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## Review

# Recent insights for the emerging COVID-19: Drug discovery, therapeutic options and vaccine development

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### ABSTRACT

SARS-CoV-2 has been marked as a highly pathogenic coronavirus of COVID-19 disease into the human population, causing over 5.5 million confirmed cases worldwide. As COVID-19 has posed a global threat with significant human casualties and severe economic losses, there is a pressing demand to further understand the current situation and develop rational strategies to contain the drastic spread of the virus. Although there are no specific antiviral therapies that have proven effective in randomized clinical trials, currently, the rapid detection technology along with several promising therapeutics for COVID-19 have mitigated its drastic transmission. Besides, global institutions and corporations have commenced to parse out effective vaccines for the prevention of COVID-19. Herein, the present review will give exhaustive details of extensive researches concerning the drug discovery and therapeutic options for COVID-19 as well as some insightful discussions of the status of COVID-19.

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## 1. Introduction

This century has witnessed the worldwide spread of several hitherto unknown coronaviruses. The rapid alteration of ecology and urbanization along with vulnerable public health systems have facilitated more frequent emerging of epidemics, which have become more and more intractable for us to prevent and contain. In December 2019, a new

coronavirus (CoV) correlated with human respiratory disease was firstly reported causing pneumonia and death [1]. Soon afterward, the disease cases continued to expand and soared dramatically worldwide. The causative virus, named as severe acute respiratory syndrome CoV-2 (SARS-CoV-2), was identified as the pathogen leading to the CoV disease COVID-19. Infections with SARS-CoV-2 are now swift and violent, and as of 28 May 2020, over 5.5 million cases have been confirmed in more than 215 countries, with over 353,334 deaths. To date,

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SARS-CoV-2 has most closest relation to SARS and relevant viruses that circulate in bats, as evidenced by viral genome analysis as well as the research probing into the proximal origin of such virus [2].

In general, CoVs, subfamily coronavirinae, are a cluster of highly diversified, enveloped, positive-sense and single-stranded viruses ((+)-ssRNA virus) that can induce enteric, respiratory, hepatic as well as neurological disorders of discrepant severity in a wide range of animal species, encompassing humans [3,4]. As the largest RNA viruses ever discovered, CoVs can be categorized into  $\alpha$ -,  $\beta$ -,  $\delta$ - and  $\gamma$ -CoVs. Among these genera, the  $\beta$  group can be subdivided into A, B, C and D lineages [5]. In the past 17 years, three neoteric  $\beta$ -CoVs, SARS-CoV, Middle East respiratory syndrome CoV (MERS-CoV), along with SARS-CoV-2 have emerged, engendering severe human diseases. Although the origin of SARS-CoV-2 outbreak is not yet clear, recent studies have deduced that it might be transmitted through bats because it is highly similar to the bat SARS-CoV-like coronaviruses [6]. Later on, genomic and evolutionary proofs of the occurrence of Pangolin-CoV indicated that pangolin species might be the potential intermediate host for SARS-CoV-2 [7,8]. Unlike human CoVs, zoonotic viruses hold the capacity of infecting both animals and humans, leading to severe respiratory diseases (i.e., acute respiratory distress syndrome (ARDS) and pneumonia) [9,10]. Clinical data revealed that the COVID-19 symptoms are far more severe among the elders with comorbidities, while asthma, allergic illnesses, as well as chronic obstructive pulmonary disease are also risk factors [11,12]. Despite the continuous improvement of the prevention strategy and the disease surveillance system, the lack of efficacious drug treatment and correlated high morbidity cases of the SARS-CoV-2 along with its potentiality to induce pandemics, highlighting the urgent demand for neoteric drug discovery. In this review, we will first briefly describe the status of epidemiology, mechanism and diagnosis of COVID-19. In addition to the discussion of current management strategies for COVID-19, the emphasis has been given to the treatment options and drug discovery of COVID-19. We will refer to the drug screening progress and the development of vaccines for the COVID-19 therapy. Ultimately, we will delineate the overarching challenges in the clinical invention of new anti-coronavirus agents and offer some insights toward the prevention of such disease based on the understanding of its epidemic dynamics in real-time.

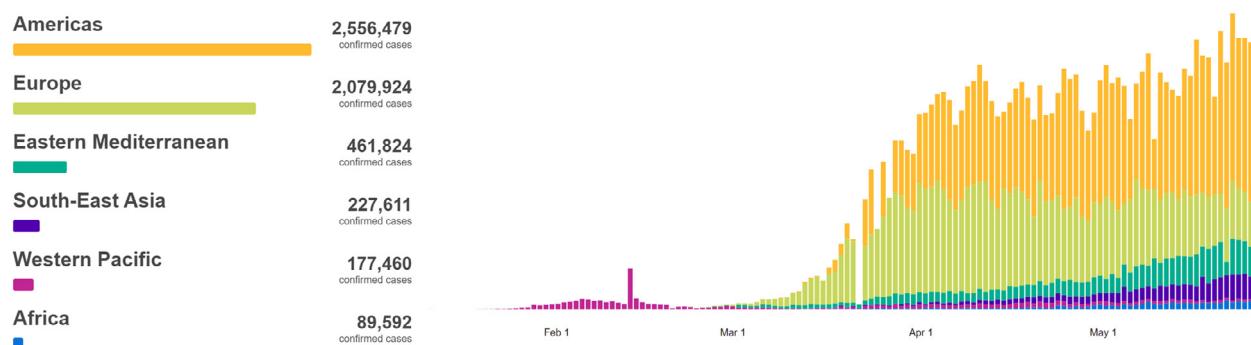
## 2. The epidemiology of COVID-19

Spread mainly through respiratory droplets or close contact, SARS-CoV-2 induced diseases has been growing dramatically in accordance with the published data from the World Health Organization (WHO) [13]. Although the number of confirmed cases in China has decreased a lot from late February 2020, and there is no report of COVID-19 deaths on 6th April, the confirmed cases of CoVs worldwide are still expanding with a vengeance. The spectrum of illness presentation or severity profile also affects triage and diagnostic decision-making, along with the therapeutic options and prognostic

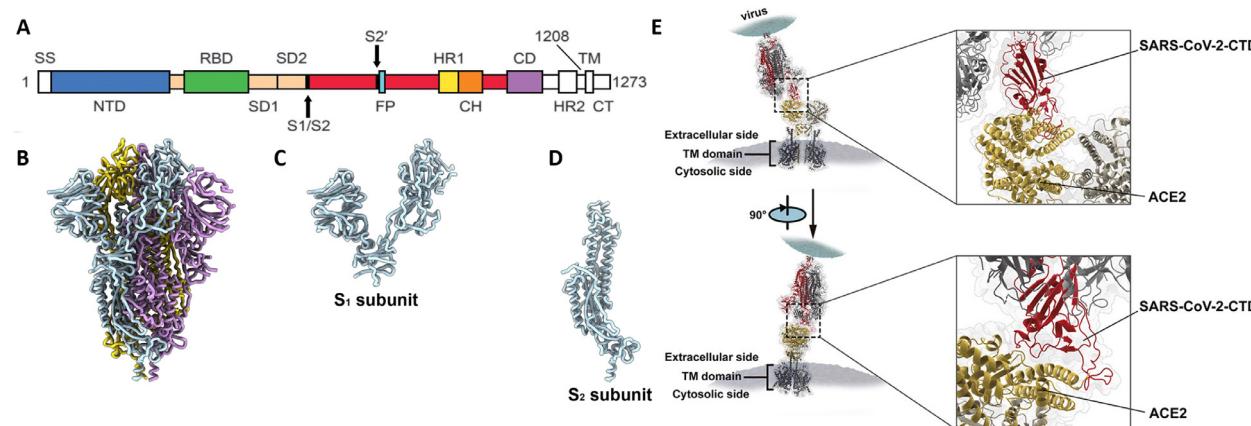
expectations [14]. Till now, the exact source of the current outbreak of COVID-19 remains unclear, but the dynamic model is similar to the classic zoonotic emergence to human-to-human transmission [15]. The mortality rate of SARS-CoV-2 (~3.8%) is lower compared to that of MERS-CoV (37.1%) or SARS-CoV (10%), but the number of infections is more than ten times higher [16]. With respect to the lack of evidence that companion animals might be a source of infection, patients with COVID-19 are the prime source of infection, and those with severe conditions are more infectious than those with mild conditions. Intriguingly, asymptotically infected persons or patients in incubation has also been demonstrated to shed the infectious virus, serving as a potential infection source to drive the transmission of the COVID-19 [17]. In addition, researches focused on the follow-up of recovered patients revealed that the tested samples of rehabilitees continuously showed a positive RT-PCR result, implicating that asymptomatic infection during incubation or recovery from COVID-19 may pose a daunting challenge to disease control and prevention [18].

The incubation period refers to the time between exposure to the virus and initial symptoms. A research report on the early propagation dynamics of COVID-19 unveiled that the average incubation period of COVID-19 was around 5 d, and its 95% distribution was 12.5 d [19]. Another study analyzing the travel history and symptoms in 88 confirmed cases revealed a similar average incubation period of around 6 days [20]. In addition, there was an unusual case with an incubation period of up to 19 d [21]. Although such a long incubation period may be a low probability event (the condition of 14 d was suggested by experts for quarantine), the longer incubation time indicates the adjustment of screening and control policies [22].

The early outbreak data of COVID-19 largely follow exponential growth. Disparate models based upon the clinical progression of the disease had been proposed to assess the basic reproductive ratio  $R_0$ . A retrospective analysis of the first 425 identified cases demonstrated that in the early stages of COVID-19, the  $R_0$  was assessed to be 2.2 [19]. Nevertheless, deterministic compartmental models based upon the likelihood and a model analysis revealed that the control reproduction number  $R_c$  might be as high as 6.5 due to the estimation of four generations of viral transmission and serried social contacts [23]. In this regard, it is noteworthy that  $R_0$  estimates may vary in the light of numerous biologics, social behavior, and environmental factors [24]. In general, the basic  $R_0$  assessed by the majority of researches ranges between 2 and 4 [25]. According to the WHO data updated on May 28, 2020, more than 215 countries have reported 5593,631 confirmed cases, including 353,334 deaths (Fig. 1). The grand total case fatality rate of global cases outside China is 1.31% [26]. The US had also recorded the largest number of coronavirus deaths in a day, with more than 1810 deaths reported on April 7th, according to data from Johns Hopkins University. Given the condition that the population of all races and ages is generally susceptible, there is an urgent need to further implementing the timely diagnosis, along with efficient isolation of patients, to cut down the  $R_0$  of SARS-CoV-2 and control the epidemic outbreak.



**Fig. 1 – Cumulatively identified cases of COVID-19 worldwide, as of 28 May 2020 [26].** As indicated in this figure, the overall data still presents a slow upward trend, suggesting that the epidemic has not been effectively alleviated.



**Fig. 2 – Structure of SARS-CoV-2 S in the pre-fusion conformation and the genome, along with the crystal structure of the C-terminal domain of SARS-CoV-2 (SARS-CoV-2-CTD) S protein in complex with human ACE2.** (A) Schematic of SARS-CoV-2 S primary structure colored by domain. SS, signal sequence; S2', S2' protease cleavage site; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail. Arrows denote protease cleavage sites. (B) Ribbon diagrams of the SARS-CoV-2 S ectodomain cryoEM structures. (C) The SARS-CoV-2 S1 subunits. (D) The SARS-CoV-2 S2 subunits. (E) A hACE2-binding mode of SARS-CoV-2. Reproduced with permission [32–34].

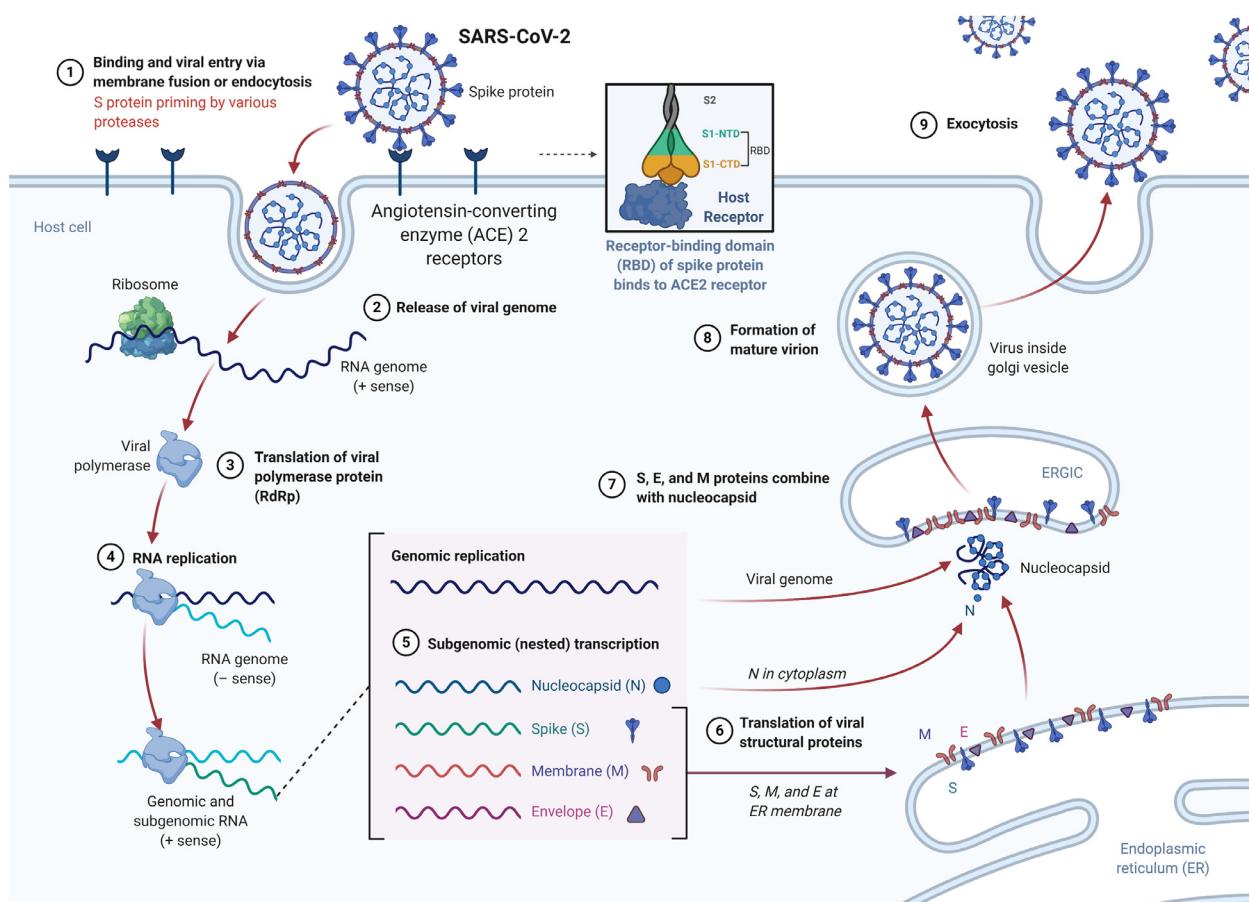
### 3. Virological characteristics of SARS-CoV-2

As previously mentioned, SARS-CoV-2, belonging to the coronaviridae family, is the causative pathogen of COVID-19. Closely resembled other  $\beta$ -CoVs, the SARS-CoV-2 virion with a genome size of  $\sim 3$  kb has a nucleocapsid consisted of genomic RNA and phosphorylated nucleocapsid (N) protein [27]. Of note, the nucleocapsid is embedded in a phospholipid bilayer and is encased by two disparate types of spike proteins: spike glycoprotein trimmer present in all CoVs and the hemagglutinin-esterase shared merely in some CoVs. The spike (S) protein plays a pivotal role in binding to receptors and is the key to determine host tropism and transmission capacity (Fig. 2). The matrix protein (M) along with the viral envelop (E) are also located in the viral envelope [28]. Genome analysis showed that the SARS-CoV-2 possesses 5' and 3' terminal sequences, with a gene order 5' -replicase open reading frame (ORF) 1ab-S-envelope(E)-membrane(M)-N-3' [29]. The virus particles are 60–100 nm in diameter, and are round or oval [30]. It can be inactivated by ultraviolet

light or heated at 56 °C for 30 min and is sensitive to most disinfectants (i.e., ether, 75% ethanol, peracetic acid, chlorine, as well as chloroform) [31].

Accumulating evidence has revealed that SARS-CoV-2 and SARS-CoV have the same human cell receptor, the angiotensin-converting enzyme 2 (ACE2), while MERS-CoV hinges on dipeptidyl peptidase 4 for host cell entry [35]. ACE2 is a type I membrane protein expressed in the lungs, hearts, kidneys, and intestines, mainly associated with cardiovascular diseases [35]. A recent study analyzed the cryogenic electron microscopy structure of the SARS-CoV-2 S protein unveiled that it exhibits around 10 to 20-fold higher binding affinity to ACE2 in comparison with SARS-CoV [36]. The overall replication cycle of SARS-CoV-2 is depicted in Fig. 3.

As for the phylogenetic network analysis of SARS-CoV-2 genomes, after analysis of the sample from across the world, researchers have found three central variants differentiated through amino acid alterations, denoted A, B, and C, with A being the ancestral type in line with the bat outgroup CoV. Intriguingly, types A and C account for a considerable



**Fig. 3 – The possible viral entry and replication mechanism of SARS-CoV-2.** When the S protein of SARS-CoV-2 binds to the cellular receptor ACE2, it begins its life cycle. After the receptor is bound, the conformational change of the S protein helps the viral envelope to fuse with the cell membrane through the endosome pathway. Then, SARS-CoV-2 releases the RNA into the host cell. Genomic RNA is translated into viral replicate polyproteins pp1a and 1ab, which are then cleaved into small products by viral proteases. The polymerase generates a series of subgenomic mRNAs through discontinuous transcription, which is ultimately translated into related viral proteins. Viral proteins and genomic RNA are subsequently assembled into virions in the ER and Golgi, and then transported through vesicles and released from the cells. ERGIC, ER-Golgi intermediate compartment. Created with BioRender.com.

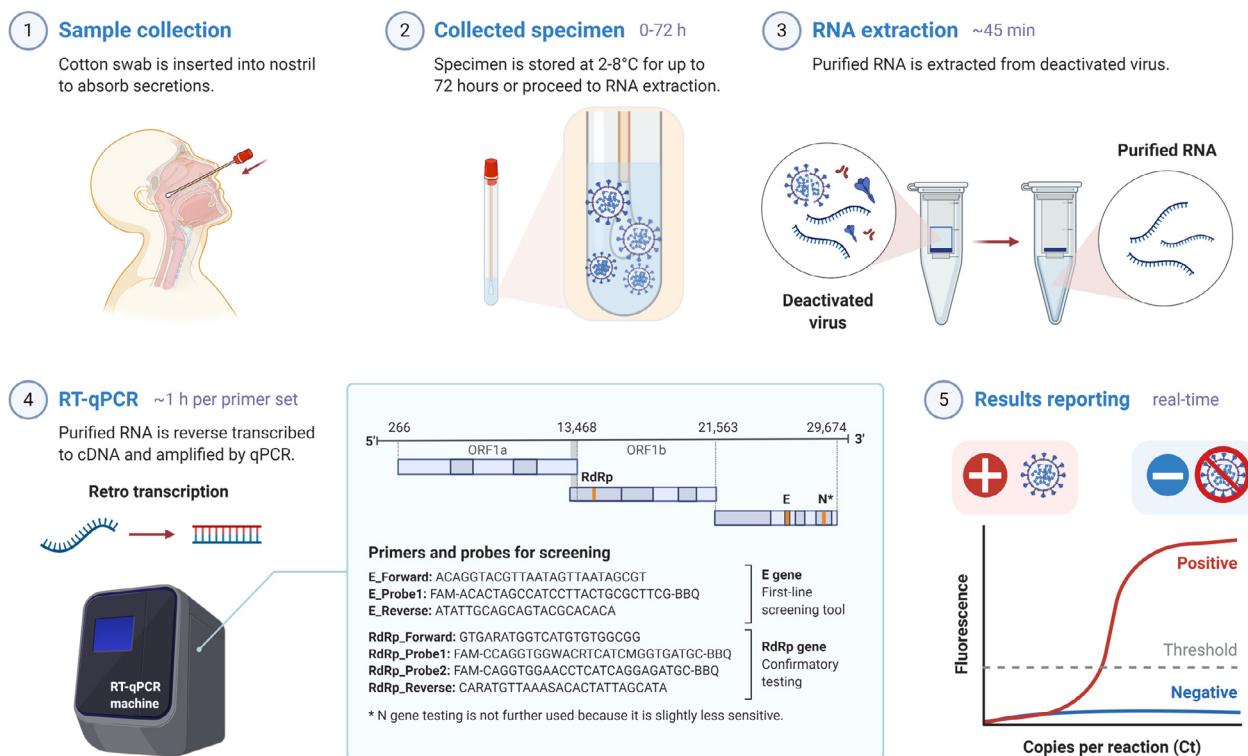
proportion outside East Asia (Europe and the United States) [37]. By comparison, B is the most common type in East Asia, and its ancestors' genomes do not appear to spread outside East Asia without first mutating into a derived type B. Hence, SARS-CoV-2 genomes were found to be closely correlated, and evolutionary selection took place in human hosts, sometimes with parallel evolutionary events, where the same viral mutation occurs in two disparate human hosts [37]. Owing to the erratic nature of RNA viruses as well as its high contagiousness, the continuous monitoring of SARS-CoV-2 from humans or animal species is of extreme significance for pandemic control.

#### 4. Diagnosis and pathogenesis of SARS-CoV-2

##### 4.1. Diagnostic testing for COVID-19

Rapid and accurate diagnosis of COVID-19 is of considerable significance for controlling outbreaks in the communities

and hospitals [38]. Technologies such as polymerase chain reaction (PCR), reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR (rRT-PCR), and reverse transcription loop-mediated isothermal amplification (RT-LAMP) have been leveraged as ideal diagnostic tests for CoVs [39,40]. To date, the frontline reaction to the SARS-CoV-2 outbreak has been PCR testing. As the gold standard for diagnosing the source of infection, PCR holds the preponderance that the primers required for such assays can be generated relatively quickly once the viral sequence is identified (Fig. 4) [41]. Soon after the virus was identified, the first quantitative RT-PCR assays to detect SARS-CoV-2 were inaugurated and distributed in January 2020 by WHO. Nevertheless, this test protocol was complicated and high-priced, and is primarily applicable for large centralized diagnostic laboratories. As for the diagnostic criteria currently formulated by the China National Health Commission, nasopharyngeal cancer and oropharyngeal swab tests have ripened into the standard evaluation for the diagnosis of COVID-19 infection. So far, three new RT-PCR tests targeting



**Fig. 4 – COVID-19 diagnostic test by RT-PCR. First, cotton swab is deployed to collect the secretion sample from the patient's nose or throat. The virus particles in the sample are then deactivated along with the separation of RNA strands. Then, the purified RNA strands are copied by utilizing reverse transcription and amplified by RT-PCR to detect the presence of virus-specific gene sequences. Created with BioRender.com.**

the RNA-dependent RNA polymerase (RdRp)/helicase (Hel), nucleocapsid, and spike genes of SARS-CoV-2 had been inaugurated, with extremely lower detection limit *in vitro* [42]. The SARS-CoV E gene detection was superior to the RdRp gene test combined with the one-step RT-PCR system. The E gene PCR was adequate for diagnosing SARS-CoV-2 infection, but the RdRp protocol was endorsed to verify positive results [43]. Remarkably, a new FDA-authorized COVID-19 test using the Abbott ID NOW diagnostics platform has been developed, which can produce results in just 5 minutes cutting down on wait times both in terms of getting tested and receiving a diagnosis. As gene detection of SARS-CoV-2 might provide false negative results, it can be complemented by antibody detection, especially to better screen asymptomatic patients.

Clinically, for those who are recently suffering from fever, fatigue, sore throat, cough or dyspnea due to exposure, the diagnosis of COVID-19 infection should be conducted with typical chest computerized tomography (CT) characteristics regardless of negative RT-PCR outcomes [44].

Most of the COVID-19 cases shared similar characteristics on CT images, presenting bilateral distribution of patchy shadows and ground-glass opacity, sometimes presenting a circular shape and peripheral lung distribution [45]. Some of the data published from China showed that in 21 primal chest CT scans, a large proportion of patients (86%) developed frosted glass opacity, affecting more than one lung lobe (71%) (bilateral involvement) [46]. It is also worth noting that lung cavitation, pleural effusions, discrete pulmonary nodules,

along with lymphadenopathy were absent [46]. In addition to imaging technology, a recent study displayed that the Cas13-based SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) platform can be harnessed for diagnosis of SARS-CoV-2 [47]. In this system, RNA-targeting Cas13 enzyme is deployed to identify specific genetic targets. The Cas13 can cleave nearby RNAs, a 'collateral' feature useful for amplifying a reporter signal in the diagnostic test. However, such a system needs to be further verified in clinical tests. Overall, combined with immunochromatography, colloidal gold, and other biotechnologies, associative detection strategies have been progressed swiftly.

#### 4.2. Pathogenesis of SARS-CoV-2

With regard to the transmission of SARS-CoV-2, primary viral replication is assumed to occur in the mucosal epithelium of the upper respiratory tract and further multiplicates in the lower respiratory tract and gastrointestinal mucosa, inducing a mild viremia [48]. At this point, very few infections are under control and remain asymptomatic. Some patients may also suffer non-respiratory symptoms (*i.e.*, acute liver and heart injury, renal failure, diarrhea), suggesting multiple organ involvement [49,50]. Since ACE2 is extensively expressed in the nasal mucosa, bronchus, lung, heart, and kidney, etc., many human organs are vulnerable to SARS-CoV-2 [51]. Specifically, the S protein plays a critical role in determining the cell tropism and hence interspecies transmission of

SARS-CoV-2 since it hitches the virus to a cellular receptor and subsequently prompts virus entry via membrane fusion [32]. After binding to the receptor, the spike protein can catalyze the viral fusion process, allowing the viral genome to enter the cytoplasm. A prerequisite for this procedure is the division of S to subunits, a process known as priming (Fig. 3). The work of Hoffmann et al. unraveled that SARS-CoV-2 utilizes the ACE2 receptor for entry and the serine protease TMPRSS2 for S protein priming [52]. Hence, TMPRSS2 inhibitors approved for clinical use may block the entrance and may give rise to an underlying treatment option. Notably, the ability that S can readily obtain new protease cleavage sites and the fact that miscellaneous proteases can perform the same task suggests that this virus can easily adapt to the proliferation in several cell types [33]. Further, a panel of murine monoclonal antibodies (mAbs) and polyclonal antibodies (pAbs) against SARS-CoV-S1/receptor-binding domain (RBD) had been reported to be unable to interplay with S protein, implicating conspicuous discrepancies in antigenicity between SARS-CoV-2 and SARS-CoV [34].

According to the pathological findings, the first report on the pathological results of severe COVID-19 revealed that diffuse alveolar injury on both sides of the lung was accompanied by cellular fibromyxoid exudates [53]. The right lung displayed significant lung cell shedding and hyaline membrane formation, suggesting ARDS. Moreover, the left lung tissue showed pulmonary edema and hyaline membrane formation, implying early ARDS. Interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes, were found in both lungs. Another study reported that acute kidney injury and proteinuria might also occur during the progression of COVID-19 disease. ACE2 was seen to be upregulated in COVID-19 patients, and immunostaining with the SARS-CoV nucleoprotein antibody was positive in tubules [54]. Additionally, only a few interstitial mononuclear inflammatory infiltrates were found in the heart tissue, meaning that this virus may not directly induce heart impairment [53]. Aside from the acute respiratory distress syndrome, exuberant inflammatory responses during the infection process were also observed in clinical, giving rise to unrestrained pulmonary inflammation. Of note, the virus-induced ACE2 downregulation, rapid virus replication and cell damage, and antibody-dependent enhancement may lead to aggressive inflammation aroused by SARS-CoV-2 [55]. The initial stage of rapid viral replication would induce a large number of epithelial and endothelial cell death, thereby facilitating the generation of raging pro-inflammatory cytokines and chemokines (Fig. 5) [56]. Intriguingly, a recent research compared the transcriptional responses of SARS-CoV-2 with other respiratory viruses to identify the transcriptional features that might form the biological basis of COVID-19 [57]. Their work demonstrated that the overall transcriptional induction of SARS-CoV-2 is abnormal. Despite viral replication, the host's response to SARS-CoV-2 failed to initiate powerful responses of type I and III interferons (IFN-I and -III), and at the same time induced high levels of chemokines required to recruit effector cells. In other words, this unique inflammatory response was defined by low levels of IFN-I and -III juxtaposed to elevated

chemokines and high expression of IL-6. The reduced innate antiviral defenses coupled with exuberant inflammatory cytokine production may be the defining and driving features of COVID-19 [57]. Moreover, because the weakened immune response will further enable sustained viral replication, this critical finding may explain why severe cases of COVID-19 are more frequently observed in patients with comorbidities [58].

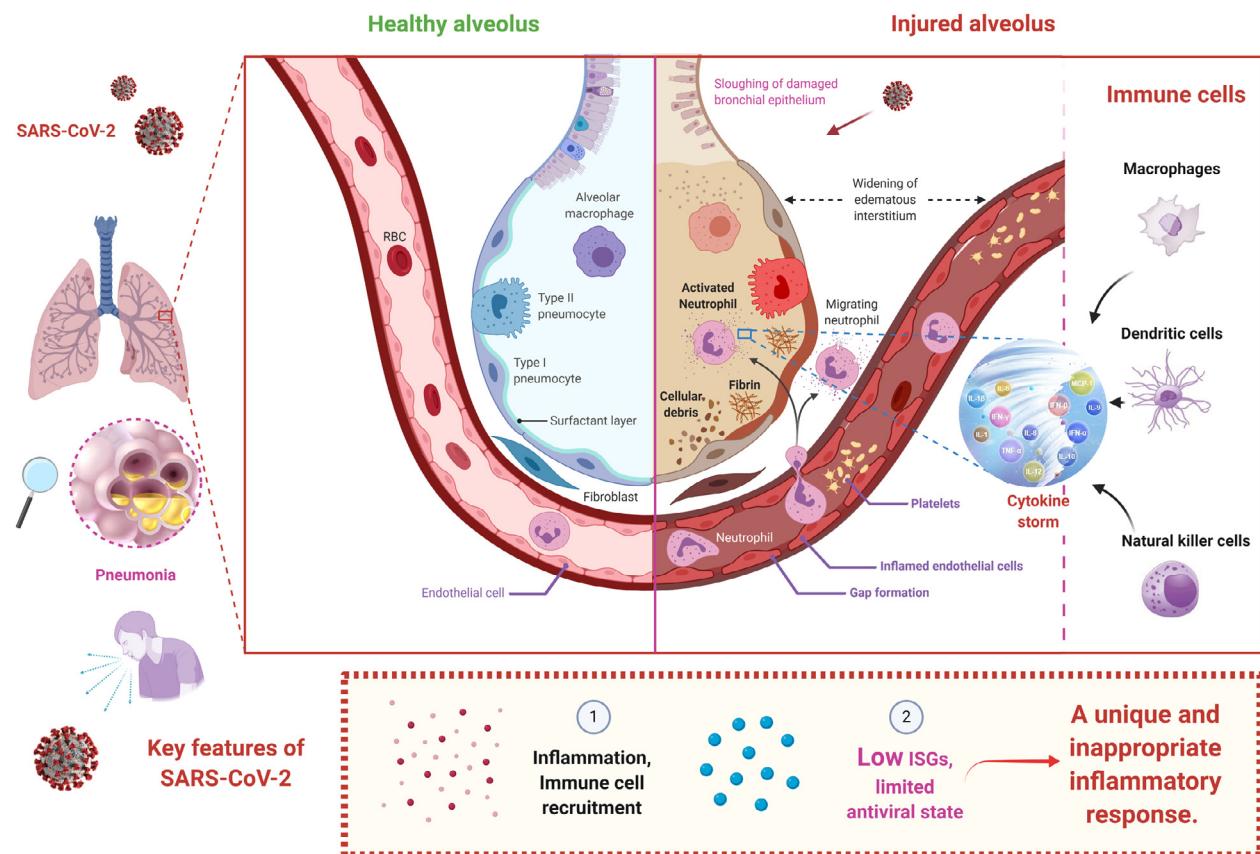
In addition to the cytokine storm, several experimentations had unraveled that lymphopenia is a customary characteristic of COVID-19, which may also accounts for severity and mortality [49,57].

## 5. Treatment strategy of COVID-19

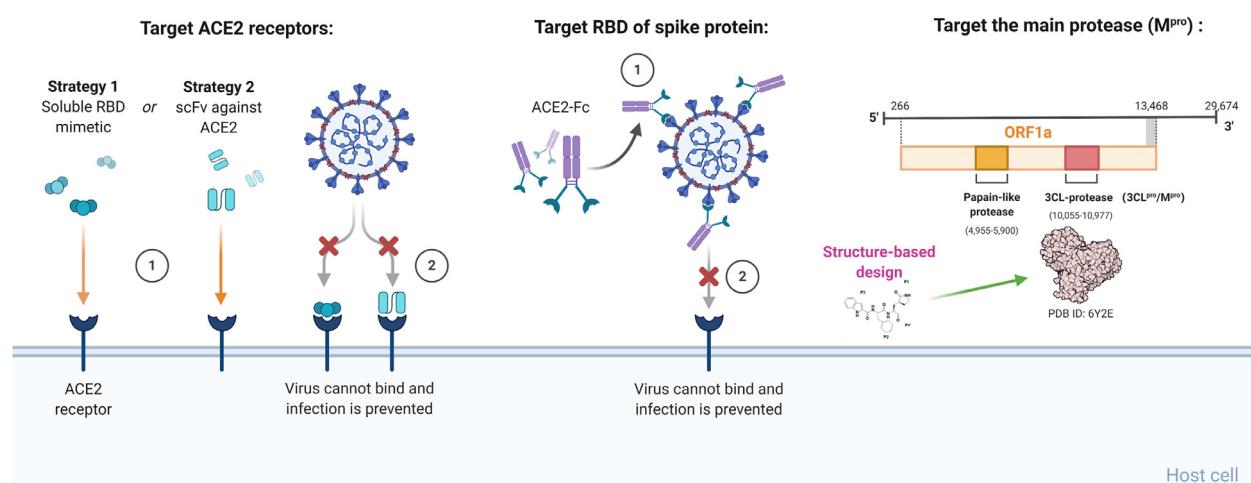
### 5.1. Crucial SARS-CoV-2 targets for novel drug development

The preceding overview of the virology of SARS-CoV-2, as well as the sundry potential mechanisms of damage to the host, lay the foundation for developing specific targeted treatment and prevention. A general idea of pivotal targets for drug discovery is shown in Fig. 6. In consideration of the role of the surface structural S in the virus-cell receptor interplay, it is of particular interest for the antiviral development. mAbs against the S1 subunit RBD and fusion inhibitors targeting the S2 subunit possess potential anti-SARS-CoV-2 capacity *in vitro* or *in vivo* [59]. Besides, since ACE2 is the key functional host receptor of SARS-CoV-2 to determine the pathogenicity, mAbs, or molecules targeting the host receptor are effective anti- SARS-CoV-2 drugs, as long as they do not elicit immunopathological effects in animal models [4]. A recent study also probed into the COVID-19 S protein-binding site to the cell-surface receptor (known as Glucose Regulated Protein 78 (GRP78)). Their outcomes unveiled that the binding between regions III and IV of the S protein model and GRP78 was more favorable. In this regard, region IV is the major tractive force for GRP78 binding, and these 9 residues can be leveraged to design therapeutics specific against this disease [60]. Of note, although inhibitors of the proteases that prime S for fusion possess antiviral activity, multiple inhibitors are needed because S can utilize a variety of proteases for priming [61]. In addition, agents directly targeting the highly conserved S2 subunit may be potential treatment candidates.

For SARS-CoV-2, the large replicase polyprotein 1a (pp1a) and pp1ab encoded by the ORF1a/b are subjected to two viral proteases, papain-like protease (PL<sup>PRO</sup>) and 3C cleavage-like protease (3CL<sup>PRO</sup>) (also known as M<sup>PRO</sup>), for producing non-structural proteins (i.e., RdRp, helicases) which are correlated to viral transcription and replication (Fig. 3) [4]. Therefore, enzyme inhibitors targeting these proteins may exhibit anti-SARS-CoV-2 activity *in vitro*. A recent study has uncovered that the M<sup>PRO</sup> of SARS-CoV-2, which is the translated polyproteins of ORF 1a/b, is a crucial enzyme that mediates viral replication and transcription [62]. Specifically, a Gln-residue almost always requires a substrate at P1 (an amino acid in substrates). There is currently no human homologous for M<sup>PRO</sup>, which makes it a promising antiviral target (Fig. 6) [63]. Dai and coworkers had conducted the structure-based design of antiviral agents targeting this protease by parsing out the



**Fig. 5 – The potential mechanism of SARS-CoV-2 inducing cytokine storm. Increased cytokine levels (IL-6, IL-10, and TNF- $\alpha$ ) are associated with severe COVID-19. ISGs, IFN-stimulated genes. Parts of this figure created with BioRender.com.**



**Fig. 6 – Crucial SARS-CoV-2 targets for novel antiviral drug development. ACE2 receptor, receptor-binding domain along with the main protease M<sup>pro</sup> can be leveraged as antiviral targets. Created with BioRender.com.**

substrate-binding pocket of M<sup>pro</sup> [62]. In this regard, further study targeting such a protease may give rise to certain antiviral drug candidates.

Another notable drug target may be the cellular enzymes that attach fatty acids to a cluster of cysteines in the cytoplasmic tail of S due to the fact that fatty acids are

essential for the fusion of host cell and assembly of the virus, like the description of other S proteins, such as hemagglutinin for influenza viruses. The enzyme that connects the acyl chain to S has not yet been discovered, but the cellular protein will undergo acetylation by the members from the ZDHHC family with unique, only partially overlapping substrate specificities.

If a few of them may be acetylated in the airway cells of the lungs, their blockage may suppress the viral replication, and the acylation of cellular proteins will rarely be damaged. In this regard, targeting acyltransferases may be promising, because the cysteine group is existed in all CoV genus S, in spite of their source [64]. However, in consideration of the palmitoylation of crucial proteins in the innate immunity, if the proteins of the innate immune response are modified with the same enzymes as viral proteins, the acylation inhibitor may be limited.

In addition, Bojkova et al. recently identified the host cell pathways modulated by SARS-CoV-2 infection and revealed that suppression of these pathways may prevent viral replication in human cells [65]. Of note, SARS-CoV-2 infection profile was determined by translatome3 and proteome proteomics at different times after infection, suggesting that this virus could reshape central cellular pathways (e.g., translation, splicing, carbon metabolism and nucleic acid metabolism) [65]. Accordingly, spliceosome inhibitors and glycolysis inhibitors may also be the potential targets.

The last point of view is the application of small interfering RNAs (siRNAs). SARS-CoV-2 can disintegrate in host cells, releasing the nucleocapsid and viral RNA into the cytoplasm, and then translate ORF1a/b into pp1a and pp1ab for genomic RNA replication [66]. Hence, siRNAs targeting structural genes may play a role in the SARS-CoV-2 infections, and further optimizing the delivery of siRNAs *in vivo* may make them clinically valuable.

### 5.2. Drug discovery approaches toward anti-SARS-CoV-2 drug screening

The drug discovery strategy bears the brunt of the COVID-19 outbreak is the test of existing broad-spectrum antiviral agents that have been harnessed to cure other viral infections via utilizing standard tests for measuring the effects of drugs on the cytopathy, virus production along with plaque generation in live and pseudotyped CoVs [67]. Drug discovery utilizing this method encompass interferon  $\alpha$ , interferon  $\beta$ , interferon  $\gamma$ , arbidol, ribavirin, along with cyclophilin inhibitors [65,66,68]. These drugs possess the distinct preponderance of easy access to known pharmacokinetic and pharmacodynamic features, dose regimens, and adverse effects [69]. Nevertheless, they have no specific anti-SARS-CoV-2 effect and may be correlated with severe untoward effects.

In addition to the test of existing broad-spectrum antiviral agents, another anti-SARS-CoV-2 drug discovery approach might be the chemical libraries screening, which involves a good deal of existing compounds or databases with the information on transcriptional signatures in disparate cell lines [68,69]. Such a method holds the potential of offering a prompt, high-throughput screening of many off-the-shelf composites, which can thereby be further assessed via antiviral detection test. Importantly, a variety of different types of agents have been discovered among these agent repurposing programs, incorporating many that with significant physiological and immune effects (i.e., those affecting the lipid or sterol metabolism, regulation of neurotransmitters, kinase signaling, estrogen receptors,

and DNA synthesis/repair) [70–73]. It is noteworthy that this method has the main drawback that most agents are not clinically useful in virtue of their underlying immunosuppressive effects or anti-SARS-CoV-2 half-maximal effective concentration values that significantly surpass the peak serum concentration levels that are obtainable at the therapeutic dose [74]. An interesting exception is the anti-HIV protease inhibitor lopinavir-ritonavir, which has been reported to be effective for SARS-CoV in both non-human primate models and in non-randomized clinical trials [75]. In addition, nelfinavir, a selective inhibitor of HIV protease, which has been demonstrated to possess a sound suppression of SARS-CoV, suggestive of a potential drug candidate for COVID-19 [76].

The most critical method for anti-SARS-CoV-2 drug discovery includes the de novo development of new, specific drugs based upon the genomic and biophysical understanding of this virus. For instance, the determination of key SARS-CoV-2 targets may bring about the production of siRNA molecules or inhibitors against specific viral enzymes correlated with viral replication. Additionally, mAb targeting host receptors, inhibitors of host cell proteases, host cell endocytosis viruses, along with humanized mAb targeting the RBD, and antiviral peptides targeting the S2 subunit offer various methodology and options for the design and development of possible therapeutics. With the emerging outbreak of the COVID-19 pandemic, the above-mentioned methods are critical for identifying candidate drug composites that can be widely categorized into virus-based and host-based therapy options.

### 5.3. Therapeutics options for SARS-CoV-2 in clinical

During the outbreak of the pandemic COVID-19, considerable efforts are underway to discover novel therapeutic drugs for CoV infections. A wide variety of agents has been selected as therapeutic options for SARS-CoV-2 in clinical trials (Table 1).

#### 5.3.1. Antiviral agents

##### (I) Nucleoside Analogs

Nucleoside analogs can interfere with cellular nucleotide synthesis pathways and terminate viral genome replication through accumulating mutations and cutting off the entry of natural nucleotides [83]. Since nucleosides and nucleotides are the basic components of viral nucleic acids, nucleoside analogs serve as viral RNA synthesis inhibitors in a wide spectrum of RNA viruses. With the view of the targeting ability toward RdRp, nucleoside analogs are responsible for viral RNA replication [84].

Ribavirin is a guanine analog among approved nucleoside analogs used to treat hepatitis c virus and respiratory syncytial virus infections and has been harnessed to cure patients with SARS [85]. Ribavirin was extensively leveraged for patients with or without concomitant usage of steroids during the SARS outbreak in 2003 [86]. When combined with IFN- $\beta$ , it can exert the synergistical inhibition effect on SARS-associated CoV replication *in vitro* [87]. However, the efficacy and safety of this agent remain uncertain, and it may arouse adverse reactions like anemia in high doses [77]. In COVID-19 therapy, ribavirin was utilized with pegylated interferon for

**Table 1 – List of candidate therapeutic drugs for SARS-CoV-2 therapy in clinical trials.**

Category	Candidate therapeutics	Modality	Manufacturer	Status of clinical trials
Nucleoside Analogs	Pegylated interferon with ribavirin [77]	/	Valeant	Under clinical trial for COVID-19 (ChiCTR2000029387)
	Favipiravir (T-705) [78]	A guanine analog for the treatment of influenza virus infections	Toyama	Under clinical trial for COVID-19 (ChiCTR2000029548)
	Remdesivir [79] (GS-5734)	An adenine analog with a similar chemical structure to the approved HIV reverse transcriptase inhibitor tenofovir alafenamide.	Gilead	Phase II clinical trial for Ebola (NCT03719586); Under phase III clinical trials for COVID-19 (NCT04252664)
Type I interferons	IFN- $\beta$ 1 [80]	Used as a treatment for multiple sclerosis	Multiple companies	Treatment of COVID-19 in the early stage
Protease inhibitors	Lopinavir and ritonavir [76]	Protease inhibitors approved as anti-HIV drugs	Abbott	Under clinical trials for SARS; Under clinical trial for COVID-19 (ChiCTR2000029539)
Chloroquine	Chloroquine and hydroxychloroquine [81]	Oral prescription drugs for treatment of malaria and certain inflammatory conditions	Multiple companies	Under investigation in clinical trials for pre-exposure or post-exposure prophylaxis of SARS-CoV-2 infection
Antibodies	CR3022 [82]	A SARS Cov-specific human monoclonal antibody	Multiple companies	Experimental phase

stimulating the innate antiviral reaction at a relatively lower dose to curtail side effects.

In addition, another promising guanine analog is favipiravir (T-705), which has been approved in Japan for the therapy of influenza virus infections and has also been demonstrated to suppress the replication of Ebola, yellow fever, enterovirus and norovirus [78]. Recently, Wang and coworkers suggested that favipiravir may also be a potential candidate for COVID-19 therapy, which showed effective antiviral activity in Vero E6 cells with an EC50 value of around  $61\mu M$ . To improve the condition of COVID-19 patients, favipiravir was utilized with other antiviral drugs like baloxavir marboxil [88].

As an adenine analogue with a similar chemical structure to the approved HIV reverse transcriptase inhibitor tenofovir alafenamide, remdesivir (GS-5734) exhibits broad-spectrum antiviral activity against several RNA viruses and has the capacity of competing with RdRp (Fig. 7) [89]. It also possesses outstanding *in vitro* antiviral activity compared with lopinavir and ritonavir [90]. In the United States, the first case of SARS-CoV-2 infection was reported, and remdesivir was administered. The patient's clinical condition improved after only one day of remdesivir treatment [79]. A newly released research offered remdesivir for COVID-19 inpatients on the basis of sympathy. In the cohort of patients admitted for treatment of severe COVID-19, patients treated with sympathetic remdesivir achieved relatively good clinical improvement (36 of 53 patients (68%)) [91]. However, recently, leaked data from a crucial remdesivir investigation suggests this potent CoV agent may not be effective. Accordingly, the chief medical officer of Gilead Sciences said that the summary post online might include inappropriate characterizations of the study and it was terminated early due to low enrollment

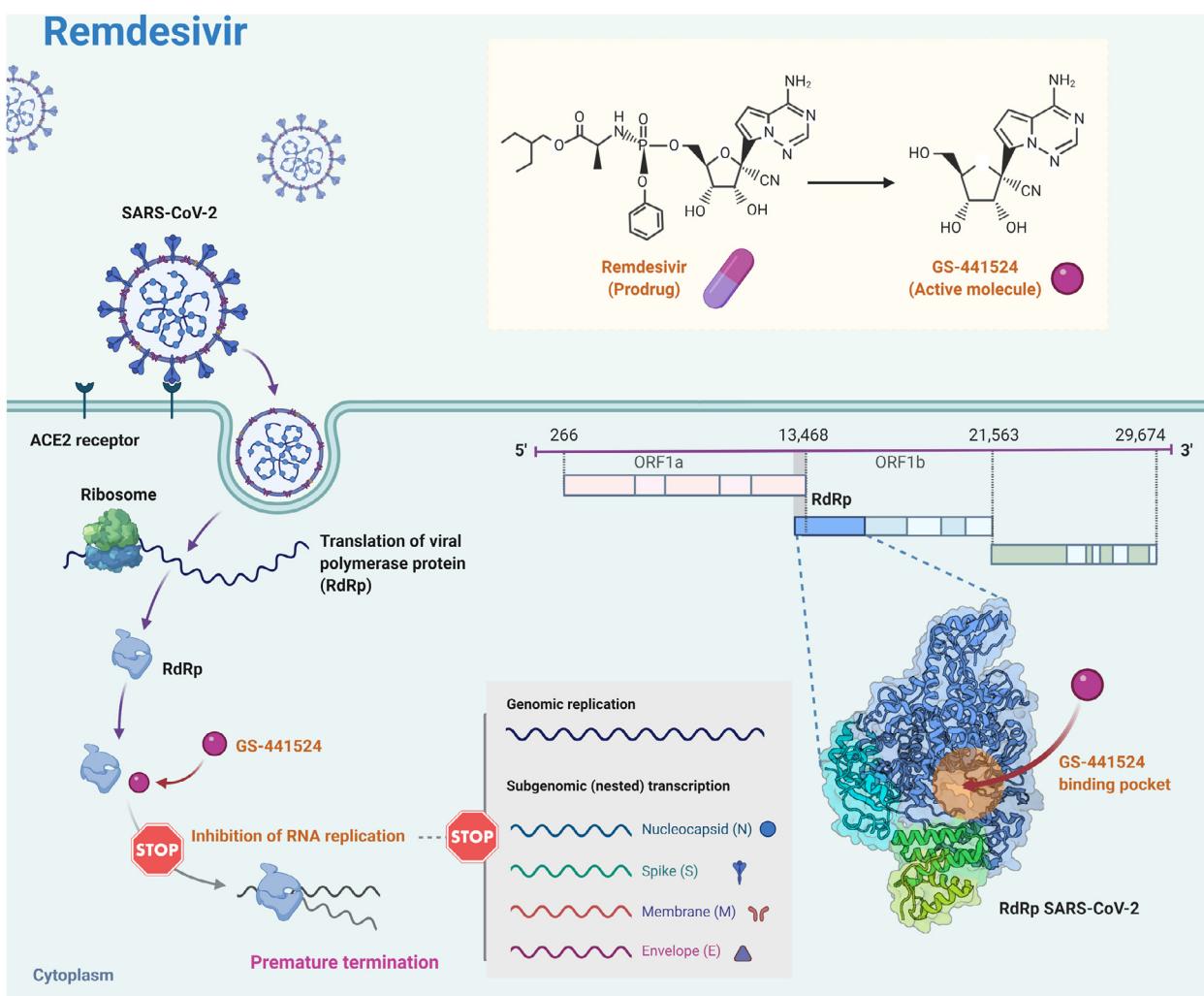
[92]. Paradoxically, the drug's maker announced that in its own trial, more than half of 400 participants with severe COVID-19 had recovered from their illness within two weeks of receiving treatment [93]. However, this study lacked a placebo-controlled arm. Moreover, the outcomes from the National Institute of Allergy and Infectious Diseases (NIAID) further demonstrated that this drug can stop some patients from becoming critically ill [93]. In this regard, these findings suggest that differences in races and differences in clinical trials may lead to disparate experimental outcomes. The real anti-SARS-CoV-2 activity of remdesivir need to be further studied in the long term.

### (II) Type I interferons

Type I interferons designate a cluster of antiviral cytokines consisting of the omnipresent  $\alpha$  and  $\beta$  subtypes, along with the  $\omega$ ,  $\epsilon$  and  $\kappa$  subtypes, inducing large numbers of proteins that can undermine viral replication in host cells [94]. Former researches have revealed that IFN- $\beta$  was superior against SARS-CoV in comparison with IFN- $\alpha$  [95]. As for the clinical trial of type I interferons, in China, therapy guidelines for COVID-19 recommend the management of 5 million U of IFN- $\alpha$  via vapor inhalation in patients twice daily with ribavirin as combined therapy [96]. In the detailed study of Sallard et al., they reported that IFN- $\beta$ 1 might constitute a safe and easy to upscale therapy against COVID-19 in the early stages of the disease. Besides, *in vitro* data indicated that this virus might be significantly more sensitive to IFN-I than other CoVs [80].

### (III) Protease inhibitors

Protease inhibitors are promising candidates for antiviral agents, and can block the replication of viral genes via binding to enzymes that are responsible for proteolysis



**Fig. 7 – Potential antiviral mechanism of remdesivir against SARS-CoV-2. The active molecule metabolized from remdesivir prodrug (GS-441524) may intercept RdRp early in viral replication, thereby interfering with the downstream steps of the SARS-CoV-2 replication cycle. Created with BioRender.com.**

[97]. Lopinavir and ritonavir, both protease inhibitors, have been approved as HIV medicines and have been found to possess antiviral activity against SARS and MERS. For the sake of destroying the SARS-CoV-2, clinical trials had begun to parse out the antiviral property of HIV protease inhibitors among patients. Notwithstanding, the antiviral effect of such inhibitors in coronavirus proteases remains controversial. Notably, a study regarding the comparison of the efficacy of prophylactic remdesivir and therapeutic remdesivir in combination with lopinavir, ritonavir, and interferon  $\beta$  against MERS-CoV unveiled that remdesivir was more effective than the combination therapy in reducing viral load and improving the degree of pathological changes in lung tissue. Aside from the gastrointestinal adverse reactions aroused by lopinavir-ritonavir, it is worth noting that lopinavir-ritonavir treatment alone may fail to offer benefits in comparison with standard care alone. The median time for clinical improvement was 16 d, and the reduction in viral RNA load among patients with severe SARS-CoV-2 did not appear to differ in both cases [98]. Despite the disheartening outcomes, a marginally

lower number of deaths were observed among the patients with lopinavir-ritonavir treatment in the late stage of this disease in contrast to the standard-care group. Further, Baden and Rubin unraveled that lopinavir simply was not particularly potent against SARS-CoV-2. The concentration necessary to suppress viral replication is relatively high as compared with the serum levels found in patients treated with lopinavir-ritonavir [99]. Besides, nelfinavir, which is a selective inhibitor of HIV protease, has also been displayed to possess a robust suppression of SARS-CoV, indicating an alternative therapeutic option for COVID-19 [76].

### 5.3.2. Chloroquine and hydroxychloroquine

Chloroquine, as a drug extensively utilized in anti-malaria and autoimmune diseases, has been found to be a potential broad-spectrum antiviral agent [100]. It can prevent viral infections via elevating the endosomal pH needed for virus-cell fusion and disturbing the glycosylation of SARS-CoV cell receptors [101]. Gao et al. revealed that chloroquine was effective in the therapy of COVID-19-associated pneumonia [102]. Wang

and coworkers also conducted in vitro study, and they found that it is an ideal candidate antiviral drug against SARS-CoV-2 infection in Vero E6 cells with EC<sub>50</sub> value of around 1 μM [88]. Although several trials had verified that chloroquine suppresses the exacerbation of COVID-19, the optimal dosage of chloroquine will require to be evaluated in future trials [102].

Hydroxychloroquine is an analog of chloroquine, and there are few studies on its interaction [103]. In previous SARS outbreaks, hydroxychloroquine was found to possess anti-SARS-CoV capacity in vitro [104]. In line with the research of Yao et al. by applying a physiologically based pharmacokinetic model, they found that hydroxychloroquine is more effective than chloroquine in Vero cells infected with SARS-CoV-2 [105]. Of note, it has been revealed that cytokines IL-6 and IL-10 are elevated in response to SARS-CoV-2 infection, which may induce the cytokine storm (Fig. 5), which in turn cause multiple organ failure and death [49]. Both chloroquine and hydroxychloroquine possess immunomodulatory effects and can inhibit such immune responses [106]. Hence, Chinese hospitals and Oxford University had initiated 21 clinical studies to evaluate the efficacy of these drugs in COVID-19 infection. The further crucial study may relate with the determination of whether the benefit of chloroquine treatment is hinged on the age of the patients as well as the clinical manifestations [107]. Recently, a multinational registry analysis of the application of hydroxychloroquine or chloroquine unraveled that there might be an increased frequency of ventricular arrhythmias during the treatment of COVID-19 diseases [108]. The WHO has also suspended hydroxychloroquine treatment in COVID-19 solidarity trial in terms of the outcomes of in-hospital mortality and de-novo ventricular arrhythmias [109].

### 5.3.3. Monoclonal or polyclonal antibodies

Monoclonal or polyclonal antibodies have been recommended as tools for preventing and treating viral infections. Considering the relatively high RBD in SARS-CoV-2, the cross-reactivity of anti-SARS-CoV antibodies with the COVID-19 S protein was evaluated. Tian et al. determined the potent binding of S protein via a SARS CoV-specific human mAb CR3022 [82]. Unfortunately, other SARS-CoV RBD directed mAbs (i.e., 230, m396, and 80R) cannot bind to the COVID-19 RBD [32]. In this respect, CR3022 may be a promising therapeutic candidate, either alone or in combination with other neutralizing mAbs, for the therapy of COVID-19 disease. In addition, tocilizumab is a monoclonal antibody for the therapy of RA exacerbation. It was designed to suppress the binding of IL-6 to its receptors, hence mitigating cytokine release syndrome. At present, it is also being trailed for the COVID-19 therapy [110]. Interestingly, Shi et al. reported a human neutralizing antibody targets the receptor binding site of SARS-CoV-2 [111]. In their work, they successfully isolated 2 specific human mAbs (named as CA1 and CB6) from a convalescent COVID-19 patient. CA1 and CB6 demonstrated potent SARS-CoV2-specific neutralization activity in vitro against SARS-CoV-2. Moreover, CB6 could suppress SARS-CoV-2 infection in rhesus monkeys at both prophylactic and treatment settings. The following structural studies unraveled that CB6 could recognize an epitope that overlaps with ACE2-

binding sites in SARS-CoV-2 receptor binding domain, thus interfering with the virus/receptor interactions by both steric hindrance and direct interface-residue competition [111]. Another recent study revealed the isolation and characterization of 206 RBD-specific mAbs derived from single B cells of eight SARS-CoV-2 infected individuals [112]. The researchers identified antibodies with potent anti-SARS-CoV-2 neutralization capacity that correlated with their competitive capacity with ACE2 for RBD binding. Of note, crystal structure analysis of RBD-bound antibody uncovered steric hindrance that suppressed viral engagement with ACE2 and thereby blocked viral entry [112]. These findings implicate that anti-RBD antibodies may be viral species-specific inhibitors.

Besides, most patients with severe COVID-19 suffered a lot from the cytokine storm (Fig. 5) [53]. So, neutralizing antibodies against other pro-inflammatory cytokines may be another promising strategy to dampen the inflammatory responses, thus responding well to treat the COVID-19. In a clinical trial conducted in Anhui, China, IL-6 receptor-targeted mAb tocilizumab was utilized to treat 21 patients with severe COVID-19. Clinical data showed quick fever control and improved respiratory function [113]. Overall, the development of COVID-19-specific antibodies takes a long time, meaning the difficulty in applying antibodies for neoteric pathogens to clinical practice in a brief period.

### 5.3.4. Corticosteroids

As a class of drug that lowers inflammation in the body, corticosteroids have been utilized in several serious viral respiratory infections such as SARS-CoV and MERS-CoV with limited benefits. However, in some cases, there is proof of delayed viral clearance and increased rates of secondary infection and mortality [114]. In a study of 41 COVID-19 patients, 22% were given corticosteroids, which inhibit inflammation in the lungs [49]. However, according to the current WHO interim guidance, glucocorticoids are not recommended for routine treatment unless otherwise indicated, as other coronaviruses and influenza studies have identified possible injury and increased risk of death from glucocorticoid therapy [115]. Despite the potential antiviral activity toward COVID-19, corticosteroids should not be given principally, and corticosteroid pulse therapy should be conducted with caution. In addition, corticosteroid therapy for the SARS therapy yielded adverse effects such as psychosis, diabetes, and avascular necrosis [116]. In general, the treatment with corticosteroids may be harmful, but such agents can be prescribed to the right patient at the proper time.

### 5.3.5. Convalescent plasma transfusion

Convalescent plasma has also been utilized as a last resort to improve the survival in patients with miscellaneous viral infections (i.e., SARS, H5N1, H1N1, and Ebola virus infection) [117]. The theoretical basis for the therapeutic effect of plasma in the recovery phase is that immunoglobulin Abs in the plasma of patients recovering from the viral infection may inhibit viremia. To date, in the previous SARS therapy, convalescent plasma given early after the onset of symptoms reduced overall mortality after treatment

compared to placebo or no treatment [118]. For the test of convalescent plasma effect on COVID-19 infection, Zhou et al. found that the SARS-CoV-2 isolated from bronchoalveolar lavage fluid in a severe patient could be neutralized by the serum of several patients [6]. Additionally, another study of Shen et al. showed that improvements in clinical conditions were observed following plasma transfusions, improvements included normalization of body temperature within three days, a decrease in Sequential Organ Failure Assessment score, resolution of ARDS, and decline in viral loads [119]. During the emerging of COVID-19 infection in China, the National Health Commission of China also called on convalescent patients to donate blood for COVID-19 therapy. Despite the difficulty in collecting the plasma, convalescent plasma therapy showed great therapeutic potential for the COVID-19 therapy.

#### 5.3.6. Herbal medications

Based on data mining, the Traditional Chinese Medicine was also regarded as an alternative method for the treatment of COVID-19 disease in high-risk populations. During the COVID-19 outbreak in China, Chinese herbal drugs such as *Astragali Radix*, *Glycyrrhizae Radix Et Rhizoma*, *Atractylodis Macrocephalae Rhizoma*, *Saposhnikoviae Radix*, *Lonicerae Japonicae Flos* and *Fructus Forsythiae* were commonly harnessed for improving the treatment effect of COVID-19 infections. Herbal medications can offer a more effective and personalized treatment by adjusting the specific medicine for each patient based on the disparate syndromes [120]. Previous studies also indicated that the patients with SARS-CoV infection have benefited from TCM treatment [121], involving amelioration of adverse effects of traditional therapeutics. Some TCM such as Lianhuaqingwen capsules exert anti-viral replication and anti-inflammatory activity against SARS-CoV-2 in vitro [122]. However, the experimental study on the efficacy of herbal medicines lags behind the clinical application of traditional Chinese medicine in the COVID-19 treatment [123]. Hence, rigorous clinical trials on large populations should be carried out to identify the potential therapeutic efficacy of herbal medications.

#### 5.3.7. Mesenchymal stem cell therapy

Stem cell therapy is also making its way into COVID-19 disease treatment. Recently, some researches have demonstrated that the intravenous transplantation of mesenchymal stem cells (MSCs) was safe and efficient for COVID-19 pneumonia, especially for critically ill patients [124]. As mentioned, COVID-19 infection may induce uncontrolled inflammatory innate reactions along with undermined adaptive immune reactions, subsequently leading to detrimental tissue damage. MSC-based immunomodulation treatment is able to counteract the cytokine storm aroused by the immune system and foster endogenous repair via reparative attributes of the stem cells [125]. As it is unveiled by the recent study of China, seven COVID-19 patients (1 critically ill patient, 4 critically ill patients, and 2 patients with mild symptoms) received intravenous injection of bone marrow MSCs. In all cases, the patients were cured, while the 3 patients in the placebo control group all suffered from severe illness, 1 died, 1 developed ARDS, and 1 was in

stable condition [126]. This study uncovered that MSCs could reclaim the lung microenvironment, protect the alveolar epithelial cells, block pulmonary fibrosis, and treat pulmonary dysfunction [124]. Notably, the FDA has also opened the way to the compassionate use of MSCs intravenous infusions in patients with COVID-19 ARDS and very dismal prognosis [127]. Although such a kind of treatment is an ideal option for COVID-19 therapy, it has limitations regarding the supply of clinical-grade MSCs and the rate of preparation for subsequent clinical use.

#### 5.3.8. Other therapies

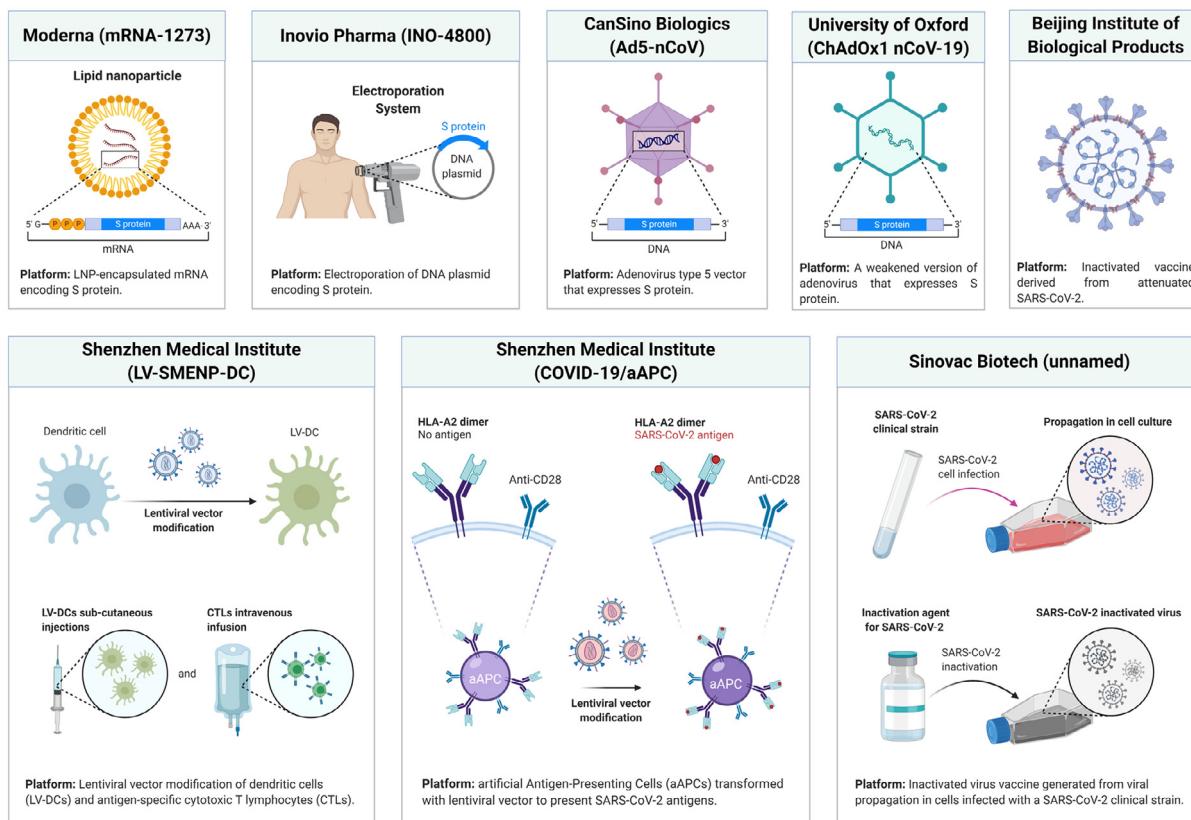
Hydrogen peroxide ( $H_2O_2$ ) appears to be another potential therapeutic option for COVID-19. Of note, health experts have said that this compound could help prevent the virus from spreading across the body and from causing damage [128]. A recent study illustrated that even just 0.5% of  $H_2O_2$  could kill human CoVs, such as those that caused SARS and MERS [129]. Inhaling the vapor with a nebulizer has been the most convenient way to receive  $H_2O_2$  to fight viral infections. The microscopic mist can easily penetrate the nostrils, sinuses and lungs, which are commonly affected by respiratory diseases like COVID-19. Besides, molecular hydrogen has been verified to favorably modulate the generation of both  $O_2^-$  and NO through influencing NADPH oxidase, and the NOS enzymes [130]. According to the National Health Commission of China, the conditional use of mixed inhalation of hydrogen and oxygen ( $H_2/O_2$ : 66.6%/33.3%) treatment may improve the symptoms [131].

Lung transplantation can be performed in advanced patients with respiratory failure owing to COVID-19-related pulmonary fibrosis. As it is reported in a clinical study, lung transplantation may offer the ultimate treatment option for severe patients to avoid certain deaths, while at the same time protecting transplantation doctors and medical staff appropriately [132].

## 6. Development of SARS-CoV-2 vaccines

In terms of controlling the epidemic aroused by emerging viruses, rapid diagnosis and effective vaccines serve as a complementation to antiviral therapy. Preventive and therapeutic SARS-CoV-2 vaccines will be of fundamental value as the most conspicuous way to mitigate the pandemic crisis [133]. Fortunately, published data on the SARS-CoV-2 genetic sequence has sparked a global campaign to inaugurate a vaccine against the infections. The scope of the impact of the COVID-19 pandemic on humanitarianism and the economy is also prompting the assessment of the next-generation vaccine technology platform through new paradigms. On March 16th, 2020, the first trial of COVID-19 vaccine candidate was launched in record speed. Besides, recently, Chen and her team developed and assessed the safety, tolerability and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine [134]. Their clinical data further revealed that the Ad5 vectored COVID-19 vaccine was tolerable and immunogenic at 28 days post-vaccination [134]. Moreover, the Coalition for Epidemic Preparedness Innovations (CEPI) is also

## Clinical Phase Vaccine Candidates for COVID-19



**Fig. 8 – Clinical phase vaccine candidates for COVID-19. aAPC, artificial antigen-presenting cell; MHC, major histocompatibility complex class; VLP, virus-like particle; DC, dendritic cell; LV, lentiviral vector; CTLs, cytotoxic T lymphocytes; HLA-A, a group of human leukocyte antigens (HLA) that are coded for by the HLA-A locus. Created with BioRender.com.**

combining efforts to espouse the development of vaccines against COVID-19. Fig. 8 summarizes the current cross-sectional COVID-19 vaccine candidates which have entered the clinical phase.

As for the vaccine development of SARS-CoV-2, the pivotal and tangible avenues can be divided into four aspects: (1) Selection of antigen epitope; (2) Overcoming the antibody-dependent enhancement (ADE) issue; (3) Weighing humoral immunity and cellular immunity; (4) Selection of technical route befittingly. Up till now, structural epitope mapping by homology modeling has uncovered the immunoreactive antigen epitopes of SARS-CoV-2 [135]. The mainstream of the vaccine development is based upon the S protein in virtue of its essential role in the viral infectivity. Other subsequent developments can constrain focus on other viral proteins (i.e., the N protein, and E protein). Further, the titers of neutralizing antibodies that were variable among different patients were associated with the spike-binding Abs targeting S1, RBD, and S2 regions [136]. In this regard, we should also pay more attention to the titers of neutralizing antibodies.

In addition, researchers need to know whether the vaccine will induce the same type of immune system failures that have been observed previously. In some cases, the vaccine-primed immune system seems to initiate a shoddy response

to natural infections [137]. Additionally, allergic inflammation aroused by Th2 immunopathology should be taken into consideration, according to theCoV experts [137]. Therefore, animal and human clinical trials of COVID-19 candidate vaccines should encompass a rigorous assessment of possible immune complications before putting into use.

According to the previous study on SARS-CoV, SARS-specific IgG Ab may ultimately fade away, and the peripheral memory B cell response cannot be detected in recovered SARS patients. In stark contrast, the memory response of specific T cells lasted at least six years, implicating the significance of cellular immunity for preventing the recurrence epidemics [138]. With regard to the technical routes, we can see efforts to espouse ‘quick-fix’ programs for the purpose of developing vaccines against COVID-19 worldwide [139]. There is a desperate need for selecting effective technical routes to develop different kinds of vaccines (i.e., live-attenuated vaccines, inactivated vaccines, nucleic acid vaccines, subunit, recombinant, polysaccharide and conjugate vaccines) in a quicker and safer manner [140].

As announced by the WHO, there are now more than 70 potential vaccines under development, with three already in clinical trials [141]. The following section will describe the status of vaccine development against this crisis by

miscellaneous methods. The potential vaccine candidates for COVID-19 are summarized in [Table 2](#).

### 6.1. DNA-based vaccines

DNA vaccines provide a precise and flexible tactic to deliver antigens to the immune system and can contain additional sequences of coding molecules to manipulate the results [\[147\]](#). So far, a variety of DNA vaccine platforms have been exploited to enhance the vaccine efficacy through electroporation to deliver plasmids and addition of adjuvants, yielding improved the immune responses [\[148\]](#). Inovio Pharmaceuticals have begun pre-clinical trials of a DNA vaccine (named as INO-4800) against COVID-19 [\[149\]](#). This vaccine can induce T cell activation via transferring DNA plasmids that express the SARS-CoV-2 S proteins [\[150\]](#). This vaccine platform holds the preponderance of producing therapeutic antibodies and activating immune cells to deliver the vaccine to patients through the skin. Recently, Yu and colleagues developed a series of DNA vaccine candidates expressing different forms of SARS-CoV-2 S protein and assessed them in 35 rhesus macaques [\[151\]](#). Vaccinated animals developed humoral and cellular immune responses, including neutralizing antibody titers in comparison with those found in convalescent humans and macaques infected with SARS-CoV-2. Surprisingly, all animals were challenged with SARS-CoV-2 after vaccination, and the vaccine encoding the full-length S protein resulted in over 3.1 and over 3.7 log<sub>10</sub> reductions in median viral loads in bronchoalveolar lavage and nasal mucosa, respectively, as compared with sham controls [\[151\]](#). Their data indicated vaccine protection against SARS-CoV-2 in nonhuman primates [\[152\]](#). Hence, in the near future, with the support of CEPI, these findings will boost the vaccine development and the corporation is making provision for the first phase of trials in the United States and China [\[153\]](#).

### 6.2. mRNA-based vaccines

The mRNA vaccine is a hopeful alternative to traditional vaccine methods in virtue of its high efficiency, rapid development capabilities, and the potential for low-cost manufacturing [\[154\]](#). Recently, Moderna, Inc. has launched phase I clinical trials for mRNA-1273, which encodes S protein of SARS-CoV-2. This mRNA-vaccine was fabricated with the cooperation of the National Institute of Allergy and Infectious Diseases [\[155\]](#). mRNA-1273, which encodes a prefusion-stabilized form of the SARS-CoV-2 spike, is under test for a broad dosing range (25–250 micrograms) during its phase 1 submission [\[143\]](#). The firm further announced that although commercial vaccines are unlikely to be marketed in at least 12 to 18 months, in an emergency, possibly in the fall of 2020, some people, including medical professionals, may obtain the vaccine [\[146,147\]](#).

### 6.3. Recombinant subunit vaccines

Subunit vaccines are superior to other types of vaccines since they are highly safe and possess fewer adverse effects via eliciting the immune system without drawing into any

infectious viruses [\[9\]](#). It has also been reported that the enhancement of T cell responses and generation of high titer neutralizing Abs were observed *in vivo* during the vaccine development researches [\[157\]](#). Clover Biopharmaceuticals was pre-clinically testing a recombinant subunit vaccine in the light of the S-Trimer of the SARS-CoV-2 [\[158\]](#). The researchers detected the antigen-specific neutralizing Abs in the sera of fully recovered patients [\[158\]](#). Besides, GlaxoSmithKline (GSK) disclosed a vaccine which can elicit a protective immune response against SARS. The vaccine contains an S protein immunogen, which was combined with the emulsion adjuvant, GSK2, yielding elevated level of anti-SARS-CoV IgG2a/IgG2b Ab responses. Recently, GSK and Clover Biopharmaceuticals announced a partnership to enhance immune response via introducing GSK's adjuvant system to S-Trimer [\[159\]](#). In addition, the team of the University of Queensland is also designing subunit vaccines via the transformative technology, known as "molecular clamp" [\[160\]](#). Molecular clamps are peptides that stabilize surface proteins, improve the recognition of correct antigens, and lead to a more robust immune response. This vaccine platform can be easily applied to a variety of enveloped viruses and rapidly manufacture their products [\[160\]](#).

Further, patent application US20060002947 disclosed the fabrication of hybrid peptides comprised of three elements: (1) invariant chain; (ii) essential peptide; (2) chemical structure linking the Ii to the antigenic epitope; (3) antigen epitope binding MHC II molecule. This theory was harnessed to produce Ii-Key/MHC II SARS hybrids. In this respect, Generex reported that they will employ its Ii-Key immune system activation biotechnology to generate a COVID-19 viral peptide vaccine for human clinical practice [\[161\]](#).

### 6.4. Other vaccine approaches

Aside from the aforementioned approaches, Genexine Inc. is exploring a new vaccine via utilizing the Hyleukin-7 platform technology [\[162\]](#). Such a platform may improve the immune responses fusing IL-7 with hyFc, aiming to hybridize IgD and IgG4 for the long-term effect of Fc fusion proteins [\[163–165\]](#). Specifically, IgD possesses a flexible hinge structure and can maximize the biological activity [\[153,154\]](#). IgG4 possesses an unexposed junction site that mitigates immunogenicity via preventing antibody-dependent cellular cytotoxicity [\[155,156\]](#). This corporation reported enhanced vaccine efficacy, lung T cell accumulation, and plasmacytoid dendritic cell growth after Fc-fused IL-7 treatment in the virus infection models [\[166\]](#).

## 7. Conclusions and inspirations

Despite prodigious global efforts made to contain SARS-CoV-2, the current COVID-19 epidemic has expanded into a full-blown pandemic, yielding outright panic and economic slowdown. Health-care systems in many regions are overburdened and under-resourced, impelling medical workers and governors to make formerly unthinkable

**Table 2 – The potential vaccine candidates for COVID-19 [135–145].**

	Candidate	Vaccine characteristics	Lead developer/sponsor	Current status
Clinical-phase	INO-4800	DNA plasmid encoding S protein delivered via electroporation	Inovio Pharmaceuticals	Phase I (NCT04336410)
	mRNA-1273	LNP-encapsulated mRNA vaccine encoding S protein	Moderna	Phase I (NCT04283461)
	LV-SMENP-DC	DCs modified with lentiviral vector expressing synthetic minigene based upon domains of selected viral proteins;	Shenzhen Geno-Immune Medical Institute	Phase I (NCT04276896)
	/	Recombinant novel coronavirus (2019-CoV) vaccine (adenoviral vector)	Institute of Military Medicine under the Academy of Military Sciences of the People's Liberation Army of China	Phase II (ChiCTR2000030906)
	/	Inactivated vaccine	Beijing Institute of Biological Products/Wuhan Institute of Biological Products	Phase I (ChiCTR2000031809)
	ChAdOx1	Attenuated adenovirus capable of producing the S protein of SARS-CoV-2	University of Oxford	Phase I/II (NCT04324606)
	Ad5-nCoV	Adenovirus type 5 vector that expresses S protein	CanSino Biologicals	Phase I (NCT04313127)
	Pathogen-specific aAPC	aAPCs modified with lentiviral vector expressing synthetic minigene based upon domains of selected viral proteins	Shenzhen Geno-Immune Medical Institute	Phase I (NCT04299724)
	/	Single-dose intranasal replication-defective adenovirus vector vaccine incorporating the SARS-CoV-2 S protein	Altimmune	Phase 1 trial planned for mid-August.
	BNT162	mRNA vaccine expressing codon-optimized undisclosed SARS-CoV-2 proteins	BioNTech	Clinical testing to begin late April
Experimental-phase	STARR	Self-transcribing and replicating RNA vaccine expressing undisclosed epitopes	Arcturus	Manufacturing stage
	/	Protamine-complexed mRNA-based vaccine expressing undisclosed SARS-CoV-2 protein(s)	CureVac	Phase 1 planned in June or July
	/	Undisclosed SARS-CoV-2-derived synthetic peptide conjugated to the key moiety of the MHC II-associated invariant chain	Generex Biotechnology	Human trials planned in June
	/	Modified vaccinia Ankara VLP vaccine based upon Wuhan strain of SARS-CoV-2	GeoVax	Candidates in animal studies
	/	Electroporated linear DNA vaccine based on S protein and selected epitopes	LineaRx	Four candidates for testing by the beginning of May or June preclinical testing ongoing with clinical trials to begin summer 2020
	/	Undisclosed recombinant SARS-CoV-2 protein VLP produced in tobacco	Medicago	Preclinical testing ongoing with clinical trials to begin summer 2020
	/	Recombinant subunit vaccine of SARS-CoV-2 S protein locked in prefusion conformation by polypeptide moiety (molecular clamp)	University of Queensland	Preclinical as of mid-March
	/	Oral recombinant adenovirus 5 vector vaccine of undisclosed SARS-CoV-2 proteins for mucosal immune response	Vaxart	Preclinical as of mid-March

aAPC, artificial antigen-presenting cell; MHC, major histocompatibility complex class; VLP, virus-like particle; DC, dendritic cell.

judgments concerning the allotment of medical care. In the wake of such a severe situation, a number of researches have trailed strategies to tackling the direct impact of COVID-19, either by modeling studies of the viral activity or via parsing out potential therapeutic options and finding a vaccine to end the coronavirus pandemic. Although the development of therapeutics and vaccines for the COVID-19 therapy is still in its middle stage, some marked advances have been made from complete genome sequencing of SARS-CoV-2 to the clinical practice of COVID-19 vaccines.

Rapid genome-wide association of SARS-CoV-2, along with international sharing of information, enabled us to generate rapid and more proper diagnostic tools. As set forth, the major fashioned diagnostic tools are qRT-PCR-based approaches, requiring a long time for specimen preparation and analysis, thus putting off the imperative actions for COVID-19 infections. Of note, in acute respiratory infections, RT-PCR is commonly utilized for detecting pathogenic viruses in respiratory secretions. The positive rate of PCR from oropharyngeal swabs is not very high, implicating the requirement of more swab testing are need to confirm the diagnosis. With the continuous spread of SARS-CoV-2, there is a desperate demand to develop rapid diagnostic methods that can be tested more efficiently.

As the report goes, certain drugs are known to effectively treat patients with COVID-19. Nevertheless, the lack of clinical data may render the clinical prognosis difficult to predict. In addition to the judicious design of novel therapeutics that target viral replication or immunopathology, currently, rapid screening of therapeutic agents to repurpose FDA-approved and well-characterized agents might be a more workable method. Considering the severity of the recent zoonotic coronavirus outbreaks, therapeutic drugs for pan-coronavirus should be carried out to cope with future outbreaks.

With respect to the detailed exploitation of COVID-19 vaccines, several pharmaceutical corporations and institutions have also launched the project for vaccine development. Notwithstanding, the commercial market for vaccines, especially vaccines for emerging infectious diseases, is confined by the high cost and time required for vaccine development along with the uncertainty of profitability. Accordingly, CEPI is combining efforts to encourage the progress of vaccines against COVID-19. Besides, in the near future, the production of a lot of vaccines may bring about the challenge in scaling up manufacturing quickly, because the infrastructure needed will differ in virtue of the vaccine type. Another concern regarding the vaccine development may be the antibody-dependent enhancement issue.

In addition, this global epidemic crisis indicated that host-species expansion or interspecies transmission of neoteric coronavirus to humans might be unavoidable. Clinicians and researchers should combine efforts to swiftly evolve our perception of all facets of SARS-CoV-2 infections and fill in the gaps concerning the emergence of this virus. Ultimately, we can also learn from this epidemic that we need to improve our disease monitoring and surveillance system to prevent such a serious disease outbreak. Digital technologies such as big-data analytics, artificial intelligence, and blockchain technology may also need to be fully exploited and developed, thereby

enabling real-time data collection at scale and modeling risk associations immediately to contain the pathophoresis.

Overall, the challenges posed by 21st century epidemics are real and changing: future epidemics will be fueled by various internal and external causes. In our response, we must view pandemics as interconnected cycles, not isolated events, and while we cannot forecast specific outbreaks, we can make provision for them.

## Conflict of interest

The authors assert that there is no conflict of interests concerning the publication of this review.

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