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REVIEW

Insights into the Origin, Transmission and Outbreak of Coronavirus Disease (Covid 19): A Recent Study

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Present pandemic situation due to the appearance of COVID-19 has put the world in a miserable condition. More than 2.5 million people have been infected with the causal strain of coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). The first case of COVID-19 infection was reported in China in December 2019. Scientists are searching the effective tool to combat this virus. The study has been undertaken by the scientists towards finding effective medicine, vaccine as well as robust techniques to detect this virus. Besides development of new treatment, the application of clinical data analytics has also been observed for the off-label use of the already available medications. As a part of the real time application of science and technology, several clinical trials has been in process to extract the best answer against COVID-19. In present article, a comprehensive review has been carried out to conscripting the available knowledge about discovery, genomic structure, mechanism of infection and clinical features of SARS-CoV-2. Presently available procedures for detection of this virus have been highlighted. Besides these, available treatments which have been explored by the researchers worldwide, includes precisely convalescent plasma therapy, monoclonal antibody therapy as well as antiviral medications along with their regulatory status, have been discussed elaborately which will definitely enrich the global understanding as well as proficient ability to combat this pathogens.

Keywords: COVID-19, SARS-CoV-2, SARS-CoV, MERS-CoV, Genomic architecture, Pathogenesis, Transmission, Therapy.

INTRODUCTION

At the end of late December, 2019, an outbreak of a novel coronavirus (SARS-CoV-2) that causes a severe acute respiratory illness was reported from Wuhan city in the Hubei Province of China. The disease known as "COVID-19" (coronavirus disease 2019) gradually affected more than 190 countries worldwide with total confirmed cases of around 5,044,814 with a mortality of around 327,448 as of 20.5.2020. Due to the rapid spread of this virus across the countries worldwide, World Health Organization (WHO) declared COVID-19 as pandemic on dated 11th March 2020. Similar such outbreaks happened earlier on 2002-2003 in the form of Severe Acute Respiratory Syndrome (SARS) and again in 2011 in the form of Middle East Respiratory Syndrome (MERS) caused by some newly discovered coronaviruses (SARS-CoV and MERS-CoV) belonging to the genus Betacoronavirus having zoonotic origin. The genome of SARS-CoV-2 virus is fully sequenced [1] having similar, but distinct genomic composition of MERS-CoV and SARS-CoV. Since it is a novel virus, its future course is largely unknown. Hence, it will take many more years of research to fully understand the characteristics of this virus, its origin and symptoms along with the immune response associated with the host in combating this viral infection. Based on the previous data available for the two viruses, SARS-CoV and MERS-CoV, one can have an idea about the host immune response against this particular SARS-CoV-2 virus and also mechanisms of the virus uses to evade the host immune response. This review will highlight some important characteristics of the virus along with the recent advancements in therapeutics for the treatment against this novel coronavirus based on the available data.

Discovery of a novel β-CoV strain (SARS-CoV-2): The corona viruses are enveloped viruses containing a positive sense, single-stranded RNA genome with size ranging from 26-32 kb in length [2] and can be broadly classified into four distinct genera such as α, β, γ, δ-CoVs. Among them, α-coronavirus (HCoV-229E and NL63) and β-coronavirus (HCoV-OC43 and HCoV-HKU1, MERS-CoV, SARS-CoV) are known to infect humans [3]. The β-CoVs (HCoV-OC43 and HCoV-OC43) and α-CoVs (HCoV-NL63 and HCoV-229E) have low pathogenic potential causing mild respiratory symptoms. In comparison, SARS-CoV and MERS-CoV, the two other β-CoVs having high pathogenicity causes severe and fatal respiratory tract infections [4]. In December 2019, an unknown microbial infection was reported in Wuhan, China affecting individuals who were suffering from cough, fever and dyspnea with acute respiratory distress syndrome (ARDS). Subsequent viral genome sequencing from the patients with pneumonia revealed that the unidentified microbial infection is caused by an unknown β-CoV strain [5]. By analyzing the genome sequence of this novel β-CoV, it was found that the virus is most closely related to bat CoV RaTG13 having 96.3% genome sequence identity followed by about 88% genome sequence identity with the two bat derived SARS-like coronavirus, bat-SL-CoVZC45 and bat-SL-CoVZXC21 and is distantly related to MERS-CoV and SARS-CoV with about 50% and 79.5% genome sequence identity, respectively [5-8]. The international Virus Classification Commission named this novel unknown β-CoV strain as 'SARS-CoV-2' virus on February 11, 2020 [9]. Further phylogenetic classification revealed that SARS-CoV-2 virus (29.8 kb) belonged to the subgenus Sarbecovirus of genus Betacoronavirus surrounded by an envelope containing single stranded positive sense RNA genome [5,6].

Genomic architecture of SARS-CoV-2: The genomes of coronaviruses consist of 6-11 open reading frames (ORFs). About two-third of the viral RNA are found in the first ORF (ORF1a/b). The viral RNAs are mainly translated into pp1a and pp1b, two large polyproteins. The polyproteins are subsequently processed (proteolytically cleaved) into 16 non-structural proteins (nsps) forming the replicase transcriptase complex in the virus [4,10]. The main role of the nsps is to rearrange the membranes that originate from the rough endoplasmic reticulum into double membrane vesicles. The viral replication and transcription mainly takes place in these double membrane vesicles [6,9]. The remaining one-third part of the viral genome containing the other ORFs encodes four essential structural proteins such as spike glycoprotein (S), envelope (E), nucleocapsid protein (N) and matrix proteins (M) [11]. Apart from that several accessory proteins are also encoded by these ORFs. The coronaviruses mainly binds to the receptor of the host cell through the spike surface glycoprotein (S) thereby determines host tropism [12,13]. The spike glycoproteins of SARS-CoV and MERS-CoV binds to the different host receptors through different receptor binding domains *e.g.*, ACE2 is the main receptor for SARS-CoV [14] along with CD209L, which acts as the alternative receptor [15], while DPP4, dipeptidyl peptidase 4 is the primary receptor for MERS-CoV. Recently based on the comparison of genomes of three novel SARS-CoV-2 strains (Wuhan/IVDC-HB-01/2019 (GISAID accession ID: EPI_ISL_402119) (HB01), Wuhan/IVDC-HB-05/2019 (EPI_ISL_402121) (HB05) and Wuhan/IVDC-HB-04/2019 (EPI_ISL_402120) (HB04), it was found that the genomes of these strains are very much identical with some minor differences, mainly five nucleotide differences in the 29.8 kb genome [16]. Furthermore, it was shown that the genome of SARS-CoV-2 was annotated to possess 14 open reading frames (ORF) that encodes 27 proteins. Among them orf1ab and orf1a genes encodes pp1ab and pp1a polyproteins. The pp1ab and pp1a are further processed into 15 non-structural proteins (nsps) including nsp1 to nsp10 and nsp12 to nsp16. On the other hand the 3′-terminus of SARS-CoV-2 genome mainly contains four structural proteins (S, E, M and N) and eight accessory proteins. The eight accessory proteins are 3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) [16]. The SARS-CoV-2 is very much similar to that of another coronavirus, SARS-CoV at the level of amino-acid but there also exist some differences. The 8a protein is absent in SARS-CoV-2, whereas it is present in SARS-CoV virus. On the other hand, the 8b protein in 121 amino acid long it SARS-CoV-2 in compared to SARS-CoV where it is 84 amino acid long. Differences also exist in case of 3b protein; in case of SARS-CoV-2 it is 22 amino acid long whereas it is 154 amino acids in case of SARS-CoV [16]. From the whole genome sequencing data it was found that the SARS-CoV-2 is very much closely related to SARS-like bat CoVs and is less related to the the MERS-CoVs. From phylogenetic analysis of the encoded proteins such as pp1ab, pp1a, matrix, accessory protein 7a envelope, and nucleo-capsid genes it was also shown that SARS-CoV-2 is very much closest SARS-like bat CoVs. In terms of spike glycoprotein gene the SARS-CoV-2 showed less than 75% genome sequence identity to bat-SL-CoVZC45 and bat-SL-CoVZXC21 and human SARS-CoV [17]. Furthermore, the spike glycoprotein of SARS-CoV-2 consists of S1 and S2 domains are longer than that of the spike proteins in SARS-CoVs. The spike glycoprotein is essential for the determination of host tropism and transmission of the virus [5-8]. The S2 subunit of the spike glycoprotein has 99% sequence identity with SARS-CoV. In the carboxyl terminal domain of S1 subunit, the receptor binding domain (RBD) is located which interacts with the human receptor. Recent homology modeling data suggests that SARS-CoV-2 has similar receptor binding domain structure like that of SARS-CoV virus inspite of having 70% genome sequence identity of SARS-CoV-2 virus with that of SARS-CoV in the S1 domain [5,6,18,19]. It was also found that SARS-CoV-2 uses the same receptor (ACE2) in the ACE-2 expressing cells (like type II alveolar cells in lungs) for entry into the host cell [14,15]. The interaction of the virus with the host cell receptor is vital for the pathogenesis. In terms of the other accessory genes such as 3a and 8b, SARS-CoV-2 is related to the SARS-CoVs [16]. As there exist a close relationship among SARS-CoV-2 and SARS-like bat CoVs, a closer look into the amino acid substitutions in different proteins pointed out how the SARS-CoV-2 is structurally and functionally different from SARS-CoVs [16]. In case of nsp7, nsp13, envelope, matrix or accessory proteins 8b and p6 there is no amino acid substitution whereas in case of nsp2 and nsp3 there are 61 and 102 amino acid substitutions, respec-

tively. Additionally, there are 27 amino acid substitutions on the spike glycoprotein region (six substitutions in the receptor binding domain between region 357-528 amino acids and six substitutions in the underpinning domain between the region 569-655 amino acids) [16]. Another study also indicate that there are four amino acid substitutions (Q560L, S570A, F572T, and S575A) in the carboxyl terminal of the receptor binding subunit S1 domain that lies on the two peptides that were known to be antigens of SARS-CoV [21]. Finally, the genomic sequence of SARS-CoV-2 isolated from various patients have 99.9% genome sequence identity [5,6,18,19], which infers that the SARS-CoV-2 may have originated from one source and hence could be rapidly detected. Recently, a study also on the population genetic analysis on 103 SARS-CoV-2 genomes pointed out that SARS-CoV-2 virus evolved into two main types such as L type and S type. Among them L type (70%) is the most prevalent type than S type (30%). During the early stages of the outbreak of SARS-CoV-2, L type, that was derived from S type was most prevalent and further human intervention put more selective pressure on the L type making it more aggressive and as a result the virus have spread very quickly among the population [22].

Replication and entry of virus: The spike glycoprotein (S) plays a major role in the entry of coronavirus into the host cells [23]. It was confirmed that SARS-CoV-2 virus uses the same receptor, ACE2 for cellular entry just like SARS-CoV [24]. The spike glycoprotein on the surface of the coronavirus binds to the cellular ACE2 receptor on the human cells [25]. Initial reports pointed out that the direct membrane fusion between the virus and the plasma membrane is responsible for the SARS-CoV entry into the host cells [26]. This membrane fusion is mediated by critical proteolytic cleavage on S glycoprotein of SARS-CoV at position, S2' thereby increasing virulence [27]. Apart from that clathrin dependent and independent endocytosis mechanisms also played a role in the entry of SARS-CoV virus into the host cell [28,29]. The spike (S) glycoprotein is composed of two subunits, S1 and S2 [30]. S2 subunit consists heptad repeat 1 (HR1) and heptad repeat 2 (HR2), two tandem domains that mediates the fusion of virus with the host cell membrane while S1 subunit contains the receptor binding domain and determines host specificity and cellular tropism [31,32]. Following the membrane fusion of the virus with the cell membrane, the viral genome RNA is released into the cytoplasm. The uncoated RNA was then translated into pp1a and pp1ab, two viral polyproteins which are subsequently processed by viral encoded chymotrypsin like protease ($3CL^{pro}$) or main protease (M^{pro}) and one or two papain like protease into 16 non-structural proteins (nsps) forming the replicase transcriptase complex (RTC) in double membrane vesicles [5,7-9,33-35]. This is followed by the synthesis of a nested set of various subgenomic RNAs (encodes various accessory and structural proteins) by RTC through discontinuous transcription [35,36]. The envelope glycoproteins that are newly synthesized are then inserted into the membrane of endoplasmic reticulum or Golgi apparatus. On the other hand, the viral nucleocapsid is formed from nucleocapsid proteins and viral genomic RNA. Ultimately, the virus germinates into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). At last, the virion containing vesicles fuse with the plasma membrane thereby releasing the virus [2,37]. From a Cryo-EM study, it was found that the interaction between the S-protein and the ACE2 receptor in SARS-CoV-2 virus is about 10-20 fold higher than that of SARS-CoV virus [38]. In one study, it was shown that furin preactivation along with the proteins cathepsin and transmembrane protease serine 2 (TMPRSS2) enhance the entry of SARS-CoV-2 pseudovirus *in vitro* in different cell lines such as lung fibroblast and epithelial cell lines [39]. However in SARS-CoV, entry of the virus is facilitated by TMPRSS2 and cathepsins [40,41] but not by furin pre-activation [42]. This preactivation of furin makes the SARS-CoV-2 virus less dependent on the target cells especially with cells expressing low amounts of TMPRSS2 and/or lysosomal cathepsins [42]. Similar type of event has also been observed in case of avian influenza viruses [43]. Further detailed mechanism for the entry of virus into the host cell could be very much useful in the development of therapeutics against SARS-CoV-2 virus.

Transmission of the virus: Back in 2003, a viral outbreak causing Severe Acute Respiratory Syndrome (SARS) was reported in the Guangdong province in China reportedly caused by a virus belonging to the betacoronavirus subgroup. The virus was later named as SARS-CoV [44,45]. The disease gradually spread rapidly from Guangdong, China to other parts of the world with more than 8000 infected individuals and 776 deceased. The patients who got infected with the SARS-CoV virus were diagnosed with a diffused injury in the alveoli of the lungs ultimately leading to acute respiratory distress syndrome (ARDS). Again in 2012, another member belonging to the betacoronavirus subgroup, MERS-CoV (Middle East Respiratory Syndrome Coronavirus (MERS-CoV) caused similar kind of viral outbreak in Saudi Arabia. According to the World Health Organization (WHO) more than 2428 individuals was infected with MERS-CoV with 838 associated deaths [46]. MERS-CoV is phylogenetically more diverse in contrast to other human CoVs. A mild upper respiratory injury mainly initiates the MERS-CoV infection which gradually progress ultimately leading to severe respiratory distress. The MERS-CoV patients also suffer from pneumonia, ARDS and renal failure just like SARS-CoV [47]. By the end of 2019, China reported another outbreak of unknown acute respiratory tract infections in the Wuhan province [48,49]. The outbreak can be traced in the Hunan sea-food market, in the Wuhan province in China affecting more than 50 individuals. The live animals like bats, frogs, snakes, birds, marmots and rabbits were sold in the Hunan sea-food market [48]. The further details about the vital epidemic were reported by the National Health Commission of China on January 12, 2020 suggesting viral pneumonia. The virus was later identified as a novel coronavirus (SARS-CoV-2) [48]. Initially, it was believed that the individuals who got infected with SARS-CoV-2 might have visited the Hunan sea-food market where live animals were sold or may have consumed virus infected animals as a source of food. Reports also claimed that the bats are the potential reservoir of SARS-CoV-2 viruses [7,49]. However due to the

lack of evidence it could not be proved that the SARS-CoV-2 origin was from the Hunan sea food market. On the other hand, bats are the natural host of a number of CoVs that includes SARS-CoV and MERS-CoV [50-52]. Although SARS-CoV-2 and Bat CoV RaTG13 showed almost 96.2% genome sequence similarity [53], bats were not sold in the Hunan sea-food market [54]. Several phylogenetic analysis and protein sequence alignment data revealed that similar residues of receptor was also observed in some other species suggesting that there are some alternative intermediate hosts like snakes, pangolins and turtles [55]. Further investigations also revealed that some individuals also got infected with the novel coronavirus in spite of not visiting the sea-food market suggesting human to human transmission capability of the virus which in turn affected 185 countries around the world. The transmission of the virus in humans mainly takes place when an individual comes is exposed to the coughing, sneezing or aerosols of the infected patients. Finally the aerosols can enter the human body through the inhalation of nose or mouth followed by penetration into the lungs [56-60]. According to the National Health Commission of China, among the people who are non-residents of Wuhan, 31.3% of the individuals got infected with the virus travelled to Wuhan province in China and 72.3% got infected with the virus coming in contact with the people of Wuhan. Apart from that 3.8% cases occurred through the transmission of the virus among health care workers [61]. It was postulated that the transmission of the virus to humans had taken place due to the close contact of the individuals with the intermediate hosts or through consumption of wild animals. Some studies also pointed out that 40% of the confirmed COVID-19 cases were initially asymptomatic in nature but later develop clinical symptoms of the disease [62]. But however, it remains difficult to find out the source and transmission of the SARS-CoV-2 virus to humans.

Clinical features: COVID-19, an acute respiratory disease caused by the novel coronavirus, SARS-CoV-2 mainly spreads at a much lower infective dose through the respiratory tract by direct contact, aerosol droplets and respiratory secretions [59,63]. Reports also suggests multiple transmission routes of the virus due to the presence of these novel virus in blood as well as in fecal swabs [64]. The presence of ACE2 receptor in the alveolar epithelial cells of the lung and the enterocytes of the small intestine as well as in the arterial and venous endothelial cells and arterial smooth muscle cells in many organs (stomach, small intestine, oral and nasal mucosa, nasopharynx, lung, colon, lymph nodes, bone marrow, spleen, liver, kidney, brain and skin thymus) suggests the various possible routes of virus infection and spread throughout the human body [42]. After an incubation of approx 5.2 days, the symptoms of COVID-19 start appearing [54]. Studies suggest that the intermediate period between the onsets of symptoms to death is approximately 6 to 41 days with a median of about 14 days [50]. The people with some underlying disease and elderly individuals are very much prone to this deadly COVID-19 disease. It was also found out the patients who got infected with the disease has a median age of 47-59 years of which 4.9-45.7% are females [58,65]. Most of the COVID-19 patients have similar symptoms such as fever, cough, headache, malaise, vomiting, myalgia, anosmia, diarrhea, asthenia, blue coloration in face and lips, persistent pain or pressure in the chest and breathing problems [66-69]. Though in most of the cases it is found that the adults and the children who got infected with this virus develop some mild flu-like symptoms but some patients are also in critical stage with symptoms like respiratory distress, respiratory failure, multiple organ failure and in some extreme cases even death occurred [68,70]. Recent findings also link various dermatological symptoms with the COVID-19 disease. As per study, 18 Italian patients infected with COVID-19 shows various cutaneous manifestations such as erythematous rashes (14 patients), urticarial rashes (3 patients) and chicken pox like vesicular rashes (1 patient) on the trunk. Among the 18 patients, 8 patients develop the symptoms before onset and 10 patients after hospitalization [71,72]. Recently, from case history of a 23 year-old-man infected with COVID-19, it was found that the patients developed acute, infiltrated, violaceous, and painful plaques on the toes and on the lateral position of the feet known as chilblains. It was hypothesized that the chilblains may be one of the early symptoms of the COVID-19 disease where fever and dry cough is almost minimal and absent [73].

Clinical diagnosis of COVID-19: The clinical diagnosis of the COVID-19 disease is primarily based on factors such as clinical manifestations, epidemiological track record, *etc*. In order to diagnose the disease some diagnostic procedures were mainly conducted such as nucleic acid detection in the nasal, throat swab or other respiratory tract samplings by real time PCR techniques followed by further confirmation through next generation sequencing, Enzyme Linked Immuno-sorbent Assay (ELISA), CT scan and blood culture method. Recently another assay, DETECTR assay can also prove to be an efficient diagnostic approach [74]. As the symptoms (cough, fever, pneumonia, respiratory distress and dyspnea) associated with the individuals suffering from COVID-19 is highly atypical, these auxiliary examinations are very much essential in the detection of the virus along with the epidemiological track record of the patient. Preliminarily, the viral research institution of China identified the novel coronavirus, SARS-CoV-2 through classical Koch's postulates, finally observing the morphological characteristics of the virus using electron microscopy techniques [44]. Real-time quantitative polymerase chain reaction (RT-qPCR) and high throughput sequencing are the two most commonly use techniques that are mainly employed for the detection of nucleic acid of SARS-CoV-2 virus. The main identification method through which SARS-CoV-2 can be detected is through virus blood culture and whole genome sequencing by high throughput technologies but due to the limited applications of high-throughput sequencing in clinical diagnosis it cannot be routinely used in the detection of the virus. The main limitations of these high throughput sequencing is primarily high cost and dependence on the equipment. Hence, to overcome this situation, RT-qPCR is mainly applied for the detection of pathogenic viruses in blood and respiratory secretions [75]. Various companies in China launched the RT-qPCR test kits for routine clinical diagnosis soon after the outbreak of the SARS-CoV-2 virus. The primers and probes that are recommended by the Chinese Centre for Disease Control and Manage-

ment for clinical diagnosis in RT-qPCR techniques are mainly designed against the ORF1ab and N gene regions of the SARS-CoV-2 viral genome. When both the target comes out to be positive in RT-qPCR techniques the patient is confirmed to be positive. In one separate study two one step RT-qPCR assays (Taqman based method) was designed in which two different regions of the viral genome ORF1b and N genes were detected separately. Two separate SARS-CoV-2 patients were detected using this method using respiratory specimens whereas the negative control samples were confirmed to be negative [76]. Another report also suggest that when the saliva is used as a specimen sample of the infected individuals for clinical diagnosis using RT-qPCR analysis (SYBR based method), the rate of positive detection of virus is around 91.7%, suggesting that saliva is a promising non-invasive specimen for the diagnosis, monitoring and also infection control of patients suffering from COVID-19 [77]. Some reports also pointed out the shortcomings of this method. In one case five individuals infected with COVID-19 was tested negative using RT-qPCR technique but they are later confirmed to be infected with the virus by positive chest CT scan reports followed by repeated swab tests using RT-qPCR techniques [78]. Apart from that the method also suffers from few more disadvantages such as laborious nucleic acid detection method, long waiting time for the results and the associated biological safety hazards linked with the patient samples. However based on the available data, sensitivity of these RT-qPCR techniques is only 50-79% and depends on several factors such as number of clinical samples collected and the type of sample used [79]. Due to the false negative results of the RT-qPCR techniques, some prefer CT scans of the patients for clinical diagnosis of SARS-CoV-2 because of its sensitivity. The suspected individuals whose clinical results comes out to be negative by RT-qPCR analysis was tested again using chest CT scan and repeated RT-qPCR tests. High-resolution CT (HRCT) proved beneficial for the detection and disease severity of virus in patients suffering from COVID-19 [80]. The CT scan report of one of the patients infected with COVID-19 who was later admitted to Chinese hospital shows peripheral ground-glass opacities in both the lungs at multiple positions without sparing the subpleural position [81]. In another study, nine patients infected with COVID-19 shows distinct CT scan patterns. Out of the nine patients, three (33.3%) had parenchymal abnormalities that were detected by chest radiography. It was also shown that most of abnormalities were peripheral consolidations. It was also revealed from the chest CT scan images that a total of 77 lung parenchymal lesions were observed in the nine patients, among them eight patients had bilateral lung parenchymal lung abnormalities. The right lower lobe was most frequently involved followed by the left upper and lower lobes. Also among the 77 pulmonary lesions, it was found that 39% lesions were patchy, 13% lesions were confluent in nature and 48% were small nodular lesions. The posterior lung was involved in 67% of the lesions and 78% lesions were found in the peripheral lung. The lesions are ill-defined and are patchy to confluent or nodular. The patchy to confluent type of lesions are mainly found in the pleura of the lungs and the nodular lesions are mainly found in the bronchovascular bundles [82]. Similar type of results was also observed from patients suffering from SARS-CoV and MERS-CoV infections [83,84]. Hence CT scan proved to be vital technique for the clinical detection of the virus but it also suffers from few disadvantages. Firstly, the CT scan cannot differentiate between SARS-CoV-2 induced pneumonia from other types of viral pneumonia and secondly, hysteresis associated with the abnormal CT imaging. Some companies also developed some ELISA kits and POCT of IgM/IgG kits for the detection of SARS-CoV-2 virus. The detection rate of these kits is much higher compared to the nucleic acid based detection but there is unavailability of the kits in the markets and also published research articles. In one of the study, the serum samples of the patients infected with COVID-19 were tested with two assay kits; one of them is Enzyme Linked Immuno-Sorbent Assay (ELISA) IgM and IgG detection kits and the other one is colloidal gold-immunochromatographic assay (GICA) IgM and IgG detection kits. It was shown that the combined ELISA IgM and IgG detection kit is 87.3% effective and the combined GICA IgM and IgG detection kit is 82.4% sensitive [85]. Some studies also indicate that the sensitivity of the SARS-CoV N-based IgG ELISA kits (94.7%) is much higher than the SARS-CoV S- based IgG ELISA kits (58.9%) [86]. Apart from that the sensitivity of SARS-CoV-2 IgG/IgM remains to be evaluated. Recently, a method based on CRISPR-Cas12 lateral flow (DETECTR) assay was devised that provides easy detection of the virus from respiratory swab RNA extracts. The assay is very much sensitive with almost 95% efficiency in comparison to SARS-CoV-2 real-time RT-PCR assays. The SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) assay performs both the steps of reverse transcription and isothermal amplification simultaneously using loop-mediated amplification (RT-LAMP) from the nasopharyngeal and oropharyngeal swabs RNA extracts in universal transport medium (UTM). This is followed by the detection of Cas12 of the predefined sequences of coronavirus, after which the cleavage of the reporter molecule finally confirmed the detection of virus. The primers that are used in this assay were specifically designed against the E (envelope) and N (nucleoproteins) genes of the SARS-CoV-2 but were modified as per requirements of the LAMP assay. The N1 and N3 regions of the viral genome were not targeted as they are devoid of suitable protospacer adjacent motifs for Cas12 guide RNA (gRNAs). Next, the Cas12 gRNAs were designed to detect three separate SARS-like corona viruses such as (SARS-CoV-2 (accession NC_045512), SARS-CoV (accession NC_004718) and bat SARS like coronavirus (bat-SL-CoVZC45, accession MG772933) in the E gene and can detect only the SARS-CoV-2 in the N gene. It was finally demonstrated using synthetic, *in vitro* transcribed SARS-CoV-2 RNA of the gene targets that CRISPR–Cas12-based assay can only detect SARS-CoV-2 with almost no cross-reactivity reactions for related coronavirus strains using N gene guide RNA. Although there is expected cross-reactivity reactions for E gene guide RNA. The method was further optimized for the SARS-CoV-2 DETECTR assay on the N gene and E gene with human RNase P gene as control. The optimized protocol consists of RT-LAMP reaction at 62 ºC

for 20-30 min followed by the detection of Cas12 at 37 ºC for 10 min. The assay can be conducted in approximately 30-40 min followed by the visualization on a lateral flow strip. The assay is confirmed positive when there is a detection of both N and E genes. The assay is presumptive positive when there is a detection of either of the N or E genes. This data is consistent with the US-FDA EUA guidelines and also point-of-care diagnostics under the EUA. The FAM-biotin reporter molecule is used for the visualization of Cas12 detection. In order to capture the labeled nucleic acid lateral flow strips were mainly used. The reporter molecules which remained uncleaved are captured along the first detection line termed as control line. On the other hand, the Cas12 cleavage activity generates a detectable signal at the second detection line termed as test line. The signals generated by Cas12 were compared using both the lateral flow and fluorescence. The Cas12 signal by lateral flow was detected within 5 min and by fluorescent signal by < 1 min. The DETECTR assay is highly sensitive and has several advantages like isothermal signal amplification, target specificity with a single nucleotide precision, easy to use technology and rapid detection time. The technology is currently undergoing clinical validation [74]. Due to the wide spread nature of disease other sensitive methods should be developed immediately for the clinical diagnosis of COVID-19.

Available treatments: Presently, there is no specific antiviral agent or vaccines against COVID-19 infections. The use of supportive treatments like oxygen therapy, conservation fluid management along with some broad spectrum antibiotics to prevent secondary bacterial infections are currently being used in the treatment of SARS-CoV-2 viral infections [5,87]. In this section, potential therapeutic measures that are currently being tested against this novel coronavirus is briefly discussed.

Convalescent plasma therapy: The convalescent plasma treatment is currently being used as a treatment against SARS-CoV-2 infections. The patients who recovered from COVID-19 have specific antibodies against the virus. The plasma is collected from these recovered patients through simple processes, which are very much similar to blood donation. The plasma of contain containing antibodies is simply transfused to the patient who is currently suffering from COVID-19 infection. In theory, the plasma will boast the immune system of the patients suffering from COVID-19 thereby helping them to recover from the disease. Previously similar type of therapy is also being reported to combat against SARS-CoV and MERS-CoV infections. Previously similar type of therapy is also being reported to combat against SARS-CoV and MERS-CoV infections [88,89]. Six patients who were suffering from COVID-19 disease has been given the convalescent plasma therapy. The patients did not suffer from any side-effects of this therapy. It was further shown that 5 patients who were treated with this convalescent plasma therapy leads to a resolution of ground glass opacities and consolidation in the lungs. It was also shown that two patients the plasma therapy leads to a complete elimination of the virus. The increase in anti-SARS-CoV-2 antibody titers was reported in two patients by serological analysis. This preliminary study indicates that the therapy is highly effective and specific against COVID-19 and has special medical significance [90]. In another study, 5 critically ill patients suffering from COVID-19 received convalescent plasma cont-aining high amounts of neutralizing antibodies. All the patients improved clinically without any adverse side effects. But due to the small sample size and study design the therapy still needs to be evaluated properly. The therapy also needs to be evaluated in clinical trials [91].

Monoclonal antibody therapy: The monoclonal antibodies against targets various surface proteins on the viruses have also proved to be an effective therapy against wide range of viruses [92,93]. In one study, it is reported that CR3022, a SARS-CoV receptor binding domain (RBD) specific human monoclonal antibody has a potential to inhibit SARS-CoV-2 viral infections by binding to the receptor binding domain (RBD) of SARS-CoV-2 virus [94]. In another study, 47D11, a human monoclonal antibody neutralize the SARS-CoV-2 viral infections by binding to the conserved epitope on the spike receptor binding domain (RBD) in cell culture system. Hence, the antibodies whether alone or in combination with other therapies can prove to be an effective therapy against SARS-CoV-2 infections in the future [95].

Antiviral therapeutics: Currently, there is no effective vaccine or drug approved for use in the treatment of SARS-CoV-2 infection. Various drugs and vaccines are currently being evaluated and their clinical trials are going on. In one study, the efficiency of five FAD approved drugs, namely, penciclovir ribavirin, nitazoxanide, nafamostat, chloroquine and two antiviral drugs remdesivir (GS-5734), and favipiravir (T-750) were tested against SARS-CoV-2 infection *in vitro* on Vero E6 cell line [96]. Among them, remdesivir (GS-5734) and chloroquine showed effective results against the virus at low-micromolar concentration with minimum cytotoxicity. The effective concentration (EC_{50}) necessary to block the infections *in vitro* for remsedivir and chloroquinine were 1.13 μM and 0.77 μM, respectively [96]. Remdesivir, an antiviral nucleotide prodrug has shown potent invitro antiviral activity against a wide range of viruses and is currently being developed clinically for the treatment of Ebola virus infection. It has also shown both *in vivo* and *in vitro* activity both against MERS-CoV and SARS-CoV [97-100]. Remdesivir exerts its effect by inhibiting viral replication by binding to the viral RNA-dependent RNA polymerase (RdRp) [100,101]. Pre-clinical studies in mice show that remdesivir reduces SARS-CoV virus levels in mice [98,102]. Remdesivir also reduces levels of MERS-CoV virus and also lung injury when given as therapy or prophylaxis in mice model. Furthermore remdevisir also prevented MERS-CoV clinical disease when given as prophylaxis in a rhesus macaque model [103]. Remdesivir was also reported to be successful in treating the first COVID-19 patient in United States [104]. Similarly chloroquine and hydeoxychloroquine (an analogue of chloroquinine) also emerged as a potent therapeutic agent to treat COVID-19 infection. Chloroquine was previously used in the treatment of malaria but it's mode of action against many viral infections is not properly known [105]. Both the drugs exerts its effect by inhibiting the fusion of host cell membrane and the SARS-CoV-2 virus by increasing the endosomal pH *in vitro* [45]. Chloroquine interferes with the interaction of the SARS-CoV virus with the host cell receptor

by inhibiting the glycosylation of the cellular angiotensin converting enzyme- 2 (ACE2) receptor and also functions in both entry and post-entry stages of the COVID-19 infections *in vitro* on Vero-E6 cell lines [45,106]. *in vitro* Studies demonstrated that the drugs prevent the release of the viral genome into the host by blocking the transport of SARS-CoV-2 virus from early endosomes to endolysosomes [107]. Moreover, chloroquine also showed good immunomodulatory effect by suppressing the release of TNF-α and IL-6 [108]. Choloroquine, being a novel autophagy inhibitor interferes with viral replication [109]. Both remdesivir and chloroquine has proven to be effective against COVID-19 infections *in vitro* [2]. Currently, remdesivir and chloroquine are under phase-3 clinical trial and open-label trial against SARS-CoV-2 infections [110]. Wong *et al*. [2] also suggested that apart from remdesivir and chloroquine, nefamostat (a synthetic serine protease inhibitor which is active against MERS-CoV, influenza and ebola infections [111,112] and nitazoxanide (an antiprotozoal agent having an antiviral property against human and animal coronaviruses) [113-115] also inhibited the SARS-CoV-2 virus at a relatively low-micromolar concentration *in-vitro* [2]. The protease inhibitors, lopinavir and ritonavir used in the treatment of HIV [116] has also proved to be effective against SARS-CoV [117] and MERS-CoV infections [118]. During replication of SARS-CoV-2, the polyproteins were cleaved into RNA-dependent RNA polymerase and a helicase through the action of two proteases, 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro) [119]. In this respect, two drugs lopinavir and ritonavir showed promising activity against SARS-CoV infection *in vitro* by inhibiting the 3CLpro. This chymotrypsin-like protease (3CLpro) is also highly conserved in SARS-CoV-2 virus [120, 121]. Although *in vitro* studies of these two drugs against SARS-CoV showed good results but during *in vivo* studies higher than tolerable levels of these drugs is required to achieve good results [122]. Recently the amount of SARS-CoV-2 virus was significantly reduced in patient suffering from COVID-19 after the treatment with lopinavir/ritonavir (Kaletra®, AbbVie, North Chicago, USA) [123]. Nucleoside analogs (ribavirin and favipiravir) [119,124,125] may have some clinically potential against SARS-CoV-2 infection. Ribavirin, a guanine analog is used in the treatment of RSV and HCV. It was also tested experimentally against other viral infections such as Lassa fever and hemorrhagic fever [126,127]. Recently, ribavirin, has also been evaluated in patients that are infected with SARS [128]. Ribavarin exerts its effect through number of distinct mechanisms that includes inhibition of inosinemonophosphate dehydrogenase (IMPDH) thereby reducing the cellular pools of guanosine triphosphate (GTP), an immunomodulatory effect through the maintenance of T-helper type 1 immune response, inhibiting the mRNA capping activity, inhibition of the viral polymerases within the host and increased incorporation of ribavirin into the newly synthesized genomes of the viruses thereby causing mutations in the viral genome ultimately leading to catastrophe [129]. However, when used in high doses, the drug exhibits serious side effects that include anaemia [128]. Hence, it is not certain whether the drug can show its effect in treating SARS-CoV-2 viral infections. Another guanine analogue, favipiravir (T-705), which was originally approved in the treatment of influenza, also shows its effect against a wide range of RNA viruses such as chikungunya, ebola, yellow fever, norovirus and enterovirus through the inhibition of RNA-dependent RNA polymerases of the RNA viruses [130]. Recently favipiravir is also shown to be effective against SARS-CoV-2 virus *in vitro* on Vero E6 cells (EC_{50} = 61.88 mM) [2]. Recently, favipiravir was evaluated in patients suffering from COVID-19 in an open-label comparative controlled study. It was shown in the study that COVID-19 patients, who were treated with favipiravir showed faster clearance of the virus and better chest imaging change in comparison to the patients who were treated with lopinavir/ritonavir [131].

Arbidol (ethyl-6-bromo-4-[(dimethylamino) methyl]-5-hydroxy-1-methyl-2-[(phenylthio)methyl]-indole-3-carboxylate hydrochloride monohydrate), has also been proposed in the treatment of COVID-19. Arbidol has a broad spectrum antiviral activity against a wide range of enveloped and non-enveloped viruses [132]. Arbidol affects the various stages of the viral life cycle by directly targeting with the viral proteins or viral associated host factors [133]. Arbidol blocks the entry of the virus into the target host cells by blocking the fusion of the virus with the target host membrane [132]. Recently, arbidol effectively inhibited the SARS-CoV-2 infection *in-vitro* by blocking the entry of the virus by impeding the attachment of viruses and also mediates release from the ELs [134]. Arbidol was tested against patients suffering from COVID-19 along with lopinavir/ritonavir (Kaletra®) and Shufeng Jiedu Capsule (traditional Chinese medicine) at Shanghai Public Health Clinical Center in China. All the COVID-19 patients gained marked improvement in pneumonia associated symptoms after the therapeutic regime [65,135]. The type II transmembrane (TMSPSS2) inhibitor and imatinib, BCR-ABL kinase inhibitor can also prove to be a potential therapeutic agent against SARS-CoV-2 virus. One study indicates that in order to enter the target cells, SARS-CoV-2 uses ACE2 receptor and cellular protease TMPRSS2. Hence TMPRSS2 inhibitor (camostat mesylate) can be an essential therapeutic option against COVID-19 [136-140]. On the other hand, imatinib blocks SERS-CoV and MERS-CoV viral replication through the inhibition of coronavirus fusion with the endosomal membrane [141]. Hence, it can also be a good therapeutic agent against SARS-CoV-2 infection. In COVID19 patients, it was observed that a large number of macrophages and T lymphocytes were activated resulting in the production of a cytokine, interleukin-6 (IL-6). IL-6 binds to the receptor on the target cells causing severe inflammatory response in various organs and tissues. Tocilizumab (TCZ), a monoclonal antibody against interleukin-6 (IL-6) has reduced the severity of infection in COVID-19 patients [142]. The protease inhibitor, disulfiram used in alcohol aversion therapy has also been reported to act against SARS-CoV and MERS-CoV. Disulfiram acts as a non-competitive inhibitor of MERS-CoV papain-like protease and also acts as a competitive inhibitor of SARS-CoV papain-like protease. The drug forms a covalent adduct at the active site of the papain like protease in SARS-CoV [143]. The drug is currently evaluated against SARS-CoV-2 virus. Loperamide, an antidiarrheal agent has been

shown to inhibit MERS-CoV mediated cytopathic effects *in vitro* [144,145]. It is also currently evaluated against SARS-CoV-2 infections [146]. Other drugs such as nucleoside analog, ganciclovir [147] and neuraminidase inhibitor, oselta-mivir [148] have also been used in the treatment of COVID-19 infections [62]. Due to the rapid spread of the virus a wide spread research is going not only to identify a novel therapeutic agent against COVID-19 infections but also to understand the host-virus interaction that would provide vital information in understanding the virus even better (Table-1).

Conclusion

The world economy has been intensely affected due to the COVID-19 pandemic. Recently, the United Kingdom has laun-

TABLE-1
POTENTIAL ANTIVIRAL THERAPEUTIC AGENTS AGAINST SARS-COV-2

Antiviral drugs	Mode of action	Target diseases	Current regulatory status	Ref.
Remdesivir (GS-5734) (nucleotide analog prodrug)	Binds to the RNA dependent RNA polymerase (RdRp) inhibiting viral replication.	Ebola, MERS, SARS, showed good antiviral activity against COVID-19 infections *in vitro.*	Authorized in US, India, Singapore, Japan, UK for use under an Emergency Use Authorization (EUA).	[23,96-100, 104,110, 136,138]
Chloroquine	Increases endosomal pH thereby inhibiting the fusion of host cell membrane and the virus, inhibits the glycosylation of the cellular angiotensin-converting enzyme 2 (ACE2) receptor thereby interfering with the interaction of the host cell receptor and the virus, prevent the release of the viral genome into the host by blocking the transport of virus from early endosomes to endolysosomes, a novel autophagy inhibitor interfering with viral replication, immunomodulatory effects.	Malaria, some autoimmune disorders, showed good antiviral activity against COVID-19 infections *in vitro.*	Old and approved drug in every country almost. It is in the list of essential medicines (WHO).	[45-47, 105, 108-110]
Nefamostat (synthetic serine protease inhibitor)	Reduces the release of a protease, cathepsin B thereby prevents the fusion of viral membrane and the endosome.	MERS, Influenza virus, Ebola showed good antiviral activity against COVID-19 infections *in vitro.*	Identified as a potential therapy for COVID-19. CT will be conducted in Japan	[45,112]
Nitazoxanide (antiprotozoal agent having antiviral property)	Blocks the maturation of the viral hemagglutinin post-translationally, inhibits the replication of broad range viruses.	Antiviral property against wide range of viruses such as respiratory syncytial virus, parainfluenza virus, coronavirus, rotavirus human immunodeficiency virus *etc*.	Approved as a broad-spectrum antiviral drug, used for the treatment of various helminthic, protozoal, viral infections and under investigation for COVID-19 treatment	[45,113-115]
Lopinavir/Ritonavir (protease inhibitor)	Lopinavir/Ritonavir showed promising activity against COVID-19 infections *in vitro* by inhibiting the 3CLpro inhibiting replication. Inhibits HIV-1 protease that is necessary for protein cleavage.	HIV, SARS, MERS	Approved as a FDC medication for treatment and prevention of HIV. CT is conducted by Israel for COVID-19 effectiveness	[116-118, 122,123,135]
Ribavirin (guanosine analog)	Inhibition of inosine monophosphate dehydrogenase (IMPDH) thereby reduces the cellular pools of guanosine tri-phosphate (GTP), an immunomodulatory effect through the maintenance of T-helper type 1 immune response, Inhibition the mRNA capping activity, Inhibition of the viral polymerases within the host and increased incorporation of ribavirin into the newly synthesized viral genomes thereby causing mutations.	RSV, HCV, SARS, MERS tested experimentally against other viral infections such as Lassa fever and hemorrhagic fever.	Approved to treat RSV infection, hepatitis C and some viral hemorrhagic fevers. The University of Hong Kong is conducting CT for COVID-19	[119, 125-127]
Favipiravir (T-705) (nucleoside analog)	Inhibition of RNA-dependent RNA polymerases (RdRp) of the RNA viruses	Influenza virus, wide range of RNA viruses such as chikungunya, Ebola, yellow fever, norovirus and enterovirus. Showed good antiviral activity against COVID-19 infetions *in vitro.* (EC_{50} = 61.88 mM)	Approved in Japan and China for the treatment of influenza. However it has potential teratogenicity. Remain unapproved in USA and UK. China and Japan is going to conduct CT to evaluate the efficacy for COVID-19	[45,119, 125,131]

Umifenovir	Affects various stages of the viral life cycle by directly targeting with the viral proteins or viral associated host factors, blocks the entry of the virus into the target host cells by blocking the fusion of the virus with the target host membrane, Umifenovir effectively inhibit the SARS-CoV-2 infection *in vitro* by blocking the entry of the virus by impeding the attachment of viruses and also mediates release from the ELs.	Wide range of enveloped and non-enveloped viruses, effectively inhibit the COVID-19 infections *in vitro.*	Approved for influenza infection in Russia and China. Still unapproved in USA. CT is being conducted by the National Health Commission of China, with darunavir as a potential treatment during the COVID-19	[132-135]
Camostat mesylate (TMPRSS2 inhibitor)	Inhibits cellular protease TMPRSS2 preventing the entry of virus into the target cells.	Potential therapeutic agent against COVID-19 infections.	Approved in Japan, India for the treatment of chronic pancreatitis and post-operative reflux esophagitis, not approved by US-FDA	[136-140]
Imatinib	Inhibition of coronavirus fusion with the endosomal membrane resulting in the inhibition of viral replication.	SARS, MERS.	CTs are being conducted in USA for understanding efficacy for COVID-19	[141]
Tocilizumab (monoclonal antibody against interleukin-6)	In viral infected cells large number of macrophages and T lymphocytes were activated resulting in the production of a cytokine, interleukin-6 (IL-6). IL-6 binds to the receptor on the target cells causing severe inflammatory response in various organs and tissues.	Reduction of the severity of COVID-19 infections.	Approved by US-FDA, EMA and Japan. China approved for treatment of inflammation in patients for COVID-19	[142]
Disulfiram (protease inhibitor)	Non-competitive inhibitor of MERS-CoV papain-like protease, a competitive inhibitor of SARS-CoV papain-like protease. The drug forms a covalent adduct at the active site of the papain like protease in SARS-CoV.	SARS, MERS, currently being evaluated against COVID-19 infections.	Research is going (USA) on for finding out its effectiveness for management of inflammation	[143]
Loperamide (antidiarrheal agent)	Inhibits the MERS-CoV replication, at low micromolar (4–6 μM) the drug also inhibits the growth of other CoVs.	MERS, currently being evaluated against COVID-19 infections.	No efficacy found against COVID-19	[144-146]
Ganciclovir (nucleoside analog)	Selective and potent inhibition of the replication of the viral DNA in the form of ganciclovir triphosphate.	Cytomegalovirus infections, used in the treatment of COVID-19 infections.	Approved for treatment cytomegalovirus (CMV) infections.	[62,147]
Oseltamivir (neuraminidase inhibitor)	Inhibits viral neuraminidase activity, preventing the cleavage of new budding virions from the host's cell surface.	Influenza viruses A, used in the treatment of COVID-19 infections.	Still no evidence in supporting effectiveness in treating COVID-19	[62,148]
Dexamethasone (9-fluoro-glucocorticoid)	Anti-inflammatory properties.	Asthma, arthritis, multiple sclerosis and many severe allergies, proved to be an effective therapeutic agent against COVID-19 during RECOVERY trial.	In June 2020, recovery trial showed that this drug improves the survival rate for patients with ventilator support, WHO stated it should be reserved for the seriously ill and critical patients receiving COVID-19 treatment in a hospital setting.	[149-152]

MERS: Middle east respiratory syndrome; SARS: Severe acute respiratory syndrome; COVID-19: Corona Virus Disease 2019; HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; RSV: Respiratory syncytial virus

ched a special trial program named the randomized evaluation of COVID-19 (recovery) trial to test whether the existing or new drugs can prove to be effective in the treatment of COVID-19 [149,150]. In that particular trial, dexamethasone, a steroid known to reduce inflammation proved to be highly effective in the treatment of COVID-19 [149-151]. It has been shown that early administration of dexamethasone reduces the mortality rate and also the duration of mechanical ventilation in acute respiratory distress patients suffering from COVID-19 [152]. Efforts have been made to fight against it - be the development of a new drug, or the usefulness of existing drugs against it, have been studied meticulously. Numerous clinical studies have been conducted to find the evidence of effectiveness of these drugs. The urge to increase the R & D expenses has become reality now which is also pressurizing the scientists as well as researchers for showcasing the real time application of science and technology, which may facilitate the unmet need of available therapy against COVID-19.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. F. Wu, S. Zhao, B. Yu, Y.M. Chen, W. Wang, Z.G. Song, Y. Hu, Z.-W. Tao, J.-H. Tian, Y.-Y. Pei, M.-L. Yuan, Y.-L. Zhang, F.-H. Dai, Y. Liu, Q.-M. Wang, J.-J. Zheng, L. Xu, E.C. Holmes and Y.-Z. Zhang, *Nature*, **579**, 265 (2020); https://doi.org/10.1038/s41586-020-2008-3
2. S. Su, G. Wong, W. Shi, J. Liu, A.C.K. Lai, J. Zhou, W. Liu, Y. Bi and G.F. Gao, *Trends Microbiol.*, **24**, 490 (2016); https://doi.org/10.1016/j.tim.2016.03.003
3. S.J. Perlman and J. Netland, *Nat. Rev. Microbiol.*, **7**, 439 (2009); https://doi.org/10.1038/nrmicro2147
4. Y. Yin and R.G. Wunderink, *Respirology*, **23**, 130 (2018); https://doi.org/10.1111/resp.13196
5. R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y. Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W.J. Liu, D. Wang, W. Xu, E.C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi and W. Tan, *Lancet*, **395**, 565 (2020); https://doi.org/10.1016/S0140-6736(20)30251-8
6. J.F.-W. Chan, K.-H. Kok, Z. Zhu, H. Chu, K.K.-W. To, S. Yuan and K.-Y. Yuen, *Emerg. Microbes Infect.*, **9**, 221 (2020); https://doi.org/10.1080/22221751.2020.1719902
7. D. Paraskevis, E.G. Kostaki, G. Magiorkinis, G. Panayiotakopoulos, G. Sourvinos and S. Tsiodras, *Infect. Genet. Evol.*, **79**, 104212 (2020); https://doi.org/10.1016/j.meegid.2020.104212
8. P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.-. Si, Y. Zhu, B. Li, C.-L. Huang, H.-D. Chen, J. Chen, Y. Luo, H. Guo, R.-D. Jiang, M.-Q. Liu, Y. Chen, X.-R. Shen, X. Wang, X.-S. Zheng, K. Zhao, Q.-J. Chen, F.Deng, L.-L. Liu, B. Yan, F.-X. Zhan, Y.-Y. Wang, G.-F. Xiao and Z.-L. Shi, *Nature*, **579**, 270 (2020); https://doi.org/10.1038/s41586-020-2012-7
9. C. Wu, Y. Liu, Y. Yang, P. Zhang, W. Zhong, Y. Wang, Q. Wang, Y. Xu, M. Li, X. Li and M. Zheng, L. Chen and H. Li, *Acta Pharm. Sin. B*, **10**, 766 (2020); https://doi.org/10.1016/j.apsb.2020.02.008
10. C. Drosten, S. Günther, W. Preiser, S. van der Werf, H.-R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R.A.M. Fouchier, A. Berger, A.-M. Burguière, J. Cinatl, M. Eickmann, N. Escriou, K. Grywna, S. Kramme, J.-C. Manuguerra, S. Müller, V. Rickerts, M. Stürmer, S. Vieth, H.-D. Klenk, A.D.M.E. Osterhaus, H. Schmitz and H.W. Doerr, *N. Engl. J. Med.*, **348**, 1967 (2003); https://doi.org/10.1056/NEJMoa030747
11. J. Cui, F. Li and Z.L. Shi, *Nat. Rev. Microbiol.*, **17**, 181 (2019); https://doi.org/10.1038/s41579-018-0118-9
12. F. Li, *Annu. Rev. Virol.*, **3**, 237 (2016); https://doi.org/10.1146/annurev-virology-110615-042301
13. Z. Zhu, Z. Zhang, W. Chen, Z. Cai, X. Ge, H. Zhu, T. Jiang, W. Tan and Y. Peng, *Infect. Genet. Evol.*, **61**, 183 (2018); https://doi.org/10.1016/j.meegid.2018.03.028
14. W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M. Somasundaran, M.A. Berne, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, H. Choe and M. Farzan, *Nature*, **426**, 450 (2003); https://doi.org/10.1038/nature02145
15. Y, Zhao, Z. Zhao, Y. Wang, Y. Zhou, Y. Ma and W. Zuo, bioRxiv 2020.2001.2026.919985 (2020); https://doi.org/10.1101/2020.01.26.919985
16. A. Wu, Y. Peng, B. Huang, X. Ding, X. Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, J. Sheng, L. Quan, Z. Xia, W. Tan, G. Cheng and T. Jiang, *Cell Host Microbe*, **27**, 325 (2020); https://doi.org/10.1016/j.chom.2020.02.001
17. WHO, WHO Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. https://www.who.int/dg/speeches/detail/who-director-general-sremarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020.
18. C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y, Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang and B. Cao, *Lancet*, **395**, 497 (2020); https://doi.org/10.1016/S0140-6736(20)30183-5
19. R. Wang, X. Zhang, D.M. Irwin and Y. Shen, *J. Infect.*, **80**, 350 (2020); https://doi.org/10.1016/j.jinf.2020.01.017
20. F. Yu, R. Jia, Y. Tang, J. Liu and B. Wei, *Stem Cell Res.*, **46**, 101859 (2020); https://doi.org/10.1016/j.scr.2020.101859
21. J.P. Guo, M. Petric, W. Campbell and P.L. McGeer, *Virology*, **324**, 251 (2004); https://doi.org/10.1016/j.virol.2004.04.017
22. X. Tang, C. Wu, X. Li, Y. Song, X. Yao, X. Wu, Y. Duan, H. Zhang, Y. Wang, Z. Qian, J. Cui and J. Lu, *Natl. Sci. Rev.*, **7**, 1012 (2020); https://doi.org/10.1093/nsr/nwaa036
23. E. de Wit, N. van Doremalen, D. Falzarano and V.J. Munster, *Nat. Rev. Microbiol.*, **14**, 523 (2016); https://doi.org/10.1038/nrmicro.2016.81
24. P.S. Masters, *Adv. Virus Res.*, **66**, 193 (2006); https://doi.org/10.1016/S0065-3527(06)66005-3
25. M.A. Tortorici and D. Veesler, *Adv. Virus Res.*, **105**, 93 (2019); https://doi.org/10.1016/bs.aivir.2019.08.002
26. G. Simmons, J.D. Reeves, A.J. Rennekamp, S.M. Amberg, A.J. Piefer and P. Bates, *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 4240 (2004), https://doi.org/10.1073/pnas.0306446101
27. S. Belouzard, V.C. Chu and G.R. Whittaker *Proc. Natl. Acad. Sci. U.S.A.*, **106**, 5871 (2009); https://doi.org/10.1073/pnas.0809524106
28. H. Wang, P. Yang, K. Liu, F. Guo, Y. Zhang, G. Zhang and C. Jiang, *Cell Res.*, **18**, 290 (2008); https://doi.org/10.1038/cr.2008.15
29. K. Kuba, Y. Imai, T. Ohto-Nakanishi and J.M. Penninger, *Pharmacol. Ther.*, **128**, 119 (2010), https://doi.org/10.1016/j.pharmthera.2010.06.003
30. N. Zhang, S. Jiang and L. Du, *Expert Rev. Vaccines*, **13**, 761 (2014); https://doi.org/10.1586/14760584.2014.912134
31. S. Xia, Y. Zhu, M. Liu, Q. Lan, W. Xu, Y. Wu, T. Ying, S. Liu, Z. Shi, S. Jiang and L. Lu, *Cell. Mol. Immunol.*, **17**, 765 (2020); https://doi.org/10.1038/s41423-020-0374-2
32. F. Yu, L. Du, D.M. Ojcius, C. Pan and S. Jiang, *Microbes Infect.*, **22**, 74 (2020); https://doi.org/10.1016/j.micinf.2020.01.003
33. A.H. De Wilde, E.J. Snijder, M. Kikkert and M.J. van Hemert, *Curr. Top. Microbiol. Immunol.*, **419**, 1 (2018); https://doi.org/10.1007/82_2017_25
34. S.G. Sawicki and D.L. Sawicki, *Curr. Top. Microbiol. Immunol.*, **287**, 31 (2005); https://doi.org/10.1007/3-540-26765-4_2
35. J. Ziebuhr, A.E. Gorbalenya and E.J. Snijder, *J. Gen. Virol.*, **81**, 853 (2000); https://doi.org/10.1099/0022-1317-81-4-853
36. S. Hussain, J. Pan, Y. Chen, Y. Yang, J. Xu, Y. Peng, Y. Wu, Z. Li, Y. Zhu, P. Tien and D. Guo, *J. Virol.*, **79**, 5288 (2005); https://doi.org/10.1128/JVI.79.9.5288-5295.2005
37. A. Perrier, A. Bonnin, L. Desmarets, A. Danneels, A. Goffard, Y. Rouille, J. Dubuisson and S. Belouzard, *J. Biol. Chem.*, **294**, 14406 (2019); https://doi.org/10.1074/jbc.RA119.008964
38. W. Song, M. Gui, X. Wang and Y. Xiang, *PLoS Pathog.*, **14**, e1007236 (2018); https://doi.org/10.1371/journal.ppat.1007236
39. J. Shang, Y. Wan, C. Luo, G. Ye, Q. Geng, A. Auerbach and F. Li, *Proc. Natl. Acad. Sci. USA*, **117**, 11727 (2020); https://doi.org/10.1073/pnas.2003138117
40. G. Simmons, D.N. Gosalia, A.J. Rennekamp, J.D. Reeves, S.L. Diamond and P. Bates, *Proc. Natl. Acad. Sci. USA*, **102**, 11876 (2005); https://doi.org/10.1073/pnas.0505577102
41. Q. Li, X. Guan, P. Wu, X. Wang, L. Zhou, Y. Tong, R. Ren, K.S.M. Leung, E.H.Y. Lau, J.Y. Wong, X. Xing, N. Xiang, Y. Wu, C. Li, Q. Chen, D. Li, T. Liu, J. Zhao, M. Liu, W. Tu, C. Chen, L. Jin, R. Yang, Q. Wang, S. Zhou, R. Wang, H. Liu, Y. Luo, Y. Liu, G. Shao, H. Li, Z. Tao, Y. Yang, Z. Deng, B. Liu, Z. Ma, Y. Zhang, G. Shi, T.T.Y. Lam, J.T. Wu, G.F. Gao, B.J. Cowling, B. Yang, G.M. Leung and Z. Feng, *N. Engl. J. Med.*, **382**, 1199 (2020); https://doi.org/10.1056/NEJMoa2001316

42. I. Hamming, W. Timens, M.L. Bulthuis, A.T. Lely, G. Navis and H. van Goor, *J. Pathol.*, **203**, 631 (2004); https://doi.org/10.1002/path.1570
43. D. Wrapp, N. Wang, K.S. Corbett, J.A. Goldsmith, C.-L. Hsieh, O. Abiona, B.S. Graham and J.S. McLellan, *Science*, **367**, 1260 (2020); https://doi.org/10.1126/science.abb2507
44. J. Peiris, Y. Guan and K. Yuen, *Nat. Med.*, **10(S12)**, S88 (2004); https://doi.org/10.1038/nm1143
45. K. Pyrc, B. Berkhout and L. Van Der Hoek, *Expert Rev. Anti Infect. Ther.*, **5**, 245 (2007); https://doi.org/10.1586/14787210.5.2.245
46. A. Rahman and A. Sarkar, *Am. J. Public Health*, **109**, 1288 (2019); https://doi.org/10.2105/AJPH.2019.305186
47. Z.A. Memish, A.I. Zumla, R.F. Al-Hakeem, A.A. Al-Rabeeah and G.M. Stephens, *N. Engl. J. Med.*, **368**, 2487 (2013); https://doi.org/10.1056/NEJMoa1303729
48. C. Wang, P.W. Horby, F.G. Hayden and G.F. Gao, *Lancet*, **395**, 470 (2020); https://doi.org/10.1016/S0140-6736(20)30185-9
49. M. Giovanetti, D. Benvenuto, S. Angeletti and M. Ciccozzi, *J. Med. Virol.*, **92**, 518 (2020); https://doi.org/10.1002/jmv.25699
50. T. Hampton, *JAMA*, **294**, 2291 (2005); https://doi.org/10.1001/jama.294.18.2291
51. A. Banerjee, K. Kulcsar, V. Misra, M. Frieman and K. Mossman, *Viruses*, **11**, 41 (2019); https://doi.org/10.3390/v11010041
52. W. Li, Z. Shi, M. Yu, W. Ren, C. Smith, J.H. Epstein, H. Wang, G. Crameri, Z. Hu, H. Zhang, J. Zhang, J. McEachern, H. Field, P. Daszak, B.T. Eaton, S. Zhang and L.-F. Wang, *Science*, **310**, 676 (2005); https://doi.org/10.1126/science.1118391
53. V.D. Menachery, B.L. Yount Jr., A.C. Sims, S.S. Agnihothram, K. Debbink, L.E. Gralinski, R.L. Graham, T. Scobey, J.A. Plante, S.R. Royal, J. Swanstrom, T.P. Sheahan, R.J. Pickles, D. Corti, S.H. Randell, A. Lanzavecchia, W.A. Marasco and R.S. Baric, *Proc. Natl. Acad. Sci. USA*, **113**, 3048 (2016); https://doi.org/10.1073/pnas.1517719113
54. A. Bermingham, M.A. Chand, C.S. Brown, E. Aarons, C. Langrish, C. Tong, K. Hoschler, K. Brown, M. Galiano, R. Myers, R.G. Pebody, H.K. Green, N.L. Boddington, R. Gopal, N. Price, W. Newsholme, C. Drosten, R.A. Fouchier and M. Zambon, *Euro Surveill.*, **17**, 20290 (2012).
55. Z. Liu, X. Xiao, X. Wei, J. Li, J. Yang, H. Tan, J. Zhu, Q. Zhang, J. Wu and L. Liu, *J. Med. Virol.*, 92, 595 (2020); https://doi.org/10.1002/jmv.25726
56. L.T. Phan, T.V. Nguyen, Q.C. Luong, T.V. Nguyen, H.T. Nguyen, H.Q. Le, T.T. Nguyen, T.M. Cao and Q.D. Pham, *N. Engl. J. Med.*, **382**, 872 (2020); https://doi.org/10.1056/NEJMc2001272
57. J. Riou and C.L. Althaus, *Euro Surveill.*, **25**, 2000058 (2020); https://doi.org/10.2807/1560-7917.ES.2020.25.4.2000058
58. J. Parry, China coronavirus: cases surge as official admits human to human transmission. British Medical Journal Publishing Group; 2020.
59. Laboratory diagnostics for novel coronavirus. WHO 2020 https://www.who.int/health-topics/coronavirus/laboratory-diagnostics-for-novel-coronavirus.
60. B. Kan, M. Wang, H. Jing, H. Xu, X. Jiang, M. Yan, W. Liang, H. Zheng, K. Wan, Q. Liu, B. Cui, Y. Xu, E. Zhang, H. Wang, J. Ye, G. Li, M. Li, Z. Cui, X. Qi, K. Chen, L. Du, K. Gao, Y. Zhao, X. Zou, Y.-J. Feng, Y.-F. Gao, R. Hai, D. Yu, Y. Guan and J. Xu, *J. Virol.*, **79**, 11892 (2005); https://doi.org/10.1128/JVI.79.18.11892-11900.2005
61. W.J. Guan, Z.Y. Ni, Y. Hu, W.H. Liang, C.Q. Ou, J.X. He, L. Liu, H. Shan, C.-L. Lei, D.S.C. Hui, B. Du, L.-J. Li, G. Zeng, K.-Y. Yuen, R.-C. Chen, C.-L. Tang, T. Wang, P.Y. Chen, J. Xiang, S.-Y. Li, J.-L. Wang, Z.-J. Liang, Y.-X. Peng, L. Wei, Y. Liu, Y.-H. Hu, P. Peng, J.-M. Wang, J.-Y. Liu, Z. Chen, G. Li, Z.-J. Zheng, S.-Q. Qiu, J. Luo, C.-J. Ye, S.-Y. Zhu, and N.-S. Zhong, *N. Engl. J. Med.*, **382**, 1708 (2020); https://doi.org/10.1056/NEJMoa2002032
62. N. Chen, M. Zhou, X. Dong, J.M. Qu, F.Y. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei, J. Xia, T. Yu, X. Zhang and L. Zhang, *Lancet*, **395**, 507 (2020); https://doi.org/10.1016/S0140-6736(20)30211-7
63. P.I. Lee and P.R. Hsueh, *J. Microbiol. Immunol. Infect.*, **53**, 365 (2020); https://doi.org/10.1016/j.jmii.2020.02.001
64. W. Zhang, R.H. Du, B. Li, X.S. Zheng, X.L. Yang, B. Hu, Y.-Y. Wang, G.-F. Xiao, B. Yan, Z.-L. Shi and P. Zhou, *Emerg. Microbes Infect.*, **9**, 386 (2020); https://doi.org/10.1080/22221751.2020.1729071
65. D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B. Wang, H. Xiang, Z. Cheng, Y. Xiong, Y. Zhao, Y. Li, X. Wang and Z. Peng, *JAMA*, **323**, 1061 (2020); https://doi.org/10.1001/jama.2020.1585
66. S.M. Poutanen, D.E. Low, B. Henry, S. Finkelstein, D. Rose, K. Green, R. Tellier, R. Draker, D. Adachi, M. Ayers, D.M. Skowronski, A.K. Chan, I. Salit, A.E. Simor, A.S. Slutsky, P.W. Doyle, M. Krajden, M. Petric, R.C. Brunham and A.J. McGeer, *N. Engl. J. Med.*, **348**, 1995 (2003); https://doi.org/10.1056/NEJMoa030634
67. A.L. Phelan, R. Katz and L.O. Gostin, *JAMA*, **323**, 709 (2020); https://doi.org/10.1001/jama.2020.1097
68. H. Tezer and T.B. Demirdag, *Turk. J. Med. Sci.*, **50**, 592 (2020); https://doi.org/10.3906/sag-2004-174
69. M. Montero-Odasso, D.B. Hogan, R. Lam, K. Madden, C. MacKnight, F. Molnar and K. Rockwood, *Can. Geriatr. J.*, **23**, 152 (2020); https://doi.org/10.5770/cgj.23.452
70. M.E. Hartman, R.A. Hernandez, K. Patel, T.E. Wagner, T. Trinh, A.B. Lipke, E.T. Yim, J.N. Pulido, J.M. Pagel, S.J. Youssef and J.L. Mignone, *ASAIO J.*, **66**, 603 (2020); https://doi.org/10.1097/MAT.0000000000001177
71. E.E. Freeman, D.E. McMahon, M.E. Fitzgerald, M. Rosenbach, L.P. Fox, J. Takeshita, L.E. French, B.H. Thiers and G.J. Hruza, *J. Am. Acad. Dermatol.*, **83**, 509 (2020); https://doi.org/10.1016/j.jaad.2020.04.045
72. S. Recalcati, *J. Eur. Acad. Dermatol. Venereol.*, **34**, e212 (2020); https://doi.org/10.1111/jdv.16387
73. A. Kolivras, F. Dehavay, D. Delplace, F. Feoli, I. Meiers, L. Milone, C. Olemans, U. Sass, A. Theunis, C.T. Thompson, L. Van De Borne and B. Richert, *JAAD Case Rep.*, **6**, 489 (2020); https://doi.org/10.1016/j.jdcr.2020.04.011
74. J.P. Broughton, X. Deng, G. Yu, C.L. Fasching, V. Servellita, J. Singh, X. Miao, J.A. Streithorst, A. Granados, A. Sotomayor-Gonzalez, K. Zorn, A. Gopez, E. Hsu, W. Gu, S. Miller, C.-Y. Pan, H. Guevara, D.A. Wadford, J.S. Chen and C.Y. Chiu, *Nat. Biotechnol.*, **38**, 870 (2020); https://doi.org/10.1038/s41587-020-0513-4
75. V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K.W. Chu, T. Bleicker, S. Brünink, J. Schneider, M.L. Schmidt, D.G.J.C. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J.-L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M.P.G. Koopmans and C. Drosten, *Euro Surveill.*, **25**, 23 (2020); https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
76. D.K.W. Chu, Y. Pan, S.M.S. Cheng, K.P.Y. Hui, P. Krishnan, Y. Liu, D.Y.M. Ng, C.K.C. Wan, P. Yang, Q. Wang, M. Peiris and L.L.M. Poon, Clin. Chem., **66**, 549 (2020); https://doi.org/10.1093/clinchem/hvaa029
77. K.K. To, O.T. Tsang, C.C.-Y. Yip, K.-H. Chan, T.-C. Wu, J.M.-C. Chan, W.-S. Leung, T.S.-H. Chik, C.Y.-C. Choi, D.H. Kandamby, D.C. Lung, A.R. Tam, R.W.-S. Poon, A.Y.-F. Fung, I.F.-N. Hung, V.C.-C. Cheng, J.F.-W. Chan and K.-Y. Yuen, *Clin. Infect. Dis.*, **71**, 841 (2020); https://doi.org/10.1093/cid/ciaa149
78. X. Xie, Z. Zhong, W. Zhao, C. Zheng, F. Wang and J. Liu, *Radiology*, **296**, E41 (2020); https://doi.org/10.1148/radiol.2020200343
79. W.C. Yam, K.H. Chan, L.L. Poon, Y. Guan, K.Y. Yuen, W.H. Seto and J.S.M. Peiris, *J. Clin. Microbiol.*, **41**, 4521 (2003); https://doi.org/10.1128/JCM.41.10.4521-4524.2003
80. Y. Pan, H. Guan, S. Zhou, Y. Wang, Q. Li, T. Zhu, Q. Hu and L. Xia, *Eur. Radiol.*, **30**, 3306 (2020); https://doi.org/10.1007/s00330-020-06731-x
81. J. Lei, *Radiology*, **295**, 18 (2020); https://doi.org/10.1148/radiol.2020200236
82. S.H. Yoon, K.H. Lee, J.Y. Kim, Y.K. Lee, H. Ko, K.H. Kim, C.M. Park, and Y.-H. Kim, *Korean J. Radiol.*, **21**, 494 (2020); https://doi.org/10.3348/kjr.2020.0132

83. G.C. Ooi, P.L. Khong, N.L. Muller, W.C. Yiu, L.J. Zhou, J.C.M. Ho, B. Lam, S. Nicolaou and K.W.T. Tsang, *Radiology*, **230**, 836 (2004); https://doi.org/10.1148/radiol.2303030853
84. A.M. Ajlan, R.A. Ahyad, L.G. Jamjoom, A. Alharthy and T.A. Madani, *AJR Am. J. Roentgenol.*, **203**, 782 (2014); https://doi.org/10.2214/AJR.14.13021
85. J. Xiang, M. Yan, H. Li, T. Liu, C. Lin, S. Huang, C. Shen, medRxiv 2020.02.27.20028787 (2020); https://doi.org/10.1101/2020.02.27.20028787
86. P.C. Woo, S.K. Lau, B.H. Wong, H. Tsoi, A.M.Y. Fung, R.Y.T. Kao, K. Chan, J.S.M. Peiris and K. Yuen, *J. Clin. Microbiol.*, **43**, 3054 (2005); https://doi.org/10.1128/JCM.43.7.3054-3058.2005
87. B. Down, S. Kulkarni, A.H.A. Khan, B. Barker and I. Tang, *Ann. Med. Surg*., **55**, 24 (2020); https://doi.org/10.1016/j.amsu.2020.05.014
88. J. Mair-Jenkins, M. Saavedra-Campos, J.K. Baillie, P. Cleary, F.-M. Khaw, W.S. Lim, S. Makki, K.D. Rooney, J.S. Nguyen-Van-Tam and C.R. Beck, *J. Infect. Dis.*, **211**, 80 (2015); https://doi.org/10.1093/infdis/jiu396
89. K.L. Koenig, *West. J. Emerg. Med.*, **16**, 619 (2015); https://doi.org/10.5811/westjem.2015.7.27915
90. M. Ye, D. Fu, Y. Ren, F. Wang, D. Wang, F. Zhang, X. Xia and T. Lv, *J. Med. Virol.*, (2020); https://doi.org/10.1002/jmv.25882
91. C. Shen, Z. Wang, F. Zhao, Y. Yang, J. Li, J. Yuan, F. Wang, D. Li, M. Yang, L. Xing, J. Wei, H. Xiao, Y. Yang, J. Qu, L. Qing, L. Chen, Z. Xu, L. Peng, Y. Li, H. Zheng, F. Chen, K. Huang, Y. Jiang, D. Liu, Z. Zhang, Y. Liu and L. Liu, *JAMA*, **323**, 1582 (2020); https://doi.org/10.1001/jama.2020.4783
92. P. Prabakaran, Z. Zhu, X. Xiao, A. Biragyn, A.S. Dimitrov, C.C. Broder and D.S. Dimitrov, *Expert Opin. Biol. Ther.*, **9**, 355 (2009); https://doi.org/10.1517/14712590902763755
93. E.O. Saphire, S.L. Schendel, B.M. Gunn, J.C. Milligan and G. Alter, *Nat. Immunol.*, **19**, 1169 (2018); https://doi.org/10.1038/s41590-018-0233-9
94. X. Tian, C. Li, A. Huang, S. Xia, S. Lu, Z. Shi, L. Lu, S. Jiang, Z. Yang, Y. Wu and T. Ying, *Emerg. Microbes Infect.*, **9**, 382 (2020); https://doi.org/10.1080/22221751.2020.1729069
95. C. Wang, W. Li, D. Drabek, N.M.A. Okba, R. van Haperen, A.D.M.E. Osterhaus, F.J.M. van Kuppeveld, B.L. Haagmans, F. Grosveld and B.-J. Bosch, *Nat. Commun.*, **11**, 2251 (2020); https://doi.org/10.1038/s41467-020-16256-y
96. M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong and G. Xiao, *Cell Res.*, **30**, 269 (2020); https://doi.org/10.1038/s41422-020-0282-0
97. M.K. Lo, R. Jordan, A. Arvey, J. Sudhamsu, P. Shrivastava-Ranjan, A.L. Hotard, M. Flint, L.K. McMullan, D. Siegel, M.O. Clarke, R.L. Mackman, H.C. Hui, M. Perron, A.S. Ray, T. Cihlar, S.T. Nichol and C.F. Spiropoulou, *Sci. Rep.*, **7**, 43395 (2017); https://doi.org/10.1038/srep43395
98. T.P. Sheahan, A.C. Sims, R.L. Graham, V.D. Menachery, L.E. Gralinski, J.B. Case, S.R. Leist, K. Pyrc, J.Y. Feng, I. Trantcheva, R. Bannister, Y. Park, D. Babusis, M.O. Clarke, R.L. Mackman, J.E. Spahn, C.A. Palmiotti, D. Siegel, A.S. Ray, T. Cihlar, R. Jordan, M.R. Denison and R.S. Baric, *Sci. Transl. Med.*, **9**, eaal3653 (2017); https://doi.org/10.1126/scitranslmed.aal3653
99. T.K. Warren, R. Jordan, M.K. Lo, A.S. Ray, R.L. Mackman, V. Soloveva, D. Siegel, M. Perron, R. Bannister, H.C. Hui, N. Larson, R. Strickley, J. Wells, K.S. Stuthman, S.A. Van Tongeren, N.L. Garza, G. Donnelly, A.C. Shurtleff, C.J. Retterer, D. Gharaibeh, R. Zamani, T. Kenny, B.P. Eaton, E. Grimes, L.S. Welch, L. Gomba, C.L. Wilhelmsen, D.K. Nichols, J.E. Nuss, E.R. Nagle, J.R. Kugelman, G. Palacios, E. Doerffler, S. Neville, E. Carra, M.O. Clarke, L. Zhang, W. Lew, B. Ross, Q. Wang, K. Chun, L. Wolfe, D. Babusis, Y. Park, K.M. Stray, I. Trancheva, J.Y. Feng, O. Barauskas, Y. Xu, P. Wong, M.R. Braun, M. Flint, L.K. McMullan, S.-S. Chen, R. Fearns, S. Swaminathan, D.L. Mayers, C.F. Spiropoulou, W.A. Lee, S.T. Nichol, T. Cihlar and S. Bavari, *Nature*, **531**, 381 (2016); https://doi.org/10.1038/nature17180
100. E. Tchesnokov, J. Feng, D. Porter and M. Götte, *Viruses*, **11**, 326 (2019); https://doi.org/10.3390/v11040326
101. C.J. Gordon, E.P. Tchesnokov, E. Woolner, J.K. Perry, J.Y. Feng, D.P. Porter and M. Götte, *J. Biol. Chem*., **295**, 6785 (2020); https://doi.org/10.1074/jbc.RA120.013679
102. A. Shannon, N.T.-T. Le, B. Selisko, C. Eydoux, K. Alvarez, J.-C. Guillemot, E. Decroly, O. Peersen, F. Ferron and B. Canarda, *Antiviral Res*., **178**, 104793 (2020); https://doi.org/10.1016/j.antiviral.2020.104793
103. E. de Wit, F. Feldmann, J. Cronin, R. Jordan, A. Okumura, T. Thomas, D. Scott, T. Cihlar and H. Feldmann, *Proc. Natl. Acad. Sci. USA*, **117**, 6771 (2020); https://doi.org/10.1073/pnas.1922083117
104. M.L. Holshue, C. DeBolt, S. Lindquist, K.H. Lofy, J. Wiesman, H. Bruce, C. Spitters, K. Ericson, S. Wilkerson, A. Tural, G. Diaz, A. Cohn, L. Fox, A. Patel, S.I. Gerber, L. Kim, S. Tong, X. Lu, S. Lindstrom, M.A. Pallansch, W.C. Weldon, H.M. Biggs, T.M. Uyeki and S.K. Pillai, *N. Engl. J. Med.*, **382**, 929 (2020); https://doi.org/10.1056/NEJMoa2001191
105. A.C.C. Aguiar, E. Murce, W.A. Cortopassi, A.S. Pimentel, M. Almeida, D.C.S. Barros, J.S. Guedes, M.R. Meneghetti and A.U. Krettli, *Int. J. Parasitol. Drugs Drug Resist.*, **8**, 459 (2018); https://doi.org/10.1016/j.ijpddr.2018.10.002
106. M.J. Vincent, E. Bergeron, S. Benjannet, B.R. Erickson, P.E. Rollin, T.G. Ksiazek, N.G. Seidah and S.T. Nichol, *Virol. J.*, **2**, 69 (2005); https://doi.org/10.1186/1743-422X-2-69
107. J. Liu, R. Cao, M. Xu, X. Wang, H. Zhang, H. Hu, Y. Li, Z. Hu, W. Zhong and M. Wang, *Cell Discov.*, **6**, 16 (2020); https://doi.org/10.1038/s41421-020-0156-0
108. E. Schrezenmeier and T. Dörner, *Nat. Rev. Rheumatol.*, **16**, 155 (2020); https://doi.org/10.1038/s41584-020-0372-x
109. E.B. Golden, H.Y. Cho, F.M. Hofman, S.G. Louie, A.H. Schonthal and T.C. Chen, *Neurosurg. Focus*, **38**, E12 (2015); https://doi.org/10.3171/2014.12.FOCUS14748
110. G. Li and E.D. Clercq, *Nat. Rev. Drug Discov.*, **19**, 149 (2020); https://doi.org/10.1038/d41573-020-00016-0
111. H.P. Hsieh and J.T. Hsu, *Curr. Pharm. Des.*, **13**, 3531 (2007); https://doi.org/10.2174/138161207782794248
112. H. Nishimura and M. Yamaya, *Tohoku J. Exp. Med.*, **237**, 45 (2015); https://doi.org/10.1620/tjem.237.45
113. J.F. Rossignol, *Antiviral Res.*, **110**, 94 (2014); https://doi.org/10.1016/j.antiviral.2014.07.014
114. J. Cao, J.C. Forrest and X. Zhang, *Antiviral Res.*, **114**, 1 (2015); https://doi.org/10.1016/j.antiviral.2014.11.010
115. J.F. Rossignol, *J. Infect. Public Health*, **9**, 227 (2016); https://doi.org/10.1016/j.jiph.2016.04.001
116. R.S. Cvetkovic and K.L. Goa, *Drugs*, **63**, 769 (2003); https://doi.org/10.2165/00003495-200363080-00004
117. C.M. Chu, V.C. Cheng, I.F. Hung, M.M. Wong, K.H. Chan, K.S. Chan, R.Y.T. Kao, L.L.M. Poon, C.L.P. Wong, Y. Guan, J.S.M. Peiris and K.Y. Yuen, *Thorax*, **59**, 252 (2004); https://doi.org/10.1136/thorax.2003.012658
118. Y.M. Arabi, A.Y. Asiri, A.M. Assiri, H.A. Aziz Jokhdar, A. Alothman, H.H. Balkhy, S. AlJohani, S. Al Harbi, S. Kojan, M. Al Jeraisy, A.M. Deeb, Z.A. Memish, S. Ghazal, S. Al Faraj, F. Al-Hameed, A. AlSaedi, Y. Mandourah, G.A. Al Mekhlafi, N.M. Sherbeeni, F.E. Elzein, A. Almotairi, A. Al Bshabshe, A. Kharaba, J. Jose, A. Al Harthy, M. Al Sulaiman, A. Mady, R.A. Fowler, F.G. Hayden, A. Al-Dawood, M. Abdelzaher, W. Bajhmom and M.A. Hussein, *Trials*, **21**, 8 (2020); https://doi.org/10.1186/s13063-019-3846-x
119. A. Zumla, J.F. Chan, E.I. Azhar, D.S. Hui and K.Y. Yuen, *Nat. Rev. Drug Discov.*, **15**, 327 (2016); https://doi.org/10.1038/nrd.2015.37
120. M. Tahir ul Qamar, S.M. Alqahtani, M.A. Alamri and L. Che, *J. Pharm. Anal*., **10**, 313 (2020); https://doi.org/10.1016/j.jpha.2020.03.009
121. X. Liu and X.J. Wang, *J. Genet. Genomics*, **47**, 119 (2020); https://doi.org/10.1016/j.jgg.2020.02.001
122. F. Chen, K.H. Chan, Y. Jiang, R.Y.T. Kao, H.T. Lu, K.W. Fan, V.C.C. Cheng, W.H.W. Tsui, I.F.N. Hung and T.S.W. Lee, *J. Clin. Virol.*, **31**, 69 (2004); https://doi.org/10.1016/j.jcv.2004.03.003

123. J. Lim, S. Jeon, H.Y. Shin, M.J. Kim, Y.M. Seong, W.J. Lee, K.-W. Choe, Y.M. Kang, B. Lee and S.-J. Park, *J. Korean Med. Sci.*, **35**, e79 (2020); https://doi.org/10.3346/jkms.2020.35.e79
124. A. Biswas, U. Bhattacharjee, A. K. Chakrabarti, D. N. Tewari, H. Banu, S. Dutta, *Crit. Rev. Microbiol.*, **46**, 182 (2020); https://doi.org/10.1080/1040841X.2020.1739001
125. E. De Clercq, *Chem. Asian J.*, **14**, 3962 (2019); https://doi.org/10.1002/asia.201900841
126. J.B. McCormick, I.J. King, P.A. Webb, C.L. Scribner, R.B. Craven, K.M. Johnson, L.H. Elliott and R. Belmont-Williams, *N. Engl. J. Med.*, **314**, 20 (1986); https://doi.org/10.1056/NEJM198601023140104
127. M.S. Bronze and R.A. Greenfield, *Curr. Opin. Investig. Drugs*, **4**, 172 (2003).
128. V.M. Corman, N.L. Ithete, L.R. Richards, M.C. Schoeman, W. Preiser, C. Drosten and J.F. Drexler, *J. Virol.*, **88**, 11297 (2014); https://doi.org/10.1128/JVI.01498-14
129. C.L. Yeager, R.A. Ashmun, R.K. Williams, C.B. Cardellichio, L.H. Shapiro, A.T. Look and K.V. Holmes, *Nature*, **357**, 420 (1992); https://doi.org/10.1038/357420a0
130. S. Gierer, S. Bertram, F. Kaup, F. Wrensch, A. Heurich, A. Kramer-Kuhl, K. Welsch, M. Winkler, B. Meyer, C. Drosten, U. Dittmer, T. von Hahn, G. Simmons, H. Hofmann and S. Pohlmann, *J. Virol.*, **87**, 5502 (2013); https://doi.org/10.1128/JVI.00128-13
131. Q. Cai, M. Yang, D. Liu, J. Chen, D. Shu, J. Xia, X. Liao, Y. Gu, Q. Cai, Y. Yang, C. Shen, X. Li, L. Peng, D. Huang, J. Zhang, S. Zhang, F. Wang, J. Liu, L. Chen, S. Chen, Z. Wang, Z. Zhang, R. Cao, W. Zhong, Y. Liu and L. Liu, *Engineering*, (2020); https://doi.org/10.1016/j.eng.2020.03.007
132. Y.S. Boriskin, I.A. Leneva, E.I. Pecheur and S.J. Polyak, *Curr. Med. Chem.*, **15**, 997 (2008); https://doi.org/10.2174/092986708784049658
133. J. Blaising, S.J. Polyak and E.I. Pecheur, *Antiviral Res.*, **107**, 84 (2014); https://doi.org/10.1016/j.antiviral.2014.04.006
134. X. Wang, R. Cao, H. Zhang, J. Liu, M. Xu, H. Hu, Y. Li, L. Zhao, W. Li, X. Sun, X. Yang, Z. Shi, F. Deng, Z. Hu, W. Zhong and M. Wang, *Cell Discov.*, **6**, 28 (2020); https://doi.org/10.1038/s41421-020-0169-8
135. Z. Wang, X. Chen, Y. Lu, F. Chen and W. Zhang, *Biosci. Trends*, **14**, 64 (2020); https://doi.org/10.5582/bst.2020.01030
136. M. Hoffmann, H. Kleine-Weber, N. Krüger, M. Müller, C. Drosten and S. Pöhlmann, bioRxiv 2020.01.31.929042 (2020); https://doi.org/10.1101/2020.01.31.929042
137. I. Glowacka, S. Bertram, M.A. Muller, P. Allen, E. Soilleux, S. Pfefferle, I. Steffen, T.S. Tsegaye, Y. He, K. Gnirss, D. Niemeyer, H. Schneider, C. Drosten and S. Pöhlmann, *J. Virol.*, **85**, 4122 (2011); https://doi.org/10.1128/JVI.02232-10
138. N. Iwata-Yoshikawa, T. Okamura, Y. Shimizu, H. Hasegawa, M. Takeda and N. Nagata, *J. Virol.*, **93**, e01815-18 (2019); https://doi.org/10.1128/JVI.01815-18
139. M. Kawase, K. Shirato, L. van der Hoek, F. Taguchi and S. Matsuyama, *J. Virol.*, **86**, 6537 (2012); https://doi.org/10.1128/JVI.00094-12
140. Y. Zhou, P. Vedantham, K. Lu, J. Agudelo, R. Carrion Jr., J.W. Nunneley, D. Barnard, S. Pöhlmann, J.H. McKerrow, A.R. Renslo and G. Simmons, *Antiviral Res.*, **116**, 76 (2015); https://doi.org/10.1016/j.antiviral.2015.01.011
141. C.M. Coleman, J.M. Sisk, R.M. Mingo, E.A. Nelson, J.M. White and M.B. Frieman, *J. Virol.*, **90**, 8924 (2016); https://doi.org/10.1128/JVI.01429-16
142. X. Xu, M. Han, T. Li, W. Sun, D. Wang, B. Fu, Y. Zhou, X. Zheng, Y. Yang, X. Li, X. Zhang, A. Pan and H. Wei, *Proc. Natl. Acad. Sci. USA*, **117**, 10970 (2020); https://doi.org/10.1073/pnas.2005615117
143. M.H. Lin, D.C. Moses, C.H. Hsieh, S.-C. Cheng, Y.-H. Chen, C.-Y. Sun and C.-Y. Chou, *Antiviral Res.*, **150**, 155 (2018); https://doi.org/10.1016/j.antiviral.2017.12.015
144. A.H. De Wilde, D. Jochmans, C.C. Posthuma, J.C. Zevenhoven-Dobbe, S. van Nieuwkoop, T.M. Bestebroer, B.G. van den Hoogen, J. Neyts and E.J. Snijder, *Antimicrob. Agents Chemother.*, **58**, 4875 (2014); https://doi.org/10.1128/AAC.03011-14
145. T. Pillaiyar, S. Meenakshisundaram and M. Manickam, *Drug Discov. Today*, **25**, 668 (2020); https://doi.org/10.1016/j.drudis.2020.01.015
146. S. Jeon, M. Ko, J. Lee, I. Choi, S. Young Byun, S. Park, D. Shum and S. Kim, *Antimicrob. Agents Chemother.*, **67**, e00189 (2020); https://doi.org/10.1128/AAC.00819-20
147. T. Matthews and R. Boehme, *Rev. Infect. Dis.*, **10(Suppl 3)**, S490 (1988); https://doi.org/10.1093/clinids/10.Supplement_3.S490
148. B. McQuade and M. Blair, *Am. J. Health.*, **72**, 112 (2015); https://doi.org/10.2146/ajhp140390
149. Biggest COVID-19 Trial Tests Repurposed Drugs First, *Nat. Biotechnol.*, **38**, 510 (2020); https://doi.org/10.1038/s41587-020-0528-x
150. E. Wilkinson, *BMJ*, **369**, m1626 (2020); https://doi.org/10.1136/bmj.m1626
151. E. Mahase, *BMJ*, **368**, m1252 (2020); https://doi.org/10.1136/bmj.m1252
152. J. Villar, C. Ferrando, D. Martínez, A. Ambrós, T. Muñoz, J.A. Soler, G. Aguilar, F. Alba, E. González-Higueras, L.A. Conesa, C. Martín-Rodríguez, F.J. Díaz-Domínguez, P. Serna-Grande, R. Rivas, J. Ferreres, J. Belda, L. Capilla, A. Tallet, J.M. Añón, R.L. Fernández, J.M. González-Martín, G. Aguilar, F. Alba, J. Álvarez, A. Ambrós, J.M. Añón, M.J. Asensio, J. Belda, J. Blanco, M. Blasco, L. Cachafeiro, R. del Campo, L. Capilla, J.A. Carbonell, N. Carbonell, A. Cariñena, D. Carriedo, M. Chico, L.A. Conesa, R. Corpas, J. Cuervo, F.J. Díaz-Domínguez, C. Domínguez-Antelo, L. Fernández, R.L. Fernández, C. Ferrando, J. Ferreres, E. Gamboa, E. González-Higueras, R.I. González-Luengo, J.M. González-Martín, D. Martínez, C. Martín-Rodríguez, T. Muñoz, R. Ortiz Díaz-Miguel, R. Pérez-González, A.M. Prieto, I. Prieto, R. Rivas, L. Rojas-Viguera, M.A. Romera, J. Sánchez-Ballesteros, J.M. Segura, P. Serna-Grande, A. Serrano, R. Solano, J.A. Soler, M. Soro, A. Tallet and J. Villar, *Lancet Respir. Med.*, **8**, 267 (2020); https://doi.org/10.1016/S2213-2600(19)30417-5