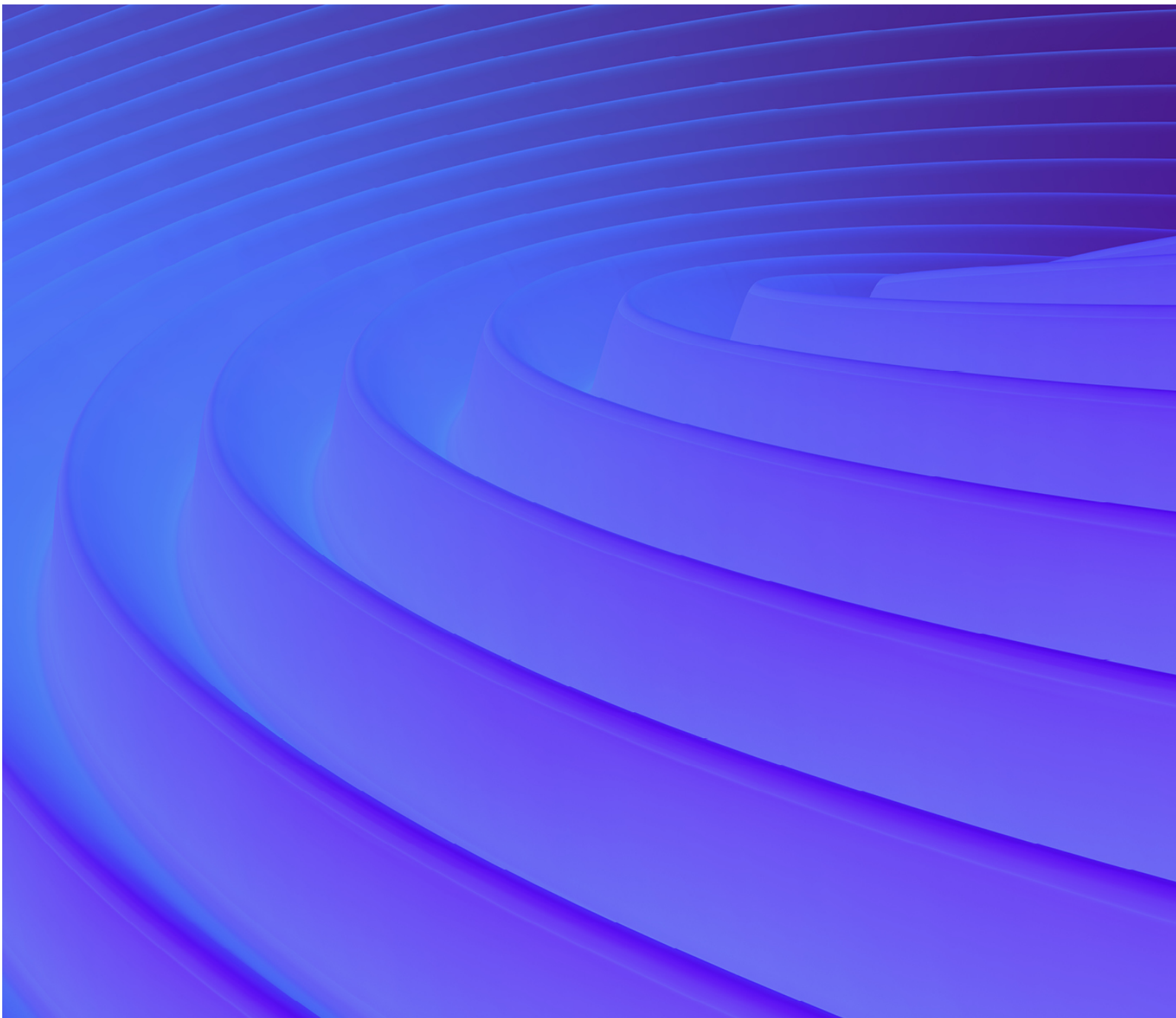


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Disease Briefing: Coronaviruses



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Coronavirus: Disease Briefings

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 chicken. 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 (Berry, M. et al (2015); Su, S. et al (2016)).

Coronaviruses have long been recognized as important veterinary pathogens, causing respiratory and enteric diseases in mammals as well as in birds. Before 2019, only six coronaviruses had been 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)). The first four are endemic locally; they have been associated mainly with mild, self-limiting disease, whereas the latter two can cause severe illness (Song, Z. et al (2019); Paules, C.I. et al (2020)). SARS-CoV and MERS-CoV are betacoronaviruses (Chen, Y. et al (2020)), and 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 represent an ongoing threat to human health (Hui, D.S. et al (2020); Zhu, N. et al (2020)). This fact again became 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 respiratory disease, including potentially fatal pneumonia, in Wuhan, China (**WHO statement regarding cluster of pneumonia cases in Wuhan, China (World Health Organization, January 9, 2020); Emergencies: Novel coronavirus 2019 (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.

Important RNA viruses and the diseases they produce in humans

Family/Characteristics	Viruses	Diseases
Orthomyxoviruses (Orthomyxoviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; segmented genome)	Influenza A and B virus	Upper respiratory infection, croup
Paramyxoviruses (Paramyxoviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; nonsegmented genome)	Parainfluenza 1-3 virus	Upper respiratory infection, croup
	Respiratory syncytial virus	Upper respiratory infection, croup
	Measles virus	Measles
	Mumps	Aseptic meningitis
Coronaviruses (Coronaviridae) Single-stranded RNA, enveloped (No DNA step in replication; positive-sense genome)	Human coronaviruses	Upper respiratory infection
Rhabdoviruses (Rhabdoviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; nonsegmented genome)	Rabies virus	Rabies
Picornaviruses (Picornaviridae) Single-stranded RNA, nonenveloped	Rhinoviruses	Common cold
	Hepatitis A virus	Hepatitis
	Enteroviruses: - Polioviruses - Coxsackie A24 viruses - Coxsackie B viruses - Coxsackie B1-5 viruses - Coxsackie A9 viruses - Echoviruses	Paralysis Acute hemorrhagic conjunctivitis Myocarditis, pericarditis Aseptic meningitis Aseptic meningitis Aseptic meningitis, encephalitis
Caliciviruses (Caliciviridae) Single-stranded RNA, nonenveloped	Norwalk virus	Gastroenteritis
Hepeviruses (Hepeviridae) Single-stranded RNA, nonenveloped	Hepatitis E	Hepatitis
Togaviruses (Togaviridae) Single-stranded RNA, enveloped (No DNA step in replication; positive-sense genome)	Alphaviruses (Group A arboviruses)	Encephalitis, hemorrhagic fever, chikungunya
	Rubivirus	Rubella
Flaviviruses (Flaviviridae) Single-stranded RNA, enveloped (No DNA step in replication; positive-sense genome)	Group B arboviruses	Encephalitis, hemorrhagic fever
	Hepatitis C virus	Hepatitis

sense genome)	Dengue virus	Dengue fever
	Zika virus	Zika
Bunyaviruses (Bunyaviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; segmented genome)	Some arboviruses Hantavirus	Encephalitis, hemorrhagic fevers Fever, renal involvement
Reoviruses (Reoviridae) Double- stranded RNA, nonenveloped	Human rotaviruses	Gastroenteritis
Arenaviruses (Arenaviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; segmented genome)	Lymphocytic choriomeningitis (LCM virus) Lassa virus	Meningitis Hemorrhagic fever
Retroviruses (Retroviridae) Single- stranded RNA, enveloped (DNA step in replication)	HTLV-I, HTLV-II HIV-1, HIV-2	T cell leukemia, lymphoma, paresis AIDS

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 spike (S) glycoprotein radiating from the virus lipid envelope (Chan, J.F. et al (2015); Chen, Y. et al (2020)).

There are 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 (Tseng, Y.T. et al (2010)). The viral genome is associated with the basic phosphoprotein nucleocapsid (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 and MERS can cause severe acute respiratory disease and multi-organ failure (Zumla, A. et al (2016)).

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 newly identified 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 new 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 Guangdong in November 2002, and was first reported at the beginning of 2003 in Asia, followed by reports of a similar disease in North America and Europe (Heymann, D.L. et al (2013)). Worldwide, 33 countries and regions on five continents reported SARS cases, but the most severely affected were mainland China and Hong Kong. In spring 2003, SARS became a global health threat. The rapid spread of the virus to different continents after the initial outbreak underscored the ease with which infectious diseases can be spread internationally among members of our highly mobile global population (Cleri, D.J. et al (2010); Heymann, D.L. et al (2013)).

Although the disease has been absent since 2003, the rapid global spread of SARS demonstrated 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 need for 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 (Cheng, V.C. et al (2013); Al-Tawfiq, J.A. et al (2014); Zumla, A. et al (2015)). 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)).

Causative Agent: SARS Coronavirus

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 (Falsey, A.R. et al (2003); Berry, M. et al (2015)).

The new coronavirus was named "Urbani SARS-associated coronavirus" in honor of Dr. Carlo Urbani, a WHO scientist who first reported the disease and subsequently died from SARS on March 29, 2003 (Cleri, D.J. et al (2010); Felkai, P. (2018)). Evidence based on many different methods, such as cell culture, microscopy, microarray data, serologic tests and PCR, supported the hypothesis that this new coronavirus was the causative agent of SARS (Gerberding, J.L. (2003)).

The absence of antibodies against the SARS virus in healthy people indicated that the virus had not previously circulated in the human population, providing additional supporting data for the possibility that SARS was caused by a new virus. The SARS virus was likely to have originated in animals, followed by either mutation or recombination events that facilitated infection of humans (Zumla, A. et al (2016)).

Investigators in both the U.S. and the Netherlands developed a model system of infection in monkeys in order to fulfill Koch's postulates. Experiments conducted at the Erasmus Medical Center of the University of Rotterdam gave the ultimate evidence that the SARS-CoV was the causative agent of SARS (Fouchier, R.A.M. et al (2003); Kuiken, T. et al (2003)).

SARS-CoV Morphology, Structure and Replication

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.

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)). SARS-CoV entry into target cells is inhibited by polyanion compounds that have antiviral activity against other enveloped viruses. This data indicates that the SARS-CoV envelope proteins may have positive charges interacting with negative charges on the heparan sulfate proteoglycans present on the surface of target cells (Vicenzi, E. et al (2004)). The SARS-CoV requires 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 (Perlman, S. et al (2009); Berry, M. et al (2015); Abdel-Moneim, A.S. (2014)).

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)).

Origin of SARS-CoV

The fact that different animal coronaviruses are able to recombine their RNA to originate new viruses led to the hypothesis that SARS-CoV may have arisen as a result of a recombination event between an animal and a human virus (Chan, P.K. et al (2013)).

How the virus became infectious for humans is unknown. The lack of sequence homology with any of the known human coronavirus strains makes a recombination event among human pathogens a remote possibility. By using methods such as Bayesian phylogenetic interference, it has been shown that the SARS-CoV genome has a recombination breakpoint within the RNA

polymerase gene, and that the 5' region is related to mammalian and the 3' region to avian coronaviruses (Rest, J.S. et al (2003)).

In May 2003, scientists in Hong Kong reported the discovery of a virus virtually identical to the virus causing SARS in a rare species of civet (*Civettictis civetta*), a tree-dwelling cat. Yuen Kwok-Yung, a microbiologist at Hong Kong University, reported that the coronavirus had been found in the feces of masked palm civets, a nocturnal species found from Pakistan to Indonesia. The masked palm civet is considered to be a delicacy in southern China. Some of the first known cases of SARS occurred in November 2002 among food handlers who handled, killed and sold animals for food and chefs in Guangdong Province who were involved in the preparation of wild game for banquets. Infected civets are asymptomatic. The Hong Kong University team was able to culture a coronavirus almost identical to the SARS coronavirus from all 25 of the masked palm civets, representing eight different species that were tested (Enserink, M. (2003)).

Another team detected SARS-CoV-like viruses in live animals sold for food in the Guangdong province. The presence of the virus was confirmed in the Himalayan palm civet (*Paguma larvata*) and was found in a raccoon dog (*Nyctereutes procyonoides*) (Chan, P.K. et al (2013)). Sequence analysis showed a phylogenetic distinction between animal and human viruses, making passage from humans to the analyzed animals unlikely. This finding points to the possibility of interspecies transmission route within animals held in the market, making the identification of the natural reservoir even more difficult. Subsequent studies suggested that the SARS-CoV had not been circulating in civets for long, and thus that some other species may be acting as a natural reservoir (Hui, D.S. et al (2010)); later investigations identified bats as the reservoir species for SARS-CoV as well as closely related coronaviruses (Song, Z. et al (2019)).

By serological analysis about 40% of wild animal traders and 20% of people employed in the slaughter of animals for market in the affected region had SARS-CoV antibodies, although none had SARS-like symptoms (Berry, M. et al (2015)). Therefore a SARS-like coronavirus had been present in the area at least two years before the SARS-outbreak. The virus, initially not infectious in the human population, may have evolved and adapted to humans to give rise to the SARS-CoV.

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 (Loeb, M. et al (2004); Cleri, D.J. et al (2010); Chen, W.Q. et al (2009)). Nosocomial spread was reduced through use of surgical masks, gloves and gowns (Seto, W.H. et al (2003)).

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 (McDonald, L.C. et al (2004); Cleri, D.J. et al (2010)). 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. A superspreading event was believed to be involved in the rapid propagation of the virus in the Amoy Gardens apartment building

outbreak, where more than 300 residents were infected, presumably by a single patient (Cleri, D.J. et al (2010)). Other superspreading events were reported in the Hotel Metropole in Hong Kong, among passengers on Air China flight 112 from Hong Kong to Beijing, and in an acute care hospital in Toronto, Canada (Braden, C.R. et al (2013)). 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)).

Viral RNA may persist long after seroconversion, and could be detected in respiratory secretions, plasma and feces for some weeks (Drosten, C. et al (2003)). The SARS outbreak revealed the susceptibility of modern hospitals to nosocomial infections and emphasized the importance of implementing measures to reduce the risk of hospital infections (Gopalakrishna, G. et al (2004)).

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 includes high fever ($>38^{\circ}\text{C}$) 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 are not easily distinguishable from other common respiratory infections, and fever may be 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 has been reported (Zhang, D.M. et al (2008)). Symptomatically, SARS generally followed a triphasic pattern that accompanies each of the phases in tissues. 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 (Yang, J.K. et al (2006)), 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

The WHO reported a total of 8,096 SARS cases and 774 resulting deaths worldwide during the period of the major outbreak between November 1, 2002 and August 7, 2003. Mainland China was hardest hit, with at least 5,327 cases and 349 deaths (66% and 45% of the total, respectively) (Zhang, Y. et al (2013)). 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. It has been estimated that the worldwide economic cost of the SARS epidemic was about USD 30 billion. The 6% annual

economic growth of East Asia in 2003 was reduced to 5% during the epidemic (Kondro, W. (2003)). 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)). Globally, the economic cost of the epidemic was estimated at up to 100 billion (Paules, C.I. et al (2020)).

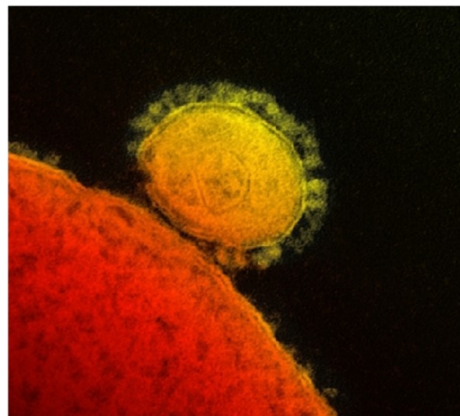
The rapid and effective containment of SARS just months after its international recognition was achieved thanks to an unprecedented international collaboration between researchers, healthcare providers and health authorities (Braden, C.R. et al (2013)). However, factors and circumstances that caused 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 (Lau, E.H. et al (2010); Berry, M. et al (2015)).

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 now 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 (Perlman, S. et al (2013)) and to use a different host-cell receptor, identified as dipeptidyl peptidase 4 (DPP4, also known as CD26) (Raj, V.S. et al (2013); 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)).

MIDDLE EAST RESPIRATORY SYNDROME
CORONAVIRUS (MERS-COV)



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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 the first member of the betacoronavirus genus known to infect humans, and is more closely related to bat coronaviruses such as HKU4 and HKU5 than it is to SARS-CoV (Banik, G.R. et al (2015); Chan, J.F. et al (2015)). 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 is 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)). 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 April 10, 2019). 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 Saudi Arabia, and confirmed that the virus can be transmitted between humans during close contact (Assiri, A. et al (2013); Zumla, A. et al (2015)). At the end of December 2019, the World Health Organization had been notified of 2,499 laboratory-confirmed human cases of infection with the virus and 861 related deaths (Middle East respiratory syndrome coronavirus (MERS-CoV) (World Health Organization), consulted February 18, 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 (R_0) 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)).

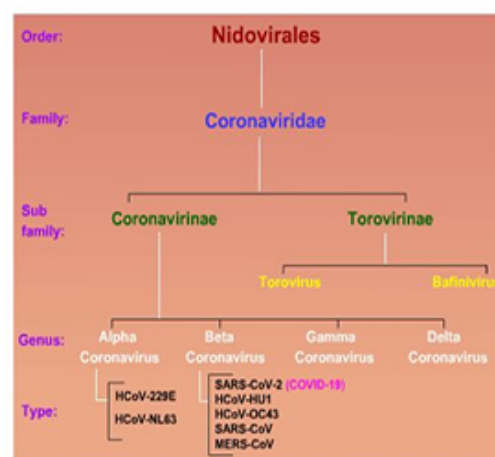
For more epidemiology information, consult the Incidence and Prevalence Database (IPD): [IPD: Middle East respiratory syndrome coronavirus \(MERS-CoV\).](#)

Facts about COVID-19

In late 2019, a new coronavirus began causing febrile respiratory illness in mainland China; two months later, the rapidly spreading disease was officially christened Coronavirus Disease 2019 (Covid-19) by WHO. 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)). In January, the Chinese Center for Disease Control and Prevention (China CDC) acknowledged that only 22% of the 198 confirmed Covid-19 cases included in its outbreak analysis could be traced to a Huanan market-related exposure (Wu, J.T. et al (2020)).

The causative virus—originally termed 2019-nCoV—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)).

Coronavirus Taxonomy



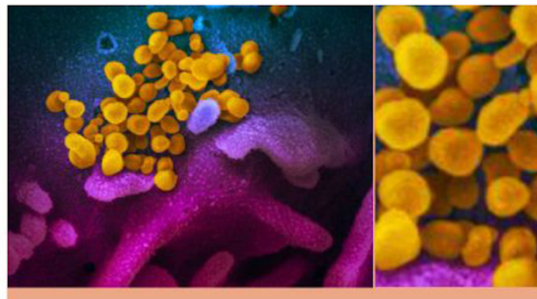
Pillaiyer, T., Menonshchandran, S., Marickam, M. Recent discovery and development of inhibitors targeting coronaviruses, *Drug Discov Today*, 2020: Advanced publication 2020.
Su, S., Wong, G., Shi, W., et al. Epidemiology, genetic recombination, and pathogenesis of Coronaviruses. *Trends Microbiol* 2016, 24(5): 490-502.

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SARS-CoV-2 Morphology, Structure and Replication

The viral genome is a single-stranded, positive-sense RNA encoding for structural (especially spike [S]) and nonstructural (3-chymotrypsin-like protease, papain-like protease, helicase, and RNA-dependent RNA polymerase) proteins as well as accessory proteins (Li, G. et al (2020); Zhang, L. et al (2020)). Like SARS-CoV, the new virus is believed to use ACE2 as its binding receptor (Wan, Y. et al (2020)), deploying the S protein for virus-cell receptor interaction during viral entry (Li, G. et al (2020)). Due to these similarities, 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 sold at the Huanan market is believed to have served as an amplifying intermediate host (Perlman, S. (2020); Lu, R. et al (2020)), as bat-derived coronaviruses cannot directly infect humans (Wang, R. et al (2020)).

Coronavirus (2019-nCoV; SARS-CoV-2)



Source: NIAID-RML NIAID had produced images of the novel coronavirus (SARS-CoV-2, previously known as 2019-nCoV) on its scanning and transmission electron 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

Transmission of the virus during the viremic stage of disease is primarily via respiratory secretions (droplets) or direct contact (Lai, C.C. et al (2020)), and infection can be detected in oral swabs. At later stages of infection, however, 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)).

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 (R_0) of 2.2 (Li, Q. et al (2020)); later studies with more data suggested a higher R_0 of 2.24-3.58 (Lai, C.C. et al (2020)). In contrast, the R_0 for both SARS-CoV and MERS-CoV is less than 1 (Wu, J.T. et al (2020)). It soon became apparent that infected individuals might be capable of transmitting the disease during the prodromal period (Heymann, D.L. 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 pre-symptomatic 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)).

Symptoms and Disease

Following an incubation period ranging from 2-14 days (mean 6.5 days), Covid-19 manifests as respiratory illness ranging 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)).

In an early description of 41 clinical cases, patients had serious, sometimes fatal, Pneumonia. Clinical presentations were very similar to those of SARS. The most severely ill patients developed acute respiratory distress syndrome, a syndrome characterized by the acute onset of hypoxemic respiratory failure with bilateral infiltrates, requiring ICU admission and oxygen therapy. The mortality rate in this early patient set was approximately 15% (Huang, C. et al (2020)), and primarily involved older patients with serious underlying diseases or conditions. A later analysis of a larger group of 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)).

Patients hospitalized in Wuhan, China with suspected SARS-CoV-2 infection were screened by RT-PCR and next-generation sequencing. Among those identified with SARS-CoV-2 infection, 32% had underlying diseases including diabetes, hypertension and cardiovascular disease. Up to 66% had been exposed to the Huanan seafood market, including one family cluster. Other diagnosing criteria were fever (98%), cough (76%) and myalgia or fatigue (44%). All patients had pneumonia with abnormal findings on chest CT. Critically ill patients admitted to intensive care unit showed high 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 (Huang, C. et al (2020); Zumla, A. 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)).

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) specifically detect viral RNA. RT-PCR was the only 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)). Detection of viral RNA increased and peaked after about 10 days from the onset of the disease. The virus remained detectable in respiratory secretions for more than one month in some patients, but after three weeks could not be recovered for culture. In the initial phase that occurred in the first week postinfection, the virus could be detected in nasopharyngeal aspirates, throat swabs and sputum samples, while in later phases viral RNA was more easily detected in stool samples (Chan, K.H. et al (2004)).

RT-PCR is currently the only rapid diagnostic test that can give the necessary sensitivity and specificity that are required for a routine clinical diagnostic tool; 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 (Shanghai GeneoDx Biotech, Daan Gene, Shanghai ZJ Bio-tech, Huada Biotechnology and Sansure Biotech) 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**). 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 results. 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, Ares Genetics is collaborating with BGI Group to make real-time fluorescence PCR tests for the new coronavirus, producing results in several hours.

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))

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 (see Poxviruses), 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. Travel history and contact tracing will help to inform the diagnosis (Tian, X.L. et al (2020)).

Prevention

Without effective drugs or vaccines against the infectious agents (Li, G. et al (2020)), social distancing strategies such as isolation, quarantine and community containment are the most effective means of controlling a coronavirus outbreak with epidemic potential (Wilder-Smith, A. et al (2020)). Authorities are often reluctant to impose these measures because of their economic and social impact; however, without other means of control of the epidemic spread of SARS, there was no alternative. The success of these measures was demonstrated in Singapore, where application of infection control measures resulted in a decrease in R_0 (secondary infection rate) from 7 at week 1 to <1 after week 2 (Cleri, D.J. et al (2010)). In Taiwan, the application of Level A quarantine (that of potentially exposed contacts of suspected SARS patients) resulted in the prevention of approximately 461 additional cases and 62 additional deaths; the use of Level B quarantine (that of travelers arriving from affected areas), in contrast, reduced the number of new cases and deaths by only about 5% (Hsieh, Y.H. et al (2007)). CDC recommends use of airborne infection isolation procedures in the care of all confirmed MERS infections in that country (Al-Tawfiq, J.A. et al (2014)). 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 $R_0 = 2.68$), WHO-linked epidemiology experts recommend stringent controls in order to prevent independent, self-sustaining outbreaks in major cities around the world (Wu, J.T. et al (2020)).

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. SARS and other 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 for up to 48 hours at 20°C and for 24 hours at 30°C (Chan, J.F. et al (2015)). Personal protective equipment, including eye protection, is 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 trade of wild animals (Wang, R. et al (2020)).

Vaccines

The successful containment of coronavirus epidemics in farm animals by vaccines, based on either killed or attenuated virus, points to the potential success of vaccine programs.

The S protein is currently considered to be one of the most promising targets for coronavirus vaccine development (Song, Z. et al (2019)), and is being targeted for the development of anti-MERS-CoV vaccines (Ma, C. et al (2014); Zhang, N. et al (2015)), including mucosal vaccine for intranasal administration (Ma, C. et al (2014)). This research 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)). Human MERS-CoV vaccines are also now in development, including DNA vaccines, vector-based, live attenuated and protein subunit vaccines (Cho, H. et al (2018); Schindewolf, C. et al (2019)); many of these vaccines target the S protein (Li, F. et al (2019); Song, Z. et al (2019)).

During the SARS outbreak, research by scientists at the University of Pittsburgh School of Medicine and the Graduate School of Public Health in collaboration with CDC showed that an adenoviral-based vaccine could induce both SARS-CoV-specific T cell and virus-neutralizing antibody responses (Gao, W. et al (2003)). Both responses have been found important for lasting protection. In long-term studies of recovered SARS patients, antibody responses waned after approximately six years, while T-cell responses persisted, suggesting that the latter is required for long-lasting immunity (Zumla, A. et al (2015)).

Upon emergence of SARS-CoV-2, 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)).

The following table presents an up-to-date overview of the development of potential coronavirus vaccines.

Experimental coronavirus vaccines in active preclinical and clinical development

Drug name	Organisations	Description	Phase
<u>BVRS-GamVac-Combi</u>	Ministry Healthcare Russian Federation	Middle East respiratory syndrome coronavirus (MERS - CoV) vaccine comprising a combined heterologous adenoviral vector	Phase I/II
<u>GLS-5300</u>	Inovio Pharmaceuticals; GeneOne Life Science	Middle East Respiratory Syndrome DNA vaccine using the SynCon (TM) technology, encoding MERS spike protein	Phase I/II
<u>LV-SMENP-DC</u>	Shenzhen Genoimmune Medical Institute	Human COVID - 19 coronavirus (SARS - CoV - 2) vaccine consisting of dendritic cells modified with lentiviral vectors (NHP/TYF) expressing	Phase I/II

		<p>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)</p>	
<u>ChAdOx1 MERS</u>	Vaccitech Ltd.; University of Oxford	<p>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)</p>	Phase I
<u>MVA-MERS-S</u>	Ludwig-Maximilians- Univ. Muenchen	<p>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</p>	Phase I
<u>mRNA-1273</u>	Moderna	Human COVID - 19 coronavirus (SARS - CoV - 2) mRNA vaccine	Phase I
<u>GREVAX/MERS</u>	Greffex	<p>Recombinant adenoviral vector developed using GREVAX Universal Platform (GREVAX vector) encoding Middle East respiratory syndrome coronavirus (MERS - CoV) antigens</p>	Preclinical
<u>INO-4800</u>	Inovio Pharmaceuticals	Human COVID - 19 coronavirus (SARS -	Preclinical

CoV - 2) DNA vaccine			
<u>IR-101C</u>	Immune Response BioPharma	Coronavirus vaccine consisting of depleted coronavirus spike glycoprotein without outer envelope inactivated with beta propiolactone and gamma irradiation; propagated in HUT78 cells	Preclinical
<u>LUNAR-COV19</u>	Duke University; Arcturus Therapeutics	Human COVID - 19 coronavirus (SARS - CoV - 2) 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
<u>MVA-MERS-S DF1</u>	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
<u>TNX-1800</u>	Tonix Pharmaceuticals	Human COVID - 19 coronavirus (SARS - CoV - 2) vaccine comprising an engineered, live replicating strain of horsepox virus (HPXV) encoding a protein of COVID - 19 virus	Preclinical
<u>1080103</u>	CureVac	Human COVID - 19 coronavirus (SARS - CoV - 2) mRNA vaccine	Preclinical
<u>1080229</u>	Jenner Vaccine Foundation	Human COVID - 19 coronavirus (SARS - CoV - 2) vaccine comprising replication - deficient simian adenovirus vector ChAdOx1 encoding spike glycoprotein of COVID - 19 virus	Preclinical

<u>1082107</u>	Novavax	Human COVID - 19 coronavirus (SARS - CoV - 2) vaccine comprising recombinant COVID - 19 spike glycoprotein; encapsulated in nanoparticles	Preclinical
<u>1083739</u>	Mitsubishi Tanabe Pharma	Human COVID - 19 coronavirus (SARS - CoV - 2) virus - like particle (VLP) vaccine	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)). Supportive care is the mainstay of treatment for patients with severe disease (Momattin, H. et al (2019); Murthy, S. et al (2020)). Treatment approaches, all of which are investigational, are targeted either at the virus itself or the host response.

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 Kaletra (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 atorvastatin 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)).

Interim guidelines for the appropriate care of patients in whom Covid-19 is suspected have been issued by WHO, CDC and other organizations (see Links to Guidelines).

Broad-Spectrum Antiviral Agents

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 used for the treatment of SARS patients, with variable results (Cleri, D.J. et al (2010)). 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- α 2b 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- α 2a, rIFN- α 2b or rIFN- β 1a), 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 are cited as one factor against its use in patients with Covid-19 (Li, G. et al (2020)).

The antimalarial agents chloroquine and hydroxychloroquine have broad-spectrum antiviral activity, including anti-SARS-CoV-2 activity in vitro, as well as antiinflammatory effects. Chloroquine acts by increasing endosomal pH required for fusion of the virus with the host cell, as well as by interfering with glycosylation of SARS-CoV-2 cellular receptors. Based on this promising profile, low cost and easy availability of the drug, a clinical trial evaluating chloroquine for the treatment of Covid-19 pneumonia has been initiated (Gao, J. et al (2020); Colson, P. et al (2020)). Chinese experts have issued a consensus statement regarding the use and appropriate dosing of chloroquine (Unknown Author (2020)).

Viral Enzyme Inhibitors

The process of coronavirus replication is well understood. Several unique steps have been identified as potential targets for antiviral drugs. Viral fusion with the host cell could potentially be blocked by entry inhibitors or membrane fusion inhibitors, similar to antivirals used for HIV infection. Viral protease inhibitors 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)); a number of host proteases have been shown to proteolytically process the S protein, which determines viral entry. These include cathepsin, furin and trypsin (Millet, J.K. et al (2015); Kilianski, A. et al (2014)). The S protein can also be activated by other host proteases including type II transmembrane serine protease (TMPRSS2), which is considered a promising antiviral drug target (Kilianski, A. et al (2014); Li, F. et al (2019)).

The 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)).

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 randomized controlled trial was quickly initiated to assess the efficacy and safety of the combination to treat Covid-19 (Huang, C. et al (2020)).

The RNA-directed RNA polymerase 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 late February, the U.S. NIH announced that a randomized, controlled clinical trial to evaluate the safety and efficacy of remdesivir in hospitalized adults diagnosed with Covid-19 has begun at the University of Nebraska Medical Center in Omaha.

Favipiravir, a nucleoside analogue that is approved for use in treating influenza A and B, is being evaluated as a potential broad-spectrum antiviral for use in the Covid-19 outbreak. 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)). However, favipiravir was not highly active against SARS-CoV-2 in vitro (Wang, M. et al (2020)).

Elements of the viral replication process have also been identified as potential therapeutic targets, including viral helicase, features of which are highly conserved among different coronaviruses (Adedeji, A.O. et al (2014)). Other potential antiviral drug targets include virus entry, assembly and exocytosis, which enables the release of virus from host cells. Despite a good understanding of viral targets and the identification of potential antiviral agents in vitro and in animal models, however, these findings have not translated into efficacy in humans (Zumla, A. et al (2016); Chen, Y. et al (2020)).

Monoclonal Antibodies

Monoclonal antibodies (MAbs) often represent the first line of investigation and defense against emerging diseases. Neutralizing MAbs, including murine, chimeric and fully human antibodies have been tested; the latter are preferred due to their reduced immunogenicity (Jin, Y. et al (2017)).

In the case of the SARS outbreak, MAb directed at the binding receptor ACE2 were investigated. Neutralization of Middle East respiratory syndrome coronavirus has been attempted using this strategy. In a collaborative study by U.S. and Chinese researchers, three MAbs targeting the receptor (CD26/DPP4) binding domain of the MERS-CoV spike glycoprotein were identified from a large library of candidate antibodies and were evaluated in vitro. The MAb 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 have 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)).

The anti-CCR-5 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.

Interferons

The host immune response, including the innate interferon response, is crucial for controlling viral replication. Coronaviruses suppress this response in order to evade the immune system. However, they may be responsive to treatment with interferons, particularly recombinant forms (Zumla, A. et al (2016)). 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)). In vitro, MERS-CoV has been shown to be 50-100 times more susceptible than SARS-CoV to treatment with IFN-alfa (Abdel-Moneim, A.S. (2014)). In early 2020, a controlled trial was launched in China to test the efficacy of lopinavir/ritonavir in combination with IFN-alfa-2b in hospitalized patients with SARS-CoV-2 infection.

Immunomodulators

During the SARS epidemic, the Chinese government granted approval for use of immune system enhancers such as SciClone's Zadaxin (thymosin alpha 1), an immune system enhancer that is marketed in mainland China for hepatitis B, to treat patients with SARS. Zadaxin works by stimulating the production of white blood cells, enhancing the body's ability to fight off infections (Goldstein, A.L. et al (2009)). Because ribavirin decreases the release of proinflammatory cytokines in mice infected with the mouse hepatitis coronaviruses, it may also act as an immunomodulator (Peiris, J.S. et al (2003)). In vitro studies indicate that ribavirin concentrations that inhibit other viruses are not sufficient to inhibit the replication of the SARS-CoV (Normile, D. (2003)). Therefore some of its benefits may be due to its immunomodulatory activity (Mazzulli, T. et al (2004)). Other treatment options with immunomodulating properties were also used during the SARS epidemic, including i.v. immunoglobulins and convalescent-phase plasma (Tai, D.Y. (2007); Mair-Jenkins, J. et al (2015)).

During the MERS-CoV outbreak in 2015, some Korean patients were treated with convalescent plasma, i.e. passive immunotherapy entailing the infusion of blood plasma from patients who had overcome the infection. 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 has concluded that this approach is 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 antibody titers (Arabi, Y. et al (2016)).

In March 2020, Takeda announced that it was initiating the development of an anti-SARS-CoV-2 polyclonal hyperimmune globulin (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. These convalescent donors have developed antibodies to the virus that could potentially mitigate severity of illness in COVID-19 patients and possibly prevent it.

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)).

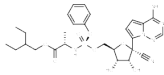
Based on previous experience with SARS and MERS, routine use of corticosteroids was not recommended in patients with Covid-19 (Huang, C. et al (2020)). However, precise use of corticosteroids, adhering strictly to the most recent treatment guidelines, may be warranted to treat appropriately selected 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)).

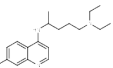
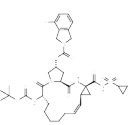
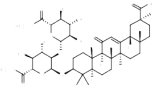
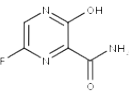
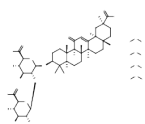
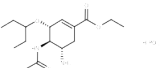
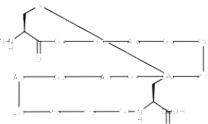
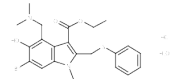
Current Coronavirus Pipeline

Consult the tables below for an overview of all products mentioned in this review, including drugs, biologics and diagnostic agents that have been marketed or are under active development for this indication. Tables may also include drugs not covered in the preceding sections because their mechanism of action is unknown or not well characterized.

Drugs and biologics in development for the treatment and prevention of coronavirus infections

Drug	Organization	Mechanism	Phase	Structure
<u>ASC-09/ritonavir</u>	Ascleptis	HIV Protease Inhibitors; HIV-1 Protease Inhibitors; Tumor Necrosis Factor Receptor Superfamily Member 6 (CD95)/PLC-gamma-1 Interaction Inhibitors	Phase III	

<u>Remdesivir</u>	Gilead	RNA-Directed RNA Polymerase (Viral) Inhibitors	Phase III	
<u>Lopinavir/ritonavir</u>	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	
<u>BVRS-GamVac-Combi</u>	Ministry Healthcare Russian Federation		Phase I/II	
<u>GLS-5300</u>	Inovio Pharmaceuticals; GeneOne Life Science	Spike Glycoprotein (S) (MERS-CoV)-Directed Immunity Inducers	Phase I/II	
<u>LV-SMENP-DC</u>	Shenzhen Genoimmune Medical Institute	Envelope Protein (E) (SARS-CoV-2; COVID-19 Virus)-Directed Immunity Inducers; Membrane Glycoprotein (M) (SARS-CoV-2; COVID-19 Virus)-Directed Immunity Inducers; Nucleocapsid Phosphoprotein (N) (SARS-CoV-2; COVID-19 Virus)-Directed Immunity Inducers; Spike Glycoprotein (S) (SARS-CoV-2; COVID-19 Virus)-Directed Immunity Inducers	Phase I/II	
<u>[131I]-Metuximab injection</u>	Fourth Military Medical University	Anti-CD147; Signal Transduction Modulators	Phase I/II	
<u>ChAdOx1 MERS</u>	Vaccitech Ltd.; University of Oxford	Spike Glycoprotein (S) (MERS-CoV)-Directed Immunity Inducers	Phase I	
<u>MVA-MERS-S</u>	Ludwig-Maximilians-Univ. Muenchen	Spike Glycoprotein (S) (MERS-CoV)-Directed Immunity Inducers	Phase I	
<u>REGN-3048</u>	Regeneron	Anti-Spike Glycoprotein (MERS-CoV Coronavirus)	Phase I	
<u>REGN-3051</u>	Regeneron	Anti-Spike Glycoprotein (MERS-CoV Coronavirus)	Phase I	
<u>SAB-301</u>	SAB Biotherapeutics	Anti-Spike Glycoprotein (MERS-CoV Coronavirus)	Phase I	

<u>mRNA-1273</u>	Moderna	Human Coronavirus (SARS-CoV-2; COVID-19 Virus) Proteins-Directed Immunity Inducers	Phase I	
<u>APN-01</u>	Apeiron Biologics		Clinical	
<u>Chloroquine phosphate</u>	Guangdong Zhongsheng Pharmaceutical	Apoptosis Inducers; Histamine N-methyltransferase (HNMT) Inhibitors	Clinical	
<u>Danoprevir</u>	Asclepis	Serine Protease NS3/Non-Structural Protein 4A (NS3/NS4A) (HCV) Inhibitors	Clinical	
<u>Darunavir/cobicistat</u>	Shanghai Public Health Clinical Center	Cytochrome P450 CYP3A4 Inhibitors; HIV Protease Inhibitors	Clinical	
<u>Diammonium glycyrrhizinate</u>	Sino Biopharmaceutical	Microbiome Modulators	Clinical	
<u>Favipiravir</u>	Sihuan Pharmaceutical	RNA-Directed RNA Polymerase (Influenza A Virus H1N1) Inhibitors; RNA-Directed RNA Polymerase (NS5B) (HCV) Inhibitors	Clinical	
<u>Magnesium isoglycyrrhizinate</u>	Sino Biopharmaceutical		Clinical	
<u>Oseltamivir phosphate</u>	Wuhan Tongji Hospital	Neuraminidase (Sialidase) (Influenza Virus) Inhibitors	Clinical	
<u>Solnatide</u>	APEPTICO	Epithelial Sodium Channel (ENaC) Activators; Protein Kinase PKC alpha Inhibitors; Signal Transduction Modulators	Clinical	
<u>Umifenovir hydrochloride</u>	Wuhan Tongji Hospital	Capsid Assembly (Hepatitis B Virus) Modulators; Viral Entry Inhibitors	Clinical	

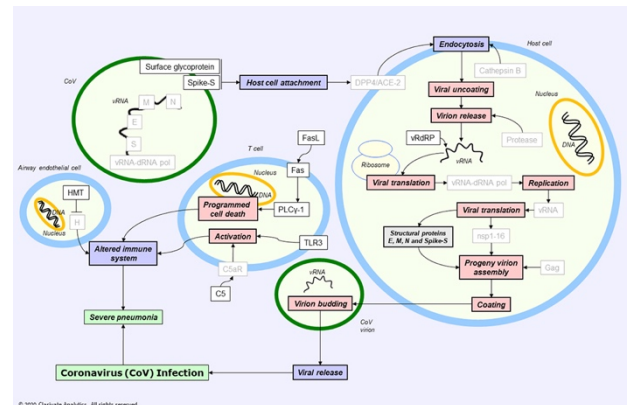
<u>IFX-1</u>	Staidson (Beijing) Biopharmaceuticals	Anti-C5 (Complement 5)	IND Filed
<u>Leronlimab</u>	CytoDyn	Anti-CD195 (CCR5); Signal Transduction Modulators; Viral Entry Inhibitors	IND Filed
<u>GREVAX/MERS</u>	Greffex		Preclinical
<u>Human leukocyte interferon alpha</u>	AIM ImmunoTech		Preclinical
<u>INO-4800</u>	Inovio Pharmaceuticals	Human Coronavirus (SARS- CoV-2; COVID-19 Virus) Proteins-Directed Immunity Inducers	Preclinical
<u>IR-101C</u>	Immune Response BioPharma		Preclinical
<u>LCA-60</u>	Vir Biotechnology	Anti-Spike Glycoprotein (MERS-CoV Coronavirus)	Preclinical
<u>LUNAR-COV19</u>	Duke University; Arcturus Therapeutics	Human Coronavirus (SARS- CoV-2; COVID-19 Virus) Proteins-Directed Immunity Inducers	Preclinical
<u>MVA-MERS-S DF1</u>	Universitaetsklinikum Hamburg-Eppendorf	Spike Glycoprotein (S) (MERS-CoV)-Directed Immunity Inducers	Preclinical
<u>Rintatolimod</u>	AIM ImmunoTech	Signal Transduction Modulators; Toll-Like Receptor 3 (TLR3) Agonists	Preclinical
<u>TNX-1800</u>	Tonix Pharmaceuticals	Human Coronavirus (SARS- CoV-2; COVID-19 Virus) Proteins-Directed Immunity Inducers	Preclinical
<u>1080229</u>	Jenner Vaccine Foundation	Spike Glycoprotein (S) (SARS- CoV-2; COVID-19 Virus)- Directed Immunity Inducers; Viral Entry Inhibitors	Preclinical

<u>1080103</u>	CureVac	Human Coronavirus (SARS-CoV-2; COVID-19 Virus) Proteins-Directed Immunity Inducers	Preclinical
<u>1083739</u>	Mitsubishi Tanabe Pharma	Drugs Targeting Human Coronavirus (SARS-CoV-2; COVID-19 Virus) Proteins	Preclinical
<u>1082107</u>	Novavax	Spike Glycoprotein (S) (SARS-CoV-2; COVID-19 Virus)-Directed Immunity Inducers; Viral Entry Inhibitors	Preclinical

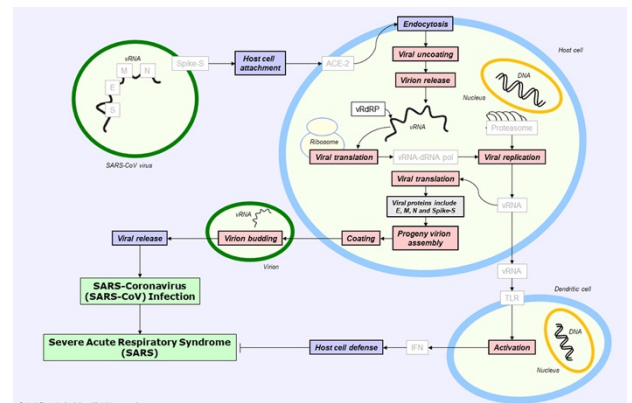
Targets for Therapeutic Intervention

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.

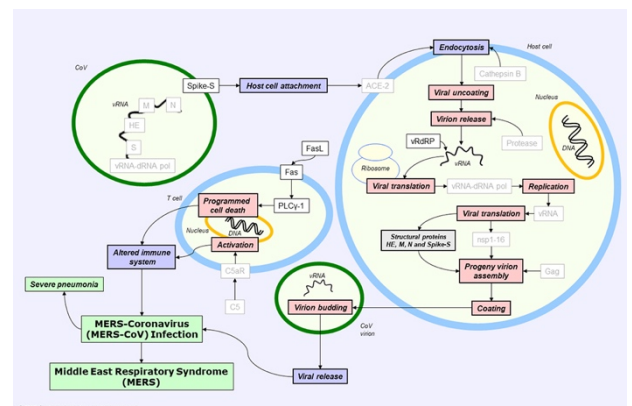
Coronavirus (CoV) Infection Targetscape



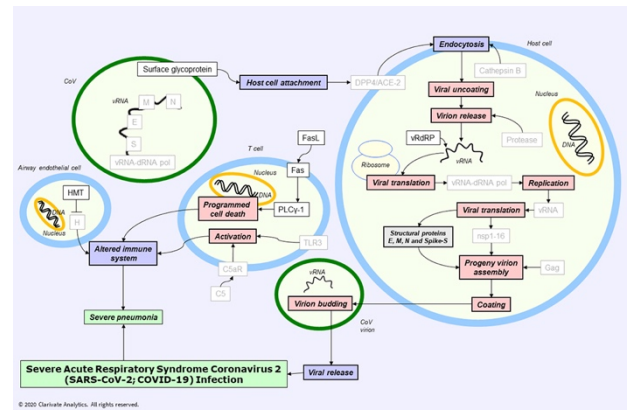
Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) Infection Targetscape



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



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



Latest Headlines

13-Mar-2020

BeyondSpring submits patent application for BPI-002 as antiviral treatment

BeyondSpring announced the submission of a provisional U.S. patent application for its pipeline asset BPI-002, for methods of treating viral infections, including COVID-19, when administered alone or in combination with a vaccine. BPI-002 is a novel orally administered small molecule agent that is a potent T-cell co-stimulator, which has the potential to target two main effects. BPI-002 can potentially activate the adaptive immune system to directly attack and kill virally infected cells, including RNA viruses, such as those causing COVID-19. Additionally, if combined with a vaccine, including a COVID-19 vaccine, BPI-002 can potentially function as an adjuvant to provide improved long-term humoral protection against future viral infection (BeyondSpring News Release).

13-Mar-2020

Medicago announces advances in the production of vaccine candidate for COVID-19

Medicago announced that the company has successfully produced a virus-like particle (VLP) of the coronavirus just 20 days after obtaining the SARS-CoV-2 gene. Production of the VLP is the first step in developing a vaccine for COVID-19, which will now undergo preclinical testing for safety and efficacy, and once this is completed, the company expects to enter discussions with the appropriate health agencies to initiate human trials of the vaccine by summer 2020. In collaboration with the Laval University's Infectious Disease Research Centre, headed by researchers who have helped develop a vaccine and treatment for Ebola, Medicago is also using its technology platform to develop antibodies against SARS-CoV-2, which could potentially be used to treat people infected by the virus. This research is being funded, in part, by the Canadian Institute for Health Research (CIHR). Medicago is a leader in plant-based technology, having previously demonstrated its capability to be a first responder in a flu pandemic. The company's first product, a seasonal recombinant quadrivalent VLP vaccine for active immunization against influenza, is currently under review by Health Canada following the completion of a robust safety and efficacy clinical program involving over 25,000 patients (Medicago News Release).

13-Mar-2020

PLX cells to be developed for the potential treatment of complications caused by COVID-19

Pluristem Therapeutics has signed a collaborative agreement with the BIH Center for Regenerative Therapy (BCRT) and the Berlin Center for Advanced Therapies (BeCAT) at Charite University of Medicine Berlin to expand its existing framework and research agreement and conduct a joint project evaluating the therapeutic effects of Pluristem's patented PLX cell product candidates for the treatment of the respiratory and inflammatory complications associated with the COVID-19 coronavirus. PLX cells are allogeneic mesenchymal-like cells that have immunomodulatory properties that induce the immune system's natural regulatory T cells and M2 macrophages, and as such may prevent or reverse the dangerous overactivation of the immune system. Consequently, PLX cells may have the potential to reduce the fatal symptoms of COVID-19-induced pneumonia and pneumonitis. Previous preclinical studies with PLX cells demonstrated significant therapeutic effects in animal models of pulmonary hypertension, lung fibrosis, acute kidney injury and gastrointestinal injury, which are possible complications of the severe COVID-19 infection. In a clinical setting, the PLX cells have shown strong immunomodulatory potency in patients post major surgery. Taken together, PLX cells' potential therapeutic effects along with the safety profile seen in clinical trials involving hundreds of

patients worldwide show promise of these cells as therapy for mitigating the tissue-damaging effects of COVID-19 (Pluristem Therapeutics News Release).

13-Mar-2020

AbCellera and Eli Lilly establish collaboration to codevelop antibody products for COVID-19

AbCellera and Eli Lilly have entered into an agreement to codevelop antibody products for the treatment and prevention of COVID-19, the disease caused by the SARS-CoV-2 novel coronavirus. The collaboration will leverage AbCellera's rapid pandemic response platform, developed under the DARPA Pandemic Prevention Platform (P3) program, and Lilly's global capabilities for rapid development, manufacturing and distribution of therapeutic antibodies. Within 1 week of receiving a blood sample from one of the first U.S. patients who recovered from COVID-19, AbCellera screened over 5 million immune cells and identified over 500 unique fully human antibody sequences, the largest panel of anti-SARS-CoV-2 antibodies reported. Next, these antibodies will be screened to identify those most effective in neutralizing SARS-CoV-2. Many of these antibodies will be expressed in collaboration with partners at the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, and pending agreement with NIAID will be tested for their ability to neutralize the virus. Under the agreement with Lilly, AbCellera and Lilly have committed to equally share initial development costs towards a product, after which Lilly will be responsible for all further development, manufacturing and distribution (Eli Lilly News Release).

13-Mar-2020

Emergent BioSolutions begins development of hyperimmune-based candidates for COVID-19

Emergent BioSolutions has initiated development of two product candidates for the treatment and prevention of coronavirus disease, COVID-19. These product candidates are being developed on Emergent's hyperimmune platforms that have a well-established safety database. COVID-HIG, manufactured from human plasma with antibodies to SARS-CoV-2, will be developed as a potential treatment for severe hospitalized patients as well as protection for at-risk individuals. In parallel, COVID-EIG, manufactured from plasma of immunized horses with antibodies to SARS-CoV-2, will be developed as a potential treatment for severe hospitalized patients. Emergent has initiated plasma collection efforts for both human and equine platforms with a goal of manufacturing clinical material within the next 4-5 months in anticipation of beginning a clinical study as early as the third quarter of this year. The company's hyperimmune specialty plasma product manufacturing platform has been used to create multiple products that have obtained FDA and Health Canada approval (Emergent BioSolutions News Release).

13-Mar-2020

Chinese trial studies utility of ASC-09F for COVID-19 pneumonia

In China, Tongji Hospital is evaluating treatment with oseltamivir plus ASC-09F (ASC-09/ritonavir) or ritonavir for COVID-19 pneumonia (ClinicalTrials.gov Identifier NCT04261270). Sixty patients with confirmed infection with SARS-CoV-2 and newly diagnosed respiratory discomfort will be enrolled in this randomized, open, controlled phase III study to receive either oseltamivir (75 mg once a day), oseltamivir with ASC-09F (one ASC-09F tablet [400 mg/tablet] twice a day for 14 days) or oseltamivir with ritonavir (three ritonavir tablets [100 mg/tablet] twice a day for 14 days). The trial will evaluate time of clinical remission, and the rates of comprehensive adverse outcome; no fever; no cough; no dyspnea; no need for oxygen inhalation; undetectable viral RNA; mechanical ventilation; and ICU admission (ClinicalTrials.gov Website).

11-Mar-2020

Mesoblast to evaluate remestemcel-L for treatment of COVID-19 lung disease

Mesoblast announced that it plans to evaluate its allogeneic mesenchymal stem cell (MSC) product candidate remestemcel-L in patients with acute respiratory distress syndrome (ARDS) caused by coronavirus (COVID-19) in the U.S., Australia, China and Europe. ARDS is the principal cause of death in COVID-19. Mesoblast is in active discussions with various government and regulatory authorities, medical institutions and pharmaceutical companies to implement these activities. Remestemcel-L has potential for use in the treatment of ARDS, which supported by recently published results from an investigator-initiated clinical study conducted in China. The results of this trial demonstrated that allogeneic MSCs cured or significantly improved functional outcomes in all 7 treated patients with severe COVID-19 pneumonia. Additionally, in post-hoc analyses of randomized controlled study in 60 patients with chronic obstructive pulmonary disease (COPD), remestemcel-L infusions were well tolerated and significantly reduced inflammatory biomarkers, as well as significantly improved pulmonary function in those patients with elevated inflammatory biomarkers. Since the same inflammatory biomarkers are also elevated in COVID-19, these data suggest that remestemcel-L could be useful in the treatment of patients with ARDS due to COVID-19. Remestemcel-L is being studied in numerous clinical trials across several inflammatory conditions, including in elderly patients with lung disease and adults and children with steroid-refractory acute graft-versus-host disease (aGVHD). The candidate is currently being reviewed by the FDA for potential approval in the treatment of children with steroid-refractory aGVHD (Mesoblast News Release).

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11-Mar-2020

Brilacidin to be tested against COVID-19

Innovation Pharmaceuticals informed shareholders that management has been advised testing of the company's flagship defensin mimetic drug candidate, brilacidin, as a novel experimental treatment against the latest coronavirus is scheduled to commence at 1 of the 12 U.S. Regional Biocontainment Labs (RBLs) during the week of March 16, 2020. Scientists at the RBL will be evaluating brilacidin against SARS-CoV-2, the specific coronavirus responsible for COVID-19. Brilacidin is a promising candidate for COVID-19 due to its unique qualities to mimic the human innate immune system and mechanism of action that includes disruption of the membrane of pathogens, leading to cell death. Throughout laboratory and clinical studies, brilacidin has demonstrated potent activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), as well as coverage against Gram-negative bacteria. Research supports the ability of brilacidin to swiftly penetrate a peptidoglycan layer in these difficult bacteria, which is key to rapid pathogen destruction and decreases the likelihood of drug resistance emerging. Since SARS-CoV-2 is an enveloped virus, the ability of brilacidin to penetrate cell walls gives promise in this opportunity to meet an urgent medical need in treating coronaviruses like SARS-CoV-2. While penetrating the SARS-CoV-2 envelope could prove beneficial in designing a new drug or vaccine against the virus, this would have to be confirmed through extensive laboratory and clinical research. Innovation hopes to disclose the RBL's research agenda for brilacidin in the coming days, with the upcoming research to be funded by third parties independent of the company. Innovation is only responsible for providing brilacidin and any requested data. The RBL now has the brilacidin on site (Innovation Pharmaceuticals News Release).

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11-Mar-2020

Tiziana accelerates development of TZLS-501 for COVID-19

Tiziana Life Sciences is expediting development of TZLS-501 (formerly NI-1201), a novel, fully human anti-IL-6 receptor (anti-IL6R) monoclonal antibody, for the treatment of patients infected with coronavirus SARS-CoV-2, which causes COVID-19. Tiziana plans to administer TZLS-501 using a proprietary formulation technology. TZLS-501 binds to both the membrane-bound and soluble forms of IL-6R and rapidly depletes circulating levels of IL-6 in the blood. An excessive

production of IL-6 is regarded as a key driver of chronic inflammation and is believed to be associated with severe lung damage observed with COVID-19 and acute respiratory illness. Early clinical studies conducted by doctors in China suggest that anti-IL6R monoclonal antibodies may be used in clinical practice for treatment of COVID-19, and the recommendation by China's National Health Commission of the use of Actemra (tocilizumab; Roche) for treatment of patients with COVID-19 with serious lung damage and elevated IL-6 levels also supports the utility of anti-IL6R monoclonal antibodies. Tiziana entered into a worldwide license for composition of matter of TZLS-501 with Novimmune in 2017 (Tiziana Life Sciences News Release).

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10-Mar-2020

CEL-SCI applies its LEAPS peptide technology to develop immunotherapy against COVID-19

CEL-SCI Corporation announced that it is developing an immunotherapy with the potential to treat the COVID-19 coronavirus using its patented Ligand Antigen Epitope Presentation System (LEAPS) peptide technology, with the LEAPS peptides utilizing conserved regions of coronavirus proteins to stimulate protective cell-mediated T-cell responses and reduce viral load. The LEAPS peptide technology can be used to construct immunotherapeutic peptides that exhibit both antiviral and antiinflammatory properties, therefore, these products not only target the virus infection against which they are directed, but also elicit the appropriate protective responses against it. Predictions of success using the LEAPS peptides against COVID-19 coronavirus are based on previous studies conducted in collaboration with the National Institutes for Allergies and Infectious Diseases (NIAID) with another respiratory virus, pandemic influenza (H1N1). In these studies, LEAPS peptides elicited protection of mice from morbidity and mortality after the introduction of infection by activating appropriate T-cell responses rather than an inflammatory response. Previous studies have also shown that LEAPS immunogens can prevent lethal infection by herpes simplex virus (HSV) and stop the inflammatory disease progression of rheumatoid arthritis in animal models. LEAPS peptides against HSV demonstrated that the T cell response was sufficient to prevent viral disease, and if there was residual virus production, antiviral antibody was generated to further control the spread of the virus. The currently proposed LEAPS peptides are directed towards antigens within the NP protein of COVID-19 that elicit cytolytic T-cell responses. Unlike glycoprotein spike antigens which are important for antibody based vaccines, these antigens are less variable between viral strains and less likely to change in response to antibodies elicited by prior infection or other vaccines (CEL-SCI Corporation News Release)

Suggested reading

Related websites

- [Centers for Disease Control and Prevention \(CDC\) – 2019 novel coronavirus, Wuhan, China](#)
- [Centers for Disease Control and Prevention \(CDC\) – SARS information](#)
- [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 \(SARS\) \(World Health Organization\)](#)

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 \(Science\)](#)
- [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 \(Public Health England, January 2020\)](#)

[Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: Experts 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 \(World Health Organization, January 25, 2020\)](#)

[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 \(World Health Organization, January 2018\)](#)

[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, clinicians, and public health authorities - January 2015 \(Centers for Disease Control and Prevention, January 30, 2015\)](#)

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