

The pharmacological development of direct acting agents for emerging needed therapy against severe acute respiratory syndrome coronavirus-2

Sheng-Fan Wang^{a,b,c}, Kuan-Hsuan Chen^{a,d}, Szu-Yu Wang^a, Aliaksandr A. Yarmishyn^e, Wei-Yi Lai^{c,e}, Yi-Ying Lin^{c,e}, Mong-Lien Wang^{e,f,g}, Shih-Jie Chou^{c,e}, Yi-Ping Yang^{c,e,g}, Yuh-Lih Chang^{a,c,d,*}

^aDepartment of Pharmacy, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; ^bSchool of Pharmacy, Taipei Medical University, Taipei, Taiwan, ROC; ^cInstitute of Pharmacology, National Yang-Ming University, Taipei, Taiwan, ROC; ^dFaculty of Pharmacy, National Yang-Ming University, Taipei, Taiwan, ROC; ^eDepartment of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; ^fSchool of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC; ^gInstitute of Food Safety and Health Risk Assessment, School of Pharmaceutical Sciences, National Yang-Ming University, Taipei, Taiwan, ROC

Abstract: Recently, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was quickly identified as the causal pathogen leading to the outbreak of SARS-like illness all over the world. As the SARS-CoV-2 infection pandemic proceeds, many efforts are being dedicated to the development of diverse treatment strategies. Increasing evidence showed potential therapeutic agents directly acting against SARS-CoV-2 virus, such as interferon, RNA-dependent RNA polymerase inhibitors, protease inhibitors, viral entry blockers, neuraminidase inhibitor, vaccine, antibody agent targeting the SARS-CoV-2 RNA genome, natural killer cells, and nucleocytoplasmic trafficking inhibitor. To date, several direct anti-SARS-CoV-2 agents have demonstrated promising *in vitro* and clinical efficacy. This article reviews the current and future development of direct acting agents against SARS-CoV-2.

Keywords: COVID-19; Direct therapy; Pharmacological development; SARS-CoV-2

1. INTRODUCTION

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was quickly confirmed as the causal pathogen leading to the outbreak of SARS-like illness (later named coronavirus disease 2019 [COVID-19]) in China.¹ As for now, COVID-19 has become a pandemic that led to healthcare crisis all over the world. SARS-CoV-2 belongs to *Betacoronavirus* genus of coronaviruses (CoVs) that are known to cause multiple respiratory infections in humans. The history of CoVs can trace back to the middle of nineteenth century.² The CoV particles are enveloped and contain a single-stranded positive-sense RNA genome. The CoV genome encodes both structural (such as envelope [E] protein, transmembrane [M] glycoprotein, spike [S] glycoprotein and nucleocapsid [N] protein) proteins responsible for virus replication and virus entry and nonstructural proteins that are involved in genome replication and transcription. The viral membrane harbors the S protein that plays an important role in virus entry and is responsible for inducing host immune response.³ The

CoV infection begins with the interaction of the receptor binding domain (RBD) of the viral S protein and the receptor on the cell surface such as dipeptidyl peptidase-4 for MERS-CoV and angiotensin-converting enzyme 2 (ACE2) for SARS-CoV.⁴ According to the recent results, SARS-CoV-2 utilizes a similar host receptor, ACE2 for its attachment and entry. Therefore, the treatment strategy designed for SARS-CoV can be potentially applied to use against SARS-CoV-2.⁵ After the entry, the virus gets uncoated and translates its open reading frames 1a and 1b (ORF1 and ORF1b) into polyproteins pp1a and pp1ab. Subsequently, these polyproteins are cleaved into several nonstructural proteins by several proteases, which further assemble and form the transcription-replication complex. The RNA-dependent RNA polymerase (RdRp) transcribes the positive strand of RNA to negative strand, which is further transcribed into positive-strand subgenomic mRNAs. These subgenomic mRNAs are translated to make new accessory and structural proteins. Interfering with any specific step of the virus replication cycle would be a potential therapeutic target to combat SARS-CoV-2. Increasing evidence reveals potential therapeutic agents acting directly against SARS-CoV-2, such as interferon (IFN), RdRp inhibitors, protease inhibitors, coronaviral protease inhibitor, viral entry blocker, neuraminidase inhibitor, vaccine, antibody, agent targeting the SARS-CoV-2 RNA genome, natural killer cells, and nucleocytoplasmic trafficking inhibitors. Some of them have already demonstrated both *in vitro* and clinical efficacy.

2. PHARMACOLOGICAL DEVELOPMENT OF DIRECT-ACTING AGENTS AGAINST SARS-COV-2

The drug discovery process to develop new antiviral agents and obtaining the clinical approval usually takes a long period of

*Address Correspondence: Dr. Yuh-Lih Chang, Pharmacy Department, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail address: ylchang@vghtpe.gov.tw (Y.-L. Chang).

Author Contributions: Dr. Sheng-Fan Wang and Dr. Kuan-Hsuan Chen contributed equally to this work.

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2020) 83: 712-718.

Received April 28, 2020; accepted April 28, 2020.

doi: 10.1097/JCMA.0000000000000353.

Copyright © 2020, the Chinese Medical Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

time. Until now, no significantly effective antiviral drugs are clinically approved for treating coronavirus infections. To enhance the progress of potential treatment for coronaviruses especially for SARS-CoV and MERS-CoV, repurposing of broadly acting antiviral drugs such as IFNs and ribavirin that have been used for other viral infections or other indications is usually employed. These drugs have the significant superiority for their well-known characteristics of pharmacokinetic and pharmacodynamics. Another approach for discovery of anti-CoV drugs includes the *de novo* development of ideal, novel agents according to the biochemical understanding of the specific coronavirus. The novel specific anti-SARS-CoV-2 agents might comprise inhibitors interfering with the viral replication cycle, antibody targeting the host receptor and virus S protein, and inhibitors of host cellular proteases involved in the virus endocytosis pathway.

2.1. Interferon

IFNs are a group of cytokine mediators that are induced in response to virus infection. IFNs are classified into subtypes I and II, both of which are involved in innate immunity and adaptive immune response. Several effector antiviral mechanisms of IFN are known, such as mRNA translation inhibition, enhancement of RNA degradation, RNA editing, targeting viral nucleocapsids, and inhibiting RNA synthesis.⁶ Among the type I IFN, IFN- α is quickly stimulated in innate immune response to initial virus infection. IFN- α and β inhibit the replication of SARS-CoV.^{7,8} However, IFN- γ was identified to lack antiviral activity against SARS-CoV.⁹ Previous study demonstrated that SARS-CoV inhibited IFN transcription in infected cells and the additional IFN could partly resume innate immunity against SARS-CoV.¹⁰ Pegylated IFN- α 2b, a licensed drug for chronic hepatitis B and C, showed the anti-SARS-CoV activity by decreasing viral replication and lung damage.^{11,12} IFN- α is a candidate medication for SARS and MERS treatment.^{13,14} Therefore, the current clinical evidence showed that IFNs might be used for the development of novel anti-SARS-CoV-2 therapy.

2.2. RdRp inhibition

Ribavirin is a broad-spectrum antiviral agent that has previously been used for hepatitis C patients treatment. It is a guanosine derivative and can target RdRp enzyme to inhibit the synthesis of viral RNA and capping of mRNA. During the previous SARS epidemic, ribavirin was widely used for patients in China.¹⁵ However, evidence showed ribavirin might have no significant antiviral activity against SARS-CoV *in vitro* and had significant toxicity concern in clinical practice.^{16,17} Hence, ribavirin usage for SARS-CoV is questionable and has been criticized.¹⁸ Earlier evidence demonstrated that the combination of ribavirin and IFN- β had a synergistic effect for inhibiting the replication of SARS coronavirus in animal and human cell lines.⁷ Regarding the adverse reactions and absence of the *in vitro* efficacy for SARS-CoV, the usage of ribavirin against SARS-CoV-2 should be carefully considered.

Remdesivir, by far, is the most promising drug among all ongoing clinical trials. Due to its structural similarity to adenosine, it can inhibit the RdRp by incorporating into viral RNA, thus halting viral genome replication. Recently, the first experience of remdesivir usage in the United States for the COVID-19-infected patient was reported.¹⁹ The *in vitro/in vivo* antiviral activity of remdesivir and IFN- β was found to be superior to the combination of lopinavir/ritonavir/LPV/RTV/IFN- β for MERS-CoV.²⁰ The most updated cohort study showed clinical improvement observed in 36 of 53 patients (68%).²¹ Therefore, remdesivir alone or together with IFN- β might be the potential regimen for the therapy of COVID-19.

Favipiravir (FPV) is a guanine analog, also selectively inhibiting RdRp (like remdesivir) that has been approved for the treatment of influenza.²² The clinical evaluation for its efficacy and safety in the therapy of COVID-19 is ongoing (ChiCTR2000029600).

2.3. Protease inhibition

A co-formulation of lopinavir and ritonavir, which was reported to have *in vitro* activity against the SARS-CoV and was revealed to have some activity against MERS-CoV *in vivo*, has been tested for the therapy of COVID-19.²³⁻²⁵ The component of lopinavir can bind to HIV-1 protease and block the cleavage of viral Gag-Pol precursor polyprotein into specific proteins essential for HIV infection, resulting in dysfunctional viral particles. Another component, ritonavir, can increase the plasma level of lopinavir by inhibiting the CYP3A-mediated metabolism of the latter. It has been used for the treatment of COVID-19 patients.²⁶⁻²⁸ However, lopinavir-ritonavir treatment might not have benefit in the severe COVID-19 hospitalized patients.²⁹ Nelfinavir, another HIV protease inhibitor, has previously been shown to inhibit the replication of SARS-CoV.³⁰ In addition, nelfinavir has revealed rational binding conformation with the viral main protease of SARS-CoV-2. (BioRxiv doi:10.1101/2020.01.28.922922.) Therefore, nelfinavir might be a potential therapeutic option for COVID-19.

2.4. Coronaviral protease inhibitors

Coronaviruses encode two proteases, chymotrypsin-like and papain-like proteases, which are responsible for the viral replication and interfering with the host innate immunity. Hence, targeting specific coronaviral proteases is a potential treatment option against coronaviruses.³¹ Cinanserin, an old serotonin receptor antagonist, inhibits the chymotrypsin-like protease and might be a potential inhibitor of replication of SARS-CoV.³² In addition, some flavonoids, which have inhibitory activity on chymotrypsin-like protease might be used against SARS-CoV.^{33,34} Moreover, evidence showed that diarylheptanoids, the natural products derived from Japanese alder (*Alnus japonica*), can inhibit the papain-like protease and restore the host innate immunity response against SARS-CoV through maintaining the function of IFNs.³¹ Therefore, specific coronaviral proteases might be good candidate targets for developing new drugs to fight COVID-19.

2.5. Viral entry inhibitors

Hydroxychloroquine and chloroquine are aminoquinolines, which have been used to treat autoimmune diseases and malaria. They are weak diprotic bases that can elevate the pH of the endosomes, which in turn prevent viral entry fusion.³⁵ Chloroquine can also interfere with ACE2 glycosylation of the cellular receptor of SARS-CoV and SARS-CoV-2. *In vitro* tests revealed its capability to reduce viral copy number. Its *in vivo* antiviral capability is now under clinical trial (open-label trial ChiCTR2000029609). The preliminary study in France evaluated the efficacy of hydroxychloroquine in SARS-CoV-2 patients. The results showed that the virologic cure rate was significantly higher in hydroxychloroquine/azithromycin-treated group ($p = 0.001$).³⁶ However, the comprehensive evaluation of safety and clinical efficacy in chloroquine/hydroxychloroquine therapy for COVID-19 patients is still under investigation. Hence, the benefits and risk of chloroquine or hydroxychloroquine therapy for COVID-19 remains to be carefully elucidated.

2.6. New coronavirus entry blockers

In a recent study, it was shown that SARS-CoV-2 recognizes ACE2 more efficiently than SARS-CoV.³ Therefore, interfering

with the interaction between S protein of SARS-CoV-2 and ACE2 might be a potential approach for antiviral therapy. Recently, the COVID-19 high-performance computing (HPC) Consortium has been established for the development of anti-SARS-CoV-2 therapy and prediction of the COVID-19 pandemic. Using the world's most powerful supercomputer, SUMMIT, the high-throughput screening of the possible docking models and identification of novel small molecules which can bind to the viral S protein or S protein-human ACE2 interface has been proposed. At present, the initial 77 novel compounds were identified for the subsequent clinical efficacy tests against SARS-CoV-2 (ChemRxiv. Preprint. <https://doi.org/10.26434/chemrxiv.11871402.v4>).

In addition to the ACE2, it has been revealed that S protein-derived cell entry also depends on transmembrane protease serine 2 (TMPRSS2).³⁷ Hence, camostat mesylate, a TMPRSS2 inhibitor, can eliminate SARS-CoV-2 infection *in vitro* assay. On the other hand, the heptad repeat (HR) of SARS-CoV-2 S protein is also involved in the process of viral entry.³⁸ Moreover, anti-HR peptides possess anti-SARS-CoV-2 activity through inhibition of viral fusion.³⁸ The pH- and receptor-dependent endocytosis are also involved in coronavirus entry mechanism.^{39,40} Adaptor protein complex 2-associated protein kinase 1 is a host kinase responsible for regulation of clathrin-mediated endocytosis.⁴¹ Recent evidence suggested that baricitinib, a Janus kinase inhibitor and also an adaptor protein complex 2-associated protein kinase 1-binding drug identified by artificial intelligence technology research, might be a novel candidate drug for the future COVID-19 treatment.⁴² Targeting novel coronavirus entry mechanisms might provide an alternative potential strategy for future anti-SARS-CoV-2 drug development.

2.7. Neuraminidase inhibition

Oseltamivir, an antiviral medication used for influenza A/B through neuraminidase inhibition, has gained considerable attention and has been proposed for evaluation of its efficacy for COVID-19 treatment. There is no evidence that oseltamivir has significant anti-SARS-CoV activity *in vitro*.¹⁶ The efficacy of oseltamivir against coronavirus is still unknown in the clinic.⁴³ Coronaviruses do not utilize neuraminidase and thus there is no specific enzyme to be targeted by oseltamivir or any other neuraminidase inhibitor. Hence, the *in vitro* data have suggested that neuraminidase inhibitors such as baloxavir would exert activity against SARS CoV-2 or other coronaviruses should be carefully interpreted.⁴⁴

Amantadine, indicated for influenza and Parkinson disease, has showed the antiviral activity against human coronavirus OC43 and SARS-CoV.^{9,45} SARS-CoV-2 entry into host cells might be dependent on interacting with the S protein and host cellular receptor. Subsequently, cathepsin L and cathepsin B (CTSL/CTSB), the host cell proteases responsible for cleavage of the spike protein, play an important role in the developing process. It provides potential target for COVID-19 therapy development. Recently, amantadine was screened for the possibility to downregulate the expression of CTSL/CTSB through high-throughput drug screen gene expression analysis (doi: <https://doi.org/10.1101/2020.04.05.026187>). However, other evidence indicated its poor activity against SARS-CoV.¹⁶ At present, the amantadine and its analog are not recommended for influenza due to influenza A resistance and side effect on lactation due to decrease of serum prolactin.⁴⁶ Although amantadine and its analog are not commonly discussed, this aspect whatever used for adjuvant or single treatment might be worthy to reassess in the future development for anti-SARS-CoV-2 therapy.

2.8. Vaccine

Effective vaccines are important to prevent and control viral pandemics such as influenza vaccines. Unfortunately, even after numerous studies on coronaviruses, the licensed vaccines are still lacking. Theoretically, the S protein of SARS-CoV-2 is a promising target for vaccine development. Recently, Wrapp et al.⁴⁷ found that the cryogenic electron microscopy structure of SARS-CoV-2 S trimer provides additional protein engineering information and thus can speed the progress of vaccine development. In addition, discovering the unique pentapeptides of SARS-CoV-2 S protein provides several candidate epitopes for vaccine development.⁴⁸ Due to high similarity between SARS-CoV-2 and SARS-CoV, a researcher identified the SARS-CoV-derived B- and T-cell epitopes were identically mapped to SARS-CoV-2 proteins which might be helpful for the initial phase of vaccine development.⁴⁹ Inactive vaccines derived from SARS, might be tested for SARS-CoV-2. Besides, rhesus θ -defensin and protein cage nanoparticles, which are innate immunomodulators, exhibit high anti-SARS-CoV potency.^{50,51} Protein cage nanoparticles designed vaccine for SARS-CoV might be further evaluated for their antiviral activity against SARS-CoV-2.

To date, despite the fact that the sequence information of SARS-CoV-2 has been revealed, Moderna's mRNA-1273 is the pioneer vaccine candidate on the market that has stepped its foot into clinical trials.⁵² If the synthetic strand of mRNA could prove its safety in humans and pass through the phase I trial, evaluation of its clinical benefits would be subsequently executed. It is highly expected for its antiviral response against SARS-CoV-2 through targeting S protein (NCT04283461). Numerous potential SARS-CoV-2 vaccines are being developed, including viral-like particles, nucleic acid-based vaccine, adenoviral vector-based vaccine, recombinant subunits vaccine, recombinant influenza viral vector vaccine, inactivated vaccine, and so on.⁵³ Upon current development progress, further work is needed to develop safe and effective vaccines for the control of the SARS-CoV-2 ongoing epidemics.

2.9. Therapeutic antibodies

Passive immunity by antibody therapy might be another strategy to control the SARS-CoV-2 pandemic. Passive immunization with the antibody, which can neutralize SARS-CoV-2 virus, might inhibit the virus replication and mitigate the disease severity. According to the prior experience in treating MERS, SARS, Ebola, and influenza viral infections with convalescent plasma from recovered patients that contains high antibody titers against virus, convalescent plasma could ameliorate the viral level and mortality.⁵⁴⁻⁵⁷ However, the challenges such as unknown viral kinetics of SARS-CoV-2 and insufficient donors with suitable clinical status still exist.

The usage of monoclonal antibodies for infectious disease is a new area, which addresses many concerns related to serum therapy and intravenous immunoglobulin medication in terms of better specificity, purity, safety, and reducing risk of contamination. Like the SARS-CoV, SARS-CoV-2 also targeted ACE2 for its attachment and entry into host cells. Hence, several neutralizing monoclonal antibodies targeting SARS-CoV by recognized S1 and S2 subunits, and amino acid residues might be applied to SARS-CoV-2.⁵⁸ A recent study showed that CR3022, a neutralizing antibody from a recovered SARS patient, might have the cross-reactive activity between SARS-CoV-2 and SARS-CoV through structural modeling with highly conserved distal epitope from the RBD.⁵⁹ Nevertheless, evidence showed that even SARS-CoV-2 spike protein displayed high (about 75%) homology with SARS-CoV, the novel epitopes only contributed to about 85% of the RBD antibody epitopes, implied concerned alterations in their antigenicity.⁶⁰ This result might explain the

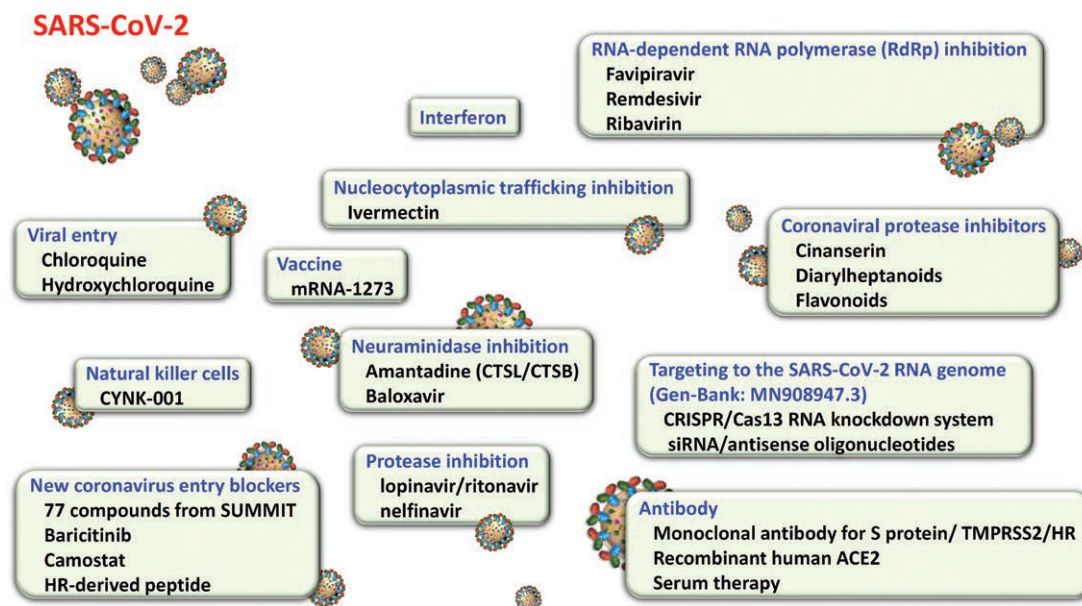


Fig. 1 Potential direct-acting agents against SARS-CoV-2.

reason for failure of some anti-SARS-CoV spike protein antibodies against SARS-CoV-2 and indicate that the developments of new antibody and specific vaccine for SARS-CoV-2 are necessary and urgent.⁶¹

On the other hand, the rationale of ACE2 receptor as a specific target against SARS-CoV-2 has been proposed.⁶² It is reasonable to use the ACE2 receptor as a target for neutralizing the virus. Currently, recombinant human ACE2 (rhACE2; GSK2586881) is being investigated for evaluation of its clinical effects in COVID-19 patients (NCT04287686). Moreover, in order to target ACE2 against SARS-CoV-2, it would be advisable to convert the soluble ACE2 receptor to the immunoadhesin form, which can be achieved by binding with the immunoglobulin Fc domain (ACE2-Fc). This modification will extend its lifespan and recruit immune response against the virus in the future antibody development. Nevertheless, the SARS-CoV-2 S protein-derived cell entry not only depends on ACE2 but also on the host cellular serine protease TMPRSS2.³⁷ Moreover, the HR loops including HR1 and HR2 domains on the S2 fragment of SARS-CoV-2 are involved in viral entry mechanism.³⁸ Furthermore, ultimately, the cocktail antibodies strategy might be required to pursue full SARS-CoV-2 protection coverage for specific population, which would elevate the complexity for formulation and manufacturing. Hence, antibodies targeting the fusion/entry mechanism of SARS-CoV-2 are still ongoing investigated and be worthy to expected in the future.

2.10 Targeting the SARS-CoV-2 RNA genome

Beyond targeting the S protein of SARS-CoV-2, directly targeting the viral RNA genome through inducing its degradation might be a reasonable strategy. SARS-CoV-2 RNA genome sequence has been recently identified (Gen-Bank: MN908947.3), and using RNA interference by small interfering RNA (siRNA) or antisense oligonucleotides against the virus might be another potential treatment strategy.⁶³ However, several challenges still exist, first, the conserved RNA sequence domains of SARS-CoV-2 are still unclear; second, it is unclear whether the oligonucleotides can be efficiently delivered into the lungs. By using such tools as liposomes, it is possible to deliver oligonucleotides to some extent; however, it is still unclear whether sufficient

amount of oligonucleotides could be effectively delivered into the lung lesions and could eliminate the virus in clinical settings.⁶⁴ Therefore, there still exists an uncrossed gap for immediate and quick development of anti-SARS-CoV-2 therapy using oligonucleotides.

Targeting the RNA genome of SARS-CoV-2 by a novel CRISPR RNA knockdown system might be another option. Recent evidence revealed that the CRISPR/Cas13 RNA knockdown system might be used against SARS-CoV-2 RNA genome.⁶⁵ The CRISPR/Cas13d system encompasses Cas13d protein and guides RNA specifically targeting the SARS-CoV-2 RNA genome. It is believed that the CRISPR/Cas13 system might be delivered to the SARS-CoV-2-infected lung tissue by adeno-associated virus vector. Therefore, the CRISPR system against the SARS-CoV-2 might be a potential developmental strategy in the future.

2.11 Natural Killer Cells

Until now, no well-established evidence has been revealed for the details of the immunological response in SARS-CoV-2 infected patients. However, we can initially glean some information from previous CoV studies such as SARS-CoV and MERS-CoV. After virus entering the host cell, it activates several receptors such as RIG-I-like receptor, NOD-like receptor, toll-like receptor, and C-type lectin-like receptors. Subsequently, it stimulates the expression of several inflammatory factors, dendritic cell maturation, and synthesis of type I IFNs which can control the viral spread and eliminate virus via phagocytosis by macrophages.⁶⁶ Learning to recognize the virus and raising a defense immune system may take several days to weeks. During this period, natural killer (NK) cells might initially be an important gate keeper for exogenous virus.

Recent evidence suggested that NK cells might be helpful against particular viruses *in vivo*, but there were not any clearly successful clinical results against virus infection.⁶⁷ Hence, Celularity, a New Jersey-based therapeutics company declared that, "CYNK-001, a cancer treatment is under clinical trial against COVID-19 awaiting for FDA permission." However, several challenges exist such as novel viral evading mechanisms of immune system including N protein of SARS-CoV.⁶⁸ Another

important challenge is cytokine storm, a characteristic developed by some COVID-19 patients. Therefore, the clinical testing should proceed with extreme caution and carefully monitor the clinical status to see whether it over-activates immune system and further harms healthy lung cells.

2.12 Nucleocytoplasmic trafficking inhibition

Ivermectin, an approved antiparasitic medication have been recognized as an antiviral agent against several viruses such as influenza, HIV, dengue virus, West Nile virus, and *Venezuelan equine encephalitis virus in vitro*.⁶⁹⁻⁷² Initially, the inhibitory activity of ivermectin on interaction of virus integrase protein (IN) and the importin (IMP) $\alpha/\beta 1$ heterodimer was proposed as the antiviral mechanism because various RNA viruses are dependent on IMP $\alpha/\beta 1$ for infection.⁷³⁻⁷⁵ Previous studies demonstrated that IMP $\alpha/\beta 1$ was involved in a signal-dependent SARS-CoV nucleocapsid protein nucleocytoplasmic trafficking during SARS-CoV infection and could affect host cell division.⁷⁶⁻⁸⁰ Besides, the SARS-CoV accessory protein can evade innate immune antiviral response through sequestering IMP $\alpha/\beta 1$ on the rough ER/Golgi membrane.⁸¹ The evidence suggests that ivermectin might be used against SARS-CoV-2 through nuclear transport inhibitory activity. Recently, an *in vitro* experiment showed that a single treatment with ivermectin resulted in about 5000-fold reduction in SARS-CoV-2 virus.⁸² The next step of drug development is to clarify the dosing regimen. Based on its *in vitro* efficacy and a well-known safety information, ivermectin might be worthy to further evaluate as a potential anti-SARS-CoV-2 drug.

In conclusion, several direct anti-SARS-CoV-2 agents have demonstrated some promising *in vitro* and clinical efficacy (Fig. 1). However, most of them are case reports or preliminary data from clinical trials with small sample sizes; therefore, further clinical trials are required. According to the current knowledge about the pandemic of SARS-CoV-2, the pharmacological development of direct-acting agents against COVID-19 is an urgent issue and a critical challenge for all of the pharmaceutical scientists and healthcare professionals.

REFERENCES

- Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020;395:507-13.
- Pillaiyar T, Meenakshisundaram S, Manickam M. Recent discovery and development of inhibitors targeting coronaviruses. *Drug Discov Today* 2020;25:668-88.
- Du L, Yang Y, Zhou Y, Lu L, Li F, Jiang S. MERS-CoV spike protein: a key target for antivirals. *Expert Opin Ther Targets* 2017;21:131-43.
- Raj VS, Mou H, Smits SL, Dekkers DH, Müller MA, Dijkman R, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 2013;495:251-4.
- Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol* 2020;94:e00127-20.
- Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev* 2001;14:778-809.
- Morgenstern B, Michaelis M, Baer PC, Doerr HW, Cinatl J Jr. Ribavirin and interferon-beta synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines. *Biochem Biophys Res Commun* 2005;326:905-8.
- Turner RB, Felton A, Kosak K, Kelsey DK, Meschievitz CK. Prevention of experimental coronavirus colds with intranasal alpha-2b interferon. *J Infect Dis* 1986;154:443-7.
- Chen F, Chan KH, Jiang Y, Kao RY, Lu HT, Fan KW, et al. In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. *J Clin Virol* 2004;31:69-75.
- Kuri T, Zhang X, Habjan M, Martínez-Sobrido L, García-Sastre A, Yuan Z, et al. Interferon priming enables cells to partially overturn the SARS coronavirus-induced block in innate immune activation. *J Gen Virol* 2009;90(Pt 11):2686-94.
- Haagmans BL, Kuiken T, Martina BE, Fouchier RA, Rimmelzwaan GF, van Amerongen G, et al. Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nat Med* 2004;10:290-3.
- Bijlenga G. Proposal for vaccination against SARS coronavirus using avian infectious bronchitis virus strain H from The Netherlands. *J Infect* 2005;51:263-5.
- Loutfy MR, Blatt LM, Siminovitch KA, Ward S, Wolff B, Lho H, et al. Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. *JAMA* 2003;290:3222-8.
- Mustafa S, Balkhy H, Gabere MN. Current treatment options and the role of peptides as potential therapeutic components for Middle East Respiratory Syndrome (MERS): a review. *J Infect Public Health* 2018;11:9-17.
- Wenzel RP, Edmond MB. Managing SARS amidst uncertainty. *N Engl J Med* 2003;348:1947-8.
- Tan EL, Ooi EE, Lin CY, Tan HC, Ling AE, Lim B, et al. Inhibition of SARS coronavirus infection in vitro with clinically approved antiviral drugs. *Emerg Infect Dis* 2004;10:581-6.
- Booth CM, Matukas LM, Tomlinson GA, Rachlis AR, Rose DB, Dwosh HA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 2003;289:2801-9.
- Cyranoski D. Critics slam treatment for SARS as ineffective and perhaps dangerous. *Nature* 2003;423:4.
- Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al.; Washington State 2019-nCoV Case Investigation Team. First case of 2019 novel coronavirus in the United States. *N Engl J Med* 2020;382:929-36.
- Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat Commun* 2020;11:222.
- Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of remdesivir for patients with severe Covid-19. *N Engl J Med* 2020;NEJMoa2007016. doi: 10.1056/NEJMoa2007016
- Furuta Y, Takahashi K, Kuno-Maekawa M, Sangawa H, Uehara S, Kozaki K, et al. Mechanism of action of T-705 against influenza virus. *Antimicrob Agents Chemother* 2005;49:981-6.
- Groneberg DA, Poutanen SM, Low DE, Lode H, Welte T, Zabel P. Treatment and vaccines for severe acute respiratory syndrome. *Lancet Infect Dis* 2005;5:147-55.
- Chan JF, Yao Y, Yeung ML, Deng W, Bao L, Jia L, et al. Treatment with lopinavir/ritonavir or interferon- $\beta 1b$ improves outcome of MERS-CoV infection in a Nonhuman Primate Model of Common Marmoset. *J Infect Dis* 2015;212:1904-13.
- Han Q, Lin Q, Jin S, You L. Coronavirus 2019-nCoV: a brief perspective from the front line. *J Infect Dis* 2020;80:373-7.
- Lim J, Jeon S, Shin HY, Kim MJ, Seong YM, Lee WJ, et al. The author's response: case of the index patient who caused tertiary transmission of coronavirus disease 2019 in Korea: the application of lopinavir/ritonavir for the treatment of COVID-19 pneumonia monitored by quantitative RT-PCR. *J Korean Med Sci* 2020;35:e89.
- Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA* 2020;323:1488-94.
- Wang Z, Chen X, Lu Y, Chen F, Zhang W. Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. *Biosci Trends* 2020;14:64-8.
- Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. *N Engl J Med* 2020;382:1787-99.
- Yamamoto N, Yang R, Yoshinaka Y, Amari S, Nakano T, Cinatl J, et al. HIV protease inhibitor nelfinavir inhibits replication of SARS-associated coronavirus. *Biochem Biophys Res Commun* 2004;318:719-25.
- Park JY, Jeong HJ, Kim JH, Kim YM, Park SJ, Kim D, et al. Diarylheptanoids from *Alnus japonica* inhibit papain-like protease of severe acute respiratory syndrome coronavirus. *Biol Pharm Bull* 2012;35:2036-42.
- Chen L, Gui C, Luo X, Yang Q, Günther S, Scandella E, et al. Cinanserin is an inhibitor of the 3C-like proteinase of severe acute respiratory

- syndrome coronavirus and strongly reduces virus replication in vitro. *J Virol* 2005;79:7095–103.
33. Jo S, Kim S, Shin DH, Kim MS. Inhibition of SARS-CoV 3CL protease by flavonoids. *J Enzyme Inhib Med Chem* 2020;35:145–51.
 34. Jo S, Kim H, Kim S, Shin DH, Kim MS. Characteristics of flavonoids as potent MERS-CoV 3C-like protease inhibitors. *Chem Biol Drug Des* 2019;94:2023–30.
 35. Mauthe M, Orhon I, Rocchi C, Zhou X, Luhr M, Hijlkema KJ, et al. Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. *Autophagy* 2018;14:1435–55.
 36. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020:105949.
 37. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;181:271–80.e8.
 38. Xia S, Zhu Y, Liu M, Lan Q, Xu W, Wu Y, et al. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mol Immunol* 2020;1–3. doi: 10.1038/s41423-020-0374-2
 39. Inoue Y, Tanaka N, Tanaka Y, Inoue S, Morita K, Zhuang M, et al. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. *J Virol* 2007;81:8722–9.
 40. Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, et al. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res* 2008;18:290–301.
 41. Neveu G, Ziv-Av A, Barouch-Bentov R, Berkerman E, Mulholland J, Einav S. AP-2-associated protein kinase 1 and cyclin G-associated kinase regulate hepatitis C virus entry and are potential drug targets. *J Virol* 2015;89:4387–404.
 42. Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet* 2020;395:e30–1.
 43. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497–506.
 44. McCreary EK, Pogue JM. Coronavirus Disease 2019 Treatment: A Review of Early and Emerging Options. *Open Forum Infect Dis* 2020;7:ofaa105.
 45. Brison E, Jacomy H, Desforges M, Talbot PJ. Novel treatment with neuroprotective and antiviral properties against a neuroinvasive human respiratory virus. *J Virol* 2014;88:1548–63.
 46. Anderson PO. Breastfeeding and respiratory antivirals: coronavirus and influenza. *Breastfeed Med* 2020;15:128.
 47. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020;367:1260–3.
 48. Lucchese G. Epitopes for a 2019-nCoV vaccine. *Cell Mol Immunol* 2020;17:539–40.
 49. Ahmed SF, Quadeer AA, McKay MR. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. *Viruses* 2020;12:E254.
 50. Wohlford-Lenane CL, Meyerholz DK, Perlman S, Zhou H, Tran D, Selsted ME, et al. Rhesus theta-defensin prevents death in a mouse model of severe acute respiratory syndrome coronavirus pulmonary disease. *J Virol* 2009;83:11385–90.
 51. Wiley JA, Richert LE, Swain SD, Harmsen A, Barnard DL, Randall TD, et al. Inducible bronchus-associated lymphoid tissue elicited by a protein cage nanoparticle enhances protection in mice against diverse respiratory viruses. *PLoS One* 2009;4:e7142.
 52. Jackson NAC, Kester KE, Casimiro D, Gurunathan S, DeRosa F. The promise of mRNA vaccines: a biotech and industrial perspective. *NPJ Vaccines* 2020;5:11.
 53. Pang J, Wang MX, Ang IYH, Tan SHX, Lewis RF, Chen JJ, et al. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): a systematic review. *J Clin Med* 2020;9:E623.
 54. Mupapa K, Massamba M, Kibadi K, Kuvula K, Bwaka A, Kipasa M, et al. Treatment of ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. *J Infect Dis* 1999;179 (Suppl 1):S18–23.
 55. Yeh KM, Chiueh TS, Siu LK, Lin JC, Chan PK, Peng MY, et al. Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a Taiwan hospital. *J Antimicrob Chemother* 2005;56:919–22.
 56. Luke TC, Kilbane EM, Jackson JL, Hoffman SL. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? *Ann Intern Med* 2006;145:599–609.
 57. Mair-Jenkins J, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, et al.; Convalescent Plasma Study Group. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *J Infect Dis* 2015;211:80–90.
 58. Shanmugaraj B, Siriwardananon K, Wangkanont K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for coronavirus disease-19 (COVID-19). *Asian Pac J Allergy Immunol* 2020;38:10–8.
 59. Yuan M, Wu NC, Zhu X, Lee CD, So RTY, Lv H, et al. A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. *Science* 2020;368:630–3.
 60. Zheng M, Song L. Novel antibody epitopes dominate the antigenicity of spike glycoprotein in SARS-CoV-2 compared to SARS-CoV. *Cell Mol Immunol* 2020;17:536–8.
 61. Tian X, Li C, Huang A, Xia S, Lu S, Shi Z, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg Microbes Infect* 2020;9:382–5.
 62. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med* 2020;46:586–90.
 63. Qureshi A, Tantray VG, Kirmani AR, Ahangar AG. A review on current status of antiviral siRNA. *Rev Med Virol* 2018;28:e1976.
 64. Youngren-Ortiz SR, Gandhi NS, España-Serrano L, Chougule MB. Aerosol delivery of siRNA to the lungs. Part 1: rationale for gene delivery systems. *Kona* 2016;33:63–85.
 65. Nguyen TM, Zhang Y, Pandolfi PP. Virus against virus: a potential treatment for 2019-nCoV (SARS-CoV-2) and other RNA viruses. *Cell Res* 2020;30:189–90.
 66. Ben Addi A, Lefort A, Hua X, Libert F, Communi D, Ledent C, et al. Modulation of murine dendritic cell function by adenine nucleotides and adenosine: involvement of the A(2B) receptor. *Eur J Immunol* 2008;38:1610–20.
 67. Tripathy SK, Keyel PA, Yang L, Pingel JT, Cheng TP, Schneeberger A, et al. Continuous engagement of a self-specific activation receptor induces NK cell tolerance. *J Exp Med* 2008;205:1829–41.
 68. Lu X, Pan J, Tao J, Guo D. SARS-CoV nucleocapsid protein antagonizes IFN- β response by targeting initial step of IFN- β induction pathway, and its C-terminal region is critical for the antagonism. *Virus Genes* 2011;42:37–45.
 69. Götz V, Magar L, Dornfeld D, Giese S, Pohlmann A, Höper D, et al. Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. *Sci Rep* 2016;6:23138.
 70. Lundberg L, Pinkham C, Baer A, Amaya M, Narayanan A, Wagstaff KM, et al. Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan Equine Encephalitis virus replication. *Antiviral Res* 2013;100:662–72.
 71. Tay MY, Fraser JE, Chan WK, Moreland NJ, Rathore AP, Wang C, et al. Nuclear localization of dengue virus (DENV) 1-4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin. *Antiviral Res* 2013;99:301–6.
 72. Wagstaff KM, Sivakumaran H, Heaton SM, Harrich D, Jans DA. Ivermectin is a specific inhibitor of importin α/β -mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem J* 2012;443:851–6.
 73. Wagstaff KM, Rawlinson SM, Hearn AC, Jans DA. An AlphaScreen®-based assay for high-throughput screening for specific inhibitors of nuclear import. *J Biomol Screen* 2011;16:192–200.
 74. Caly L, Wagstaff KM, Jans DA. Nuclear trafficking of proteins from RNA viruses: potential target for antivirals? *Antiviral Res* 2012;95:202–6.
 75. Jans DA, Martin AJ, Wagstaff KM. Inhibitors of nuclear transport. *Curr Opin Cell Biol* 2019;58:50–60.
 76. Rowland RR, Chauhan V, Fang Y, Pekosz A, Kerrigan M, Burton MD. Intracellular localization of the severe acute respiratory syndrome coronavirus nucleocapsid protein: absence of nucleolar accumulation during infection and after expression as a recombinant protein in vero cells. *J Virol* 2005;79:11507–12.

77. Timani KA, Liao Q, Ye L, Zeng Y, Liu J, Zheng Y, et al. Nuclear/nucleolar localization properties of C-terminal nucleocapsid protein of SARS coronavirus. *Virus Res* 2005;114:23–34.
78. Wulan WN, Heydet D, Walker EJ, Gahan ME, Ghildyal R. Nucleocytoplasmic transport of nucleocapsid proteins of enveloped RNA viruses. *Front Microbiol* 2015;6:553.
79. Wurm T, Chen H, Hodgson T, Britton P, Brooks G, Hiscox JA. Localization to the nucleolus is a common feature of coronavirus nucleoproteins, and the protein may disrupt host cell division. *J Virol* 2001;75:9345–56.
80. Hiscox JA, Wurm T, Wilson L, Britton P, Cavanagh D, Brooks G. The coronavirus infectious bronchitis virus nucleoprotein localizes to the nucleolus. *J Virol* 2001;75:506–12.
81. Frieman M, Yount B, Heise M, Kopecky-Bromberg SA, Palese P, Baric RS. Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. *J Virol* 2007;81:9812–24.
82. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res* 2020;178:104787.