating suppression of the adaptive immune response (Pedersen, S.F. et al (2020); Zhang, W. et al (2020)). These patients progress rapidly to cardiovascular collapse, multiorgan dysfunction, sepsis and death (Luo, P. et al (2020)).

With an increased understanding of these processes, several therapeutic approaches targeting the cytokine storm in Covid-19 are now being evaluated. These agents, which address aspects of the disease that may not improve with antiviral drug therapy, include NSAIDs, glucocorticoids, immunosuppressants, antagonists/inhibitors of proinflammatory cytokines, Janus kinase (JAK) inhibitors (Zhang, W. et al (2020); Yi, Y. et al (2020)) and complement inhibitors.

The proinflammatory cytokine interleukin-6 (IL-6) has been identified as a primary driver of the cytokine storm, and several reports in the literature describe compassionate use or small trials of

monoclonal antibodies (MAbs) targeting IL-6 or its receptor (Sanders, J.M. et al (2020)). In a retrospective observational study conducted in China, a single dose of the humanized anti-IL-6R MAb tocilizumab was administered to 15 patients with various degrees of disease severity and/or comorbidities. Following the treatment, disease stabilized in 10 patients, worsened in 2, and was unable to prevent the deaths of 3 critically ill patients. Nonetheless, the investigators concluded that further evaluation is warranted to determine the appropriate dose and timing of administration, as well as the profile of patients who would benefit from the treatment (Luo, P. et al (2020)). Based on these and other positive findings, a multicenter, large-scale clinical trial was initiated in China (ChiCTR2000029765) and has resulted in the treatment of approximately 500 severe or critically patients (Fu, B. et al (2020)). Moreover, according to Chinese Covid-19 treatment guidelines, tocilizumab can be used to reduce Covid-19 mortality in patients with extensive bilateral lung lesions (i.e., ground-glass opacity) or in severe or critical patients who have elevated laboratory detected IL-6 levels (Fu, B. et al (2020)).

In the U.S., Sanofi and Regeneron have initiated the randomized, double-blind, placebo-controlled, phase III COVACTA study to evaluating the safety and efficacy of tocilizumab added to standard of care compared to placebo plus standard of care in hospitalized adults with severe pneumonia. Note that tocilizumab is already approved for marketing in many countries, including the U.S., where its approved indications include the treatment of severe life-threatening cytokine release syndrome caused by chimeric antigen receptor T-cell (CART) immunotherapy.

Another clinical program is evaluating the anti-IL-6R antibody sarilumab in U.S. patients hospitalized with severe Covid-19 infection (Sanders, J.M. et al (2020)). The randomized, double-blind, placebo-controlled phase II/III trial is assessing the safety and efficacy of adding sarilumab to usual supportive care, compared to supportive care plus placebo. The trial incorporated an adaptive design to evaluate the MAb in adults hospitalized with laboratory-confirmed Covid-19 that is classified as severe or critical, or who are suffering from multiorgan dysfunction. In April, following a review by the trial's independent data monitoring committee of all available phase II and phase III data and the observation of negative trends in the severe group, the trial was amended so that only patients classified as critical would continue to be enrolled.

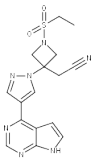
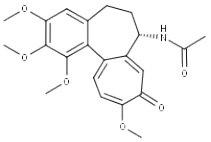
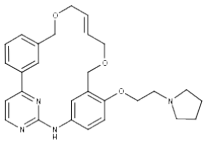
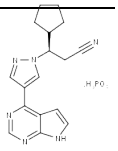
The anti-CCR5 MAb leronlimab (PRO-140) is being evaluated as a potential treatment for patients infected with SARS-CoV-2. Leronlimab has the potential to enhance the cellular immune response by suppressing Treg cells that, in turn, inhibit the antiviral T-cell responses and the potential to repolarize macrophage activity. Leronlimab has shown no drug-related serious adverse events in 9 clinical trials involving more than 800 patients, and has been previously used in combination with protease inhibitors used in HIV therapy, which could be potentially used to treat Covid-19. Preliminary results from the first 10 patients treated in the study suggested significant improvements in several important immunologic biomarkers in 8 of the 10 severely ill patients, with improvements in cytokines and IL-6, and a trend toward normalization of the CD4/CD8 ratio.

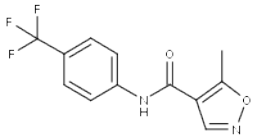
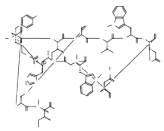
The complement system comprises more than 30 members, which are implicated in the host defense response to infection and injury. This family of serum molecules mediates inflammation and opsonization of antigens and microorganisms in addition to controlling lysis of pathogens or cells sensitized with antibody. These serum molecules may be activated via the classical pathway involving activation by immune complexes binding to C1q subcomponent of C1, which has six Fc binding sites, or by an alternative pathway that can involve activation in the presence of suitable surface molecules. Complement components interact with each other, so that a small stimulus can result in a cascade of activity (Gralinski, L.E. et al (2018)). Complement activation has also been implicated in the formation of diffuse thrombotic microangiopathy and end organ dysfunction, both of which are associated with increased morbidity and mortality in Covid-19 patients. Inhibition of the complement system has been identified as a potential method of treating patients with severe disease due to SARS-CoV-2 infection (Diurno, F. et al (2020); Campbell, C.M. et al (2020)).

The NLRP3 inflammasome has been identified as a potential pathophysiological component determining the clinical course of patients with Covid-19. Inflammasomes are large multiprotein

complexes composed of members of the NOD-like receptor family (NLR) such as NLRP3, PYCARD, CASP1 and possibly CASP4 and CASP5. These complexes are responsible for the activation of inflammatory process and innate immune responses associated with host defense. Inflammasomes can rapidly detect invading pathogenic microbes and eliminate them. They are assembled in response to microbial or endogenous products released from damaged or dying cells and the composition of an inflammasome is dependent on the activator that initiates its assembly. Dysregulation of inflammasomes has been associated with several autoinflammatory and autoimmune disorders, including gout; NLRP3 inflammasomes are also implicated in the pathogenesis of acute respiratory distress syndrome in patients with Covid-19. The marketed uricosuric drug colchicine is a nonselective inhibitor of NLRP3 inflammasomes with an established safety profile; as such, it has been selected for clinical evaluation for the prevention of complications in patients with laboratory-confirmed Covid-19 (Jamilloux, Y. et al (2020); Deftereos, S.G. et al (2020)).

Drugs and biologics targeting the cytokine storm for treatment of Covid-19

Drug name	Organizations	Description	Phase	Structure
Baricitinib	National Institute Allergy Infect Dis	Jak1 Inhibitors; Jak2 Inhibitors; Signal Transduction Modulators	Phase III	
Canakinumab	Novartis	Anti-IL-1beta; Signal Transduction Modulators	Phase III	
Colchicine	Montreal Heart Institute (MHI)	Antimitotic Drugs; Microtubule Destabilizers (Tubulin Polymerization Inhibitors); NLRP3 Inflammasome Inhibitors	Phase III	
Lenzilumab	Humanigen	Anti-GM-CSF; Signal Transduction Modulators	Phase III	
Pacritinib	CTI BioPharma	Angiogenesis Inhibitors; Cyclin-Dependent Kinase 2 (CDK2) Inhibitors; FLT3 (FLK2/STK1) Inhibitors; Interleukin-1 Receptor-Associated Kinase 1 (IRAK-1) Inhibitors; Jak2 Inhibitors; Macrophage Colony-Stimulating Factor 1 Receptor (CSF1R; CD115; c-Fms) Inhibitors; Signal Transduction Modulators	Phase III	
Ruxolitinib phosphate	Novartis; Incyte	Jak1 Inhibitors; Jak2 Inhibitors; Signal Transduction Modulators; Tyk2 Inhibitors	Phase III	
Anakinra	Swedish Orphan Biovitrum	IL-1 Receptor Antagonists; Signal Transduction Modulators	Phase II/III	

IFX-1	InflaRx	Anti-C5 (Complement 5)	Phase II/III
Olokizumab	R-Pharm	Anti-IL-6; Signal Transduction Modulators	Phase II/III
RPH-104	R-Pharm	IL-1 Inhibitors; Signal Transduction Modulators	Phase II/III
Gimsilumab	Roivant Sciences	Anti-GM-CSF; Signal Transduction Modulators	Phase II
Sirukumab	Janssen	Anti-IL-6; Signal Transduction Modulators	Phase II
APL-9	Apellis Pharmaceuticals	Complement C3 Inhibitors	Phase I/II
1086612	Chongqing Sidemu Biotechnology	Anti-GM-CSF; Drugs Acting on NKG2D; Drugs Targeting Angiotensin-I Converting Enzyme-Related Carboxypeptidase (ACE2); Signal Transduction Modulators; Viral Fusion Inhibitors	Phase I/II
Leflunomide	University of Chicago	Angiogenesis Inhibitors; Dihydroorotate Dehydrogenase (DHODH) Inhibitors; Jak3 Inhibitors; PDGFR Family Inhibitors; Signal Transducer and Activator of Transcription 6 (STAT6) Inhibitors; Signal Transduction Modulators	Phase I 
TD-0903	Theravance Biopharma	Janus Kinase (Jak) Inhibitors; Signal Transduction Modulators	Phase I
TJ-003234	I-Mab Biopharma	Anti-GM-CSF; Signal Transduction Modulators	Phase I
Compstatin 40	Amyndas Pharmaceuticals	Complement C3 Inhibitors	Clinical 
Conestat alfa	Pharming	Complement C1s Subcomponent (C1S) Inhibitors; Serine Protease Inhibitors	Clinical
Eculizumab	Alexion Pharmaceuticals	Anti-C5 (Complement 5)	Clinical

Jaktinib dihydrochloride monohydrate	Suzhou Zelgen Biosciences	Jak2 Inhibitors; Signal Transduction Modulators	Clinical
Namilumab	Izana Bioscience	Anti-GM-CSF; Signal Transduction Modulators	Clinical

Targeting Coagulation Disorders

Thrombotic and thromboembolic disease have emerged as potential complications of Covid-19. Potentially due to excessive inflammation, platelet activation, endothelial dysfunction and/or stasis, SARS-CoV-2 infection may predispose to thrombosis in the venous or arterial circulation, manifesting as stroke or venous thromboembolism (Kollias, A. et al (2020); Connors, J.M. et al (2020)). Stroke has been reported as a presenting symptom of Covid-19, including in younger patients without underlying conditions (Oxley, T.J. et al (2020)). Moreover, some investigational agents being tested in patients with Covid-19 may carry the risk of thrombotic events, or may interact with antiplatelet agents, in patients who were taking them prior to becoming infected (Connors, J.M. et al (2020)).

Covid-19-associated coagulopathy (CAC) appears to be linked to the systemic hyperinflammatory host response, rather than a procoagulatory effect of the virus itself. In response to the viral infection, the innate immune system initiates a complex systemic immune cascade which results in the activation of coagulation systems and generation of thrombin, a phenomenon known as immunothrombosis (Bikdeli, B. et al (2020); Levi, M. et al (2020)). Elevated D-dimer level is associated with poor prognosis, and disseminated intravascular coagulation is thought to be involved in the majority of Covid-19 deaths (Tang, N. et al (2020); Kollias, A. et al (2020)).

WHO treatment guidelines recommend pharmacological prophylaxis (low-molecular-weight heparin or heparin) to prevent venous thromboembolism in severely ill adolescents and adults without contraindications, both during acute illness in the hospital and following discharge (Clinical management of severe acute respiratory infection (SARI) when Covid-19 disease is suspected - Interim guidance (World Health Organization, updated March 13, 2020)). Higher than the standard therapeutic dose may be required in Covid-19 patients, particularly those who are obese. Current data do not support the use of anticoagulants to treat microvascular thrombosis (Bikdeli, B. et al (2020)).

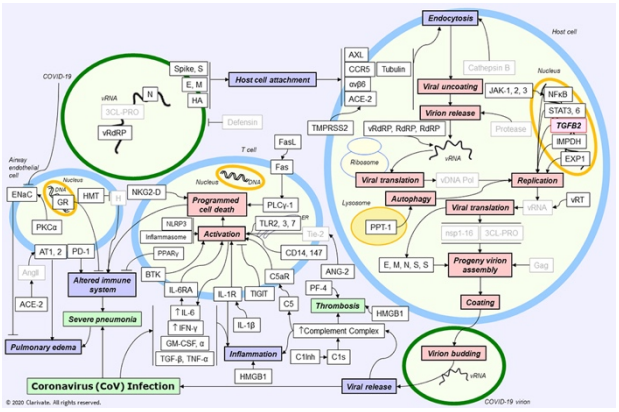
Several investigational agents targeting Covid-19-associated coagulopathy are being evaluated in the clinic, including dociparstat sodium, a non-anticoagulant heparin derivative (2-O, 3-O desulfated heparin) that retains antiinflammatory activity. The drug is being evaluated in phase II/III clinical trials at Chimerix for the treatment of acute lung injury (ALI) in patients with severe Covid-19. The mechanistic rationale supporting dociparstat's potential in ALI patients with Covid-19 is two-fold. The first is based in its potential to decrease inflammation/immune cell infiltration in Covid-19 patients with ALI, and the second in its potential to alleviate the underlying causes of coagulation disorders by inhibiting HMGB1 and platelet factor 4 (PF4) activities. HMGB1, an endogenous damage-associated molecular pattern (DAMP) molecule, has been identified as a therapeutic target for Covid-19 (Andersson, U. et al (2020)).

Targets for Therapeutic Intervention

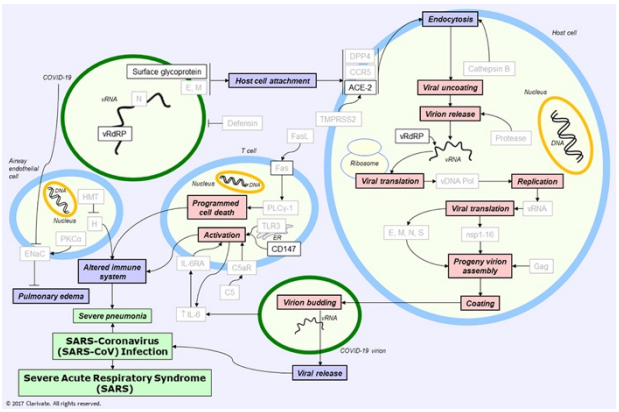
For an overview of validated therapeutic targets for this indication, consult the targetscape below. The targetscape shows an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of the condition and their biological actions. An arrow indicates a positive effect; a dash indicates a negative effect. Gray or lighter symbols are protein targets that are not validated (i.e., not under active development [UAD]). Pink text boxes with red borders indicate validated gene targets. Yellow text boxes are gene targets not UAD. Purple and pink text boxes indicate extracellular and

intracellular effects, respectively. Green text boxes indicate a related disease/condition/symptom. For in-depth information on a specific target or mechanism of action, see the corresponding section in this report.

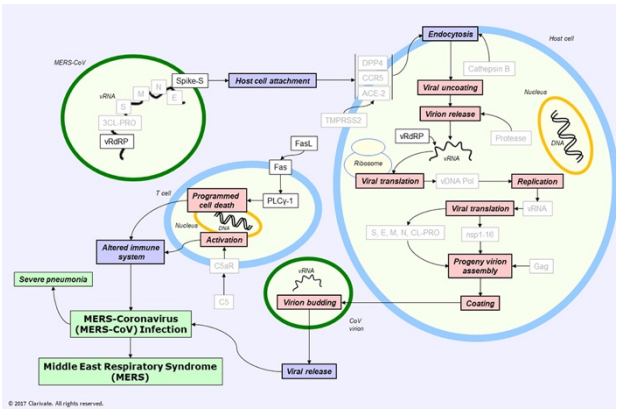
Coronavirus (CoV) Infection Targetscape



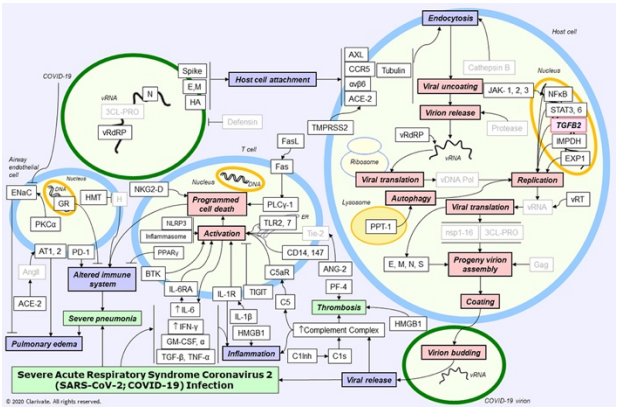
Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) Infection Targetscape



Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Infection Targetscape



Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2; COVID-19) Infection Targetscape



Latest Headlines

05-Jun-2020

ValiRx collaborates with Oncolytika and Black Cat Bio to explore combination treatment for COVID-19

ValiRx has entered into a collaboration agreement with Oncolytika and Black Cat Bio to explore the use of VAL-201 in a combination treatment for patients suffering a hyperimmune response to coronavirus SARS-CoV-2 infection. Many patients infected with coronavirus SARS-CoV-2 exhibit more severe symptoms, with significant damage believed to be caused by an excessive response of the immune system, even after the viral infection has been reduced. This is known as a hyperimmune response. Oncolytika has proposed a combination therapy to overcome the hyperimmune response seen in many patients infected with coronavirus SARS-CoV-2, consisting of a selective SRC kinase inhibitor (which inhibits a potential oncogenic pathway) in combination with one or two complementary treatments. Oncolytika and Black Cat have filed a patent to protect the proposed use of the combination therapy, with ValiRx providing samples of its proprietary SRC kinase inhibitor, VAL-201, for preclinical testing, as well as access to safety and tolerability data collected in the recently completed clinical trial in men with prostate cancer. No cash funding is committed to the project by ValiRx under this agreement. Subject to successful outlicencing, ValiRx will receive 40% of all licensing income generated. The collaboration addresses an emergent and immediate unmet medical need, and details the commencement of a short-term experimental plan, with the agreement covering a maximum of 2 years (ValiRx News Release).

05-Jun-2020

Inovio and partners to initiate phase I/II study of COVID-19 DNA vaccine in South Korea

Inovio, the International Vaccine Institute (IVI), and Seoul National University Hospital announced a partnership to initiate a phase I/II clinical trial of Inovio's COVID-19 vaccine INO-4800 in South Korea. Initially, 40 healthy adults (19-50 years old) are expected to enroll in this 2-stage trial, designed to assess the safety, tolerability and immunogenicity of INO-4800, and which will further expand to enroll an additional 120 people (19-64 years old). This will be the first clinical study of COVID-19 vaccine in Korea. The trial is funded by the Coalition for Epidemic Preparedness Innovations (CEPI) through Inovio and is supported by the Korea Center for Disease Control and Prevention/Korea National Institute of Health. The study is expected to initiate later in June. In normal circumstances, it would generally take several years to start clinical trials of a new vaccine, however, in the midst of the COVID-19 pandemic, the trial in Korea will be conducted just 2 months after a similar clinical study began in the U.S. in early April 2020 (ClinicalTrials.gov Identifier NCT04336410). The speedy regulatory approval was made possible with support from the Korean Ministry of Food and Drug Safety, following its adoption of a fast-track approval process for clinical trials of COVID-19 vaccines and therapeutics that are developed with a proven safety platform, as announced in April. Such vaccines, including DNA vaccines, can be exempt from toxicology tests leveraging the available preclinical data using the DNA platform, and expediting clinical trial review process. The DNA vaccine of U.S.-based Inovio to be tested was one of the first technologies to receive support from CEPI, greatly accelerating the development process of the COVID-19 vaccine. IVI and SNU Hospitals have collaborated in the past to conduct phase I/IIa trials for a MERS coronavirus vaccine (INO-4700/GLS-5300), which was developed by Inovio and South Korea's GeneOne Life Science. INO-4700 has achieved promising results in clinical trials conducted to date (Inovio News Release; IVI News Release).

05-Jun-2020

Intravacc announces partnership to develop intranasal COVID-19 vaccine

Intravacc has announced that an intranasal vaccine against COVID-19 will be developed through a newly established public private partnership that combines the vaccine development technology from Intravacc, the viral vector technology and animal technologies from Wageningen Bioveterinary Research (WBVR), and the coronavirus expertise from Dutch Utrecht University. The vaccine will consist of a Newcastle disease virus (NDV) vector that expresses the immunogenic spike (S) protein of SARS-CoV-2, which is an important target for neutralizing antibodies. NDV has been shown to be safe for intranasal/intratracheal delivery in mammals, including nonhuman primates. The advantage of a nasal vaccination is that it induces both mucosal and systemic immunity, while an intramuscular vaccination primarily induces an antibody response. Intranasal vaccination may also confer protection against infections at other mucosal sites, such as the lungs, intestines and genital tract. Additionally, administration through the nasal cavity is easily accessible. Intravacc will develop a scalable vaccine production process using its FDA-approved Vero cell platform, in preparation of GMP productions. WBVR has developed a technique called reverse genetics, which allows the genetic modification of NDV, resulting in the development of NDV as a vaccine vector against human and animal infectious diseases. This vector technology will now be used to generate a vaccine against COVID-19 (Intravacc News Release).

05-Jun-2020

PharmAust reports preliminary data for monepantel in models of SARS-CoV-2 infection

PharmAust provided an update on its preliminary work evaluating the effects of monepantel and monepantel sulfone in cells infected with SARS-CoV-2 in tissue culture. These experiments have been undertaken for PharmAust by the Walter and Eliza Hall Institute of Medical Research virologists in accredited and controlled safety facilities at the Institute in Melbourne. The findings of these in vitro studies, which were conducted using a model of SARS-CoV-2 viral infection in African Green Monkey VERO cells, demonstrated that both infectivity and replication of SARS-CoV-2 virus particles could be suppressed by between 50%-95% in cell cultures, as determined by the median tissue culture infectious douse (TCID50) assay. For the qPCR individual assays, the degree of suppression was up to approximately 55%. These preliminary experiments suggest both monepantel and monepantel sulfone have the ability reduce the capacity of SARS-CoV-2 to replicate and to mature into infectious virus particles. Interestingly, relatively low concentrations of monepantel blocked the infectious capacity of SARS-CoV-2 in tissue culture. Based on the above findings, PharmAust has moved to broaden and extend its intellectual property in the area of antiviral activity through the filing of a patent application specifically covering monepantel in the treatment of COVID-19. The company plans further validation of these preliminary results as soon as possible (PharmAust News Release). Monepantel is in early clinical development at PharmAust for the treatment of solid tumors.

05-Jun-2020

Identification of inhibitors of the main protease of SARS-CoV-2

The 33.8-kDa main protease (Mpro) of SARS-CoV-2 constitutes an attractive drug target because of its essential role in viral replication and transcription and its lack of closely related homologues in humans. Researchers from ShanghaiTech University and colleagues have applied a strategy combining structure-assisted drug design, virtual drug screening, and high-throughput screening to repurpose drugs to target viral Mpro. By computer-aided drug design, the team identified a mechanism-based irreversible inhibitor (N3) and determined the crystal structure of the Mpro-N3 complex. Next, they used structure-based virtual and high-throughput screening of around 10,000 compounds (including approved drugs, drug candidates under clinical

development, and other pharmacologically active compounds) to identify Mpro inhibitors. Six of them showed inhibitory activity with half-maximal inhibitory concentration values between 0.67 to 21.4 mcM. They evaluated the antiviral activity of these six compounds in SARS-CoV-2-infected Vero cells, with 10 mcM ebselen (EC50 of 4.67 mcM) and N-3 (EC50 of 16.8 mcM) showing the strongest antiviral effects. Therefore, ebselen, an organoselenium compound with anti-inflammatory, antioxidant and cytoprotective properties and very low cytotoxicity, may show potential for COVID-19 treatment. Moreover, these results support the utility of this screening strategy for the rapid discovery of drugs against new infectious diseases (Jin, Z. et al. Nature 2020, Advanced publication).

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05-Jun-2020

DMX-200 to be studied for COVID-19 within REMAP-CAP program

Dimerix's DMX-200 (propagermanium/irbesartan) has been selected for inclusion in the protocol as a new treatment arm in the global program, REMAP-CAP (Randomised, Embedded, Multifactorial Adaptive Platform trial for Community-Acquired Pneumonia; ClinicalTrials.gov Identifier NCT02735707), aimed at treating patients with acute respiratory distress syndrome (ARDS) as a result of COVID-19. The REMAP-CAP program, funded by a consortium of government and nongovernment organizations, is endorsed by the World Health Organization (WHO) and is designated as a Pandemic Special Study. REMAP-CAP (and the companion platform REMAP-COVID) is an international adaptive platform trial studying a number of diverse interventions in hospitalized patients with proven or suspected COVID-19, enrolling in both ICU and non-ICU settings. The overall REMAP-CAP study, currently registered as a phase IV study, plans to include over 7,000 patients at over 200 already initiated study sites across Asia-Pacific, Europe and North America. The study has several existing treatment domains, including antiviral, immune-modulation and immunoglobulin treatment arms. Now, a domain within REMAP-CAP/COVID is being developed to study the effects of renin-angiotensin system inhibition, which includes DMX-200. Pending study approvals in each territory, the adaptive study aims to compare directly the treatment effect of a number of study treatment options on the clinical outcomes of COVID-19 patients requiring hospital care. Dimerix will work with REMAP-CAP to rapidly obtain the necessary regulatory and ethics approvals, before providing DMX-200 to sites from its existing pharmaceutical grade manufactured supply. All of the other selected candidates for this study are a repurposing of existing approved drugs with potential for COVID-19. Dimerix has been working on DMX-200 as a renal therapy to reduce damage from inflammatory cells by blocking their signaling and limiting subsequent onset of fibrosis. DMX-200 may also benefit ARDS patients with COVID-19 by reducing the inflammatory response in the lungs and thus reducing inflammation and subsequent fibrosis. Multiple publications have recently reported a SARS-CoV-2-induced elevation of MCP-1, the proinflammatory ligand acting on CCR2, the receptor targeted by DMX-200. Separately, phase II studies with DMX-200 in kidney disease continue on track, with read-out anticipated around the middle of this year (Dimerix News Release).

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04-Jun-2020

FDA gives FSD Pharma permission to submit IND for phase IIa trial of FSD-201 for COVID-19

The FDA has given FSD Pharma permission to submit an IND application for the use of FSD-201 (ultramicronized palmitoylethanolamide [PEA]) to treat COVID-19. FSD Pharma is developing FSD-201 for its anti-inflammatory properties to avoid the cytokine storm associated with acute lung injury in hospitalized COVID-19 patients. On the basis of FDA feedback, the planned trial is expected to be a randomized, controlled, double-blind, U.S., multicenter phase IIa study to assess the efficacy and safety of FSD-201 dosed at 600 mg or 1200 mg twice daily plus standard of care (SOC) versus SOC alone in symptomatic patients with clinical presentation compatible with COVID-19. Eligible patients will present with symptoms consistent with influenza/coronavirus signs and/or newly documented positive COVID-19 disease. The primary endpoint is to determine if FSD-201 plus SOC provides a significant improvement in clinical status (i.e., shorter time to symptom relief). Key secondary objectives include determining if

FSD-201 plus SOC demonstrates additional benefit in terms of safety, objective assessments such as length of time to normalization of fever, length of time to improvement of oxygen saturation and length of time to clinical progression, including time to mechanical ventilation or hospitalization, and length of hospital stay. The exploratory endpoint is cytokine clearance as measured by ELISA. The treatment period is expected to be 14 days. All patients who experience clinical benefit are expected to continue to receive their assigned treatment until study completion. PEA downregulates hyperactive mast cells, inhibits iNOS expression and nuclear NF-kappaB translocation. It is theorized that coronavirus activates the cellular IKK/NF-kappaB signaling pathway for replication; therefore, PEA as a PPAR-alpha agonist may ameliorate oxidative/nitrosative stress induced by NF-kappaB and may be a suitable agent for antiviral intervention. PEA has also been shown to downmodulate excess immune response activity that contributes to the physiologic derangement induced by viruses and help mitigate the pathogenesis of the cytokine storm. Some Italian physicians have been using ultramicronized PEA to treat COVID-19 patients in Italy on a compassionate use basis. FSD Pharma acquired worldwide rights (ex-Italy and Spain) to ultramicronized PEA from Epitech, which markets ultramicronized PEA as a prescription-based food for special medical purposes in Italy under the brand name Normast for several chronic pain and inflammatory conditions, including sciatic pain and diabetic neuropathy. FSD is developing FSD-201 for its anti-inflammatory properties, and a first-in-human safety and tolerability study is currently progressing in Australia (FSD Pharma News Release).

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03-Jun-2020

Description of a potential COVID-19 mouse model presenting human ACE2 and TMPRSS2 genes

Infections with SARS-CoV-2 have been responsible for over 370,000 deaths across the world since its appearance in December 2019. Numerous studies are in progress in search for a new effective treatment or vaccine to contain the pandemic. Nevertheless, to date there is no appropriate murine model to conduct in vivo experiments, complicating research advances. Previously, researchers developed mouse models for SARS-CoV based on the humanization of angiotensin-converting enzyme 2 (ACE2), which is necessary for the virus entry into the host cell. However, these models were unable to precisely mimic the human disease. Now it is known that a second protein interferes with ACE2 for SARS-CoV-2 host-cell invasion: the transmembrane protease/serine subfamily member 2 (TMPRSS2). Hence, investigators from the Institute of Gene Biology of the Russian Academy of Sciences propose a new mouse model in which both human ACE2 (hACE2) and human TMPRSS2 (hTMPRSS2) would be introduced in the murine genome using CRISPRcas9 technology. The researchers intend to place LoxP sites in front of the hTMPRSS2, creating an inducible hACE2/hTMPRSS2 expression to control the sensitiveness of the mice to the infection. In this way, the new mouse model would be safer for research conditions. This murine model, presenting two human genes that are essential for cell invasion of the virus, would better mimic the human syndrome and could also represent a tool for in vivo drug screening of compounds that inhibit TMPRSS2 (Soldatov, V.O. et al. Res Results Pharmacol 2020, 6(2): 1).

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03-Jun-2020

FDA clears IND for phase I/II study of Altimmune's T-COVID

The FDA has cleared Altimmune's IND for a phase I/II trial of T-COVID, an investigational agent for the treatment of early COVID-19. Patient enrollment is expected to commence this month. The double-blind trial will evaluate the potential of T-COVID to prevent clinical worsening in patients with early COVID-19. The trial is expected to enroll approximately 100 patients age 35 years and older, randomized 1:1 to receive either intranasal T-COVID or placebo administered in an outpatient setting within 48 hours of onset of symptoms and 24 hours of diagnosis. The study will be enrolled in three cohorts of increasing risk factors for severe COVID-19, with the final cohort enrolling patients of all ages and risk factors. The primary efficacy endpoint is the proportion of patients with clinical worsening, defined as a 4% decrease in pulse oxygen

saturation (SpO2), or need for hospitalization. Data readout is anticipated in the fourth quarter of this year. Treatment with T-COVID administered as a single intranasal dose to patients with an early onset of symptoms and recent diagnosis of COVID-19 may prevent the progression to severe lung inflammation and thereby decrease the development of severe COVID-19 and the need for hospitalization. The company believes the mechanism of action underlying T-COVID has the potential to be studied for both pre- and post-exposure prophylaxis for higher-risk individuals, such as frontline healthcare workers. T-COVID is based on the same replication-deficient adenovirus 5 (RD-Ad5) vector technology behind Altimmune's intranasal vaccine candidates, which include NasoVAX for influenza, NasoShield for anthrax, and AdCOVID for COVID-19, but it acts through a different mechanism. In preclinical studies sponsored by the National Institute of Allergy and Infectious Diseases, intranasal administration of RD-Ad5 vectors modulated the innate immune response to lethal challenge with a respiratory virus in mice and protected them from death. The immunomodulatory effects resulted in significantly decreased cellular inflammation and lower concentrations of IL-6 and other inflammatory cytokines in the lungs of treated animals compared to controls. Excessive production of inflammatory cytokines such as IL-6 has been associated with lung pathology and death in COVID-19. The protective effects were independent of any specific immunity or vaccine effects against the challenge virus. These protective effects were only observed with intranasal administration of RD-Ad5, and intramuscular administration provided no survival benefit. The FDA has agreed that the company may use its existing lot of RD-Ad5-based NasoVAX influenza vaccine for the planned T-COVID trial (Altimmune News Release).

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03-Jun-2020

Intravacc and EpiVax collaborate to advance novel COVID-19 vaccine

Intravacc and EpiVax have entered into a collaboration agreement to advance a novel vaccine against COVID-19 based on Intravacc's proprietary Outer Membrane Vesicles (OMV) technology platform. Under this joint research project, Intravacc will combine its immunogenic OMV delivery platform with synthetically produced COVID-19 epitopes, designed and optimized by EpiVax, in order to generate a safe and effective T-cell response against SARS-CoV-2 and related coronaviruses. Preclinical studies will start immediately to select the best candidate peptides for the vaccine. Intravacc will utilize its in-house pilot-scale facility for the GMP production of the OMV-peptide vaccine, for phase I studies expecting to start in the fourth quarter of this year (Intravacc News Release).

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Suggested reading

Related websites

- Centers for Disease Control and Prevention (CDC) -- Coronavirus (Covid-19)
- Centers for Disease Control and Prevention (CDC) -- SARS information
- Coalition for Epidemic Preparedness Innovation (CEPI)
- Coronavirus Global Health Emergency (United Nations)
- Covid 19 (Infectious Diseases Data Observatory)
- European Centre for Disease Prevention and Control -- Novel coronavirus
- European Commission - Public health - COVID-19 resources
- MEDLINEplus: Coronavirus infections
- Middle East respiratory syndrome coronavirus (MERS-CoV) (World Health Organization)
- National Institute of Allergy and Infectious Diseases
- NCBI web resource: Severe Acute Respiratory Syndrome (SARS)
- SARS information - Health Canada
- Severe acute respiratory syndrome (SARS) (World Health Organization)
- The Covid-19 host genetics initiative

Related articles

- Coronavirus (Covid-19) (New England Journal of Medicine)
- Coronavirus disease 2019 (COVID-19) (JAMA Network)
- Coronavirus: Latest news and resources (The BMJ)
- Coronavirus: Research, commentary, and news (Science)
- COVID-19 resource centre (The Lancet)
- Nature.com collection: Coronavirus
- Novel coronavirus (COVID-19) resource center (Center for Infectious Disease Research and Policy, University of Minnesota)
- SARS Reference by B.S. Kamps and C. Hoffman (Eds.)

Guidelines

A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version) (February 2020)

Clinical management of severe acute respiratory infection when Middle East respiratory syndrome coronavirus (MERS-CoV) infection is suspected - Interim guidance (World Health Organization, 2019)

Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected - Interim guidance (World Health Organization, January 12, 2020)

Collection: Novel coronavirus (2019-nCoV) guidance for health professionals (Public Health England, January 2020)

Coronavirus disease 2019 (Covid-19) treatment guidelines (National Institutes of Health, April 2020)

COVID-19 rapid guideline: Managing suspected or confirmed pneumonia in adults in the community (National Institute for Health and Care Excellence, April 2020)

COVID-19 rapid guideline: Managing symptoms (including at the end of life) in the community (National Institute for Health and Care Excellence, April 2020)

Diagnosis and treatment protocol for novel coronavirus pneumonia (trial version 7) (National Health Commission & State Administration of Traditional Chinese Medicine, March 2020)

Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: Expert consensus statement (February 2020)

Infection prevention and control during health care for probable or confirmed cases of novel coronavirus (nCoV) infection - Interim guidance (World Health Organization, May 6, 2013)

Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected - Interim guidance (World Health Organization, updated March 2020)

Initial public health response and interim clinical guidance for the 2019 novel coronavirus outbreak -- United States, December 31, 2019-February 4, 2020 (Centers for Disease Control and Prevention, February 5, 2020)

Interim infection prevention and control recommendations for patients with known or patients under investigation for 2019 novel coronavirus (2019-nCoV) in a healthcare setting (Centers for Disease Control and Prevention, January 2020)

Management of asymptomatic persons who are RTPCR positive for Middle East respiratory syndrome coronavirus (MERS-CoV) - Interim guidance (World Health Organization, January 2018)

Surviving Sepsis Campaign: Guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19) (Surviving Sepsis Campaign, March 2020)

Treatment of MERS-CoV: Information for clinicians - Clinical decision-making support for treatment of MERS-CoV patients (Public Health England, July 2014)

Update on the epidemiology of Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection, and guidance for the public, clinicans, and public health authorities - January 2015 (Centers for Disease Control and Prevention, January 30, 2015)

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