

Confronting the threat of SARS-CoV-2: Realities, challenges and therapeutic strategies (Review)

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Abstract. The novel coronavirus (SARS-CoV-2) appeared in 2019 in Wuhan, China, and rapidly developed into a global pandemic. The disease has affected not only health care systems and economies worldwide but has also changed the lifestyles and habits of the majority of the world's population. Among the potential targets for SARS-CoV-2 therapy, the viral spike glycoprotein has been studied most intensely, due to its key role in mediating viral entry into target cells and inducing a protective antibody response in infected individuals. In the present manuscript the molecular mechanisms that are responsible for SARS-CoV-2 infection are described and a progress report on the status of SARS-CoV-2 research is provided. A brief review of the clinical symptoms of the condition and current diagnostic methods and treatment plans for SARS-CoV-2 are also presented and the progress of preclinical research into medical intervention against SARS-CoV-2 infection are discussed.

Contents

1. Introduction
2. Virology
3. Epidemiology
4. Mechanism of SARS-CoV-2 infection
5. Clinical manifestations and diagnosis
6. Treatments
7. Vaccines
8. Conclusions and outlook

1. Introduction

Following the emergence of severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, a novel coronavirus pneumonia epidemic, named COVID-19 by the World Health Organization (WHO) (1), resulted from SARS-CoV-2 infection in Wuhan, China, and was identified in late December 2019 (2). Subsequently, this disease spread rapidly to all parts of the world. As the number of patients with SARS-CoV-2 infection increased globally, the WHO declared COVID-19 a pandemic on 12th March 2020 (3). To date >37 million people have contracted this disease and as of 10th October 2020, the death toll stands at >1,070,355 (4).

SARS-CoV-2 is a member of the nested viroid family *coronoviridae* and the coronavirus genus. It is the 7th coronavirus known to infect humans and the third coronavirus known to be transmitted from animal to animal, animal to human and human to human (5,6). COVID-19 has been suggested to be a disease of the nicotinic cholinergic system (7) and SARS-CoV-2 most commonly causes a lower respiratory infection or pneumonia (8). High levels of mortality are noticed in elderly individuals infected with SARS-CoV-2, with the risk of death in individuals aged <65 years 15 to 100 fold lower compared with that in older individuals in developed countries, including Germany, Canada, France, Italy and the USA (9). Oxidative stress and inflammatory cytokine production in elderly individuals cause a chronic low level of inflammation and increase the severity of viral infections (10). In view of the adverse consequences of the current COVID-19 epidemic, it is necessary to develop effective treatment strategies to deal with the lack of effective drugs, high mortality

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Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; IL-6, interleukin-6; LH, Lianhua qingwen; MERS-COV, Middle East respiratory syndrome coronavirus; RBD, receptor-binding domain; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2

Key words: coronavirus disease 2019, COVID-19, severe acute respiratory syndrome coronavirus 2

and the possibility of further epidemics caused by the virus. The present manuscript describes the situation, clinical characteristics and current detection and treatment methods for SARS-CoV-2 infection and discusses the developing strategies for the treatment of COVID-19.

2. Virology

The first strain of SARS-CoV-2 was isolated from Wuhan, China on 24th January 2020 after the outbreak of COVID-19 (11). In similarity to other coronaviruses, SARS-CoV-2 consists of four structural proteins, namely the spike (S), envelope, membrane/matrix (M) and nucleocapsid proteins (12). Non-structural proteins are produced after RNA genome expression in the host cell during formation of new virus particles (12). On the surface of mature coronavirus, the S protein usually forms a crown-like trimer, an important morphological feature which differentiates coronaviruses from other viruses (11). Similarly, SARS-CoV-2 viruses isolated in South Korea have also been identified by typical corona-like structure formed by the S protein (13).

Based on phylogenetic analysis [Global Initiative on Sharing All Influenza Data (GISAID) accession no. EPI_ISL_402124] (14), SARS-CoV-2 is a lineage B betacoronavirus and shares high sequence identity with SARS-CoV and the bat SARS-like coronavirus (SL-CoV) (15). Like MERS-CoV and SARS-CoV, SARS-CoV-2 is a positive-sense single-stranded RNA virus and shows a similar pattern of infection (16). Using full-length genome sequencing, SARS-CoV-2 and a bat coronavirus BatCoV RaTG13 were found to be 96% identical (14), suggesting an origin of the SARS-CoV-2 infection in bats. Comprehensive sequence analysis with relative synonymous codon usage bias demonstrated that SARS-CoV-2 may be derived from recombination between a bat coronavirus and another coronavirus from snakes (17). Additionally, mink have been suggested as a potential host of SARS-CoV-2 (18). Interspecies transmissions of viruses between animals and humans may result in unpredictable pathogenic potential and transmissible COVID-19 disease (19).

3. Epidemiology

A cluster of patients with atypical pneumonia was reported in Wuhan, China on December 31, 2019 (20). During the subsequent 6 weeks, several cases were reported in more than 37 countries, including the USA, Japan, Iran and South Korea (1). The infection rapidly spread across the globe, threatening global public health. Chinese authorities locked-down Wuhan city and suspended transport to and from Wuhan to control the spread (21). Imposing mobility restrictions as fast as possible is thought to be an effective way to avoid an outbreak (22). Numerous patients were diagnosed and treated, and the epidemic situation in China has been gradually controlled (23). Various countries have instituted measures, such as practicing good hygiene (washing hands), wearing a mask and quarantine (24). Meanwhile, understanding of the epidemiological characteristics of COVID-19 have developed and immunotoxicity of chemicals and drugs and immunodeficiency caused by the environment and lifestyle are thought to contribute to COVID-19 (24). Nevertheless, uncertainties remain regarding both the virus-host interaction and the evolution of the epidemic, leaving the epidemic situation worldwide severe.

4. Mechanism of SARS-CoV-2 infection

The SARS-Cov-2 virus binds to the angiotensin-converting enzyme 2 (ACE2) on human cells via the receptor binding domain (RBD) located on the S1 subunit of the S protein-homotrimer of the S protein (25). This leads to clathrin-mediated endocytosis (26,27), release of viral RNA and induction of viral replication. The viral genome codes for non-structural proteins containing polypeptides, nucleoproteins, RNA polymerase, 3-chymotrypsin-like protease, papain-like protease and helicase in the host cell in order to form new virus particles (12). The newly manufactured virus emerges via exocytosis where it binds again to ACE2, thus entering a vicious cycle of infecting other cells (12).

As a glycoprotein, the S protein is generally composed of 1,160-1,400 amino acid residues and contains multiple N-glycosylation sites, which are important for proper folding and modulating accessibility to host proteases. The S protein exists in a metastable prefusion conformation, where the S1 and S2 subunits remain noncovalently bound in the prefusion conformation (28). The S1 subunit, comprising the RBD on the surface, is responsible for recognizing and binding to the receptor of the host cell. The S2 subunit, embedded in the envelope, mediates membrane fusion during viral assembly (29). It has been proposed that the S protein is activated for membrane fusion via extensive irreversible conformational changes (30,31). When the S1 subunit binds to a susceptible cell receptor, S1/S2 cleavage is triggered, which enables the S1 RBD to undergo hinge-like conformational movements and the S2 subunit transits to a highly stable post fusion conformation. This S1/S2 cleaved site harbors several arginine residues, rendering it multibasic (32). SARS-CoV-2 S harbors a furin cleavage site (33), a feature conserved among the 144 SARS-CoV-2 isolates sequenced to date, but not found in the closely related bat virus RaTG13 S (14), at the S1/S2 boundary, which is processed during biosynthesis. It can be hypothesized that the almost ubiquitous expression of furin-like proteases participates in expanding SARS-CoV-2 cell and tissue tropism, relative to SARS-CoV, and increases viral transmissibility and/or alters its pathogenicity.

Proteases are necessary for host cell entry and promoting virus-cell fusion (32,34). When SARS-CoV-2 targets host cells (13), the serine protease transmembrane protease serine 2 (TMPRSS2) is employed for S protein priming (Fig. 1) (12,32,35). Notably, all ACE2-expressing pulmonary cells are also TMPRSS2-positive (35).

Similarly to SARS-CoV infection, the S protein of SARS-CoV-2 binds to the human ACE2 receptor, which partially explains the efficient transmission of SARS-CoV-2 in humans (34). The binding ability of SARS-CoV-2 S protein to ACE2 is stronger than that of SARS-CoV (36), which may explain why SARS-CoV-2 is more transmissible than SARS-CoV. The expression level and tissue distribution of ACE2 determine the cell tropism and pathogenicity of SARS-CoV-2 (37). Coronavirus infection and the induced cytokine storm are able to enhance the expression of ACE2 in host cells, further accelerating infection and transmission of the virus (38). The expression of ACE2 in human lung cells was analyzed using single cell RNA sequencing analysis technology and the results indicated that the expression of ACE2

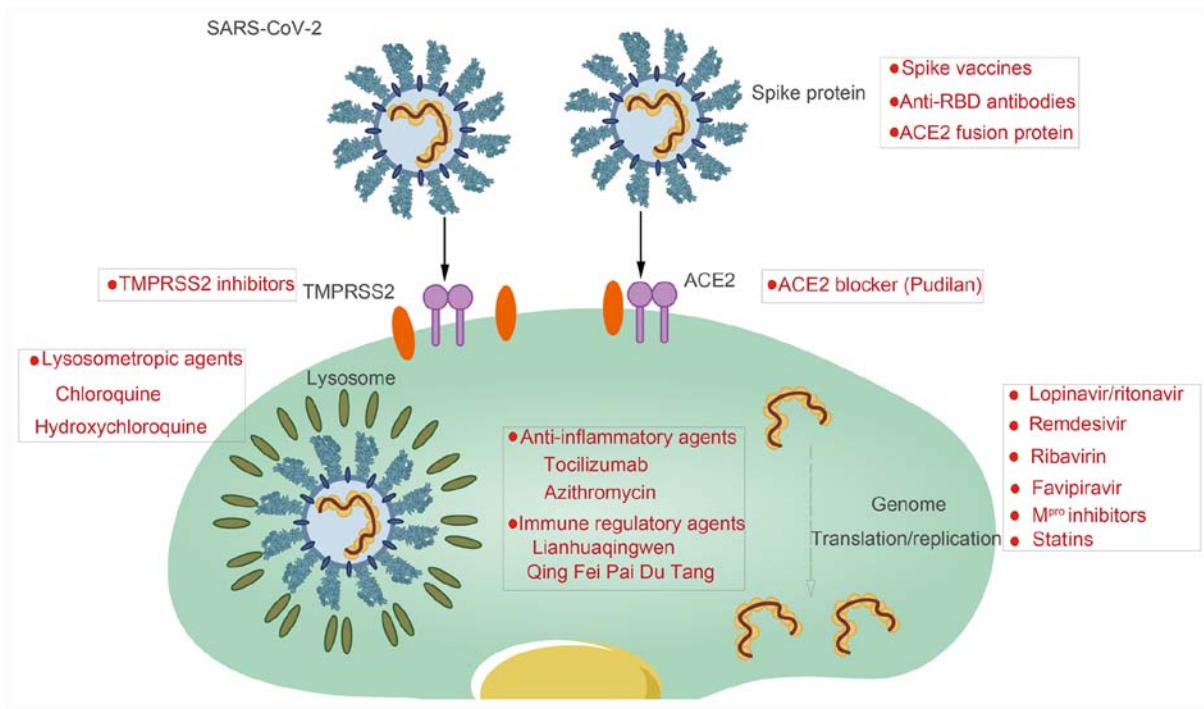


Figure 1. Mechanism by which SARS-CoV-2 infects host cells. The S protein on the surface of the SARS-CoV-2, a newly emerged pathogen spreading world-wide, binds with high affinity to the human ACE2 receptor to gain entry into target cells, including those of lung and gastrointestinal tissues. SARS-CoV-2 employs the serine protease TMPRSS2 for S protein priming. The pharmaceutical agents which disrupt the COVID-19 infection of host cells are exhibited in red based on the mechanism and the specific site of action. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2; S, spike; COVID-19, coronavirus disease 2019; RBD, receptor binding domain.

was concentrated in a small group of type II alveolar epithelial cells in the lung (37), suggesting that these cells may be the target of SARS-CoV-2. Recent studies have also indicated that ACE2 is highly expressed not only in lung cells, esophageal epithelium and stratified epithelial cells, but also in the absorbing intestinal epithelial cells of the ileum and colon (39), suggesting that the digestive system is also a potential pathway for SARS-CoV-2 infection.

5. Clinical manifestations and diagnosis

Clinical manifestations. The most frequent clinical feature in patients infected with SARS-CoV-2 appears to be pneumonia. Early COVID-19 is characterized primarily by the symptoms of fever, myalgia, cough and sore throat, all of which are common in other acute respiratory virus infections (8). Most cases appear to be mild, and most hospitalized patients have pneumonia with bilateral infiltration upon chest imaging (8,40). Notably, the period from infection to the appearance of symptoms varies in patients with SARS-CoV-2 infection, and there are also great differences in symptoms among individuals (41). There are patients with COVID-19 with no detectable fever or other clinical symptoms (5).

SARS-CoV-2 is believed to be transmitted through large respiratory droplets and close contact. Positive reverse transcription (RT-PCR) results from stool specimens from patients with COVID-19 suggested that stool or sewage might serve as another vehicle for viral transmission (42).

PCR detection. Currently, the preferred method for detection of COVID-19 is via molecular testing. Multiple specimen

types are recommended due to the unknown sensitivity and specificity of tests. The PCR detection method was designed to detect a variety of targets in SARS-CoV-2 genome and is the most widely used molecular diagnostic method to analyze samples such as sputum, nasal swab, pharyngeal swab, bronchoalveolar lavage and blood plasma (43,44). A significant portion of suspected patients who have tested negative for viral RNA indeed fit the diagnosis based on clinical and chest CT findings (45). PCR detection involves RNA extraction and preservation, and the degradation and contamination of RNA samples could result in missed detection or false positive results (46). Moreover, the time, manpower and economic (advanced PCR equipment and expensive reagents) costs are such that it is difficult to meet the huge testing demand in the rapid outbreak of COVID-19 (45).

Serological tests. Serological testing is method that is considered, at the present time, as complementary to nucleic acid detection (47). IgM testing has been designed and validated, but currently limited information is available about the performance of these tests (48). Some tests may produce inaccurate results (49), suggesting gold immunochromatography assay and ELISA methods should be used to eliminate or reduce the impact of cross-reaction. Serological testing is helpful for preliminary screening of suspected and high-risk groups (50).

Previously, numerous methods for rapid detection and diagnosis of coronavirus infection based on the S protein have been developed. Thachil *et al* (51) established an indirect ELISA with the S1 subunit of the S protein of porcine delta coronavirus as the coating antigen, with a sensitivity of 91% and specificity of 95% that was able to detect the specific IgG

specific antibody against porcine delta coronavirus in serum samples. Zhao *et al* (52) established a set of ELISA detection methods for antibodies against the S1 subunit of horse coronavirus, which effectively diagnoses infection. Moreover, Sunwoo *et al* (53) developed a bispecific monoclonal antibody against the S1 antigen on the surface of SARS-CoV, which can be used in clinical diagnosis of suspected SARS patients. Since the S protein is the most important antigenic determinant for coronavirus (54), ELISA methods based on the S protein antigen-antibody reaction could be effective. Additionally, ELISA detection is much faster and easier in comparison with PCR detection (46). Therefore, developing an effective ELISA kit for SARS-CoV-2 detection would be helpful in the current epidemic.

6. Treatment

Overview. Numerous compounds have been proven effective against SARS-CoV and MERS-CoV which have not been tested widely for the newly emerged SARS-CoV-2. At present, drugs that inhibit the process of virus replication, assembly and fusion with host cells are under research.

Lopinavir/ritonavir. Lopinavir/ritonavir, protease inhibitors that have been widely used for the treatment of human immunodeficiency virus-1 infection (55), are usually used in combination with azithromycin to increase the half-life of lopinavir by inhibiting cytochrome P450. A previous study suggested that patients with SARS treated with a combination of lopinavir/ritonavir and ribavirin had lower risk of developing acute respiratory distress syndrome or death (56). The combination of lopinavir/ritonavir and interferon- β improved outcomes in MERS-CoV infection (57). However, the lopinavir/ritonavir combination provided little benefit in improving the clinical outcomes in patients with mild and moderate COVID-19 (58). There were no benefits of lopinavir/ritonavir beyond the standard treatment in a trial performed on patients with severe COVID-19 in China, but a slightly lower number of deaths was observed in the group receiving lopinavir/ritonavir in the late stage of SARS-CoV-2 infection compared with the standard-treatment group (59).

Remdesivir. As a new nucleoside analogue (60,61), remdesivir has a broad-spectrum antiviral capacity against filoviruses, paramyxoviruses, pneumoviruses, and human and bat derived coronaviruses (62-65), which makes it a promising agent for COVID-19 treatment. Against the Ebola virus, remdesivir has completed the phase I clinical trial, and the pharmacokinetics and safety in the human body have relatively complete data (66). A study reported that the replication of virus in human primary cell was significantly inhibited (67,68), because the triphosphate cannot be removed by non-structural protein 14 N-terminal exoribonuclease (69). Remdesivir effectively reduced the virus titer in the lungs of rhesus macaques infected with MERS-CoV and it improved the degree of lung tissue damage in comparison with that of the control group (70). New England Journal of Medicine recently published a case of SARS-CoV-2 infection in the United States treated with remdesivir (71). A patient infected with SARS-CoV-2 was administered remdesivir and the clinical

status improved within 24 h without any noticeable adverse effect (71). It is worth noting that uncertainties about adverse effects and clinical efficacy of remdesivir have been reported recently, such as nausea, vomiting, rectal hemorrhaging and hepatic toxicity (72).

Favipiravir. Favipiravir, which selectively and potently inhibits RNA-dependent RNA polymerase (73-75), has been administered to patients infected with Ebola virus (76) and has been approved in Japan for influenza treatment and in China for the treatment of COVID-19 (77). Preliminary studies on 80 patients with COVID-19 in China have demonstrated that favipiravir exerts an antiviral action more potent than lopinavir/ritonavir, and no serious adverse reactions have been reported (77).

Chloroquine and hydroxychloroquine. The antimalarial drugs chloroquine and hydroxychloroquine are used in Korea and China for the treatment of COVID-19 (78,79). Chloroquine presented an encouraging anti-SARS-CoV-2 profile in early clinical trials (68,80). Additionally, the chloroquine hydroxyl derivative hydroxychloroquine (available as an antirheumatic drug under the name 'Plaquenil') demonstrated a stronger *in vitro* anti-COVID-19 effect than chloroquine (81,82). A study found no evidence of clinical benefit of the combination of hydroxychloroquine and azithromycin for the treatment of 11 patients with severe COVID-19 (83). However, patients with COVID-19 in France treated with hydroxychloroquine (600 mg per day) were significantly improved, even in combination with azithromycin (84). In the wake of this evidence, hydroxychloroquine was included in guidelines for COVID-19 therapy in Belgium (85) and Italy (86). Recently, chloroquine was found not to inhibit human lung cancer cells infection with SARS-CoV-2 although this study used human lung epithelial cells, not the renal cell line selected in previous experiments with TMPRSS2 protease (87). However, chloroquine/hydroxychloroquine has an antiviral effect because there is another enzyme, cathepsin L (catl), in renal cells that can process the S protein of the novel coronavirus, and its function is affected by cell pH (87). Chloroquine/hydroxychloroquine limits the function of catl by regulating pH, thus indirectly inhibiting the invasion of SARS-CoV-2 (87). Moreover, a study from French scientists indicated that hydroxychloroquine cannot reduce viral load nor improve clinical symptoms, prevent or treat COVID-19, regardless of the dosage and timing (88). Thus, the application of chloroquine/hydroxychloroquine requires further study.

Tocilizumab. When COVID-19 progresses from severe to critical, patients may develop a cytokine storm immune reaction. Consequently, the treatment of the cytokine storm is an important part of rescuing severe patients (89). Since interleukin-6 (IL-6) is one of the key cytokines involved in infection-induced cytokine storm, an IL-6 receptor (IL-6R) antagonist would be a promising drug for patients with COVID-19. Tocilizumab, a monoclonal antibody with activity against the IL-6R was developed for the treatment of rheumatoid arthritis (90) and has been approved by the US FDA for the treatment of cytokine release syndrome (91). For COVID-19, it has been tested on 6 patients in Italy who experienced rapid improvement in their health only 24-48 h after administration (92). As

tocilizumab blocks the IL-6-mediated immune response in COVID-19, it was approved to treat pneumonia and the severe cytokine release syndrome induced by the immune system in patients with coronavirus as an 'off label' usage in China (93).

Dexamethasone. Dexamethasone, a synthetic glucocorticoid with anti-inflammatory and immunosuppressive properties, inhibits growth of myeloma and lymphoma cells (94). Upon binding to the glucocorticoid receptor, a ligand activated transcription factor, dexamethasone regulates the expression of a diverse sets of genes, resulting in resolution of inflammation. Dexamethasone inhibits the activity of inflammatory cells, including neutrophils, macrophages and lymphocytes, and suppresses pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukins, and other genes such as cyclooxygenase-2 and inducible nitric oxide synthase (95).

A randomized, controlled clinical trial in the United Kingdom found that dexamethasone reduced deaths by about one-third in patients with COVID-19 who were on ventilators (96). Administered at a low-to-moderate dose of 6 mg dexamethasone per day for 10 days improved the clinical outcomes in patients on ventilators (96). Those who were receiving oxygen therapy but were not on ventilators also saw improvement, but no effect on patients who were not receiving oxygen therapy or ventilation was observed. Some reports suggested that well-timed, higher doses of 0.5-1.0 mg/kg methylprednisolone per day improved outcomes in those patients with respiratory failure, severe illness, and cytokine storm (97,98). Notably, steroidal drugs can be associated with numerous adverse effects, including diabetes/hyperglycemia, osteopenia, cataracts, avascular necrosis, fluid retention, hypertension and infection (94,99). A meta-analysis of 15 studies with 5,270 patients with SARS-CoV-2 indicated that corticosteroids were associated with higher rates of bacterial infection, longer hospital stay and higher mortality (100). These reports suggest that high-dose steroidal agents may be beneficial in later stages of severe SARS-CoV-2 infection and/or impending cytokine storm (94,99). Therefore, patients with non-severe, non-cytokine storm SARS-CoV-2 infection are not recommended for dexamethasone treatment, and the possible side effects of dexamethasone drug used must be considered.

Traditional Chinese medicine (TCM). Lianhua Qingwen (LH), is produced from a mixture of herbs, including *Forsythia suspensa*, *Lonicera japonica* Thunberg, *Ephedra sinica*, *Prunus armeniaca*, *Isatis indigotica*, *Dryopteridis crassirhizoma*, *Houttuynia cordata*, *Pogostemon cablin*, *Rhodiola rosea* and *Glycyrrhiza inflanta*, along with menthol and a traditional Chinese mineral medicine containing calcium sulfate, Gypsum Fibrosum. LH is used to treat influenza and chronic obstructive pulmonary disease (101). Patients with influenza complicated by bronchial pneumonia that received LH capsules displayed greater symptom improvement than those in a control group (101). A prospective multicenter open-label, randomized, controlled trial of LH in 284 patients with COVID-19 (142 each in the treatment and control groups) demonstrated that the recovery rate was significantly higher in the treatment group who received the usual antiviral or antibiotics treatments alone based on the protocol for diagnosis and treatment of novel coronavirus pneumonia

(4th edition) or in combination with LH (4 capsules, three times per day) for 14 days, compared with that in the control group (102). Additionally, LH treatment shortened the time to symptom recovery, and improved the recovery of chest radiologic abnormalities with no adverse reactions observed, in comparison with that in the control group (102). Though there were no differences in the rate of conversion to severe cases or viral assay findings, LH capsules may be beneficial in cases of COVID-19, although this requires more randomized, controlled trials in a larger patient population. LH combined with ribavirin, a guanosine nucleoside analog, has been used in the treatment of viral upper respiratory tract infection (103).

Qing Fei Pai Du Tang (QFPDT), a TCM containing 21 herbs, including *Ephedra sinica*, *Bupleurum chinense*, *Pogostemon cablin*, *Cinnamomum cassia* and *Scutellaria baicalensis*, is thought to act in the lung (104,105). By regulating a series of proteins co-expressed with ACE2 and signaling pathways closely related to the occurrence and development of diseases, it may play a role in reducing inflammation (104). QFPDT may act as an antiviral agent by targeting ribosomal proteins that are necessary for viral replication, thus inhibiting viral mRNA translation and inhibiting a group of proteins that interact with viral proteins (103). When QFPDT was administered to 214 patients with COVID-19 in China, the majority of patients (60%) displayed improved symptoms, where the illness of the 30% patients were stabilized (106). An additional 701 patients with COVID-19 were treated with QFPDT and, of these, 130 patients (18.5%) were symptom free after treatment, 51 patients (7.27%) recovered from their fever and cough, symptoms were improved in 268 patients (38.2%) and stabilized in 212 patients (30.2%) (106). Administration of QFPDT along with Western medical therapy (the antiviral medicines interferon, lopinavir or arbidol) revealed a tendency to mitigate the extent of multiorgan impairment in 63 patients with confirmed COVID-19 (107), providing evidence that QFPDT combined with antiviral drugs for the treatment of COVID-19 may be beneficial.

Statins. Statins, used conventionally for lowering cholesterol and for their anti-thrombotic properties, also have anti-viral activity (108,109). Statins block the infectivity of enveloped viruses through inhibition of glycoprotein processing. Reiner *et al* (110) performed docking studies, which revealed that statins interact directly with the main protease enzyme of SARS-CoV-2. Among them, promising statins, including pitavastatin, rosuvastatin, lovastatin and fluvastatin, might be useful in COVID-19 treatment (110). A recent retrospective study involving 13,981 patients with COVID-19 indicated a lower risk for 28-day all-cause mortality in the matched statin group of 1,219 patients, suggesting the safety of statins or the combination of a statin with an angiotensin-converting enzyme (ACE) inhibitor/angiotensin receptor blocker for treatment of patients with COVID-19 (111). As statins may induce the expression of ACE2, resulting in an increased risk of SARS-CoV-2 viral entrance (111), further research is urgently needed to validate the utility of statins to combat the mortality of COVID-19.

Viral main protease (M^{pro}) inhibitors. M^{pro} , a key coronavirus enzyme playing an important role in proteolytic

maturation (110), has been investigated as a potential protein target to prevent infection expansion (112).

Carmofur (1-hexylcarbamoyl-5-fluorouracil), an antineoplastic agent used to treat colorectal cancer, breast, gastric and bladder cancers, is shown to inhibit the SARS-CoV-2 M^{pro}. Carmofur inhibits viral replication in SARS-CoV-2 infected Vero E6 cells and shows promise for its successful use as a new antiviral treatment for COVID-19 (113).

Jin *et al* (114) identified 7 compounds (N3, ebselen, disulfiram, tideglusib, carmofur, shikonin and PX-12) from >10,000 compounds, including approved drugs, drug candidates in clinical trials and other pharmacologically active compounds, that could inhibit M^{pro}. Following further study, ebselen and N3 demonstrated the strongest antiviral effects against SARS-CoV-2. Among these, N3 is a mechanism-based inhibitor developed using computer-aided drug design, which specifically inhibits M^{pro} from multiple coronaviruses, including SARS-CoV and MERS-CoV (114). Ebselen is an organoselenium compound with anti-inflammatory, anti-oxidant and cytoprotective properties and has previously been investigated for the treatment of multiple diseases, including bipolar disorder and has a low cytotoxicity (114).

Gupta *et al* (115) reported that, by using a combination of molecular docking, scoring functions and molecular dynamics simulations, C1 (1E,6E)-1,2,6,7-tetrahydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione and C2 (4Z,6E)-1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one, identified from among 267 compounds in *Curcuma longa* L. (*Zingiberaceae* family), bound strongly to the catalytic core of the M^{pro} protein with higher efficacy than lopinavir, a standard M^{pro} inhibitor.

RBD-targeting antibodies. As the S protein plays the most important role in viral attachment, fusion and entry, much of the development of monoclonal antibodies, entry inhibitors and vaccines are focused on the S protein (28). Specifically, the 193 amino acid length (N318-V510) receptor binding domain (RBD) within the S protein is a critical target for neutralizing antibodies (116). 206 RBD-specific monoclonal antibodies derived from single B cells of eight SARS-CoV-2 infected individuals displayed potent anti-SARS-CoV-2 neutralization activity but did not cross-react with SARS-CoV or MERS-CoV RBDs (117), suggesting that anti-RBD antibodies are viral species-specific inhibitors. According to a study by Robbiani *et al* (118), most convalescent plasma collected from individuals who recover from COVID-19 does not contain high levels of neutralizing activity but has anti-SARS-CoV-2 RBD antibodies, suggesting that humans are intrinsically capable of generating potent anti-RBD antibodies that neutralize SARS-CoV-2. In addition, 2 specific human monoclonal antibodies, named CA1 and CB6, from a convalescent COVID-19 patient demonstrated potent *in vitro* SARS-CoV-2-specific neutralization activity against SARS-CoV-2 (119).

ACE2-targeting agents. ACE2, identified as an important drug target for the treatment of cardiovascular and kidney diseases, is a key functional receptor for coronavirus infection (120). A fusion protein containing an ACE2 mutant with low catalytic activity, which has a high binding affinity for the

receptor-binding domains of SARS-CoV and SARS-CoV-2, has broad neutralizing activity against SARS-CoV and SARS-CoV-2 *in vitro* and exhibits desirable pharmacological properties in mice (121), suggesting that an ACE2 fusion protein have potential applications in the development of vaccines for SARS-CoV-2 treatment.

Pudilan (PDL), a TCM including *Isatis indigotica*, *Corydalis bungeana*, *Taraxacum mongolicum* and *Scutellaria baicalensis* has been used as an anti-SARS-CoV-2 agent in China (121). By conducting network pharmacology analysis, PDL might also have therapeutic potential for COVID-19, it may prevent SARS-CoV-2 entry into cells by blocking the ACE2 receptor and by regulating cytokines and chemokines to moderate the immune response (121). However, the targets predicted by bioinformatics and network pharmacology tools require further investigation to confirm.

TMPRSS2 inhibitors. TMPRSS2 facilitates viral particle entry into host cells and its inhibition blocks viral fusion with ACE2 (32). Withanone (Wi-N), a natural compound derived from *Withania somnifera* and used in Indian Ayurvedic medicine, can bind and stably interact at the catalytic site of TMPRSS2 (122). Having strong interactions with TMPRSS2 catalytic residues, Wi-N may decrease the endogenous expression of TMPRSS2 (122), suggesting that Wi-N probably confers therapeutic effects against COVID-19 by blocking the entry of SARS-CoV-2 into host cells.

TMPRSS2 targets were screened from the natural compounds library Natural Product Activity and Species Source, a freely accessible database containing 30,927 compounds, using a ligand-based pharmacophore approach and a molecular docking-based screen in the Molecular Operating Environment software (123). The 12 compounds with the most favorable structural features were studied for physicochemical and absorption, distribution, metabolism, excretion and toxicity properties. The results suggested that the compound NPC306344, with a low-molecular-weight, interacted significantly with the active site residues of TMPRSS2 (123). However, *in vitro* and *in vivo* studies should be conducted to confirm the preventive effect of NPC306344.

Probiotics. Several patients with COVID-19 experienced dysbiosis, characterized by lower levels of *Lactobacillus* and *Bifidobacterium*. Prebiotic and probiotic intake for these patients reduces the risk of secondary infection due to bacterial translocation (124), suggesting that probiotics could be a promising strategy for the treatment of SARS-CoV-2.

Scientific data supports the action of probiotics that help maintain or restore the balance of the intestinal microbiome, consequently enhancing the immune response to viral infections, such as SARS-CoV and MERS-CoV, suggesting that probiotics could be beneficial in the treatment of viral infections (125,126). The regulatory role of probiotics on the gut-lung axis and the mucosal immune system for the potential antiviral mechanisms revolves around the competitive inhibition of the growth of pathogenic bacteria. In addition, the secretion of antimicrobial peptides, the action of metabolites, and nucleosidase activity also is responsible for the potential antiviral ability of probiotics (125,127).

Antibiotics. SARS-CoV-2 infection impairs the host immune system via damaging lymphocytes, especially B cells, T cells and NK cells (128), which may be the main promoter for coinfection with bacteria and fungi (129,130). A single-center, retrospective case series of 221 patients with COVID-19 suggested that the bacterial coinfection rate was 7.7%, and the fungal coinfection rate was 3.2% (131). A report of postmortem needle autopsy in 10 COVID-19 cases indicated that the pulmonary pathological changes of fatal COVID-19 include signs of diffuse alveolar damage and, in some cases, bacteria and fungi were detected (132), suggesting a serious bacterial or fungal infection secondary to the diffuse alveolar damage. In another 44 nasopharyngeal test samples, 38 varieties of bacteria and 9 varieties of fungi were found (133). A patient with COVID-19 recovered after combination therapy against the virus, bacteria and fungi, and respiratory support. Therefore, antibiotic treatment for COVID-19 patients seemed to be a basic requirement (130,134).

Azithromycin, a macrolide antibiotic with excellent tissue penetration and anti-inflammatory effects, downregulates pathways involving serine proteases TMPRSS2 and TMPRSS11D required for SARS-CoV-2 activation, indicating that azithromycin may hinder SARS-CoV-2 infection (135). In combination with hydroxychloroquine, azithromycin was shown to inhibit the replication of SARS-CoV-2 (136). As of 28th April 2020, there are 21 clinical trials registered on ClinicalTrials.gov for azithromycin related to COVID-19 (137).

However, the most appropriate antibacterial agent must be chosen based on the clinical symptoms of patients with COVID-19 and microbiological results; otherwise, clinicians must halt the misapplication of antibiotics (138).

7. Vaccines

According to the latest WHO vaccine candidate research and development report (139), as of 1st October, 2020, there were 187 global COVID-19 vaccines under development, including five known types of vaccine: Inactivated vaccine, attenuated live vaccine, recombinant protein vaccine, nucleic acid vaccine (RNA and DNA vaccine) and virus vector vaccine. Of these vaccines, 38 are in the human trial phase. The progress of vaccine research is summarized in Table I.

Inactivated viral vaccines. Inactivated vaccines use cell culture to create virus particles and destroy pathogenicity, by physical or chemical means, so that the viral particles retain only antigenicity (140). The commonly used cell lines for vaccine production are the canine renal epithelial (MDCK) cells and the African green monkey kidney (Vero) cells (141). A SARS-CoV-2 candidate vaccine PiCoVacc induced a specific neutralizing antibody against SARS-CoV-2 in mice, rats and rhesus monkeys (142), where the inactivated vaccine was safe and reliable. At present, there are nine inactivated vaccine projects. Of these, four are in phase I or II clinical trials. The blind clinical phase III trial of the Vero inactivated vaccine from Sinopharm Group Co., Ltd. has started in Abu Dhabi (143). The results of completed phase I/II suggested that there were no serious adverse reactions in the vaccinated group and that the antibody positive rate reached 100% according to the 0- and 28-day vaccination procedures. The clinical phase I/II of

CoronaVac inactivated vaccine developed by SinoVac Biotech Ltd. (Beijing Kexing Zhongwei Biological Technology Co., Ltd.) also demonstrated no serious adverse reactions (144,145). The positive conversion rate of neutralizing antibody was >90% after 14 days of whole immunization.

Live attenuated viral vaccine. Live attenuated vaccine is obtained by passing several generations of the virus until it retains only weak pathogenicity in the human host (139). There are three COVID-19 live attenuated vaccine projects under research and development (139): i) A recombinant live attenuated vaccine jointly developed by Codagenix, Inc. and the Serum Institute of India Pvt. Ltd.; ii) a vaccine developed by Griffith University of Australia and India Immunologicals Ltd. (ILL), and iii) the viral vector vaccine and attenuated measles vaccine targeting the S protein and N protein developed by the German Center for Infection Research (Deutsches Zentrum für Infektionsforschung) and IDT Biologika GmbH. Due to the long development time of live attenuated vaccine, the three projects have not yet entered the clinical trial stage. Notably, a study suggested that the live attenuated SARS vaccine could produce toxic viral protein again after multiple generations of replication in mice (146), indicating that the live attenuated vaccine still has substantial safety concerns.

Recombinant protein vaccine. The recombinant protein vaccine, a genetically engineered vaccine, is considered to be safe type of vaccine (140). These vaccines are produced by integrating specific antigen viral gene into expression vectors and transforming the expression vectors into bacteria, yeast or animal cells, inducing expression of antigen proteins (140). However, due to the selection of different cells as vectors, the expressed antigen may be different from the natural antigen of the virus, so the immunogenicity is weak (147). There are two methods to solve this problem: Using virus like particles and adding adjuvants (147). A vaccine candidate with M matrix adjuvant of Novavax has entered clinical trial phase II (148). A vaccine developed by Clover Biopharmaceuticals Inc./GSK/Dynavax with an S-trimer, a protein highly similar to the SARS-CoV-2 S, developed using the patented technology of trimer tag and genetic engineering, has been proven to bind to the specific antibody in the serum of convalescent patients (149). A phase I clinical trial was also completed to evaluate the safety and immunogenicity of the S-trimer candidate (149).

Nucleic acid vaccines. Nucleic acid vaccines are also known as gene vaccines, including DNA and mRNA vaccines (150). These vaccines use intramuscular injection of plasmid or naked DNA, RNA or mRNA gene of a certain antigen to induce antigen protein expression in host body, thereby eliciting an immune response (151,152). At present, there are no human nucleic acid vaccines on the market, partially because of some of the technical difficulties in delivering a precise and accurate vaccine. A DNA vaccine may be integrated into the genome because it needs to enter the nucleus to express the antigen (146). Although the mRNA can avoid the risk of host genome integration, it has some disadvantages, such as instability (152). Some methods to improve the stability and protein production of mRNA and to improve the delivery effect have

Table I. Anti-COVID-19 vaccines in clinical evaluation (138,139).

Immunization strategy				Clinical Stage				Advantages/ limitations of the vaccine platform
Vaccine platform	Vaccine developer	Schedule	Route	Phase I	Phase I/II	Phase II	Phase III	
Inactivated virus	Inactivated virus	Sinovac Biotech Ltd.	2 doses	i.m		NCT04383574 NCT04352608 NCT04551547 ChiCTR2000031809	NCT04456595 669/ UN6.KEP/EC/2020 ChiCTR2000034780	Compared with the live attenuated vaccines, the route is mature, the preparation is simple and fast and has the pre-existing technology for development. The immunization period is short but incidence of serious adverse reactions is high.
	Inactivated virus	Wuhan Institute of Biological Products Co., Ltd.	2 doses	i.m				
	Inactivated virus	Beijing Institute of Biological Products Co., Ltd.	2 doses	i.m		ChiCTR2000032459	ChiCTR2000034780	
	Inactivated virus	Chinese Academy of Medical Sciences	2 doses	i.m	NCT04412538			
Inactivated virus	Inactivated virus	Research Institute for Biological Safety Problems (Republic of Kazakhstan)	2 doses	i.m		NCT04530357		
	Inactivated virus	Bharat Biotech International Ltd.	2 doses	i.m		NCT04471519		
	Virus-like particles	Medicago, Inc.	2 doses	i.m	NCT04450004			Its composition is clear, safe and stable.
	DNA	Inovio Pharmaceuticals, Inc./International Vaccine Institute	2 doses	i.d		NCT04447781 NCT04336410		It is produced only by sequence of pathogens, and the manufacturing process is simple. It may be integrated into the genome.
DNA	DNA plasmid vaccine+ electroporation	Osaka University/ AnGes, Inc./Takara Bio, Inc.	2 doses	i.m		NCT04463472		
	DNA plasmid vaccine	Cadila Healthcare Ltd.	3 doses	i.d		CTRI/2020/07/026352 NCT04527081 NCT04445389		
	DNA vaccine	Genexine Consortium	2 doses	i.m				

Immunization strategy			Clinical Stage				Advantages/ limitations of the vaccine platform	
Vaccine platform	Vaccine developer	Schedule	Route	Phase I	Phase I/II	Phase II	Phase III	
RNA	LNP-encapsulated mRNA	2 doses	i.m	NCT04283461		NCT04405076	NCT04470427	It is produced only by sequence of pathogens and the manufacturing process is simple. Instability.
	3 LNP-mRNAs	2 doses	i.m		2020-001038-36 ChiCTR2000034825 NCT04537949		NCT04368728	
	mRNA	2 doses	i.m			NCT04515147		
	mRNA	2 doses	i.m	NCT04449276	NCT04480957			
Viral vector-based	RNA	2 doses	i.m	ISRCTN17072692				It induces strong humoral and cellular immune responses. The body will interfere with the prestored immune response of the virus vector.
	RNA	2 doses	i.m	ChiCTR2000034112				
	Replicating viral vector	2 doses	i.m	ChiCTR2000037782				
	Replicating viral vector	1 dose	i.m	NCT04497298				
	Non-replicating viral vector	1 dose	i.m		PACTR202006922165132 2020-001072-15	2020-001228-32	ISRCTN89951424 NCT04516746 NCT04520393 NCT04526990 NCT04540419	
	Non-replicating viral vector	1 dose	i.m	ChiCTR2000030906		ChiCTR2000031781		

Table I. Continued.

Vaccine platform	Vaccine developer	Immunization strategy		Clinical Stage			Advantages/ limitations of the vaccine platform
		Schedule	Route	Phase I	Phase I/II	Phase II	Phase III
Non-replicating viral vector	Gamaleya Research Institute of Epidemiology and Microbiology	2 doses	i.m	NCT04436471 NCT04437875			NCT04530396
		2 doses	i.m				
	Janssen Pharmaceuticals Inc.	1 dose	i.m	NCT04528641			
	ReiThera Srl/Leukocare AG/Univercells SA	2 doses	i.m/mucosal	NCT04552366			
	Institute of Biotechnology/PLA Academy of Military Sciences	2 doses	i.m				
Protein subunit vaccine	Novavax, Inc.	2 doses	i.m				
	Anhui Zhifei Longcom	2 doses or 3 doses	i.m	NCT04445194			
	Biologic Pharmacy Co., Ltd./Institute of Microbiology (Chinese Academy of Sciences)	2 doses	i.m				
	Kentucky Bioprocessing, Inc	2 doses	i.m				
	Sanofi SA/GlaxoSmithKline	2 doses	i.m				
	Sichuan Clover Biopharmaceuticals Inc./GlaxoSmithKline/Dynavax Technologies	2 doses	i.m	NCT04405908			
	Vaxine Pty Ltd./Medvtox, Inc.	1 dose	i.m	NCT04453852			

Vaccine platform	Vaccine developer	Immunization strategy		Clinical Stage			Advantages/limitations of the vaccine platform	
		Schedule	Route	Phase I	Phase I/II	Phase II		Phase III
	University of Queensland/CSL Ltd.	2 doses	i.m	ACTRN12620000674932p				
	Medigen Vaccine Biologics Corporation/ National Institute of Health/Dynavax Technologies	2 doses	i.m	ISRCTN51232965 NCT04487210				
	Instituto Finlay de Vacunas (Cuba)	2 doses	i.m	IFV/COR/04				
	FBRI SRC	2 doses	i.m	NCT04527575				
	VB VECTOR, Rospotrebnadzor (Russia)							
	Sichuan University	2 doses	i.m					
	University Hospital Tübingen	1 dose	sc	ChiCTR2000037518 NCT04546841				
	COVAX	2 doses	i.m	NCT04545749				

academic focus on an mRNA vaccine (152). These methods include the use of modified nucleotides and the development of nanoparticle delivery systems, which can stabilize mRNA, enhance cell uptake and improve the bioavailability of mRNA after it enters the cell (152).

The first COVID-19 vaccine approved for clinical trial was the mRNA-1273 vaccine developed by Moderna, Inc., which is currently in phase III clinical trials (153). In addition, the mRNA vaccine led by BioNTech SE is in phase III clinical trials (154). Viral vector vaccines. A recombinant virus vector vaccine is a vaccine that takes the replicative activity or non-replicating virus as a carrier and recombines antigenic genes into the viral genome (140). The adenovirus vector (Ad5) vaccine developed by Beijing Institute of Biotechnology and CanSino Biologics, Inc. has entered phase II clinical trials (139). A total of 108 healthy volunteers aged 18-60 were recruited to phase I clinical trials to determine the human tolerance of different doses of the vaccine by observing the safety of vaccine use (155). The results indicated that the Ad5 NCoV vaccine is well tolerated and can induce an immune response to SARS-CoV-2 (156). Compared with the phase I clinical trials, the phase II clinical trials opened the upper age limit, to further analyze and confirm the preliminary efficacy and safety of the vaccine in the population, and to determine the immune program and dose of the vaccine. In addition, the ChAd0x1-s vector vaccine, led by Oxford University, has also entered phase III of clinical trials (156).

8. Conclusions and outlook

SARS-CoV-2 is a new virus and its source, transmission mode, pathogenesis and clinical manifestations are not well-known. In addition, SARS-CoV-2 is highly infectious, creating an outbreak that has spread rapidly to all parts of the world (2). The novel coronavirus has been prevalent all over the world since SARS-CoV-2 was first identified in patients who were exposed at a seafood market in Wuhan City, Hubei Province, China in December 2019 (11). Similar to findings related to SARS-CoV and MERS-CoV, SARS-CoV-2 is believed to have crossed species and initiated primary human infections (5,6). As SARS-CoV-2 will evolve through frequent recombination of its genomes and through mutations (157), the propensity to infect multiple species and the increasing human-animal interface presents unknown problems.

Practicing good hygiene and wearing masks seem to be effective methods to restrict the spread of COVID-19 (24,158). However, prevention and treatment of COVID-19 with drugs remain urgent issues to be resolved. Non-specific antiviral therapy (oseltamivir, ganciclovir), antibacterial therapy (moxifloxacin, ceftriaxone, azithromycin) and glucocorticoid therapy are being improvised by combination with broad-spectrum antivirals such as remdesivir, chloroquine, and lopinavir/ritonavir (159). Plasma therapy shows promise as a beneficial treatment. The deliberate infection of healthy volunteers with SARS-CoV-2 were proposed, as it may shorten the time required for the development of COVID-19 vaccines (160).

During the current outbreak, the development of clinical drugs against coronaviruses has been challenging, and the recent advances in understanding the molecular mechanisms of

infection and transmission facilitate more rapid diagnoses and treatment of COVID-19. Aside from traditional drug therapy during the ongoing SARS-CoV-2 epidemic, vigorous efforts on the vaccine development are urgently needed. Finding a short section or sections of viral protein sequence suitable for a synthetic vaccine and antagonists against COVID-19 can be useful for preliminary design proposals. The S protein, a glycoprotein encoded by coronavirus genome RNA that is usually cut into spherical S1 subunit and rod-shaped S2 subunit during virus assembly, is an important component of coronavirus to recognize and infect host cells. Thus, identification of immunogenic targets against the important SARS-CoV-2 proteins, such as the S glycoprotein, will provide crucial advances towards the development of sensitive diagnostic tools and potential vaccine candidates. Moreover, the therapeutic options currently under investigation, such as inhibitors targeting the S protein, require confirmation in clinical trials prior to recommendation. Moving forward there are treatment options available that could be utilized clinically during the ongoing SARS-CoV-2 epidemic, as these agents have shown significant effects against COVID-19 in preclinical trials. Based on knowledge of the mechanism of SARS-CoV-2 replication and infection, a broad-range of combinational therapies should also be evaluated. Computational techniques combined with the mechanistic studies of SARS-CoV-2 can aid in the design and development of predicted antiviral agents. Follow-up experimental studies will test novel candidates for drug repurposing, which will be hopefully be translated into clinical practice.

The present review summarizes the latest findings related to the clinical features, diagnosis, and management of COVID-19. The aim was to provide the most up-to-date understanding of SARS-CoV-2, with ongoing guidance for COVID-19 prevention and control. As there is a dynamic and a large volume of emerging therapeutic approaches for COVID-19, only articles/publications/translations from English (Pubmed database; <https://www.ncbi.nlm.nih.gov/>) and Chinese (China National Knowledge Infrastructure database; <https://www.cnki.net/>) of the adult population between December 2019 and September 2020, and realize that some relevant international data might be missing.

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Authors' contributions

RW prepared the draft manuscript. RW, XL, FL and SL critically revised and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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