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Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases

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Since the outbreak of the novel coronavirus disease COVID-19, caused by the SARS-CoV-2 virus, this disease has spread rapidly around the globe. Considering the potential threat of a pandemic, scientists and physicians have been racing to understand this new virus and the pathophysiology of this disease to uncover possible treatment regimens and discover effective therapeutic agents and vaccines. To support the current research and development, CAS has produced a special report to provide an overview of published scientific information with an emphasis on patents in the CAS content collection. It highlights antiviral strategies involving small molecules and biologics targeting complex molecular interactions involved in coronavirus infection and replication. The drug-repurposing effort documented herein focuses primarily on agents known to be effective against other RNA viruses including SARS-CoV and MERS-CoV. The patent analysis of coronavirus-related biologics includes therapeutic antibodies, cytokines, and nucleic acid-based therapies targeting virus gene expression as well as various types of vaccines. More than 500 patents disclose methodologies of these four biologics with the potential for treating and preventing coronavirus infections, which may be applicable to COVID-19. The information included in this report provides a strong intellectual groundwork for the ongoing development of therapeutic agents and vaccines.

BACKGROUND

The outbreak of the novel coronavirus disease, COVID-19, caused by the new coronavirus 2019-nCoV that is now officially designated as severe acute respiratory syndrome-related coronavirus SARS-CoV-2, represents a pandemic threat to global public health.[1,2] Although the epicenter of the COVID-19 outbreak in December of 2019 was located in Wuhan, China, this disease has spread to more than 100 countries (Figure 1) with over 100 000 confirmed cases and over 3,800 confirmed deaths worldwide (Figure 2) as of March 9, 2020.[3] In addition, millions of people's lives have been affected as a result of mandatory isolations/quarantines. The ripple effect of the COVID-19 outbreak could potentially bring major challenges to worldwide health systems and have far-reaching consequences on the global economy if the spread of the virus is not effectively controlled.[1,2,4]

Coronaviruses (CoVs) are relatively large viruses containing a single-stranded positive-sense RNA genome encapsulated within a membrane envelope. The viral membrane is studded with glycoprotein spikes that give coronaviruses their crown-like appearance (Figure 3). While coronaviruses infect both humans and animals, certain types of animals such as bats that host the largest variety of coronaviruses appear to be immune to coronavirus-induced illness.[5] There are four classes of coronaviruses designated as alpha, beta, gamma, and delta. The beta-coronavirus class includes severe acute respiratory syndrome (SARS) virus (SARS-CoV), Middle East respiratory syndrome (MERS) virus (MERS-CoV), and the COVID-19 causative agent SARS-CoV-2. Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 attacks the lower respiratory system to cause viral pneumonia, but it may also affect the gastrointestinal system, heart, kidney, liver, and central nervous system leading to multiple organ failure.[6,7] Current information indicates that SARS-CoV-2 is more transmissible/contagious than SARS-CoV.[8]

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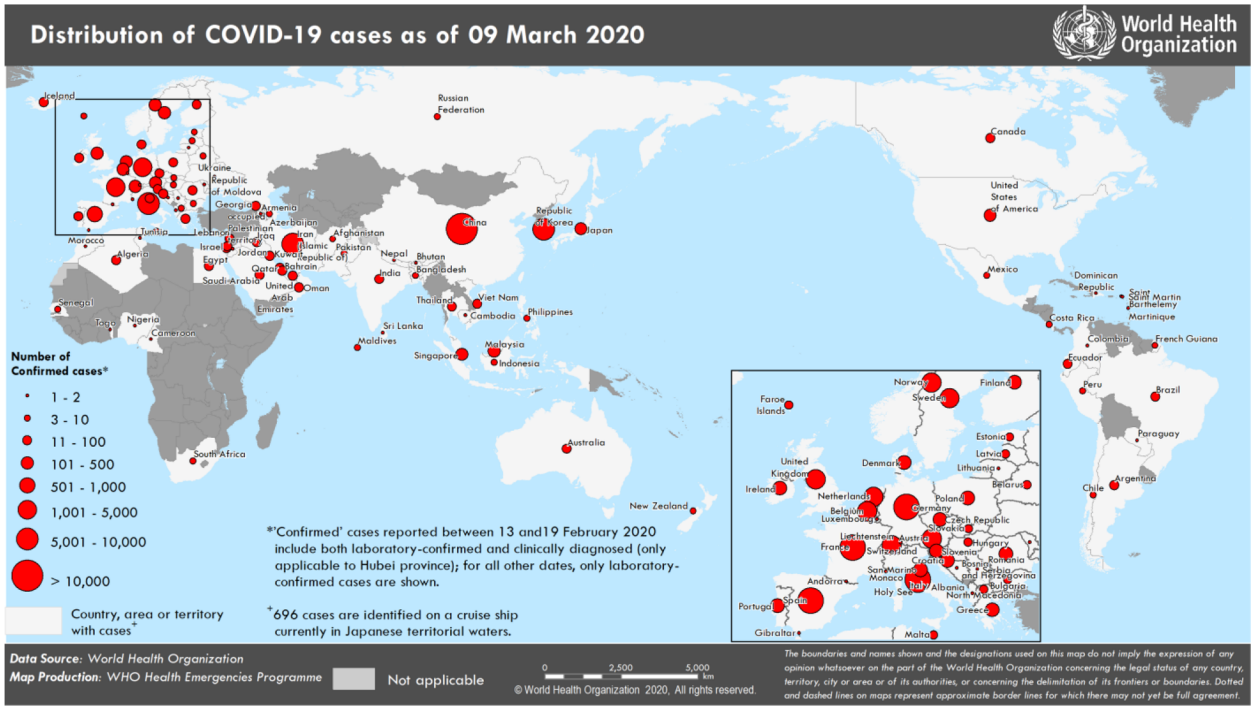

Figure 1. Global distribution of confirmed COVID-19 cases. (Map was reproduced from WHO Coronavirus Disease (COVID-2019) Situation Reports.[3] Used with permission from ref 3. Copyright 2020 World Health Organization.)

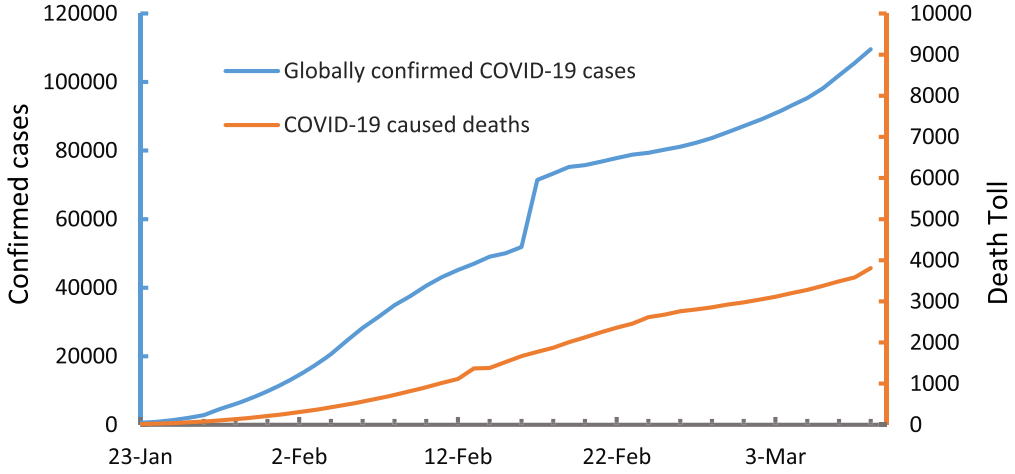

Figure 2. Global trend of confirmed COVID-19 cases and associated deaths from January 23 through March 9, 2020. (Data were obtained from WHO Coronavirus Disease (COVID-2019) Situation Reports[3]).

The betacoronavirus genome encodes several structural proteins, including the glycosylated spike (S) protein that functions as a major inducer of host immune responses. This S protein mediates host cell invasion by both SARS-CoV and SARS-CoV-2 via binding to a receptor protein called angiotensin-converting enzyme 2 (ACE2) located on the surface membrane of host cells.[9−11] A recent study also revealed that this invasion process requires S protein priming which is facilitated by the host cell-produced serine protease TMPRSS211. In addition, the viral genome also encodes several nonstructural proteins including RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro), and papain-like protease (PLpro).[12,13] Upon entrance to the host cells, the viral genome is released as a single-stranded positive RNA. Subsequently, it is translated into viral polyproteins using host cell protein translation machinery, which are then cleaved into effector proteins by viral proteinases 3CLpro and PLpro.[12,13] PLpro also behaves as a deubiquitinase that may deubiquinate certain host cell proteins, including interferon factor 3 and NF-κB, resulting in immune suppression.[13,14] RdRp synthesizes a full-length negative-strand RNA template to be used by RdRp to make more viral genomic RNA.

The interaction between viral S protein and ACE2 on the host cell surface is of significant interest since it initiates the infection process. Cryo-EM structure analysis has revealed that the binding affinity of SARS-CoV-2 S protein to ACE2 is about 10−20 times higher than that of SARS-CoV S protein.[10,15] It is speculated that this may contribute to the reported higher transmissibility and contagiousness of SARS-CoV-2 as compared to SARS-CoV.[8]

The prospect also exists for discovery of therapeutic agents targeting the highly conserved proteins associated with both SARS-CoV and SARS-CoV-2.[15−18] RdRp and 3CLpro protease of SARS-CoV-2 share over 95% of sequence similarity with

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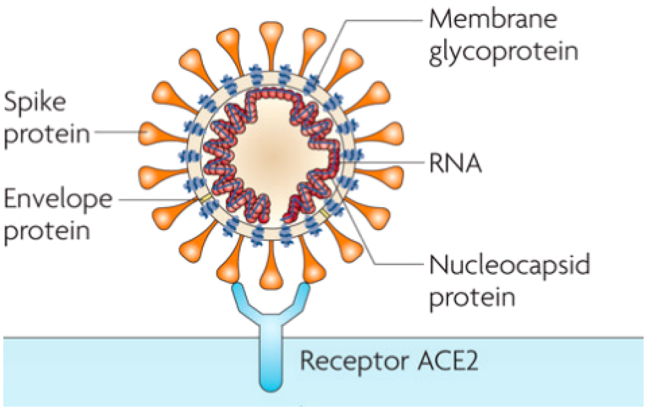

Figure 3. Cartoon illustration of the coronavirus structure and viral receptor ACE2 on the host cell surface. (Image was reproduced with permission from ref 9, Nature Reviews Microbiology 7(3), 226–236. Copyright 2009 Springer Nature.)

those of SARS-CoV despite the fact that these two viruses demonstrate only 79% sequence similarity at the genome level.[15−18] On the basis of sequence alignment and homology modeling, SARS-CoV and SARS-CoV-2 share a highly conserved receptor-binding domain (RBD), a domain of S protein, and 76% of sequence similarity in their S proteins.[15−18] In addition, although the PLpro sequences of SARS-CoV-2 and SARS-CoV are only 83% similar, they share similar active sites.[16]

To date, there are no SARS-CoV-2-specific antiviral agents. Researchers have been racing to find possible treatments to save lives and produce vaccines for future prevention. To support research and development efforts to discover effective therapeutic and preventive agents for COVID-19, CAS, a division of the American Chemical Society specializing in scientific information solutions, has analyzed scientific data related to the development of therapeutic agents and vaccines for human coronaviruses since 2003. The analyses presented in this report are based on the CAS content collection, a scientist-curated data collection covering published scientific literature and patents from over 60 patent authorities worldwide. For a subset of the analyses, both CAS and MEDLINE data were collectively analyzed.

SCIENTIFIC LITERATURE AND PATENTS RELATED TO COVID-19, SARS, AND MERS

Trend in Scientific Publications Related to COVID-19.

Since the outbreak of COVID-19, this new disease and its causative virus have drawn major global attention. Scientists and physicians worldwide have been conducting a major campaign to understand this new emergent disease and its epidemiology in an effort to uncover possible treatment regimens, discover effective therapeutic agents, and develop vaccines. Figure 4 shows the total number of journal articles related to COVID-19 or SARS-CoV-2 published each week from the last week of 2019 through the week of February 24, 2020. Over 500 journal articles were published electronically or in print during this period, and the number of published articles has increased each week since the week of January 13, 2020. Although a large portion of these articles are about clinical manifestations and treatment options, an increasing number of studies are focused on elucidation of virus structure, virus transmission mechanisms/dynamics, as well as identification of antiviral agents and accurate diagnostics for virus detection. These trends reflect immense interest and desire from the scientific community, including both academic and industrial

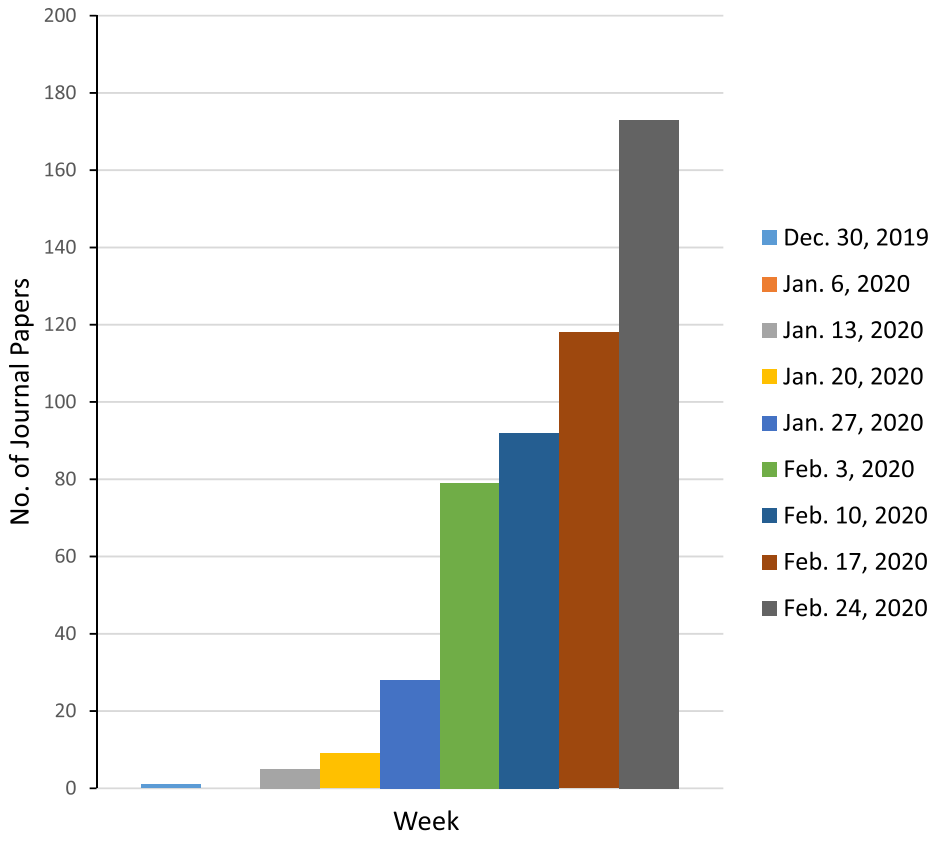

Figure 4. Number of journal articles related to COVID-19 published each week.

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organizations as well as clinicians, to identify new methods to halt the progression of this epidemic disease and to prevent infection and transmission in the future.

Notable Journal Articles Related to COVID-19 and SARS-CoV-2. Table 1 lists some journal articles published from December 30, 2019 through February 23, 2020. These articles were selected based on collective use of factors such as journal impact factor, citation, and type of study. For example, the No. 8 article listed about the characterization of the SARS-CoV-2 genome has greatly facilitated the global effort to develop a vaccine for prevention of COVID-19. Also shown in this table are journal articles pertaining to potential antiviral drug candidates such as remdesivir, baricitinib, and chloroquine for the treatment of this disease.

Over 500 journal articles were published in the first two months of 2020, and the number of published articles has increased each week since the week of January 13, 2020.

Distribution of patents related to SARS and MERS. As mentioned earlier, COVID-19 is caused by SARS-CoV-2, a new type of coronavirus in the same genus as SARS-CoV and MERS-CoV. Viral proteins responsible for SARS-CoV-2 entry into host cells and replication are structurally similar to those associated with SARS-CoV. Thus, research and development on SARS and MERS may offer insights that would be beneficial to the development of therapeutic and preventive agents for COVID-19. This report identified pertinent data from patents related to these two coronaviruses. Figure 5 shows the distribution of patents in the CAS content collection related to SARS (A) and MERS (B). The number of patents related to SARS is almost 12 times the number related to MERS, probably because the SARS outbreak occurred about 10 years before the MERS outbreak. Among SARS patents, about 80% are related to the development of therapeutics, 35% are related to vaccines, and 28% are related to diagnostic agents or methods. Because an individual patent may cover any two or more areas, the sum of percentage values is greater than 100%. A similar distribution pattern was also observed for patents related to MERS. Thus, for both diseases, more patents have been devoted to the development of therapeutic agents as opposed to diagnostic methods and vaccines.

RESEARCH AND DEVELOPMENT IN SMALL MOLECULE ANTIVIRAL AGENTS FOR COVID-19 AND RELATED CORONAVIRUS DISEASES

Key Proteins and Their Roles in Viral Infection. Identification of targets is important for identifying drugs with high target specificity and/or uncovering existing drugs that could be repurposed to treat SARS-CoV-2 infection. Table 2 lists potential targets, their roles in viral infection, and representative existing drugs or drug candidates that reportedly act on the corresponding targets in similar viruses and thus are to be assessed for their effects on SARS-CoV-2 infection. 3CLpro and PLpro are two viral proteases responsible for the cleavage of viral peptides into functional units for virus replication and packaging within the host cells. Thus, drugs that target these proteases in other viruses such as HIV drugs, lopinavir and ritonavir, have been explored.[19] RdRp is the RNA polymerase responsible for viral RNA synthesis that may be blocked by existing antiviral drugs or drug candidates, such as remdesivir.[19] Conceivably, the interaction of viral S protein with its receptor ACE2 on host cells, and subsequent viral endocytosis into the cells, may also be a viable drug target. For example, the broad-spectrum antiviral drug Arbidol, which functions as a virus-host cell fusion inhibitor to prevent viral entry into host cells against influenza virus,[20] has entered into a clinical trial for treatment of SARS-CoV-2.[21,22] The protease TMPRSS2 produced by the host cells plays an important role in proteolytic processing of S protein priming to the receptor ACE2 binding in human cells.[11] It has been shown that camostat mesylate, a clinically approved TMPRSS2 inhibitor, was able to block SARS-CoV-2 entry to human cells, indicating its potential as a drug for COVID-19.[11]

ACE2 involvement with coronavirus infection is of further interest since ACE2 is a potent negative regulator restraining overactivation of the renin-angiotensin system (RAS) that may be involved in elicitation of inflammatory lung disease in addition to its well-known role in regulation of blood pressure and balance of body fluid and electrolytes.[23,24] It catalyzes degradation of angiotensin II to angiotensin (1−7). The balance between angiotensin II and angiotensin (1−7) is critical since angiotensin II binds to angiotensin AT1 receptor to cause vasoconstriction, whereas angiotensin (1−7) elicits vasodilation mediated by AT2.[25−27] Although the notion that ACE2 mediates coronavirus invasion is largely accepted, it remains unclear how the levels or activities of ACE2, AT1 receptors, and AT2 receptors are altered in coronavirus-induced diseases due to the limited number of studies.[23,24] Therefore, it is yet to be determined whether some drugs or compounds that target any of these proteins (e.g., L-163491 as a partial antagonist of AT1 receptor and partial agonist of AT2 receptor) may alleviate coronavirus-induced lung injury.[28]

Patents and Potential Drug Candidates Related to Key Protein Targets. The CAS content collection contains patents related to coronavirus key proteins listed above. Table 3 lists the number of patents related to each protein target and associated therapeutic compounds with a CAS Registry Number (CAS RN) reported in these patents. CAS data show that targets 3CLpro and RdRp attracted more attention than other targets, and more compounds with therapeutic potential were identified for these targets, probably due to the work done for SARS-CoV which also contains 3CLpro and RdRp.

Existing Drugs with Potential Therapeutic Applications for COVID-19. Since SARS-CoV-2 is a newly discovered pathogen, no specific drugs have been identified or are currently available. An economic and efficient therapeutic strategy is to repurpose existing drugs. On the basis of genomic sequence information coupled with protein structure modeling, the scientific community has been able to rapidly respond with a suggested list of existing drugs with therapeutic potential for COVID-19. Table 4 provides a summary of such drugs together with potential mechanisms of actions for their activities. Barcitinib was proposed because of its anti-inflammatory effect and possible ability to reduce viral entry.[35] A fixed dose of the anti-HIV combination, lopinavir−ritonavir, is currently in clinical trials with Arbidol or ribavirin.[22] Remdesivir, developed by Gilead Sciences Inc., was previously tested in humans with Ebola virus disease and has shown promise in animal models for MERS and SARS. The drug is currently being studied in phase III clinical trials in both China and the USA. Favipiravir, a

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Table 1. Notable Journal Articles on COVID-19 and/or SARS-CoV-2 Published as of February 23, 2020[a]

no.	journal	paper title	publication date	organization
1	The New England Journal of Medicine	A novel coronavirus from patients with pneumonia in China, 2019	January 24, 2020	NHC Key Laboratory of Biosafety, China, and National Institute for Viral Disease Control, Chinese Center for Disease Control and Prevention, Beijing, China[b]
2	Lancet	Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China	January 24, 2020	Department of Pulmonary and Critical Care Medicine, China-Japan Friendship Hospital, Beijing, China; NHC Key Laboratory of Systems Biology of Pathogens and Christophe Merieux Laboratory, Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China[b]
3	The New England Journal of Medicine	Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia	January 29, 2020	Chinese Center for Disease Control and Prevention, Beijing, China; School of Public Health, University of Hong Kong, Hong Kong; Hubei Center for Disease Control and Prevention, Wuhan, Hubei, China[b]
5	Journal of Virology	Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS	January 29, 2020	Department of Epidemiology, University of North Carolina, Chapel Hill, NC, USA
6	Lancet	Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study	January 30, 2020	Tuberculosis and Respiratory Department, Wuhan Jinyintan Hospital, Wuhan, China
7	The New England Journal of Medicine	First case of 2019 novel coronavirus in the United States	January 31, 2020	The Washington State Department of Health Public Health Laboratories, WA, USA[b]
8	Lancet	Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding	January 30, 2020	NHC Key Laboratory of Biosafety, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China, Central Theater, People's Liberation Army General Hospital, Wuhan, China, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Beijing, China[b]
9	Lancet	Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study	January 31, 2020	School of Public Health, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China[b]
10	Nature	A new coronavirus associated with human respiratory disease in China	February 3, 2020	Shanghai Public Health Clinical Center & School of Public Health, Fudan University, Shanghai, China[b]
11	Nature	A pneumonia outbreak associated with a new coronavirus of probable bat origin	February 3, 2020	Key Laboratory of Special Pathogens, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, China[b]
12	Lancet	Baricitinib as potential treatment for 2019-nCoV acute respiratory disease	February 4, 2020	BenevolentAI, London, UK and Department of Surgery and Cancer, Imperial College London, UK
13	Cell Research	Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro	February 4, 2020	State Key Laboratory of Virology, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, China, and National Engineering Research Center for the Emergency Drug, Beijing Institute of Pharmacology and Toxicology, Beijing, China[b]
14	Emerging Microbes & Infections	RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak	February 5, 2020	State Key Laboratory of Virology, Modern Virology Research Center, College of Life Sciences, Wuhan University, Wuhan, China[b]
15	The Journal of the American Medical Association	Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China	February 7, 2020	Department of Critical Care Medicine, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, China
16	Cell Host & Microbe	Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China	February 7, 2020	National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China; Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, USA; Center for Systems Medicine, Institute of Basic Medical Sciences & Peking Union Medical College, Beijing, China[b]
17	Cellular & Molecular Immunology	Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein	February 11, 2020	Key Laboratory of Medical Molecular Virology, School of Basic Medical Sciences, Fudan-Jinbo Joint Research Center, Fudan University, Shanghai, China

[a]Note: The publication date is the date for electronic publication. [b]Only corresponding organization(s) is/are listed for papers published by multiple organizations.

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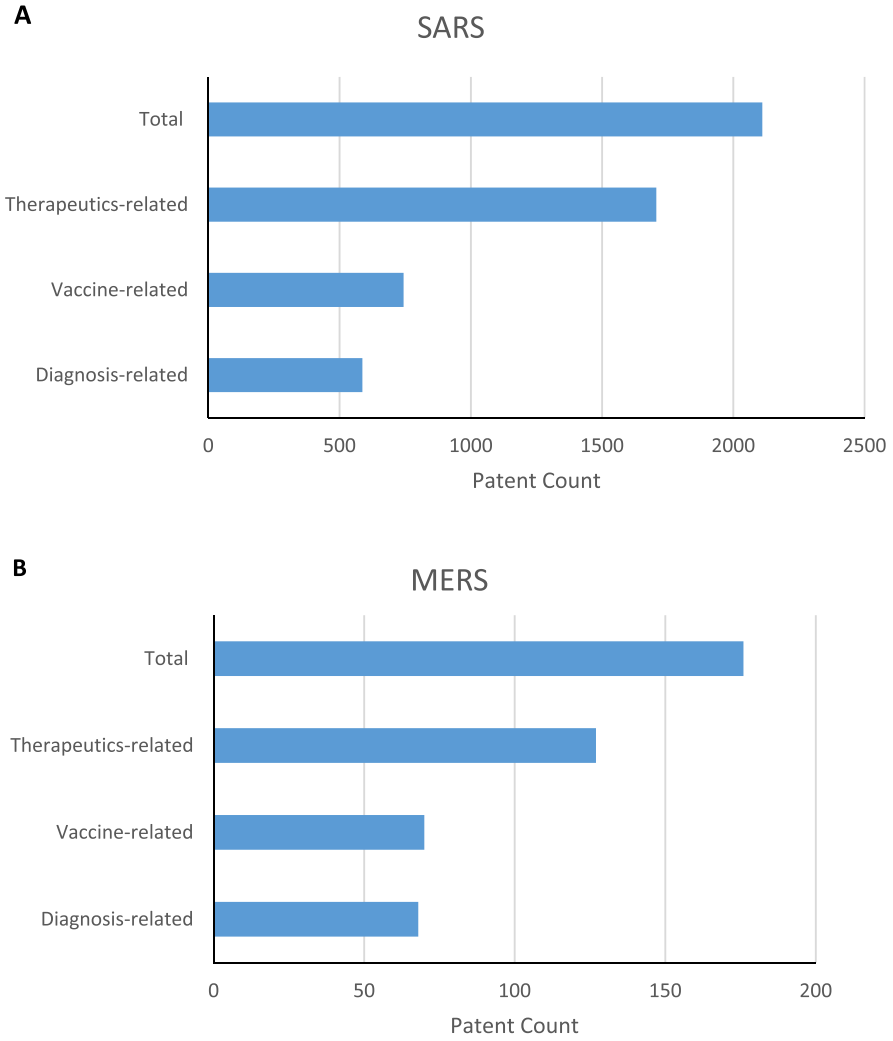

Figure 5. Distribution of patents related to SARS (A) and MERS (B) based on application purpose.

Table 2. Key Proteins and Their Roles during the Viral Infection Process

target candidate	full name	role during viral infection	drug candidate
3CLpro	coronavirus main protease 3CLpro	a protease for the proteolysis of viral polyprotein into functional units	lopinavir[19,30]
PLpro	papain-like protease PLpro	a protease for the proteolysis of viral polyprotein into functional units	lopinavir[19,30]
RdRp	RNA-dependent RNA polymerase	an RNA-dependent RNA polymerase for replicating viral genome	remdesivir,[19,29,32] ribavirin[16,29,31]
S protein	viral spike glycoprotein	a viral surface protein for binding to host cell receptor ACE2	Arbidol[20,22,33a]
TMPRSS2	transmembrane protease, serine 2	a host cell-produced protease that primes S protein to facilitate its binding to ACE2	camostat mesylate[11]
ACE2	angiotensin-converting enzyme 2	a viral receptor protein on the host cells which binds to viral S protein	Arbidol[20,22,33a]
AT2	angiotensin AT2 receptor	an important effector involved in the regulation of blood pressure and volume of the cardiovascular system	L-163491[28]

[a]An inhibitor of viral entry to host cells. Its direct action on S protein and ACE2 is yet to be confirmed.

purine nucleoside leading to inaccurate viral RNA synthesis,[36] was originally developed by Toyama Chemical of Japan, and has recently been approved for a clinical trial as a drug to treat COVID-19.[30] Chloroquine, an antimalarial drug, has proven effective in treating coronavirus in China.[32] In addition to the above-mentioned, many other antiviral drugs are also listed.

Selected Patents Related to Promising Small Molecule Drug Candidates. Table 5 shows selected patents associated with the aforementioned potential drugs, together with patents disclosing small molecules for treatment of SARS or MERS. The selection was based on the presence of important terms in CAS-indexed patents as well as the presence of the synthetic preparation role assigned by CAS scientists during document indexing. Patent applications WO2009114512 and WO2014028756 disclose preparation of compounds active as JAK inhibitors, one of which was later named as baricitinib and developed for reducing inflammation in rheumatoid arthritis. Patent application JP5971830 discloses preparation of polycyclic pyridone compounds and their use as endonuclease inhibitors. Patent applications US20160122374 and US20170071964

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Table 3. Key Protein Targets and Related Patents in the CAS Content Collection and Potential Drug Candidates in CAS REGISTRY of Chemical Substances

target	no. of patents	no. of potential drug candidates
3CLpro	49	2178
PLpro	4	189
RdRp	26	570
S protein	46	333
ACE2	5	97
AT2	2	38

disclose preparation of the nucleotide analog drug remdesivir that was later developed as a therapeutic agent for Ebola and Marburg virus infections (Patent US20170071964). Because of its promising results in at least two COVID-19 patients, remdesivir has now entered into phase III clinical trials.

Patent application WO2013049382 discloses both structures and syntheses of compounds from various structure classes (peptidyl aldehydes, peptidyl α-ketoamides, peptidyl bisulfite salts, and peptidyl heterocycles), as well as certain formulation compositions, developed to inhibit viral 3C protease or 3C-like protease (i.e., 3CLpro).

Patent application WO2018042343 presents both preparation methods and biological assay results for compounds capable of inhibiting the SARS virus proteases. These compounds appeared to exhibit good enzyme-inhibiting activity ($pIC_{50} \approx 7$

Table 4. Existing Drugs with Therapeutic Potentials for COVID-19 (Drug Repurposing)

drug candidate	CAS RN	target	possible mechanism of action on COVID-19	disease indication
baricitinib[35]	1187594-09-7	JAK kinase	a JAK inhibitor that may interfere with the inflammatory processes	approved drug for rheumatoid arthritis
lopinavir[19a]	192725-17-0	viral proteases: 3CLpro or PLpro	protease inhibitors that may inhibit the viral proteases: 3CLpro or PLpro	lopinavir and ritonavir are approved drug combination for HIV infection
ritonavir[19,37c]	155213-67-5			
darunavir[33]	206361-99-1			approved drug for HIV infection
favipiravir (favilavir)[29,36]	259793-96-9	RdRp	a purine nucleoside that acts as an alternate substrate leading to inaccurate viral RNA synthesis	viral infections
remdesivir[19,29,32a]	1809249-37-3		a nucleotide analogue that may block viral nucleotide synthesis to stop viral replication	Ebola virus infection
ribavirin[16,29−31a]	36791-04-5			RSV infection, hepatitis C, some viral hemorrhagic fevers
galidesivir[34b]	249503-25-1			hepatitis C, Ebola virus, Marburg virus
BCX-4430 (salt form of galidesivir)[34b]	222631-44-9			hepatitis C, Ebola virus, Marburg virus
Arbidol[22,33a]	131707-23-8	S protein/ ACE2[d]	an inhibitor that may disrupt the binding of viral envelope protein to host cells and prevent viral entry to the target cell	influenza antiviral drug
chloroquine[29,32]	54-05-7	endosome/ ACE2	a drug that can elevate endosomal pH and interfere with ACE2 glycosylation	malarial parasite infection
nitazoxanide[29]	55981-09-4	N/A	a drug that may inhibit viral protein expression	various helminthic, protozoal, and viral infection-caused diarrhea

[a]Drugs under clinical trials for treating COVID-19 (repurposing). [b]Drugs under clinical trials for other virus-induced diseases. [c]Ritonavir is a pharmacokinetic profile enhancer that may potentiate the effects of other protease inhibitors due to its ability to attenuate the degradation of those drugs by the liver enzyme CYP3A4 and thus is used in combination with antiviral Lopinavir.[37] [d]An inhibitor of viral entry to host cells. Its direct action on S protein and ACE2 is yet to be confirmed.

Table 5. Selected Patents Associated with Potential Drugs (Repurposing) for COVID-19 or Small Molecules for Treatment of SARS or MERS

patent no.	priority date	title	organization
WO2009114512	20080311	Preparation of azetidine and cyclobutane derivatives as JAK inhibitors	Incyte Corporation, USA
WO2014028756	20140220	Deuterated baricitinib	Concert Pharmaceuticals, Inc., USA
JP5971830	20150428	Preparation of polycyclic pyridone derivatives as cap-dependent endonuclease (CEN) inhibitors and prodrugs thereof	Shionogi and Co., Ltd., Japan
US20160122374	20141029	Preparation of nucleosides and methods for treating Filoviridae virus infections	Gilead Sciences, Inc., USA
US20170071964	20160916	Preparation of amino acid-containing nucleotides and methods for treating arenaviridae and coronaviridae virus infections	Gilead Sciences, Inc., USA
WO2007075145	20070704	Preparation of benzopyranone derivatives as anti-coronaviral agents	Singapore Polytechnic, Singapore; Shanghai Institute of Materia Medica Chinese Academy of Sciences, China
WO2005021518	20050310	Preparation of 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid derivatives as cysLT2 receptor antagonists for treatment of respiratory diseases	Ono Pharmaceutical Co., Ltd., Japan
WO2007120160	20071025	Preparation of N-heterocyclic acetamides useful for viral inhibition	Novartis AG, USA
WO2009119167	20091001	Aniline derivative having anti-RNA viral activity	KinoPharma, Inc., Japan
WO2013049382	20130404	Broad-spectrum antivirals against 3c or 3c-like proteases of picornavirus-like supercluster: picornaviruses, caliciviruses and coronaviruses	Kansas State University Research Foundation; The Ohio State University; Wichita State University - all in USA
WO2018042343	20180308	Preparation of peptides that inhibit 3C and 3CL proteases and methods of use thereof	GlaxoSmithKline, UK
WO2007067515	20070614	Five-membered iminocyclitol derivatives as selective and potent glycosidase inhibitors: new structures for antivirals and osteoarthritis therapeutics	Academia Sinica, Taiwan

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or $IC_{50} \approx 0.1\ \mu M$) and antiviral activity, which was assessed by host cell viability using cultured human lung fibroblast MRC-5 cells infected with a specified virus (e.g., MERS virus) expressing the viral S protein. Drug administration routes were also mentioned in this patent.

Small Molecule Compounds in Research and Development with Potential Effects on Key Protein Targets for Human Coronavirus-Induced Diseases. Besides various commercialized antiviral drugs, there are also small molecule compounds currently in research and development that have shown significant inhibitory effects on many key proteins from similar coronaviruses such as SARS-CoV and MERS-CoV (Table 6). These drug candidates mostly inhibit viral enzymes including proteases and components for RdRp. Since 3CLpro protease has a high level of sequence homology between SARS-CoV and SARS-CoV-2, inhibitors against 3CLpro of SARS-CoV may also be applicable to SARS-CoV-2. Compounds, including benzopurpurin B, C-467929, C-473872, NSC-306711 and N-65828, which may inhibit the activity of viral NSP15, poly(U)-specific endoribonuclease, were tested for reduced SARS-CoV infectivity in cultured cells with IC_{50} of 0.2−40 μM.[38] Compound C-21 and CGP-42112A are two AT2 agonists, whereas L-163491 has dual functions as a partial agonist for AT2 receptor and a partial antagonist of AT1 receptor. Since AT1 and AT2 are important effectors in the RAS system to which ACE2 belongs, it has been speculated that these compounds may be used to adjust the balance between AT1 and AT2, which may be affected by coronavirus infection and to alleviate viral-induced lung injury during the infection.[24]

> Besides various commercialized antiviral drugs, there are also small molecule compounds currently in R&D that have shown significant inhibitory effects on many key proteins from other viruses.

Small Molecules Identified by Structure Similarity, Lipinski's Rule of 5, and CAS-Indexed Pharmacological Activity and/or Therapeutic Usage. Besides the aforementioned antiviral drugs, there may be additional small molecule compounds with therapeutic or pharmacological potential against viruses such as SARS-CoV and MERS-CoV. Compounds listed in Tables 4 and 6 were subjected to a Tanimoto similarity search in CAS REGISTRY using CAS proprietary fingerprints.[a] Those substances with at least 60% structural similarity match and meeting Lipinski's rule of 5 were identified. Table 7 lists selected compounds that were also identified to have a pharmacological activity or therapeutic usage role. Compound name and CAS RN are provided for each compound. The second column lists the number of compounds that met the structure similarity and Lipinski's rule criteria. Although more work remains to be done in this regard, the methodology and results mentioned here point to a strategy that may help streamline the process of drug discovery for COVID-19.

BIOLOGICS FOR CORONAVIRUS-ASSOCIATED DISEASES

Distribution of Biologics Patents Related to SARS and MERS. The new coronavirus SARS-CoV-2 related to SARS and MERS viruses is causing serious and ongoing epidemiological problems around the world. Since there is limited clinical and basic research information at this time, treatment options for COVID-19 currently comprise investigational drugs and management of symptoms. As biologics have the potential to broaden the spectrum of the treatment options for coronavirus-induced diseases, leveraging prior knowledge and practices used to address the SARS and MERS outbreaks provides a practical strategy for developing new target-specific therapeutic agents for SARS-CoV-2. To this end, an analysis of biologics from patents contained in the CAS content collection was performed. The patent analysis included information related to therapeutic antibodies, cytokines, interfering and other therapeutic RNAs, and vaccines for potential treatment and/or prevention of SARS-related diseases from patents published from 2003 to the present. Figure 6 shows more than 500 patents that disclose the use of these four biologics classes to treat and prevent SARS and MERS. Of these patents, vaccine development was the largest class (363), followed by therapeutic antibodies (99), interfering RNAs (35), and cytokines (22). Given the indispensable role of vaccines in viral disease prevention, detailed analysis of vaccines will be presented in a later section.

Antibodies. Ninety-nine patents containing information about antibodies with therapeutic and/or diagnostic potential for SARS and MERS were identified. Of these, 61 patents claimed preparation of SARS-specific antibodies (23), MERS-specific antibodies (17), or antibodies with diagnostic application (21). Similar to SARS-CoV, the receptor-binding domain (RBD) in the S protein of SARS-CoV-2 binds to human ACE2 receptor in order to gain access into host cells.[42] In viral infection, the S protein, but not the other structural proteins, M, E, and N in SARS-CoV, elicits an immune response.[43] Table 8 shows the target analysis of patents related to development of therapeutic antibodies for SARS. Over 90% of these antibodies are directed against S protein including its RBD. The data indicate that the S protein is a putative target for SARS-CoV-2 antibody development.

> More than 500 patents that disclose the use of four biologics classes such as therapeutic antibodies, cytokines, RNA therapies, and vaccines to treat and prevent SARS and MERS have been analyzed for this study.

An additional 38 patents contained information pertaining to other types of antiviral antibodies that were useful for SARS and MERS therapies. These included neutralizing antibodies or antibodies designed to target proteins such as IL-6/IL-6R, TLR3 (Toll-like receptor 3), CD16, ITAM (immunoreceptor tyrosine-based activation motif), DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin), ICAM-3 (intercellular adhesion molecule 3), or IP-10/CXCL10 (interferon γ-inducible protein 10). Cytokine storm has been reported to correlate with disease severity in SARS-CoV-2 infection. Patients admitted to an ICU had higher concentrations of proinflammatory cytokines and chemokines, particularly G-CSF, IP-10/CXCL10, MCP1 (monocyte chemoattractant protein 1), and TNFα, as well as elevated cytokines from T helper 2 cells such as IL-4 and IL-10.[44] Patent application

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Table 6. Small Molecule Compounds in Research and Development with Therapeutic Potential for COVID-19

CAS RN	small molecule compound	target	possible mechanism of action on COVID-19
4431-00-9	aurine tricarboxylic acid	RNA-dependent RNA polymerase (RdRp)	an inhibitor that may bind to viral RdRp, as tested against SARS-CoV in cell culture[16]
502960-90-9	4-methyl-*N*-[(1*S*)-2-oxo-2 [[(1*S*,2*E*)-1-(2-phenylethyl)-3-(phenylsulfonyl)-2-propen-1-yl] amino]-1-(phenylmethyl)ethyl]- 1-piperazinecarboxamide	viral proteases: 3CLpro and PLpro	an inhibitor that may disrupt the function of 3CLpro and PLpro, which was tested against SARS-CoV[16,39,40]
1851279-09-8	4-(1,1-dimethylethyl)-*N*-[(1*S*)-2-oxo-2-[[(1*S*,2*E*)-1-(2-phenylethyl)-3-(phenylsulfonyl)-2-propen-1-yl]amino]-1-(phenylmethyl)ethyl]- 1-piperazinecarboxamide		
1851280-00-6	4-(2-methoxyethyl)-*N*-[(1*S*)-2-oxo-2-[[(1*S*,2*E*)-1-(2-phenylethyl)-3-(phenylsulfonyl)-2-propen-1-yl]amino]-1-(phenylmethyl)ethyl]- 1-piperazinecarboxamide		
223537-30-2	rupintrivir		a cysteine protease inhibitor that may disrupt the function of 3CLpro and PLpro[41]
2409054-43-7	(α*R*)-α-[[3-(4-chloro-2-fluorophenyl)-1-oxo-2-propen-1-yl]amino]-*N*-[(1*R*)-1-methyl-2-(2-oxo-3-pyrrolidinyl)ethyl]- benzenepropanamide	viral proteases: 3CLpro or PLpro	an inhibitor that may disrupt the function of 3CLpro or PLpro, as tested against SARS-CoV or MERS-CoV[39,40]
452088-38-9	5-[(4-methyl-1-piperidinyl)sulfonyl]-1*H*-indole-2,3-dione		
2409054-44-8	3-hydroperoxy-4-[2-hydroxy-3-[3-(4-hydroxyphenyl)-1-oxo-2-propen-1-yl]-6-methoxyphenyl]-2-butanone		
41137-87-5	hirsutenone		
992-59-6	benzopurpurin B	NSP15 (poly(U)-specific endoribonuclease)	chemical inhibitors that may suppress viral infectivity by inhibiting endoribonuclease NSP15, as tested against SARS-CoV in cultured cells[38]
351891-58-2	C-467929		
331675-78-6	C-473872		
813419-93-1	NSC-306711		
501444-06-0	N-65828		
477775-14-7	C-21	AT2	an angiotensin AT2 receptor agonist that may alleviate the virus-induced lung injury[24]
127060-75-7	CGP-42112A		
170969-73-0	L-163491		a dual-property molecule that functions as angiotensin AT1 partial antagonist and AT2 agonist which may alleviate the virus-induced lung injury[24]

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Table 7. Examples of Similar Molecules with Possible Therapeutic Effects Identified by Structural Similarity, Lipinski's Rule of 5, and Pharmacology/Therapeutic Role Assigned by CAS Scientists during Document Indexing

query substance name (CAS RN)	no. of substances with >60% similarity	example of selected similar substance	Registry Number of selected similar substance
ribavirin (36791-04-5)	1520	viramidine	119567-79-2
galidesivir (249503-25-1)	502	(2*R*,3*S*,5*R*)-5-(4-amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3-hydroxy-2-pyrrolidinemethanol	1610426-50-0
		(2*S*,4*R*,5*S*)-5-(4-amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-4-hydroxy-2-pyrrolidinemethanol	872534-76-4
		(2*R*,3*R*,4*S*,5*S*)-5-(4-amino-5*H*-pyrrolo[3,2-*d*] pyrimidin-7-yl)-3-hydroxy-4-methoxy-2-pyrrolidinemethanol	1610426-51-1
chloroquine (54-05-7)	21176	hydroxychloroquine	118-42-3
		(±)-chloroquine diphosphate	50-63-5
		chloroquine hydrochloride	3545-67-3
		chloroquine sulfate	132-73-0
favipiravir (259793-96-9)	309	6-bromo-3,4-dihydro-3-oxo-2-pyrazine-5-d-carboxamide	1476773-04-2
		6-fluoro-3,4-dihydro-3-oxo-2-pyrazine-5-d-carboxamide	1492021-26-7
2-butanone, 3-hydroperoxy-4-[2-hydroxy-3-[3-(4-hydroxyphenyl)-1-oxo-2-propen-1-yl]-6-methoxyphenyl] (2409054-44-8)	63195	xanthoangelol D	132998-83-5

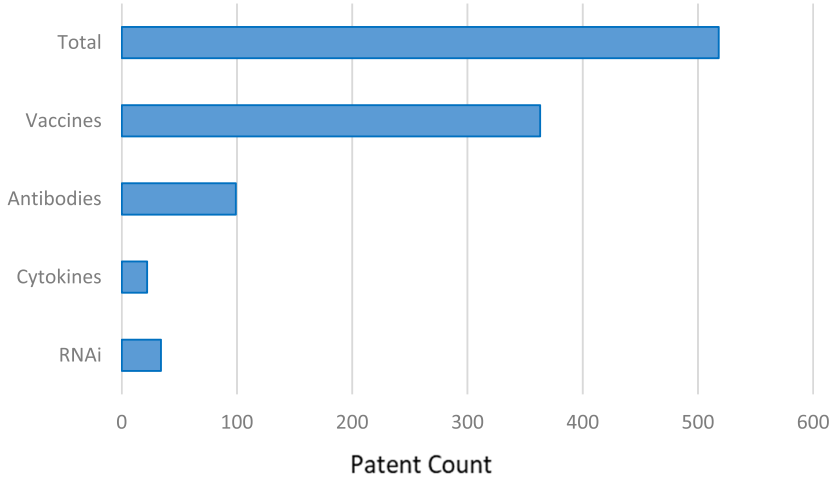

Figure 6. Distribution of biologics patents related to SARS and MERS.

WO2005058815 discloses human anti-IP-10 antibodies, including bispecific molecules and immunoconjugates that bind to IP-10 with high affinity, for treating inflammation, autoimmune disease, neurodegenerative disease, bacterial infection, and viral infection. Patent application WO2017095875 discloses the preparation of human antibodies and immunoconjugates specifically targeting chemokine IP-10, including an anti-IP-10 antibody shown to suppress free serum IP-10 in about 3 days at 0.5 mg/kg and in approximately 10 days at 10 mg/kg in Cynomolgus macaques.

In addition, DC-SIGN/CD209, a type II transmembrane adhesion molecule with C-type lectin function, is mainly expressed on interstitial dendritic cells and lung alveolar macrophages.[45] DC-SIGN functions as an entry cofactor in transferring SARS-CoV to susceptible cells such as pneumocytes.[46] Patent application WO200505824 claims the production of a humanized anti-DC-SIGN antibody that interfered with the interaction of DC-SIGN with its receptor, ICAM-3. The antibody effectively blocked viral binding, infection, and transmission for viral infections/diseases, including SARS.

Cytokines. Cytokines are low-molecular-weight proteins that act as chemical signals in the immune response to pathogen invasion. The production of various cytokines in response to an invading pathogen such as a virus contributes to the host organism's ability to eliminate the pathogen. Specific types of cytokines, including chemokines, interferons (IFNs), interleukins, and lymphokines, have been reported and characterized in the literature over the past 40 years. By early 2020, the CAS Lexicon contained over 700 terms for specific types of cytokines associated with 76 724 documents, including 11 837 patents.

During a viral infection, the most prominent cytokines produced are IFNs, which interfere with viral replication. IFNs are classified as type I (IFN-α, IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-ν, IFN-τ, and IFN-ω), type II (IFN-γ), or type III (IFN-λ) based on the receptor complex used for signaling as well as sequence homology.[47] Because of their ability to interfere with viral replication, interferons and interferon fusion proteins have been utilized as therapeutic agents for treatment of viral infections for the past 20 years. A few patents disclosing these proteins and their use for treating SARS are described below.

rSIFN-co. Patent applications WO2011072487 and WO2016180335 describe the cloning of a recombinant interferon (rSIFN-co, CAS RN 2043378-94-3) as well as a method for determining its potency that was effective for treating

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Table 8. Target Analysis of Patents on Developing Therapeutic Antibodies for SARS

patent number	antigen of SARS antibody	patent title	organization	priority date
EP2112164	lipid attachment signals or GPI	Antiviral peptides linked to a lipid attachment signals or GPI against enveloped virus such as HIV, avian flu, SARS or Ebola virus	Institute Pasteur of Shanghai	20080229
WO2009128963	spike protein	Cross-neutralizing human monoclonal antibodies to SARS-CoV and methods of use thereof	Institute for Research In Biomedicine	20080117
WO2009128963	spike protein	Cross-neutralizing human monoclonal antibodies to spike protein of SARS coronavirus and methods of use thereof	Humab, LLC	20080117
WO2008035894	viral infection	Preparation of antiviral antibody 3D8 fragments and their use in treatment of viral infection	Sung Kyun Kwan University; Ajou University; Invitroplant Co., Ltd.	20060919
WO2008060331	spike protein	Antibodies to SARS coronavirus	Amgen Inc.	20060519
WO2007044695	spike protein	Neutralizing monoclonal anti-spike protein antibodies for diagnosis and treatment of SARS-coronavirus-associated disease and screening of vaccine or anti-SARS agent	Dana-Farber Cancer Institute	20051007
CN1911963	RBD of S protein	Method for preparing neutralizing monoclonal antibody against severe acute respiratory syndrome coronavirus and its application	Chinese Academy of Sciences	20050810
CN1903878	spike protein	Fab fragment of human antibody IgG against SARS coronavirus	Fudan University	20050726
WO2006095180	S2 protein	Human monoclonal antibodies against SARS-associated coronavirus and treatment of patients with SARS	Ultra Biotech Ltd.; University of California	20050310
WO2006086561	spike protein	Neutralizing monoclonal antibodies against severe acute respiratory syndrome-associated coronavirus	New York Blood Center, Inc.	20050208
CN1664100	spike protein	Preparation of heavy chain and light chain variable regions of anti-SARS coronavirus antigen antibodies and their diagnostic and therapeutic uses thereof	Chen Zhinan	20050204
CN1660912	IL-8	Sequences of monoclonal antibodies against human interleukin 8 and therapeutic use	Ye Qingwei	20041208
WO2006051091	spike protein	Compositions against SARS-coronavirus and uses thereof	Crucell Holland BV	20041111
WO2006051091	spike protein	Compositions against SARS-coronavirus comprising at least two immunoglobulins reacting with non-competing epitopes, and therapeutic and diagnostic uses thereof	Crucell Holland BV	20041111
CN1673231	spike protein	Monoclonal antibody of SARS coronavirus N protein and its use in treatment of SARS virus infections	Chinese Academy of Sciences	20040715
US20060240551	spike protein	Neutralizing monoclonal antibodies against severe acute respiratory syndrome-associated coronavirus	New York Blood Center, Inc.	20040602
WO2005054469	spike protein	Anti-SARS-coronavirus monoclonal antibodies, and diagnostic, therapeutic and vaccine preparation uses	Health Canada	20031205
WO2005060520	spike protein	Antibodies specific to SARS-CoV spike protein for diagnosis and therapy of SARS and for screening of epitopic vaccines or anti-SARS therapeutics	Dana-Farber Cancer Institute, Inc.	20031125
US20050106563	spike protein	Epitope profiles of SARS coronavirus for use in antigen detection, antibody production, and defense against infection	Genesis Biotech Inc.	20030908
US20050069869	spike protein	SARS coronavirus codon-optimized sequences for spike (S) protein expression, anti-S human monoclonal antibodies, and therapeutic and diagnostic uses thereof	University of Massachusetts	20030804
WO2005012360	S and N proteins	Antibody binding molecules specific for SARS coronavirus	Crucell Holland BV	20030722
CN1566155	S, N, and M proteins	Antibody library-derived human monoclonal anti-SARS virus antibodies for treating severe acute respiratory syndrome	Igcon Therapeutics Co., Ltd.; Genetastix Corporation	20030710
WO2005007671	spike protein	Compositions and methods for treating SARS using peptides derived from SARS virus E2 N-terminal-alpha helix or C-terminal-alpha helix and related monoclonal antibody	Epitomics, Inc.	20030429

various viral infections/diseases, including SARS. The invention relates that rSIFN-co has an identical amino acid sequence to Infergen (118390-30-0), but it has an altered spatial conformation and different biological potency. The rSIFN-co not only has an antiviral activity that is 20 times stronger than the clinically available interferon, but also has significantly stronger antitumor properties against breast cancer and cervical cancer than other recombinant human α-interferons. The invention further relates that rSIFN has reduced toxic and side effects and can be safely used in large doses (each dose can be >10 million IU), making it possible to successfully treat some viral diseases or tumors that require large doses of interferon.

IFN-ω. Patent application WO2004096852 discloses the amino acid sequence for recombinant human interferon ω (rhIFN-ω) (RN 791910-34-4) that was shown to have an anti-SARS viral activity similar to that of IFN-β. IFN-ω effectively decreased disease severity and inhibited proliferation of coronavirus strain BJ01 in monkeys.

IL-28A (IFN-λ2), IL-28B (IFN-λ3), and IL-29 (IFN-λ1) Variants. Patent application WO2005097165 claims a method for treating SARS viral infection using IL-28A, IL-28B, and IL-29 cysteine variants conjugated to polymers (e.g., polyethylene glycol) and discloses the amino acid and nucleic acid sequences for these cysteine variants. Of these variants, MetIL-29C172S-PEG (RN 867228-40-8) was specifically shown to inhibit viral replication.

Interferon-Human Serum Albumin Fusion Protein. Patents applications US20090053173 and CN101942026 both disclose long-lasting fusion proteins (HSA-IFN) with each of them being composed of an interferon fused with human serum albumin-binding peptide for treatment of a wide range of diseases, including SARS. Specific HSA-IFN fusion proteins were constructed using five different interferons (IFN-α1b, IFN-α2b, IFN-β, IFN-ω, IFN-γ) with corresponding CAS RNs 1122730-20-4, 1122730-23-7, 1122730-25-9, 1122730-27-1, and 1122730-29-3, respectively. These HSA-IFN fusion proteins significantly lengthened the plasma half-life of interferons (e.g., from 10 h to 12 days for HSA-IFN-α2b) due to slower free interferon release into the plasma and thus may prolong the effects of interferon for each injection.

RNA Therapies. RNA interference (RNAi) is a biological process wherein small complementary RNA duplexes target

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Table 9. Representative siRNA Data from Chinese Patent CN1648249

siRNA	sense strand (CAS RN)	antisense strand (CAS RN)
No. 8*	5′-cgucgcagcguguaggcacua-3′ (RN 874840-18-3)	5′-cagugccuacacgcugcgacg-3′ (RN 874840-32-1)
No. 51*	5′-aacgguuuacgucuacucgca-3′ (RN 874840-19-4)	5′-cgcgaguagacguaaaccguu-3′ (RN 874840-47-8)
No. 56*	5′-aacguacugccacaaaacagc-3′ (RN 874840-20-7)	5′-acuguuuuguggcaguacguu-3′ (RN 874840-46-7)

Table 10. Representative siRNA Data from US20050004063

siRNA	sense strand	CAS RN	target region or gene
SARSi-1	5′-gugaacucacucgugagcuctt-3′	821121-35-1	512−531 bp of replicase A1 region
SARSi-2	5′-guacccucuugauugcauctt-3′	821121-36-2	586−604 bp of replicase A1 region
SARSi-3	5′-gagucgaagagaggugucutt-3′	821121-37-3	916−934 bp of replicase A1 region
SARSi-4	5′-gcacuugucuaccuugaugtt-3′	821121-38-4	1194−1213 of replicase A1 region
SARSi-5	5′-ccuccagaugaggaagaagtt-3′	821121-39-5	3028−3046 bp of replicase A region
SARSi-6	5′-gguguuuccauuccaugugtt-3′	821121-40-8	5024−5042 bp of replicase A region
SARSi-7	5′-cacugauuccguucgagauctt-3′	821121-41-9	S gene
SARSi-8	5′-cguuucggaagaaacagguactt-3′	821121-42-0	E gene
SARSi-9	5′-caagccucuucucgcuccuctt-3′	821121-43-1	N gene
SARSi-10	5′-guggcuuagcuacuucguugtt-3′	821121-44-2	M gene
SARSi-11	5′-ugcuugcugcugucuacagtt-3′	821121-45-3	M gene

and neutralize specific mRNA molecules, resulting in inhibition of gene expression or genetic translation. Interfering RNAs include microRNAs and small interfering RNAs (siRNAs) that are generally about 21−25 nucleotides in length. Short hairpin RNAs (shRNAs) are artificial synthetic RNA molecules designed to fold into a tight hairpin conformation that allows them to silence their target genes, and can serve as precursors of siRNAs. The expression of shRNAs in cells is typically accomplished by their delivery via plasmids or viral or bacterial vectors.[48] Although microRNAs are noncoding and naturally found in plants, animals, and some viruses, synthetic versions are currently being used to silence a variety of genes.[49] The ability to chemically synthesize modified analogues of microRNAs as well as siRNAs, which are capable of altering disease-related gene expression or inhibiting pathogen gene expression, has created a host of new therapeutic options.[50]

In contrast to the microRNAs and siRNAs, antisense RNAs are single-stranded RNAs which are naturally occurring or synthetic and usually around 19−23 nucleotides in length with a sequence complementary to that of a protein coding mRNA, allowing it to hybridize and block protein translation.[48]

Since the discovery of RNAi in the late 1990s, it has become a well-known method for silencing/suppressing target genes associated with virulence and pathogenesis. Thirty-five patents in the CAS content collection disclose the use of RNAi in treating SARS, with 28 patents using siRNA molecules, three patents using antisense oligonucleotides, two patents using RNA aptamers, one patent using a ribozyme, and one patent using a microRNA inhibitor. Supporting Information Table S1 provides a high-level view of these 35 patents including the specific RNAi targets. A few of these patents are further discussed below.

siRNAs Targeting Coronavirus Proteins M, N, or E. Patent application CN101173275 discloses two double-stranded RNAs (dsRNAs) designed to specifically target two separate regions of the SARS protein M mRNA. The siRNA-M1 sequences targeting the 220−241 region of protein M mRNA correspond to CAS RNs 1023405-01-7 and 1023405-02-8, while siRNA-M2 sequences targeting the 460−480 region correspond to CAS RNs 1023405-03-9 and 1023405-04-0. The interference efficiency of these two siRNAs on SARS M protein gene expression was greater than 70%.

Patent application CN1648249 discloses sequences associated with siRNAs specifically designed to target the M, N, and E genes of SARS. Three of these siRNAs (Nos. 15, 58, and 90) were shown to inhibit expression of GFP-M, GFP-N, and GFP-E fusion proteins, respectively. An additional three siRNAs (Nos. 8*, 51*, and 56*) were prepared which contained a mutation at the 3′ end (bold letter) of the sense strand. These modified siRNAs (shown below) demonstrated increased inhibition of SARS virus gene expression compared to the original siRNAs.

siRNAs Targeting Replicase and RNA Polymerase Region. Patent application US20050004063 discloses six siRNAs (SARSi-1 to SARSi-6) targeting the replicase 1A region of SARS that were shown to inhibit virus infection and replication, with SARSi-4 being the most efficient. The sense strand sequences and corresponding CAS RNs are shown in the table below. This invention also disclosed SARSi-7 to SARSi-11, which target the S, E, N, and M genes.

The authors demonstrated that SARSi-2, SARSi-3, SARSi-4, and SARSi-7-11 inhibited coronavirus infection and replication in FRhk-4 cells. SARSi-4 was the most effective with nearly complete inhibition, followed by SARSi-2 and SARSi-3.

Patent application CN1569233 discloses siRNAs, shown in Table 11, that target SARS genes encoding RNA-dependent RNA polymerase, helicase, nucleoprotein N, and proteolytic enzymes. These siRNAs were able to inhibit or kill 50−90% of the SARS virus BJ01 strain, with the proteolytic enzyme-targeting siRNAs being the most effective.

RNA Aptamers. Two Korean patents describe the use of RNA aptamers for inhibition of SARS viruses. Patent application KR2009128837 identifies RNA aptamers as anti-SARS agents capable of binding to and inhibiting the double-stranded DNA unwinding of the SARS virus helicase. Related patent application KR 2012139512 describes RNA aptamers with distinct affinity for the nucleocapsid of SARS-CoV for potential pharmaceutical use.

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Table 11. Representative siRNA Data from CN1569233

sequence	CAS RN	gene target	% inhibition of SARS virus
5′-caucauccggugaugcuac-3′	872062-80-1	RNA-dependent RNA polymerase	~50
5′-uaguguauacggcaugcuc-3′	872062-81-2	helicase	~70
5′-gugcgugcagacgguucgu-3′	872062-82-3	nucleoprotein N	~95
5′-cguagucgcgguaauucaa-3′	872067-98-6	proteolytic enzyme	~90

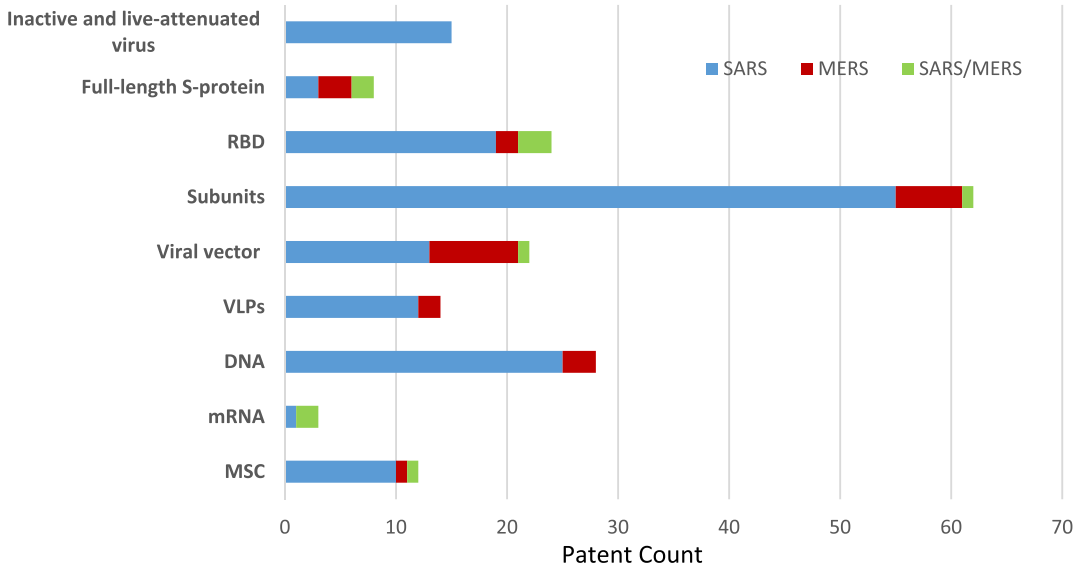

Figure 7. Distribution of vaccine-related patent associated to SARS and MERS.

A total of 188 patents are directly associated with anti-SARS and anti-MERS vaccines with a demonstrated immune response. Supporting Information Table S2 contains additional information on these SARS/MERS vaccine-related patents.

Ribozymes. Patent application JP2007043942 describes a therapeutic RNA/DNA chimeric ribozyme designed to recognize and cleave conserved common regions and regions with loop structures in the genes of coronaviruses, including SARS. This ribozyme specifically recognizes the GUC in viral genes with loop conformations.

Antisense Oligonucleotides. Antisense oligonucleotides have also been developed to reduce the severity of SARS virus infections and to prevent or treat SARS virus-associated disease, to detect the virus in human samples, and to diagnose SARS virus-associated diseases. Patent application WO2005023083 published by Ionis Pharmaceuticals describes hybrid DNA/RNA antisense oligonucleotides designed to disrupt the pseudoknot in the frameshift site of the SARS coronavirus RNA. In addition to directly targeting the virus, antisense oligonucleotides may be used to target disease-related proteins involved in the inflammatory process.

VACCINES

It is crucial to develop safe and effective vaccines to control the COVID-19 pandemic, eliminate its spread, and ultimately prevent its future recurrence. Since the SARS-CoV-2 virus shares significant sequence homology with two other lethal coronaviruses, SARS and MERS, the vaccines identified in these patents related to SARS and MERS viruses could potentially facilitate the design of anti-SARS-CoV-2 vaccines.

Distribution of Patents Related to SARS and MERS among Vaccine Types. Antiviral vaccines generally fall into one of the following types: inactive or live-attenuated viruses, virus-like particle (VLP), viral vectors, protein-based, DNA-based, and mRNA-based vaccines. There are 363 patents in the CAS content collection related to vaccine development to prevent viral disorders/diseases, including SARS and MERS. Of these, 175 patents disclose vaccines for non-coronaviruses that may have relevance to SARS and MERS, while 188 patents are directly associated with anti-SARS and anti-MERS vaccines with a demonstrated immune response. Supporting Information Table S2 contains additional information on these SARS/MERS vaccine-related patents.

Figure 7 reveals the distribution of patents among these vaccine types related to SARS and MERS. As can be seen, 15 patents disclose information about inactive and live-attenuated virus vaccines, 28 patents describe DNA vaccines, 21 patents disclose information on viral vector vaccines, 13 patents disclose information on VLP vaccines, and three patents are focused on mRNA vaccines.

It was reported that viral S protein subunit vaccines produced higher neutralizing antibody titers and more complete protection than live-attenuated SARS-CoV, full-length S protein, and DNA-based S protein vaccines.[51] Unsurprisingly, about half of the patents focused on protein vaccines comprising the S protein subunit vaccine and vaccines specifically targeting the receptor binding domain (RBD) of the S1 subunit of the viral S protein. Collectively, S protein/gene is the preferred target

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site in SARS/MERS vaccine development, and the same strategy can be potentially useful in developing SARS-CoV-2 vaccines. A condensed report on several patents that describe vaccines for generating immunity to SARS and MERS follows.

Attenuated Virus Vaccines. Patent application US20060039926 discloses live attenuated coronavirus or torovirus vaccines. Introduction of a mutation (Y6398H) into the Orf1a/b polyprotein (p59/nsp14/ExoN) was shown to completely attenuate virulence of mouse coronavirus (MHV-A59). The attenuated MHV virus exhibited reduced replication in mice at day five following intracerebral inoculation.

DNA-Based Vaccines. Patent application WO2005081716 discloses compositions and methods for inducing/enhancing immune responses, particularly antigen-specific CD8+ T cell-mediated responses, against antigens of the SARS coronavirus. An enhancement of the immune response involving particularly cytotoxic T cell immune responses is induced in vivo by chimeric nucleic acids that encode an endoplasmic reticulum chaperone polypeptide (e.g., calreticulin) linked to at least one antigenic polypeptide or peptide from SARS-CoV. Using gene gun delivery of DNA-coated gold particles, vaccination of mice against a calreticulin−nucleocapsid fusion protein resulted in potent nucleocapsid-specific humoral and T cell-mediated immune responses. Vaccinated animals were capable of significantly reducing the titer of a challenging vaccinia vector expressing the N protein of the SARS virus.

Patent application WO2015081155 discloses immunogens, which comprise consensus proteins derived from the MERS-CoV spike protein, for use in DNA-based vaccines targeting MERS-CoV. The consensus spike protein significantly induced both humoral and cellular immune responses, including increased titers of IgG and neutralizing antibodies. The induced cellular immune response involved increased CD3+CD4+ and CD3+CD8+ T cell responses that produced IFN-γ, TNF-α, IL-2, or both IFN-γ and TNF-α. On March 3, 2020, Inovio Pharmaceutical, Inc. announced they had designed the DNA vaccine called INO-4800 to be planned for human trials in the United States in April.[57]

Protein-Based Vaccines. Patent application WO2010063685 by GlaxoSmithKline (GSK) discloses a vaccine capable of provoking a protective immune response against SARS. The vaccine comprises an S protein immunogen and an oil-in-water emulsion adjuvant. An engineered ectodomain immunogen (soluble S protein), in combination with the emulsion adjuvant, GSK2, induced high levels of anti-SARS-CoV IgG2a or IgG2b antibody responses and neutralizing antibody responses in animal models. In late February 2020, GSK announced a collaboration with Chinese firm Clover Biopharmaceuticals to assess a coronavirus (COVID-19) vaccine candidate.[52] This collaboration will involve the use of Clover's protein-based coronavirus vaccine candidate (COVID-19 S-Trimer) with GSK's adjuvant system. By applying their Trimer-Tag technology, Clover has manufactured an S-Trimer subunit vaccine using a rapid mammalian cell culture-based expression system. The Trimer-Tag is an advanced drug development platform, which enables the production of novel, covalently trimerized fusion proteins that can better target previous undruggable pathways.

Patent application US20070003577 discloses immunogenic compositions and vaccines associated with the S protein of SARS coronavirus. A TriSpike SARS coronavirus vaccine was prepared from a recombinant full-length trimeric S protein. The recombinant protein was shown to (1) exhibit native antigenicity as shown by reactivity with convalescent SARS patient sera; (2) exhibit specific binding to soluble ACE2 receptor; (3) promote antibody-dependent viral entry in otherwise refractory human Raji B cells; and (4) elicit protection against a challenge infection in an animal model.

Patent application US20060002947 (Antigen Express, Inc., a subsidiary of Generex) discloses the preparation of hybrid peptides composed of three elements, including (a) an invariant chain (Ii) key peptide for antigen presentation enhancing activity, (b) a chemical structure linking the Ii to the antigenic epitope, and (c) an antigenic epitope that binds to a MHC class II molecule. The methodology was used to create Ii-Key/MHC II SARS hybrids. Recently, Generex announced that it is developing a COVID-19 vaccine following a contractual agreement with a Chinese consortium comprised of China Technology Exchange, Beijing Zhonghua Investment Fund Management, Biology Institute of Shandong Academy of Sciences, and Sinotek-Advocates International Industry Development. The company will utilize its Ii-Key immune system activation technology to produce a COVID-19 viral peptide for human clinical trials.[53]

Virus-like Particle Vaccines. In 2015, patent application WO2015042373 by Novavax disclosed an immunogenic composition composed of MERS-CoV nanoparticle VLPs containing at least one trimer of a S protein, produced by baculovirus overexpression in Sf9 cells. This VLP preparation induced a neutralizing antibody response in mice and transgenic cattle, when administered along with their proprietary adjuvant Matrix M (RN 1235341-17-9). In addition, preparations of sera from vaccinated cattle (SAB-300 or SAB-301) were injected into Ad5-hDPP4 transduced BALB/c mice prior to challenge with MERS-CoV. Both SAB-300 and SAB-301 were able to protect these mice from MERS-CoV infection with a single prophylactic injection. Novavax announced on February 26, 2020[54] that it was beginning animal testing on potential COVID-19 vaccine candidates due to their previous experiences working with other coronaviruses, including both MERS and SARS. Their COVID-19 candidate vaccines targeting the S protein of SARS-CoV-2 were developed using their recombinant nanoparticle vaccine technology along with their proprietary adjuvant Matrix-M.

mRNA-Based Vaccines. The potential advantages of an mRNA approach to prophylactic vaccines include the ability to mimic natural infection to stimulate a more potent immune response as well as the ability to combine multiple mRNAs into a single vaccine. Patent application WO2017070626 by Moderna discloses mRNA vaccines composed of mRNAs encoding antigenic viral full-length S, S1, or S2 proteins from SARS-CoV and MERS-CoV virus, formulated in cationic lipid nanoparticles. They show that mice vaccinated with mRNA encoding coronavirus full-length S protein generated much higher neutralizing antibody titers compared to mRNA encoding the S protein S2 subunit. New Zealand white rabbits immunized with MERS-CoV mRNA vaccine encoding the full-length S protein reduced more than 90% of the viral load in the lungs of the rabbits and induced a significant amount of neutralizing antibody against MERS-CoV. Moderna announced on February 24, 2020[55] that it has released the first batch of mRNA-1273 against SARS-CoV-2 for use in humans, prepared using methods and strategies outlined in their previous patents. Vials of mRNA-1273 have been shipped to the National Institute of Allergy and Infectious Diseases (NIAID), a division of the National Institutes of Health (NIH), to be used in the planned Phase 1 study in the United States. Moderna reports

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that mRNA-1273 is an mRNA vaccine targeting a prefusion-stabilized form of the S protein associated with SARS-CoV-2, which was selected by Moderna in collaboration with investigators at the NIAID Vaccine Research Center. Manufacture of this batch was funded by the Coalition for Epidemic Preparedness Innovations.

Patent application WO2018115527 describes vaccines comprising mRNA encoding at least one antigen of a MERS coronavirus, preferably a S protein or a S protein fragment (S1), an envelope protein (E), a membrane protein (M), or a nucleocapsid protein (N), all of which were effective in inducing an antigen-specific immune response. Intradermal administration into mice of a lipid nanoparticle (LNP)-encapsulated mRNA mixture encoding MERS-CoV S proteins was shown to result in translation in vivo and induction of humoral immune responses.

SUMMARY AND PERSPECTIVES

This report provides an overview of published information on global research and development of coronavirus-related therapeutic agents and preventive vaccines based on the extensive CAS content collection, with a focus on patents. It includes an overview of coronavirus morphology, biology, and pathogenesis with a particular focus on antiviral strategies involving small molecule drugs, as well as biologics targeting complex molecular interactions involved in coronavirus infection and replication. The drug-repurposing effort summarized in this report is focused primarily on agents currently known to be effective against other RNA viruses including SARS-CoV, MERS-CoV, influenza, HCV, and Ebola as well as anti-inflammatory drugs. The potential impact of biologics for treatment of coronavirus infections is promising and includes a wide variety of options including bioengineered and vectored antibodies, cytokines, and nucleic acid-based therapies targeting virus gene expression as well as various types of vaccines.

The information provided in this report provides a strong intellectual groundwork for support of ongoing research and development for discovery and development of therapeutic agents and vaccines for treatment of COVID-19 and coronavirus-related diseases. Because of limited space, this report devotes minimal attention to current efforts involved in advancing more efficient and accurate COVID-19 diagnosis methods and products.

Novel infectious diseases resulting from RNA viruses subject to mutation and genetic recombination, as well as cross-species transmission, will continue to present a serious global health threat, as exemplified by COVID-19. Despite two former major outbreaks of coronavirus infections causing the SARS and MERS respiratory illnesses, the world remains underprepared to effectively manage the current COVID-19 outbreak, as evidenced by the fact that COVID-19 has resulted in thousands of deaths worldwide.

A concerted effort to develop effective drugs and vaccines against existing and potential future coronavirus infections and other highly pathogenic virus outbreaks is necessary to reduce overwhelming impacts on human life and worldwide healthcare systems. Given the costly and arduous process involved with clinical drug development, the outbreak of COVID-19 further highlights the value of developing relatively broad-spectrum antiviral drugs and the importance of applying innovative approaches such as artificial intelligence to facilitate drug discovery. Given the lengthy process of new drug development, the current strategy of drug repurposing has become one of the chosen solutions for immediate treatment of SARS-CoV-2 infected individuals. Long-term drug development goals for the pharmaceutical industry include identification of inhibitors aimed at the replication or infection processes associated with SARS-CoV-2 or other related coronaviruses, as well as the symptomatic results of their infections leading to severe disease and/or death. The summarized lists, contained in this report, of small molecule compounds, and additional descriptions of biologics with properties suitable for inhibiting several key coronavirus proteins, could serve as information starting points for drug development. Since vaccines are crucial for prevention of coronavirus-related epidemic diseases in the future, it is reassuring that a number of innovative strategies are already being deployed. Four MERS coronavirus DNA vaccine candidates began phase 1 clinical trials in September of 2019,[56] and Moderna Inc. released its first batch of mRNA-1273 in February of 2020, which is an mRNA vaccine against SARS-CoV-2 ready for phase 1 study in the United States.[55]

Additional collaboration in the areas of antiviral discovery processes and clinical trial performance will enhance patients' access to drug candidates with improved therapeutic potential and ideally reduce the amount of time required to bring these drugs to market. The abundance of publications and the rapid publication rate associated with the SARS-CoV-2 virus-related disease outbreak, as illustrated in this report, are indicative of the intense effort by research institutes and pharmaceutical industries to address both molecular mechanisms and therapeutic routes useful for treating current and future coronavirus outbreaks.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.0c00272.

Tables of distribution of RNAi patents related to SARS and MERS in the CAS content collection and distribution of vaccine patents related to SARS and MERS in the CAS content collection (PDF)

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Notes

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■ ADDITIONAL NOTE

[a]To learn more about CAS proprietary fingerprints: https://www.cas.org/resources/case-studies/data-quality-impacts-machine-learning

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