Review article

Progress in SARS-CoV-2, diagnostic and clinical treatment of COVID-19

Yang Li [a,b,1], Si-Ming Lu [c,d,e,1], Jia-Long Wang [a,b,1], Hang-Ping Yao [a,b,**], Li-Guo Liang [a,b,f,*]

[a] *State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China*
[b] *National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China*
[c] *Department of Laboratory Medicine, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China*
[d] *Zhejiang Key Laboratory of Clinical in Vitro Diagnostic Techniques, Hangzhou, China*
[e] *Institute of Laboratory Medicine, Zhejiang University, Hangzhou, China*
[f] *Centre for Clinical Laboratory, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China*

ARTICLE INFO

ABSTRACT

Background: Corona Virus Disease 2019(COVID-19)is a global pandemic novel coronavirus infection disease caused by Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Although rapid, large-scale testing plays an important role in patient management and slowing the spread of the disease. However, there has been no good and widely used drug treatment for infection and transmission of SARS-CoV-2.
Key findings: Therefore, this review updates the body of knowledge on viral structure, infection routes, detection methods, and clinical treatment, with the aim of responding to the large-section caused by SARS-CoV-2. This paper focuses on the structure of SARS-CoV-2 viral protease, RNA polymerase, serine protease and main proteinase-like protease as well as targeted antiviral drugs.
Conclusion: In vitro or clinical trials have been carried out to provide deeper thinking for the pathogenesis, clinical diagnosis, vaccine development and treatment of SARS-CoV-2.

1. Introduction

At the end of 2019, a new type of coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2(SARS-CoV-2) rapidly swept the world. According to the report of World Health Organization (WHO), as of May 25th, 2024, there were 704,433,855 confirmed cases (Fig. 1A) and 7,007,868 deaths (Fig. 1B) of COVID-19 in 223 countries or regions worldwide, with a fatality rate of 1.07 % (https://covid19.who.int/). and it showed that according to amino acid sequence and phylogenetic analysis, SARS-CoV-2, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome Coronavirus (MERS-CoV) were all β coronaviruses (Fig. 1C) (GISAID accession no. EPI_ISL_402124) [1,2].

---

* Corresponding author. Centre for Clinical Laboratory, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China
** Corresponding author. State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China
E-mail addresses: yaohangping@zju.edu.cn (H.-P. Yao), lianglg@zju.edu.cn (L.-G. Liang).
[1] Yang Li, Si-Ming Lu and Jia-Long Wang contribute equally to the study presented in this article.
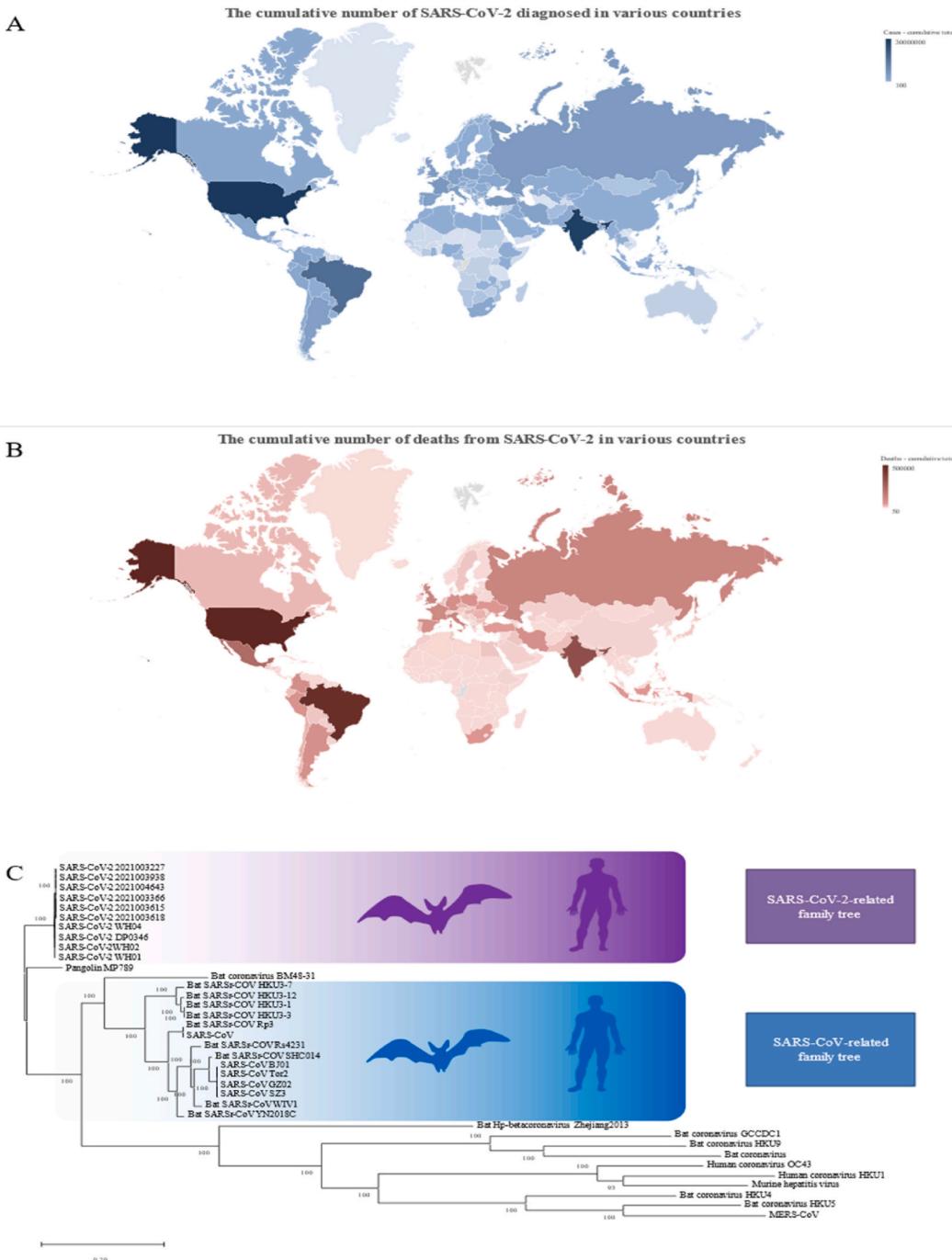
Fig. 1. Incidence, mortality, and phylogenetic tree analysis of COVID-19 A. Cumulative number of SARS-CoV-2 diagnosed in various countries. B. Cumulative number of deaths from SARS-CoV-2 in various countries. C. Cluster analysis of coronaviruses.

Similar to the symptoms of SARS-CoV patients, many clinical cases have shown that acute respiratory distress syndrome (ARDS) caused by cytokine storm and secondary hemophagocytic lymphohistiocytosis (sHLH) leading to multiple system damage, both of which are related to CRS (cytokine release syndrome) [3]. SARS-CoV-2 acts on the angiotensin-converting enzyme 2 (ACE2) receptors on oral epithelial cells of the respiratory system, alveolar type II epithelial cells, and other respiratory tract cells, infecting host cells [4, 5]. Single-cell RNA sequencing results from TCGA, FANTOM5 and other databases showed that ACE2 was highly expressed in a variety of cells such as alveolar type II cells, esophagus lamellar cells, ileocolonic cells, and proximal tubule cells of the renal [6]. The site provides the possibility for SARS-CoV-2 infection and metastasis. After invading the respiratory system, SARS-CoV-2, with intracellular

self-replication, leads to leukocytes chemotaxis to the infection site, resulting in vasodilation, increased permeability of alveolar wall, decreased pulmonary surfactant, and loss of alveolar gas exchange function [1,3].

2. Genomic structure and infection pathway of SARS-CoV-2

The SARS-CoV-2 belongs to the β-coronaviruses. It is an unsegmented single-stranded positive stranded RNA virus, each of which has a genome length of about 30,000 nucleotides. About 40 % of the viral genome consists of nucleotides G and C, encoding 9860 amino acids. The genome contains two flanking untranslated regions (UTR) and an open reading frame (ORF) encoding the entire part of poly protein. The ORF1a and ORF1b genes, which account for about two-thirds of the entire genome, encode 16 nonstructural proteins. Its genome composition is the same as that of other coronaviruses, with a ORFs, including replicase (ORF 1a, ORF 1b), spike protein (S), envelope (E), stroma egg-self (M), nuclear protein (N) [7] (Fig. 2A). Specifically, S protein is the most important membrane protein on the surface of coronavirus. It consists of two domains. The part near the N terminal forms a spherical domain, and the part near the C terminal forms a rod-like structure through the membrane. N protein is another important structural protein in coronavirus. In the coronavirus particle, the N protein is located at the core of the particle and occurs in the form of Genomic RNA. M protein is a transmembrane protein, and its binding with S protein is related to the assembly of virus particles. The binding of M protein and N protein enables the structural stability of virus RNP. The E protein, thought to be a viral porin, can disrupt ion concentration
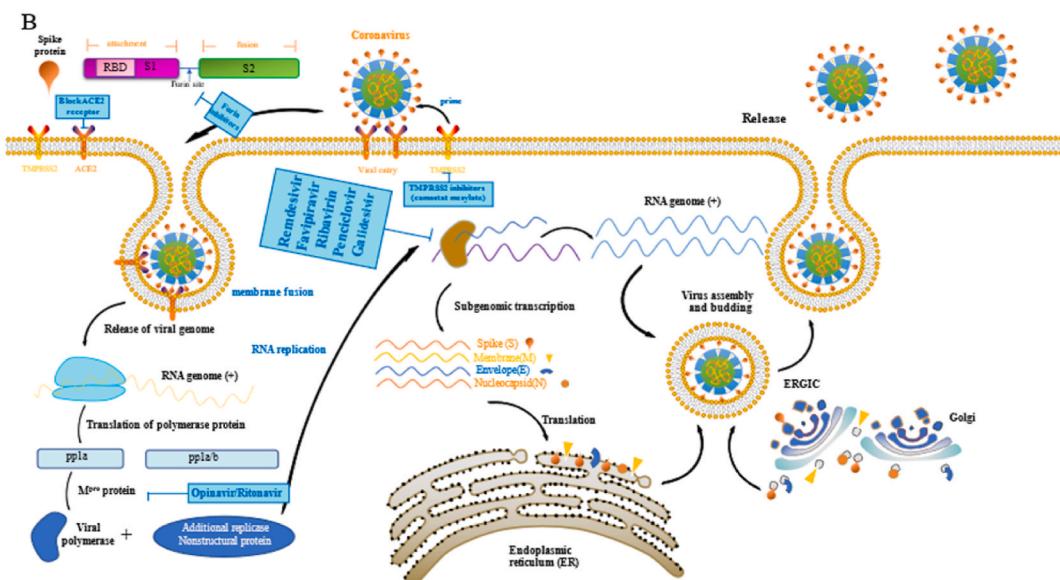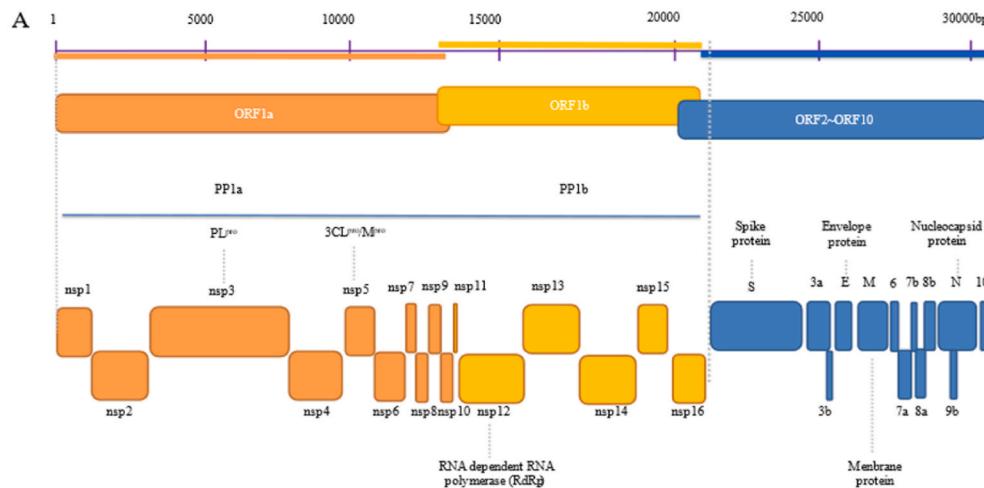

Fig. 2. Structure (A) and invasion pathway (B) of SARS-CoV-2.

homeostasis in cells. Its RNA is tightly packed with nucleocapsid 9(N protein) Coated with phospholipid bilayer and spike protein (S protein) [8]. SARS-CoV-2 enters the cell and completes the process of self-replication, which mainly includes: The S protein on the membrane surface is composed of S1 and S2 subunits. It is activated by transmembrane protease serine 2(TMPRSS2) and binds to the receptor ACE2, facilitating the entry of the virus into the substance [9,10]. Secondly, after entering the cell, furin-mediated cleavage of the S2 subunit releases hydrophobic fusion peptide, phospholipid bilayer fuses with the host cell membrane, and releases the membrane core capsid N protein, positive strand RNA and open reading frame 1a/b(ORF1a/b) bind to express a variety of proteins, including pp1a and pp1ab. Cleavage by two viral proteases, papain-like protease (PL^{pro}) and main chytrypsin-like protease (M^{pro}) to produce non-structural proteins [11,12], in which RNA-dependent RNA polymerase (RdRp) enzymes and spinases are used to participate in viral transcription and replication. SARS-CoV-2 uses full-length positive-strand genomic RNA as a template to replicate to form full-length negative-strand RNA, and then synthesizes a large amount of positive-strand RNA, which is assembled with N protein to form nucleocapsid, and then exocytosis with other structural proteins to form virus released into the extracellular [2] (Fig. 2B). Three aspects of viral spike proteins, RNA-dependent RNA polymerase, and chymotrypsin are described below.

2.1. S protein

S protein, the medium through which coronavirus entering target cells, is a major immunogenic antigen composed of a receptor binding domain (RBD) S1 and carboxyl-terminal membrane fusion domain S2. It binds specifically to amino terminal ACE2 to form a trimeric structure [13]. The biophysical analysis of expressed and purified S protein by cryo-electron microscopy showed the binding capacity of SARS-CoV-2 and its target receptor ACE2 is 4~20 times that of SARS-CoV [14].

Protease cleavage sites at the S1–S2 junction are critical for activating membrane fusion, virus entry, and syncytia formation. The furanase on which the cleavage depends is a proprotein convertases (PC) located in the TGN (trans-Golgi network, TGN). Furanase is usually expressed in the lung, which is one reason for the entry of respiratory viruses into cells [15]. Depending on the joint action of transmembrane TMPRSS2 and furanase, the S protein is activated by host furanase and cleaved into S1 and S2 subunits. RBD binds to the host receptor with the help of transmembrane TMPRSS2 and triggers the conformational change of S2 subunit, making the virus fuse with the cell membrane [15].

2.2. RdRP

After the coronavirus infects host cells, RdRP, also known as NSP12 encoded by the coronavirus itself, forms an important component of the subsequent replication and transcription complex (RTC), which with NSP (non-structural proteins, NSP), selects matching mRNA templates to determine transcription of mRNA. It is involved in the 5′cap or 3′polyadenylic acid tail of the product mRNA [16], occupying one of the core positions in the virus replication and transcription.

The structure of the mini-RTC was analyzed using cryo-electron microscopy. The results showed the structure was assembled from RdRP, viral RNA, NSP7 and NSP8, and two helicase molecules NSP13-1 and NSP13-2 [17,18]. There are two different structures of the micro-RTCs, NSP13-1 and NSP13-2 interact to stabilize the overall structure of the micro-RTC. During viral RNA reproduction, NSP 13-2 anchors the 5′-amplifed RNA template and interacts with NSP7-NSP8. NSP12-RNA interactions form RTC to complete the virus life cycle [16,18].

Coronavirus encodes RTC (assembled into 16 NSPs) that promote viral replication and transcription. Among them, NSP12 binds to RdRP and viral RNA, while NSP8 and NSP7 give NSP12 RNA processing ability [17]. When RdRP holoenzyme (comprised of NSP7, NSP8, and NSP12) synthesizes SARS-CoV-2 RNA, the NSP13 amino-terminal domain identified as a helicase binds to the amino-terminal of NSP8 to catalyze the virus, and the double-stranded oligonucleotides melt into single chain. The presence of RdRP can increase the catalytic rate of NSP13 [16].

2.3. M^{pro} protease

In the process of coronavirus expression varying multiple proteins, the main chymotrypsin-like protease (M^{pro}, also known as 3C-like Proteinase, 3CL^{pro}) and papain-like protease (PL^{pro}) process the virus, and the multi-protein translation of RNA is very important [19,20]. M^{pro} has at least 11 cleavage sites in the RdRp precursor of the virus, replicase 1 ab (~790 kDa), and most of the sites are identified by Leu-Gln↓ (Ser, Ala, Gly) (↓ means cutting Site). The domains I and II of M^{pro} are six-strand anti-parallel β-barrels forming a substrate binding site. The five helices form a globular cluster of domain III, which is involved in regulating the dimerization of M^{pro} [21] by forming a salt bridge mainly through the Glu290 site of one protomer and Arg4 of the other protomer. The dimerization of M^{pro} is essential for catalytic activity. Each -NH2 residue in the dimer interacts with each Glu166 in another protomer, thus helping to form the substrate binding site capsule [22]. Previous studies have shown that replacing Ser284, Thr285 and Ile286 with Ala residues in SARS-CoV-2 M^{pro} can increase the catalytic activity of the protease by 3.6 times. At the same time, the accumulation of two domains III of the dimer increased the activity of Mpro. However, the M^{pro} catalytic efficiency of SARS-CoV-2 ($k_{cat}/K_m = 3426.1 \pm 416.9 s^{-1} M^{-1}$) is only slightly higher than that of SARS-CoV ($k_{cat}/K_m = 3011.3 \pm 294.6 s^{-1} M^{-1}$). Meanwhile, studies based on SARS-CoV show that M^{pro} has different crystal structures at different pH values, and has high catalytic activity in the structure of pH 7.3–8.5 [22].

3. Clinical features and diagnosis of COVID-19

3.1. Clinical symptoms

The gender of susceptible populations for COVID-19 has not yet been determined, and according to the statistics, the difference is small (48.6 % female) [23]. However, according to various studies, the population who are susceptible to COVID-19 of is range in age from 40 to 60 [23–25].

Infected patients improves within 2 weeks, with fever, cough, and dyspnea as the main symptoms [23]. Besides, muscle pain, fatigue, and spitting are also more common [24,26]. Less common symptoms include nasal congestion, sore throat, headache, hemoptysis, and diarrhea. Some patients have kidney injury and acute myocardial injury [23,27].

3.2. Diagnostic technology

The most effective response to the spread of COVID-19 has been proven to be rapid detection, isolation, and treatment. Pathogen detection can be divided into imaging, and viral isolation and culture detection, viral RNA molecular biology detection and immunological detection based on viral antigen and antibody reaction. Herein, the imaging diagnosis, molecular biological and ELISA detection techniques of viral will be introduced in detail.

3.2.1. CT/PET

Chest imaging, especially chest CT, is one of the most sensitive imaging techniques for detecting lung abnormalities. Quantitative CT analysis provides useful information for predicting disease progression in the management of COVID-19 patients plays a key role. For example, one study compared the impact of the COVID-19 pandemic on 2-[^{18}F] FDG PET/CT imaging workflows during three waves in health facilities in southern Italy [28]. This study showed that PET/CT based technology can well assist patients in disease diagnosis. An evaluation report based on chest CT in COVID-19 patients has shown that the use of structured radiological reports in COVID-19 patients can improve referring physician satisfaction, optimize reporting time, and provide more and higher quality information in the report compared to conventional analysis [29]. In a prospective study, 89 volunteers who had been vaccinated against COVID-19 were included in ultrasound examinations to assess the association between lymphadenopathy and side effects of vaccination. The results showed that CT can be a good auxiliary judge of vaccine-induced lymphadenopathy, and confirmed that there was no statistical difference between vaccine side effects and lymphadenopathy [30]. Post-vaccine AIH-like syndrome raises worries about
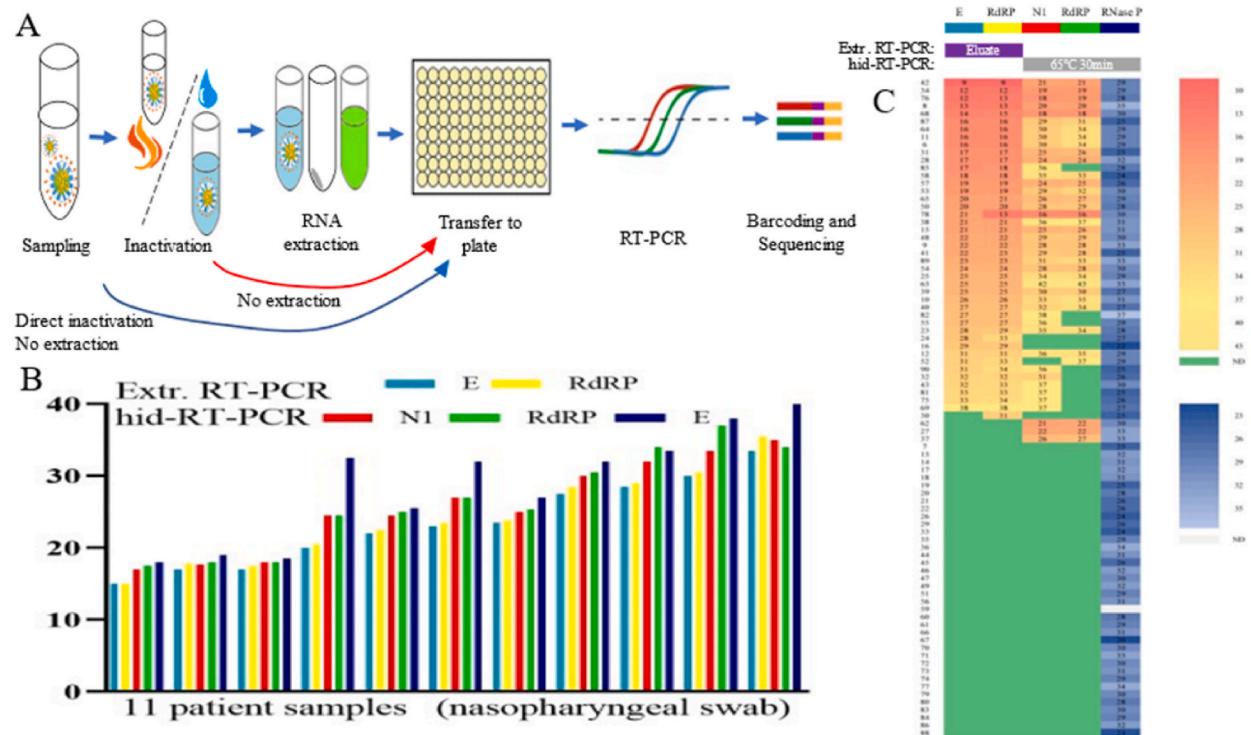

Fig. 3. N, E, and RdRP genes of SARS-CoV-2 were detected by RT-PCR A. Heat-inactivated and lysed samples were assayed separately for PCR amplification; B. Targeted detection of the E, N and RdRP genes of novel coronaviruses; C. Heatmap analysis of the effects of different treatments for gene amplification of viral samples.

a risk of unprecedented immunological side effects, especially in individuals predisposed to autoinflammatory disorders [31]. COVID-19 vaccine-induced AIH is extremely uncommon and has an exceptional prognosis. This also suggests that hepatologists may be able to use CT ultrasound diagnosis in combination with indicators such as jaundice or elevated liver enzymes to analyze possible side effects of vaccination. It is worth noting that with the mutation of the virus, the virulence of pneumonia is decreasing, and the characteristics and evolution of CT images of COVID-19 have certain limitations. It further suggests that perhaps artificial intelligence based on deep learning can provide better help for clinicians in diagnosis.

3.2.2. RT-PCR

Timely quantitative detection of SARS-CoV-2 is of great significance for controlling the spread of COVID-19, including in the social settings, and in the public health response [32,33]. The recent spread of SARS-CoV-2 has shown the urgent need for accurate and rapid diagnostic tests to promote clinical and public health interventions, and reverse transcriptase polymerase chain reaction (RT-PCR) tests are used to rule out infection in high-risk populations [34]. In one study, a method was described circumventing RNA extraction in COVID-19 testing by performing RT-PCR directly on heat-inactivated or lysed samples (Fig. 3A). This study designed detection primers targeting the E, N and RdRP genes of the novel coronavirus (Fig. 3B), and conducted 85 clinical samples and heat map analysis to evaluate the effectiveness of the method [35] (Fig. 3C). Swab samples have been used in the analysis as a cost reduction strategy. 32, 466 RT-PCR reactions were used to detect 133,816 samples, with an overall efficiency of 4121 RT-PCR reactions, saving 101,350 (76 %) RT-PCR reactions [36] results of heat-inactivated nasopharyngeal swab samples (65 °C, 30min) were similar to those RT-PCR results, which could greatly save time and cost. This may help expand COVID-19 testing [35].

3.2.3. LAMP

Compared with RT-PCR, the detection of Loop-mediate Isothermal Amplification (LAMP) technology shows great potential. Some SARS-CoV-2 RT-qPCR and RT-LAMP nasopharyngeal and oropharyngeal swabs can be nucleic acid tests without prior RNA purification or extraction steps [37,38]. The stability of exposed RNA was tested with swab samples collected with specific medium (Aimes), and the IVT RNA molecules of the viral N gene were quantitatively titrated against the swab samples of COVID-19 negative control subjects. The effects of reagents and pyrolysis are similar to previous reports for other RNA viruses, as well as tests for thermal inactivation using swab specimens for direct RT-qPCR analysis [39,40]. These results suggest that both untreated cotton swab and high-temperature pyrolysis cotton swab samples can be suit to detect SARS-CoV-2 RNA in cotton swab samples of potential individuals. In one study, a two-color RT-LAMP assay protocol for detecting SARS-CoV-2 RNA was test [41]. RNA samples isolated from 95 pharyngeal swab specimens were analyzed by the RT-LAMP assay using a 96-well plate (Fig. 4A). After a 30-min incubation at 65 °C, quantification of the red-to-yellow color change in all wells using spectrophotometric OD measurements (Fig. 4B), the results showed that the RT-LAMP assay reliably detected SARS-CoV-2 RNA with an RT-qPCR cycle threshold (CT) number of up to 30, with a sensitivity of 97.5 % and a specificity of 99.7 %. In another study, Cap-iLAMP which combined a hybridization capture-based RNA extraction of gargle lavage samples with an improved colorimetric RT-LAMP assay (Fig. 4C) and smartphone-based color scoring was
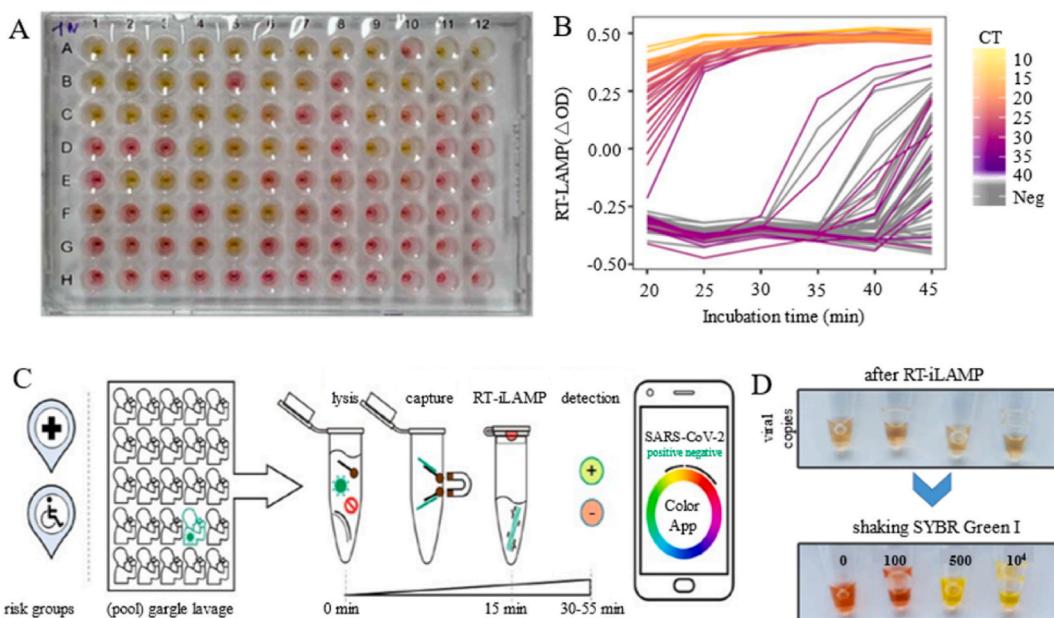
Fig. 4. Detection of SARS-CoV-2 RNA in throat swab samples from clinical individuals using by RT-LAMP A. RT-LAMP assay for SARS-CoV-2 RNA testing using a 96-well plate; B. Cap-iLAMP combines hybridisation capture-based RNA extraction of mouthwash samples with improved colourimetric RT-LAMP analysis; D. Colour reaction test for detection of viral copy number.

presented (Fig. 4D). This method can complete sample detection in less than 1 h. The sensitivity and specificity of another colorimetric RT-LAMP detection of SARS-CoV-2 RNA in throat swab samples from clinical individuals showed the colorimetric RT-LAMP detection pair with high-speed, low price and low equipment requirements is as high as the sample with CT ∼ 30 has good sensitivity [42].

3.2.4. RPA

Nucleic acid testing is mainly based on high sensitivity and high specificity, but it is time-consuming and expensive. There have been many innovative applications of LAMP technology in COVID-19 diagnosis in recent years [38,41,43], due to the global demand for SARS-CoV-2 detection, Enhanced Recombinase Polymerase Amplification (eRPA) is more isothermal than other applications. The improvement of technology has greater potential development advantages.

In one study, single-tube probe RT-RPA has shown 100 % diagnostic sensitivity and specificity. The detection limit was 7.74 copies per reaction, and high-concentration samples could be obtained within 7 min [44]. Therefore, this assay is one of the fastest nucleic acid-based SARS-CoV-2 detection methods. To extract viral nucleic acid from clinical swabs has become a limiting. Recent studies have shown that thermal lysis without RNA extraction has proven to be a rapid method for lysing and inactivating viruses for diagnostic tests [41,45]. To select reverse transcriptase in RT-RPA based on the method will affect the enlargement efficiency of recombinant enzyme polymerase. Superscript IV reverse transcriptase is used in combination with RNaseH, both of which are designed to have minimal RNaseH activity and can improve target synthesis ability, robustness and synthesis rate [46]. TCEP was added to a new crown oropharyngeal swab buffer and EDTA and engineered enzyme were heated at 75 °C [47]. In another study, a molecular diagnostic test for SARS-CoV-2 based on an enhanced recombinase polymerase amplification (eRPA) reaction was reported. SARS-CoV-2 RNA was amplified by eRPA using primers targeting the N (Fig. 5A) or S (Fig. 5B) gene and reactions were read out by lateral flow strip (Fig. 5C) [48]. Compared with RT-PCR, all samples with more than 5 molecules per reaction obtained consistent results. The assay is also highly specific, showing no cross-reactivity (0 of 80 tests) with 10,000 copies of RNA from other coronaviruses, i.e., MERS, SARS-CoV, CoV-HKU1, or CoV-229E (Fig. 5D). These important improvements in RPA indicate the potential of using isothermal amplification to detect SARS-CoV-2 in daily life.

3.2.5. ELSIA

In addition to the molecular nucleic acid tests mentioned, enzyme-linked immunosorbent assay (ELISA) is used to detect antibodies to help determine whether a patient is infected with the virus [49–51]. Antibody tests for COVID-19 are more reliable after 2 weeks of symptoms. Recently, a reliable, rapid, and quantitative point-based detection (POCT) technique for SARS-CoV-2 specific antibodies has been described [52]. It can simultaneously detect SARS-CoV-2 IgM and IgG antibodies in the blood of infected patients within minutes, and can distinguish patients at different stages of the disease. The detection limits of IgG and IgM against SARS-CoV-2 virus were 0.6
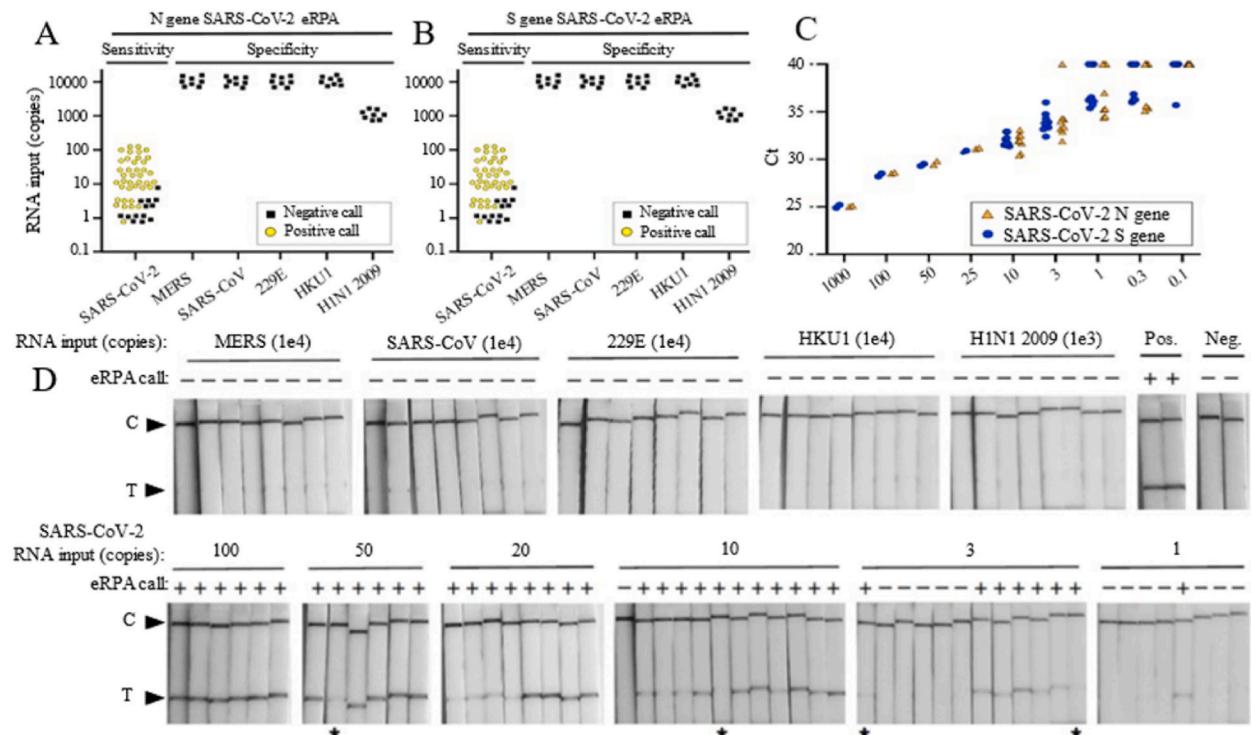

Fig. 5. N and S genes of SARS-CoV-2 analysis using by eRPA method Amplification of SARS-CoV-2 RNA by eRPA using primers targeting the N (A) or S (B) genes. Ct values gradually increased with decreasing assay concentration (C).Cross-reactivity tests with other coronaviruses(D).

ng/mL and 0.3 ng/mL, respectively, which can be rapidly detected by fingertip blood. In a study, a panel of 123 plasma samples from a COVID-19 outbreak study population, preselected by semiquantitative anti-SARS-CoV-2 IgG testing, was used to assess the relationship between the novel quantitative ELISA (IgG) and a microneutralization assay [53].These results substantiate the implementation of the QuantiVac ELISA to assess protective immunity following infection or vaccination. In another study, a semi-stable mammalian episomal expression system was used to produce high quantities of the receptor-binding domain-RBD of SARS-CoV-2 [54]. The recombinant antigen was tested in an in-house IgG ELISA for COVID-19 with a panel of human sera. Thus, this serological test can be an attractive and inexpensive option in scenarios of limited resources to face the COVID-19 pandemic. However, the ELISA test has several problems that make it inappropriate sometimes. These problems include false negatives, noise responses from samples, nonspecific reactions because of improper plate cleaning, time-consuming, differences in reagent concentrations in prepared ELISA kits, high prices, and operator skills that trigger immunoassays, use ELISA readers and other associated equipment, and count exact amounts of antigens or antibodies.

3.2.6. Microfluidic chip

Several serological tests such as POCT and Assure have gained emergency use authorization (EUA) from the U.S. Food and Drug Administration (FDA), including enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay and neutralization test. It is reliable [55], but needs analysis and determination by trained operators after hours or even days. On the other hand, faster detection methods (such as lateral flow analysis) are more conducive to the operation of the experimenter and provide accurate results, but only provide qualitative results [56]. Therefore, it is urgent to develop rapid, economical, and accurate detection methods.

In one study, optical microfluidic sensing platform for gold nanorods uses SARS-CoV-2 anti-S protein to bind to receptors, and the wavelength of the local surface plasmon resonance (LSPR) peak of the gold nanostructure in the microfluidic device is shifted [57]. Shift correlation provides high-precision detection for the analysis of complex samples such as plasma or serum containing fibrinogen, globulin, etc. The label-free microfluidic platform reached the detection limit of 0.08 ng/mL (0.5Pm), which was within the clinically relevant concentration range [57]. Kim et al., propose an innovative method to detect SARS-CoV-2 using isothermal amplification of nucleic acids on a mesh containing multiple microfluidic pores (Fig. 6A) [58]. In short, templates were self-assembled to form an asymmetric dumbbell shape (Fig. 6B), and primers were immobilized on the nylon mesh surface (Fig. 6C). Templates were hybridized with primers immobilized on nylon mesh surface. When the template on the nylon-mesh surface hybridizes with a target pathogen (Fig. 6D), the template was ligated to form a closed-loop template. DNA hydrogel formation by COVID-19 template DNA (Fig. 6E). The
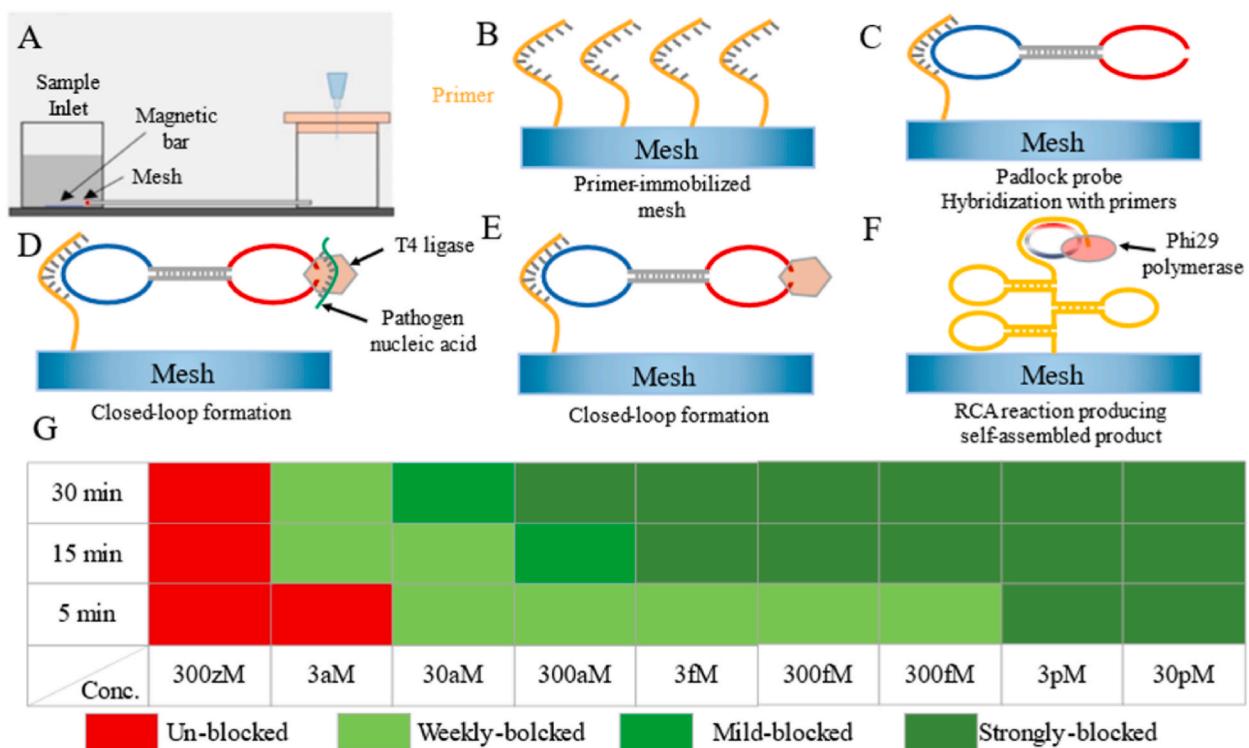
Fig. 6. A microfluidic system to detect SARS-CoV-2 using a mesh-based RCA process (A) An explanatory schematic of the experimental setup for COVID-19 detection (B) Templates with primers immobilized on the nylon mesh surface are self-assembled to form an asymmetric dumbbell shape. (C) The templates are hybridized to the primers immobilized on the surface of the nylon mesh. (D) The template is ligated to form a closed loop template when the template hybridizes to the nylon mesh surface with a target pathogen (E). (F) Formation of DNA hydrogels from COVID-19 template DNA. (G)Heat-map for COVID-19 detection in terms of pathogen concentration and incubation time.

hybridization of pathogen DNA and immobilized probes is amplified by rolling circles to form DNA hydrogels (Fig. 6F). The detection limit (LOD) of SARS-CoV-2 was determined to be 15 min and 0.7 Am (Fig. 6G). These results indicated rapid, easy, and effective detection with a moderate-sized LOD of the target pathogen by remote point-of-care testing. Many microfluidic platforms provide sustainable and timely detection tools to fill the gaps in standard serological detection and make the qualitative and quantitative diagnosis of SARS-CoV-2 more accurate and cheaper.

4. Prophylaxes

4.1. Vaccines

Vaccination is the most effective method to control SARS-CoV-2 and amount of things such as mRNA vaccine, Attenuated live vaccine, recombinant protein subunit vaccine to against it [24,59]. As of June 21, 2021, 287 vaccine candidates for SARS-CoV-2 had been reported and 102 were in clinical trials (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines). Many SARS-CoV-2 vaccine candidates are in phase II testing, and some in phase III testing. China vaccine targets with RBD area S protein antigen selected new recombinant protein subunit vaccine, has completed phase II clinical trial, the stage is conducting clinical trials and clinical trials showed the vaccine has good tolerance and immunogenicity, and minor adverse reactions, the safety and immunogenicity data support III phase of clinical trial with 25 (including g vaccination program, 3 times. To further evaluate safety and efficacy on a large scale [60]. The United States announced that two COVN mRNA vaccine clinical trials met the

Table 1
The latest on the use of vaccines.

Type	Name	Manufacturer	Efficiency	Untoward effect
Virus vaccines	CoronaVac	Sinovac Research and Development Co.	/	Bell's palsy Encephalopathy Anaphylaxis Thromboembolic Guillain barre syndrome
	BBIBP-CorV	Sinopharm + China National Biotec Group Co + Beijing Institute of Biological Products	79.34 %	Dizziness Fatigue Headache Nausea Vomiting Allergic Dermatitis Fever
Viral-vector vaccines	Ad26.COV2. S	Janssen Pharmaceutical by Johnson & Johnson	/	Thrombosis with thrombocytopenia Guillain-Barre syndrome
	AZD122 (ChAdOx1-S)/ Vaxzervria/CoviShield	AstraZeneca + University of Oxford	/	Thrombocytopenia syndrome Capillary leak syndrome Myocarditis Pericarditis Anaphylaxis Guillain- Barre syndrome
Nucleic-acid vaccines	BNT162b2/Comirnaty	Pfizer/BioNTech + Fosun Pharma	95 %	Anaphylaxis Myocarditis Lymphadenopathy Appendicitis Herpes zoster infection Pericarditis Bell's palsy
	mRNA-1273/Spikevax	Moderna + National Institute of Allergic and Infectious diseases	94 %	Anaphylaxis Myocarditis Pericarditis
Protein-based vaccines	NVX-CoV2373	Novavax	/	Injection site reaction Pain Tenderness Erythema Swelling Fever Headache Fatigue Malaise Myalgia Arthralgia Nausea

primary efficacy endpoint requirements, with protective efficacy of 94.1 % and 95.0 %, respectively, and consistent efficacy in different age, gender, and ethnic groups [61]. Another clinical trials of the vectored vaccine(ChAdOx1) developed in the United States found that two doses of immunity were better than one dose, in a phase I/II trial (randomized control), 1077 experimental participants produced neutralizing antibodies targeting SARS-CoV-2 after receiving the second vaccine injection, and the experiment proved that the second injection The interval of eight weeks is better than four weeks, and it's no side effects also prove its reliability [62].

Vaccination has helped the whole population build up an immune barrier (Detail in Table 1). However, in the battle against the virus in the future, human beings still need to create a more rigorous environment through scientific research and innovation to wear down the claws of the novel coronavirus. For example, to prevent future mutations of other sites in terms of drug resistance and environmental resistance of the novel coronavirus, drug research and development should have a broader perspective, and to develop novel coronavirus drugs with multiple mechanisms of action should be encouraged to avoid the generation of drug resistance caused by a single drug target.

5. Treatments for COVID-19

Up to now, only a few specific drugs have been approved to treat SARS-CoV-2. While many drug trials registered with the WHO, most of them are still in the experimental and clinical confirmation stage. Based on previous studies, the drugs used to treat COVID-19 are divided into several categories: interfering with virus recognition of ACE2 into cells; inhibiting RNA replication, synthesis, gene expression; broad-spectrum antiviral drugs; and vaccines etc. (Fig. 7).

### 5.1. Inhibit virus recognition of host ACE2

According to the entry of SARS-CoV-2 into the recipient cell needs ACE2 to bind to RBD, and after TMPRSS2 activation, furin protease cleavage and membrane fusion, therapeutic strategies can be divided into the following categories.

5.1.1. Blocking ACE2 receptor

It is proved that S protein is a protective antibody based on SARS-CoV and SARS-CoV-2 specific binding to ACE2 [63,64], its binding capacity is 10–20 times that of SARS-CoV [14]. In addition, SARS-CoV down-regulates express ACE2 in recipient cells causing local lung injury [65,66]. S protein specific antibodies or ACE2 small molecule inhibitors can be designed to inhibit infect recipient cells. For example, a bacteriophage display library of SARS-CoV-2 S protein genome fragment (SARS-CoV-2 GFPDL) was used to analyze vaccine-induced antibody libraries to identify immunodominant epitopes in the S1, S1-RBD, and S2 domains. Also, these analyses showed that RBD immunogen had a 5-fold increase in affinity for ACE2 compared to other spike antigens, leading to higher antibody expression, and the best binding of their antibody to the target antigen [67]. However, some studies have shown that SARS-CoV polyclonal antibody can inhibit the entry of SARS-CoV S protein into cells, but not inhibit SARS-CoV-2 pseudovirus particles [68].

5.1.2. Administration of exogenous soluble ACE2

ACE2 is a membrane-anchored protein with very low solubility in blood [69]. It has been proved in cell culture that soluble ACE2
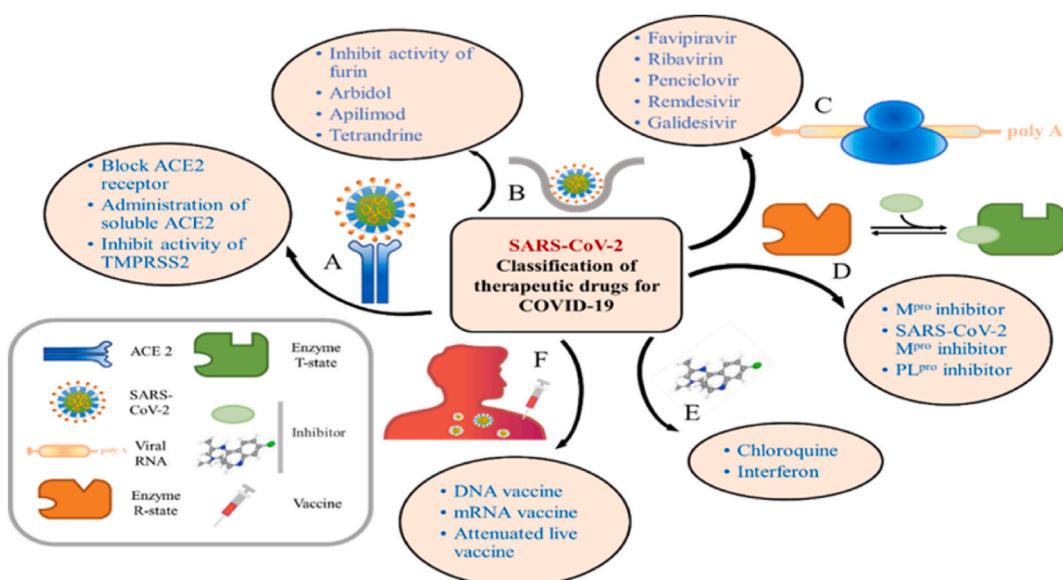
Fig. 7. Classification of therapeutic drugs for COVID-19.

fused with Ig can inhibit the infection of SARS-CoV-2 and S protein pseudovirus [63]. hrsACE-2 can inhibit SARS-CoV-2 infection in a dose-dependent manner, and hrsACE2 has been test in phase 1 and phase 2 clinical trial [70], and is considered as a potential treatment for COVID-19 [71]. Purified viral RNA was used in Vero E6 cells to culture as a reproduction marker, and each group of cells was washed and with different concentrations of hrsACE2. The results showed that clinical-grade hrsACE2 could reduce the growth rate of the SARS-CoV-2 in Vero cells by 1000–5000 times [72]. And, exogenous ACE2 negatively regulates the renin-angiotensin system (RAS) and relieves lung damage [66]. Therefore, injection of hrsACE2 has emerged as a potential treatment for acute COVID-19 infection.

5.1.3. Inhibition of TMPRSS2

There is no binding active site between ACE2 and S protein itself, and the activation of S protein needs TMPRSS2 to start [9,73]. Camostat mesylate is a potent serine protease inhibitor, and its effectiveness has been demonstrated in *vitro* to significantly reduce to express SARS-CoV-2 in cells [9]. Complete inhibition was achieved when camostat mesylate and carbenicillinase inhibitor (E−64d) were added, and camostat mesylate had no cytotoxic effect [74]. Therefore, a similar compound may be considered for temporarily treatment of patients infected with SARS-CoV-2. According to data from ClinicalTrials.gov, there are currently 21 clinical trials on camostat. (https://clinicaltrials.gov/ct2/results?cond = camostat+%20mesilate&term = &cntry = &state = &city = &dist =). One study showed that Nafamostat (intravenous formulation) and camostat (oral formulation) have been discussed as TMPRSS2 inhibitors. They also analyzed whether it was associated with a reduced risk of 30-day all-cause mortality in adults with COVID-19. Due to the number of available patients and enrolled patients meeting the primary outcome were small, the RCT evidence is inconclusive to determine whether there is a mortality reduction and safety with either nafamostat or camostat for the treatment of adults with COVID-19 [75]. A small-molecule compound, N-0385, has been identified and characterized by Shapira et al., which exhibits low nanomolar potency and a selectivity index of higher than 10^6 in inhibiting SARS-CoV-2 variants of concern B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma) and B.1.617.2 (Delta) [76]. Notably, in the K18-human ACE2 transgenic mouse model of severe COVID-19, N-0385 affords a high level of prophylactic and therapeutic benefit after multiple administrations or even after a single administration. That result indicates N-0385 provides an effective early treatment option against COVID-19 and emerging SARS-CoV-2 variants of concern.

5.2. Inhibition of viral membrane fusion

Furin is a ubiquitous host calcium-dependent membrane-anchored protease. It activates the precursor protein by cleavage of the basic residue congruent sequence [77]. It is involved in many physiological and pathological processes, such as the inhibition of dengue virus, west nile virus and hepatitis B virus. Replication of viruses and many other viruses [78,79] makes it possible to inhibit the enzyme to control bacterial infections.

Experiments showed that to combine MI-701, oseltamivir, ribavirin, and other inhibitors of peptidofuran can inhibit furan, and long-term blocking of the enzyme may cause toxicity to the body [15]. In addition, Arbidol, an indole derivative, binds to the hydrophobic lumen of the hemagglutinin (HA) trimer, which is located at the distal end of the antigen-conserved epitope to which the neutralizing antibody binds. Arbidol can form hydrophobic interaction with the binding site, and cause certain conformational rearrangements, such as salt bridges, stabilize HA pre-fusion conformation, inhibit virus-mediated membrane fusion, and inhibit virus entry into cells [72]. Clinical trials demonstrated that the mortality of patients treated with arbidol (12 cases) was lower than that of the untreated group (6 cases) [80].Data from ClinicalTrials.gov shows that there are currently 14 clinical trials on Arbidol. (https://clinicaltrials.gov/ct2/results?cond = &term = Arbidol+&cntry = &state = &city = &dist =)

5.3. Inhibition of viral RNA synthesis

Based on viral RNA synthesis required for SARS-CoV-2 exocytosis, therapeutic strategies are divided into the following categories.

5.3.1. Nucleotide analogues inhibit SARS-CoV-2 mRNA synthesis

RdRp is a viral enzyme in the life cycle of RNA viruses. Nucleoside analogues in the form of adenine or guanine derivatives can target and bind to the viral RdRp, block the synthesis of viral nascent template RNA strands on a broad spectrum, and reduce viral replication [81,82]. It can be considered that various nucleotide analogues mRNA synthesis inhibitors, including hepatitis C virus (HCV), Zika virus (ZIKV) and coronavirus (CoVs), can also act on SARS-CoV-2 [83–85].Targeting experiments are conducted using different anti-polymerase drugs that have been approved for a variety of viruses on the market. The results showed that Ribavirin, Renzivir, Sofosbuvir, Galidevir, and Tenofovir are effective drugs against SARS-CoV-2 due to binding closely to RdRp. In addition, the results showed that guanosine derivatives (IDX-184), Setrobuvir, and YAK are the first choice for antiviral therapy, with potential against SARS-CoV-2 strains [86,87]. These drugs do not require toxicity measurement prior to FDA approval because of previous toxicology experiments. Molnupiravir is an antiviral drug with anti- RNA polymerase activity and currently is under investigation for the treatment of patients with COVID-19 [88,89]. In a cohort study, treatment with molnupiravir during the first five days of infection was found to significantly reduce the risk of PASC in patients with SARS-CoV-2 infection who had at least one risk factor for progression to severe covid-19 compared to patients who did not receive treatment [90]. Similarly, another study confirmed that adding molnupiravir to routine care reduced COVID-19-related hospitalizations and deaths in this population, but it did not reduce the frequency of COVID-19-related hospitalizations or deaths among high-risk vaccinated adults in the community [91].

Remdesivir is an adenosine analogue that can integrate into the nascent viral RNA strand and cause it to terminate prematurely. It is shown that remdesivir covalently binds to the 3′ end of the viral RNA primer strand at the first base pair of viral RNA replication,

resulting in termination of the non-mandatory RNA strand and inhibiting viral RdRp activity [92]. Currently, the effectiveness of remdesivir remains controversial. A single-arm sympathetic drug study based on 61 patients showed that 68 % of patients had clinical improvement [93]. While a randomized, open-label study based on 397 patients showed no significant difference between a 5-day course of desivir and the 7-day course of desivir treatment in Switzerland. Due to the trial did not have a placebo control group, the extent of benefit could not be determined [94]. So far, the data of ClinicalTrials.gov has included 85 clinical trials related to remdesivir.

(https://clinicaltrials.gov/ct2/results?cond=&term=remdesivir&cntry=&state=&city=&dist=)

5.3.2. CRISPR-Cas13d system inhibits SARS-CoV-2 mRNA synthesis

Most clinical vaccine trials are performed by the body's immune system to recognize the S protein of SARS-CoV-2 or to attenuate the virus and reduce the work of the virus to enter cells [95,96], while another system, CRISPR, that can identify and degrade the viral genome in the cell Can significantly inhibit SARS, IVA, IVB and other respiratory viruses [97,98].

A type 2 VI-D CRISPR-Cas13d system derived from Rumen coccus aureus XPD3002 combines its customized 22-nt spacer sequence with CRISPR-crRNA and Cas13d proteins to guide specific RNA molecules to target RNA degradation. Cas13d has a small size (2.8 kb), highly effective target and high catalytic rate, and shows high catalytic activity on SARS-CoV-2 nucleic acid degradation and gene sequence expression inhibition in A549 cells, so Cas13d can be used as a new molecule A level approach to provide extensive pan-coronavirus protection against multiple viruses in the same family at the same time [98,99].The stable A549 cell line expressing Cas13d and co-expressing the mCherry marker was infected by lentivirus, using the SARS-CoV-2 strain and the conserved region between the SARS-CoV and MERS-CoV genomes: RdRP gene and N gene as reporter genes After crRNA pool transduction, A549 cells were infected with SARS-CoV-2. The results show that most crRNA pools targeting RdRP and N genes can inhibit to express the reporter gene to a certain extent compared to the control, and the difference in the ability of different crRNA pools to inhibit the SARS-CoV-2 reporter gene may be due to SARS-CoV −2 The inherent RNA secondary structure of genomic fragments or the binding affinity of each crRNA sequence is different [99].

5.4. Inhibition of translation maturity

The proteases M^{pro} and PL^{pro} are the key enzyme bodies for coronavirus replication, as well as the necessary proteases involved in the subsequent RNA transcription and translation of SARS-CoV-2. They are responsible for packaging polypeptides into proteins. The M^{pro} of SARS-CoV-2 is highly like the M^{pro} of SARS-CoV-2. Therefore, drugs can be screened based on the design of SARS-CoV M^{pro}. Currently approved M^{pro} protease inhibitors mainly include nirmatrelvir/ritonavir (Paxlovid), Ensitrelvir, lopinavir/ritonavir tablets, etc., because of its antiviral activity against SARS-CoV and MERS-CoV, it is considered as a treatment at present means.

Previous studies predicted the SARS-CoV-2 M^{pro} protease drug trial has identified two related protease inhibitors, lopinavir and ritonavir, as potential drug candidates, and multiple studies have shown that the triple combination of lopinavir, interferon 1β (IFN-1β) and ribavirin (ribavirin) is more effective than the single medication [100]. However, another study confirmed that lopinavir and ritonavir were inactive and cytotoxic against SARS-CoV-2 Mpro in the FRET assay. They are weak inhibitors of SARS-CoV M^{pro} and do not act as inhibitors of M^{pro} [101]. Six inhibitors including Nirmatrelvir, Ensitrelvir, Boceprevir, GC-376, Calcineurin inhibitor II, and XII were evaluated as inhibitors of the SARS-CoV-2 major protease (M^{pro}) in some studies reporting favorable results with good inhibition of SARS-CoV-2 virus replication [102–104]. First-generation therapeutics have improved clinical outcomes in patients infected with SARS-CoV-2. First-generation therapeutics have improved clinical outcomes in patients infected with SARS-CoV-2 [105]. Ensitrelvir (formerly S-217622), as a potential treatment for SARS-CoV-2 has been reported [106–108]. Ensitrelvir demonstrates strong in *vitro* antiviral activity against the SARS-CoV-2 Omicron subvariants BA.4 and BA.5. As the world's first coronavirus oral drug approved by the FDA, Paxlovid has attracted much attention since its birth [109–111]. In one study, researchers used population-based real-world data to evaluate the effectiveness of Paxlovid [112]. In 180,351 eligible patients, Paxlovid treatment or vaccination resulted in a significant reduction in the incidence or mortality from severe COVID-19. Meanwhile, the National Institutes of Health has conducted a prospective, double-blind, placebo-controlled study of Paxlovid that will evaluate the SARS-CoV-2 antiviral drug nirmatrelvir/ritonavir (Paxlovid) as a potential treatment for long-term COVID [113]. The above-mentioned drugs are current potential treatments. In the treatment of COVID-19, early treatment of lopinavir and ritonavir tablets can shorten the time of virus shedding [114]. However, a further double-blind randomized trial showed that there was no significant difference in clinical improvement or mortality or viral RNA load between the lopinavir ritonavir tablet group (99 cases) and the standard treatment group (100 cases). However, due to adverse reactions, 13.8 % Lopinavir ritonavir tablets treatment group patients stopped the drug at an early stage [115]. Lopinavir and ritonavir currently have 93 clinical trials.(https://clinicaltrials.gov/ct2/results?cond = COVID&term = lopinavir %2Fritonavir&cntry = &state = &city = &dist =)

The SARS-CoV-2 papain-like protease (PL^{pro}) mediates the cleavage of viral polyprotein as well as modulates the host innate immune response upon viral infection, rending it a promising antiviral drug target [116]. In addition, PL^{pro} protease is not only involved in the process of viral protein translation and maturation, but also has deubiquitylation enzyme activity, which can inhibit host immune response and inhibit the PL^{pro} activity related proteases [117]. Together with main protease (Mpro), PL^{pro} is responsible for processing the viral replicase polyprotein into functional units. For example, many studies have reported that GRL-0617 inhibits viral replication of SARS-CoV-2 because its binding site and mode of inhibition are almost identical to that of SARS-CoV PL^{pro}, and these results further consolidate the position of PLpro as a drug target for antiviral therapy of COVID-19 [11,12,117–120]. Therefore, it is an attractive target for antiviral drug development [12,121–123]. For example, Zhao et al., designed an approach using a

SARS-CoV-2 PL^{pro} inhibition activity as the primary screening tool for the discovery of the small molecule inhibitors, with a secondary cell-based assay for evaluation of their antiviral activity. Some compounds (YM155, cryptotanshinone, tanshinone I) that strongly inhibit SARS-CoV-2 PL^{pro} were screened of over 6000 compounds [124]. However, in another study, many compounds, including YM155, cryptotanshinone, tanshinone I, dihydrotanshinone I, Tanshinone IIA, SJB2-043, 6-thioguanine, and 6-mercaptopurine, were shown to be ineffective. Thus, more efforts are needed to find effective and specific SARS-CoV-2 PL^{pro} inhibitors [125]. Shen et al., leveraged the cooperativity of multiple shallow binding sites on the PL^{pro} surface yielding novel 2phenylthiophenes with nanomolar inhibitory potency [126]. With slow off-rates, improved binding affinities, and low micromolar antiviral potency in SARS-CoV-2-infected human cells. This binding cooperativity translates to the most potent PL^{pro} inhibitors. It is worth noting that the papain like protease (PL^{pro}) small molecule inhibitor HL-21 jointly developed by Professor He Wei of the School of Pharmacy of Tsinghua University has obtained the "Drug Clinical Trial Approval Notice", becoming the world's first PL^{pro} inhibitor drug to enter clinical development. These analogues can be used as potential targeted therapeutic agents for SARS-CoV-2. A machine learning-driven discovery of a potent, selective, and orally available SARS-CoV-2 PL^{pro} inhibitor based on the discovery of a lead compound, PF-07957472 (4), with strong efficacy in a mouse-adapted model of COVID-19 infection is shown in a recent study [127]. In the efficacy study, mice were infected 4 h prior to the first dose. They were dosed BID for four days, and then viral lung titers were measured. Compound 4 caused a statistically significant reduction in lung viral titers at four days post-infection for both the 50 and 150 mg/kg dose groups. This reduction occurred at unbound systemic exposures (Cmin) that maintained or exceeded the dNHBE EC90 throughout the dosing period. In a recent study, Wang et al. designed and synthesised 85 non-covalent PLpro inhibitors and revealed the interaction patterns of PLpro with eight lead compounds. In particular, the in vivo lead Jun 1, 2682 inhibited SARS-CoV-2 and its variants, including nirmatrelvir-resistant strains, with EC50s ranging from 0.44 to 2.02 µM. In a mouse model of SARS-CoV-2 infection, oral administration of Jun 1, 2682 increased survival and reduced viral load and lesions in the lungs, suggesting that PLpro inhibitors are promising oral antiviral candidates for SARS-CoV-2 [128].

5.5. Peptide inhibitors

Besides the development of small-molecule therapeutics that target viral proteases, there is also interest molecular tools to inhibit the initial event of viral attachment of the SARS-CoV-2 Spike protein to host ACE2 surface receptor [129]. For example, using peptide arrays, Chopra et al., developed an in *vitro* peptide inhibitor of the Spike-ACE2 interaction. That study highlights the utility of systematic peptide arrays as a platform for the development of coronavirus protein inhibitors [130]. In another study, yang et al., performed structural studies of the HR1HR2 bundle, and designed an extended HR2 peptide that achieves single-digit nanomolar inhibition of SARS-CoV-2 in cell-based and virus-based assays. The peptide also strongly inhibits all major SARS-CoV-2 variants to date [131]. Xia et al., developed a pan-coronavirus fusion inhibitor, EK1, which targeted the HR1 domain and could inhibit infection by divergent human coronaviruses tested, including SARS-CoV and MERS-CoV. One lipopeptide namely EK1C4, which is derived from EK1, was the most potent fusion inhibitor against SARS-CoV-2 S protein-mediated membrane fusion and pseudovirus infection with IC50s of 1.3 and 15.8 Nm. Mouse infection studies showed that EK1C4 can protect mice from infection [132]. Thijssen et al., used messenger RNA (mRNA) display under a reprogrammed genetic code to find a spike-targeting macrocyclic peptide (S1b3inL1) that inhibits SARS-CoV-2 Wuhan strain infection and pseudoviruses containing spike proteins of SARS-CoV-2 variants or related sarbecoviruses. The broadly active macrocyclic peptide S1b3inL1 described, as well as the new druggable site it reveals, demonstrates the power of in *vitro* selection technologies to provide alternative solutions to those offered by small molecules or biologicals [133].

5.6. Broad-spectrum

5.6.1. Chloroquine

Chloroquine is a broad-spectrum drug against malaria and autoimmune disease. According to research reports, chloroquine can effectively inhibit the replication of avian influenza virus H5N1 and influenza B virus [134]. Some studies have shown that chloroquine can inhibit the SARS-CoV-2 in vitro. Recent experimental studies have shown that oral chloroquine has an EC90 of 6.90 µmol/L for SARS-CoV-2 in Vero. It influences the invasion and post-invasion stages of SARS-CoV-2 infection in Vero E6 cells. At the same time, the severity, antipyretic phenomenon, lung imaging improvement time, negative transformation time and viral nucleic acid transfer rate of patients treated with chloroquine were all better than those in the control group, which could shorten the course of disease. In addition, chloroquine phosphate has been found to be effective against COVID-19. In addition to this activity, chloroquine is based on the ability to regulate the immune system and can cooperate with other cells in the body to enhance resistance to viruses [135]. However, a multi-center study based on 14,888 patients showed that neither chloroquine nor hydroxychloroquine would be beneficial to patient outcomes, and the study was withdrawn due to insufficient sample information [136]. There are currently 92 clinical trials on chloroquine. However, it is necessary to expand the scope of clinical trials to further verify this conclusion. Further, the specific mechanism of chloroquine on the novel coronavirus needs further research. (https://clinicaltrials.gov/ct2/results?cond = covid&term = chloroquine&cntry = &state = &city = &dist =).

5.6.2. Interferon

Both viral and host factors influence the outcome of IFN signal transduction. IFN signal transduction may be a threat to patients because of its virus-mediated systemic pro-inflammatory effects [137]. And whether the IFN response is protective or pathogenic in SARS and MERS depends on the environment in which IFN signals are induced. Its inhibitory effect has been demonstrated in various animal models [138,139].

According to previous reports, compared with the infection of Influenza Viruses (IAV), IFN-α and interferon-stimulated gene (ISG) are delayed in induction of SARS-CoV and MERS-CoV infection [140]. While in the cohort of COVID-19 patients, the IFN-α and ISG levels correlated with viral load. The quantity is a function of the seriousness of the disease. Serious infections can cause elevated IFN signals, but cannot reduce the viral load. At the same time, studies have shown that SARS-CoV can inhibit the production of type I interferon, which leads to the activation of pro-inflammatory mononuclear macrophages and cytokines in the lungs, resulting lung injury. The virus also inhibits the transduction of interferon signals and increases interference. Besides, in most studies, all patients received interferon therapy [141,142], so it is impossible to decide whether the efficacy comes from interferon or other drugs or the result of the disease itself.

5.7. Chinese medicine

Traditional Chinese medicine has achieved good results in treating infectious diseases [143]. During China's fight against the disease, 92 percent of confirmed COVID-19 cases received integrated treatment with traditional Chinese and western medicine, and more than 90 percent responded effectively to treatment or showed significant improvement [144–147]. For patients with mild to moderate disease, early intervention with traditional Chinese medicine has been shown to be effective in preventing the disease from transitioning to severe and critical conditions. In severe cases, TCM can help stabilize the condition and extend the treatment window. In China's fight against COVID-19, six TCM prescriptions - JHQG, LHQW, XFBD, HSBD, XBJ and LCDD - have proved to be the most effective in treating COVID-19 patients. These ingredients may play a therapeutic role in treating COVID-19 by targeting ACE2, 3CLpro and IL-6. Among them, Jinhua Qinggan Granules and Lianhua Qingwen capsules are recommended for the treatment of patients under medical observation/in the early stage of disease development with fatigue and fever as the main clinical manifestations [148,149]. Qingfei Detoxification Decoction is recommended to treat moderately and severely infected patients; Xuanfeihuadu granules are recommended to treat moderate cases; Huashuifengdu granules and Xuebijing are recommended for the treatment of severe cases (novel coronavirus pneumonia). Therefore, the key active ingredients in TCM compounds and the molecular mechanisms that drive the therapeutic effect of COVID-19 deserve further study to help better manage this devastating disease.

6. Conclusions

The COVID-19 pandemic, which broke out in December 2019, has continued to ravage the world, causing huge losses in production and life. There are still a lot of issues that we need know of, and many difficulties in developing antiviral drugs; Whether the special furin site is one reasons for the strong infectiousness of SARS-CoV-2. Whether protease action can be ignored for S protein-dependent endocytosis; Whether there is a faster, simpler and more economical method for detecting SARS-CoV-2; The variability of SARS-CoV-2 single-stranded RNA makes it difficult for inhibitors and specific monoclonal antibodies to function; Whether some treatment methods based on clinical small sample studies, such as plasma, antibody infusion, interferon, etc. can pass clinical RCT research; And the issue of is most concern: to develop various SARS-CoV-2 vaccines, there are still many problems. Due to the global pandemic and the variability of the COVID-19 epidemic, their development faces both opportunities and challenges. Although many targeted drug research and clinical trials for SARS-CoV-2 have laid a solid foundation for the treatment of COVID-19. What we need to be wary of is that, assuming the initial success of vaccines, the SARS-CoV-2 may come back due to the emergence of subtypes and mutations of the virus or even become a seasonal epidemic. It is worth noting that while the scientific community has developed several therapeutic monoclonal antibodies and small molecule drugs for clinical use over the past four years or so of the COVID-19 pandemic, it cannot be ignored that continued viral transmission, ongoing evolution and increasing selective pressures have the potential to generate viral variants that are resistant to these interventions [150,151]. This also leads to viruses capable of becoming resistant to antiviral drugs. This is exacerbated by the fact that in vitro and in vivo resistance has been reported for almost all licensed or approved SARS-CoV-2 therapeutic antivirals. Some drug-resistant variants exist before these drugs enter the human body and can spread [104]. Therefore, subsequent concerted efforts by the scientific community are needed to monitor resistance variants and to develop more protease inhibitors and other antiviral drugs with different mechanisms of action and resistance profiles for combination therapy.

Data availability statement

Data availability is not applicable to this article as no new data were created or analyzed in this study.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Yang Li: Writing – original draft, Data curation. **Si-Ming Lu:** Writing – original draft, Conceptualization. **Jia-Long Wang:** Writing – original draft, Investigation, Formal analysis, Data curation. **Hang-Ping Yao:** Writing – review & editing, Funding acquisition. **Li-Guo Liang:** Writing – review & editing, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. 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, Z.L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (7798) (2020) 270–273.

[2] 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, W. Tan, Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (10224) (2020) 565–574.

[3] D. Blanco-Melo, B.E. Nilsson-Payant, W.C. Liu, S. Uhl, D. Hoagland, R. Moller, T.X. Jordan, A. Oishi, M. Panis, D. Sachs, T.T. Wang, R.E. Schwartz, J.K. Lim, R. A. Albrecht, B.R. tenOever, Imbalanced host response to SARS-CoV-2 drives development of COVID-19, Cell 181 (5) (2020) 1036–1045 e9.

[4] H. Zhang, M.R. Rostami, P.L. Leopold, J.G. Mezey, S.L. O'Beirne, Y. Strulovici-Barel, R.G. Crystal, Expression of the SARS-CoV-2 ACE2 receptor in the human airway epithelium, Am. J. Respir. Crit. Care Med. 202 (2) (2020) 219–229.

[5] C. Blume, C.L. Jackson, C.M. Spalluto, J. Legebeke, L. Nazlamova, F. Conforti, J.M. Perotin, M. Frank, J. Butler, M. Crispin, J. Coles, J. Thompson, R.A. Ridley, L.S.N. Dean, M. Loxham, S. Reikine, A. Azim, K. Tariq, D.A. Johnston, P.J. Skipp, R. Djukanovic, D. Baralle, C.J. McCormick, D.E. Davies, J.S. Lucas, G. Wheway, V. Mennella, A novel ACE2 isoform is expressed in human respiratory epithelia and is upregulated in response to interferons and RNA respiratory virus infection, Nat. Genet. 53 (2) (2021) 205–214.

[6] I. Hamming, W. Timens, M.L. Bulthuis, A.T. Lely, G. Navis, H. van Goor, Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis, J. Pathol. 203 (2) (2004) 631–637.

[7] V. Coronaviridae Study Group of the International Committee on Taxonomy of, The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2, Nat Microbiol 5 (4) (2020) 536–544.

[8] X. Xue, H. Yu, H. Yang, F. Xue, Z. Wu, W. Shen, J. Li, Z. Zhou, Y. Ding, C. Zhao, X.C. Zhang, M. Liao, M. Bartlam, Z. Rao, Structures of two coronavirus main proteases: implications for substrate binding and antiviral drug design, J. Virol. 82 (5) (2008) 2515–2527.

[9] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Muller, C. Drosten, S. Pohlmann, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell 181 (2) (2020) 271–280 e8.

[10] J. Shang, G. Ye, K. Shi, Y. Wan, C. Luo, H. Aihara, Q. Geng, A. Auerbach, F. Li, Structural basis of receptor recognition by SARS-CoV-2, Nature 581 (7807) (2020) 221–224.

[11] Z. Fu, B. Huang, J. Tang, S. Liu, M. Liu, Y. Ye, Z. Liu, Y. Xiong, W. Zhu, D. Cao, J. Li, X. Niu, H. Zhou, Y.J. Zhao, G. Zhang, H. Huang, The complex structure of GRL0617 and SARS-CoV-2 PLpro reveals a hot spot for antiviral drug discovery, Nat. Commun. 12 (1) (2021) 488.

[12] D. Shin, R. Mukherjee, D. Grewe, D. Bojkova, K. Baek, A. Bhattacharya, L. Schulz, M. Widera, A.R. Mehdipour, G. Tascher, P.P. Geurink, A. Wilhelm, G.J. van der Heden van Noort, H. Ovaa, S. Muller, K.P. Knobeloch, K. Rajalingam, B.A. Schulman, J. Cinatl, G. Hummer, S. Ciesek, I. Dikic, Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity, Nature 587 (7835) (2020) 657–662.

[13] A.C. Walls, Y.J. Park, M.A. Tortorici, A. Wall, A.T. McGuire, D. Veesler, Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein, Cell 181 (2) (2020) 281–292 e6.

[14] C. Liu, Q. Zhou, Y. Li, L.V. Garner, S.P. Watkins, L.J. Carter, J. Smoot, A.C. Gregg, A.D. Daniels, S. Jervey, D. Albaiu, Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases, ACS Cent. Sci. 6 (3) (2020) 315–331.

[15] M.S. Sarac, A. Cameron, I. Lindberg, The furin inhibitor hexa-D-arginine blocks the activation of Pseudomonas aeruginosa exotoxin A in vivo, Infect. Immun. 70 (12) (2002) 7136–7139.

[16] L. Yan, Y. Zhang, J. Ge, L. Zheng, Y. Gao, T. Wang, Z. Jia, H. Wang, Y. Huang, M. Li, Q. Wang, Z. Rao, Z. Lou, Architecture of a SARS-CoV-2 mini replication and transcription complex, Nat. Commun. 11 (1) (2020) 5874.

[17] A.J. te Velthuis, J.J. Arnold, C.E. Cameron, S.H. van den Worm, E.J. Snijder, The RNA polymerase activity of SARS-coronavirus nsp12 is primer dependent, Nucleic Acids Res. 38 (1) (2010) 203–214.

[18] L. Neufeld, E. Yeini, S. Pozzi, R. Satchi-Fainaro, 3D bioprinted cancer models: from basic biology to drug development, Nat. Rev. Cancer 22 (12) (2022) 679–692.

[19] D. Chimene, R. Kaunas, A.K. Gaharwar, Hydrogel bioink reinforcement for additive manufacturing: a focused review of emerging strategies, Adv. Mater. 32 (1) (2020) e1902026.

[20] R. Hilgenfeld, From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design, FEBS J. 281 (18) (2014) 4085–4096.

[21] J. Shi, J. Song, The catalysis of the SARS 3C-like protease is under extensive regulation by its extra domain, FEBS J. 273 (5) (2006) 1035–1045.

[22] A. Awad, C.M. Madla, L.E. McCoubrey, F. Ferraro, F.K.H. Gavins, A. Buanz, S. Gaisford, M. Orlu, F. Siepmann, J. Siepmann, A.W. Basit, Clinical translation of advanced colonic drug delivery technologies, Adv. Drug Deliv. Rev. 181 (2022) 114076.

[23] J.Y. Song, J.G. Yun, J.Y. Noh, H.J. Cheong, W.J. Kim, Covid-19 in South Korea - challenges of subclinical manifestations, N. Engl. J. Med. 382 (19) (2020) 1858–1859.

[24] 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, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (10223) (2020) 497–506.

[25] G. Chen, D. Wu, W. Guo, Y. Cao, D. Huang, H. Wang, T. Wang, X. Zhang, H. Chen, H. Yu, X. Zhang, M. Zhang, S. Wu, J. Song, T. Chen, M. Han, S. Li, X. Luo, J. Zhao, Q. Ning, Clinical and immunological features of severe and moderate coronavirus disease 2019, J. Clin. Invest. 130 (5) (2020) 2620–2629.

[26] A.J. Rodriguez-Morales, J.A. Cardona-Ospina, E. Gutierrez-Ocampo, R. Villamizar-Pena, Y. Holguin-Rivera, J.P. Escalera-Antezana, L.E. Alvarado-Arnez, D. K. Bonilla-Aldana, C. Franco-Paredes, A.F. Henao-Martinez, A. Paniz-Mondolfi, G.J. Lagos-Grisales, E. Ramirez-Vallejo, J.A. Suarez, L.I. Zambrano, W. E. Villamil-Gomez, G.J. Balbin-Ramon, A.A. Rabaan, H. Harapan, K. Dhama, H. Nishiura, H. Kataoka, T. Ahmad, R. Sah, a.h.w.l.o C.-R.E, Latin American Network of Coronavirus Disease, Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis, Trav. Med. Infect. Dis. 34 (2020) 101623.

[27] 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, Z. Peng, Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in wuhan, China, JAMA 323 (11) (2020) 1061–1069.

[28] S. Maurea, C. Bombace, C.G. Mainolfi, A. Annunziata, L. Attanasio, A. Stanzione, E. Matano, B. Mucci, A. D'Ambrosio, C. Giordano, M. Petretta, S. Del Vecchio, A. Cuocolo, Impact of COVID-19 pandemic on 2-[(18)F]FDG PET/CT imaging work-flow in a single medical institution: comparison among the three Italian waves, Heliyon 8 (1) (2022) e08819.

[29] A. Stanzione, A. Ponsiglione, R. Cuocolo, M. Rumolo, M. Santarsiere, R. Scotto, G. Viceconte, M. Imbriaco, S. Maurea, L. Camera, I. Gentile, A. Brunetti, Chest CT in COVID-19 patients: structured vs conventional reporting, Eur. J. Radiol. 138 (2021) 109621.

[30] V. Romeo, A. Stanzione, D. D'Auria, L. Fulgione, F. Giusto, S. Maurea, A. Brunetti, COVID-19 vaccine-induced lymphadenopathies: incidence, course and imaging features from an ultrasound prospective study, J Ultrasound 25 (4) (2022) 965–971.

[31] C. Sgamato, A. Rocco, D. Compare, S. Minieri, S.A. Marchitto, S. Maurea, G. Nardone, Autoimmune liver diseases and SARS-CoV-2, World J. Gastroenterol. 29 (12) (2023) 1838–1851.

[32] Y.W. Tang, J.E. Schmitz, D.H. Persing, C.W. Stratton, Laboratory diagnosis of COVID-19: current issues and challenges, J. Clin. Microbiol. 58 (6) (2020).

[33] 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, N.S. Zhong, C. China Medical Treatment Expert Group for, Clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (18) (2020) 1708–1720.

[34] A.V. Dora, A. Winnett, L.P. Jatt, K. Davar, M. Watanabe, L. Sohn, H.S. Kern, C.J. Graber, M.B. Goetz, Universal and serial laboratory testing for SARS-CoV-2 at a long-term care skilled nursing facility for veterans - Los Angeles, California, MMWR Morb. Mortal. Wkly. Rep. 69 (21) (2020) 651–655, 2020.

[35] I. Smyrlaki, M. Ekman, A. Lentini, N. Rufino de Sousa, N. Papanicolaou, M. Vondracek, J. Aarum, H. Safari, S. Muradrasoli, A.G. Rothfuchs, J. Albert, B. Hogberg, B. Reinius, Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR, Nat. Commun. 11 (1) (2020) 4812.

[36] N. Barak, R. Ben-Ami, T. Sido, A. Perri, M. Rivkin, T. Licht, A. Peretz, J. Magenheim, I. Fogel, A. Livneh, Y. Daitch, E. Oiknine-Djian, G. Benedek, Y. Dor, D.G. Wolf, M. Yassour, C.-D.T. Hebrew University-Hadassah, Lessons from applied large-scale pooling of 133,816 SARS-CoV-2 RT-PCR tests, Sci. Transl. Med. 13 (589) (2021).

[37] Y. Zhang, Y. Xu, A. Simon-Masseron, J. Lalevée, Radical photoinitiation with LEDs and applications in the 3D printing of composites, Chem. Soc. Rev. 50 (6) (2021) 3824–3841.

[38] S. You, Y. Xiang, H.H. Hwang, D.B. Berry, W. Kiratitanaporn, J. Guan, E. Yao, M. Tang, Z. Zhong, X. Ma, D. Wangpraseurt, Y. Sun, T.Y. Lu, S. Chen, High cell density and high-resolution 3D bioprinting for fabricating vascularized tissues, Sci. Adv. 9 (8) (2023) eade7923.

[39] N. Chotiwan, C.D. Brewster, T. Magalhaes, J. Weger-Lucarelli, N.K. Duggal, C. Ruckert, C. Nguyen, S.M. Garcia Luna, J.R. Fauver, B. Andre, M. Gray, W.C. t. Black, R.C. Kading, G.D. Ebel, G. Kuan, A. Balmaseda, T. Jaenisch, E.T.A. Marques, A.C. Brault, E. Harris, B.D. Foy, S.L. Quackenbush, R. Perera, J. Rovnak, Rapid and specific detection of asian- and african-lineage Zika viruses, Sci. Transl. Med. 9 (388) (2017).

[40] A.S. Fomsgaard, M.W. Rosenstierne, An alternative workflow for molecular detection of SARS-CoV-2 - escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020, Euro Surveill. 25 (14) (2020).

[41] V.L. Dao Thi, K. Herbst, K. Boerner, M. Meurer, L.P. Kremer, D. Kirrmaier, A. Freistaedter, D. Papagiannidis, C. Galmozzi, M.L. Stanifer, S. Boulant, S. Klein, P. Chlanda, D. Khalid, I. Barreto Miranda, P. Schnitzler, H.G. Kräusslich, M. Knop, S. Anders, A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples, Sci. Transl. Med. 12 (556) (2020).

[42] L. Bokelmann, O. Nickel, T. Maricic, S. Paabo, M. Meyer, S. Borte, S. Riesenberg, Point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP, Nat. Commun. 12 (1) (2021) 1467.

[43] B.A. Rabe, C. Cepko, SARS-CoV-2 detection using isothermal amplification and a rapid, inexpensive protocol for sample inactivation and purification, Proc. Natl. Acad. Sci. U.S.A. 117 (39) (2020) 24450–24458.

[44] O. Behrmann, I. Bachmann, M. Spiegel, M. Schramm, A. Abd El Wahed, G. Dobler, G. Dame, F.T. Hufert, Rapid detection of SARS-CoV-2 by low volume real-time single tube reverse transcription recombinase polymerase amplification using an exo probe with an internally linked quencher (Exo-IQ), Clin. Chem. 66 (8) (2020) 1047–1054.

[45] C. Myhrvold, C.A. Freije, J.S. Gootenberg, O.O. Abudayyeh, H.C. Metsky, A.F. Durbin, M.J. Kellner, A.L. Tan, L.M. Paul, L.A. Parham, K.F. Garcia, K.G. Barnes, B. Chak, A. Mondini, M.L. Nogueira, S. Isern, S.F. Michael, I. Lorenzana, N.L. Yozwiak, B.L. MacInnis, I. Bosch, L. Gehrke, F. Zhang, P.C. Sabeti, Field-deployable viral diagnostics using CRISPR-Cas13, Science 360 (6387) (2018) 444–448.

[46] D. Zucha, P. Androvic, M. Kubista, L. Valihrach, Performance comparison of reverse transcriptases for single-cell studies, Clin. Chem. 66 (1) (2020) 217–228.

[47] R. Dehbandi, M.A. Zazouli, Stability of SARS-CoV-2 in different environmental conditions, The Lancet Microbe 1 (4) (2020).

[48] J. Qian, S.A. Boswell, C. Chidley, Z.X. Lu, M.E. Pettit, B.L. Gaudio, J.M. Fajnzylber, R.T. Ingram, R.H. Ward, J.Z. Li, M. Springer, An enhanced isothermal amplification assay for viral detection, Nat. Commun. 11 (1) (2020) 5920.

[49] D.O. Andrey, P. Cohen, B. Meyer, G. Torriani, S. Yerly, L. Mazza, A. Calame, I. Arm-Vernez, I. Guessous, S. Stringhini, P. Roux-Lombard, L. Fontao, T. Agoritsas, J. Stirnemann, J.L. Reny, C.A. Siegrist, I. Eckerle, L. Kaiser, N. Vuilleumier, Diagnostic accuracy of Augurix COVID-19 IgG serology rapid test, Eur. J. Clin. Invest. 50 (10) (2020) e13357.

[50] F. Krammer, V. Simon, Serology assays to manage COVID-19, Science 368 (6495) (2020) 1060–1061.

[51] M. Lisboa Bastos, G. Tavaziva, S.K. Abidi, J.R. Campbell, L.P. Haraoui, J.C. Johnston, Z. Lan, S. Law, E. MacLean, A. Trajman, D. Menzies, A. Benedetti, F. Ahmad Khan, Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis, Bmj 370 (2020) m2516.

[52] W. Xu, D. Song, J. Liu, X. Han, J. Xu, A. Zhu, F. Long, Development of chemiluminescent lab-on-fiber immunosensors for rapid point-of-care testing of anti-SARS-CoV-2 antibodies and evaluation of longitudinal immune response kinetics following three-dose inactivation virus vaccination, J. Med. Virol. 95 (1) (2023) e28190.

[53] R. Dolscheid-Pommerich, E. Bartok, M. Renn, B.M. Kümmerer, B. Schulte, R.M. Schmithausen, B. Stoffel-Wagner, H. Streeck, S. Saschenbrecker, K. Steinhagen, G. Hartmann, Correlation between a quantitative anti-SARS-CoV-2 IgG ELISA and neutralization activity, J. Med. Virol. 94 (1) (2022) 388–392.

[54] L. Villafañe, L.G. Vaulet, F.M. Viere, L.I. Klepp, M.A. Forrellad, M.M. Romano, M.I. Romano, G. Magistrelli, M.R. Fermepin, F. Bigi, Development and evaluation of a low cost IgG ELISA test based in RBD protein for COVID-19, J. Immunol. Methods 500 (2022) 113182.

[55] A.E. Muruato, C.R. Fontes-Garfias, P. Ren, M.A. Garcia-Blanco, V.D. Menachery, X. Xie, P.-Y. Shi, A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation, Nat. Commun. 11 (1) (2020).

[56] L.J. Carter, L.V. Garner, J.W. Smoot, Y. Li, Q. Zhou, C.J. Saveson, J.M. Sasso, A.C. Gregg, D.J. Soares, T.R. Beskid, S.R. Jervey, C. Liu, Assay techniques and test development for COVID-19 diagnosis, ACS Cent. Sci. 6 (5) (2020) 591–605.

[57] R. Funari, K.Y. Chu, A.Q. Shen, Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto-microfluidic chip, Biosens. Bioelectron. 169 (2020) 112578.

[58] H.S. Kim, N. Abbas, S. Shin, A rapid diagnosis of SARS-CoV-2 using DNA hydrogel formation on microfluidic pores, Biosens. Bioelectron. 177 (2021) 113005.

[59] P. Mehta, D.F. McAuley, M. Brown, E. Sanchez, R.S. Tattersall, J.J. Manson, COVID-19: consider cytokine storm syndromes and immunosuppression, Lancet 395 (10229) (2020) 1033–1034.

[60] S. Yang, Y. Li, L. Dai, J. Wang, P. He, C. Li, X. Fang, C. Wang, X. Zhao, E. Huang, C. Wu, Z. Zhong, F. Wang, X. Duan, S. Tian, L. Wu, Y. Liu, Y. Luo, Z. Chen, F. Li, J. Li, X. Yu, H. Ren, L. Liu, S. Meng, J. Yan, Z. Hu, L. Gao, G.F. Gao, Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials, Lancet Infect. Dis. 21 (8) (2021) 1107–1119.

[61] F.P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J.L. Perez, G. Perez Marc, E.D. Moreira, C. Zerbini, R. Bailey, K.A. Swanson, S. Roychoudhury, K. Koury, P. Li, W.V. Kalina, D. Cooper, R.W. Frenck Jr., L.L. Hammitt, O. Tureci, H. Nell, A. Schaefer, S. Unal, D.B. Tresnan, S. Mather, P. R. Dormitzer, U. Sahin, K.U. Jansen, W.C. Gruber, C.C.T. Group, Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine, N. Engl. J. Med. 383 (27) (2020) 2603–2615.

[62] P.M. Folegatti, K.J. Ewer, P.K. Aley, B. Angus, S. Becker, S. Belij-Rammerstorfer, D. Bellamy, S. Bibi, M. Bittaye, E.A. Clutterbuck, C. Dold, S.N. Faust, A. Finn, A.L. Flaxman, P. Heath, D. Jenkin, R. Lazarus, R. Makinson, A.M. Minassian, K.M. Pollock, M. Ramasamy, H. Robinson, M. Snape, R. Tarrant,

M. Voysey, C. Green, A.D. Douglas, A.V.S. Hill, T. Lambe, S.C. Gilbert, A.J. Pollard, J. Aboagye, K. Adams, A. Ali, E. Allen, J.L. Allison, R. Anslow, E.H. Arbe-Barnes, G. Babbage, K. Baillie, M. Baker, N. Baker, P. Baker, I. Baleanu, J. Ballaminut, E. Barnes, J. Barrett, L. Bates, A. Batten, K. Beadon, R. Beckley, E. Berrie, L. Berry, A. Beveridge, K.R. Bewley, E.M. Bijker, T. Bingham, L. Blackwell, C.L. Blundell, E. Bolam, E. Boland, N. Borthwick, T. Bower, A. Boyd, T. Brenner, P. D. Bright, C. Brown-O'Sullivan, E. Brunt, J. Burbage, S. Burge, K.R. Buttigieg, N. Byard, I. Cabera Puig, A. Calvert, S. Camara, M. Cao, F. Cappuccini, M. Carr, M.W. Carroll, V. Carter, K. Cathie, R.J. Challis, S. Charlton, I. Chelysheva, J.-S. Cho, P. Cicconi, L. Cifuentes, H. Clark, E. Clark, T. Cole, R. Colin-Jones, C. P. Conlon, A. Cook, N.S. Coombes, R. Cooper, C.A. Cosgrove, K. Coy, W.E.M. Crocker, C.J. Cunningham, B.E. Damratoski, L. Dando, M.S. Datoo, H. Davies, H. De Graaf, T. Demissie, C. Di Maso, I. Dietrich, T. Dong, F.R. Donnellan, N. Douglas, C. Downing, J. Drake, R. Drake-Brockman, R.E. Drury, S.J. Dunachie, N. J. Edwards, F.D.L. Edwards, C.J. Edwards, S.C. Elias, M.J. Elmore, K.R.W. Emary, M.R. English, S. Fagerbrink, S. Felle, S. Feng, S. Field, C. Fixmer, C. Fletcher, K.J. Ford, J. Fowler, P. Fox, E. Francis, J. Frater, J. Furze, M. Fuskova, E. Galiza, D. Gbesemete, C. Gilbride, K. Godwin, G. Gorini, L. Goulston, C. Grabau, L. Gracie, Z. Gray, L.B. Guthrie, M. Hackett, S. Halwe, E. Hamilton, J. Hamlyn, B. Hanumunthadu, I. Harding, S.A. Harris, A. Harris, D. Harrison, C. Harrison, T. C. Hart, L. Haskell, S. Hawkins, I. Head, J.A. Henry, J. Hill, S.H.C. Hodgson, M.M. Hou, E. Howe, N. Howell, C. Hutlin, S. Ikram, C. Isitt, P. Iveson, S. Jackson, F. Jackson, S.W. James, M. Jenkins, E. Jones, K. Jones, C.E. Jones, B. Jones, R. Kailath, K. Karampatsas, J. Keen, S. Kelly, D. Kelly, D. Kerr, S. Kerridge, L. Khan, U. Khan, A. Killen, J. Kinch, T.B. King, L. King, J. King, L. Kingham-Page, P. Klenerman, F. Knapper, J.C. Knight, D. Knott, S. Koleva, A. Kupke, C. W. Larkworthy, J.P.J. Larwood, A. Laskey, A.M. Lawrie, A. Lee, K.Y. Ngan Lee, E.A. Lees, H. Legge, A. Lelliott, N.-M. Lemm, A.M. Lias, A. Linder, S. Lipworth, X. Liu, S. Liu, R. Lopez Ramon, M. Lwin, F. Mabesa, M. Madhavan, G. Mallett, K. Mansatta, I. Marcal, S. Marinou, E. Marlow, J.L. Marshall, J. Martin, J. McEwan, L. McInroy, G. Meddaugh, A.J. Mentzer, N. Mirtorabi, M. Moore, E. Moran, E. Morey, V. Morgan, S.J. Morris, H. Morrison, G. Morshead, R. Morter, Y.F. Mujadidi, J. Muller, T. Munera-Huertas, C. Munro, A. Munro, S. Murphy, V.J. Munster, P. Mweu, A. Noé, F.L. Nugent, E. Nuthall, K. O'Brien, D. O'Connor, B. Oguti, J.L. Oliver, C. Oliveira, P.J. O'Reilly, M. Osborn, P. Osborne, C. Owen, D. Owens, N. Owino, M. Pacurar, K. Parker, H. Parracho, M. Patrick-Smith, V. Payne, J. Pearce, Y. Peng, M.P. Peralta Alvarez, J. Perring, K. Pfafferott, D. Pipini, E. Plested, H. Pluess-Hall, K. Pollock, I. Poulton, L. Presland, S. Provstgaard-Morys, D. Pulido, K. Radia, F. Ramos Lopez, J. Rand, H. Ratcliffe, T. Rawlinson, S. Rhead, A. Riddell, A.J. Ritchie, H. Roberts, J. Robson, S. Roche, C. Rohde, C.S. Rollier, R. Romani, I. Rudiansyah, S. Saich, S. Sajjad, S. Salvador, L. Sanchez Riera, H. Sanders, K. Sanders, S. Sapaun, C. Sayce, E. Schofield, G. Screaton, B. Selby, C. Semple, H.R. Sharpe, I. Shaik, A. Shea, H. Shelton, S. Silk, L. Silva-Reyes, D.T. Skelly, H. Smee, C.C. Smith, D.J. Smith, R. Song, A.J. Spencer, E. Stafford, A. Steele, E. Stefanova, L. Stockdale, A. Szigeti, A. Tahiri-Alaoui, M. Tait, H. Talbot, R. Tanner, I.J. Taylor, V. Taylor, R. Te Water Naude, N. Thakur, Y. Themistocleous, A. Themistocleous, M. Thomas, T.M. Thomas, A. Thompson, S. Thomson-Hill, J. Tomlins, S. Tonks, J. Towner, N. Tran, J.A. Tree, A. Truby, K. Turkentine, C. Turner, N. Turner, S. Turner, T. Tuthill, M. Ulaszewska, R. Varughese, N. Van Doremalen, K. Veighey, M. K. Verheul, I. Vichos, E. Vitale, L. Walker, M.E.E. Watson, B. Welham, J. Wheat, C. White, R. White, A.T. Worth, D. Wright, S. Wright, X.L. Yao, Y. Yau, Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial, Lancet 396 (10249) (2020) 467–478.

[63] D. Wrapp, N. Wang, K.S. Corbett, J.A. Goldsmith, C.L. Hsieh, O. Abiona, B.S. Graham, J.S. McLellan, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation, Science 367 (6483) (2020) 1260–1263.

[64] R. Yan, Y. Zhang, Y. Li, L. Xia, Y. Guo, Q. Zhou, Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2, Science 367 (6485) (2020) 1444–1448.

[65] K. Kuba, Y. Imai, S. Rao, H. Gao, F. Guo, B. Guan, Y. Huan, P. Yang, Y. Zhang, W. Deng, L. Bao, B. Zhang, G. Liu, Z. Wang, M. Chappell, Y. Liu, D. Zheng, A. Leibbrandt, T. Wada, A.S. Slutsky, D. Liu, C. Qin, C. Jiang, J.M. Penninger, A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury, Nat. Med. 11 (8) (2005) 875–879.

[66] K. Kuba, Y. Imai, T. Ohto-Nakanishi, J.M. Penninger, Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters, Pharmacol. Ther. 128 (1) (2010) 119–128.

[67] M. Hasso, S. Perusini, A. Eshaghi, E. Tang, R. Olsha, H. Zhang, E. Lau, A. Sullivan, K. Cronin, S. Lee, J. Obando, C. DeLima, S. Nagra, T. Braukmann, V. R. Duvvuri, M. Richard-Greenblatt, A. Corbeil, J.V. Kus, A. Majury, S. Patel, J.B. Gubbay, Monkeypox virus detection in different clinical specimen types, Emerg. Infect. Dis. 28 (12) (2022) 2513–2515.

[68] X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Z. Xiang, Z. Mu, X. Chen, J. Chen, K. Hu, Q. Jin, J. Wang, Z. Qian, Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV, Nat. Commun. 11 (1) (2020) 1620.

[69] P. Serfozo, J. Wysocki, G. Gulua, A. Schulze, M. Ye, P. Liu, J. Jin, M. Bader, T. Myohanen, J.A. Garcia-Horsman, D. Batlle, Ang II (angiotensin II) conversion to angiotensin-(1-7) in the circulation is POP (Prolyloligopeptidase)-Dependent and ACE2 (Angiotensin-Converting enzyme 2)-independent, Hypertension 75 (1) (2020) 173–182.

[70] A. Khan, C. Benthin, B. Zeno, T.E. Albertson, J. Boyd, J.D. Christie, R. Hall, G. Poirier, J.J. Ronco, M. Tidswell, K. Hardes, W.M. Powley, T.J. Wright, S. K. Siederer, D.A. Fairman, D.A. Lipson, A.I. Bayliffe, A.L. Lazaar, A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome, Crit. Care 21 (1) (2017) 234.

[71] H. Zhang, J.M. Penninger, Y. Li, N. Zhong, A.S. Slutsky, Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target, Intensive Care Med. 46 (4) (2020) 586–590.

[72] V. Monteil, H. Kwon, P. Prado, A. Hagelkruys, R.A. Wimmer, M. Stahl, A. Leopoldi, E. Garreta, C. Hurtado Del Pozo, F. Prosper, J.P. Romero, G. Wirnsberger, H. Zhang, A.S. Slutsky, R. Conder, N. Montserrat, A. Mirazimi, J.M. Penninger, Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2, Cell 181 (4) (2020) 905–913 e7.

[73] N. Iwata-Yoshikawa, T. Okamura, Y. Shimizu, O. Kotani, H. Sato, H. Sekimukai, S. Fukushi, T. Suzuki, Y. Sato, M. Takeda, M. Tashiro, H. Hasegawa, N. Nagata, Acute respiratory infection in human dipeptidyl peptidase 4-transgenic mice infected with Middle East respiratory syndrome coronavirus, J. Virol. 93 (6) (2019).

[74] O. Bardou, A. Menou, C. Francois, J.W. Duitman, J.H. von der Thusen, R. Borie, K.U. Sales, K. Mutze, Y. Castier, E. Sage, L. Liu, T.H. Bugge, D.P. Fairlie, M. Konigshoff, B. Crestani, K.S. Borensztajn, Membrane-anchored serine protease matriptase is a trigger of pulmonary fibrogenesis, Am. J. Respir. Crit. Care Med. 193 (8) (2016) 847–860.

[75] M.P. Hernandez-Mitre, S.C. Morpeth, B. Venkatesh, T.E. Hills, J. Davis, R.K. Mahar, G. McPhee, M. Jones, J. Totterdell, S.Y.C. Tong, J.A. Roberts, TMPRSS2 inhibitors for the treatment of COVID-19 in adults: a systematic review and meta-analysis of randomized clinical trials of nafamostat and camostat mesylate, Clin. Microbiol. Infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 30 (6) (2024) 743–754.

[76] T. Shapira, I.A. Monreal, S.P. Dion, D.W. Buchholz, B. Imbiakha, A.D. Olmstead, M. Jager, A. Desilets, G. Gao, M. Martins, T. Vandal, C.A.H. Thompson, A. Chin, W.D. Rees, T. Steiner, I.R. Nabi, E. Marsault, J. Sahler, D.G. Diel, G.R. Van de Walle, A. August, G.R. Whittaker, P.L. Boudreault, R. Leduc, H. C. Aguilar, F. Jean, A TMPRSS2 inhibitor acts as a pan-SARS-CoV-2 prophylactic and therapeutic, Nature 605 (7909) (2022) 340–348.

[77] M. Hoffmann, H. Kleine-Weber, S. Pohlmann, A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells, Mol. Cell. 78 (4) (2020) 779–784 e5.

[78] R.X. Lei, H. Shi, X.M. Peng, Y.H. Zhu, J. Cheng, G.H. Chen, Influence of a single nucleotide polymorphism in the P1 promoter of the furin gene on transcription activity and hepatitis B virus infection, Hepatology 50 (3) (2009) 763–771.

[79] J. Kouretova, M.Z. Hammamy, A. Epp, K. Hardes, S. Kallis, L. Zhang, R. Hilgenfeld, R. Bartenschlager, T. Steinmetzer, Effects of NS2B-NS3 protease and furin inhibition on West Nile and Dengue virus replication, J. Enzym. Inhib. Med. Chem. 32 (1) (2017) 712–721.

[80] Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study, BMJ 368 (2020) m1295.

[81] G. Kokic, H.S. Hillen, D. Tegunov, C. Dienemann, F. Seitz, J. Schmitzova, L. Farnung, A. Siewert, C. Hobartner, P. Cramer, Mechanism of SARS-CoV-2 polymerase stalling by remdesivir, Nat. Commun. 12 (1) (2021) 279.

[82] A.E. Wroblewski, I.E. Glowacka, D.G. Piotrowska, 1'-Homonucleosides and their structural analogues: a review, Eur. J. Med. Chem. 118 (2016) 121–142.

[83] A.A. Elfiky, Zika viral polymerase inhibition using anti-HCV drugs both in market and under clinical trials, J. Med. Virol. 88 (12) (2016) 2044–2051.

[84] A.A. Elfiky, A. Ismail, Molecular dynamics and docking reveal the potency of novel GTP derivatives against RNA dependent RNA polymerase of genotype 4a HCV, Life Sci. 238 (2019) 116958.

[85] A.A. Elfiky, W.M. Elshemey, IDX-184 is a superior HCV direct-acting antiviral drug: a QSAR study, Med. Chem. Res. 25 (5) (2016) 1005–1008.

[86] A.A. Elfiky, Remdesivir Ribavirin, Galidesivir Sofosbuvir, Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): a molecular docking study, Life Sci. 253 (2020) 117592.

[87] E.J. Giamarellos-Bourboulis, M.G. Netea, N. Rovina, K. Akinosoglou, A. Antoniadou, N. Antonakos, G. Damoraki, T. Gkavogianni, M.E. Adami, P. Katsaounou, M. Ntaganou, M. Kyriakopoulou, G. Dimopoulos, I. Koutsodimitropoulos, D. Velissaris, P. Koufargyris, A. Karageorgos, K. Katrini, V. Lekakis, M. Lupse, A. Kotsaki, G. Renieris, D. Theodoulou, V. Panou, E. Koukaki, N. Koulouris, C. Gogos, A. Koutsoukou, Complex immune dysregulation in COVID-19 patients with severe respiratory failure, Cell Host Microbe 27 (6) (2020) 992–1000 e3.

[88] F. Pourkarim, S. Pourtaghi-Anvarian, H. Rezaee, Molnupiravir: a new candidate for COVID-19 treatment, Pharmacol Res Perspect 10 (1) (2022) e00909.

[89] D. Roberfroid, V. Jespers, F. Hulstaert, Molnupiravir for covid-19 in nonhospitalized patients, N. Engl. J. Med. 386 (13) (2022) e32.

[90] Y. Xie, T. Choi, Z. Al-Aly, Molnupiravir and risk of post-acute sequelae of covid-19: cohort study, BMJ 381 (2023) e074572.

[91] C.C. Butler, F.D.R. Hobbs, O.A. Gbinigie, N.M. Rahman, G. Hayward, D.B. Richards, J. Dorward, D.M. Lowe, J.F. Standing, J. Breuer, S. Khoo, S. Petrou, K. Hood, J.S. Nguyen-Van-Tam, M.G. Patel, B.R. Saville, J. Marion, E. Ogburn, J. Allen, H. Rutter, N. Francis, N.P.B. Thomas, P. Evans, M. Dobson, T. A. Madden, J. Holmes, V. Harris, M.E. Png, M. Lown, O. van Hecke, M.A. Detry, C.T. Saunders, M. Fitzgerald, N.S. Berry, L. Mwandigha, U. Galal, S. Mort, B. D. Jani, N.D. Hart, H. Ahmed, D. Butler, M. McKenna, J. Chalk, L. Lavallee, E. Hadley, L. Cureton, M. Benysek, M. Andersson, M. Coates, S. Barrett, C. Bateman, J.C. Davies, I. Raymundo-Wood, A. Ustianowski, A. Carson-Stevens, L.M. Yu, P. Little, P.T.C. Group, Molnupiravir plus usual care versus usual care alone as early treatment for adults with COVID-19 at increased risk of adverse outcomes (PANORAMIC): an open-label, platform-adaptive randomised controlled trial, Lancet (London, England) 401 (10373) (2023) 281–293.

[92] D. Gurwitz, Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics, Drug Dev. Res. 81 (5) (2020) 537–540.

[93] J. Grein, N. Ohmagari, D. Shin, G. Diaz, E. Asperges, A. Castagna, T. Feldt, G. Green, M.L. Green, F.X. Lescure, E. Nicastri, R. Oda, K. Yo, E. Quiros-Roldan, A. Studemeister, J. Redinski, S. Ahmed, J. Bernett, D. Chelliah, D. Chen, S. Chihara, S.H. Cohen, J. Cunningham, A. D'Arminio Monforte, S. Ismail, H. Kato, G. Lapadula, E. L'Her, T. Maeno, S. Majumder, M. Massari, M. Mora-Rillo, Y. Mutoh, D. Nguyen, E. Verweij, A. Zoufaly, A.O. Osinusi, A. DeZure, Y. Zhao, L. Zhong, A. Chokkalingam, E. Elboudwarej, L. Telep, L. Timbs, I. Henne, S. Sellers, H. Cao, S.K. Tan, L. Winterbourne, P. Desai, R. Mera, A. Gaggar, R.P. Myers, D.M. Brainard, R. Childs, T. Flanigan, Compassionate use of remdesivir in patients with severe covid-19, N. Engl. J. Med. 382 (24) (2020) 2327–2336.

[94] S. Antinori, M.V. Cossu, A.L. Ridolfo, R. Rech, C. Bonazzetti, G. Pagani, G. Gubertini, M. Coen, C. Magni, A. Castelli, B. Borghi, R. Colombo, R. Giorgi, E. Angeli, D. Mileto, L. Milazzo, S. Vimercati, M. Pellicciotta, M. Corbellino, A. Torre, S. Rusconi, L. Oreni, M.R. Gismondo, A. Giacomelli, L. Meroni, G. Rizzardini, M. Galli, Compassionate remdesivir treatment of severe Covid-19 pneumonia in intensive care unit (ICU) and Non-ICU patients: clinical outcome and differences in post-treatment hospitalisation status, Pharmacol. Res. 158 (2020) 104899.

[95] C. Keech, G. Albert, I. Cho, A. Robertson, P. Reed, S. Neal, J.S. Plested, M. Zhu, S. Cloney-Clark, H. Zhou, G. Smith, N. Patel, M.B. Frieman, R.E. Haupt, J. Logue, M. McGrath, S. Weston, P.A. Piedra, C. Desai, K. Callahan, M. Lewis, P. Price-Abbott, N. Formica, V. Shinde, L. Fries, J.D. Lickliter, P. Griffin, B. Wilkinson, G.M. Glenn, Phase 1-2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine, N. Engl. J. Med. 383 (24) (2020) 2320–2332.

[96] K.J. Chappell, F.L. Mordant, Z. Li, D.K. Wijesundara, P. Ellenberg, J.A. Lackenby, S.T.M. Cheung, N. Modhiran, M.S. Avumegah, C.L. Henderson, K. Hoger, P. Griffin, J. Bennet, L. Hensen, W. Zhang, T.H.O. Nguyen, S. Marrero-Hernandez, K.J. Selva, A.W. Chung, M.H. Tran, P. Tapley, J. Barnes, P.C. Reading, S. Nicholson, S. Corby, T. Holgate, B.D. Wines, P.M. Hogarth, K. Kedzierska, D.F.J. Purcell, C. Ranasinghe, K. Subbarao, D. Watterson, P.R. Young, T.P. Munro, Safety and immunogenicity of an MF59-adjuvanted spike glycoprotein-clamp vaccine for SARS-CoV-2: a randomised, double-blind, placebo-controlled, phase 1 trial, Lancet Infect. Dis. 21 (10) (2021) 1383–1394.

[97] A.A. Smargon, D.B.T. Cox, N.K. Pyzocha, K. Zheng, I.M. Slaymaker, J.S. Gootenberg, O.A. Abudayyeh, P. Essletzbichler, S. Shmakov, K.S. Makarova, E. V. Koonin, F. Zhang, Cas13b is a type VI-B CRISPR-associated RNA-guided RNase differentially regulated by accessory proteins Csx27 and Csx28, Mol. Cell. 65 (4) (2017) 618–630 e7.

[98] W.X. Yan, S. Chong, H. Zhang, K.S. Makarova, E.V. Koonin, D.R. Cheng, D.A. Scott, Cas13d is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein, Mol. Cell. 70 (2) (2018) 327–339 e5.

[99] T.R. Abbott, G. Dhamdhere, Y. Liu, X. Lin, L. Goudy, L. Zeng, A. Chemparathy, S. Chmura, N.S. Heaton, R. Debs, T. Pande, D. Endy, M.F. La Russa, D.B. Lewis, L.S. Qi, Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza, Cell 181 (4) (2020) 865–876 e12.

[100] I.F.-N. Hung, K.-C. Lung, E.Y.-K. Tso, R. Liu, T.W.-H. Chung, M.-Y. Chu, Y.-Y. Ng, J. Lo, J. Chan, A.R. Tam, H.-P. Shum, V. Chan, A.K.-L. Wu, K.-M. Sin, W.-S. Leung, W.-L. Law, D.C. Lung, S. Sin, P. Yeung, C.C.-Y. Yip, R.R. Zhang, A.Y.-F. Fung, E.Y.-W. Yan, K.-H. Leung, J.D. Ip, A.W.-H. Chu, W.-M. Chan, A.C.-K. Ng, R. Lee, K. Fung, A. Yeung, T.-C. Wu, J.W.-M. Chan, W.-W. Yan, W.-M. Chan, J.F.-W. Chan, A.K.-W. Lie, O.T.-Y. Tsang, V.C.-C. Cheng, T.-L. Que, C.-S. Lau, K.-H. Chan, K.K.-W. To, K.-Y. Yuen, Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial, Lancet 395 (10238) (2020) 1695–1704.

[101] C. Ma, H. Tan, J. Choza, Y. Wang, J. Wang, Validation and invalidation of SARS-CoV-2 main protease inhibitors using the Flip-GFP and Protease-Glo luciferase assays, Acta Pharm. Sin. B 12 (4) (2022) 1636–1651.

[102] L. Fu, F. Ye, Y. Feng, F. Yu, Q. Wang, Y. Wu, C. Zhao, H. Sun, B. Huang, P. Niu, H. Song, Y. Shi, X. Li, W. Tan, J. Qi, G.F. Gao, Both Boceprevir and GC376 efficaciously inhibit SARS-CoV-2 by targeting its main protease, Nat. Commun. 11 (1) (2020) 4417.

[103] C. Ma, M.D. Sacco, B. Hurst, J.A. Townsend, Y. Hu, T. Szeto, X. Zhang, B. Tarbet, M.T. Marty, Y. Chen, J. Wang, Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease, Cell Res. 30 (8) (2020) 678–692.

[104] S.A. Moghadasi, E. Heilmann, A.M. Khalil, C. Nnabuife, F.L. Kearns, C. Ye, S.N. Moraes, F. Costacurta, M.A. Esler, H. Aihara, D. von Laer, L. Martinez-Sobrido, T. Palzkill, R.E. Amaro, R.S. Harris, Transmissible SARS-CoV-2 variants with resistance to clinical protease inhibitors, Sci. Adv. 9 (13) (2023) eade8778.

[105] M.W. McCarthy, Ensitrelvir as a potential treatment for COVID-19, Expet Opin. Pharmacother. 23 (18) (2022) 1995–1998.

[106] N. Higashi-Kuwata, K. Tsuji, H. Hayashi, H. Bulut, M. Kiso, M. Imai, H. Ogata-Aoki, T. Ishii, T. Kobayakawa, K. Nakano, N. Takamune, N. Kishimoto, S. I. Hattori, D. Das, Y. Uemura, Y. Shimizu, M. Aoki, K. Hasegawa, S. Suzuki, A. Nishiyama, J. Saruwatari, Y. Shimizu, Y. Sukenaga, Y. Takamatsu, K. Tsuchiya, K. Maeda, K. Yoshimura, S. Iida, S. Ozono, T. Suzuki, T. Okamura, S. Misumi, Y. Kawaoka, H. Tamamura, H. Mitsuya, Identification of SARS-CoV-2 M(pro) inhibitors containing P1' 4-fluorobenzothiazole moiety highly active against SARS-CoV-2, Nat. Commun. 14 (1) (2023) 1076.

[107] H. Mukae, H. Yotsuyanagi, N. Ohmagari, Y. Doi, H. Sakaguchi, T. Sonoyama, G. Ichihashi, T. Sanaki, K. Baba, Y. Tsuge, T. Uehara, Efficacy and safety of Ensitrelvir in patients with mild-to-moderate coronavirus disease 2019: the phase 2b part of a randomized, placebo-controlled, phase 2/3 study, Clin. Infect. Dis. 76 (8) (2023) 1403–1411.

[108] M. Sasaki, K. Tabata, M. Kishimoto, Y. Itakura, H. Kobayashi, T. Ariizumi, K. Uemura, S. Toba, S. Kusakabe, Y. Maruyama, S. Iida, N. Nakajima, T. Suzuki, S. Yoshida, H. Nobori, T. Sanaki, T. Kato, T. Shishido, W.W. Hall, Y. Orba, A. Sato, H. Sawa, S-217622, a SARS-CoV-2 main protease inhibitor, decreases viral load and ameliorates COVID-19 severity in hamsters, Sci. Transl. Med. 15 (679) (2023) eabq4064.

[109] B. Amani, B. Amani, Efficacy and safety of nirmatrelvir/ritonavir (Paxlovid) for COVID-19: a rapid review and meta-analysis, J. Med. Virol. 95 (2) (2023) e28441.

[110] A.S. Anderson, P. Caubel, J.M. Rusnak, E.-H.T. Investigators, Nirmatrelvir-ritonavir and viral load rebound in covid-19, N. Engl. J. Med. 387 (11) (2022) 1047–1049.

[111] Y. Duan, H. Zhou, X. Liu, S. Iketani, M. Lin, X. Zhang, Q. Bian, H. Wang, H. Sun, S.J. Hong, B. Culbertson, H. Mohri, M.I. Luck, Y. Zhu, X. Liu, Y. Lu, X. Yang, K. Yang, Y. Sabo, A. Chavez, S.P. Goff, Z. Rao, D.D. Ho, H. Yang, Molecular mechanisms of SARS-CoV-2 resistance to nirmatrelvir, Nature 622 (7982) (2023) 376–382.

[112] R. Najjar-Debbiny, N. Gronich, G. Weber, J. Khoury, M. Amar, N. Stein, L.H. Goldstein, W. Saliba, Effectiveness of Paxlovid in reducing severe coronavirus disease 2019 and mortality in high-risk patients, Clin. Infect. Dis. 76 (3) (2023) e342–e349.

[113] M.W. McCarthy, Paxlovid as a potential treatment for long COVID, Expet Opin. Pharmacother. 24 (17) (2023) 1839–1843.

[114] A.K. Mishra, T.J. Wallin, W. Pan, A. Xu, K. Wang, E.P. Giannelis, B. Mazzolai, R.F. Shepherd, Autonomic perspiration in 3D-printed hydrogel actuators, Sci. Robot. 5 (38) (2020).

[115] B. Cao, Y. Wang, D. Wen, W. Liu, J. Wang, G. Fan, L. Ruan, B. Song, Y. Cai, M. Wei, X. Li, N. Chen, J. Xiang, T. Yu, T. Bai, X. Xie, L. Zhang, C. Li, Y. Yuan, H. Chen, H. Li, H. Huang, S. Tu, F. Gong, Y. Liu, Y. Wei, C. Dong, F. Zhou, X. Gu, J. Xu, Z. Liu, Y. Zhang, H. Li, L. Shang, K. Wang, K. Li, X. Zhou, X. Dong, Z. Qu,

S. Lu, X. Hu, S. Ruan, S. Luo, J. Wu, L. Peng, F. Cheng, L. Pan, J. Zou, C. Jia, J. Wang, X. Liu, S. Wang, X. Wu, Q. Ge, J. He, H. Zhan, F. Qiu, L. Guo, C. Huang, T. Jaki, F.G. Hayden, P.W. Horby, D. Zhang, C. Wang, A trial of lopinavir-ritonavir in adults hospitalized with severe covid-19, N. Engl. J. Med. 382 (19) (2020) 1787–1799.

[116] H. Tan, Y. Hu, P. Jadhav, B. Tan, J. Wang, Progress and challenges in targeting the SARS-CoV-2 papain-like protease, J. Med. Chem. 65 (11) (2022) 7561–7580.

[117] B.T. Freitas, I.A. Durie, J. Murray, J.E. Longo, H.C. Miller, D. Crich, R.J. Hogan, R.A. Tripp, S.D. Pegan, Characterization and noncovalent inhibition of the deubiquitinase and deISGylase activity of SARS-CoV-2 papain-like protease, ACS Infect. Dis. 6 (8) (2020) 2099–2109.

[118] D.J. Calleja, G. Lessene, D. Komander, Inhibitors of SARS-CoV-2 PLpro, Front. Chem. 10 (2022) 876212.

[119] X. Gao, B. Qin, P. Chen, K. Zhu, P. Hou, J.A. Wojdyla, M. Wang, S. Cui, Crystal structure of SARS-CoV-2 papain-like protease, Acta Pharm. Sin. B 11 (1) (2021) 237–245.

[120] J. Osipiuk, S.A. Azizi, S. Dvorkin, M. Endres, R. Jedrzejczak, K.A. Jones, S. Kang, R.S. Kathayat, Y. Kim, V.G. Lisnyak, S.L. Maki, V. Nicolaescu, C.A. Taylor, C. Tesar, Y.A. Zhang, Z. Zhou, G. Randall, K. Michalska, S.A. Snyder, B.C. Dickinson, A. Joachimiak, Structure of papain-like protease from SARS-CoV-2 and its complexes with non-covalent inhibitors, Nat. Commun. 12 (1) (2021) 743.

[121] N. Agrawal, S. Pathak, A. Goyal, Potential papain-like protease inhibitors against COVID-19: a comprehensive in silico based review, Comb. Chem. High Throughput Screen. 25 (11) (2022) 1838–1858.

[122] A. Tiwari, G. Singh, G. Choudhir, M. Motiwale, N. Joshi, V. Sharma, R.K. Srivastava, S. Sharma, M. Tutone, P.K. Singour, Deciphering the potential of pre and pro-vitamin D of mushrooms against Mpro and PLpro proteases of COVID-19: an in silico approach, Molecules 27 (17) (2022).

[123] T. Klemm, G. Ebert, D.J. Calleja, C.C. Allison, L.W. Richardson, J.P. Bernardini, B.G. Lu, N.W. Kuchel, C. Grohmann, Y. Shibata, Z.Y. Gan, J.P. Cooney, M. Doerflinger, A.E. Au, T.R. Blackmore, G.J. van der Heden van Noort, P.P. Geurink, H. Ovaa, J. Newman, A. Riboldi-Tunnicliffe, P.E. Czabotar, J.P. Mitchell, R. Feltham, B.C. Lechtenberg, K.N. Lowes, G. Dewson, M. Pellegrini, G. Lessene, D. Komander, Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2, EMBO J. 39 (18) (2020) e106275.

[124] Y. Zhao, X. Du, Y. Duan, X. Pan, Y. Sun, T. You, L. Han, Z. Jin, W. Shang, J. Yu, H. Guo, Q. Liu, Y. Wu, C. Peng, J. Wang, C. Zhu, X. Yang, K. Yang, Y. Lei, L. W. Guddat, W. Xu, G. Xiao, L. Sun, L. Zhang, Z. Rao, H. Yang, High-throughput screening identifies established drugs as SARS-CoV-2 PLpro inhibitors, Protein Cell 12 (11) (2021) 877–888.

[125] C. Ma, J. Wang, Validation and invalidation of SARS-CoV-2 papain-like protease inhibitors, ACS Pharmacol. Transl. Sci. 5 (2) (2022) 102–109.

[126] Z. Shen, K. Ratia, L. Cooper, D. Kong, H. Lee, Y. Kwon, Y. Li, S. Alqarni, F. Huang, O. Dubrovskyi, L. Rong, G.R.J. Thatcher, R. Xiong, Design of SARS-CoV-2 PLpro inhibitors for COVID-19 antiviral therapy leveraging binding cooperativity, J. Med. Chem. 65 (4) (2022) 2940–2955.

[127] M.C.R. Michelle R. Garnsey, Luong T. Nguyen, Rhonda Cardin, Joseph Tillotson, Ellene Mashalidis, Aijia Yu, Lisa Aschenbrenner, Amanda Balesano, Amin Behzadi, Britton Boras, Jeanne S. Chang, Heather Eng, Andrew Ephron, Tim Foley, Kristen K. Ford, James M. Frick, Scott Gibson, Li Hao, Brett Hurst, Amit S. Kalgutkar, Magdalena Korczynska, Zsofia Lengyel-Zhand, Liping Gao, Hannah R. Meredith, Nandini C. Patel, Jana Polivkova, Devendra Rai, Colin R. Rose, Hussin Rothan, Sylvie K. Sakata, Thomas R. Vargo, Wenying Qi, Huixian Wu, Yiping Liu, Irina Yurgelonis, Jinzhi Zhang, Yuao Zhu, Lei Zhang, Alpha A. Lee, Discovery of SARS-CoV-2 papain-like protease (PLpro) inhibitors with efficacy in a murine infection model, bioRxiv (2024) 1–12.

[128] B. Tan, X. Zhang, A. Ansari, P. Jadhav, H. Tan, K. Li, A. Chopra, A. Ford, X. Chi, F.X. Ruiz, E. Arnold, X. Deng, J. Wang, Design of a SARS-CoV-2 papain-like protease inhibitor with antiviral efficacy in a mouse model, Science (New York, N.Y.) 383 (6690) (2024) 1434–1440.

[129] D. Schutz, Y.B. Ruiz-Blanco, J. Munch, F. Kirchhoff, E. Sanchez-Garcia, J.A. Muller, Peptide and peptide-based inhibitors of SARS-CoV-2 entry, Adv. Drug Deliv. Rev. 167 (2020) 47–65.

[130] A. Chopra, A.H. Shukri, H. Adhikary, V. Lukinovic, M. Hoekstra, M. Cowpland, K.K. Biggar, A peptide array pipeline for the development of Spike-ACE2 interaction inhibitors, Peptides 158 (2022) 170898.

[131] K. Yang, C. Wang, A.J.B. Kreutzberger, R. Ojha, S. Kuivanen, S. Couoh-Cardel, S. Muratcioglu, T.J. Eisen, K.I. White, R.G. Held, S. Subramanian, K. Marcus, R. A. Pfuetzner, L. Esquivies, C.A. Doyle, J. Kuriyan, O. Vapalahti, G. Balistreri, T. Kirchhausen, A.T. Brunger, Nanomolar inhibition of SARS-CoV-2 infection by an unmodified peptide targeting the prehairpin intermediate of the spike protein, Proc. Natl. Acad. Sci. U.S.A. 119 (40) (2022) e2210990119.

[132] S. Xia, M. Liu, C. Wang, W. Xu, Q. Lan, S. Feng, F. Qi, L. Bao, L. Du, S. Liu, C. Qin, F. Sun, Z. Shi, Y. Zhu, S. Jiang, L. Lu, Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion, Cell Res. 30 (4) (2020) 343–355.

[133] V. Thijssen, D.L. Hurdiss, O.J. Debski-Antoniak, M.A. Spence, C. Franck, A. Norman, A. Aggarwal, N.J. Mokiem, D.A.A. van Dongen, S.W. Vermeir, M. Liu, W. Li, M. Chatziandreou, T. Donselaar, W. Du, I. Drulyte, B.J. Bosch, J. Snijder, S.G. Turville, R.J. Payne, C.J. Jackson, F.J.M. van Kuppeveld, S.A.K. Jongkees, A broad-spectrum macrocyclic peptide inhibitor of the SARS-CoV-2 spike protein, Proc. Natl. Acad. Sci. U.S.A. 120 (26) (2023) e2303292120.

[134] Y. Yan, Z. Zou, Y. Sun, X. Li, K.F. Xu, Y. Wei, N. Jin, C. Jiang, Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model, Cell Res. 23 (2) (2013) 300–302.

[135] M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, Cell Res. 30 (3) (2020) 269–271.

[136] M.R. Mehra, S.S. Desai, F. Ruschitzka, A.N. Patel, RETRACTED: hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis, Lancet. 20 (2020) 31324–31326.

[137] R. Karki, B.R. Sharma, S. Tuladhar, E.P. Williams, L. Zalduondo, P. Samir, M. Zheng, B. Sundaram, B. Banoth, R.K.S. Malireddi, P. Schreiner, G. Neale, P. Vogel, R. Webby, C.B. Jonsson, T.D. Kanneganti, Synergism of TNF-alpha and IFN-gamma triggers inflammatory cell death, tissue damage, and mortality in SARS-CoV-2 infection and cytokine shock syndromes, Cell 184 (1) (2021) 149–168 e17.

[138] R. Channappanavar, A.R. Fehr, J. Zheng, C. Wohlford-Lenane, J.E. Abrahante, M. Mack, R. Sompallae, P.B. McCray Jr., D.K. Meyerholz, S. Perlman, IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes, J. Clin. Invest. 129 (9) (2019) 3625–3639.

[139] R. Channappanavar, A.R. Fehr, R. Vijay, M. Mack, J. Zhao, D.K. Meyerholz, S. Perlman, Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice, Cell Host Microbe 19 (2) (2016) 181–193.

[140] V.D. Menachery, A.J. Eisfeld, A. Schafer, L. Josset, A.C. Sims, S. Proll, S. Fan, C. Li, G. Neumann, S.C. Tilton, J. Chang, L.E. Gralinski, C. Long, R. Green, C. M. Williams, J. Weiss, M.M. Matzke, B.J. Webb-Robertson, A.A. Schepmoes, A.K. Shukla, T.O. Metz, R.D. Smith, K.M. Waters, M.G. Katze, Y. Kawaoka, R. S. Baric, Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses, mBio 5 (3) (2014) e01174. -14.

[141] S. Wan, Y. Xiang, W. Fang, Y. Zheng, B. Li, Y. Hu, C. Lang, D. Huang, Q. Sun, Y. Xiong, X. Huang, J. Lv, Y. Luo, L. Shen, H. Yang, G. Huang, R. Yang, Clinical features and treatment of COVID-19 patients in northeast Chongqing, J. Med. Virol. 92 (7) (2020) 797–806.

[142] H. Qiu, J. Wu, L. Hong, Y. Luo, Q. Song, D. Chen, Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study, Lancet Infect. Dis. 20 (6) (2020) 689–696.

[143] K. Huang, P. Zhang, Z. Zhang, J.Y. Youn, C. Wang, H. Zhang, H. Cai, Traditional Chinese Medicine (TCM) in the treatment of COVID-19 and other viral infections: efficacies and mechanisms, Pharmacol. Ther. 225 (2021) 107843.

[144] F. Jiang, N. Xu, Y. Zhou, J. Song, J. Liu, H. Zhu, J. Jiang, Y. Xu, R. Li, Contribution of traditional Chinese medicine combined with conventional western medicine treatment for the novel coronavirus disease (COVID-19), current evidence with systematic review and meta-analysis, Phytother Res. 35 (11) (2021) 5992–6009.

[145] Y. Zhang, Y. Liu, M. Li, S. Feng, X. Li, Z. Gao, Y. Yang, X. Tong, Timely treatment and higher compliance to traditional Chinese medicine: new influencing factors for reducing severe COVID-19 based on retrospective cohorts in 2020 and 2021, Pharmacol. Res. 178 (2022) 106174.

[146] J.L. Ren, A.H. Zhang, X.J. Wang, Traditional Chinese medicine for COVID-19 treatment, Pharmacol. Res. 155 (2020) 104743.

[147] Y. Li, Y. Qin, N. Chen, L. Ge, Q. Wang, T. Aboudou, J. Han, L. Hou, L. Cao, R. Li, M. Li, N. Mi, P. Xie, S. Wu, L. Hu, X. Li, Z. Song, J. Ji, Z. Zhang, K. Yang, Use of traditional Chinese medicine for the treatment and prevention of COVID-19 and rehabilitation of COVID-19 patients: an evidence mapping study, Front. Pharmacol. 14 (2023) 1069879.

[148] Y. Zhao, B. Zhao, Y.B. Liang, Successful treatment of subacute thyroiditis after recombinant COVID-19 vaccination using traditional Chinese medicine: a case report, Cureus 14 (10) (2022) e30716.

[149] Y. Ling, Y. Dong, Q. Li, D. Huang, Z. Li, M. Chen, Treatment of COVID-19 by monoclonal antibodies and the traditional Chinese medicine, Med Nov Technol Devices 15 (2022) 100159.

[150] Y. Hu, E.M. Lewandowski, H. Tan, X. Zhang, R.T. Morgan, X. Zhang, L.M.C. Jacobs, S.G. Butler, M.V. Gongora, J. Choy, X. Deng, Y. Chen, J. Wang, Naturally occurring mutations of SARS-CoV-2 main protease confer drug resistance to nirmatrelvir, ACS Cent. Sci. 9 (8) (2023) 1658–1669.

[151] S. Iketani, D.D. Ho, SARS-CoV-2 resistance to monoclonal antibodies and small-molecule drugs, Cell Chem. Biol. 31 (4) (2024) 632–657.