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CORONAVIRUS EDITION

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THE CORONAVIRUS EDITION was divided in Issue 1 (March) and 2 (June) of 2020. The aim of these editions is to make a panoramic compilation of the pandemic, presenting all aspects, discoveries, reports about the COVID-19 pandemic and SARS-CoV-2: Epidemiological issues of the COVID-19, the pathophysiology of the disease, immunological responses, gallery photos, treatments, vaccines ongoing, diagnostics of the disease, characteristics genome of the virus and so on. All the data the ISI-SENAI-CMATEC Group used for the issues of JBTH was based on review articles, systematic review, meta-analyses, clinical trials, and guidelines from the best International Centers against COVID-19, index medicus database and reports of World Health Organization (WHO).

The Group of ISI-SENAI CIMATEC and the Group of Development and Innovation Laboratory of Butantan Institute inform that all reviewed studies were available for free. Also, most of the articles is open-access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). Images, tables, figures, and graphics reproduced in our review is for academic knowledge with no commercial purposes. The content of this issue was mainly based on review articles. Some parts of the text were directly cited from the sources with the proper references, which serve for our discussion or conclusion. Nevertheless, all the images that have Copyright by the author or other Journal, we asked for the right to use in this issue. The reproduced or copy of these items should be asked for the source referred in the text. The articles presented in this issue are following the Fair Use of American law and article 46 of Brazilian law Nº 9.610 of February 19, 1998. This issue is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0).

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The ISI-SENAI-CIMATEC Group writes some articles of this issue in a partnership with the Development and Innovation Laboratory Group of Butantan Institute.

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Reflexions about the COVID-19 Pandemic

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The world has been surprisely caught by an unprecedent pandemic in the last 100 years.

Of course we are all aware of the bubonic plague in the middle age that killed a significant number of the human inhabitants at the time. Also we know the suffering imposed by recurrent pandemic smallpox which took many lives since ancient ages and from which we justly got rid of, eliminating new cases in any part of the word since 1980. As well as the cholera pandemic of the 18th century. Not to mention the swine flu in 1918, due to H1N1 virus, that supposedly killed around 50 million people.

Nevertheless in the last 100 years we have not had a very impressive pandemic, so considered the widespread occurrence of a disease, affecting all regions of the world, usually infectious and serious, not until 2009 when we had a resurgence of influenza viral pandemic of relatively mild virulence, which prompted a vaccine development against it.

And also in the 21st century threatening epidemic from coronavirus were detected causing a serious respiratory infection in China and few other countries, by SARS-CoV and in the middle orient by the MERS-CoV- in 2003 and 2012 respectively.

All this led Bill Gates to alert the world that what should be a threat to humanity would not be a nuclear war, but viral pandemics.

But the world did not pay attention to this prediction, although it came from a very serious genius of our time.

Instead it continued to pursue the processes with all aiming results in which cuts and adjustments for cost containment were in order. In light of this view storage is a part less considered.

As a result the great potencies of the world were caught short of supplies of all that matters in facing a pandemic like the COVID-19 such as mechanical ventilators, personal protection equipment -PPEs-, intensive care unit beds, health personal and even supplies.

On the other hand all of the basic scientific knowledge that was being gathered related to the two recent coronavirus epidemics alluded above, was not stimulated, and thus an opportunity to have a better preparedness regarding specific treatment was missed.

Which has much to do with the way that scientific knowledge has been developed lately always searching for an immediate, lucrative result. Which penalizes the progress in basic knowledge.

Thus, there should not have been a surprise with the COVID-19 pandemic.

The world should have been better prepared to face its explosive appearance.

We hope that at the end of this pandemic, when a vaccine arrives, the world takes the opportunity to counterbalance the suffering caused by so many life losses and turn an appreciative look of thankfulness to the health professionals that faced this war, with determination and personal suffering, including many deaths among them, and also that science should prevail among all considerations in the biological and medical arena.

And recognize, once and for all, that for humanity health is what matters most.

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Brief Report of Guidelines and Protocols for the Management of COVID-19

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In less than a year, the novel coronavirus rapidly changed the world scenario. To dealing with the fast spread of the disease, health associations coordinate data flows and issue guidelines to better mitigate the impact of the threat. Also, scientific groups around the world are working to ensure that all information about the mechanisms of the virus, transmission, and disease clinics is updated as the disease progresses. The objective of this study was to present the guidelines and recommendations for preventing, management strategies, clarifications about pandemics disinformation, and diagnosing COVID-19 infection in human specimens adopted from the main health centers and institutions in the world, such as WHO and Centers for Disease Control and Prevention (CDC). It is important to highlight that the rapid and effective enforcement of existing international and national action plans, as well as parallel review and improvisation, is facilitating the affected countries to contain transmission and possibly delay the peak of outbreak and mortality.

Keywords: Guidelines. Recommendations. WHO. CDC.

Introduction

In December 2019, a mass of pneumonia cases, caused by a newly identified β-coronavirus, occurred in Wuhan, China [1]. The World Health Organization (WHO), on 12 January 2020, initially named this coronavirus as the 2019-novel coronavirus (2019-nCoV) and, on February 11, 2020, officially named the disease as coronavirus disease 2019 (COVID-19) [2]. On the same day, The International Committee on Taxonomy of Viruses has proposed to name the causative agent of COVID-19 as severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) [2].

COVID-19 has rapidly spread to other regions of China and around the world [2]. In Brazil, more than 4 million patients have been confirmed since the first case has been identified, on February 26, 2020 [3]. WHO declared COVID-19 as a pandemic in March 2020, and the disease now poses massive health, economic, and social burden globally [4,5]. This pandemic is further complicated by the substantial risk of viral spread and transmission by asymptomatic carriers [6].

To dealing with the rapid spread of the disease, health associations coordinate data flows and issue guidelines to better mitigate the impact of the threat. Also, scientific groups around the world are working to ensure that all information about the mechanisms of the virus, transmission, and disease clinics is updated as the disease progresses. Thus, new diagnostic and therapeutic therapies can be and are being developed quickly. Despite everything, many doubts still hover about the virus-host relationship and mainly about the evolution of the pandemic [7]. The constant updating of information reports, such as protocols and guidelines, is part of the pandemic coping mechanism. Operational planning has the function of balancing the situation and responding directly to the demands of COVID-19, assisting with health services to mitigating the collapse of the health system [8].

Overburdened health systems allow for a dramatic increase in both direct mortalities from an outbreak and indirect mortality from preventable and treatable vaccine diseases. Based on this scenario, countries make decisions that are necessary to balance the demands of responding directly to COVID-19, at the same time that strategic planning for coordinated actions to maintain the provision of essential health services is carried out [9]. For this

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reason, preparing and making documents available is essential to guide and provide immediate support to countries in responding to COVID-19. [10]. Nevertheless, they should not only be directed to national governments, but also the entire population. All relevant communication must be given to the public: what is known and unknown about the disease, what is being done, and the actions that must be taken regularly. Also, community participation in following the proposed guidelines contributes to a better-controlled situation [11].

Concerning diagnosis, the guidelines and protocols also serve to safely establish the diagnosis, as well as portraying the production of vaccines and alternative treatments in force. The nucleic acid amplification tests remain the mainstay diagnostics for laboratory confirmation of SARS-CoV-2 infection, while serological antibody tests are used to aid contact tracing, epidemiological, and vaccine evaluation studies [12]. Currently, about 200 vaccines are in the process of development, some of them already in clinical trial stages. Most candidates are based mainly on viral protein, due to its essential role in viral infectivity [12,13]. Before the efficacy of such vaccines in humans, strong international coordination and collaboration between studies by pharmaceutical companies with regulatory agencies are necessary to limit further damage by SARS-CoV-2. With several COVID-19 vaccines approaching phase III trials, the Food and Drug Administration (US FDA) has launched a development and licensing guidelines for these products. The guidelines discuss possible outcomes of the immune responses that can be observed, as well as stipulate parameters such as the number of study participants, including monitoring them "ideally at least one to two years"; and on pre-licensing safety for preventive vaccines. Following these and other guidelines are important for development programs to focus on traditional approval through direct evidence of the vaccine's safety and efficacy in protecting humans from SARS-CoV-2 infection [14].

So, any follow-up of news related to COVID-19 must be documented and made available in an accessible manner, mainly to guide other countries in the reference recommendations on the pandemic. Therefore, the objective of this work was to present the guidelines and recommendations for preventing, management strategies, clarifications about pandemics disinformation and diagnosing COVID-19 infection in human specimens adopted from the main health centers and institutions in the world, such as WHO and Centers for Disease Control and Prevention (CDC). Since COVID-19 is a new infection and new scientific evidence based on expert reviews is still emerging, the contents reported in this work, such as new diagnostic methods or general tests, selection of biosafety specifications and recommendations, may undergo further updates.

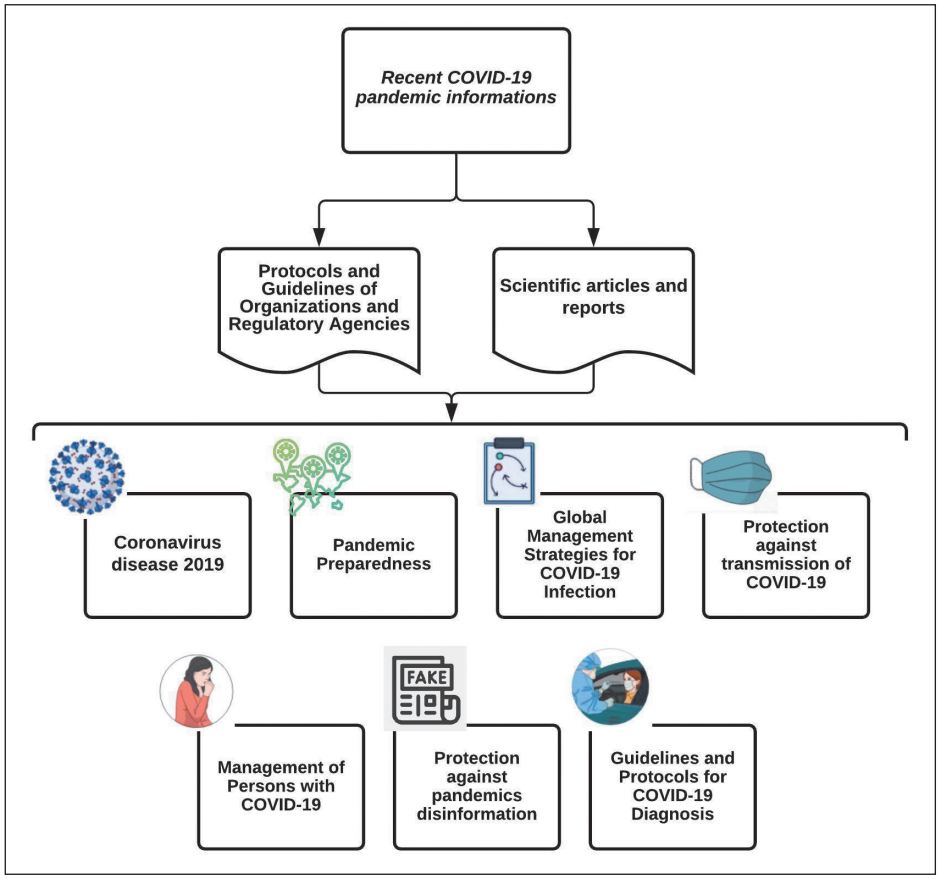
Method

The method of this review was based on guidelines and protocols of the main associations and regulatory agencies, with a focus on actions to combat COVID-19. Figure 1 demonstrates in a simplified way the methods applied for the development of this review. It is worth mentioning that for the development of this study, the most recent documents, and articles were used on prevention information, pandemic preparedness, and information on possible and potential vaccines, in addition to other information considered relevant.

Coronavirus Disease 2019

The CoVs have become the major pathogens of emerging respiratory disease outbreaks. As of September 9, there were 27,973,127 confirmed cases worldwide, with 905,426 deaths. On average, every 24 hours, 234,498 new cases appear from people infected with SARS-CoV-2. In Brazil, the disease still grows considerably. With more than 4 million confirmed cases, Brazil ranks third among the countries with the highest number of cases, behind only the USA with 6,304,181 cases and India with 4,562,414 cases [15]. It is worth mentioning that these numbers

Figure 1. The method applied for the development of this study.



are possibly an underestimate of the infected and dead due to limitations of surveillance and testing. CoV virus family are a large family of single-stranded RNA viruses (+ssRNA). SARS-CoV-2 is a virus ranging from 60 nm to 140 nm in diameter with spike-like projections on its surface giving it a crown-like appearance under the electron microscope, hence the name coronavirus [16], that can be isolated in different animal species. For reasons yet to be explained, these viruses can cross species barriers and can cause, in humans, illness ranging from the common cold to more severe diseases such as MERS and SARS. Interestingly, these latter viruses have probably originated from bats and then moving into other mammalian hosts (the Himalayan palm civet for SARS-CoV, and the dromedary camel for MERS-CoV) before jumping to humans. The dynamics of SARS-CoV-2 are currently unknown, but there is speculation that it also has an animal origin [17] that, due to evidenced genetic relations, points to the bat as the primary host [18]. Regarding symptomatology, clinical features of COVID-19 include fever, dry cough, shortness of breath, normal or low levels of peripheral white blood cells, and inflammatory changes on chest X-ray [19].

All people, regardless of age group, are susceptible to SARS-CoV-2 infection. The infection occurs through large droplets generated during the cough and sneeze of symptomatic people, however, asymptomatic people are also liable to transmit the virus [20]. Studies have shown higher viral loads in the nasal cavity compared to the throat, with no difference in viral load between symptomatic and asymptomatic people. [21]. Patients can be infectious for the duration of symptoms and even during clinical recovery. Infection is acquired either by inhalation of these droplets or touching surfaces contaminated by them and then touching the nose, mouth, and eyes. The virus is also present in the stool and

contamination of the water supply and subsequent transmission via aerosolization/feco oral route is also hypothesized [22].

Pandemic Preparedness

WHO and other leading epidemiology organizations unanimously agree on the indispensable role of pandemic preparation and a plan at global and national levels to mitigate the public health emergency of COVID-19 or any future outbreaks [23,24]. Pandemic preparation is an effort of the government and the society requiring inputs from each person susceptible to the infection agent as well as policymakers at national and international levels, frontline healthcare providers, infrastructure developers, and maintenance personnel, pharmaceutical industry and researcher community, and so forth [25]. Moreover, the pandemic preparedness plan needs constant reviewing and improvisation.

The magnitude of the COVID-19 pandemic requires worldwide action plans. The United States quickly created the United Nations Strategic Preparedness and Response Plan (SPRS) to control the transmission of the virus to delaying the spread of COVID-19, provide optimal care for all patients, and minimize the impact on healthcare systems and socioeconomic activities [26]. Several nations are well placed to implement this action plan with minimal support. However, each country has its issues that the placing authority has to adjust the guidelines and protocols for the reality of each country or district. Thus, all nation has to prepare a COVID-19 Country Preparedness and Response Plan (CPRP) against the COVID-19 [27]. These CPRPs need constant monitoring and reviewing using indicators shared by WHO and CDC, for example, updating as the situation evolves. Part of these plans is the protocols and guidelines adopted in each country [28].

Saxena, 2020 [27] reported that the success against a pandemic is grounded in the following actions, which have to be included in the protocols and guidelines:

(i) Surveillance of the pathogen: characterization, epidemiology, transmission, symptoms, pathogenesis, diagnosis and detection, infection, contact tracing, data from confirmed cases, predicting mass infection outbreak, keeping a count, and estimation of mortality.
(ii) Response management: production and supply of protective/preventive pharmaceutical interventions or non-pharmaceutical interventions; extensive test the community, education of the community about the disease and how it spreads, transmit and all information about the disease.
(iii) Facilitating timely medical help: access to hospitals/healthcare providers, personal and public hygiene, disinfection, and quarantine services.
(iv) Lessons learned from the present outbreak to facilitate future action plans and preparedness.

Global Management Strategies for COVID-19 Infection

The authorities of worldwide and Health Organizations created strategies, which including protocols and guidelines, due to the exponential transmission of SARS-CoV-2 and social-economic impact of the pandemic [24,29,30], such as social distancing, travel restrictions, implementation of personal and public hygiene (non-pharmaceutical interventions), implementation of diagnosis (clinical with symptoms and laboratory), extensive testing for community and medicines interventions. All these practices are necessary to delay the peaking of the outbreak, avoid burden on the healthcare infrastructure, and "flattening the curve" of the infected patients [25,31].

Protection against Transmission of COVID-19

According to WHO [32], individual protections against the transmission of COVID-19 between

people are: Regularly and thoroughly clean your hands with an alcohol-based hand rub or wash them with soap and water. This practice kills viruses that may be on your hands; Maintain at least 1 meter (3 feet) distance between yourself and others. Therefore, when someone coughs, sneezes, or speaks, spraying small liquid droplets from their nose or mouth which may contain the virus, you are not too close to breathe in the droplets, including the COVID-19 virus if the person has the disease; Avoid going to crowded places. Where people come together in crowds, you are more likely to come into close contact with someone that has COVID-19 and it is more difficult to maintain physical distance of 1 meter (3 feet); Avoid touching eyes, nose, and mouth. Hands touch many surfaces and can pick up viruses. Once contaminated, hands can transfer the virus to your eyes, nose, or mouth. From there, the virus can enter your body and infect you. Make sure you, and the people around you, follow good respiratory hygiene. This means covering your mouth and nose with your bent elbow or tissue when you cough or sneeze. Then dispose of the used tissue immediately and wash your hands. Droplets spread the virus. By following good respiratory hygiene, you protect the people around you from viruses such as cold, flu, and COVID-19; Stay home and self-isolate even with minor symptoms such as cough, headache, mild fever, until you recover. Have someone bring you supplies. If you need to leave your house, wear a mask to avoid infecting others. Avoiding contact with others will protect them from possible COVID-19 and other viruses; If you have a fever, cough, and difficulty breathing, seek medical attention, but call by telephone in advance if possible and follow the directions of your local health authority. National and local authorities will have the most up to date information on the situation in your area. Calling in advance will allow your health care provider to quickly direct you to the right health facility. This will also protect you and help prevent the spread of viruses and other infections.

Keep up to date on the latest information from trusted sources, such as WHO or your local and national health authorities. Local and national authorities are best placed to advise on what people in your area should be doing to protect themselves. The guidelines proposed by the CDC first preconize the knowledge about how the virus spreads [33]. The virus is thought to spread mainly from person-to-person, but can also be spread between people who are in close contact with one another, and through respiratory droplets produced when an infected person coughs, sneezes, or talks [33]. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs. It is important to highlight that some recent studies have suggested that COVID-19 may be spread by people who are not showing symptoms [6,34]. Therefore, until an effective vaccine or antiviral drug be developed, the unique way to not spread the virus is by practicing social distancing.

Some other steps are also indicated, such as: Often wash hands with soap and water for at least 20 seconds especially in a public place, or after blowing your nose, coughing, or sneezing; Avoid close contact, especially with people who are sick; Cover your mouth and nose with a cloth face cover when around others; Cover coughs and sneezes; Clean and disinfect; Monitor health by being alert for symptoms: watch for fever, cough, shortness of breath, or other symptoms of COVID-19.

Management of Persons with COVID-19

Patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can experience a range of clinical manifestations, from no symptoms to critical illness. This section of the Guidelines discusses the clinical management of patients according to illness severity. Currently, the Food and Drug Administration has not approved any drugs for the treatment of COVID-19. However, an array of drugs approved for other indications, as well as multiple investigational agents, are being studied for the treatment of COVID-19 in several hundred clinical trials around the globe. Some drugs can be

accessed through Emergency Use Authorization, expanded access programs, or compassionate use mechanisms. Available clinical data for these drugs under investigation are discussed in Antiviral Therapy and Immune-Based Therapy.

In general, adults with COVID-19 can be grouped into the following severity of illness categories, although the criteria in each category may overlap or vary across guidelines and clinical trials:

(i) Asymptomatic or Presymptomatic Infection: Individuals who test positive for SARS-CoV-2 by virologic testing using a molecular diagnostic (e.g., polymerase chain reaction) or antigen test, but have no symptoms.

(ii) Mild Illness: Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain) without shortness of breath, dyspnea, or abnormal chest imaging.

(iii) Moderate Illness: Individuals who have evidence of lower respiratory disease by clinical assessment or imaging and saturation of oxygen (SpO_2) ≥94% on room air at sea level. Severe Illness: Individuals who have respiratory frequency >30 breaths per minute, SpO_2 <94% on room air at sea level, the ratio of the arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mmHg, or lung infiltrates >50%.

(iv) Critical Illness: Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

In pediatric patients, radiographic abnormalities are common and, for the most part, should not be used as the sole criteria to define the COVID-19 illness category. Normal values for respiratory rate also vary with age in children, thus hypoxia should be the primary criterion to define severe illness, especially in younger children.

Protection against Pandemics Disinformation

Since SARS-CoV-2 is a novel virus, there is a knowledge gap that has been proven to be a breeding ground for the spread of false and/or misleading information. The Department of Evidence and Intelligence for Action in Health of the Pan American Health Organization (PAHO) has stated that the "infodemic" - an overabundance of information - make the pandemic worse since makes it hard for people to find trustworthy sources and reliable guidance when they need it, and anybody can write or publish anything on the web [35]. Moreover, it can affect decision-making processes and there is no quality control on what is published. According to PAHO [35], people can help in the fight against the COVID-19 infodemic by Identifying evidence; Avoid fake news; Support open science; Determine if the information adds up; Report harmful rumors; Confirm that the information has been shared before by other people; Participate responsibly in social conversations; and, Share information responsibly.

Aiming to fight misinformation, WHO has joined forces with the Government of the United Kingdom and is running an awareness campaign called "Stop The Spread" [36]. The campaign is about the risks of the spread of false and incorrect information about the coronavirus pandemic aiming to raise awareness about the risks of misinformation, encouraging people to double-check information with trusted sources and is also being promoted in many countries across Africa, Asia, Europe, Middle East, and Latin America.

With the same purpose, an important action adopted by the European Union is the Joint Communication "Tackling COVID-19 disinformation - Getting the facts right" focused on the response to disinformation around the coronavirus pandemic [37]. In this communication, it is highlighted that first of all, there is a need to differentiate the various forms of false or misleading information, distinguishing the illegal content from the content that is harmful

but not illegal. The next step is to determine if exists an intention to deceive or cause public harm, or to make an economic gain, qualifying it as disinformation. If there is no such intention, the content can be qualified as misinformation. The EU guides to address misinformation through grounded rebuttals and myth-busting and media literacy initiatives, differently from disinformation, that needs to be addressed through actions taken by governments. The main actions of the plan are: Strengthening Strategic Communication Within and Outside The EU; Cooperating Better Within the EU; Cooperation with Third Countries and International Partners; Greater Transparency of Online Platforms About Disinformation and Influence Operations; Ensuring Freedom of Expression and Pluralistic Democratic Debate; Empowering and Raising Citizen Awareness; Protecting Public Health and Consumers' Rights.

In Brazil, the Ministry of Health has made available a WhatsApp phone number for the population as an exclusive channel to receive viral information that will be investigated by the technical areas and officially answered if they are true or false [38].

Guidelines and Protocols for COVID-19 Diagnosis

Early recognition and rapid diagnosis are essential in interrupting the transmission chain of SARS-CoV-2. To diagnose patients, the decision must be based on clinical and epidemiological factors, linked to an analysis of the probability of infection [39]. According to the Guidelines on Response to Coronavirus Disease 2019 published by Korea Centers for Disease Control and Prevention (CDC) [40], case definitions are applied under conditions of serious alert levels, as is the case with COVID-19. They are divided into three types of definitions:

(i) Confirmed case: a case with infection confirmed by the realization of the gold standard RT-qPCR test in real-time, regardless of the clinical manifestation.

(ii) Suspected case: when the patient has a fever above 37.5°C and/or respiratory symptoms within 14 days after close contact with a confirmed case.

(iii) Patient under investigation (PUI): cases suspected of having COVID-19 based on the opinion of a doctor, or that fit the definition of a previous case or that have an epidemiological correlation with a massive domestic outbreak of COVID-19.

Currently, the COVID-19 investigation includes the techniques of molecular testing of SARS-CoV-2 nucleic acid amplification by real-time PCR preceded by reverse transcription reaction (RT-qPCR); and immunological tests (rapid test or classic serology for antibody detection). The partial or total sequencing of the viral genome, although is not a diagnostic method, can be investigatively used in epidemiological studies, when necessary [41]. The laboratory diagnosis considered the gold standard, as previously mentioned, is RT-qPCR, as recommended by the guidelines of the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) [39]. This molecular test is based on the detection of unique viral RNA sequences, with confirmation by nucleic acid sequencing, when necessary. It is worth mentioning that several protocols for this test have been proposed for the diagnosis of COVID-19, worldwide [39,42-45]. The difference between the molecular test and the serological/immunological tests is based on the application. While RT-qPCR is used for diagnosing patients with COVID-19, serological tests are not indicated for diagnostic confirmation, and are recommended for surveillance purposes only [41]. This happens due to the period of the immunological window in the initial period of infection, where the production of IgM and IgG antibodies normally only occurs after 7 days (IgM) and 14 days (IgG), which reduces the sensitivity of this type of test in the first infection days [46].

RT-qPCR is a special version used when the genetic material targeted for detection is RNA.

It is a very fast, sensitive, and reliable test, capable of producing results in 3 to 4 hours. Viral RNA is converted to DNA, copied several times using repeated temperature cycles in a PCR machine, and its detection is made by applying fluorescent markers that, when exceeding a certain level of fluorescence, indicates that the virus was present in that sample collected [46]. Originally, the method used radioactive isotope markers, however, this type of genetic marker was replaced by special markers, mostly fluorescent dyes, thus allowing almost immediate visualization of the results while the process is still in progress (real-time). The first SARS-CoV-2 detection protocol using this technique was developed by China's CDC and is based on the detection of the N and ORF1ab genes [47]. In this test, the positive result for the presence of the virus is reported when both markers are amplified [48], and a different result from this means that the test needs to be repeated for real confirmation [49,50]. The same recommendation is made by the US CDC, which if only one target is positive in the N region target, the result is considered inconclusive and needs to be re-tested [48]. Outside China, the first RT-qPCR protocol was incorporated primers targeting genes of the E, N, and RdRp, developed by Charité Institute (Germany) [43]. Also, other protocols were developed by the HKU Institute, Hong Kong, which is based on the amplification of the ORF1b-nsp and N genes. The National Institute of Infectious Diseases, Department of Virology III, Japan, for the Japan Pancorona and multiple targets, and S genes. The National Institute of Health in Thailand, for marker N and the Pasteur Institute in France, which is based on the detection of two regions in RdRp. Table 1 shows a summary regarding the interpretation of the results based on the Centers for Disease Control and Prevention's diagnostic panel [45].

The diagnostic accuracy of the RT-qPCR for the identification of patients with COVID-19 can be influenced by the type of sample used in the test, in addition to the time of evolution of the condition [51,52]. In general, sputum, nasopharyngeal swab (NP), and oropharyngeal swabs (OP) are the most common sample types taken from patients with mild to moderate illness. If both NP and OP are collected, they can be placed in the same tube and tested simultaneously to save reagents [53]. In general, the bronchoalveolar lavage (BAL) showed the highest positive rates, followed by sputum, NP, and OP in order of decreasing sensitivity [21,54,55]. Throat

Table 1. Real-Time RT-qPCR diagnostic panel.

N1	N2	RP	Result Interpretation	Report	Action
+	+	+/-	SARS-CoV-2 detected	SARS-CoV-2 positive	Issue a report and notify the responsible health agency
If only one of the two targets is positive		+/-	Inconclusive	Inconclusive	Repeat the RT-qPCR and/or extraction. If the repeated result remains inconclusive, contact for a third test
-	-	+	SARS-CoV-2 not detected	Not detected	Notify responsible health agency. Consider testing for other respiratory viruses
-	-	-	Invalid	Invalid	Repeat the extraction and RT-qPCR. If the repeated result remains invalid, consider taking a new sample from the patient

gargling samples are an alternative specimen, although they are less sensitive than sputum [55]. Considering the seasonality of respiratory viruses, this collection must be performed until the 7th day after the appearance of the first signs or symptoms [41]. It should be noted that the techniques employed in carrying out the RT-qPCR tests can also influence the diagnostic accuracy of the test [56], for this reason, the follow-up of tests classified in the guidelines of the WHO or other regulatory agencies is of paramount importance for maintaining the accuracy of the test.

Conclusion

The COVID-19 pandemic is spreading fast and new information about the disease comes up every day. It is important to believe that even during this current challenge that the world is experiencing, one cannot abdicate the principles of evidence-based medicine, recommendations, and guidelines grounded on high-level evidence, taking into account the professional obligations and social role of the responsible organizations as health providers. The rapid and effective enforcement of existing international and national action plans, as well as parallel review and improvisation, is facilitating the affected countries to contain transmission and possibly delay the peak of outbreak and mortality. Although the global economy is suffering from the pandemic. Therefore, it is important to review the current action plans and suitably improvise future action plans to mitigate the disease and avoid potential recurrences.

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The New Technologies in the Pandemic Era

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The pandemic of the new coronavirus affected people's lives by an unprecedented scale. Due to the need for isolation and the treatments, drugs, and vaccines, the pandemic amplified the digital health technologies, such as Artificial Intelligence (AI), Big Data Analytics (BDA), Blockchain, Telecommunication Technology (TT) as well as High-Performance Computing (HPC) and other technologies, to historic levels. These technologies are being used to mitigate, facilitate pandemic strategies, and find treatments and vaccines. This paper aims to reach articles about new technologies applied to COVID-19 published in the main database (PubMed/Medline, Elsevier Science Direct, Scopus, Isi Web of Science, Embase, Excerpta Medica, UptoDate, Lilacs, Novel Coronavirus Resource Directory from Elsevier), in the high-impact international scientific Journals (Scimago Journal and Country Rank - SJR - and Journal Citation Reports - JCR), such as The Lancet, Science, Nature, The New England Journal of Medicine, Physiological Reviews, Journal of the American Medical Association, Plos One, Journal of Clinical Investigation, and in the data from Center for Disease Control (CDC), National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID) and World Health Organization (WHO). We prior selected meta-analysis, systematic reviews, article reviews, and original articles in this order. We reviewed 252 articles and used 140 from March to June 2020, using the terms coronavirus, SARS-CoV-2, novel coronavirus, Wuhan coronavirus, severe acute respiratory syndrome, 2019-nCoV, 2019 novel coronavirus, n-CoV-2, covid, n-SARS-2, COVID-19, corona virus, coronaviruses, New Technologies, Artificial Intelligence, Telemedicine, Telecommunication Technologies, AI, Big Data, BDA, TT, High-Performance Computing, Deep Learning, Neural Network, Blockchain, with the tools MeSH (Medical Subject Headings), AND, OR, and the characters [,",:; /., to ensure the best review topics. We concluded that this pandemic lastly consolidates the new technologies era and will change the whole way of the social life of human beings. Also, a big jump in medicine will happen on procedures, protocols, drug designs, attendances, encompassing all health areas, as well as in social and business behaviors.
Keywords: COVID-19. SARS-CoV-2. New Technologies. AI. Big Data. BDA. Telecommunication Technologies.

Introduction

The COVID-19 pandemic is causing serious disturbances in human society and unprecedented health and economic crisis. At the same time, several new technologies are being applied to the novel pandemic of coronavirus in a global effort to mitigate the consequences of the disease, to optimize the efforts against COVID-19, and to find a drug or vaccine as quickly as possible to treat and cure people worldwide. Healthcare fields were fast to adopt digital solutions and the most advanced technology tools in response to the COVID-19 pandemic. Many of the solutions implemented now could be solidified soon, contributing to the meaning of new digital-based models of care. These technologies mainly include Artificial Intelligence (AI), Telecommunication Technology (TT), Big Data Analytics (BDA), 3D Printing Technology (3DPT), High-Performance Computing (HPC), among others. Due to the considerable studies on technological innovations in the combat and control of the COVID-19 pandemic, we decided to summarize them in Tables (Table 1-6) and Figures (1-28) and describe the main technologies and their applications to mitigate the pandemic and help in the solutions against COVID-19.

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General Use of New Technologies Against COVID-19

New technologies very often are capable of providing solutions for our daily lives during a crisis [1, 16, 29, 30]. They have the potential to

Table 1. New Technologies and the aplications in COVID-19 pandemic.

Technology	Description	Applications (Examples)
Artificial Intelligence (AI)	Artificial intelligence can be a powerful instrument when it comes to the assessment of risks of infection and screening of the population, which comes in handy in pandemic times. It works similarly to machine learning, computer vision, and natural language processing, instructing computers to use models based on big data for recognizing, explaining, and predicting patterns. Currently, the use of this technology is somewhat restricted due to lack of data, and because sometimes the data can be noisy and outlier [1,2].	It can work detecting viruses, individuals with fever, and suspected symptoms of COVID-19 through the integration of thermal imaging, AI, Computer vision, and cloud computing, and advice accordingly for appropriate treatment. Its use can bring down the time for genetic detection to minutes. [2] It can provide real-time coverage of the COVID-19 outbreak (great importance for epidemiological information) [3]. It can aid in keeping this virus within reasonable limits, and in designing new molecules in silico [4]. For automated detection and monitoring of COVID-19 patients over time, a deep learning-based analysis system of thoracic CT images has been built [5, 6]. Machine learning-based screening of SARS-CoV-2 assay designs using a CRISPR-based virus detection system was demonstrated with high sensitivity and speed [7]. For large-scale screening of COVID-19 patients, neural network classifiers were developed to work based on their distinct respiratory patterns [8]. It can be used with deep learning and neural network and radiological images (CT and x-Ray) [9,10] Lastly, Artificial intelligence can also be used to predict the immunogenic landscape of SARS-CoV-2.
Computer Vision	Computer vision is an interdisciplinary field that is devoted to using computers to develop a high-level understanding by interpreting digital images and the information present in them. It has made substantial progress in the last few years, mainly due to the success of deep learning, a sub-field of machine learning [11]	Prevention and control, when applied to CT and x-Ray images [11]
Big Data	Big data is an analytic technique using technology that can store information about a large number of patients infected by this virus. It can be quite useful to track and control the worldwide spread of COVID 19 since this technology provides the basis for faster (almost real-time) evaluation and decision making. It is helping save lives and quickly identifying effective therapies [1,2].	Storage capacity for extensive public data is provided in a format that lends itself efficiently for analysis, and as a result, appropriate measures can be taken towards the prevention of disease transmission, movement, health monitoring, and prevention system. Big data can be highly useful for analyzing and forecasting the reach and impact of coronavirus. COVID-19 trackers are able to gather nearly real-time data from sources around the world and subsequently equip scientists, doctors, epidemiologists, and policymakers with up-to-date information, which can be very helpful to make better decisions in order to neutralize the effects of the virus [1, 2]. It can be used in association with AIs: whilst Big Data enables data analysis and interpretation, the AI uncovers hidden trends and patterns, which can be used to build predictive models.

Technology	Description	Applications
Telemedicine	Telehealth or Telemedicine is the distribution of health-related services and information via electronic information and telecommunication technologies [12]. It allows long-distance patient and clinician contact, care, advice, reminders, education, intervention, monitoring, and remote admissions [13, 14].	A patient can have a consultation from well-trained professionals on their medical conditions through video calls, avoiding the need for a hospital visit and thus helping the social distancing and man-to-man contact and disease transmission. However, these remote consultations are now possible by using better telecom infrastructure with Virtual reality and Augmented reality: - Medical Care Appointments (SMAs) [15].
Blockchain	Blockchain has recently come to light as a key technology in the field of epidemic management. It is able to provide robust, transparent, and cheap means of assisting effective decision-making and, as a result, could lead to faster responses during emergencies of this kind. Blockchain has the potential to be an integral part of the global response to COVI-19 by tracking the transmission of the virus, managing insurance payments, and maintaining the sustainability of medical supply chains and donation tracking pathways [16].	Algorithms can facilitate the offer of real-time data to all the strategic partners, as well as traceability in the process of disease control, and support in the effective management of the supply chain [2].
5G + Smart Applications	5G networks are digital cellular networks, in which the service area covered by providers is divided into small geographical areas called cells. Analog signals representing sounds and images are digitized in the telephone, converted by an analog-to-digital converter, and transmitted as a stream of bits. All the 5G wireless devices in a cell communicate by radio waves with a local antenna array and low power automated transceiver (transmitter and receiver) in the cell, over frequency channels assigned by the transceiver from a pool of frequencies that are reused in other cells. The local antennas are connected with the telephone network and the Internet by a high-bandwidth optical fiber or wireless backhaul connection. As in other cell networks, a mobile device crossing from one cell to another is automatically "handed off" seamlessly to the new cell. 5G can support up to a million devices per square kilometer, while 4G supports only up to 100,000 devices per square kilometer [17, 18]. The new 5G wireless devices also have 4G LTE capability, as the new networks use 4G for initially establishing the connection with the cell, as well as in locations where 5G access is not available [19].	The high-speed network allows real-time data of video and audio quality for patient data analysis, telemedicine, medical and surgical intervention

Technology	Description	Applications
Internet of Things (IoT)	Internet of Things is an automated solution that has resulted in tremendous growth in automated manufacturing, management of assets, etc. It comprises of collection, transfer, analytics, and storage of data. Collection of data is done with the help of sensors incorporated in mobile phones, robots, etc. The data collected is then sent for analytics and decision making to the central cloud server [1, 2].	All devices are connected to the internet in the hospital and strategic locations. Thus, these connected devices help to inform the medical staff of any errors and changes of requirements during the treatment process (similar to the factories of the future). IoT is proving to be very helpful in the fight against COVID-19. For instance, drones are in use for surveillance in order to ensure the implementation of quarantine and mask-wearing. This technology can be used for tracing the origin of an outbreak. It can be helpful to the epidemiologists for searching patient zero and also in identifying the persons coming in contact with the patients. The compliance of quarantine by the patients can be ensured. The patients who breach the quarantine can be tracked down. Moreover, this technology can be beneficial in providing relief to the medical staff by remote monitoring of in-home patients [1, 2].
Drones	An unmanned aerial vehicle (UAV) (or uncrewed aerial vehicle [20], commonly known as a drone) is an aircraft without a human pilot onboard and a type of unmanned vehicle. UAVs are a component of an unmanned aircraft system (UAS); which includes a UAV, a ground-based controller, and a system of communications between the two. The flight of UAVs may operate with various degrees of autonomy: either under remote control by a human operator, autonomously by onboard computers [21] or piloted by an autonomous robot [22].	These unmanned vehicles controlled by remote location can undertake jobs of logistics provider and area surveillance and can also be used for disinfecting remote locations
Robotics	Robotics is an interdisciplinary research area at the interface of computer science and engineering [23]. Robotics involves the design, construction, operation, and use of robots. The goal of robotics is to design intelligent machines that can help and assist humans in their day-to-day lives and keep everyone safe. Robotics draws on the achievement of information engineering, computer, engineering, mechanical, engineering, electronic, engineering, and others.	Undertakes repetitive jobs with precision and reliability in the hazardous environment of infectious disease in and around the hospitals and can make an intelligent decision with inputs from the population data analyzed through AI.
Modern enterprise video communications platform	Communication technologies that provide videotelephony and online chat services through a cloud-based peer-to-peer software platform and are used for teleconferencing, telecommuting, distance education, and social relations [24, 25].	The application of the software helps in holding video and audio communications, chats, and webinars easily and quickly through large numbers of communication devices.

Technology	Description	Applications
Smartphone apps	A mobile application, also known as an app, is a computer program or software application designed to run on a mobile device such as a phone, tablet, or watch. Apps were originally intended for productivity assistance such as email, calendar, and contact databases, but the public demand for apps caused rapid expansion into other areas such as mobile games, factory automation, GPS and location-based services, order-tracking, and ticket purchases so that there are now millions of apps available. Apps are generally downloaded from application distribution platforms which are operated by the owner of the mobile operating system, such as the App Store (iOS) or Google Play Store. Some apps are free, and others have a price, with the profit being split between the application's creator and the distribution platform. Mobile applications often stand in contrast to desktop applications which are designed to run on desktop computers, and web applications that run in mobile web browsers rather than directly on the mobile device [26].	Uses a high-speed network and helps to track strategic locations, infected patients, and registering the data and modeling of disease outcomes as per the application software and other technologies can also be integrated with the software.
Virtual reality	Virtual reality (VR) is a digital technology that provides a simulated experience that is almost the same or different from the working world. Its applications include video games, 3D games, educational training, medical training, military training, etc. The environment provided by this technology presents the benefits of great comfort, creativity, and productivity. People can work together in real-time through intuitive whiteboards, the simulations can be visited, and the content can be recorded [1, 2].	In the times of the COVID-19 outbreak, the technology of virtual reality offers a great option for video calls. The most significant benefit of this medium is its ability to make people feel like they are together in the same space without the need for traveling. The extra benefit is that people can entirely focus on the task at hand without any distractions at all. VR improves efficiency, upgrades the working in a group, reduces travel costs, reduces absenteeism, and lowers the impact of the environment. So, in this time of COVID-19 disease, VR has been an excellent tool for communication and collaboration [1, 2].
Holography	Holography is 3D photography. It presents 3D views with changing perspectives. It is a contrast to photography; it records both the phase and the complex amplitude of the wave which comes from the object. The record is called the hologram. It is like a window that has memory. The hologram can reconstruct an accurate 3D image of the original object. It provides corporations with an alternative to virtualize their events without the need for webcasting. With the use of this, the businesses can launch products, add new clients, and build their brands [1,2].	The digital technology of holography has paved a new way to conduct conferences and live events. It ensures the reduced exposure of the speakers, employees, and clients to COVID-19. It feels like speakers are living from their homes or offices virtually on a real event stage regarding COVID-19. Thousands of people can attend this live streaming at the same time. Holography has now the ability to offer ultra-realism. In this time of COVID 19 outbreak, when the workers are bound to stay at home, this technology of streaming holographic events is becoming readily acceptable [1, 2].

Technology	Description	Applications
Cloud computing	Cloud computing is a digital technology that involves the delivery of computer system resources over the internet such as servers, storage, databases, networking, intelligence, etc. This technology provides faster innovation and resources which are flexible. It results in reduced operating cost and increased efficiency of running the infrastructure [1, 2].	In the present times of social isolation amid the COVID-19 outbreak, people have been able to continue their digital lives with the help of applications like Zoom video, Slack, Netflix through services such as Amazon Web Services, Microsoft Azure, and Google Cloud. Cloud computing can be helpful to fight against COVID-19 in several ways. For instance, introduced especially designed Salesforce Care solution for healthcare providers who receive a large number of requests due to COVID-19 [1, 2]. All necessary information is stored at a computing platform and made available, to enables an enormous amount of computing power to the users with the help of the internet and helps in making real-time decisions in disease modeling. The software can be employed with blockchain and other tools to model requirements of critical facilities at a different level, from the hospital to the nation Use official epidemiological data and predict the possible outcomes of the COVID-19 pandemic using based RNNs on (Recurrent Neural Networks).
Autonomous robot	An autonomous robot is used to carry out the tasks without the influence of any external agency. It can be employed to collect information about the environment. It can be used for a very long period without help. It is considered as a sub technology of robotics and artificial intelligence. It can ignore the situations which may be dangerous for human beings [1, 2].	During the present times of lockdown in the COVID-19 pandemic, an autonomous police robot can be deployed for patrolling the areas to confirm that the people are following the orders of lockdown. The autonomous police robots may also be deployed in the hospitals in order to help the medical staff to perform their duties without any disruption. It can be very helpful to enhance the performance of the medical staff and in turn, to contain the spread of the COVID-19 [1, 2].
3D Scanning	3D scanning is used to convert the physical part into CAD digital data. This technology is successful for the reverse engineering processes. In medicine, this technology is used for scanning the human body and its part as per precise dimension. 3D scanning output is used for the analysis of real-world objects for collecting data about its shape and appearance. The 3D model can then be constructed with the use of collected data. This data can be used for a large number of applications. 3D scanners are also useful in developing video games and movies [1, 2].	3D scanning is a non-contact technique that helps the thoracic chest scanning for COVID-19. Also, a useful tool to detect and quantify COVID 19 virus. Virtual reality, motion capture, robotic mapping, and industrial design are some of the other applications of this technology [1, 2]. Undertakes manufacture of personalized devices for healthcare workers and patients, using 3D printing technology for the COVID-19, whenever required.
3D Printing	3D printing is already emerging in the medical field for the manufacturing of customized part from the input of CAD digital file. This can quickly revise the previous version of the product in lesser time and cost. It helps in the design and development of ventilator parts as per the required shortage. Thus, fulfills the need for the global supply chain by manufacturing required precaution parts [1, 2].	3D printing technology can be used in some critical applications to contain the spread of COVID-19 disease. A face mask to be produced with the use of this technology is already under development. This face mask can be employed to test a large number of persons for COVID-19 in 30 min. The use of surgical masks and N95 respirators is not suitable for the environment, and it can prove to be detrimental to the ecosystem. On the other side, it is claimed that the newly developed NanoHack 3D-printed mask is recyclable and can be reused [1, 2].

Technology	Description	Applications
Biosensor	Biosensors are used for the conversion of the biological signal into an electrical signal. Some of the essential types of biosensors are optical, thermal, piezoelectric, and electrochemical biosensors. They find applications in a wide variety of fields such as medical science, the food industry, the marine sector, etc. They are stable and sensitive. In the case of biological wars, the biosensors can be employed for the support of the military. This technology of biosensor, which is entirely new to the market used effectively as a wireless device in an environment of the multi-patient hospital [1, 2].	In the present time of pandemic COVID-19, the biosensors are capable of providing devices that can be easy to employ, sensitive, cost-saving and can provide high accuracy. A glucose monitor is a perfect example of the biosensor which is used in clinical analysis and diagnosis of the disease. A single-use wireless biosensor patch 1AX is under development. This biosensor patch can be employed for the early detection and then the monitoring of the symptoms of COVD-19. The real-time recording of the temperature, ECG trace, respiration rate, etc. will be performed by this patch [1, 2]. - Lab on a chip (microfluidics) is promised technology for diagnostic COVID-19 [27].
Nanotechnology	Nanotechnology is a multidisciplinary field that makes use of nano-sized particles and devices for various applications, including diagnostics, targeted drug delivery, and the production of new therapeutic materials. Nanoparticles such as gold and silver have been used in biomedical and diagnostic applications, for the detection of viral particles for instance. Nanotechnology has been shown to help in treating viral infection by means of various mechanisms. Nanoparticles can act as antiviral drug delivery systems; they can interact and bind to a virus and thereby prevent it from attaching and entering the host cell; they can be designed to exhibit antiviral effects. Altogether, the use of nanotechnology in the development of new medicines has been recognized as a key enabling technology, capable of providing new and innovative medical solutions to address unmet medical needs [16].	Nanomedicine has already been used in drug delivery. In the case of an RNA-based vaccine, which consists of messenger RNA (ribonucleic acid) strands, lipid nanoparticles have been used to pack the RNA molecule and deliver it within the body. While no RNA vaccine has ever been licensed, a US-based biotechnology company specializing in messenger RNA therapeutics recently announced that its mRNA-based vaccine candidate (mRNA-1273) for the novel coronavirus disease (COVID-19) had just entered Phase 1 study. Novavax, meanwhile, also recently initiated the development of a vaccine candidate for COVID-19, using its proprietary recombinant nanoparticle vaccine technology [16].
Virtual screening	New programs with *in silico* methods.	Identification of novel drug candidates and repurposing of known drugs [28].

help people perform daily life work during the lockdown [31, 32] and bring benefits for mitigating effects of COVID-19 pandemic such as:

- Planning of activities regarding COVID-19;
- Providing a better experience without imposing the risks to healthcare and other workers;
- Manufacturing of items for precautionary measures related to SARS-CoV-2;
- In-time provisioning of medical items using smart supply chain;
- Using robotic based treatment for infected patients aiming at reducing risks and increasing workplace safety for health professionals;
- Using virtual and augmented reality for training purpose;
- Promoting a flexible working environment for treatment.
- Detecting of images from computerized tomography (CT) lung scans and faster RT-PCR correlation;
- Monitoring, in real-time, changes in body temperature through the use of wearable sensors;
- Providing an open-source data platform to track the spread of the disease;
- Predicting the number of potential new cases by area and which types of populations will be most at risk, as well as to evaluate and optimize strategies for controlling the spread of the epidemic;
- Detecting fake news about COVID-19 using applying machine-learning techniques, following words that are excessive or alarming, and recognize which online sources are considered eligible to believing or not.

Ting and colleagues [33] described the potential application of inter-related digital technologies for tackling COVID-19 (Table 2) by monitoring, surveillance, detection and prevention of the disease through IoT, BDA, AI, and deep learning; and mitigation of the impact on the healthcare sector indirectly related to COVID-19 through telemedicine and education.

They concluded that the successful use of digital technology to mitigate the major public health challenge in 2020 will probably positively influence the public and government of such technologies for other areas in the future. As well, according to Brohi and colleagues [34], Artificial Intelligence (AI), 3D Printing Technology (3DPT), Big Data Analytics (BDA), High-Performance Computing (HPC), and Telecommunication Technology (TT) are the five state-of-the-art technologies that could assist the scientists and researchers in mitigating and eliminating COVID-19 (Figure 1).

Artificial Intelligence (AI)

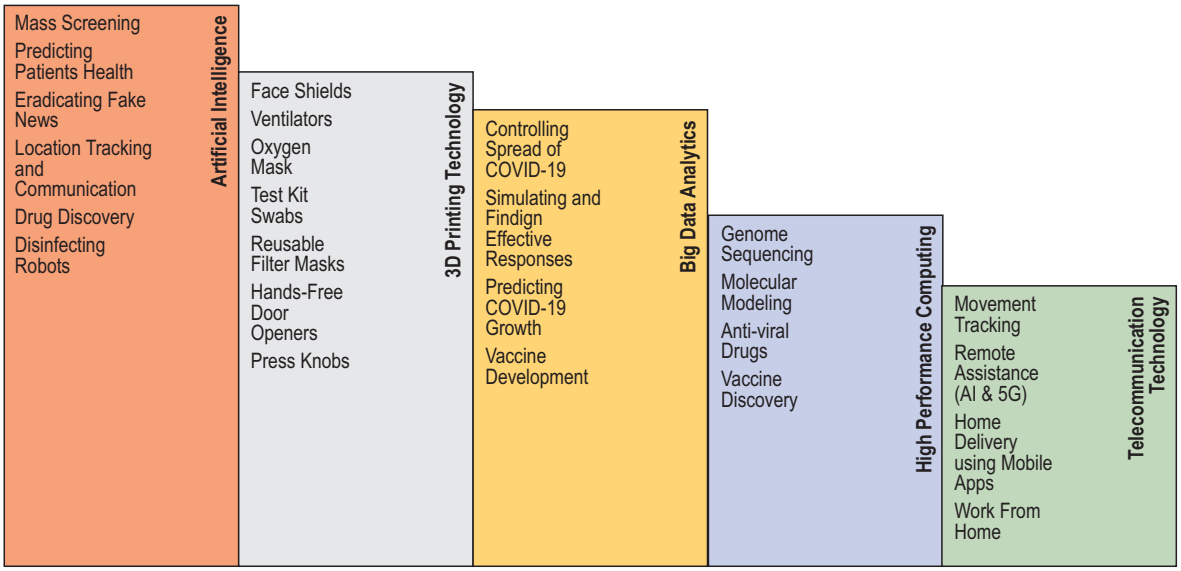
Different studies describing the use of Artificial Intelligences against COVID-19 and the issues in that regard are presented below, as well as state-of-the-art studies on AI techniques (Table 3). According to Brohi and colleagues [34], AI applications that rely on deep machine learning and neural network models are being used as a tool to help with prediction and detection of COVID-19, such as detecting COVID-19 through chest scans with remote monitoring and controlling, predicting COVID-19 threats, combating fake news, handling AI-based Apps with geolocation to follow individuals and inform communities via real-time interaction on limitations, advice, and guidelines to avoid COVID-19 hotspots, as well as in using drug discovery platforms to recognize molecules with possible effects against SARS-CoV-2, developing robots as support systems to disinfect hospitals, restaurants, public transport hubs, and possible COVID-19 locations without human intercommunication with infectious objects. Vaishya and colleagues [50] also described the quick analysis of irregular symptoms and alert patients and healthcare authorities that AI can do. Ai and colleagues [51] similarly reported that AI can give updated information in real-time that is helpful in the prevention of COVID-19, since

Table 2. Digital technologies and their impact on public-health strategies.

Digital Technologies				
Public-health measures	**IoT**	**Big Data**	**AI**	**Blockchain**
Monitoring, surveillance, detection and prevention of COVID-19 (directly related to COVID-19)	+++	+++	++	+
Examples	Real-time tracking and live updates in various online databases in the USA, UK and China	Modeling of disease activity, potential growth and areas of spread	Detection of COVID-19 from chest imaging (x-Ray) (Beijing Hospital)	Manufacturing and distribution of COVID-19 vaccines once they are available
	Live tracking of the at-risk vicinity in Korea (Coronamap.live; Wuhanvirus.kr)	Modeling of the preparedness and vulnerability of countries in fighting a disease outbreak	Prognostication of disease progression via clinical data, imaging and AI	Insurance claims from COVID-related illness and death
Mitigation of impact (indirectly related to COVID-19)	+++	++	+++	++
Examples	Virtual clinics (PingAn, China)	Business modeling on pharmaceutical supplies for various medications	AI to automatically diagnose medical conditions unrelated to COVID-19 (Zhongshan Ophthalmic Eye Center, China)	Distribution of patients' regular medication to the local pharmacy or patients' doorstep
	Public information dissemination via WhatsApp in Singaporea	Modeling of the utility of operating theaters and clinics with manpower projections	Medical 'chat bots' to address public inquiries on COVID-19	-

+++: high; ++: regular; +: low.
Credit/Source: Ting and colleagues [33].

Figure 1. Key applications of state-of-the-art technologies to mitigate and eliminate COVID-19.



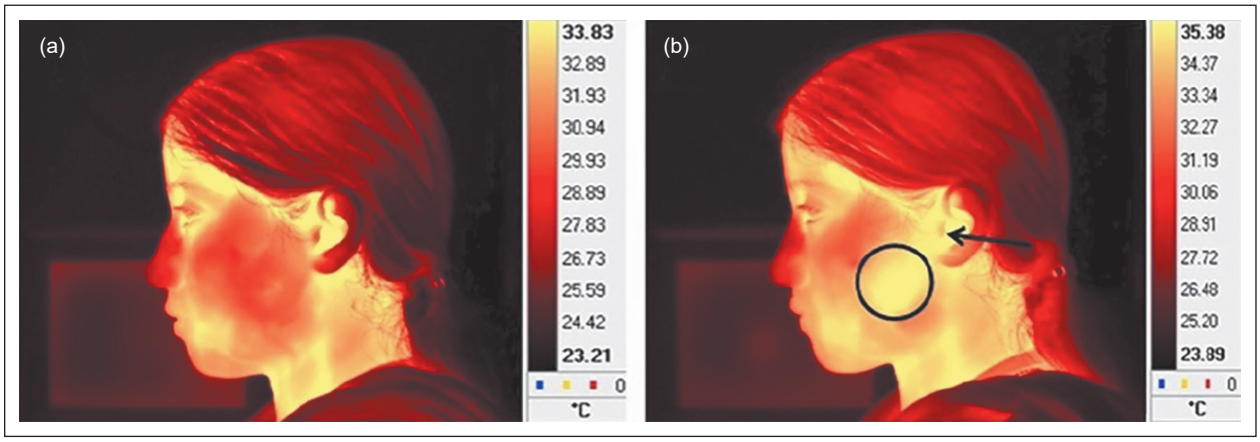
Credit/Source: Brohi and colleagues [34].

Table 3. State-of-the-art studies on AI applications for COVID-19.

Subject	Contributions	References
Detection and diagnosis	A CNN-based DeTraC framework is proposed. In particular, the transfer learning concept is used to utilize wellperformed deep models. For the pre-trained ResNet18 model, the DeTraC method achieves competitive performance, accuracy of 95.12%, sensitivity of 97.91%, and specificity of 91.87%.	[35]
	A deep CNN model for classification of COVID-19 and the dataset is designed by collecting 13, 975 chest X-ray images across 13, 870 patients. The proposed CNN model can achieve the test accuracy of 93.3%.	[36]
	Using chest CT images, 63 quantitative features of COVID-19 are analyzed by an RF model. The proposed method can obtain promising results, e.g., accuracy of 0.875 and AUC score of 0.91.	[37]
	The AI4COVID-19 framework is proposed to consider domain knowledge of medical experts. The input data is cough/sound signals, which may be recorded by smartphones. The performance is very promising, the classification accuracy of 97.91% (93.56%) is obtained for cough (COVID-19) detection.	[38]
Identifying, tracking, prevention and control, and predicting the outbreak	A time-dependent SIR model is proposed to dynamically adjust the control parameters according to the outbreak policies. The model is also extended to consider undetectable infected cases.	[39]
	A modified autoencoder framework is investigated to model the transmission dynamics of COVID-19. Using the empirical data from the WHO, the model can achieve an average error of less than 2.5%. An interesting observation is that a faster intervention can significantly reduce the numbers of infected and dead cases.	[40]
	Infrared thermography was also recommended as an early detection strategy for infected people, especially in crowns like passengers on an airport (Figure 2).	[41, 42]
Infodemiology and infoveillance	Data is collected from Sina Weibo, Baidu search engine, and Ali e-commerce 29 marketplace to evaluate public concerns/behaviors and risk perception to the COVID-19 outbreak. The result shows that fast reaction to quickly classify rumors and misinformation can well mitigate impacts of irrational behaviors.	[43]
	Applications of computer visions for combating the COVID-19 pandemic are presented. Potential use cases (e.g., risk assessment and diagnosis) and challenges (e.g., data collection and model sharing) are also discussed.	[44]
	An AI-driven system, namely α-satellite, is proposed to estimate the risk of COVID-19 in an hierarchical manner. Data is collected from heterogeneous sources, e.g., WHO, demographic and mobility data, and social platforms.	[45]
Biomedicine and pharmacotherapy	A pre-trained deep learning model is utilized to train a dataset of 4, 895 commercially available drugs. After learning and manual refinement, 10 drugs are selected as potential COVID-19 inhibitors.	[46]
	For drug repurposing, a data-driven approach is examined over 6, 000 candidate drugs. The key finding is that the inhibitor CVL218 is very promising and has a safety profile in monkeys and rats.	[47]
	A deep generative model, namely CogMol, is proposed to find potential molecules that can blind three relevant protein structures of coronavirus. Also, in silico screening experiments are conducted to assess the toxicity of the generated molecules.	[48]

Credit/Source: Pham and colleagues [49].

Figure 2. Temperature screening in process using thermal images of a subject talking on a hand-held mobile phone; (a) after 1 min and (b) after 15 min of talking. After 15 min of talking the temperature of the encircled region increased from 30.56 to 35.15 C, whereas the temperature of the ear region (indicated by an arrow) increased from 33.35 to 34.82C. Similar system can be used for fever screening.



Credit/Source: Ulhaq and colleagues [11].

it can predict the probable sites of infection, the necessity for beds, and healthcare professionals during this emergency. It recognizes features, causes, and reasons for the spread of infection helping the process of early detection, diagnosis, and decision-making. In the near future, AI will perform an essential role in affording more predictive and preventive healthcare [51, 53-54]. Moreover, AI aids in developing vaccines and treatments faster than usual and is also helpful for clinical trials [55-60]. It also reduces the workload of healthcare workers [61, 62] and adopts digital approaches with decision science [51, 63, 64].

McCall [65] pointed out some companies that it is using AI for predicting the news of the outbreak (Blue Dot, a Canadian company); and potential drugs (BenevolentAI and Imperial College London); designing new molecules that can halt viral replication (Insilico Medicine); and use imaging methods (Infervision's AI).

Unfortunately, patient-level COVID-19 data is not publicly available. However, Cosgriff and colleagues [66] described the Medical Information Mart for Intensive Care (MIMIC), which has been a model of publicly-available, anonymized electronic health record data sharing since 1996 that represents the most studied critical care cohort in the world, allowing clinicians and computer scientists to address research questions and build predictive models [67]. Sun and colleagues [3] described the online platform that provides real-time coverage of the COVID-19 outbreak in China, obtained by using crowdsourced reports from DXY.cn [68], a social network for Chinese physicians, health-care professionals, pharmacies, and health-care facilities established in 2000, by the reports linked to an online source, concluded that the news reports and social media can "help reconstruct the progression of an outbreak and provide detailed patient-level data in the context of a health emergency".

In a review study, Alimadadi and colleagues [5] report the large-scale data of COVID-19 patients that can be combined and analyzed by advanced machine learning algorithms to a better comprehension of the pattern of viral spread, further enhance diagnostic agility and precision, develop new efficient therapeutic procedures, and potentially recognize the most susceptible people based on personalized genetic and physiological properties. They presented some examples that represent a good model of the application of AI: the Allen Institute for AI in partnership with leading

research groups issued an opensource, weekly updated COVID-19 Open Research Dataset [69], which continuously documents COVID-19-related scholarly articles to accelerate novel research projects urgently requiring real-time data; the survival prediction of severe COVID-19 patients [47, 61]; the discovering potential drug candidates against COVID-19 [47, 61]; biochemistry (e.g., ACE2 expression level) and clinical data (e.g., age, respiratory pattern, viral load, and survival) of COVID-19 patients with underlying medical conditions can be analyzed by machine learning approaches to not only identify any reliable features (e.g., ACE2) for risk prediction but also further perform risk classification and prediction for a balanced preparation of ongoing disease treatment and COVID-19 defense.

However, as well as other studies, Alimadadi and colleagues [5], see a current hurdle in the availability of COVID-19-related clinical data, which would need to be managed and processed into easily accessible databases. The creation of integrating COVID-19-related clinical data such as the UK Biobank, with pre-existing data of patients, could potentialize the efforts towards a faster and feasible approach for meaningful data-mining by bioinformaticians and computational scientists. A centralized compilation of global COVID-19 patient data will be helpful for future artificial intelligence and machine learning researches to promote predictive, diagnostic, and therapeutic approaches against COVID-19 and alike pandemics in the future.

Blasiak and colleagues [70] described the use of IDentif.AI, a platform that quickly optimizes infectious disease (ID) combination therapy design applying artificial intelligence (AI). The platform IDentif.AI was realized on a 12-drug candidate therapy search set representing over 530,000 possible drug combinations. The combination therapy against SARS-CoV-2 was composed of remdesivir, ritonavir, and lopinavir, which the results proposed that the combination was a 6.5-fold improvement in efficacy than remdesivir alone. Also, IDentif.AI showed hydroxychloroquine and azithromycin to be relatively inefficient. The platform analysis was also capable to confirm clinical trial results to date without requiring any data from these trials. So, the IDentif.AI platform suggests that it may apply to the speedy development of optimal drugs for this current pandemic and future outbreaks. Ramdas and colleagues [15] presented a virtual platform for remote shared care delivery that has the potential to enhance provider capacity while mitigating transmission risks and enabling privacy in COVID-19 pandemic: identity can be withheld, voices disguised, and patient video made visible only to the clinician. This technology such as shared medical appointments (SMAs) has been used since 1999 in the US and could be very important in the pandemic especially to mitigate the transmission of COVID-19.

Thorlund and colleagues [71] developed a network of COVID-19 interventional clinical trials (completed, ongoing, and planned) by the data from the International Clinical Trials Registry Platform, including those from the Chinese Clinical Trial Registry, ClinicalTrials.gov, Clinical Research Information Service - Republic of Korea, EU Clinical Trials Register, ISRCTN, Iranian Registry of Clinical Trials, Japan Primary Registries Network, and German Clinical Trials Register. They also developed an artificial intelligence (AI)-based method for data searches to identify potential clinical studies not captured in trial registries, and used a content aggregator service, such as LitCovid, to ensure their data acquisition strategy was complete. Trials for COVID-19 were then "mapped according to geographical, trial, patient, and intervention characteristics when these data are available. Syntheses of these trials are urgently needed to assist clinicians, researchers, and policymakers to make evidence-informed decisions to minimize the morbidity and mortality due to COVID-19".

About chest images, COVID-19, and pneumonia of different natures share similar CT characteristics, which contributes to the challenges in distinguishing between them with accuracy.

Bai and colleagues [72] used AI to compare if AI assistance improved radiologists' performance in identifying COVID-19 and non-COVID-19 pneumonia on chest CT. Their AI model achieved a test accuracy of 96% (95% CI: 90-98%), sensitivity 95% (95% CI: 83-100%), and specificity of 96% (95% CI: 88-99%) with Receiver Operating Characteristic (ROC) AUC of 0.95 and Precision-Recall (PR) AUC of 0.90, concluding that the AI aided the radiologists to distinguish the chest CT with COVID-19 pneumonia from non-COVID-19 pneumonia (Figures 3).

Singh and colleagues [73] classified COVID-19-infected patients from chest CT images using multi-objective differential evolution (MODE), a novel deep learning model, and convolutional neural networks (CNN) for classification of human beings based upon whether they are affected by COVID-19 or not. A multiobjective fitness function is designed to classify COVID-19-infected patients by considering sensitivity and specificity. For that, they compared their model with other studies (Table 4).

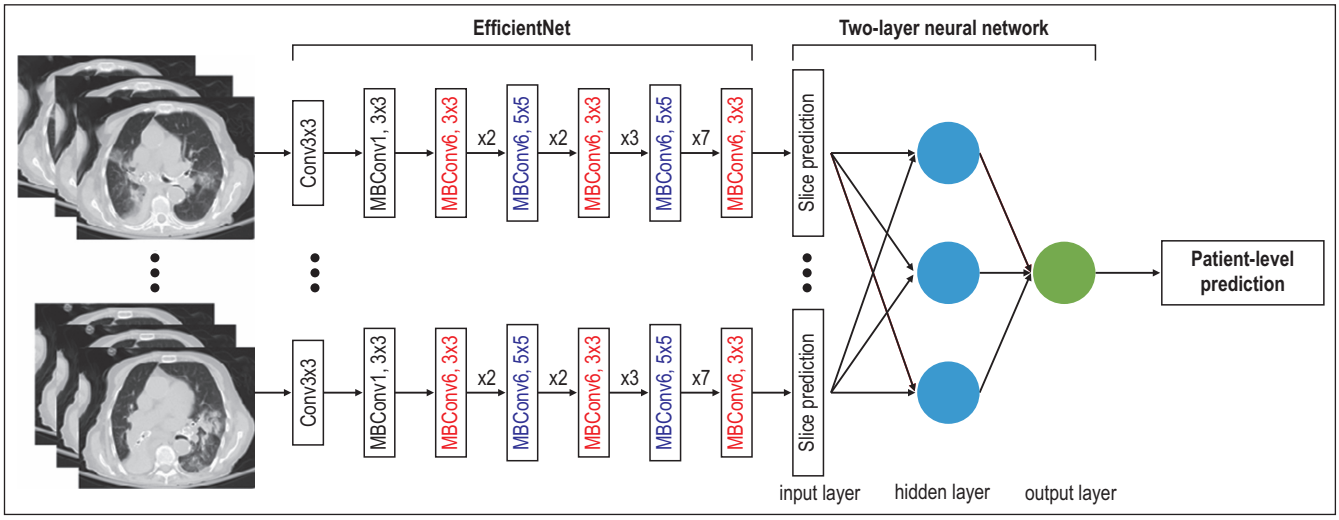
From an extensive review, it has been found that the chest CT images can be helpful in the early classification of COVID-19-infected patients. They did extensive experiments that reveal the proposed model "outperforms competitive models, i.e., adaptive neuro-fuzzy inference systems (ANFIS), artificial neural networks (ANN), and convolutional neural networks (CNN) CNN models in terms of accuracy, F-measure, sensitivity, specificity, and Kappa statistics by 1.9789%, 2.0928%, 1.8262%, 1.6827%, and 1.9276%, respectively". Therefore, the proposed model is useful for real-time COVID-19 disease classification from chest CT images, according to Singh and colleagues [73].

About chest CTs, Laghi [83] disagrees with some of the optimistic expectations about the diagnostic value of a particular algorithm applied to lung CT images as outlined by McCall because this is not yet supported by scientific evidence. According to Laghi, the little evidence that has been reported shows that approximately 50% of patients with COVID-19 infection have a normal CT scan if scanned early after the onset of symptoms [84]. This evidence is the main reason why the American College of Radiology does not consider CT imaging as a useful screening test in asymptomatic individuals [85]. On the other hand, he deeply believes that AI can and should be used to support the work of a radiologist and that "the objective quantification of the disease, expressed as a percentage of the pulmonary parenchyma involved, is currently the most interesting application of AI in COVID-19 infection, which allows monitoring the course of the disease" [86].

There are many studies using chest CT and AI. Although chest CT is an effective imaging technique for lung-related disease diagnosis, chest x-Ray is more widely available, has a lower cost, and lower ionizing radiation when compared to CT (Table 5). So, deep learning, one of the most successful AI techniques, is an effective means to assist radiologists to analyze the vast amount of chest x-Ray images, which can be critical for efficient and reliable COVID-19 screening (Figure 4). Zhang and colleagues' study [87] developed a new deep anomaly detection model for fast, reliable screening of 100 chest x-Ray images of 70 patients confirmed with COVID-19 and 1,431 additional chest X-ray images with other pneumonia (Figures 5 and 6).

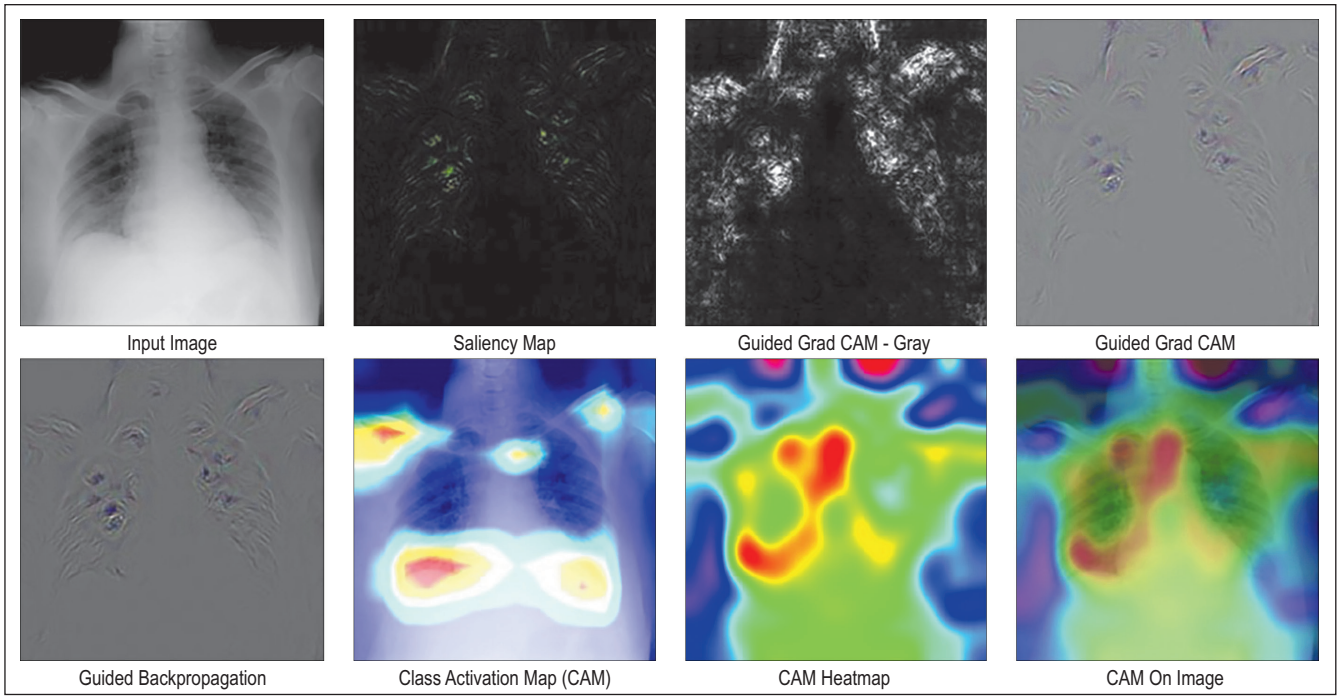
The initial experimental results show 96% sensitivity and 70.65% specificity. Shi and colleagues [88] obtained a sensitivity of 90.70% and specificity of 83.30% on a large-scale CT dataset, including 1,658 patients with COVID-19 and 1,027 with non-COVID-19 pneumonia. In this study, the model obtained the sensitivity of 90.00%, specificity of 87.84% (when T = 0:25) or the sensitivity of 96.00%, specificity of 70.65% (when T = 0:15) on the x-Ray dataset that contained 100 images from 70 COVID-19 subjects and 1,431 images from 1,008 non-COVID-19 pneumonia subjects. When compared to the CT-based screening method, Zhang and colleagues' x-Ray-based model performed relative performance. More importantly, the model only learns from 70 COVID-19 patients, which is less than 5 percent

Figure 3. COVID-19 classification neural network model.



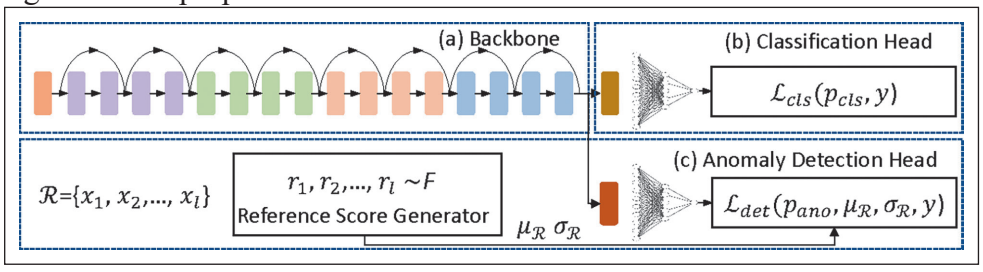
Credit/Source: Bai and colleagues [72].

Figure 4. Visualizations shown by using different saliency maps that provide additional insights diagnosis. Adapted from Ghoshal and Tucker [93].



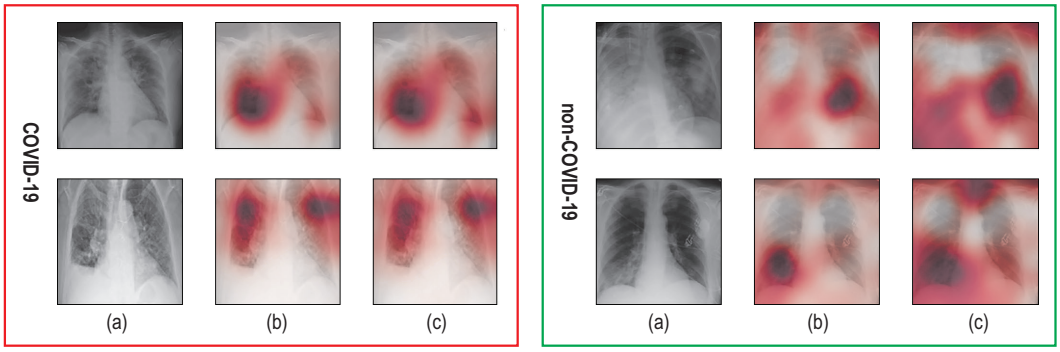
Credit/Source: Ulhaq and colleagues [11].

Figure 5. Diagram of the proposed model.



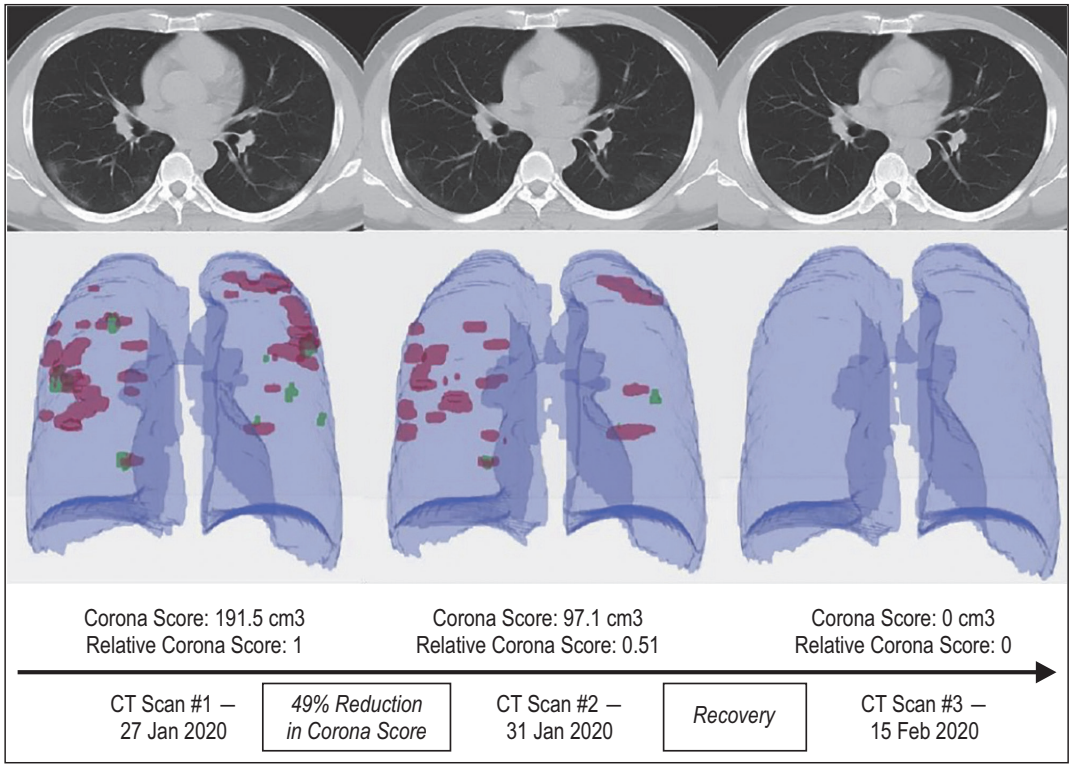
Credit/Source: Zhang and colleagues [87].

Figure 6. Visualization of the patients' chest X-Ray images (a) and the corresponding Grad-CAMs obtained by this model; (b) is the Grad-CAMs obtained by the classification head and (c) in the Grad-CAMs obtained by the anomaly detection head.



Credit/Source: Zhang and colleagues [87].

Figure 7. Corona score that is calculated by measurements of infected areas and severity of disease from CT images. It can be used for identifying patients that are critically ill so that they get immediate medical attention. Image adapted from Gozes and colleagues [61].



Credit/Source: Ulhaq and colleagues [11].

of Shi's study [88]. Hence, the proposed model that utilizes chest x-Rays can be recognized as an effective computer-aided diagnosis (CAD) tool for low-cost and fast COVID-19 screening [87].

According to Pham and colleagues [49], the AI is applicable in this pandemic in the detection and diagnosis, identification, prediction, as well as pharmacotherapy, among other areas of healthcare. They observed that the AI-based framework is highly suitable for mitigating the impact and effect of the COVID-19 pandemic as an amount of credible COVID-19 data is becoming available.

Table 4. Studies of chest CT and AI (Figure 7).

Studies	Results
Li and colleagues [74]	The authors developed a deep learning model (COVNet) to extract visual features from chest CT for detection of COVID-19. They used visual features to distinguish between community acquired pneumonia and other nonpneumonia lung diseases. However, COVNet is unable to categorize the severity of this disease.
Gozes and colleagues [61]	The authors developed an artificial intelligence-based CT analysis tool for detecting and quantification of COVID-19. The system extracted slice of opacities in the lungs automatically. The developed system achieved 98.2% sensitivity and 92.2% specificity. The output of system provides quantitative opacity measure and 3D volume display for opacities. The system is robust against pixel spacing and slice thickness.
Shan and colleagues [75]	The authors developed a deep learning-based system named VB-net for automatic segmentation of all the lung and infection sites using chest CT.
Xu and colleagues [76]	The authors developed a prediction model to discriminate COVID-19 pneumonia and influenza-A viral pneumonia using deep learning techniques. The CNN model was used for prediction. The maximum accuracy obtained from prediction model was 86.7%.
Wang and colleagues [9]	The authors investigated the radiographic changes in CT images of infected patients. They developed a deep learning-based prediction model that utilizes the modified inception transfer learning technique. The features are extracted from CT images for prior diagnosis. The accuracy of 89.5% obtained from this method is better than Xu's model [76] and saved time for diagnosis.
Narin and colleagues [77]	The authors proposed an automatic deep convolution neural network– based transfer models for prediction of COVID-19 in chest X-ray images. They used InceptionV3, Inception-ResNetV2, and ResNet50 models for better prediction. The ResNet50 pre-trained model produced accuracy of 98%, which is higher than Xu and Wang studies [9, 76].
Sethy and colleagues [78]	The authors developed a deep learning model for detecting COVID-19 from X-ray images. They extracted deep features and transferred them to support vector machine for classification. The accuracy of 95.38% obtained from the proposed model, which is better than Xu and Wang studies [9, 76].
Chen and colleagues [79]	The authors found sensitivity of 100%, specificity of 93.55%, accuracy of 95.24%, from the platform UNet++ to extract valid areas in CT images (46,096 CT images) using 289 randomly selected CT images.
Song and colleagues [80]	The authors used neural networkDRENet + ResNet50 [81], with Feature Pyramid Network (FPN)+ Attention module, with 777 CT images and results of AUC of 0.99 and recall (sensitivity) of 0.93. Accuracy of 0.86 and F-Score 0.87.
Jin and colleagues [81]	The authors used 2D CNN based AI system, model name is not specified with 970 CT with accuracy of 94.98%, an area under the receiver operating characteristic curve (AUC) of 97.91%.
Zheng and colleagues [82]	The authors used Zheng [65] 3D deep convolutional neural Network to Detect COVID-19 (DeCoVNet) from CT volumes, obtained d 0.959 ROC AUC and 0.976 PR AUC.

So, AI studies are not executed at a large scale, but they are still helpful as they can provide rapid response and meaningful information to medical staff and policymakers. However, it is still a big challenge to design AI algorithms with the current quality and quantity of COVID-19 datasets. These issues should be resolved with time, but not without constant effort from the research communities and the help from official organizations with more reliable and high-quality data.

In order to create an algorithm or a platform for AI, it is crucial to have a deep understanding of the disease (pathogenesis, genetics, the behavior of the disease, transmissibility, risk groups) and to have collected credible data. AIs need high-quality input data in order to have good results in a pandemic. It is important to point out that, regarding imaging methods (CT and x-Ray), AI is a powerful tool for radiologists, but still, it is not completely reliable by itself.

Table 5. Representative work for X-Ray based COVID-19 diagnosis.

Study	Results
Guszt´av Ga´al and colleagues [89]	The authors used 247 images with the model Attention U-Net+ adversarial+ Contrast Limited Adaptive Histogram Equalization (CLAHE) [90] with a performance of DSC of 97.5% on the JSRT dataset.
Abbas and colleagues [35]	The authors used CNN features of pre-trained models on ImageNetand ResNet+ Decompose, Transfer, and Compose (DeTraC), for the classification of COVID-19 chest X-Ray images from Japanese Society of Radiological Technology (JSRT) + Cohen JP. COVID-19 image data collection with a performance of a high accuracy of 95.12% (with a sensitivity of 97.91%, a specificity of 91.87%, and a precision of 93.36%).
Narin and colleagues [77]	The authors used the model pre-trained ResNet50 with transfer learning with an accuracy of 97% for InceptionV3 and 87% of accuracy for Inception-ResNetV2 (Images from The open source GitHub repository shared by Dr. Joseph Cohen+Chest X-Ray Images (Pneumonia) https: //www.kaggle.com/ paultimothymooney/ chest-xray-pneumonia).
Wang and colleagues [36]	The authors used the model COVID-Net: lightweight residual projection expansion projection-extension (PEPX) design pattern, with 16,756 chest radiography images across 13,645 patient cases from COVIDx dataset, with a performance of 92.4% of accurancy on COVIDx dataset.
Asnaoui and colleagues [91]	The authors used the fined tuned versions of VGG16, VGG19, DenseNet201, Inception-ResNet-V2, Inception-V3, Resnet50, MobileNet-V2 and Xception with 5856 images (4,273 pneumonia and 1,583 normal), with the following performance: Resnet50, MobileNet-V2 and Inception-Resnet-V2 show highly satisfactory performance with accuracy (more than 96%).
Sethy and colleagues [78]	The authors used Deepfeatures from Resnet50 + SVM classification and Data available in the repository of GitHub, Kaggle and Open-i as per their validated X-ray images with a following performance: resnet50 plus SVM achieved accuracy, FPR, F1 score, MCC and Kappa are 95.38%,95.52%, 91.41% and 90.76%, respectively.
Apostolopoulos and colleagues [92]	The authors used various fine-tune dmodels: VGG19, MobileNet, Inception,Inception Resnet V2, Xception (1427 X-Ray images) and the perfomace was: accuracy with Xception was the highest, 95.57, sensitivity of 0.08 and specificity of 99.99.
Ghoshal and Tucker [93]	The authors used dropweights based Bayesian Convolutional Neural Networks (BCNN) (total of 5,941 PA chest radiography images across 4 classes (Normal: 1,583, Bacterial Pneumonia: 2,786, non-COVID-19 Viral Pneumonia: 1,504, and COVID-19: 68) and a performance of 88.39% accuracy with BCNN.
Farooq and Hafeez [94]	The authors used 3-step technique to fine-tune a pre-trained ResNet-50 architecture to improve model performance (COVIDx dataset image) with an accuracy of 96.23% (on all the classes) on the COVIDx dataset.

Big Data (Figure 8)

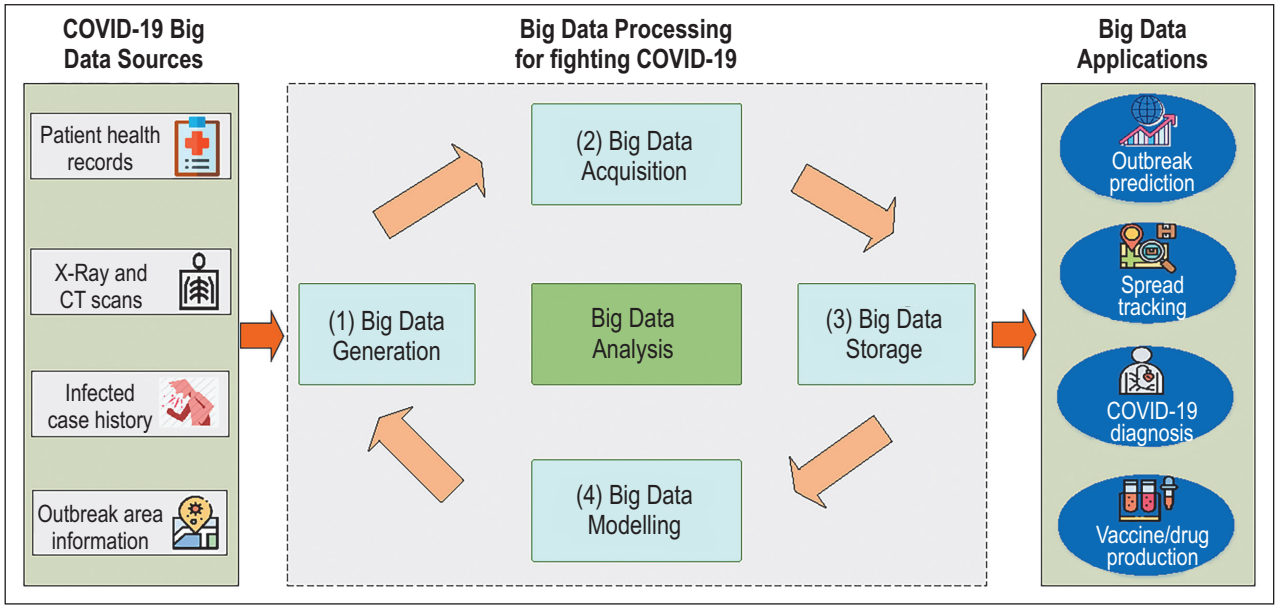
In Pham and colleagues' study, [49], they reviewed the emerging literature about Big Data and found that big data plays an important role in combating the COVID-19 pandemic through many promising applications, including outbreak prediction (Table 6) (Figures 9 and 10), the virus spread tracking, coronavirus diagnosis/treatment, and vaccine/drug discovery (Figure 11). Big data potentially allows outbreak prediction on the global scope using data analytic tools on large datasets collected from available sources such as health organizations (e.g. WHO), and healthcare institutes [95, 96]. Big data has also appeared to be a hopeful solution for coronavirus spread tracking by linking intelligent tools such as ML and DL [97] for developing prediction models, which could be extremely useful for governments in controlling the potential COVID-19 outbreak in the future. Furthermore, big data has the potential to help COVID-19 diagnosis and treatment processes. The investigation results from the literature studies show that big data can improve

many medical procedures from early diagnosis, disease analysis, and prediction of treatment outcomes [98, 99]. Finally, data learning from big datasets also aids to discover potential targets for an effective vaccine against SARS-CoV-2 [100], and combining large-scale knowledge graphs, literature, and transcriptome data, helping to identify potential drug candidates against SARS-CoV-2 [101].

BDA, AI, and TT

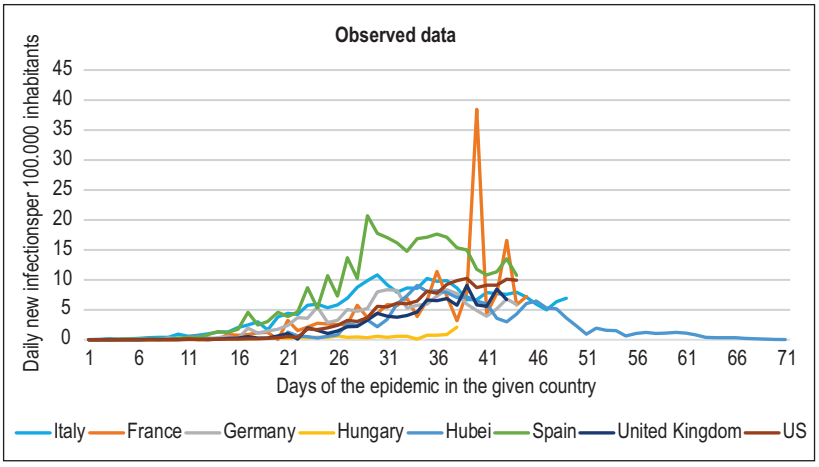
According to Brohi and colleagues [34], the Big Data Analytics (BDA) systems integrated with AI and TT can be used to perceive swift insights and undertake proactive decisions to encounter COVID-19 by extracting and analyzing data from sources such as hospitals, clinics, insurance, immigration, and national databases, travel history, and location-tracking applications. BDA systems are capable to classify individuals and communities in many categories of risk. For example, high-risk patients, such as those that came from a country with a high rate of virus spread, can be tracked down to be quarantined quickly and undergo health screening. Similarly, the authorities can "urge the low and medium risk patients such as those who have recently visited COVID-19 high-risk countries or been in potential contact with COVID-19 positive patient but are asymptomatic, to practice self-isolation". These types of interventions due to insights provided by BDA systems have the potential to reduce the weight on healthcare practitioners and control the spread of disease. Using BDA, authorities can conceive prescription models, simulate and investigate their impacts before implementation in real-life situations. Furthermore, real-time trackers such as the COVID-19 map of Johns Hopkins University, are examining enormous datasets and updating the number of deaths and cases. The information produced by the trackers could be applied to predict the COVID-19 curve and might allow insight for decisions regarding public safety and economic stability. BDA tools are also used by scientists worldwide to produce an anti-viral drug and vaccine in the combat against SARS-CoV-2. Some notable contributions and applications of BDA to mitigate COVID-19 are described in Wang and colleagues [116] and Balilla's studies [117].

Figure 8. Big data and its applications for fighting COVID-19 pandemic.



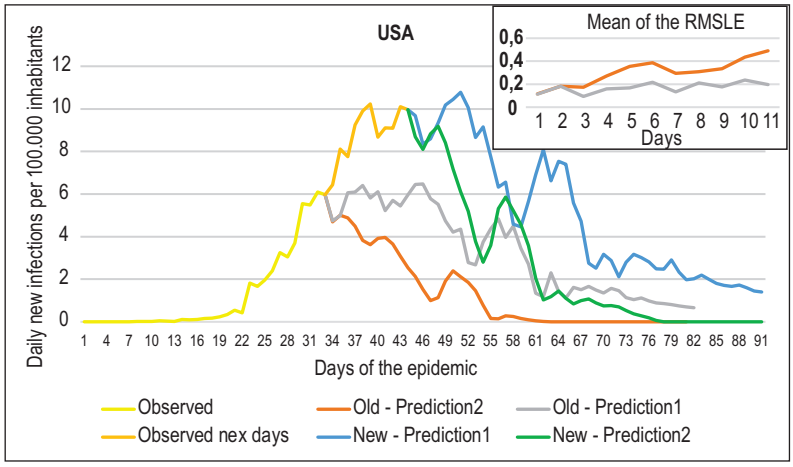
Credit/Source: Pham and colleagues [49].

Figure 9. The historical datasets from different countries (use of AI-based RNN).



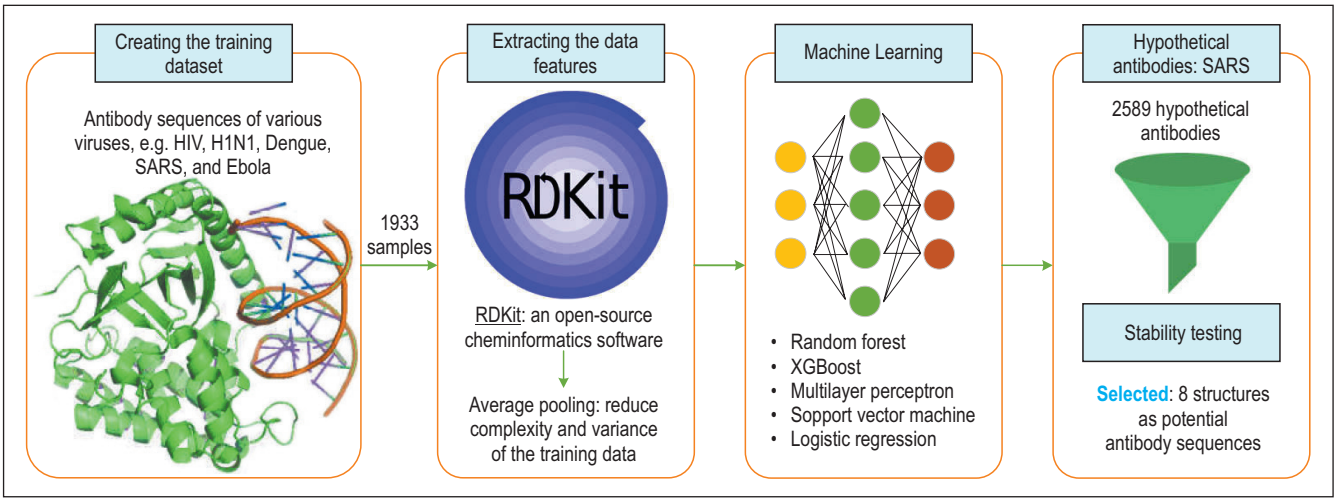
Credit/Source: Kolozsvári and colleagues [114].

Figure 10. Observation and predictions for the United States of America (USA).



Credit/Source: Kolozsvári and colleagues [114].

Figure 11. Illustration of a data-driven framework for discovering antibody sequences to treat the COVID-19 disease [115].



Credit/Source: Pham and colleagues [49].

Table 6. Summary of the state-of-the-art studies on big data applications for COVID-19.

Subjects	Contrbutions	References
Outbreak prediction	A big data platform is proposed to estimate the outbreak possibility using the huge data sets from Italian Civil Protection sources. The first trial is implemented in Wuhan to predict the population infected with COVID-19 for quarantine	[102]
	A big data-based solution is proposed to implement pandemic modeling to interpret the cumulative numbers of infected people, recovered cases in different regions, i.e., Wuhan, Beijing, and Shanghai. This scheme is able to predict the tendency of the COVID-19 outbreak in the areas at high risks of pandemic.	[103]
	A framework is introduced using a large dataset from various regions and countries such as Korea, China, to estimate the pandemic based on a logistic model that can adjudge the reliability of the predictions.	[104]
	A big data analytic method is investigated in the US with the large-scale datasets collected from American cities. The approach enables to calculate prediction errors to optimize the data modeling model for improving stimation accuracy	[105]
Virus spread tracking	A big data-based analytic methodology for tracking the COVID-19 spread is considered using a large dataset collected from China National Health Commission with 854,424 people. The analytic results show a high correlation between the positive infection cases and the population size.	[106]
	A big data-based analytic model is built using datasets collected from China, Singapore, South Korea, and Italy for virus spread tracking. This model can estimate the maximum number of infected patients in a certain área.	[99]
	A temperature-based model is proposed to evaluate the relationship between the number of infected cases and the average temperature in different countries necessary for coronavirus tracking.	[107]
	A big data-based unsupervised model is designed for COVID-19 spread tracking from online data by incorporating a basic news media coverage metric associated with confirmed COVID-19 cases. The work is in progress for coronavirus tracking tasks.	[108]
Coronavirus diagnosis/ treatment	A robust, sensitive, specific and highly quantitative solution based on multiplex polymerase chain reactions is proposed to diagnose the SARS-CoV-2. The proposed scheme has been shown to be an efficient and low-cost method to diagnose Plasmodium falciparum infections.	[109]
	A method is proposed using 6381 proteins in human cells that get infected with COVID-19 virus. This aims to analyze the data gathered from the Kyoto Genes storage to serve COVID-19 diagnosis.	[100]
	An array of clinical tests have been implemented from the big dataset, from Typical and Atypical CT/X-Ray imaging manifestation to hematology examination and detection of pathogens in the respiratory tract. These tests provide a comprehensive guideline with useful tools to serve the diagnosis and treatment of COVID-19.	[110]
Vaccine/drug discovery	A method is proposed to investigate the spike proteins of SARS-CoV, MERS-CoV and SARS-CoV-2 and four other earlier out-breaking human coronavirus strains. It enables critical screening of the spike sequence and structure from SARS-CoV-2 for vaccine development.	[111]
	A project is built using a huge dataset collected from the National Center of Biotechnology Information for facilitating vaccine production. Different peptides were proposed for developing a new vaccine against COVID-19.	[112]
	A solution is proposed based on molecular docking for drug investigations with over 2500 small molecules, which aims prompting drug repositioning against COVID-19.	[113]

Credit/Source: Pham and colleagues [49].

High-Performance Computing

Directing the issues that originate from COVID-19 demanded research contributions in areas such as bioinformatics, epidemiology, and molecular modeling, and these fields need platforms with the large computational capacity to address complex scientific obstacles and process big datasets in short timeframes. To promote the developments, the White House Office of Science and Technology Policy, the U.S. Department of Energy, and IBM developed the COVID-19 HPC Consortium consisting of the federal government, academia, and industry leaders who are offering free compute time and resources on their world-class machines [118]. Some of the consortium partners include IBM, Hewlett Packard Enterprise, Microsoft, Amazon Web Services, AMD, Google Cloud, NVIDIA, Massachusetts Institute of Technology, Rensselaer Polytechnic Institute, University of Illinois, the University of Texas at Austin, Argonne National Laboratory, Lawrence Livermore National Laboratory, San Diego Supercomputer Center and NASA. "Researchers can submit their proposals to the consortium to access small clusters and some of the largest supercomputers in the world. HPC can be utilized in genome sequencing, understanding the accurate biological structure of the virus, and modeling various treatments" (Figures 12-14). AI-driven HPC platforms can be used to discover appropriate anti-viral drugs and vaccines for COVID-19. The papers from Smith and colleagues and UCL 2020 [119, 120] have discussed the use of HPC infrastructures with supercomputers to tackle COVID-19.

Blockchain

Blockchain applications own the potential to control disease outbreaks over time by producing 'ledgers' that are both secure and updated hundreds of times per day. Also, using blockchain can promote diagnostic accuracy and treatment effectiveness, streamline the fast isolation of groups of cases, follow drug supply chains and medical supplies, control medical data, and recognize disease symptom patterns. In cases such as a virus outbreak, "blockchain can reduce uncertainty and offer computational trust, and an automated platform for recording and exchanging consistent factual information between multiple parties", as recently published by the Scientific Foresight Unit (STOA) of the European Parliamentary Research Service [16].

Lab-on-a-Chip

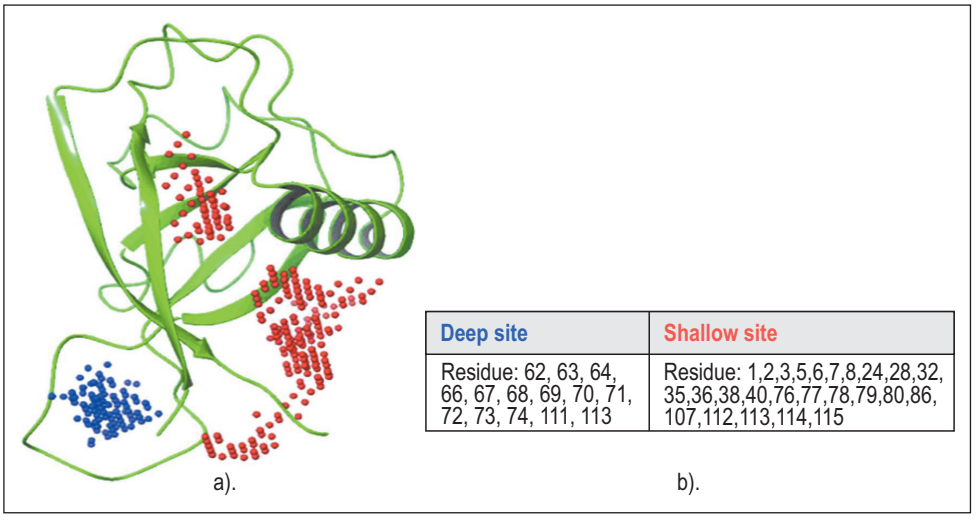
Microfluidic chips, also known as "Lab on a chip", are versatile and promising technology [121-125] that can integrate sample preparation, reactions, and detection on a micron-scale chip [126-129]. This advanced technology has both integrated and miniaturized characteristics, which can integrate a traditional laboratory into a small chip. The microfluidic technology detects viruses efficiently in real-time (reportedly 5 min) and accurately (as low as 1 copy), with few steps and no need for professional skills (Figure 15). This technology is also well-suited in Point of Care Testing (POCT) for viral detection [130]. Zhuang and colleagues [27] summarized the current shortcomings of microfluidics ("Lab on a Chip") in viral detection and proposes ideas for future developments. They presented the performance of microfluidics in virus detection over recent years (Figures 16 and 17).

According to Zhuang and colleagues [27] and Knop and colleagues [131], for Covid-2019 detection, the RT-PCR is the gold-standard method. However, the rapid development of the epidemic requires faster and more efficient tests. So, POC instruments such as microfluidic technology play an important role in diagnosing the virus during this epidemic. In their review [27], they found (among others) the ID NOW® instrument (Abbott™) that can detect positive samples in 5 min and negative results in 13 min, and had its emergency use authorized in the US by U.S. Food and Drug Administration (FDA).

Figure 12. Sequence analysis COVID-19 (Wuhan-Hu-1) Nsp1.

The Figure represents alignment between Wuhan-Hu-1 Nsp1 and SARS Nsp1 protein sequence. Red highlights consensus sequences whereas Blue highlights differences in amino-acid sequence. Important residues shown to play a role in affecting host gene expression and anti-viral signaling are highlighted in green and pink color. Green highlighting similar residues whereas Pink highlighting residues that are different in COVID-19.
Credit/Source: Sharma and colleagues [28].

Figure 13. Model of COVID-19 (Wuhan-Hu-1) Nsp1 with Deep and shallow binding site predicted by SiteMap.



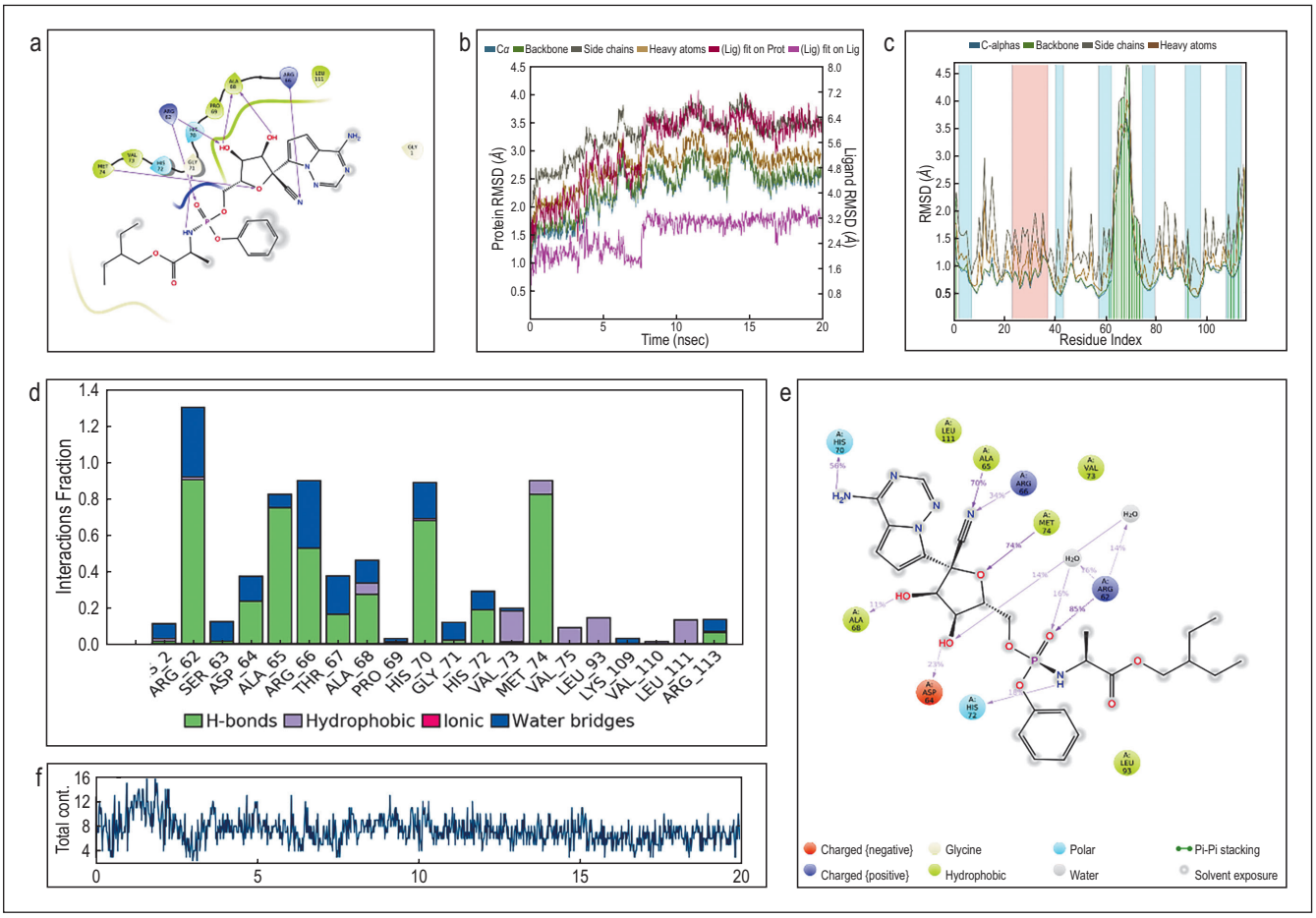
Deep site	Shallow site
Residue: 62, 63, 64, 66, 67, 68, 69, 70, 71, 72, 73, 74, 111, 113	Residue: 1,2,3,5,6,7,8,24,28,32, 35,36,38,40,76,77,78,79,80,86, 107,112,113,114,115

a. Represent COVID-19 Nsp1 model derived using Modeller 9.22, using 2hsx as a template. Red dot represents Shallow binding site consisting region of alpha-helix and beta-sheets. Blue dots represent deep binding site present in mostly loop region. b. Represents residues present in deep and shallow binding site respectively. Residue numbers are as per the structural model (Residue 1 of structure is residue 12 in the sequence)
Credit/Source: Sharma and colleagues [28].

Also Filmarray® (BioFire™), which integrates nucleic acid extraction, purification, and PCR amplification into a single chip, resulting in sequential and accurate detection; GeneXpert® (by Cepheid™; approved by the FDA in the US for COVID-19), which works integrating sample preparation, nucleic acid amplification, and detection into a small detection kit; RTisochip® (CapitalBio™) in China, which can detect 6 common respiratory viruses (influenza) including COVID-19 in a single chip within 1.5 h; And Cannon™, developed in Japan, which that can detect the SARS-CoV-2 in 35 min.

Such advanced technology in POC is bound to change current medical methods. Countries like the USA, China, and Japan, have approved their use, fully demonstrating the application value of the lab on a chip in POC. The authors also point out the challenges that microfluidic chips have yet to overcome when it comes to virus detection, "such as sample preparation integration, quantitative methods, the ability to perform throughput and

Figure 14. Docking and MD simulation results for Nsp1-deep-Remdesivir.



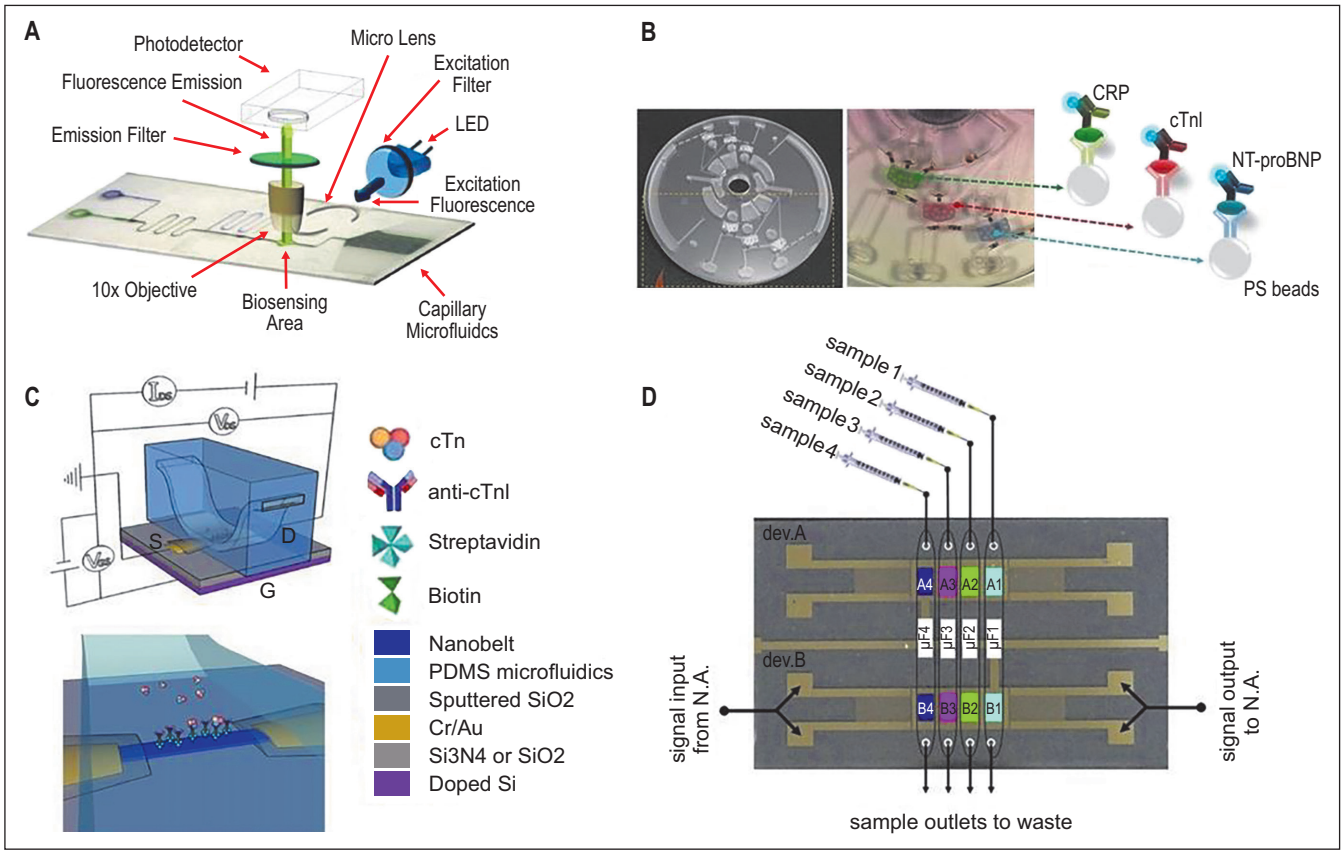
a. Remdesivir -Nsp1interactions after XP docking b. Interaction types and Interacting residues of Nsp1 with Remdesivir over simulation time. Normalized stacked bars indicate the fraction of simultion time for which a particular type of interaction was maintained. Values more than 1.0 suggest that the residue forms multiple interactions of same subtype with ligand (Remdesivir) c. RMSD plot of Nsp1 and Remdesivir. d. Nsp1-RMSF plot e. Interaction of Remdesivir atoms with Nsp1 residues along with types and duration of interactions. Interactions that persist for more than 10% of simulation time have been shown. If a residue forms multiple interaction of same type with the same atom of ligand then that residue can have more than 100% interaction. Total number of contacts (H-bonds, Water bridges, Hydrophobic, Ionic) between Nsp1 and Remdesivir over the course of MD simulation.
Credit/Source: Sharma and colleagues [28].

multiplex during a virus outbreak. The material and design of microfluidic chips, the innovation of detection methods, and the miniaturization of instruments" [27] also need to be improved. If used in association with the Biological mobile phone, Mobile detection station, or Artificial Intelligence, the potential for virus detection is greatly enhanced. In the future, microfluidic products that meet the criteria for POC proposed by WHO (which are: being affordable to those at risk of infection, containing high sensitivity, high specificity, user- friendly capabilities, being rapid and robust, equipment-free, and delivered to those who need it) will be widely available.

3D Printing Technology

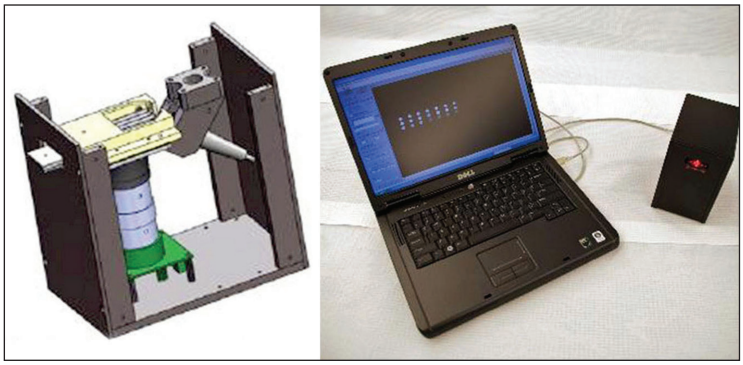
According to Brohi and colleagues [34], the rapidly expanding daily curve of COVID-19 cases has made it a challenge for countries to meet equipment demand. There is a worrying shortage of medical devices and Personal Protective Equipment (PPE)

Figure 15. Examples of LoC-based platforms for CVD biomarker detection.



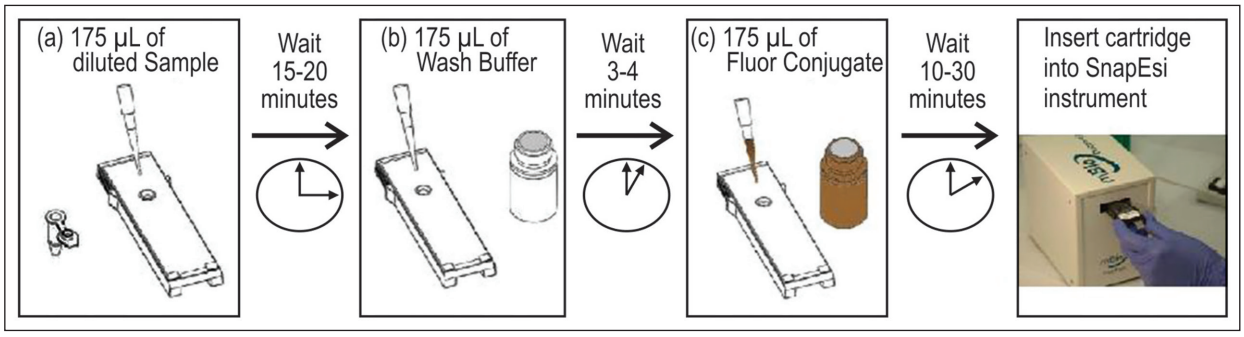
(A) An autonomous capillary microfluidic platform with embedded optics for troponin I detection [133]; (B) a lab-on-a-disc platform for fully integrated multiplex cardiac marker immunoassays [134]; (C) functionalized SnO2 nanobelt field-effect transistor sensors for label-free detection of cardiac troponin [135]; (D) detection of multiple cardiac markers with an integrated acoustic platform for cardiovascular risk assessment [136]. Figure A is reprinted from Mohammed et al., Autonomous capillary microfluidic system with embedded optics for improved troponin I cardiac biomarker detection, Biosens. Bioelectron., 61, 478–484, Copyright (2014), with permission from Elsevier; Figure B is reprinted with permission from Park et al., Lab-on-a-Disc for Fully Integrated Multiplex Immunoassays. Anal. Chem. 2012, 84, (5), 2133–2140. Copyright (2012) American Chemical Society; Figure C is reprinted from Cheng et al., Functionalized SnO2 nanobelt field-effect transistor sensors for label-free detection of cardiac troponin, Biosens. Bioelectron., 26, 4538–4544, Copyright (2011), with permission from Elsevier; Figure D is reprinted from Mitsakakis et al., Detection of multiple cardiac markers with an integrated acoustic platform for cardiovascular risk assessment, Anal. Chim. Acta, 699, 1–5, Copyright (2011), with permission from Elsevier.
Credit/Source: Wu and colleagues [132].

Figure 16. Biochip and biochip reader model for PPC/mBio Inc. (USA)



Credit/Source: Knop and colleagues [131].

Figure 17. Biochip protocol (steps).



Credit/Source: Knop and colleagues [131].

for frontline healthcare professionals. During this pandemic, 3DPT has the potential to save lives. The companies of 3DPT can design items such as face shields, face masks, test kit swabs, reusable filter masks, ventilators, oxygen masks, and other medical devices quickly. Further, the simple productions of 3DPT, such as Hands-Free Door Openers and Press Knobs, could inhibit the spread of infectious diseases such as COVID-19. For medical equipment, the FDA has released FAQs on the use of 3D printed PPE to counter COVID-19 in the country. Companies have since then massively used 3DPT to create and provide equipment to hospitals. However, the FDA has indicated technical challenges that need to be addressed for 3DPT invented PPE to be valid. For example, 3D-printed PPE may provide a physical barrier, but 3D-printed PPE is improbable to provide the same fluid barrier and air filtration protection as FDA-cleared surgical masks and N95 respirators [137]. According to Kritikos' research (representing STOA) [11], the significant benefit of this technique is that components that are needed in small quantities "can be produced at a low cost, as only one type of manufacturing machine is needed and the blueprints for designs, computer-aided design (CAD) files, can be distributed or replicated at the cost of locally-sourced materials". Due to its accessibility, tangible design, and product testing and flexibility, 3D printing becomes relevant when the supply chains of critical products are strained, such as in this pandemic where hospitals and healthcare systems around the world are facing serious deficiencies of protective equipment medical supplies. 3D printing can represent an important role in providing vital equipment when it is hard to source [11, 16]. For example, a group of Italian volunteers utilized their 3D printer to make unofficial copies of a patented valve, because it was missing at Italian hospitals, and they distributed them to a hospital in Brescia where 250 coronavirus patients required breathing machines. In addition, many companies in the US turned their 3D-printer business into a manufacturing place for face shields to be used by health workers that were performing the tests for COVID-19. Meantime, 3D manufacturers around the world are developing 3D-printed face shields, inspired by the 3Dprinted N95 mask to filter out airborne particles that could carry the virus. And, more than 5,000 pairs of 3D printed safety goggles for medical professionals were created, fabricated, and donated in China to Chinese hospitals in just two weeks [11, 16].

Telecommunication Technology

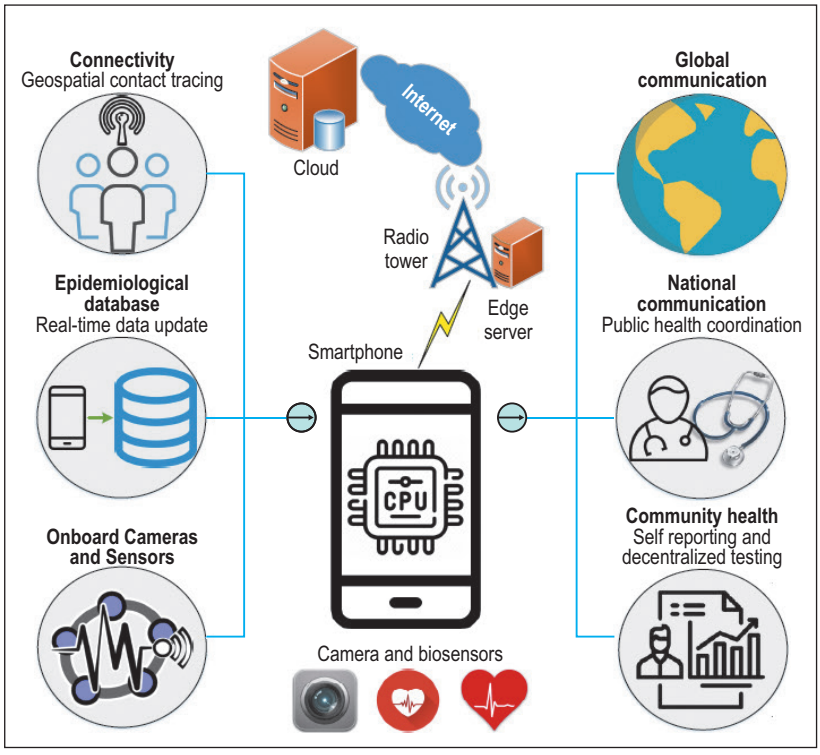
Lockdown policies were the go-to procedure in several countries in attempts to flatten the curve and contain COVID-19. Although lockdowns have severe impacts on the economy and business, it seems to be an effective process to reduce the casualties caused by the disease. Brohi and colleagues' research [34] considers Telecommunication Technologies a tool to track individuals and assist authorities to ensure

compliance with strict movement orders. It is possible to warn people from visiting COVID-19 hotspots with location-tracking applications, AI, and BDA. Healthcare providers can adopt mobile technology to assess patients with mild symptoms remotely and give them guidelines during the period of self-isolation to reduce the crowd on hospitals [11, 34]. With the union of AI and 5G technology, medical robots can monitor patients' temperature, diagnose and give them recommendations with minimal intervention of healthcare workers. TT played an important role in business continuity during this pandemic. TT has also made a pivotal role in commercial business in ordering necessary items while maintaining social distance and complying with movement control orders to stay indoors. During this pandemic, many telecommunication companies have decided to implement free services to their consumers (Figure 18) [138, 139]. Companies are complying with the Work from Home (WFH) model and managing their business, interacting with their employees using services such as Microsoft Teams, Zoom, and Skype.

Issues with the Use of AI and Big Data against COVID-19

According to Naudé [140] and Pham and colleagues [49], there is a critical difficulty to make AI and big data platforms and their applicability a trustful solution in the fight against COVID-19: a lack of standard datasets. Several AI algorithms and big data platforms have been suggested, but they are not experimented with using the same dataset. Moreover, most datasets found in the literature have been created thanks to individual efforts, e.g., the authors obtain some datasets accessible on the Internet, then consolidate them to produce their dataset and evaluate their proposed algorithms. To win this challenge, the government, health organizations (e.g., WHO and CDC), and giant firms represent a pivotal role as they can collaboratively work

Figure 18. An AI-based framework using mobile phones for COVID-19 diagnosis and surveillance.



Credit/Source: Pham and colleagues [49].

for high-quality and big datasets. Diversity of data sources can be implemented by these entities, e.g., x-Rayx-Ray and CT scans from the hospitals, personal information, satellite data, and statements from self-diagnosis apps. Adopting these datasets originated from healthcare organizations, governments, clinical labs, and patients, AI leverages intelligent analytic tools for predicting efficient and safe vaccine/drug against COVID-19, mitigating the diseases, as well as presenting a better way to socially and economical lead with the pandemic. Big data has proved its capability to tackle the COVID-19 pandemic, providing various promising solutions to help fight the COVID-19 pandemic. By combining with AI analytics, big data helps us to understand COVID-19 in terms of virus structure and disease development. It can help healthcare providers in various medical operations from early diagnosis, disease analysis to prediction of treatment outcomes. With its great potentials, the integration of AI and big data can be the key enabler for governments in fighting the potential COVID-19 outbreak in the future, according to Pham and colleagues [49]. Some recommendations that can be considered to promote COVID-19 fighting: AI and big data-based algorithms should be further optimized to enhance the accuracy and reliability of the data analytics for better COVID-19 diagnosis and treatment, and AI and big data can be used in association with other emerging technologies to offer newly effective solutions for fighting COVID-19.

Conclusion

COVID-19 crisis is promoting the implementation of digital solutions quickly and with an impression never seen before. The list of innovative digital solutions against COVID-19 is fast-growing, especially in health devices. These new innovations include video-visits, mobile-phone applications, and "chatbots", artificial-intelligence (AI) powered diagnostic tools, voice systems, or mobile sensors such as oxygen monitors, smartwatches, or thermometers. A new category of digital service is inspecting people under investigation at home in quarantine and/or large-scale population monitoring. Telemedicine and remote consultation such as Zoom, Google, Microsoft Teams, and Skype, among others, have already proven to be useful at a time when access to health services for non-COVID-19 or non-acute patients is limited, not recommended, or postponed. So, it is important to maintain the new innovations and solutions offered today to implement tomorrow's best practices and models of care and to be prepared for future pandemics.

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Immunologic Responses against SARS-CoV-2

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Coronavirus disease 2019 (COVID-19) emerged in Wuhan, China, in December 2019 and quickly spread worldwide becoming a global health problem unprecedented. The infection is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is characterized as a RNA virus with an envelope derived from host cell with glycoprotein spikes, appearing like a crown-like external structure under electron microscope. Due to the aggressive spread profile of SARS-CoV-2, the scientific community is under pressure to generate knowledge about the morphology of the virus and the immune response against SARS-CoV-2, in order to generate useful information for the development of vaccines and methods of immunological diagnosis. Previous knowledge about other coronaviruses, such as SARS-CoV-1 and MERS-CoV, were the pillars for understanding the immune response of SARS-CoV-2. Until now, we know that the anti-SARS-CoV-2 immune response in the host involves mechanisms related to innate immunity, activation of CD4+ and CD8+ T cells and production of antibodies (IgA, IgG and IgM) against the virus. In spite of being a new pathogen, the literature on SARS-CoV-2 has increased dramatically in the past few months, especially in the immunology field. Here, we review the literature on SARS-CoV-2 immunology, focusing on the innate and adaptative immune responses.

Keywords: COVID-19. SARS-CoV-2. Immunologic Response. Immunity.

Introduction

Coronavirus disease 2019 (COVID-19) emerged in Wuhan, China, in December 2019 and quickly spread to other cities and countries, being currently classified as a pandemic by the World Health Organization (WHO) [1,2]. The infection is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which became well-known for its high transmissibility and pathogenicity [3]. According to the WHO, SARS-CoV-2 has infected more than 48 million people worldwide, with more than 1 million confirmed deaths, being the United States, India and Brazil the countries most affected [4]. Despite all the efforts to control the virus spread, daily new cases and deaths related to SARS-CoV-2 are being reported worldwide.

SARS-CoV-2 is not the first coronavirus that cause human global outbreaks. In 2003 and 2012, SARS (retrospectively named SARS-CoV-1) and Middle Eastern respiratory syndrome (MERS) CoVs, respectively caused outbreaks in several countries [5,6]. Despite the genetic similarity between the three virus, SARS-CoV-1 and MERS-CoV exhibited only limited person-to-person spread, resulting in dramatically lower numbers of confirmed cases when compared to SARS-CoV-2 [7]. Due to the aggressive spread profile of SARS-CoV-2, an unprecedented economic and health crisis was caused worldwide. In this context, an urgent need arose regarding knowledge about the immunology of SARS-CoV-2, in order to generate useful information for the development of vaccines and methods of immunological diagnosis.

In spite of being a new pathogen, the literature on SARS-CoV-2 has increased dramatically in the past few months, especially in the immunology field. Here, we review the literature on SARS-CoV-2 immunology, focusing on the innate and adaptative immune responses.

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Structural Basis of SARS-CoV-2

Coronaviruses belong to Coronaviridae family of order Nidovirales and are classified into four genera that include α-, β-, γ-, and δ coronaviruses. They have a viral envelope derived from host cell with glycoprotein spikes, appearing like a crown-like external structure under electron microscope [8].

Coronaviruses have the largest RNA genomes (27 to 32 kb) among the RNA viruses. Their positive-sense single-stranded RNA genome is involved in a nucleocapsid of helical symmetry when relaxed and spherical when inside the virus. They use a nested set of mRNAs produced by the viral RNA polymerase for replication in the cytoplasm of the host cell [9, 10].

There are three types of coronaviruses that evolved to cause severe pulmonary diseases in humans since the beginning of this century which are the SARS-CoV-1, MERS-CoV and the new emerged SARS-CoV-2 [11]. The genome of SARS-CoV-2 shares about 82% sequence identity with SARS-CoV-1 and MERS-CoV and encodes four major structural proteins: spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein and nucleocapsid (N) protein. In addition, there are several accessory and non-structural proteins (nsp1-16) produced during SARS-CoV-2 infection [12].

The transmembrane homotrimer S protein forms the spike structure in the viral surface that plays an essential role in viral attachment, fusion, entry and transmission in host cells. This protein is cleaved by host cell furin-like protease into two subunits (S1 and S2). The presence of this furin-like cleavage site in SARS-CoV-2 facilitates the S protein priming what might explain the transmission efficiency improvement of SARS-CoV-2 when compared to other beta coronaviruses [13].

The S1 subunit is responsible for the receptor recognition process and can be divided into a N-terminal domain (NTD) and a C-terminal domain (CTD), that is also called receptor binding domain (RBD), and the S2 subunit, on the other hand, is correlated with virus membrane fusion process. The SARS-CoV-2 enters in host cell by the attachment of the S glycoprotein to the angiotensin-converting enzyme 2 (ACE2) receptor, expressed in lower respiratory tract cells and also in various organs such as heart, lungs, kidneys and gastrointestinal tract [10, 14] (Figure 1).

Other important SARS-CoV-2 structural component is the N protein that forms the nucleocapsid. This protein is highly phosphorylated, increasing its affinity to the viral RNA. The N protein is involved in processes related to the viral genome, the viral replication cycle and the cellular response of host cells to viral infections [15].

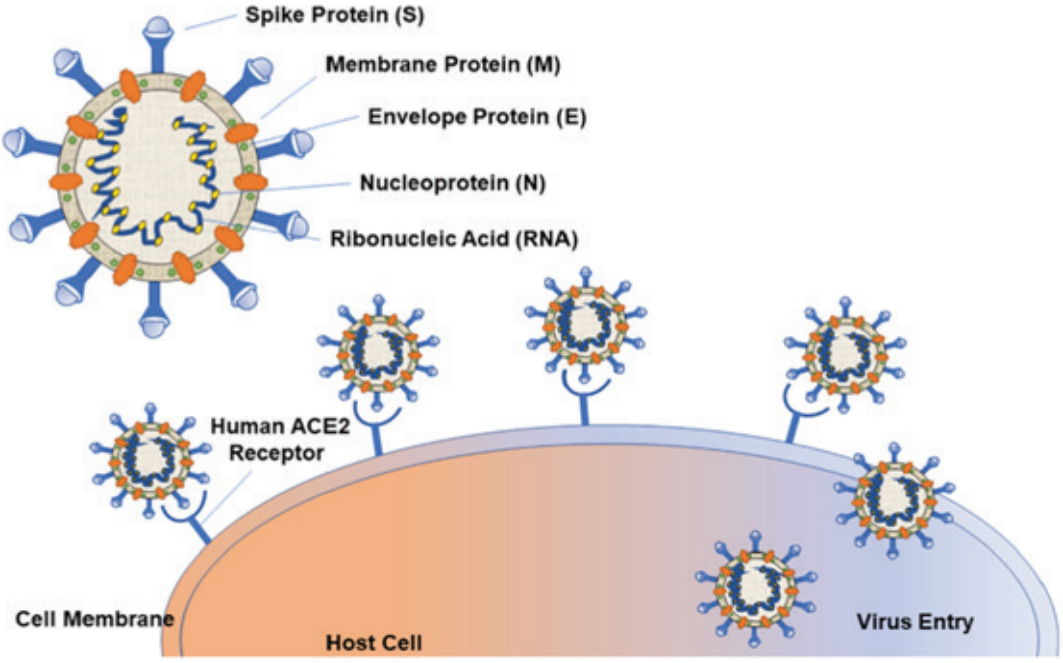
Moreover, the M protein, found in high amount at the virion structure as a transmembrane dimer, plays a role in the maintenance of membrane conformation and shape. On the other hand, the scarcely found E protein, also a transmembrane protein, may help in the virus assembly and release and has an ion channel activity that could be important to the viral pathogenesis [10].

Innate Immunity against SARS-CoV-2

The innate immunity is the first line of defense against invasive microorganisms. The response of innate immunity is not specific to a particular pathogen, but it can recognize conserved patterns among them and quickly respond to an invasion process. The innate immune response is also responsible to activate the adaptive immune system, which is a more specific response which can also keep memory and avoid a re-infection [16].

Regarding SARS-CoV-2 infection, it was described that the initial response in innate immunity is triggered by the engagement of pattern recognition receptors (PRRs) by viral genomic material by cytosolic RIG-I like receptors (RLRs) and extracellular and endosomal Toll-like receptors (TLRs), specially TLR-3, this activation starts the

Figure 1. Schematic representation of the SARS-CoV-2 structure and its mode of host entry.



Credit/Source: Naqvi and colleagues [15a].

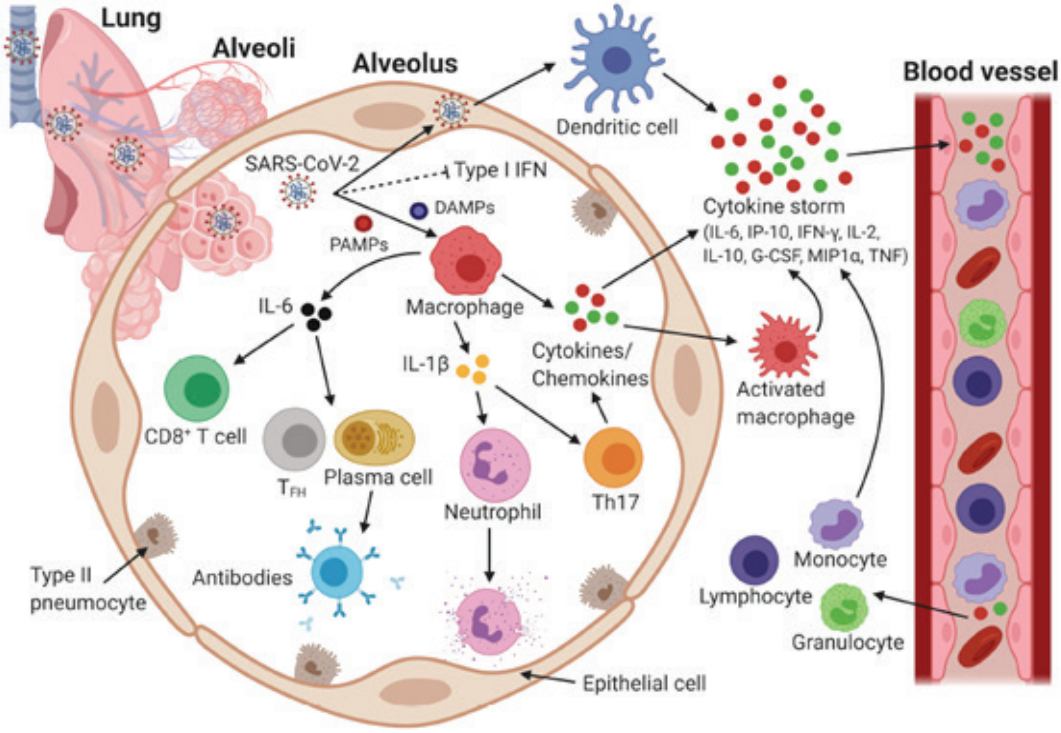
signaling cascade to produce type I/III interferons, tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6 and IL-18 (Figure 2). Together, these cytokines induce antiviral programs in immune cells and potentiate the adaptive immune response [17]. Recent studies indicate that a critical role of innate immunity to SARS-CoV-2 includes the action of Type I Interferon (IFN-I) response [18].

Type I IFNs are polypeptides secreted by infected cells after activation of pattern-recognition receptors, and play an important role in signaling the body to infection in course. Their function includes: activation of intracellular antimicrobial responses, turning the cells refractory to the infection, limiting the virus spread, modulation of cellular innate immune response, promoting antigen presentation, activation of Natural Killer cells (NK), and modulation of pro-inflammatory pathways contributing to the activation of adaptive immune system [20]. Among IFN-I molecules, IFN-α and IFN-β have an important antiviral action in SARS-CoV-2 infection. They are produced by macrophages in the first few hours of the infection and peak at the first 8 to 10 days of symptom onset, inducing the transcription of IFN-stimulated genes and displaying a strong pro-inflammatory response [21].

Analysis of bronchoalveolar lavage fluid cells (BALF) have shown that SARS-CoV-2 viruses can efficiently suppress Type I IFN induction and antagonize its effects. It was observed that when exposed to SARS-CoV-2 infection, the cells failed to induce competent IFN-I responses, favoring the escape of the virus to the immune sensing and delaying the adaptive immune response [18]. Moreover, it was described for other types of SARS-CoV that the failure to elicit an early Type I IFN response correlates with the severity of disease, which has also been observed in COVID-19 [22].

The myeloid cells of innate immunity also play an important role in COVID-19. Activated HLA-DRhiCD11chiCD14+ monocytes were found increased in patients with mild symptoms, while

Figure 2. Immune response against SARS-CoV-2.



The initial response against SARS-CoV-2 is triggered by activation of viral pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) which culminates with the release of proinflammatory molecules by endothelial cells, epithelial cells and tissue-resident macrophages, such as G-CSF, IFN- γ, IL-2, IL-6, IL-10, M1Pα and TNF. Macrophages and virus-infected dendritic cells activated by cytokines/chemokines increase the production of additional cytokine and chemokines, which is known as cytokine storm. The immune response also counts with recruitment of neutrophil, T-helper type 17 cells, CD8+ T cells and B cells.
Credit/Source: Frederiksen and colleagues [19].

monocytes characterized by low expression of HLA-DR and anti-inflammatory markers genes (CD163 and PLAC8) appeared in patients with severe COVID-19 [23].

Moreover, it was described that cells infected by SARS-CoV-2 viruses overexpressed six chemokines that belong to the human ontology "Neutrophil Chemotaxis" including CXCL1, CXCL2, CXCL3, CXCL5, IL-8 (CXCL8), and CCL20 [24]. These chemokines attract neutrophils to the lungs. In COVID-19, neutrophil recruitment and activation, and the presence of neutrophil-derived extracellular traps that cause damage to the tissue observed in hospitalized patients are markers previously reported in hyperinflammatory conditions [24, 25].

Innate lymphoid cells are also involved in the innate imune response, they work as effector cells divided into cytotoxic and non-cytotoxic groups. In the cytotoxic side the NKs are reported to act as early antiviral response. Interestingly, it was observed a reduction of resident NKs in the lung of moderate to severe COVID affected patients, while only in the severe cases new adaptive NKs have been attracted to the inflammation site and contributed to the hyper-inflammation that aggravate the symptoms. Therefore the recruitment of new adaptive NKs has been linked as a marker of a worse prognosis [26, 27].

An interesting link that can be useful to understand the role of innate immunity plays in COVID-19 regards the fact that children account for less than 5% of diagnosed cases, and from those approximately 90% are diagnosed as asymptomatic, mild or moderate for the disease [28]. As children have been less exposed to less

infections during the course of their lifetime and have not developed yet most of its adaptive immune responses, there is a strong suggestion that their more active innate immune response plays a crucial role in responding to SARS-CoV-2 infection [29, 30]. It is hypothesized that children can respond better to the cytokine storm through modulation of the levels of myeloperoxidase, IL-6, IL-10 and p-selectin, avoiding the hyper-inflammation that leads to the severity of acute respiratory distress syndrome observed in the adults [31]. Moreover, aging impairs the fully function of the cells from the immune system, decreasing TLR functions in monocytes, macrophages and dendritic cells; increasing basal cytokine production by dendritic cells; reducing cell signaling, chemotaxis; and triggering inflammatory dysregulations and persistent inflammation, potentiating and age-associated inflammatory environment leading to the complications in COVID-19 observed for the elderly patients [32].

Altogether, the studies suggest that the key to decrease disease fatality is to induce the stimulation of this first immune response that make the bridge between the innate and adaptive immunity, even though the balance and timeframe of this induction is very sensitive, since the presence of those components early in disease can be protective but if prolonged can hyper-activate the inflammatory response and become pathogenic [33].

T-Cell Immunity to SARS-CoV-2

The transition between innate and adaptive immune responses is essential for the clinical progression or control of SARS-CoV-2 infection. The antiviral response in the host involves mechanisms related to innate immunity, activation of CD4+ and CD8+ T cells and production of neutralizing antibodies against the virus [34]. In this sense, CD4+ and CD8+ T cells play a role in balancing the fight against pathogens and the risk of developing autoimmunity or hyperinflammation [35]. During the immune response to viruses, CD4+ T cells are responsible for inducing B lymphocytes to produce specific antibodies against the virus, while CD8+ T cells have the ability to destroy infected cells [36].

Severe lymphopenia is associated with the poor prognosis of COVID-19 [37, 38]. Recently, a study demonstrated that patients with the severe form of COVID-19, showed T-cell lymphopenia, associated with a decrease of regulatory T cells and increase of ratio of naive CD4+ cells in relation to memory CD4+ cells [38]. In addition, about 80% of the total inflammatory cells found in the lung tissue of patients infected with SARS-CoV-2 are CD8+ T cells type [39, 40]. In this context, a deficient immune response to prevent the replication of the virus and the elimination of infected cells, associated with the decrease in CD4+ T cells and its subset of regulatory T cells, contribute to reduction of neutralizing antibodies production and can activate a range of immune system cells, resulting in increase of production of pro-inflammatory cytokines, known as cytokine storm. This phenomenon can cause lymphocyte apoptosis and it is responsible for triggering severe acute respiratory syndrome as well as systemic disorders, such as disseminated instravascular coagulation observed in patients with the severe form of COVID-19 [40-42].

Although antibody production were undetectable, virus-specific memory T cells have been found in individuals 6 years after the recovering of SARS-CoV infection [43]. In 2016, Ng and colleagues [44] demonstrated that memory CD8+ T cells shown specific reactivity for regions of the membrane proteins and nucleocapsid of the SARS-CoV. In addition to this fact, recently it was observed that SARS-CoV-2 has a protein structure genetically similar to SARS-CoV-1 [45]. This observation, can suggest that conserved regions of the epitopes can trigger an immune response against various coronaviruses, serving as an important strategy for the development of vaccines and prevent the possibility of re-infection caused by mutant strains of the virus [46].

Humoral Immunity against SARS-CoV-2

The humoral immune response, especially the production of antibodies, plays an important role in protecting and limiting infections at later phase, and prevent future re-infection. Although SARS-CoV-2 is a novel pathogen, a robust B cell response against the virus is well characterized with the detection of virus-specific IgA, IgG, IgM and neutralizing IgG antibodies during the course of infection and after virus clearance [17]. The SARS-CoV-2 internal N protein and the external S glycoprotein are the sites of antibodies biding. Interestingly, the receptor binding domain (RBD) of Spike protein appears as target of neutralization antibodies and also shows non crossreactivity to RBD from MERS-CoV or SARS-CoV-1, being a promise tool in the diagnostic and immunization fields [47].

In order to understand the dynamic of antibody response against SARS-CoV-2, the response triggered by SARS-CoV-2 are speculated to be similar with the immune response caused by SARS-CoV-1 once they share 82% of sequence identity [48]. The antibody profile against SARS-CoV-1 virus has a typical pattern of IgM and IgG production [49]. Triggering a humoral S- and N-specific IgM response where IgM peak happens within 4 weeks and becomes undetectable 3 months post symptoms onset (PSO); and the switch to IgG occurred around day 14, and can last for a time [50-52].

In the course of SARS-CoV-2 infection, an increase in virus-specific IgM and IgA during the acute phase of the disease followed by an increase in virus-specific IgG at later phases has been observed [53, 54]. However, given the short time since the beginning of the COVID-19 pandemic, it is not well elucidated this sequence of seroconversion, as well as for how long the protecting levels of these blocking antibodies will remain active and protective [55].

The typical view on IgM responses preceding IgG responses were observed by some authors. Xiao and colleagues [56] investigated 34 SARS-CoV-2 confirmed cases and showed positive results for IgM and IgG at week 3 post symptoms onset. The decrease in IgM levels was seen at week 4; being two cases completely negative at week 5 to 6. In accordance, Zhao and colleagues [57] investigated the antibody response to SARS-CoV-2 among 173 hospitalized patients and observed a seroconversion of IgM and IgG around day-12 and day-14, respectively. Pan and colleagues[58], showed that, after the first week of symptoms onset, only 11% of 86 cases had a detectable IgM response. The seropositive response increased between 8 and 14 days after PSO for IgM and IgG. The data report low positivity for IgG after PSO and high levels at later points (more than 15 days).

However, some works demonstrated that this scenario of preceding and declining IgM, followed by a late seroconversion of IgG, seemed not to be generally applicable to SARS-CoV-2 infection [55]. The high discrepancy of the patterns of IgM and IgG seroconversions related to SARS-CoV-2 was reported by Qu and colleagues (2020) [59]. The group observed that the mediam time of seroconversion for IgG was 11 days and 14 days for IgM. Therefore, IgG seroconversion was three days earlier than that for IgM after the SARS-CoV-2 infection. Liu and colleagues (2020) [60] evaluated 23 confirmed cases of SARS-CoV-2 infection and report seroconversion at day 7 or later after the infection, with appearance of IgM and IgG in parallel and only in a few cases with either isolated IgM or IgG. These results highlight the variability of serological response, as well as appearance of IgM and IgG for SARS-CoV-2 disease.

More considerably, detectable levels of total antibodies were found in the sera of patients with undetectable levels of RNA in their respiratory tract samples. This evidence highlighted the extreme importance to combine molecular and serological tests for the exact diagnosis of COVID-19 patients at different stages of the disease [57]. The work report by Pan and colleagues [58], also demonstrated that 43.6% of PCR-negative cases showing clinical symptoms for SARS-CoV-2

found to be positive for antibodies against SARS-CoV-2. The inconsistency between the PCR and antibody results might indicate a wrong time point of taking samples for the PCR test being either too late, or that sampling or other critical steps had not been efficient.

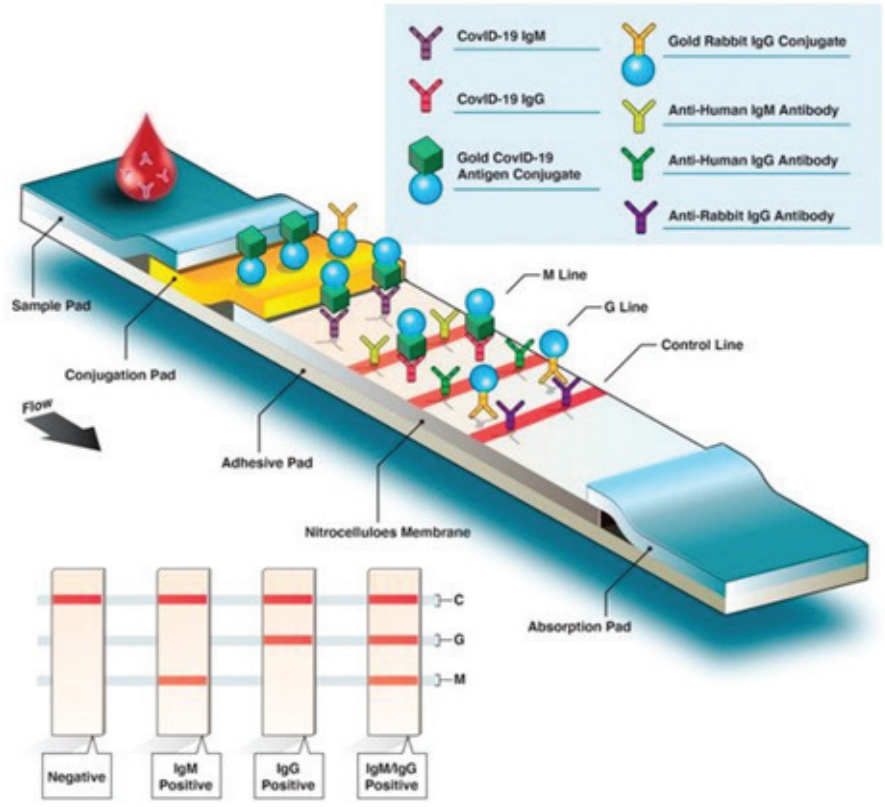
Immune Response Assessment Methods

Since the emergence of the COVID-19 pandemic, several research teams around the world have applied a huge effort to develop and improve different diagnostics methods to detect the immunological response against SARS-CoV-2 infection [61]. The evaluation of humoral and cellular response is important to understand the disease epidemiology, including the asymptomatic infection rate and the protection level in a given population. This information is pivotal to the governs make decisions regarding social distance, lockdown and to improve the public hospital capacities. Moreover, the assays to measure immune response against SARS-CoV-2 may help the governs to reopen borders and get employees back to work, limiting the economic damage [62]. The search of the presence of antibodies and T-cell response against SARS-CoV-2 are two diagnostic ways to measure the host response against the virus. The T-cell response against S-glycoprotein has been characterized and correlates to IgG and IgA antibodies and the production of interferons are known to control viral infection [63]. Interferons are widely used to treat viral infections such as hepatitis B and C, and IFN-α decreases virus titer in the lungs of SARS-CoV-infected macaques [64], suggesting it may be a pharmacological treatment for COVID-19. However, measure interferon production by T-lymphocytes is a strategy to determine previous infections, host response capability to fight against the virus and also associate the host cellular response to the clinical outcome. Actually, this diagnostic is applied to Mycobacterium tuberculosis infection, known as QuantiFERON® [65]. In this case, the blood of the patient is incubated in contact to a mix of peptide antigens from *M. tubesculosis*. In the end, the level of IFN-γ produced is determined by ELISA (enzyme-linked immunosorbent assay). For COVID-19, there is no approach regarding the measurement of IFN-γ as a readout of immune response status against SARS-CoV-2.

Nowadays, the most common diagnostic methods to evaluate the immunological response against SARS-CoV-2 are based on the detection of antibodies. Tests based on the antibody detection are important to determine if people have been infected by a such pathogen. The infection stimulates the recognition of antigens by the immune system, triggering the production of specific antibodies which will be secreted by plasm cells. It is noteworthy the SARS-CoV-2 serology may be complementary to the RT-qPCR and for epidemiological studies. The serology may be used to confirm or exclude COVID-19 in such situations: 1) consecutive negative RT-qPCR results associated to the presence of clinical symptoms; 2) for infectious control in hospitalized patient presenting more than twenty days of suggestive clinical symptoms; 3) COVID-19 atypical manifestations (Guillain-Barré syndrome, meningo-encephalitis, cutaneous vasculitis, Kawasaki disease and diarrhea); 4) pre transplant or chemotherapy treatment [66].

Serological tests include neutralization assays, chemiluminescent immunoassay (CLIA), ELISA (Figure 3) and lateral-flow tests (Figure 4) [67, 68]. To detect the presence of neutralizing antibodies in human plasm it is possible to apply technics such as replicative component virus or pseudotyped viral particle-based entry assays. However, the first one is timing consuming and must be used in a biosafety level 3 laboratory structure, while the second option is not trivial to be produced. CLIA, ELISA and lateral-flow tests, apply an enzyme, fluorophore or colloidal gold-tagged secondary antibody to detect the presence of antibodies in the patient serum. For these assays an important tool is the SARS-

Figure 3. Overview of rapid diagnostic serological test.

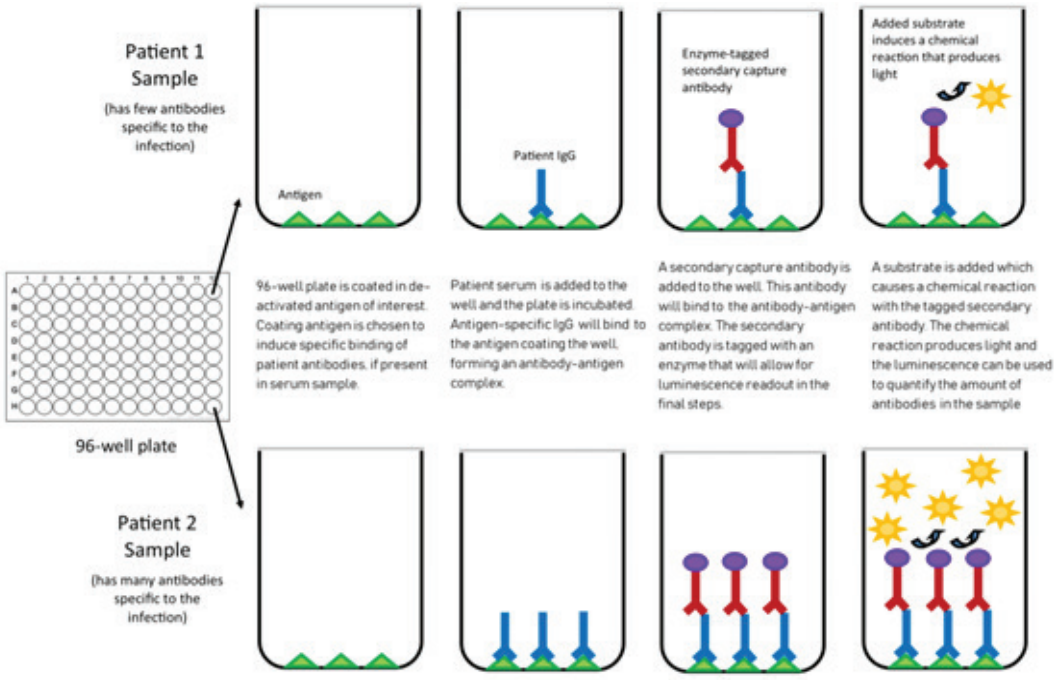


Rapid diagnostic tests (RDTs) are typically based on colorimetric lateral flow immunoassay, in which host antibodies migrate across an adhesive pad (e.g., nitrocellulose) and interact with bound virus-specific antigens and secondary antibodies (antihuman IgM/G antibodies). Conjugated SARS-CoV-2-specific antigen(s) (labeled with gold here) will bind with the corresponding host antibodies. As antibody–antigen complexes travel up the membrane, bound anti-SARS-CoV-2 IgM antibodies interact with fixed anti-IgM secondary antibodies on the M line, and anti-SARS-CoV-2 IgG antibodies interact with anti-IgG antibodies on the G line. If the blood sample does not contain SARS-CoV-2-specific antibodies, the M or G lines do not appear in the final test results; only the control (C) line will be revealed.
Source/Credit: Ghaffari [67].

CoV-2 antigens produced in laboratory. The S and N protein are usually used in these diagnostics. While the N-protein is the most abundant and immunogenic protein (then easier to detect) [69], (S) glycoprotein may elicit neutralizing antibody targeting the RBD [70]. N-protein is relatively small and highly conserved among coronavirus infecting human, allowing false positive results because of cross-reactions to other coronaviruses. On the other hand, S-glycoprotein is less conserved and may stimulate the production of more specific antibodies, as a consequence of glycosylated sites and its complex trimeric conformation. A heterogeneity in antibody development against these antigens have been observed. The anti-N antibodies were showed to appear earlier than anti-S during the infection, and the detection of both antibodies may be complementary during the serological screenings to improve the assay sensibility [71].

One of the first studies which evaluated these commercial serological assays tested the performance of 10 ELISAS, 4 CLIA and 3 lateral-flow tests, to measure the presence of IgM, IgG and IgA in 582 sera (178 and 404 positive and negative, respectively). As expected, the antibodies titer increased overtime post-symptoms and was detected by most of the

Figure 4. Schematic representation of an ELISA or CLIA assay.



Both serological tests are performed in antigen coated plates where patient serum is added. The human antibodies directed against SARS-CoV-2 antigen is detected by a seconday labeled antibodies, which could be conjugated to a horesedharsh peroxidase (ELISA) or a fluoroforo (CLIA). The signal intensity will be proportional to the antibody titer in the sample.
Credit/Source: Serology testing for COVID-19. Center for Health Security. Johns Hopkins University 2020 [68].

brands tested. Regardless the serological method applied, IgG antibodies increased after 15 days post-symptoms and generate a sensibility of 90%. Cross reaction was not observed in this study using samples from another human seasonal coronavirus and others virus infection. These same authors showed that lateral flow testes could detect 90% of IgM from infected patients and in all IgM positive assays, IgG was also present [66]. It suggests that the detection of IgM alone may be useless for the recent diagnostic of COVID-19, as determined by a longitudinal profile of IgG, IgM and IgA antibodies [72,73].

A meta-analysis performed by Bastos and colleagues [74], found that the methodology applied to conduct the accuracy of serological tests for COVID-19 are associated to high risk of patient selection bias as well as the risk regarding to the interpretation of the results. The sensitivity was higher after three weeks of symptoms onset for CLIA, ELISA and lateral flow method, in accordance to the study abovementioned. In all analysis, the sensitivity of lateral-flow based tests was the lowest detected [74]. The lateral-flow methodology is easier to perform and faster than CLIA and ELISA, then it has been commonly used around the world and is a potential point-of-care method. However, the performance of this method must be evaluated in depth. Another metanalysis searched for the accuracy of rapid tests registered in Brazil during the pandemic. This study showed this kind of test may be associated to false negative results [75].

In summary, the evidence for high performance of serological tests are weak, mainly if the methods are applied as point of care diagnosis. Then, caution is necessary when use serological tests available for clinical decision and epidemiological surveillance. Moreover, the researchers must consider the antigen used for the development of these tests, since evidences suggest the response against the N-protein or S-glycoprotein influences the prognosis of the disease [76].

Concluding Remarks

In view of the rapid spread of SARS-CoV-2 and the unknown nature of the virus, both basic science and clinical science had to intensify scientific production regarding SARS-CoV-2 in a few months in order to generate useful information for the viral infection control. Previous knowledge about other coronaviruses, such as SARS-CoV-1 and MERS-CoV, were the pillars for understanding the immune response of SARS-CoV-2. Until now, we know that the anti-SARS-CoV-2 immune response in the host involves mechanisms related to innate immunity, activation of CD4+ and CD8+ T cells and production of antibodies (IgA, IgG and IgM) against the virus. Despite the similarities with other coronoviruses, the immune response against SARS-CoV-2 has been showing some distinct characteristics that need to be better clarified, such as, for example, the kinetics of antibody production. In this fashion, we can conclude that additional studies need to be done to a better description of the immune response against SARS-CoV-2.

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Diagnostic of COVID-19: Chest Computer Tomography or RT-PCR?

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In this review article, we presented a gold-standard method to detect the SARS-CoV-2, the novel virus that is causing the COVID-19 outbreak, and the use of a computer tomography (CT) method to detect the complications of the disease. We showed the controversial analysis about which method is the best to detect the disease earlier due to the COVID-19 complications. We searched the articles in the main database (PubMed/Medline, Elsevier Science Direct, Scopus, Isi Web of Science, Embase, Excerpta Medica, UptoDate, Lilacs, Novel Coronavirus Resource Directory from Elsevier), in the high-impact international scientific Journals (Scimago Journal and Country Rank - SJR - and Journal Citation Reports - JCR), such as The Lancet, Science, Nature, The New England Journal of Medicine, Physiological Reviews, Journal of the American Medical Association, Plos One, Journal of Clinical Investigation, and in the data from Center for Disease Control (CDC), National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID) and World Health Organization (WHO). We prior selected meta-analysis, systematic reviews, article reviews, and original articles in this order. We reviewed 96 articles and used 45 from March to June 2020, using the terms coronavirus, SARS-CoV-2, novel coronavirus, Wuhan coronavirus, severe acute respiratory syndrome, 2019-nCoV, 2019 novel coronavirus, n-CoV-2, covid, n-SARS-2, COVID-19, corona virus, coronaviruses, RT-PCR, computer tomography (CT), diagnostic methods, with the tools MeSH (Medical Subject Headings), AND, OR, and the characters [,",; /., to ensure the best review topics. We concluded that chest CT plays an important role in the timely detection of lung infection abnormalities in the early phase of COVID-19 infection. However, the RT-PCR is the gold standard method to detect SARS-CoV-2.
Keywords: COVID-19. SARS-CoV-2. RT-PCR. CT.

Introduction

There is a current worldwide outbreak of a new type of coronavirus (2019-nCoV), which spreads to all over the world affecting 5,555,691 people and killing 348,541 (May 25, 2020) [1, 2]. On December 31, 2019, the China Health Authority reported the World Health Organization (WHO) to several cases of pneumonia of unknown reasons in Wuhan, Hubei province, China. The epidemiological evidence reveals that the cases originated from a seafood wholesale market in Wuhan, where poultry, snake, bats, and other live animals were on sale [3, 4]. The gene's sequence of the virus was similar to that identified in bats [5, 6]. On January 7, 2020, this disease was found to be the cause of a new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; previously known as 2019-nCoV [7] and formally named by the World Committee on Virus Classification) [8]. At the beginning of February 2020, the disease caused by this virus was named as Coronavirus Disease 2019 (COVID-19) by the World Health Organization (WHO) [9].

As the virus spreads quickly around the world affecting the lives of all people, the diagnostics play an important role in the containment of COVID-19, due to enabling the rapid implementation of control measures that limit the spread through case identification, isolation, and contact tracing [10].

The symptoms of COVID-19 are nonspecific and cannot be used for an accurate diagnosis. Many of these symptoms could be associated with other respiratory infections, such as fever, cough, fatigue, sputum production, and shortness of breath [11]. So, the gold standard method for COVID-19 is RT-PCR. However, this method brings some intrinsic problems and chest CT scans have been used for diagnosing and screening COVID-19. However, our question is about which one of these methods is the best for diagnostic COVID-19?

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This review aimed to reproduce the recent studies about the diagnostic of COVID-19, elucidating the diagnostic performance measures, including predictive values, chest CT and initial reverse transcriptase-polymerase chain reaction (RT-PCR).

The RT-PCR for COVID-19

Genome Sequence (GenBank)

The development of molecular techniques is dependent upon understanding [12] the proteomic and genomic composition of the pathogen or the induction of changes in the expression of proteins/genes in the host during and after infection [13]. The first genome sequence of SARS-CoV-2 was conducted with metagenomic RNA sequencing, an unbiased and high-throughput method of sequencing multiple genomes [14-17]. The sequence was done and added to the GenBank sequence repository on January 10, 2020 [15, 16].

Nucleic Acid Test for SARS-CoV-2

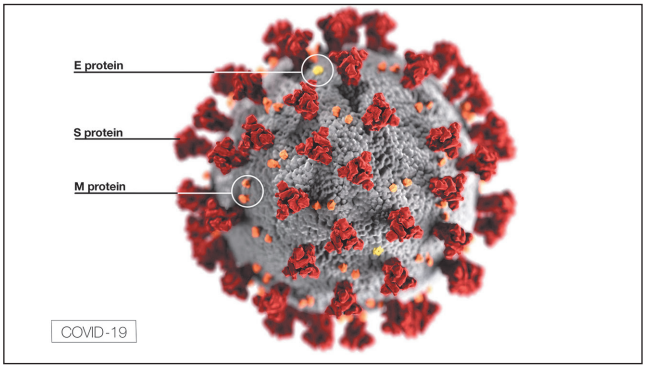
According Udugama and colleagues [14], nucleic acid testing is the primary method of diagnosing COVID-19 [18]. Several reverse transcription-polymerase chain reaction (RT-PCR) kits have been designed to detect SARS-CoV-2 genetically [14]. RT-PCR involves the reverse transcription of SARS-CoV-2 RNA into complementary DNA (cDNA) strands, followed by amplification of specific regions of the Cdna [19, 20]. The design process generally involves two main steps:

1. The sequence alignment and primer design, and
2. Assay optimization and testing.

Corman and colleagues [21] analyzed several SARS-related viral genome sequences to design a set of primers and probes. From SARS-related viral genomes, they found three regions that had conserved sequences:

1. The RdRP gene (RNA-dependent RNA polymerase gene) in the open reading frame ORF1ab region;

Figure 1. SARS-CoV-2 morphology and the proteins (conserved sequences).



Credit/Source: This illustration, created at the Centers for Disease Control and Prevention (CDC), reveals ultrastructural morphology exhibited by coronaviruses. The illness caused by this virus has been named coronavirus disease 2019 (COVID-19). (CDC Illustration).

2. The E gene (envelope protein gene), and
3. The N gene (nucleocapsid protein gene). Both the RdRP and E genes had high analytical sensitivity for detection (technical limit of detection of 3.6 and 3.9 copies per reaction), whereas the N gene provided poorer analytical sensitivity (8.3 copies per reaction) (Figure 1).

RT-PCR can be performed by one- or two-steps assay [14]:

1. One-step assay: reverse transcription and PCR amplification are consolidated into one reaction. This assay format can provide rapid and reproducible results for high throughput analysis. But, the challenge is the difficulty in optimizing the reverse transcription and amplification steps as they occur simultaneously, which leads to lower target amplicon generation. The United States Centers for Disease Control and Prevention (CDC) uses a one-step real-time (RT-PCR) assay, which provides quantitative information on viral loads, to detect the presence of SARS-CoV-2 [22].
2. Two-step assay: This assay format is more sensitive than the one-step assay, but it is more time consuming and requires optimizing additional parameters. Lastly, controls need to be carefully selected to ensure the reliability of the assay and to identify experimental errors [23,24].

Workflow for Nucleic Acid Testing for SARS-CoV-2

The National Medical Products Administration (NMPA) has approved at least 11 nucleic-acid-based methods and eight antibody detection kits in China for detecting SARS-CoV-2 [25].

Performance

The viral RNA is extracted and added to a master mix, which contains nuclease-free water, forward and reverse primers, a fluorophore-quencher probe, and a reaction mix (consisting of reverse transcriptase, polymerase, magnesium, nucleotides, and additives) [14, 18]. The master mix and extracted RNA are loaded into a PCR thermocycler, and the incubation temperatures are set to run the assay. The CDC has recommended cycling conditions for RT-PCR. During RT-PCR, the fluorophore-quencher probe is cleaved, generating a fluorescent signal. The fluorescent signal is detected by the thermocycler, and the amplification progress is recorded in real-time. This reaction takes ~45 min and can occur in a 96-well plate, where each well contains a different sample or control. There must be both a positive and negative control to interpret the final results properly when running RT-PCR. For SARS-CoV-2, the CDC provides a positive control sequence called nCoVPC [22].

Udugama and colleagues [14] listed many SARS-CoV-2 RT-PCR primers and probes from different research groups and agencies (Table 1).

CT x RT-PCR

Molecular techniques are more appropriate than chest CT scans for accurate diagnoses for COVID-19 because they can target and identify specific pathogens. The RT-PCR is the gold-standard method to detect COVID-19 [6]. However, the high false-negative rate, especially in the early stage of the outbreak, or because RT-PCR can be affected by low patient viral load and improper clinical sampling and transportation, and the lack of RT-PCR assay limited the timely diagnosis of infected patients [26-30]. Recent reviews showed that CT may have higher sensitivity (98%) of chest CT [31] for diagnosis of COVID-19 than initial RT-PCR [32,33], especially when the signs of COVID-19 pneumonia is present, such as ground-glass opacities (GGO) (presenting in 100% of cases), GGO pattern, GGO location, consolidation, multilobe involvement, bilateral distribution, location of consolidation or GGO, pulmonary nodules surrounded by GGO, interlobular septal thickening, air bronchogram, halo sign, presence of cavitation, bronchial wall thickening, bronchiectasis, perilesional vessel diameter, lymphadenopathy (defined as lymph node with short-axis > 10mm), pleural and pericardial effusion. On chest CT, ground-glass opacities (GGO) were present in 100% of patients with RT-PCR confirmed COVID-19 (Figure 2) [34-37].

Thereby, CT has become an important imaging method for the early detection of patients with COVID-19 pneumonia [28, 34]. Nevertheless, the specificity of CT is low (56%) [31] due to the nonspecific findings of COVID-19 that overlap with those of other viral pneumonia, and the images cannot distinguish between COVID-19 pneumonia and other viruses' pneumonia [34, 35]. Also, some patients present a positive RT-PCR test for COVID-19 and normal CT, as well as early negative RT-PCR and positive CT for COVID-19 pneumonia [35, 37, 38].

There is a critical issue that the physicians have to consider: the large volume of workload for hospital staff and difficulties with disinfection procedures are non-negligible issues related to the widespread use of CT as a diagnostic tool for COVID-19. Recently, the Society of Thoracic Radiology and American Society of Emergency Radiology jointly released a position statement

Table 1. Primers for SARS-CoV-2 (PCR).

Institution	Gene target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe (5'-3')
U.S. CDC [32]	N gene	**N1:** GACCCCAAAATCAGCGAAAT **N2:** TTACAAACATTGGCCGCAAA **N3:** GGGAGCCTTGAATACACCAAAA **RP-F RNAse:** AGATTTGGACCTGCGAGCG	**N1:** TCTGGTTACTGCCAGTTGAATCTG **N2:** GCGCGACATTCCGAAGAA **N3:** TGTAGCACGATTGCAGCATTG **RP-RRNAse:** GAGCGGCTGTCTCCACAAGT	**N1:** FAM-ACCCCGCATTACGTTTG GTGGACC-BHQ1 **N2** FAM-ACAATTTGCCCCCAGC GCTTCAG-BHQ1 **N3:** FAM-AYCACATTGGCACCCGC AATCCTG-BHQ1 **RP-P RNAse:** FAM-TTCTGACCTGAAGGCTC TGCGCG– BHQ-1
China CDC [105]	ORF1ab and N gene	**ORF1ab:** CCCTGTGGGTTTTACACTTAA **N:** GGGGAACTTCTCCTGCTAGAAT	**ORF1ab:** ACGATTGTGCATCAGCTGA **N:** CAGACATTTTGCTCTCAAGCTG	**ORF1ab:** FAM-CCGTCTGCGGTATGTGGAAAG GTTATGG-BHQ1 **N:** FAM-TTGCTGCTGCTTGA CAGATT-TAMRA
Charité, Germany [45]	RdRp, E, N gene	**RdRp:** GTGARATGGTCATGTGTGGCGG **E:** ACAGGTACGTTAATAGTTAATAGCGT	**RdRp:** CARATGTTAAASACACTATTAGCATA **E:** ATATTGCAGCAGTACGCACACA	**RdRp 1:** FAM-CAGGTGGAACCTCATC AGGAGATGC-BBQ **RdRp 2:** FAM-CCAGGTGGWACRTCATC MGGTGATGC-BBQ **E:** FAM-ACACTAGCCATCCTTA CTGCGCTTCG-BBQ
Hong Kong University [106]	ORF1b-nsp14, N gene	**ORF1b-nsp14:** TGGGGYTTTACRGGTAACCT **N:** TAATCAGACAAGGAACTGATTA	**ORF1b-nsp14:** AACRCGCTTAACAAAGCACTC **N:** CGAAGGTGTGACTTCCATG	**ORF1b-nsp14:** FAM-TAGTTGTGATGCWATC ATGACTAG-TAMRA **N:** FAM-GCAAATTGTGCA ATTTGCGG-TAMRA
National Institute of Infectious Diseases, Japan [107]	N gene	**N:** AAATTTTGGGGACCAGGAAC	**N:** TGGCAGCTGTGTAGGTCAAC	**N:** FAM-ATGTCGCGCAT TGGCATGGA-BHQ
National Institute of Health, Thailand [108]	N gene	**N:** CGTTTGGTGGACCCTCAGAT	**N:** CCCCACTGCGTTCTCCATT	**N:** FAM-CAACTGGCAGTAACCABQH1

Credit/Source: Udugama and colleagues [14].

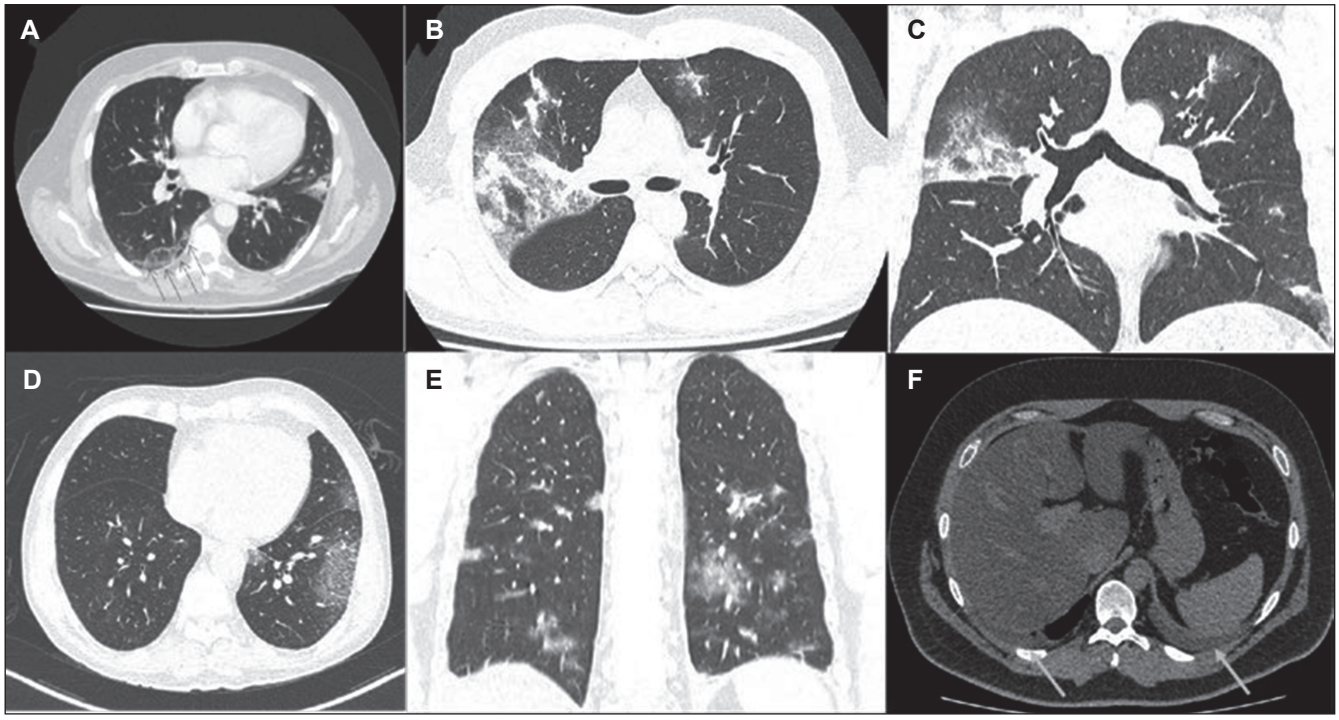
according to which routine CT screening is not recommended for the diagnosis of patients under investigation for COVID-19 [37, 39, 40].

However, besides the specificity (56%) [31] and the described issues about chest CT in this review, this new diagnostic was included in the management of patients to ensure timely treatment and isolation measures due to the delays of RT-PCR testing and the large group of patients with respiratory symptoms by COVID-19 [18, 31, 36, 37]. So, a combination of chest CT and repeat RT-PCR testing may be beneficial for the diagnosis of COVID-19 in the setting of strong clinical suspicion [36].

Discussion

Xie and colleagues [32] and Huang and colleagues [41] indicated that many positive cases for COVID-19 present initially negative RT-PCR tests, but lung abnormal lesions detected by chest CT. Fang and colleagues [33] found that 98% of the patients already presented positive CT pneumonia while 71% of the patients had initially positive RT-PCR results. They also demonstrated that the sensitivity of chest CT was higher than RT-PCR. Moreover, Ai and colleagues [34] concluded that chest CT had a high sensitivity for the diagnosis of

Figure 2. Chest CT scans presenting the spectrum of findings of COVID-19.



Credit/Source: Adapted from Chate and colleagues (2020) [38]. A-F. Besides the Figure presents many findings of COVID-19 pneumonia, in all CT images the ground-glass opacities are present.

COVID-19 than RT-PCR. They tested patients by RT-PCR assay and chest CT scanning on the same day and found that the patients who initially had negative RT-PCR results, presented positive CT abnormalities. However, they also found RTPCR-positive patients with clinical symptoms had normal CT scans. Similarly, Chung and colleagues [35] in a retrospective study found patients who showed negative findings on first-time chest CT, but positive-RT-PCR, for whom a follow-up chest CT revealed positive findings later. Furthermore, Xu and colleagues [8] reported that first-time baseline chest CT did not show any abnormalities in 23% of the patients. Similarly, Pan and colleagues [42] reported patients with first normal CT had lung abnormalities on the follow-up CT approximately 4 days later. Besides that, the chest CT was included in the new diagnostic criteria in combination with RT-PCR for confirmation of COVID-19 pneumonia diagnosis if it is narrowly indicated for fast management of the disease due to the cost-benefit for the patient [3, 7, 31, 38, 41-43].

Conclusion

We concluded that chest CT plays an important role in the timely detection of lung infection abnormalities in the early phase of COVID-19 infection. Concerning the diagnosis of COVID-19, a combination of RT-PCR screening and chest CT scanning in the highly suspected patients might be a complementary diagnostic for COVID-19. Nevertheless, RT-PCR remains the reference standard for the final diagnosis of COVID-19 infection despite the false-negative rate. The physicians should be vigilant at all times to identify patients with COVID-19 infection, who may have few or no clinical symptoms, normal chest CT, and or even initial negative PR-PCT test.

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Therapies Against COVID-19: a Running to a Treatment

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There is no specific drug or therapy against COVID-19. Since the beginning of the pandemic, scientists are running to discover a drug or therapy that can treat the disease. What we found until now are a combined drug and therapies that can mitigate the effects of the disease in the human body and how to manage the patient better. In this article, we tried to join the new discoveries and presented the drugs and therapies and their mechanisms to combat the SARS-CoV-2. We showed the immunomodulators, parasiticides, antiviral drugs (focused on Remdesivir), antimalarial drugs, anti-cytokine drugs focused on the role of IL-6, Reumathological drugs, inhibitors of cell-receptors, antiinflammatory drugs, especially the role of corticosteroids (dexamethasone), antibiotics (azithromycin), anti-thrombotic drugs, blood derivates therapies and alternative therapies currently used against COVID-19. Also, we listed the main results of clinical trials of new therapies presented by Recommended Panel Treatment Guidelines [NIAID-RML (USA)]. We searched the data in the main database (PubMed/Medline, Elsevier Science Direct, Scopus, Isi Web of Science, Embase, Excerpta Medica, UptoDate, Lilacs, Novel Coronavirus Resource Directory from Elsevier), in the high-impact international scientific Journals (Scimago Journal and Country Rank - SJR - and Journal Citation Reports - JCR), such as The Lancet, Science, Nature, The New England Journal of Medicine, Physiological Reviews, Journal of the American Medical Association, Plos One, Journal of Clinical Investigation, and in the data from Center for Disease Control (CDC), National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID) and World Health Organization (WHO). We prior selected meta-analysis, systematic reviews, article reviews, and original articles in this order. We used 302 articles from March to June 2020, using the terms coronavirus, SARS-CoV-2, novel coronavirus, Wuhan coronavirus, severe acute respiratory syndrome, 2019-nCoV, 2019 novel coronavirus, n-CoV-2, covid, n-SARS-2, COVID-19, corona virus, coronaviruses, immunomodulators, parasiticides, antiviral, antimalarial, anti-thrombotic and anti-cytokine, antiinflammatory, Reumathological drugs, inhibitors of cell-receptors, antibiotics, blood derivates therapies and alternative therapies, with the tools MeSH (Medical Subject Headings), AND, OR, and the characters [,“,; /., to ensure the best review topics. We concluded that despite there is no treatment or drugs against the COVID-19, a combined therapy can help and mitigate the effects of the disease, helping the immune system to combat the virus.
Keywords: COVID-19. SARS-CoV-2. Therapies. Treatments. New Discoveries. WHO. CDC. NIH.

Introduction

COVID-19 is defined as a sickness caused by the novel coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), which was first identified in Wuhan City, Hubei Province, China [1]. It was originally reported to the World Health Organization (WHO) on December 31, 2019, and on March 11, 2020, the WHO ed the COVID-19 outbreak as a global pandemic. The period since then has been one of the most challenging in recent history for doctors, researchers, health companies, and all the governments around the world. Scientists are running on to find treatments, drugs, and vaccines to save lives and cure people.

Notwithstanding, no drugs, biologics or vaccines have yet been approved by the health federal agencies worldwide for prevention or treatment of COVID-19. The drugs' arsenal we have available so far is destined for the management of COVID-19 patients. This article review brings a compilation of promising drugs, therapies and treatment against COVID-19, as well as the treatment guidelines panel from National Institutes of Health from the United States.

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SARS-CoV-2: Virology and Drug Targets [2] (Figures 1 and 2)

As already known, SARS-CoV-2 is able to target cells by viral structural spike (S) protein that attaches to the angiotensin-converting enzyme 2 (ACE2) receptor [3]. After the receptor attachment, the virus particle utilizes host cell receptors and endosomes to penetrate the host cells. A host type 2 transmembrane serine protease, TMPRSS2, aids cell entrance via the S protein [3]. Into the cell, viral polyproteins are synthesized that encode for the replicase-transcriptase complex. The virus then produces RNA by its RNA-dependent RNA polymerase. Structural proteins are synthesized conducting to completion of arrangement and release of viral particles [4, 5]. These steps of the viral lifecycle give possible targets for drug therapies. Assuring drug targets involve nonstructural proteins (eg, 3-chymotrypsin-like protease, papain-like protease, RNA-dependent RNA polymerase), which share homology with other novel coronaviruses (nCoVs). Added drug purposes include viral entrance and immune regulation pathways [6, 7].

The Figure 1 presents the main targets and drugs that have been used in attempt to treat the COVID-19, as well as Figure 2 presents the drugs target and immune response (Figure 3).

Immunomodulators, Drugs of ARDS/ Anticytokines and Other Investigational Therapies [8] (Table 1 attached)

Anti-TNF Agents

TNF-α is one of the most potent proinflammatory cytokines with broad spectrum of actions. Marked elevations reported in many inflammatory conditions including cytokine release syndrome. Serum TNF-α levels found elevated in COVID-19 patients with being more pronounced in more severe patients [9]. SARS-CoV viral spike protein can modulate TNF-α-converting enzyme (TACE)-dependent shedding of the ACE2 ectodomain, required for the viral entry which is coupled to TNF-α production [10]. The hypothesis is that the use of TNF inhibitors might be effective in blocking viral entry and detrimental effects of exuberant TNF-α, which is observed preclinical studies on severe respiratory syncytial virus and influenza infections [11]. Anti-TNFs lead to higher risks of bacterial, viral and fungal infections, thus their use in COVID-19 needs to be supported with preclinical and clinical studies.

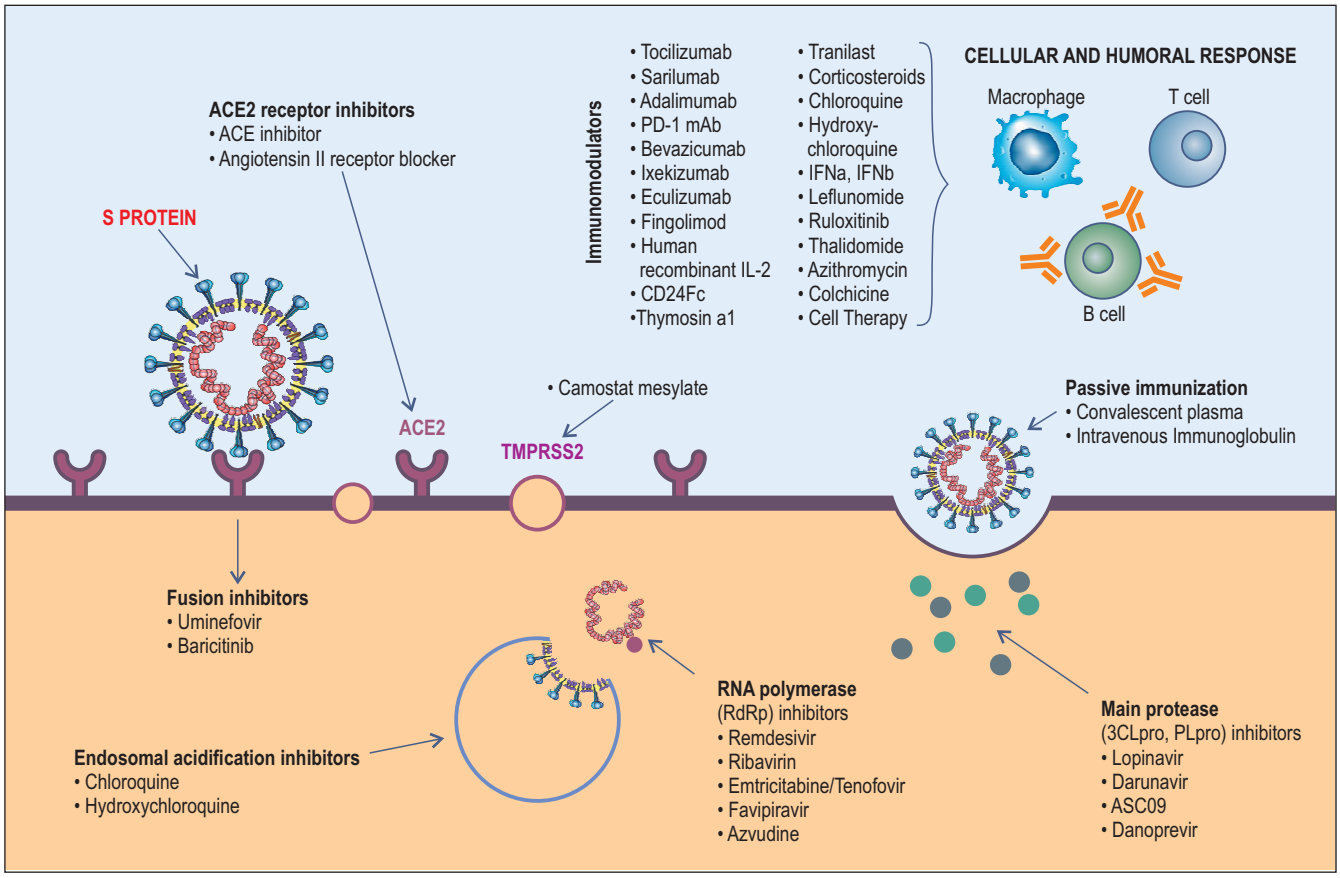
IL-1 Family Antagonists [12]

During the cytokine storm (Figure 4), the three most important cytokines in the IL-1 family are released such as IL-1β, IL-18, and IL-33 [12, 13]. Many studies have shown that a "cytokine storm" relief of IL-6, IL-1, IL-12, and IL-18, simultaneously with tumor necrosis factor-alpha (TNFα) and other inflammatory mediators. The elevated pulmonary inflammatory response may result in increased alveolar-capillary gas exchange, causing oxygenation difficult in patients with severe disease. Investigations that concentrate on the inhibition of IL-1β to decrease the cytokine storm have attracted attention. Anakinra, which is an antagonist of IL-1β, can be applied to treat the cytokine storm caused by infection. Shakoory and colleagues demonstrated that Anakira significantly improved the 28-day survival rate of patients with severe sepsis [14]. According to the Center for Disease Control Guidelines for COVID-19 [15], the data is not sufficient to recommend for or against the use of interleukin (IL)-1 inhibitors for COVID-19 treatment.

Anakira [5]

Nod-like receptor family pyrin domain-containing 3 (NLRP3) is a critical inflammasome in acute protection of the body against a wide variety of noxious stimuli, including RNA viruses [16]. NLRP3 activates caspase-1, a molecule responsible for the activation and exuberant release of IL-1β and IL-18. Previously SARS-CoV has been shown to induce NLRP3 by its ion channel-forming M protein and ORF8b [17]. SARS-CoV-2 is known to induce various cytokines, including

Figure 1. Currently tested therapeutic molecules targeting different steps of SARS-CoV-2 life cycle.



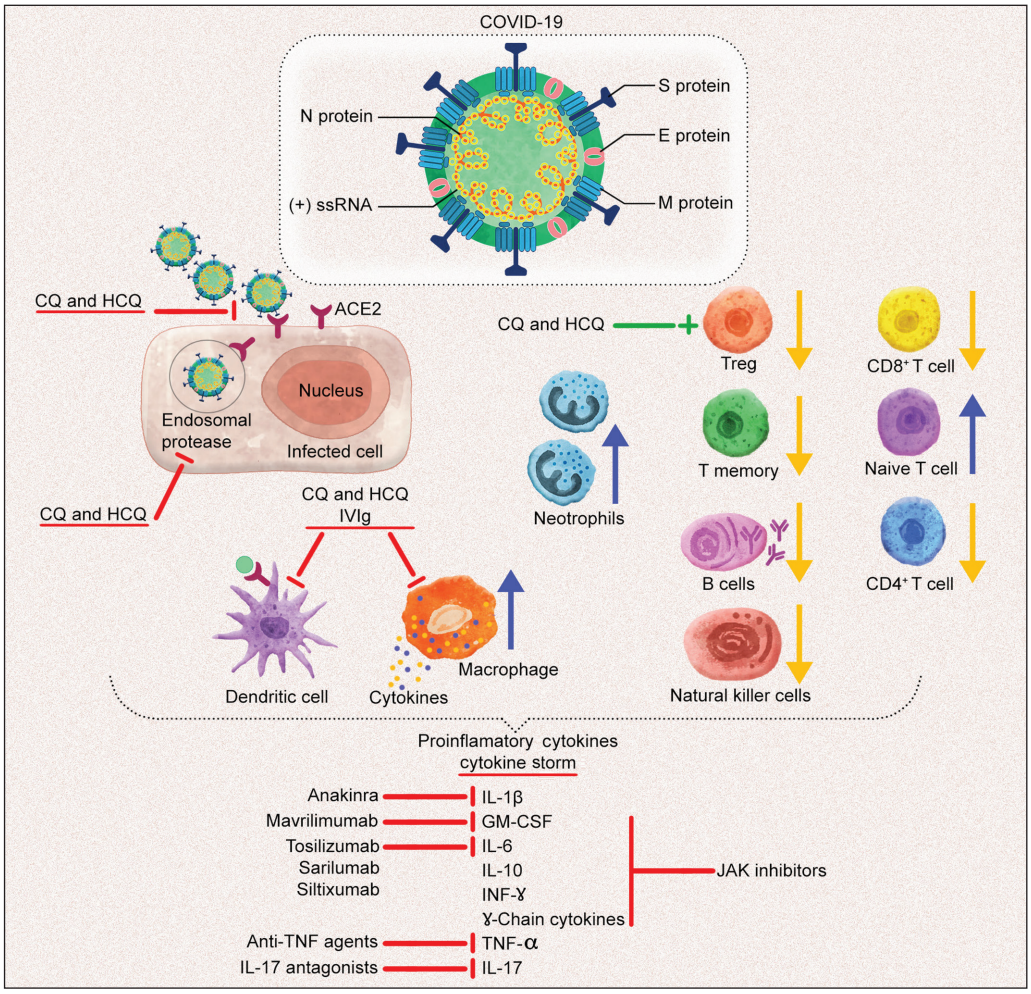
Credit/Source: Adapted from Fragkou and colleagues [7a].

the IL-1 family [18]. The IL-1 family is made up of pleiotropic cytokines, which have roles in inflammation, hematopoiesis, and fibrosis. IL-1β and TNF-α promote vascular permeability and leakage. Both IL-1β and IL-18 fuel cytokine storm and MAS and IL-1 cytokines (except IL-18) can be successfully inhibited by anakinra, as Conti and colleagues' study can attest [19].

Anakinra is a recombinant antagonist of human IL-1 and approved for the treatment of Reumatoid Arthritis (RA) and certain autoinflammatory disorders with recommended doses of 1–2 mg/kg/day with a maximum daily dose of 8 mg/kg [20, 21]. In terms of sepsis and MAS, a previous, highly cited phase III trial, anakinra did not improve 28-day survival rate in sepsis patients and was terminated earlier than expected [22]. Still, a new analysis of data from this trial suggested significant improvement in survival in patients with hepatobiliary dysfunction and disseminated intravascular coagulation (DIC) [14]. Anakinra was administered intravenously at 2 mg/kg/hr for 72 h continuously in this study without safety concerns. Careful monitoring is in order, since this dose is extremely higher than those used in rheumatology routine. There are several anakinra studies registered for COVID-19, testing 100 mg daily subcutaneous injection for 28 days to 400–600 mg/day intravenous for 5–7 days (NCT04339712, NCT04330638).

A recent study from Navarro-Millán and colleagues [23] suggested that Anakira could be benefitial in COVID-19 patients with cytokime storm syndrom when initiated early after onset of hypoxic resiratory failure. Nevertheless, due to the insufficient data on the use of interleukin (IL)-1 inhibitors, such as Anakinra, the Treatment Guidelines Panel against COVID-19 by the National Institutes of Health (NIH) from the United

Figure 2. Drugs target against COVID-19 and immune response.



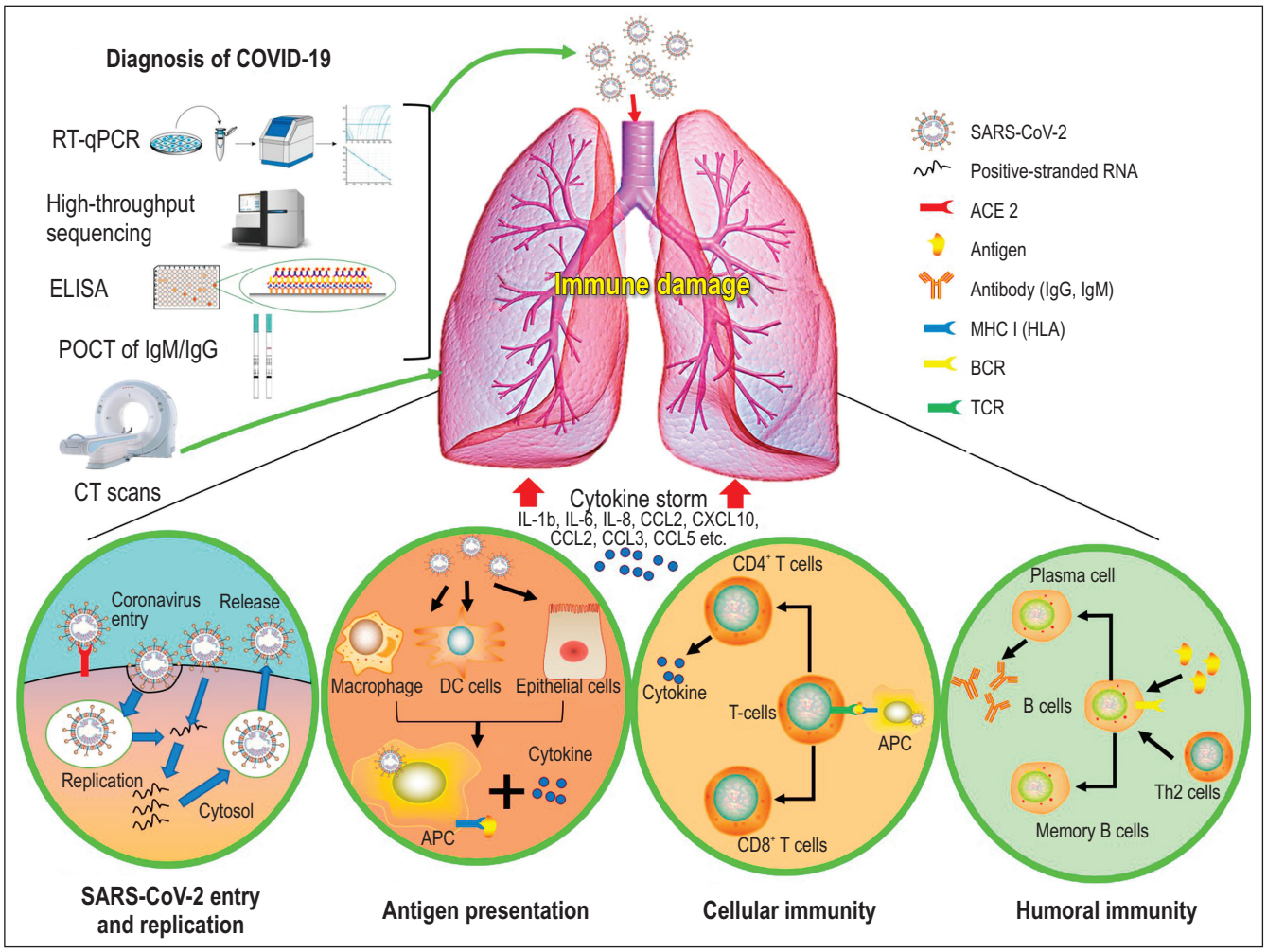
The schematic image of coronavirus (CoV). CoVs, enveloped virus, possess nonsegmented, positive (+) ssRNA genome with structural proteins: Spike (S) glycoprotein, membrane (M) protein, nucleocapsid (N) protein, and envelope (E) protein. SARS-CoV-2 S protein attaches to angiotensin-converting enzyme 2 (ACE2) receptor on the host cell to entry. After the attachment, host endosomal proteases mediate the virus membrane-endosome fusion for the release of the viral genome. Chloroquine (CQ) and hydroxycloroquine (HCQ) block the virus-receptor binding and virus-endosome fusion. Besides CQ, HCQ, and intravenous immunoglobulin (IVIg) inhibit the production of cytokines in macrophages and the antigen presentation in dendritic cells. In COVID-19, the count of neutrophils and leukosytes increase whereas the total count of lymphocytes CD4+ T cells, CD+8 T cells, regulatory T (T reg) cells, memory T cells, natural killer cells, and B cells decrease. Another beneficial effect of CQ and HCQ is increasing the activity of Treg. The aberrant proinflammatory cytokine production is observed in COVID-19. Several immunomodulatory therapies including interleukin (IL)-6 antagonists, granulocyte colony-stimulating factor (GM-CSF) inhibitor, IL-1 antagonists, IL-17 antagonists, and antitumor necrosis factor (TNF) agents might be used for this cytokine storm to resolve and limit the further inflammation and tissue damage (The yellow arrow indicates a decrease in the number of cells; the blue arrow indicates and increase in the number of cells). X (red) This agents are not recommended by FDA, CDC and WHO. Credit/Source: Tufani and colleagues [7b] This work is licensed under a Creative Commons Attribution 4.0 International License.

States does not recommend the use of interleukin (IL)-1 inhibitors against COVID-19 [15].

Anti IL-17 Antagonists

One of the cytokines found abundant in COVID-19 patients is IL-17, which is also associated with severe lung inflammation [8]. IL-17 has wide-ranging proinflammatory effects on induction of cytokines; IL-1β, IL-6, TNF-α; growth factors, G-CSF; chemokines; and matrix metalloproteinases. In a mouse model, it was found that H1N1 cause acute lung injury in an IL-17-dependent manner. It has been postulated

Figure 3. Immune resoponses against COVID-19.



Credit/Source: Li and colleagues [7c].

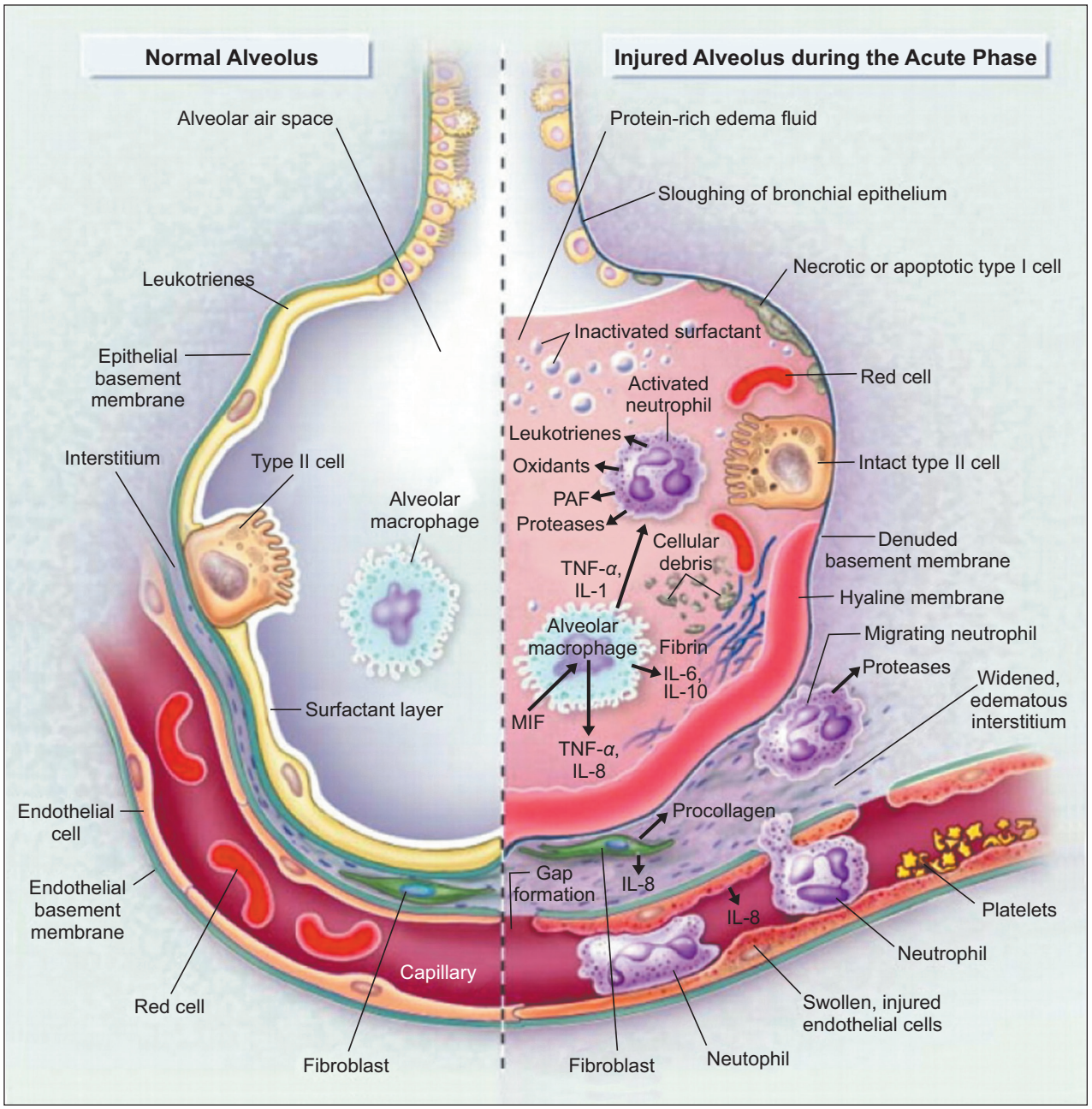
that blocking this cytokine may be effective in reducing SARS-CoV-2 related organ damage [24].

The COVID-19 Treatment Guidelines Panel recommends the use of interferons in patients with severe COVID-19 only in a clinical trial. There are insufficient data to recommend either for or against the use of interferon-beta for the treatment of COVID-19 in any phase of the disease [15].

IFN-λ[12]

IFN-λ primarily activates epithelial cells and reduces the mononuclear macrophage-mediated proinflammatory activity of IFN-αβ [13, 25, 26], and also hinders the recruitment of neutrophils to the sites of inflammation [25]. SARS-CoV and MERS-CoV mainly infect alveolar epithelial cells (AEC), and IFN- λ is able to activate the antiviral genes in epithelial cells, thereby exerting antiviral effects without overstimulating the human immune system. For this reason, IFN-λ may be an ideal treatment, according to Wang and colleagues' study [12]. Some studies have applied pegylated and non-pegylated interferons for the treatment of HCoVs, however, the effectiveness differed significantly due to the use of different treatment regimens. Early administration of interferons has certain benefits in diminishing viral load and enhances the clinical symptoms of patients to a certain limit. Nevertheless, it fails to decrease mortality rates [25, 26].

Figure 4. Cytokine storm and immune responses by SARS-CoV-2 severe infection.



The normal alveolus (Left-Hand Side) and the injured alveolus in the acute phase of acute lung injury and the acute respiratory distress syndrome (Right-Hand Side).
Credit/Source: Bakowitz and colleagues [7c].

IFN-αβ Inhibitors [12]

IFN-αβ restricts viral replication by inducing the IFN-stimulated gene. Nevertheless, IFN-αβ also intensifies diseases through intensifying the recruitment and function of mononuclear macrophages and other innate immune cells. Although an early interferon response has a protecting impact on mice infected with SARS-CoV, delayed IFN-αβ signaling produces an imbalance of the anti-SARS-CoV immune responses in humans. This event means that the

timing of IFN treatment is essential to the outcome of diseases. Based on these results, IFN-αβ receptor blockers or antagonists should be applied in the later phases of critical disease to restrict overexpressed inflammatory responses [27].

Interferon-α (IFN-α) [27, 28]

The IFN-α, a broad-spectrum antiviral drug licensed for the treatment of viral hepatitis, is applied to treat the COVID-19 at a dose of 5 million units through vapor inhalation two times a day alone or in combination with ribavirin (500 mg 2-3 times a day) and antiviral drugs lopinavir/ritonavir (400 mg/100 mg) for a period of 10 days [30]. Earlier, the combination of IFN-α 2a, ribavirin, and lopinavir/ritonavir was used as triple therapy for MERS-CoV in South Korea [31]. It was observed that the SARS-CoV-2 is more susceptible to IFNs as compared to SARS-CoV as the inhalation of IFN-α 2b decreased the infection rate significantly [28] and it can be used for prophylaxis of SARS-CoV-2 infection [33]. However, more studies should be done.

Interferon-β (IFN-β)

The other interferon, IFN-β was first developed for the chronic obstructive pulmonary disorder (COPD) and is recognized to enhance the lung's condition and improve its capacity to combat viral infections. Earlier, it was stated that a reduction in the production of INF-β is straight connected to increased susceptibility of people to develop severe respiratory diseases caused by viral infections [28]. It was also observed that the SARS-CoV-2 infection represses the production of INF-β in the body which results in protection from the immune system [32]. Recently, Synairgen, a UK biotechnology company, has been given the approval to lead a trial using IFN-β on patients with COVID-19 [33]. The advantage with IFN-β is that it can be inhaled alike to IFN-α and can be administered by patients themselves.

Monoclonal Antibodies [1]

Monoclonal antibodies affect inflammatory cytokines and other innate immune responses and represent another possible class of adjunctive therapies for COVID-19. The reason for their use is that the underlying pathophysiology of well-known organ injury in the lungs and other organs because of the overexpressed immune response and cytokine release, or "cytokine storm" [34]. IL-6 seems to be a key role in this dysregulated inflammation based on the first case reports from China [35]. So, monoclonal antibodies against IL-6 could theoretically depress this cytokine process and improve clinical outcomes.

IL-6 Antagonists

IL-6 receptors ubiquitously expressed in almost all immune cells, and IL-6 acts as a master player inducing proliferation and differentiation of immune cells. In healthy individuals, the IL-6 levels in circulation are extremely low and are in the range of 1–5 pg/mL, marked elevations reported in many inflammatory conditions including cytokine release syndrome [35]. Several therapeutic agents have been developed inhibiting the cytokine itself, the signaling via the IL-6 receptor, or its postreceptor downstream signaling pathways (JAK/STAT). Tocilizumab, sarilumab, siltuximab are IL-6 antagonists with different pharmacologic properties. Tocilizumab is approved for the treatment of Rheumatoid Arthritis (RA), juvenile idiopathic arthritis, giant cell arteritis, cytokine release syndrome, and idiopathic multicentric Castleman's disease (iMCD), whereas siltuximab received approval for iMCD and sarilumab for RA only [21].

COVID-19 patients have high plasma IL-6 levels, especially those with more severe disease presentation [36]. IL-6 production can be stimulated by SARS-CoV-2 itself or by stimulation of other immune cell [37]. Indeed, it has been shown that during COVID-19, CD4+T lymphocytes are rapidly activated to differentiate into pathogenic Th1 cells, generating GM-CSF and other proinflammatory cytokines, which further induced activation of monocytes with high expression of IL-6 [38]. In clinical view, there is striking correlation between serum IL-6 levels and SARS-CoV-2 RNAaemia, which strongly indicates worse outcome [38].

Besides the cytokine storm, recent studies in experimentally infected animals suggest a crucial role for virus-induced immunopathological events in causing fatal pneumonia after coronavirus infections [40]. Hence, blocking IL-6 would potentially reduce the detrimental immune response caused by SARS-CoV-2.

However, there is no robust evidence to routinely suggest IL-6 antagonists. A small clinical trial in China examined the effectiveness of tocilizumab in 21 patients who met the criteria for severe or critical COVID-19, including respiratory failure, requiring mechanical ventilation, shock, or admission to the ICU with multiple organ failures. Tocilizumab improved hypoxemia, fever, lymphopenia, CRP, and lung infiltration in most of the patients treated, without serious adverse events [41]. Recently, the favorable outcome of a patient with limited cutaneous systemic sclerosis under treatment with tocilizumab was reported [42].

Since there is an urgent need for the severe COVID-19 treatments, based on these limited data, tocilizumab is included in the treatment algorithms of many countries. The dose and timing for infusions are not determined yet. Numerous studies are ongoing to assess the efficacy of tocilizumab, sarilumab, and siltixumab in several countries. Current practice is to give tocilizumab 4–8 mg/kg (maximum 800 mg) as single infusion. After careful evaluation of disease severity and response to initial treatment a repeat infusion can be administered at the same dose after 12–24 h. IL-6 antagonists increase the risk of infections, therefore must be used in severe patients and at the end of the high viral load phase of COVID-19, along with antiviral treatments [43]. There are other side effects including intestinal perforation and opportunistic infections. Therefore, it is prudent to monitor patients for potential side effects.

According to the COVID-19 Treatment Guidelines Panel, there are insufficient data to recommend either for or against the use of interleukin-6 (IL-6) inhibitors (e.g., sarilumab, siltuximab, tocilizumab) for the treatment of COVID-19 [15].

Tocilizumab

Tocilizumab (branded as Actemra) is a humanized mAb developed by Roche and Chugai Pharmaceutical for treating RA and systemic juvenile idiopathic arthritis patients. At the time of publishing this article, ClinicalTrials.gov listed 20 planned studies that included tocilizumab treatment arm, all of them at the recruiting stage or earlier. A study published in April 2020 reported that 21 severe or critical COVID-19 patients in China were treated with the compound, with 20 of them recovered at the time of publication and 1 on the way to recovery (but still in ICU). Encouraged by these results, a larger multicenter clinical trial was launched (ChiCTR2000029765) and had about 500 patients treated with tocilizumab already enrolled [44].

Sarilumab

Sarilumab (branded as Kefraza), a humanized mAb, was developed by Regeneron Pharmaceuticals and Sanofi for treatment of rheumatoid arthritis (RA). A phase 2/3 randomized double-blind placebo-controlled clinical trial was planned by Regeneron Pharmaceuticals and Sanofi (and in partnership with Northwell Health's Feinstein Institutes for Medical Research) for March 2020 targeting to enroll 400 COVID- 19 patients, measuring percent change in C-protein (Phase 2 only) and time to improvement on a 7-point scale (based on death and type of hospitalization) in patients with serum IL-6 level above a threshold as primary endpoints.As of the time of this publication, the results of this study have not been made public [45].

Mavrilimumab

As mentioned, GM-CSF is one of the key molecules involved in cytokine storm which is excessively released in COVID-19 patients [46]. Blockage of this growth factor may halt immunopathology caused by virus. Mavrilimumab is a GM-CSF inhibitor developed for the refractory RA [48] and a new trial is investigating its efficacy in COVID-19 (NCT04337216).

Colchicine

Colchicine has been approved for gout and familial Mediterranean fever. In current years, colchicine has attracted attention in the management of cardiovascular diseases by suppressing its inflammatory segment [48]. Its mechanism of activity is thought to be the restraint of tubulin polymerization and microtubule formation and, perhaps, consequences on cellular adhesion molecules, inflammatory chemokines, and the inflammasome. Colchicine may repress the activation of NLRP3 inflammasome and additionally may inhibit directly the synthesis of TNF-α and IL-6 [49]. Trials are investigating the effectiveness of conventional therapeutic doses of colchicine for the treatment of COVID-19 (NCT04322682, NCT04328480, NCT04326790). However, Gendelman and colleagues [51] in a study from April 2020 with an overall sample of 14,520 subjects screened for SARS-CoV-2 infection and 1,317 positive, showed no significant difference in terms of rates of usage of hydroxychloroquine or colchicine between those who were found positive for SARS-CoV-2 and those who were found negative (0.23% *versus* 0.25% for hydroxychloroquine, and 0.53% *versus* 0.48% for colchicine, respectively). These findings raise doubts regarding the protective role of these medications in the battle against SARS-CoV-2 infection.

Janus Kinase (JAK) Inhibitors

JAK inhibitors are potent inhibitors of one or more of the JAK family of enzymes (JAK1, JAK2, JAK3, TYK2), thereby interfering with the JAK-STAT signaling pathway. The JAK/STAT pathway mediates the effect of many different molecules, including interleukins (IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-15, IL-21, IL-23), IFN-(α, β, γ) and growth factors (GM-CSF, TGF-β, erythropoietin and thrombopoietin) [24]. JAK inhibitors are currently approved for the treatment of RA and psoriatic arthritis and their use in other inflammatory disorders are continuously growing [51]. Many proinflammatory cytokines involved in cytokine storm of COVID-19 might be inhibited by JAK inhibitors.

Baricitinib

Besides above mentioned common properties of JAK inhibitors, baricitinib may block AP-2-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK) which are host kinases that regulate viral endocytosis, according to an artificial intelligence search of viral characteristics of SARS-CoV-2. This effect is only restricted to baricitinib among other JAK inhibitors and it may block viral entry and assembly of virus particles into pneumocytes in therapeutic doses used in RA [52]. However, these hypothetical views merit further evidence for clinical use both for cytokine storm and COVID-19. Currently, baricitinib (NCT04320277, NCT04340232, NCT04321993), tofacitinib (NCT04332042) and ruxolitinib (NCT04331665) studies are ongoing.

Inhibitors of Mononuclear Macrohage Recruitment and Function [12]

An autopsy of patients with COVID-19 showed many inflammatory cell infiltration in the lungs of the deceased [53]. One potentially powerful treatment approach is to decrease the recruitment of mononuclear macrophages to the site of inflammation by small interfering RNA (siRNA)-mediated silencing of C-C chemokine receptor type 2 (CCR2) to improve the outcome of the disease, which has been demonstrated in animal experiments [54, 55]. Toll-like receptor 7 (TLR7) agonists stimulate mononuclear macrophages to undergo a strong inflammatory response at the time of infections such as HCoV. Hence, TLR7 antagonists may be effective to relieve the storm of inflammatory factors caused by SARS-CoV-2 infection.

Intravenous Immunoglobulin (IVIg)

IVIg is a blood product containing polyclonal immunoglobulin G isolated and pooled

from healthy donors used to treat Immune Thrombocytopenic Purpura (ITP), Kawasaki disease and various inflammatory neurologic and myositis syndromes. It has immunomodulatory functions with unknown mechanism of action. One of the proposed mechanisms is the interaction of IgG-Fc with Fc gamma receptors located on almost all immune cells, resulting in pleiotropic functional consequences including the expansion of regulatory T cell population, phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), immune cell differentiation and maturation, apoptosis, expression of proinflammatory cytokines, and antigen-presentation [56]. Previous studies on SARS and MERS, found that IVIg therapy was effective thus proposing high-dose IVIg as an option for severe COVID-19 patients [57]. There are a few COVID-19 cases which reported efficacy of high dose IVIg [58]. However, its high cost and limited supply restrict its general use. Inferred from rheumatic diseases, COVID-19 patients with pregnancy, secondary infections, marked thrombocytopenia, muscular, myocardial and neurologic manifestations would be better candidates for IVIg treatment. There are several studies already registered for its use in COVID-19.

Inhibitors of Cell Entry of SARS-CoV-2 [58]

Inhibitors of TMPRSS2 Serine Protease

Results from previous studies reveal that diverse viruses, including Ebola virus, SARS-coronavirus (SARS-CoV), MERS-coronavirus (MERS-CoV) and influenza virus employ host cell proteases for activation of their envelope glycoproteins [59, 60]. Cleavage and activation of the spike protein (S protein) of SARS-CoV that is required for membrane fusion and host cell entry is mediated by transmembrane protease/ serine subfamily member 2 (TMPRSS2), an airway and alveolar cell serine protease [61, 62]. Hoffmann and colleagues [63] recently demonstrated that SARS-CoV-2 also employs TMPRSS2 for SARS-CoV-2 S protein priming and S protein-driven cell entry. Using camostat mesilate, a clinically proven and commercial serine protease inhibitor that partially blocks infection by SARS-CoV and HCoV-NL63 in HeLa cell expressing ACE2 and TMPRSS2 [64], it was shown that inhibition of TMPRSS2 in human lung Calu-3 cells by camostat mesilate significantly reduced infection with SARS-CoV-2. Camostat, (FOY-305),[N,N-dimethylcarbamoylmethyl 4-(4-guanidinobenzoyloxy)-phenylacetate] methanesulfate and camostat mesilate (Foipan™), alternatively termed camostat mesylate, (NI-03), (CAS number: 59721−28-7), constitute synthetic serine protease inhibitors that were developed decades ago for the treatment of oral squamous cell carcinoma [65], dystrophic epidermolysis [66], exocrine pancreatic enzyme inhibition [67], and chronic pancreatitis [68]. Camostat mesilate (NI-03) is manufactured as an oral drug by Nichi-Iko Pharmaceutical Co., Ltd., and Ono Pharmaceutical, Japan, with a three times daily dose recommendation of 100 mg–300 mg [69]. In a clinical trial investigating camostat mesilate against dyspepsia associated with non-alcoholic mild pancreatic disease, 95 patients received 200 mg camostat mesilate three times daily for 2 weeks and showed only mild, but no severe adverse effects [70], indicating that camostat mesilate is a well-tolerated drug.

Nafamostat Mesilate (BUIPEL™)

Nafamostat mesilate (Buipel™), (6-amidino-2-naphthyl-4-guanidino benzoate-dimethanesulfonate) (FUT-175), (CAS number: 81525−10- 2), is a clinical proven and synthetic serine protease inhibitor approved in Japan for the treatment of acute pancreatitis, disseminated intravascular coagulation and for anticoagulation in extracorporeal circulation [71, 72]. In a screening approach of about 1,100 drugs approved by the FDA, nafamostat mesilate has been identified to inhibit MERS-CoV S protein-mediated viral membrane fusion with TMPRSS2- expressing lung Calu-3 host cells by inhibiting TMPRSS2 protease activity [58]. Since the S proteins of MERS-Cov

and SARS-CoV-2 share considerable amino acid sequence homology [72, 73], nafamostat mesilate may also inhibit cell entry of SARS-CoV-2. In cell culture experiments with simian Vero E6 cells infected with SARS-CoV-2, nafamostat mesilate was shown to be inhibitive against SARS-CoV-2 infection at EC50 of 22.50 μM [74], suggesting that nafamostat mesilate is able to prevent SARS-CoV-2 infection. In a multicenter, randomized, open-label, phase 2 trial in 19 patients with severe acute pancreatitis, nafamostat mesilate was administered intravenously at a daily dose of 240 mg for 5 days without severe adverse effects.

Inhibitors of Angiotensin-Converting Enzyme 2 (ACE2), Antimalarial/Parasiticide Drugs

SARS-CoV and related coronaviruses directly interact via their S proteins with angiotensin-converting enzyme 2 (ACE2), a host cell exopeptidase and metallocarboxypeptidase that catalyses the conversion of angiotensin I to the nonapeptide angiotensin and the conversion of angiotensin II to angiotensin 1–7, to initiate S protein-mediated cell entry [75, 76]. It was demonstrated recently that also SARS-CoV-2 uses ACE2 as a receptor for S protein-driven host cell entry [62, 73]. Therefore, ACE2 constitute a molecular target to inhibit cell entry of SARS-CoV-2. Unfortunately, ACE inhibitors as standard drugs for the treatment of hypertension and chronic heart failure fail to inhibit ACE2 [77], but a number of other drugs and compounds have been shown to inhibit ACE2.

Antimalarial Drugs (Table 1 attached)

These drugs also fall under three categories based on their mode of action aryl amino-alcohol compound, antifolate compound and artemisinin. Most of these drugs are eliminated gradually from the body remaining for long periods of time after intake. A disadvantage of this drug is that antimalarial drug resistance develops for any drugs under this category [78].

Chloroquine

Chloroquine (CQ), a drug widely used in treating malarial and autoimmune diseases, also confers considerable broad-spectrum antiviral effects even against SARS-CoV [79-81]. A recent study demonstrated that CQ has anti-SARS-CoV-2 activity *in vitro* [82]. A subsequent letter in Bioscience confirmed that CQ is efficacious in treating COVID-19 pneumonia in numerous related clinical trials. CQ therapy resulted in improved pulmonary lesions, shortened disease course, and good outcomes [83]. Given the apparent efficiency displayed by CQ in clinical practice, CQ has been included in the Guidelines for the Diagnosis and Treatment of COVID-19 (7th edition) issued by the National Commission of the People's Republic of China (NHPFC, 2020).

Hydroxychloroquine

Hydroxychloroquine sulfate (HCQ) shares a similar chemical structure and mechanisms of action with CQ but with lower ocular toxicity [84] and has proven efficacious in containing SARS-CoV-2 *in vitro* [85]. CQ and HCQ exert antiviral function through various mechanisms. CQ has been shown to interfere with the glycosylation process of ACE2 in host cells, thereby inhibiting the efficiency of the binding of S protein with ACE2, in turn disrupting the virus/cell fusion process [80] CQ can increase the pH of acidic cellular organelles required for virus entry into host cells [86] In addition to its direct antiviral activity, CQ and HCQ can attenuate major "cytokine storms" (an overreaction of the immune system causing inflammatory "storms") by decreasing cytokine production (interleukin [IL]-1, IL-6, and tumor necrosis factor [TNF], etc.) [87].

Chloroquine and Hydroxychloroquine

Chloroquine phosphate (Resochin™) and its derivative hydroxychloroquine (Quensyl™, Plaquenil™, Hydroquin™, Dolquine™, Quinoric™) have been used for decades for the prophylaxis and treatment of malaria and for the treatment of chronic Q fever and various

autoimmune diseases [88], and have been demonstrated as potential broad-spectrum antiviral drugs [80].

Chloroquine phosphate inhibits terminal phosphorylation of ACE2, and hydroxychloroquine elevates the pH in endosomes which are involved in virus cell entry [89, 90], both mechanisms constitute relevant antiviral mechanisms of chloroquine and hydroxychloroquine, respectively. *In vivo*, hydroxychloroquine is metabolized into chloroquine. Chloroquine phosphate has previously been shown to inhibit SARS-CoV infection and spread *in vitro* [89, 91], and results from very recent studies reveal that chloroquine phosphate [83].

However, on June 15, 2020, the FDA removed the use authorization (EUA) for hydroxychloroquine and chloroquine provided to the Strategic National Stockpile to be applied for treating hospitalized patients with COVID-19, except for licensed clinical trials [92]. So, the FDA determined that hydroxychloroquine is unpropitious to be useful in treating COVID-19 in the EUA. Also, due to ongoing severe cardiac adverse events and toxic serious adverse effects, the known and potential advantages of hydroxychloroquine no longer exceed the known and potential risks for patients with COVID-19 [93].

While further clinical trials may proceed to evaluate possible benefits, the FDA determined in the EUA that its use was no longer appropriate.

Additionally, the NIH stopped the Outcomes Related to COVID-19 treated with Hydroxychloroquine among In-patients with symptomatic Disease (ORCHID) study on June 20, 2020. After the fourth investigation that involved more than 470 participants, the NIH data and safety monitoring board concluded that while there was no harm, the study drug was very doubtful to be advantageous to hospitalized patients with COVID-19 [NIH halts clinical trial of hydroxychloroquine. HIH Media Advisory June 20, 2020]. Hydroxychloroquine and chloroquine are widely used antimalarial drugs that obtain immunomodulatory effects and are consequently also applied to treat autoimmune conditions (eg, systemic lupus erythematosus, rheumatoid arthritis). As inhibitors of heme polymerase, they are also considered to have additional antiviral activity via alkalinization of the phagolysosome, which restrains the pH-dependent steps of viral replication. Wang and colleagues [82] reported that chloroquine efficiently inhibits SARS-CoV-2 *in vitro*. The pharmacological action of chloroquine and hydroxychloroquine was experimented with using SARS-CoV-2–infected Vero cells. Physiologically based pharmacokinetic models (PBPK) were conducted for each drug. Hydroxychloroquine was seen to be more potent than chloroquine *in vitro*. Based on PBPK models, the authors suggest a loading dose of hydroxychloroquine 400 mg PO BID, accompanied by 200 mg BID for 4 days [94].

Printed reports deriving from the beginning of the outbreak of COVID-19 have evaluated the potential application of these drugs in controlling cytokine storm in severe patients. Owing to widely differing dosage regimens, disease severity, evaluated outcomes, and the absence of control groups, effectiveness data have been widely inconclusive.

The UK RECOVERY Trial randomized 1,542 patients to hydroxychloroquine and 3,132 patients to usual care alone. Introductory results determined no significant difference in the primary endpoint of 28-day mortality (25.7% hydroxychloroquine vs 23.5% usual care; hazard ratio 1.11 [95% CI, 0.98-1.26]; $p = 0.10$). There was also no proof of useful effects on hospital stay duration or other outcomes [95].

A multicenter, randomized, open-label trial in Brazil discovered no improvement in 504 hospitalized patients with mild-to-moderate COVID-19. The application of hydroxychloroquine, alone or with azithromycin, did not change clinical status at 15 days compared with standard care. Prolonged QTc interval and raised liver enzyme levels were more frequent in patients receiving hydroxychloroquine, alone or with azithromycin than in those who were not taking either agent [96].

An observational study, conducted between March 1, 2020, and April 22, 2020, with follow-up through May 5, 2020, with 2,512 hospitalized patients in New Jersey with confirmed COVID-19 patients presented 547 deaths (22%) and 1,539 (61%) discharges; and 426 (17%) remained hospitalized. From them, who took at least one dose of hydroxychloroquine totaled 1,914 (76%), and those who received hydroxychloroquine plus azithromycin totaled 1,473 (59%). No significant differences were recognized in associated mortality among patients receiving any hydroxychloroquine during the hospitalization (HR, 0.99 [95% CI, 0.80-1.22]), hydroxychloroquine alone (HR, 1.02 [95% CI, 0.83-1.27]), or hydroxychloroquine with azithromycin (HR, 0.98 [95% CI, 0.75-1.28]). The 30-day unadjusted mortality rate in patients taking hydroxychloroquine alone, azithromycin alone, and the combination of these drugs, or neither drug was 25%, 20%, 18%, and 20%, respectively [97].

After these studies, the WHO stopped the hydroxychloroquine arm of the Solidarity Trial and then removed its application completely as of July 4, 2020 [98]. The FDA announced a safety alert for hydroxychloroquine or chloroquine use in COVID-19 on April 24, 2020, and removed its use in EUA on June 15, 2020 [99]. "The COVID-19 Treatment Guidelines Panel (the Panel) recommends against the use of chloroquine or hydroxychloroquine for the treatment of COVID-19, except in a clinical trial (AII). The Panel recommends against the use of high-dose chloroquine (600 mg twice daily for 10 days) for the treatment of COVID-19 (AI). As well as, The COVID-19 Treatment Guidelines Panel recommends against using hydroxychloroquine plus azithromycin for the treatment of COVID-19, except in a clinical trial (AIII) [15]."

Antiparasiticide

Ivermectin [100]

Ivermectin is a potent anthelmintic drug that was first discovered to hinder interaction between integrase (IN) molecule of human immunodeficiency virus (HIV)-1 and its nuclear transport receptor importin α/β [101]. Further studies exhibit its potential to prevent viral replication of a broad spectrum of viruses, including dengue virus, flavivirus, and influenza [102, 103]. Ivermectin, an antiparasitic drug, has exhibited restraint against SARS-CoV-2 up to 5,000-fold at 48h *in vitro*. Inhibition of IMPα/β1-mediated nuclear import of viral proteins is proposed as the presumable cause of its antiviral action [104]. So, it presented decrease of viral RNA in Vero-hSLAM cells 2 hours postinfection with SARS-CoV-2 clinical isolate Australia/VIC01/2020 *in vitro* [105]. This is an introductory study, which not translate to human use since the efficient dose is not established at this initial phase of discovery. More study is required to conclude if an antiviral effect would be obtained for humans, as the concentrations tested were much higher than what is realized from the normal oral dose [106].

Chaccour and colleagues [107] consider the recent findings regarding ivermectin permit rapid implementation of controlled clinical trials to evaluate efficacy against COVID-19. They also are worried about ivermectin-associated neurotoxicity, particularly in patients with a hyperinflammatory event possible with COVID-19. In addition, drug interactions with potent CYP3A4 inhibitors (eg, ritonavir) warrant careful consideration of coadministered drugs. Lastly, data infers that ivermectin plasma levels with significant action against COVID-19 might not be obtained without potentially toxic raises in ivermectin doses in humans. More data is needed to assess pulmonary tissue levels in humans [107, 108]. And, also, it seems that ivermectin does not interfere with the severity and mortality rate of patients.

Cepharanthine/Selamectin/Mefloquine Hydrochloride

The triple combination of cepharanthine (an anti-inflammatory alkaloid from Stephania cepharantha Hayata), (CAS number: 48,104,902), selamectin (an avermectin isolated from Streptomyces avermitilis and used as an anti-helminthic

and parasiticide drug in veterinary medicine), (CAS number. 220119−17-5), and mefloquine hydrochloride (Lariam™, used for the prophylaxis and treatment of malaria) [109, 110] has recently been shown to inhibit infection of simian Vero E6 cells with pangolin coronavirus GX_P2V/2017/Guangxi (GX_P2V), whose S protein shares 92.2 % amino acid identity with that of SARS-CoV-2 [111]. It has also been demonstrated that GX_P2V also uses ACE2 as the receptor for viral cell entry. Two libraries of 2406 clinically approved drugs were screened for their ability to inhibit cytopathic effects on Vero E6 cells by GX_P2V, and only the combination of cepharanthine, selamectin and mefloquine hydrochloride was identified as candidate drug combination against SARS-CoV-2 infection [111].

Neutralizing Antibodies Against SARS-CoV-2 [112]

Currently, polyclonal antibodies from recovered SARS-CoV-2-infected patients have been applied to treat SARS-CoV-2 infection, but no SARS-CoV-2-specific neutralizing mAbs have been announced. Researchers are working hard to produce such mAbs and/or their functional fragments as putative prophylactic or therapeutic agents to prevent or treat COVID- 19. Once such antibodies are conceived, the next steps will include *in vitro* testing for neutralizing and/or cross-neutralizing activity, *in vivo* evaluation in animal models for protecting efficiency, preclinical studies, and clinical trials testing the security and efficacy before they are approved for clinical application. Hence, it may take one to many years for such SARS-CoV-2 neutralizing mAbs or their fragments to be available for human use.

However, since SARS-CoV-2 is closely related to SARS-CoV and since their S proteins have high sequence identity [113], researchers have attempted to discover SARS-CoV nAbs with potential cross-reactivity and/or cross-neutralizing activity against SARS-CoV-2 infection. Notably, a SARS-CoV RBD-specific human neutralizing mAb, CR3022, could bind SARS-CoV-2 RBD with high affinity and recognize an epitope on the RBD that does not overlap with the ACE2-binding site [114]. In addition, sera from convalescent SARS patients or from animals specific for SARS-CoV S1 may cross-neutralize SARS-CoV-2 infection by reducing S protein-mediated SARS-CoV-2 entry [63]. Moreover, SARS-CoV RBD-specific polyclonal antibodies have cross-reacted with the SARS-CoV-2 RBD protein and cross-neutralized SARS-CoV-2 infection in HEK293T cells stably expressing the human ACE2 receptor, opening avenues for the potential development of SARS-CoV RBD-based vaccines that might eventually prevent SARS-CoV-2 and SARS-CoV infection [115]. It is also possible that SARS-CoV RBD-targeting nAbs might be applied for prophylaxis and treatment of SARS-CoV-2 infection in the current absence of SARS-CoV-2-specific vaccines and antibodies. However, robust testing lies ahead.

Convalescent Plasma [116] (Table 1 attached)

In this procedure, plasma or purified monoclonal antibodies generated against COVID-19 are collected from recovered patients and delivered to new patients as treatment. From 20 January to 25 March 2020, convalescent plasma treatments were administered to five critically sick COVID-19 patients in Shenzhen, China [117]. In this study, patients took convalescent plasma with a SARS-CoV-2-specific antibody between 10 and 22 days after admission. Among the five patients, four of them presented a reduced score in sequential organ failure assessments and viral loads. Their viral test also became negative within 12 days after the transfusion. These four patients were also removed from mechanical ventilation within 2 weeks of treatment. Lastly, three patients were discharged from the hospital in approximately 50 days. Although this trial has a small sample size, the results of convalescent plasma treatment are still hopeful. This method has been suggested as a treatment option in the US [118].

A Cochrane review of convalescent plasma use in patients with COVID-19 is perpetually being updated as data emerge. As of July 10, 2020, the review included 20 studies with 5,443 participants, of whom 5,211 received convalescent plasma. Among these studies was one randomized controlled trial with 103 participants (52 received convalescent plasma). The authors of this review expressed doubt as to the advantages of convalescent plasma in terms of influencing mortality at hospital discharge, prolonging time to death, or enhancing clinical symptoms at 7 or 28 days [119].

A meta-analysis of 15 controlled studies revealed a significantly lower mortality rate in patients with COVID-19 who received convalescent plasma compared with control groups. Nevertheless, the authors point out that the studies were of very low quality and a moderate or high risk of bias [120].

An open-label study (n = 103) with COVID-19 patients in Wuhan, China, who received convalescent plasma did not result in a statistically significant change in time to clinical improvement within 28 days compared with standard of care [121].

A nonrandomized study transfused patients with convalescent plasma based on supplemental oxygen needs. Supplemental oxygen requirements and survival were compared between plasma recipients and controls. Outcomes determined that convalescent plasma transfusion enhanced survival in non-intubated patients ($p = 0.015$), but not in intubated patients ($p = 0.752$) [122].

Hyperimmune Globulin Therapy [123]

Extending experimental findings of CoV in animal models [124] to infer the risk of antibody-enhanced immunopathology in humans is more nuanced. There are likely major differences between vaccine induced *vs* infection-induced antibodies. It is well known that innate immune response drives adaptive immune responses [125-127]. As SARS-CoV-2 infection is known to induce cytokine and chemokine expression, convalescent COVID-19 patients would likely produce antibodies that are qualitatively and quantitatively different to those elicited by vaccination alone. Although vaccines could also elicit innate immune responses, the magnitude would likely be significantly lower than those found in acutely ill COVID-19 patients since the disease is probably mediated by a pro-inflammatory cytokine response [50]. Moreover, the antigenic burden of wild-type SARS-CoV-2 infection can be expected to be significantly greater than those derived from vaccination. The level of such antigenic burden is known to drive adaptive immune responses, including neutralizing antibody titers [128, 129]. Consequently, extending findings from vaccine studies to infer the risk from hyperimmune globulin ignore the possibility of differences between the quality and titer of antibodies produced from infection and vaccination. This same explanation was also found to underpin how inactivated measles vaccination increases the risk of atypical pneumonia [130].

Early anecdotal reports that infusion of convalescent plasma to acutely ill COVID-19 patients suggest the potential of hyperimmune globulin as a treatment to halt the progression of infection to severe pulmonary disease. Hyperimmune globulin treatment, besides inhibiting viral infection, could also downregulate pro-inflammatory responses and reduce disease severity in COVID-19 patients. Intravenous immunoglobulin (IVIG) infusion is associated with anti-inflammatory responses [131], including those from viral infection [132]. Mechanistically, how this anti-inflammatory effect is mediated remains to be fully defined. High dose antibodies could bind a number of different inhibitory receptors, including the inhibitory Fc gamma receptor IIB (FcgRIIB) [129, 130], FcgRIIC [135, 137] or other receptors [141] to induce the anti-inflammatory response. Regardless of the mechanism, the presence of neutralizing SARS-CoV-2 antibodies as well as high concentration of total antibodies could produce anti-inflammatory rather than the postulated immunopathology enhancement effects in COVID-19 patients to improve prognosis.

Antiinflammatory Therapy [7]

Corticosteroids

Systemic corticosteroids have broad-spectrum actions on the immune system that may suppress the exuberant systemic inflammatory response that occurs in ARDS. Severe multi-source systemic inflammation is associated with adverse outcomes, so one may think that corticosteroids may of benefit with their broad spectrum immunosuppressive effects. However, evidence has shown that use of corticosteroids delayed viral clearance in SARS and MERS infections, similarly they increased secondary infection rates, mortality and complications of steroid therapy in survivors of influenza pneumonitis [142]. In a randomized controlled trial that included 16 non-ICU SARS patients, "early" (<7 days of illness) hydrocortisone therapy was associated with a higher subsequent plasma viral load. Therefore, corticosteroids should not be used early phases of disease unless there is a clear indication for their use [139]. In SARS infection, some patients showed severe inflammatory features despite reductions in viral load with subsequent seroconversion, suggestive of exuberant immune response independent of viral load [140]. In two small observational study, use of corticosteroids did not show a survival benefit in COVID-19 patients even increased mortality rates when used in high doses [141, 142]. Moreover, corticosteroid use was prolonged SARS-CoV-2 RNA shedding as observed in SARS and MERS infections [143]. In the light of preliminary data, corticosteroids are more likely to function on inflammation-mediated lung injury and interstitial fibrosis at late-stage of ARDS [144]. However, the dose, duration, and timing of corticosteroids must be individualized considering risk-benefit ratio, until results of ongoing well-designed prospective cohort studies obtained. At present, several studies are registered to assess the efficacy of corticosteroids in COVID-19 [145].

Methylprednisolone

As a potent anti-inflammatory and anti-fibrotic drug, low doses of methylprednisolone (DEPO-Medrol or SOLUMedrol) have the potential to prevent an extended cytokine response and may accelerate resolution of pulmonary and systemic inflammation in pneumonia [138, 145]. Recently, many medical researchers believe that corticosteroids, especially methylprednisolone, may improve dysregulated immune response caused by sepsis (possible complication of infection with COVID-19) and increase blood pressure when it is low [146]. Specifically, in a retrospective cohort study, 201 patients with confirmed COVID-19 who developed ARDS were treated with methylprednisolone (1–2mg/kg daily IVfor 5–7days) and the results showed that treatment with methylprednisolone may be beneficial for patients who develop ARDS in the reduction of the risk of death. Briefly, of those patients with ARDS who received methylprednisolone treatment, 23 of 50 (46%) patients died, while those who did not receive methylprednisolone, 21 of 34 (61.8%) died [147]. In another study, 46 patients with severe COVID-19 that progressed to acute respiratory failure, use of methylprednisolone was associated with improvement in clinical symptoms (i.e., fever, hypoxia) and a shortened disease course in patients who received the drug compared with those who did not [148]. Moreover, according to expert consensus statement from Chinese Thoracic Society, dosage regimen of methylprednisolone should be low to moderate (i.e., ≤ 0.5 to 1 mg/kg daily or equivalent) [149] and the most common regimens of methylprednisolone applied in China were typically 40–80 mg IV daily for a course of 3–6 days [150]. The appropriate dosage (low dose *versus* high dose), place in therapy (early *versus* late), and role for corticosteroids (cytokine storm or comorbidity management) require additional clarity. There is concern that the use of corticosteroids may have deleterious effects (i.e., inhibition of immune response and pathogen clearance) in patients with COVID-19 [138]. One study reported no effect on mortality and decreased viral clearance with the use of corticosteroids [2]. Furthermore, the Infectious Diseases Society of American recommends against the routine use

of corticosteroids in COVID-19. However, they do recommend the use of corticosteroids in the setting of ARDS in the context of a clinical trial [151]. Similarly, the Surviving Sepsis Campaign recommends against corticosteroids in mechanically ventilated patients with acute lung injury in the absence of ARDS [152]. However, they provide a recommendation for the use of corticosteroids in patients with ARDS acknowledging the weak level of evidence.

Dexamethasone (Table 1 attached)

Dexamethasone has demonstrated utility on ARDS by decreasing ventilator days and mortality on severe ARDS in patients without COVID-19 [153]. RECOVERY, a multicenter, randomized, open-label trial in hospitalized patients with COVID-19, revealed that the mortality rate was lower among patients who were randomized to take dexamethasone than with those who took the standard of care (SOC). This benefit was noted in patients who needed supplemental oxygen at enrollment. No advantage of dexamethasone was seen in patients who did not need supplemental oxygen at enrollment [154]. According to the COVID-19 Treatment Guidelines Panel [15]:

"• On the basis of the preliminary report from the Randomised Evaluation of COVID-19 Therapy (RECOVERY) trial (discussed below), the COVID-19 Treatment Guidelines Panel (the Panel) recommends using dexamethasone 6 mg per day for up to 10 days for the treatment of COVID-19 in patients who are mechanically ventilated (AI) and in patients who require supplemental oxygen but who are not mechanically ventilated (BI).

- The Panel recommends against using dexamethasone for the treatment of COVID-19 in patients who do not require supplemental oxygen (AI).
- If dexamethasone is not available, the Panel recommends using alternative glucocorticoids such as prednisone, methylprednisolone, or hydrocortisone (see Additional Considerations below for dosing recommendations) (AIII)."

Nonsteroidal Antiinflammatory Drugs (NSAIDs) [7]

An association between ibuprofen and more serious outcome in COVID-19 patients was hypothesized, however, with very low evidence [155]. In an *in vitro* study, another NSAID, indomethacin, was announced to have a direct antiviral impact on SARS-CoV by interfering with viral RNA synthesis, independent of cyclooxygenase inhibition. A trial is currently selecting patients to define the efficacy of naproxen for its potential synergy with viral nucleoproteins (NCT04325633). Hence, although evidence is insufficient, indomethacin or naproxen could be favored over other NSAIDs when indicated [156].

Antithrombotic Therapy [144]

Due to plaque rupture (i.e., type 1 MI) [158], dual antiplatelet therapy, and full-dose anticoagulation should be administered, unless there are contraindications according to the American College of Cardiology (ACC)/American Heart Association (AHA) and ESC guidelines [159, 160]. In patients with high bleeding risk, regimens with short potent antiplatelet agents, such as with clopidogrel, should be considered, since hemorrhagic complications are not rare. Special attention should be also given to drug-drug interactions between antiplatelet agents or anticoagulants and COVID-19 investigational therapies. Major interactions with parenteral antithrombotic agents and COVID-19 investigational therapies are not clearly delimited

Antibiotics

For patients with COVID-19, some physicians manage broad-spectrum antibiotics to all patients with moderate or severe hypoxemia. Other experts administer antibiotics only for specific cases, such as the presence of a lobar infiltrate on a chest x-ray, leukocytosis, high serum lactate level, microbiologic data, or shock. Gram stain

and cultures or testing of respiratory specimens are frequently not available due to concerns about aerosolization of the virus during diagnostic procedures or when processing specimens. There are no clinical trials that have estimated the use of empiric antimicrobial agents in patients with COVID-19 or other severe coronavirus infections. With influenza, empiric antibacterial treatment is strongly prescribed for patients with initial severe disease (i.e., those with extensive pneumonia, respiratory failure, hypotension, and fever) and those who worsen after initial improvement [161]. These recommendations are based on considerations that bacterial superinfections, especially those due to *Staphylococcus aureus* and *Streptococcus pneumonia*, are not unusual and have terrible consequences if not treated immediately. Whether moderate or severe COVID-19 disease should be approached like severe influenza will continue uncertain until more microbiologic and clinical data become available.

Azithromycin (Table 2 attached)

Azithromycin is an antibiotic that can be used to fight many different types of infections caused by susceptible bacteria, such as respiratory infections, skin infections, and sexually transmitted diseases [149, 150]. Moreover, it has been proven to be active *in vitro* against Zika and Ebola viruses and to prevent severe respiratory tract infections when treated to patients suffering viral infection [164, 165]. For the mechanism of action, azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome, thus inhibiting translation of mRNA. Previously, azithromycin has been used as adjunctive therapy to provide antibacterial coverage and potential immunomodulatory and anti-inflammatory effects in the treatment of some viral respiratory tract infections (e.g., influenza) [166, 167]. Currently, many trials are testing the effect of azithromycin conjunction with hydroxychloroquine on the course of disease in people with SARS-CoV-2. For example, Pfizer has announced positive data for the use of its azithromycin (Zithromax) drug, along with hydroxychloroquine, in a COVID-19 clinical trial that was performed in France. In brief, the clinical trial was conducted to assess hydroxychloroquine in 20 patients, 6 of which were co-administered with azithromycin. Compared with 16 controls and 14 hydroxychloroquine alone group, the 6 patients treated with hydroxychloroquine + azithromycin presented with highest virologic cure rate following 6-day treatment [166]. Three other clinical studies used azithromycin (500 mg on day 1, then 250 mg daily on days 2–5) co-treated with 10- day regimen of hydroxychloroquine (600 mg daily) in an open-label non-randomized study in France (6 pts) [166], open-label uncontrolled study in France (11 pts) [168], and uncontrolled observational study in France (80 pts) [168]. Specifically, Gautret and colleagues reported a 100% viral clearance in nasopharyngeal swabs in their 6 patients after co-treated of hydroxychloroquine and azithromycin [166]. But the findings reported by Molina and colleagues stand in contrast with those reported by Gautret. Molina and colleagues repeated the experiments, thought the rapid and full viral clearance was quite unexpected and found 8 of 11 patients had significant comorbidities [168]. Based on those results, data presented to date are insufficient to evaluate possible clinical benefits of azithromycin in patients with COVID-19 [168]. Furthermore, one must consider the additive cardiac toxicity of hydroxychloroquine and azithromycin. Both agents are known to prolong the QT interval and may potentiate the risk for cardiac events in a population known to have cardiac-related comorbidities.

Antiviral Drugs (Table 2 attached)

Drugs under this category usually follow either of the following three mechanisms in the virus-viral replication inhibition, ion channel inhibition and serine protease inhibition. Commercially available antiviral drugs mostly target the four major groups of viruses: human immunodeficiency virus (HIV),

herpes, hepatitis and influenza [169]. Earlier outbreak episodes of viral infections like SARS-CoV and MERS-CoV as well as hemorrhagic fever viruses like Ebola were treated with this category of drugs [170].

Ribavirin

Ribavirin is a broad-spectrum drug whose therapeutic potential was uncovered during 1972. This antiviral drug is used in the treatment of hepatitis C. It is usually used in combination with interferon α (IFN). This drug, approved by the FDA, competes for the active site of RdRp. Ribavirin scored 109.5 μM of half maximal concentration against SARS-CoV-2 [171].

Sofosbuvir

This drug is also an FDA approved drug against NS5B and acts as a nucleotide polymerase inhibitor used for the treatment of hepatitis C. It was used in combinationwith interferon or RBV. This drug was previously used for the treatment of Zika virus [172].

Remdesivir

Remdesivir is an intravenous (IV) investigational nucleotide prodrug of an adenosine analog. Remdesivir attaches to the viral RNA-dependent RNA polymerase, restraining viral replication by RNA transcription. Its activity has been presented in an *in vitro* study against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [82]. In a rhesus macaque model of SARS-CoV-2 infection, remdesivir treatment was initiated soon after inoculation; and the results showed that remdesivir-treated animals had lower virus levels in the lungs and less lung injury than the control animals [173]. According to The COVID-19 Treatment Guidelines Panel [15], remdesivir stocks are insufficient, so the Panel recommends that it be prioritized for use in hospitalized patients with COVID-19 who need supplemental oxygen but who are not on high-flow oxygen, noninvasive ventilation, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO).

On May 1, 2020, The FDA approved the use of remdesivir for severe COVID-19 (confirmed or suspected) in hospitalized adults and children [174, 175]. A phase 1b trial of an inhaled nebulized version was beginning in late June 2020 to decide if remdesivir can be utilized on an outpatient basis and at earlier phases of COVID-19 [176].

Remdesivir was studied in clinical trials for Ebola virus infections but presented a limited benefit [177]. Remdesivir has been revealed to restrain the replication of other human coronaviruses associated with high morbidity in tissue cultures, including severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. Effectiveness has been shown for SARS-CoV and MERS-CoV in animal models [178].

A phase 3 clinical trials are testing remdesivir for the treatment of COVID-19 in the United States, South Korea, and China, and positive results were presented. The drug was prescribed under an open-label use protocol, but the FDA has since changed and permit enlarged access to remdesivir, allowing established places to prescribe the product for multiple patients under protocol without inquiring permission for each. An adaptive randomized trial of remdesivir directed by the National Institute of Health (NCT04280705) was begun first against placebo, but additional therapies can be combined with the protocol as data emerges. The first experience with this study included travelers of the Diamond Princess cruise ship in quarantine at the University of Nebraska Medical Center in February 2020 after turning back to the United States from Japan following an onboard outbreak of COVID-19. Trials of remdesivir for moderate and severe COVID-19 correlated with standard of care and differing treatment durations are continuing.

The preliminary data analysis of these studies of the Adaptive COVID-19 Treatment Trial (ACTT) was declared on April 29, 2020.

The analysis included 1,063 hospitalized patients with advanced COVID-19 and lung involvement, pointing that patients who received remdesivir improved faster than related patients who received a placebo. Introductory results show that patients who received remdesivir had a 31% faster time to recovery than those who took placebo ($p< 0.001$). Especially, the median time to improvement was 11 days in patients treated with remdesivir compared with 15 days in those who took the placebo. Results also proposed a survival benefit by day 14, with a mortality rate of 7.1% in the remdesivir group, associated with 11.9% in the placebo group, but this was not statistically significant [179].

The ACTT results are different from another randomized trial conducted in China. Results from this randomized, double-blind, placebo-controlled, multicenter trial study (n = 237; 158 to remdesivir and 79 to placebo; 1 patient withdrew) observed that remdesivir was not correlated with statistically significant clinical benefits in adults hospitalized with severe COVID-19. Although not statistically meaningful, patients taking remdesivir had a numerically time faster improvement than those receiving placebo among patients with symptoms of the first 10 days or less. The authors concluded that the reduction in time to clinical improvement in those treated first needs confirmation in larger studies [180]. A phase 3, randomized, open-label trial revealed that remdesivir was associated with the higher recovery and reduced odds of death when compared with the standard of care in patients with critical COVID-19.

The recovery rate at day 14 was higher in patients who took remdesivir (n = 312) compared with those who received standard of care (n = 818) (74.4% *vs* 59%; $p< 0.001$). The mortality rate at day 14 was also lower in the remdesivir group (7.6% *vs* 12.5%; $p = 0.001$) [181].

The open-label phase 3 SIMPLE trial (n = 397) in hospitalized patients with severe COVID-19 disease not needing mechanical ventilation exhibited related improvement in the clinical state with the 5-day remdesivir regimen compared with the 10-day regimen on day 14 (OR: 0.75 [95% CI 0.51-1.12]). In this study, 65% of patients who underwent a 5-day course of remdesivir presented a clinical improvement of at least 2 points on the 7-point ordinal scale on day 14, compared with 54% of patients who took a 10-day course. After correcting for inequalities in baseline clinical status, patients taking a 10-day course of remdesivir had a distribution in clinical status at day 14 that was alike to that of patients taking a 5-day course ($p = 0.14$). The study illustrates the potential for some patients to be treated with a 5-day regimen, which could significantly enlarge the number of patients who could be treated with the current stock of remdesivir. The trial is continuing with an enrollment purpose of 6,000 patients [182].

Data presented at the virtual COVID-19 Conference in July 2020 incorporated a comparative analysis of clinical recovery and mortality outcomes from the phase 3 SIMPLE trials *versus* a real-world cohort of patients with severe COVID-19 taking the standard of care. The analysis revealed remdesivir was related to a 62% decrease in the risk of mortality compared with standard of care. Subgroup analyses obtained from these results were alike to different racial and ethnic groups. While these data are important, they need confirmation in prospective clinical trials [183.

Similarly, the phase 3 SIMPLE II trial in patients with moderate COVID-19 disease revealed that 5 days of remdesivir treatment was 65% more likely to yield clinical improvement at day 11 than the standard of care ($p = 0.18$). These data demonstrate that immediate intervention with a 5-day treatment course can significantly better outcomes [183, 184].

Favipiravir

Favipiravir (Avigan) also identified as T-705 was original approved for the treatment of influenza virus this February, and it has the capacity of inhibiting RNA-dependent RNA polymerase in RNA viruses such as SARS-CoV-2 [185]. In

February, a preliminary clinical trial on favipiravir was handled in China on 80 patients and showed better effects compared to lopinavir/ritonavir with minor adverse effects [186]. Another clinical trial with 340 patients in China resulted in highly promising results as the patients taking favipiravir compared to standard care presented released viral load in four days as compared to eleven days in patients taking standard care [187]. Another multicenter, open-labeled, randomized trial in China has compared the efficacy of favipiravir (1,600 mg × 2 on the first day followed by 600 mg × 2 for 9 days) and umifenovir (200 mg × 3 per day for 10 days) and the outcomes showed a higher recovery rate and better clinical results in the patients treated with favipiravir at day 7 [188]. A phase III trial is ongoing in Japan including 100 patients and is presumed to be completed in June.

Nevertheless, Avigan was not that much helpful in critically ill patients and did not reveal encouraging results. It seems that it has to be taken before the viral load peaks in the body. Favipiravir would need government approval for usage against COVID-19.

MK-4482

Another antiviral originally designed to fight the flu, MK-4482 (previously known as EIDD-2801) has had promising results against the new coronavirus in studies in cells and on animals. Sheahan and colleagues [189] presented that the analog ribonucleoside (β-D-N4-hydroxycytidine - NHC; EIDD-1931) has broad-spectrum antiviral activity against the coronaviruses (SARS-CoV-2, MERS-CoV, SARS-CoV), and also a better possible mutations beard of CoVs when compared to the nucleoside analog inhibitor remdesivir. The *in vivo* study with mice infected with SARS-CoV or MERS-CoV, both prophylactic and therapeutic administration of EIDD-2801 improved pulmonary function and reduced virus titer and body weight loss. Merck, which has been running the clinical trials on the drug has announced the beginning of the Phase III trial in the next September.

Umifenovir (Arbidol)

Umifenovir (arbidol) showed efficacy in the treatment of influenza virus infection. It is a viral entrance inhibitor to the target cells, without significant side effects. It has revealed highly promising action against SARS-CoV-2 *in vitro* [190]. A randomized, open-labeled, multi-centered clinical trial conducted in China compared the effectiveness and security of favipiravir and arbidol on COVID-19 patients on 7 day's clinical recovery rate. 120 patients were allocated to each group taking favipiravir and arbidol along with standard therapy. The results showed that the 7-day recovery rate for the arbidol group was 55.86% in comparison to 71.43% for the favipiravir group ($p = 0.0199$). Patients with hypertension or diabetes also showed better recovery in the favipiravir group [189]. Currently, three more phase IV clinical trials are designed for arbidol in the treatment of COVID-19. One clinical trial will compare the efficacy of arbidol on 380 patients at Jieming QU, Ruijin Hospital, China in comparison to the standard treatment [191], whereas, the other two would compare the efficacy of arbidol with oseltamivir [192] on 400 patients at Tongji Hospital, China, and carrimycin on 520 patients at Beijing Youan Hospital, China [193, 194].

Oseltamivir and Baloxavir

Oseltamivir and Baloxavir have antiviral activity against influenza. So, they were considered as possible treatment possibilities for COVID-19. This was used in excess in the initial report of the cases in Wuhan, where patients were managed with COVID-19 receiving oseltamivir in addition to broad-spectrum antimicrobials [2]. It is important to note that the application of oseltamivir was not as targeted therapy of SARS-CoV-2 but an option by the lack of knowledge of the causative pathogen at the time of treatment and the hope to empirically treat influenza. The authors do not recommend the use of oseltamivir for COVID-19 in this study, and there are no data suggesting *in vitro* activity of oseltamivir against SARS-CoV-2. The only data evaluating

oseltamivir activity against coronaviruses showed to be ineffective at inhibiting SARS-CoV-1 [195, 196]. Coronaviruses do not utilize neuraminidase, and thus there is no enzyme to be inhibited by oseltamivir.

Nitazonide

Nitazoxanide has shown potent *in vitro* action against SARS-CoV-2, with an EC50 at 48 hours of 2.12 μM in Vero E6 cells [80]. This powerful activity is compatible with EC50 values for nitazoxanide and its active metabolite, tizoxanide, against MERS-CoV in LLC-MK2 cells [197]. Nitazoxanide exhibits broad-spectrum *in vitro* antiviral action against influenza, respiratory syncytial virus, parainfluenza, rotavirus, and norovirus among others in addition to coronaviruses [196, 197]. This broad-spectrum antiviral activity is thought to be due to the mechanism of action, which is based on interference with host-regulated pathways involved in viral replication rather than virus-specific pathways [197]. Due to its broad-spectrum antiviral action, nitazoxanide is being studied for the management of influenza and other acute respiratory infections. Positive data were described in a phase 2b/3 study for the outpatient management of influenza [198]. Three-phase randomized controlled trials in uncomplicated influenza have since been completed (ClinicalTrials.gov Identifier NCT01610245 [March 2018], NCT02612922 [April 2018], and NCT03336619 [September 2019]), although results are still unavailable. Nitazoxanide declined to decrease the duration of hospitalization or the time to symptom alleviation in phase 2 randomized controlled trial in patients with severe acute respiratory illnesses needing hospitalization [199]. Although the *in vitro* activity of nitazoxanide against SARS-CoV-2 is promising, more data are clearly required to define its role in the management of COVID-19.

Anti-HIV Drugs

These drugs are classified into different categories based on their targets reverse transcription, retro-transcription, proteolytic processing, viral-cell fusion, co-receptors interactions and incorporation of proviral DNA into the host genome. Drugs that fall in these categories have been approved by the FDA (Food and Drug Administration) and are now officially used for the treatment of HIV [181, 184].

Lopinavir/Ritonavir

Lopinavir/ritonavir (LPV-r) is a co-formulated human immunodeficiency virus (HIV)-specific protease inhibitor that serves as first-line therapy for HIV [200]. Concomitant use of ritonavir could increase the plasma half-life of lopinavir through cytochrome P450 inhibition in the liver. During the 2003 SARS outbreak, LPV-r was reported to have *in vitro* inhibitory activity against SARS-CoV [201], and combination therapy of LPV-r and ribavirin provided favorable results in treating patients with SARS [202]. Triple combination therapy with LPV-r, ribavirin, and IFN-α has shown clinical effectiveness for MERS [203]. Notwithstanding, a recent open-label randomized study with 199 patients in Wuhan showed that LPV-r monotherapy did not produce any therapeutic benefits for COVID-19 patients compared with standard supportive care, which might be caused by the higher throat viral loads in the LPV-r group, concurrent pharmacologic interventions, and late treatment initiation [204]. The enrolled COVID-19 patients were critically ill, and LPV-r treatment might have been started relatively late. However, in another retrospective cohort study, combination therapy of LPV-r and arbidol was associated with improved pulmonary computed tomography images [205].

Darunavir

Darunavir (Prezista) is another antiviral drug utilized as an HIV-1 protease inhibitor that demonstrated to be hopeful anti-SARS-CoV-2 activity *in vitro* earlier in February in a test that occurred in China. Nevertheless, Johnson and Johnson stated on March 18, 2020, that there is no indication of sustaining the activity of darunavir against SARS-CoV-2.

Darunavir was approved with a boosting agent such as ritonavir or cobicistat [206]. A single-center open-labeled randomized and controlled phase III trial, conducted at Shanghai Public Health Clinical Center (SPHCC) for the efficacy of darunavir/cobicistat association on the 30th day of COVID-19 patients, revealed that the combination was not efficient in decreasing the symptoms or the duration of treatment [207].

Nelfinavir

Nelfinavir, an HIV-1 protease inhibitor, might be effective against SARS-CoV-2 based on a preprint publication that used homology modeling [208], but no clinical data exist.

Stem Cell Therapy (Table 1 attached)

The mesenchymal stem cells (MSC), a relevant portion of the stem cell family, have the potential of self-renewal and multidirectional differentiation, and also have powerful anti-inflammatory and immune regulatory functions. MSC can restrain the irregular activation of T lymphocytes and macrophages, and provoke their differentiation into regulatory T cell (Treg) subsets and anti-inflammatory macrophages, respectively. Also, it can inhibit the secretion of proinflammatory cytokines, such as IL-1, TNF-α, IL-6, IL-12, and IFN-γ, thereby decreasing the event of cytokine storms [209-211]. MSC can secrete IL-10, hepatocyte growth factor, keratinocyte growth factor, and VEGF to relieve ARDS, restore and repair injured lung tissues, and combat fibrosis [211]. Hence, many uses of MSC are presumed to make it a useful method for the treatment of COVID-19.

Other Therapies

Thalidomide

Thalidomide is an anti-inflammatory and an immunomodulatory agent. It was designed to expand T cells, treat inflammation, restrain cell proliferation, and decrease lung injury and pulmonary fibrosis [116, 213]. The main role of thalidomide in COVID-19 is to preserve the lungs from injury induced by immunological reactions. Nevertheless, thalidomide requires to be used with another antiviral agent, since it does not eliminate or suppress viral load. Wenzhou Medical University reported a case that has demonstrated that thalidomide has adjuvant effects in COVID-19 treatment.

Leflunomide [213]

Leflunomide is efficient in improving SARS-CoV-2 clearance and hospital releasing in refractory COVID-19 patients. The addition of leflunomide to SOC (standard of care) did not develop adverse events *versus* SOC. These introductory considerations emphasize a demand for a randomized clinical study of leflunomide against SARS-CoV-2 infection.

Dipeptidyl Peptidase 4 (DPP4; CD26)

Dipeptidyl peptidase 4 (DPP4) is a functional receptor for the emerging human coronavirus via S-protein, as well as ACE2 [215]. The interaction between the virus and the host cell membrane permits for viral S-protein-directed cell-cell fusion and the resultant spread of viral infections [216]. The specific role of DPP4 on COVID-19 persists to be examined. Further research is needed to use DPP-4 as a therapeutic target for COVID-19.

Aminopeptidase N (APN; CD13)

The aminopeptidase N (APN) is associated with broad receptor engagement, which promotes the cross-species transmission of COVID-19 [217]. Earlier studies recognized APN as a surface tag for cancer stem cells in the human liver [218].

Other studies also enabled the development of a poly(ethylene glycol)-poly(lysine) block copolymer-conjugate (Ubenimex) that targets APN especially [219]. So, low doses of APN inhibitors,

including Ubenimex or its derivatives, may be useful for restraining the spread of the virus.

Ulinastatin [12]

Ulinastatin is a natural anti-inflammatory element in the body. It protects the vascular endothelium by restraining the generation and discharge of inflammatory mediators. Ulinastatin is popularly used in clinical practice to treat pancreatitis and acute circulatory failure. Ulinastatin decreases the levels of proinflammatory factors such as TNF-α, IL-6, and IFN-γ, and raises the level of anti-inflammatory factor IL-10 [220]. These actions of ulinastatin promote the balance between proinflammatory and anti-inflammatory responses in humans, thus disrupting the cytokine storm. Animal studies reveal that the anti-inflammatory effect of high-dose ulinastatin is similar to that of hormones [221]. Nevertheless, unlike glucocorticoids, ulinastatin does not restrain immune functions. Consequently, ulinastatin could have a great application in the treatment of COVID-19.

Sirolimus

Sirolimus (rapamycin) is an immunosuppressant that is used to prevent organ transplant rejection and to treat lymphangioleiomyomatosis (LAM) by repressing the mammalian target of rapamycin (mTOR) kinase. It was originally isolated from the bacterium Streptomyces hygroscopicus located on Easter Island (Rapa Nui) [222] and is commercially available as Rapamune (Pfizer). But there is no data against COVID-19. mTOR, and more specifically a protein complex mTORC1 formed by mTOR, plays a key role in viral replication. In an *in vitro* experiment, sirolimus has been shown to affect PI3K/AKT/mTOR pathway which inhibited MERS-CoV activity [223]. A new randomized double-blind placebo-controlled clinical trial (SCOPE) by University of Cincinnati is planned to be conducted between April and September 2020 to test the effect of sirolimus on progression of patients hospitalized with COVID-19 to advanced respiratory support [224]. Studies of patients hospitalized with influenza can further shed light on the antiviral effect of sirolimus. In a randomized clinical trial conducted on 38 patients with confirmed H1N1 pneumonia and on mechanical ventilator support, a group treated with corticosteroids and 2 mg/day of sirolimus for 14 days (N = 19) showed significantly better clinical outcomes compared with the group treated with corticosteroids only, including shorter median duration of ventilator used [225]. Delayed oseltamivir plus sirolimus treatment in pH1N1-infected mouse model further suggested a significant association between the sirolimus treatment and improved outcomes [226]. Additionally, a new trial by the Chinese University of Hong Kong is planned to begin in August 2020 to investigate the effect of sirolimus and oseltamivir on normalization of respiratory status and changes in biomarkers (viral RNA concentration, 10 cytokines/ chemokines and pro-inflammatory mediators) and several other clinical endpoints in influenza patients [227]. At least one in silico study identified sirolimus as one of the 16 potential candidates for treating COVID-19 patients based on data from other human coronavirus infections using network-based drug repurposing model [228].

Sphingosine-1-phosphate Receptor 1 Agonist Therapy

Sphingosine-1-phosphate (S1P) is a signal lysophospholipid that promotes cytokine synthesis and secretion [229]. The S1P receptor signaling pathways significantly inhibit the pathological injury caused by the host's innate and adaptive immune responses. So, this fact reduces the cytokine storm provoked by influenza virus infection [230, 231]. In mouse models of IAV infection, sphingosine-1-phosphate receptor 1 (S1P1) signal transduction in respiratory endothelial cells modulates pathogenic inflammatory responses [231]. Agonists targeting S1P1 restrain extreme recruitment of inflammatory cells, inhibit proinflammatory cytokines and chemokines, and decrease the morbidity and

mortality of IAV [232]. SARS-CoV-2 also principally affects human lung epithelial cells and endothelial cells. Consequently, S1P1 agonists may be possible therapeutic drugs for decreasing cytokine and chemokine responses in those HCoV patients whose cells produced excessive immune responses. An S1 preceptor modulating drug, siponimod, was approved in 2019 to treat multiple sclerosis. However, clinical trials are required to further confirm whether siponimod is an alternative for the treatment of cytokine storm.

The Inhibitory Effect of Oxidized Phospholipids (OxPL)

OxPL improved the production of cytokines/chemokines in lung macrophages through the Toll-like receptor 4 (TLR4)–TIR-domain- containing adapter-inducing interferon-β signaling pathway in a mouse model of influenza A virus (IAV) infection, promoting the occurrence of ALI [234]. Eritoran is a TLR4 antagonist and has antiviral activity and strong immunomodulatory functions. Eritoran dramatically reduces the production of OxPL, inflammatory cytokines, and chemokines in IAV-infected mice, lowering death [234]. Pathogenic human coronaviruses also cause a high accumulation of OxPL in patients' lung tissues, resulting in ALI [233] Thus, it seems that eritoran and other OxPL inhibitors may also be able to alleviate HCoV-induced inflammatory responses.

Mycophenolate Mofetil (MMF)

MMF is widely used for the treatment of severe manifestations of connective tissue disorders and vasculitis syndromes. Mycophenolate exhibited strong antiviral effects on SARS-CoV and MERS-CoV as demonstrated *in vitro* studies, with its interaction with viral proteases [235]. A small clinical study reported efficacy of MMF in combination with IFN-β on MERS patients [236]. However, considering strong immunosuppressant effects of MMF, it is likely to cause more harm than benefit in COVID-19 patients.

Statins

Statins reduce the inflammatory processes of atherosclerosis [237]. Because of this, issues have risen whether statins may be helpful to reducing inflammation related to COVID-19.

This issue has been presented before the studies of patients who took statins to have acute viral infections by COVID-19. Virani [238] presents a brief review of data concerning observational and randomized controlled trials (RCTs) of statins and viral infections. Some observational studies insinuate that cardiovascular outcomes were decreased in patients taking statins hospitalized with influenza and/or pneumonia. RCTs of statins as anti-inflammatory agents for viral infections are poor, and outcomes have been confused. An important point that Virani reported is that no harm was associated with patients who took statins and had COVID-19 in previous trials of statins and viral infections, indicating that patients should adhere to their statin regimen.

Current Clinical Treatment Experience and Recommendations by World Health Organization (WHO), Center for Disease Control and Prevention (CDC), and National Institutes of Health (NIH) (Tables 1 and 2 attached)

The COVID-19 Treatment Guidelines Panel [15] had been developed to informing physicians on how to caring for patients with COVID-19. The references in these Guidelines are based on scientific data and expert evaluation. Each recommendation involves two ratings: a letter (A, B, or C) that designates the strength of the recommendation and a Roman numeral (I, II, or III) that designates the quality of the evidence that confirms the recommendation (Table 3).

Because clinical information about the optimal management of COVID-19 is developing quickly, these Guidelines will be updated periodically as announced data and other authoritative knowledge

become available. Panel members incorporate representatives from federal agencies, health care and academic institutions, and professional societies. Federal agencies and professional societies reproduced on the Panel include:

- American College of Chest Physicians
- American College of Emergency Physicians
- American Society of Hematology
- American Thoracic Society
- Biomedical Advanced Research and Development Authority
- Centers for Disease Control and Prevention
- Department of Defense
- Department of Veterans Affairs
- Food and Drug Administration
- Infectious Diseases Society of America
- National Institutes of Health
- Pediatric Infectious Diseases Society
- Society of Critical Care Medicine
- Society of Infectious Diseases Pharmacists

Alternative Therapies

We would like to mention that all about surrounding this topic need further studies and evidence. We bring them in order to include all therapeutic purposes to COVID-19 infection.

Vitamin C

Vitamin C is a vital nutrient and plays important role in the human body. It can neutralize free radicals and help to prevent or reverse cellular injury as a powerful antioxidant agent. It is also associated with some biological processes, many of which are correlated with the immune system [239]. Furthermore, vitamin C seems to be useful as an antiviral agent, especially against influenza viruses [240]. Many studies revealed that vitamin C positively influences the development and maturation of T lymphocytes and NK (natural killer) cells implicated in the immune response to viral agents. It also contributes to the restraint of reactive oxygen species (ROS) production and to the modulation of the cytokines typically involved in the systemic inflammatory syndrome [241]. Given this background, a phase II clinical trial (NCT04264533) is initiated in China to evaluate high-dose IV vitamin C in ICU patients with severe COVID-19-associated pneumonia [242]. Some hospitals have reported giving infected patients 1500 mg of vitamin C as supportive treatment. High-dose IV vitamin C has been given in the treatment of 50 moderate to severe COVID-19 patients in China [243]. The doses varied between 2 and 10 g per day, given over a period of 8-–10-h IV infusion. The oxygenation index was improved in real time and all the patients eventually recovered and were discharged [243]. Moreover, high-dose (1.5 mg/kg bodyweight) vitamin C has been used for several decades clinically and an NIH panel also documented clearly that this dose regimen is safe and has no major side effects [244].

Nitric Oxid and Epoprostenol

Since patients with pre-existing pulmonary conditions are at higher risk of COVID-19 and should be closely monitored and cared, pulmonary vasodilator agents have been used in some patients for hypoxemia refractory to conventional treatments, but no study has been performed specifically on COVID-19 patients. The Surviving Sepsis Campaign suggested a trial of inhaled pulmonary vasodilator method as rescue therapy in mechanically ventilated adults with COVID-19, severe ARDS, and hypoxemia despite optimized ventilation and other rescue strategies. Inhaled nitric oxide (iNO) and inhaled epoprostenol (iEPO, a naturally occurring prostaglandin) are two common pulmonary vasodilators that have been widely studied [245, 246]. Experience in patients with ARDS indicates that iNO can substantially reduce mean pulmonary artery pressure and improve oxygenation in such patients. Furthermore, *in vitro* evidence of direct antiviral activity against SARS-CoV was studied and the genetic similarity between SARS-CoV and SARS-CoV-2 suggests their potential effectiveness

against SARS-CoV-2 [249]. For iEPO, dosages up to 50 ng/kg per minute have been used [245, 248]-250]. Previous studies reported that to provide a clinically important increase in PaO2 and reduction in pulmonary artery pressure, the most effective and safe dosage appears to be 20– 30 ng/kg per minute in adults and 30 ng/kg per minute in pediatric patients [251]. For iNO, therapy was given for ≥ 3 days (30 ppm on day 1, followed by 20 and 10 ppm on days 2 and 3, respectively, thenweaned on day 4) in a pilot study on SARS-CoV [251]. Additionally, clinical trials evaluating iNO for treatment or prevention of COVID-19 are planned or underway (NCT04305457, NCT04306393, NCT04312243) [252]. And on March 20, 2020, FDA admitted emergency extended entrance allowing its iNO delivery system (INOpulse®) to be quickly applied for the treatment of COVID-19. Lastly, further studies are needed to appraise the potential role of iEPO and iNO in the treatment of COVID-19 patients.

Natural PAK1-Blockers

A specific vaccine is an effective cure for each viral infection. Nevertheless, it demands at least 12-18 months to be prepared according to the WHO. Until then we have to combat the coronavirus with the guidance of the major research Centers and WHO as well as supportive treatments. Also, "alternative" or "unconventional" therapeutics for coronaviral infection, many natural or synthetic PAK1-blockers readily accessible in the market are introduced.

The Bee Product "Propolis"

Propolis is one of the most popular and ancient substances used for medical supportive treatments. It has been adopted as traditional medicine since the ancient Egyptian era. Its properties are both anti-bacterial and anti-viral. In the modern era, propolis was identified as an anti-cancer medicine in the late 1980s by a group at Columbia University in NYC [253]. The major anti-cancer component in propolis turned out to be CAPE, an ester of caffeic acid [253] which downregulates RAC, thereby inactivating PAK1 [254]. Nevertheless, the anti-cancer properties of propolis vary from one product to another, depending on where bees store the extract. The principal anti-cancer ingredient in Brazilian green propolis is artepillin C (ARC), whereas those in subtropical propolis from Okinawa or Taiwan are polyphenols (Nymphaeols), which directly restrain PAK1 [255]. However, all propolis have PAK1-blockers. Since PAK1 is responsible not only for cancers but also for infection with a wide diversity of viruses such as influenza, HIV, papillomavirus, and coronavirus in generally, as well as immune-suppression [256, 257], propolis would be helpful for blocking coronavirus-induced fibrosis in lungs and stimulating the immune system as well. Nevertheless, the power of propolis alters from one product to another, depending on both the chemical nature of components and their content [256-259]. It's suggested daily dose is 1 mL (250 mg) /10 kg (body weight).

Pineal Hormone "Melatonine"

In 1953, Aaron Lerner at Yale University recognized the serotonin melatonin as an anti-melanogenic hormone that originated from pineal glands [260]. Around the last decade, it was found that melanogenesis depends on PAK1 [261]. Melatonin shares a broad diversity of other anti-PAK1 actions such as anti-infectious, anti-inflammatory, anti-cancer, immune stimulative, analgesic, sleepy, among others. Thus, melatonin, a current sleeping pill, could be useful for the therapeutic of coronaviral infection. Russel Reiter, recently highlighted that melatonin is an alternative or adjuvant COVID-19 therapeutic [262].

Glucocorticoid Hormone "Ciclesonide"

Ciclesonide is used to treat inflammatory diseases such as asthma and allergic rhinitis. Concerning the molecular mechanism of its anti-inflammatory effect, it seems that this hormone bars PAK1, mainly for the following purposes: (i)

Table 3. Recommendation rating scheme.

Strength of Recommendation	Quality of Evidence for Recommendation
A: Strong recommendation for the statement	I: One or more randomized trials with clinical outcomes and/or validated laboratory endpoints
B: Moderate recommendation for the statement	II: One or more well-designed, nonrandomized trials or observational cohort studies
C: Optional recommendation for the statement	III: Expert opinion

Credit/Source: NIAID-RML [15].

An example how the recommendation scheme works, there are for blood-derived products under evaluation for the treatment of COVID-19. According to the Papel, there is a summary recomendations: (*Last Updated: July 30, 2020*) [15].

Summary Recommendations
There are insufficient data for the COVID-19 Treatment Guidelines Panel (the Panel) to recommend either for or against the use of the following blood-derived products for the treatment of COVID-19: • COVID-19 convalescent plasma • Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulins
The Panel recommends against the use of the following blood-derived products for the treatment of COVID-19, except in a clinical trial: • Mesenchymal stem cells (AII) • Non-SARS-CoV-2-specific intravenous immunoglobulins (IVIG) (AIII). This recommendation should not preclude the use of IVIG when it is otherwise indicated for the treatment of complications that arise during the course of COVID-19.

inflammation requires PAK1 [263], and in PAK1-null mutant of mice no inflammation occurs [264], (ii) ciclesonide (10 mg/kg/day) almost completely suppresses the PAK1-dependent growth of lung cancer (A541 cell line) in immune-deficient mice [265], and (iii) this hormone was recently presented to blocking PAK1-dependent replication and pathogenesis of COVID-19 infection [266].

Triptolide from Thunder God Vine and Its Water-Soluble Derivative

The herbal called "Triptolide from thunder god vine" was observed to inactivate RAC, thereby barring PAK1 [267, 259]. In 2010, triptolide was found to defeat virus production during dengue virus infection of the human lungs by obstructing the PAK1 signaling pathway [268]. But, its water-solubility is very poor. Thus, a group at the University of Minnesota headed by Gunda George boosted its water-solubility [269]. The resultant phosphatase-sensitive prodrug of triptolide called "Minnelide" is in clinical trials for cancers. Thus, both Triptolide and Minnelide could be possibly valuable for the treatment of coronavirus infection.

Ivermectin from Soil Bacterium (Streptomyces avermitilis)

In the 1975s, a precursor of Ivermectin (Avermectin) was identified from a soil bacterium by a group headed by Satoshi Omura, but it provokes side effects. To reducing its side effect, a Merck group directed by William Campbell, decreased it to create "Ivermectin" (dihydro-Avermectin), sharing the Nobel prize in 2015. It turned a medicine by Merck in 1981. It has been used to treat many types of parasite infestations including head lice, scabies, river blindness (onchocerciasis), among others. Three decades after this discovery, it was demonstrated by a Russian group to suppress the increase of cancers

as well, and ultimately, we discovered that its anti-cancer action is due to the inactivation of PAK1 [270]. So, it could possibly work as an alternative (and inexpensive) therapeutic to abolish the PAK1-dependent coronaviral infection as well. In fact, very recent studies proved that Ivermectin blocks the COVID-19 infection in animal experiments [271]. A curiosity is that the IC50 against COVID-19 is essentially the same as IC50 against the PAK1-dependent growth of cancer cells [272], which strongly suggests the PAK1-dependency of COVID-19 replication.

Artemisinin: Anti-Malaria from an Old Chinese Medical Herb

In 2015, Youyou Tu and colleagues discovered an anti-malaria compound called "Artemisinin" (AM). This substance was originally isolated from the plant Artemisia annua, a herb used in Chinese traditional medicine around the 1970s. Although the exact molecular mechanism about its anti-malaria and anti-viral action still persists unclear, the purpose is not the pathogens (Plasmodium falciparum or virus) themselves, but the host cells, most probably PAK1 or a component essential for both malaria and viral infection [273, 274], based on the next considerations: (i) the AM defeats both RAS (upstream of PAK1) and RAF (downstream of both RAS and PAK1) in T-cells [263, 276], and (ii) the dihydro derivative of AM suppresses the increase pancreatic cancer cells by upregulation of p21 (a CDK inhibitor) whose expression is suppressed by PAK1 [263, 273].

Extract of Chinese (Sichuan) Pepper (HuaJiao)

Chinese red peppercorns from Sichuan Province called "Hua Jiao" are among traditional spices adopted for the preparation of an old spicy Chinese cuisine called "Marbo-beancurd". In 2006, it was demonstrated that 70% ethanol or hot (above 45°C) water extract of Hua Jiao inhibits PAK1 with IC50 around 10 μg/mL, and thereby defeating cyclin D1 expression in both NF1-deficient triple-negative breast cancer (MDA-MB-231) and MPNST cell lines in which PAK1-is abnormally activated [277]. Thus, the drinking of "Hua Jiao" tea (extract) could be helpful in COVID-19 infection. However, its major PAK1-blocking component has not been chemically known as yet. So, further studies are necessary to investigate the positive evidence of "Hua Jiao" in COVID-19 treatment.

FK 228 (Istodax): Blocking HDAC-PAK1 Pathway

Fujisawa Pharm Group isolated FK228 from a soil bacterium in 1995. It well restrains the increase of RAS cancers such as pancreatic and colon cancers, which carry oncogenic mutant of Ki-RAS. A few years later it was discovered that it inhibits directly HDAC (histone deacetylase) with IC50 around 1 nM. In 2005, it was found that FK228 inactivates PAK1 in several cancer cells, including Tamoxiphen-resistant breast cancers and NF1-deficient MPNST (malignant peripheral nerve sheath tumor) [277]. In 2009, it was approved by FDA for the treatment of Cutaneous T-cell Lymphoma (CTCL). Thus, due to its property of inhibits HDAC and suppresses the increase of RAS, it could be useful for the therapy of COVID-19 infection as well.

Synthetic PAK1-Blockers

Ketorolac

"Toradol" is a racemic (1:1) mix of S- and R-forms of ketorolac. Since S-form directly inhibits COX-2, it has been used against pain. Nevertheless, a few years ago, R-form was observed to down-regulate RAC, thereby inactivating PAK1 [278, 279]. Thus, "Toradol" could be applied for the treatment of PAK1-dependent coronaviral infection. The resultant potent PAK1-blocker, called 1,2,3-triazolyl 295 ester of Ketorolac (15 K), suppresses both increase and metastasis of chemo-resistant human pancreatic cancer xenografts in mice with IC50 < 0.1 mg/kg/day, and causes no side effect even at 5 mg/kg/day

298 [280]. Thus, 15 K could be applied not only for pancreatic cancer therapy but also for therapy of infectious diseases caused by a coronavirus (COVID300 19) and many other dangerous viruses in the future.

Vitamin D3 and Its Derivative (MART-10)

The most generally known pharmacological action of Vitamin D3 is calcemic, i.e., stimulating the absorption of calcium into bone tissues. Nevertheless, researchers of Melbourne in the late 1980s, discovered that Vitamin D3 can suppress the increase of tumors in mice fed with a calcium-less diet [281]. However, the therapy with Vitamin D3 against cancers has never been successful. This clinical failure is due to the inactivation of Vitamin D3 by CYP24 in the human body.

Thus, a Japanese group in 2010 developed a derivative of Vitamin D3, called "MART-10", which is very resistant to CYP24 and definitely less calcemic [283]. The "MART-10" is 1000 times more powerful than Vitamin D3 against breast and pancreatic cancers [282]. A few years ago, a German group at Tuebingen University discovered that Vitamin D3 down-regulates RAC, inactivating PAK1 and leading to depolymerization of actin filaments [283]. Independently, MART-10 also induced the depolymerization of actin in cancer cells [284]. Moreover, CYP24 expression turned out to depend on the oncogenic RAS-PAK1-NF κB/ Ets signaling pathway [285]. Thus, probably, either "MART-10" alone or in combination with Vitamin D3 and a CYP24-resistant PAK1-blocker such as propolis could be possibly helpful for the treatment of coronaviral infection.

Pythochemicals and Natural Products Targeting Coronaviruses

Natural products can inhibit various steps in viral infection and replication, and many of them have broad-spectrum antiviral effects, the mechanisms of which have not been fully characterized. They also can function as immunomodulators, suppressing inflammatory reaction responsible for the major morbidity and mortality of SARS-CoV-2 infection. Phytochemicals, especially flavonoids, which are widely distributed in food plants and botanicals, have been shown to interfere with NLRP3 inflammasome signaling [286]. The respiratory distress syndrome associated with SARS coronaviruses develops in part due to viral activation of the NLRP3 inflammasome within activated macrophages and T helper-1 lymphocytes, which causes increased production of inflammatory cytokines [287]. Several flavonoids that interfere with activation of the NLRP3 inflammasome may modulate inflammatory response to SARS beta coronaviruses: luteolin [288], myricetin [289], apigenin [290], quercetin [291] kaempferol [292], baicalin [293], and wogonoside [294].

These flavonoids have been shown to be active against a wide variety of viruses, via multiple mechanisms [295, 296], and are available as nutraceutical supplements at a daily dose ranging from 100 mg to 500 mg. Emodin (6-methyl-1,3,8-trihydroxyanthraquinone) (CAS number: 518−82-1) is an anthraquinone compound found in various Chinese herbs and is also produced by many species of fungi, including members of the genera Aspergillus, Pyrenochaeta, and Pestalotiopsis. Emodin has been shown to inhibit the interaction of SARS-CoV S protein with its receptor ACE2 in a dose-dependent manner [297]. Resveratrol (trans-3,5,4′-trihydroxystilbene) (CAS number: 501−36-0) is a stilbenoid and a natural polyphenol that is found in high concentrations in the skins of red wine grapes (Vitis vinifera), in red wine and in sprouted peanuts (Arachis hypogaea). Resveratrol has been demonstrated *in vitro* to inhibit MERS-CoV infection and to prolong cellular survival after virus infection. Further, expression of MERS-CoV nucleocapsid protein essential for virus replication as well as MERS-CoV- induced host

cell apoptosis are inhibited by resveratrol [298], suggesting that resveratrol may also be effective against SARS-CoV-2 infection.

Blood Purification Treatments

Blood purification treatments currently utilized in clinical practice are capable to eliminate some inflammatory factors. The purification system, which involves plasma replacement, adsorption, perfusion, blood/plasma filtration, and other procedures, can exclude some quantity of inflammatory factors, barring "cytokine storms", and decrease the damage from the body's inflammatory response. This treatment can be applied for critical patients in the early and middle phases of the disease. This artificial technology, which was led by Academician Li Lan-Juan, can reduce inflammatory factors on a huge scale. This technology has also been adopted to resist the cytokine storm of H7N9. The application of this treatment on COVID-19 has obtained certain efficiency [299]. Early renal replacement therapy appears to be an efficient method to control cytokine storm.

Inhibitors of Mononuclear Macrophage Recruitment and Function.

The autopsy of patients with COVID-19 revealed many inflammatory cell infiltration in the lungs of the deceased [300]. One treatment method that can be possibly efficient is to reduce the recruitment of mononuclear macrophages to the site of inflammation by small interfering RNA (siRNA)-mediated silencing of C-C chemokine receptor type 2 (CCR2) to increase the outcome of the disease [301, 302]. Toll-like receptor 7 (TLR7) agonists excite mononuclear macrophages to undergo a strong inflammatory response at the time of infection with single-stranded RNA (ssRNA) viruses such as HCoV. Hence, TLR7 antagonists may be able to mitigate the storm of inflammatory factors induced by SARS-CoV-2 infection.

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Table 1. Immune-based therapy under evaluation for treatment of COVID-19: clinical data to date. (*Last Updated: July 30, 2020*).

Drug Name	FDA-Approved Indications	Pre-Clinical Data/Mechanism of Action/Rationale for Use in COVID-19	Clinical Data for COVID-19, SARS, or MERS (Find clinical trials on ClinicalTrials.gov)
Blood-Derived Products			
COVID-19 Convalescent Plasma	• The FDA has provided recommendations for the use of COVID-19 convalescent plasma through EINDs for individual patients, traditional INDs, or expanded access INDs. The FDA has also approved a national expanded access program for the use of convalescent plasma for the treatment of patients with COVID-19. Clinicians can refer to the National COVID-19 Convalescent Plasma Project website for more information on that specific program and other trials evaluating convalescent plasma.	• Plasma donated from individuals who have recovered from COVID-19 includes antibodies to SARS-CoV-2. Thousands of U.S. patients have received convalescent plasma through clinical trials, expanded access treatment trials, and EIND applications. However, the standards and methods for screening donated plasma for SARS-CoV-2 binding and neutralizing antibodies have not been established. The variability in SARS-CoV-2 antibody levels in donor plasma may impact the product's efficacy. Clinical data are currently insufficient to evaluate the efficacy of convalescent plasma.	**For COVID-19:** • Open-Label, Randomized Clinical Trial of Convalescent Plasma in 103 Hospitalized Patients With Severe or Life-Threatening COVID-19: Investigators conducted an openlabel, randomized clinical trial of convalescent plasma *versus* SOC for patients with severe and life-threatening laboratory-confirmed COVID-19 in seven medical centers in Wuhan, China, from February 14 to April 1, 2020. The primary outcome was time to clinical improvement within 28 days, which was defined as patient discharged alive or a reduction of 2 points on a 6-point disease severity scale. Only plasma units with SARS-CoV-2 viral spike-receptor binding domain-specific IgG titer ≥ 1:640 were transfused. The median dose of ABO-compatible convalescent plasma was 200 mL. The time from symptom onset to randomization was 27 days in the treatment group and 30 days in the control group. Due to control of the COVID-19 outbreak in Wuhan, the trial was terminated early after 103 of the planned for 200 patients were enrolled. The convalescent plasma and control groups were well balanced by age (median age of 70 years vs. 69 years, respectively), but the control group had a higher proportion of men (65%) than the convalescent plasma group (52%). Baseline severity scores (45 patients had severe disease and 58 had life-threatening disease) and use of concomitant therapies were similar between the two groups. There was no significant difference between the groups in the primary outcome of time to clinical improvement within 28 days (HR 1.40; 95% CI, 0.79–2.49; P = 0.26). Among those with severe disease, 91% of the convalescent plasma recipients and 68% of the control patients improved by Day 28 (difference 23%; OR 1.34; 95% CI, 0.98–1.83; P = 0.07). Among those with lifethreatening disease, 21% of the convalescent plasma recipients and 24% of the control patients improved by Day 28 (difference -3.4%; OR 0.86; 95% CI, 0.33–2.24; P = 0.75). There was no significant difference in 28-day mortality between the groups (16% vs. 24% for the treatment and control groups, respectively; OR 0.65; 95% CI, 0.29–1.46; P = 0.30). At 24, 48, and 72 hours, the rates of negative SARS-CoV-2 viral PCR were significantly higher in the convalescent plasma group than in the control group (45% vs. 15%, P = 0.003 at 24 hours; 68% vs. 33%, P = 0.001 at 48 hours; and 87% vs. 38%, P <0.001 at 72 hours). Two transfusion-related events were reported, including 1 severe event; both events resolved with supportive care. The study's primary limitations were its open-label design and that, on average, administration of the convalescent plasma was approximately 1 month into the disease course. In addition, the study was terminated early, and thus the sample size was insufficient to detect differences in clinical outcomes. • Preliminary Safety Analysis of the First Consecutive 5,000 Patients to Receive Open Label, COVID-19 Convalescent Plasma Through a National Expanded Access Program: The Expanded Access to Convalescent Plasma for the Treatment of Patients with COVID-19 program is an

			ongoing, open-label, nonrandomized protocol primarily designed to provide patients with severe or life-threatening (critical) COVID-19 with access to convalescent plasma, which is an investigational product in the United States. Secondary objectives were to obtain safety data on the product. The protocol is sponsored by the Mayo Clinic and includes a diverse range of clinical sites. Plasma donors have documented COVID-19, with complete resolution of symptoms for at least 14 days prior to donation, and are either male, female without history of pregnancy, or female with history of pregnancy and negative HLA testing after the most recent pregnancy. SARS-CoV-2 antibody testing of donors is not mandated. ABO-compatible convalescent plasma is transfused preferentially, but in the absence of ABO-compatible plasma, patients may receive either Group A plasma or low anti-A titer Group O plasma, as available. The main safety outcomes for the safety analysis are SAEs including death; SAEs are reported at 4 hours and at 7 days after transfusion, or as they occur. The safety analysis describes the first 5,000 patients, enrolled between April 7 and May 3, 2020. Participants were adults with median age of 62 years, 63% male, and 81% had severe or life-threatening COVID-19. SAEs were reported in 36 patients (<1%) within 4 hours of transfusion; SAEs included 15 deaths, including 4 possibly or probably related to the convalescent plasma treatment. The 21 non-fatal SAEs included 7 TACO events, 11 TRALI events, and 3 severe allergic reactions. The overall 7-day mortality rate was 14.9%. In this study, COVID-19 convalescent plasma therapy was associated with a low rate (<1%) of serious transfusion-related events. The study design, which does not include a control arm, precludes an assessment of efficacy or ADE. • Retrospective, Single-Center, Case-Control Study Evaluating Convalescent Plasma Plus Standard of Care *Versus* Standard of Care Without Convalescent Plasma: Not Peer Reviewed. This case-control study reports clinical outcomes among 39 consecutive patients who received COVID-19 convalescent plasma through the FDA's single patient EIND program while hospitalized at Mount Sinai Hospital in New York City during the period March 24 to April 8, 2020. Recipients were transfused with 2 units of ABOcompatible convalescent plasma from donors with a SARS-CoV-2 antispike antibody titer of 1:320 dilution. The control group (n = 156) was identified retrospectively from the hospital's EHR database. The control patients were hospitalized during the same period as the patients in the convalescent plasma group and had confirmed COVID-19 but did not receive convalescent plasma. They were matched 4:1 to the convalescent plasma recipients using propensity scores to correct for measured confounders. Convalescent plasma recipients had a mean age of 55 years and 64% were male. At the time of transfusion, 87% of the recipients required supplemental oxygen (noninvasive) and 10% were mechanically ventilated. By Day 14, the clinical condition had worsened in 18% of the convalescent plasma patients and 24% of the control patients (P = 0.17). As of May 1, 2020, 13% of the plasma recipients and 24% of the matched control patients had died (P = 0.04, log-rank test) and 72% of the transfused patients and 67% of the control patients had been discharged. Interpretation of the study results is limited by the lack of randomization and the potential for unmeasured patient selection bias. • Other smaller, uncontrolled case series describing clinical outcomes in patients with COVID-19 have been reported and also suggest that serious AEs are uncommon following COVID-10 convalescent plasma treatment.

SARS-CoV-2 Specific Immunoglobulins	• Not approved by the FDA	• Concentrated antibody preparations derived from pooled plasma collected from individuals who have recovered from COVID-19 can be manufactured as SARS-CoV-2 immunoglobulin, which could potentially suppress the virus and modify the inflammatory response.	• No clinical data for COVID-19, SARS, or MERS
Non-SARS-CoV-2 Specific Intravenous Immunoglobulins	• Primary immune disorders • Thrombocytopenic purpura • Kawasaki disease • Motor neuropathy • Prophylaxis of various bacterial and viral infections	• Currently, only a small proportion of the U.S. population has been infected with SARS-CoV-2. Therefore, products derived from the plasma of donors without confirmation of SARS-CoV-2 infection are not likely to contain SARS-CoV-2 antibodies. Furthermore, although IVIG contains other blood components that may have general immunomodulatory effects, it is unclear if these theoretical immunomodulatory effects will benefit patients with COVID-19.	**For COVID-19:** • Not Peer Reviewed. A retrospective, nonrandomized cohort study of IVIG for the treatment of COVID-19 was conducted across eight treatment centers in China between December 2019 and March 2020. The study found no difference in 28-day or 60-day mortality between 174 patients who were treated with IVIG and 151 patients who were not treated with IVIG. Patients who received IVIG were hospitalized for longer (median stay of 24 days for IVIG group vs. 16 days for no IVIG group) and experienced longer duration of disease (median of 31 days for IVIG group vs. 23 days for no IVIG group). It should be noted that a higher proportion of IVIG-treated patients had severe disease at study entry (71 patients [41%] with critical status in the IVIG group vs. 32 patients [21%] in the non-IVIG group). A subgroup analysis that was limited to the critically patients suggested a mortality benefit at 28 days, which was no longer significant at 60 days. The results are difficult to interpret because of important limitations in the study design. In particular, patients were not randomized to receive IVIG or no IVIG, and the patients in the IVIG group were older and more likely to have coronary heart disease than those in the no IVIG group. The IVIG group also had more patients with severe COVID-19 disease at study entry. Also, patients in both groups received many concomitant therapies for COVID-19.
Mesenchymal Stem Cells (MSCs)	• Not approved by the FDA	• Multipotent adult stem cells that are present in most human tissues including the umbilical cord • It is hypothesized that MSCs could reduce the acute lung injury and inhibit the cellmediated inflammatory response induced by SARS-CoV-2. • MSCs lack the angiotensin-converting enzyme 2 receptor that SARS-COV-2 uses for viral entry into cells; therefore, MSCs are resistant to infection.	**For COVID-19:** • A pilot study of IV MSC transplantation in China enrolled 10 patients with confirmed COVID-19 categorized according to the National Health Commission of China criteria as critical, severe, or common-type disease. Seven patients (1 with critical illness, 4 with severe illness, and 2 with common-type illness) received MSCs; 3 patients with severe illness received placebo. All 7 patients who received MSCs recovered. Among the 3 severely ill control patients, 1 died, 1 developed ARDS, and 1 remained stable with severe disease. **For Other Viruses:** • In an open-label study of MSCs for the treatment of H7N9 influenza in China, 17 patients received MSC treatment plus SOC, and 44 patients received SOC only. In the MSC group, 3 patients (17.6%) died; in the control group, 24 patients (54.5%) died. The 5-year follow-up was limited to 5 patients in the MSC group. No safety concerns were identified.

Immunomodulators			
Corticosteroids			
Dexamethasone	FDA-Approved Indications: • Allergic states (e.g., severe or incapacitating asthma, dermatitis, drug HSRs) • Dermatologic diseases (e.g., bullous dermatitis, Stevens- Johnson syndrome) • Endocrine disorders (e.g., adrenocortical insufficiency) • Gastrointestinal diseases (e.g., ulcerative colitis) • Hematologic disorders (e.g., hemolytic anemia, idiopathic thrombocytopenia purpura, pure red cell aplasia) • Neoplastic diseases (e.g., palliative treatment of leukemia, lymphoma) • Nervous system disorders (e.g., multiple sclerosis, cerebral edema) • Ophthalmic diseases (e.g., temporal arteritis, uveitis) • Renal diseases (e.g., to induce diuresis or remission of proteinuria in idiopathic nephrotic syndrome) • Respiratory diseases (e.g., eosinophilic pneumonia) • Rheumatic disorders (e.g., ankylosing spondylitis, rheumatoid arthritis, systemic lupus erythematosus)	• Long-acting potent synthetic glucocorticoid with minimal mineralocorticoid activity. Glucocorticoid activity includes anti-inflammatory, immunosuppressive, anti-proliferative, and vasoconstrictive effects. • Potent anti-inflammatory effects may mitigate or prevent the systemic inflammatory response associated with severe COVID-19.	**For COVID-19:** • A preliminary, unpublished analysis from a large multicenter, randomized, open-label trial (RECOVERY) in hospitalized patients in the United Kingdom showed that those randomized to dexamethasone 6 mg daily (n = 2,104) had reduced mortality within 28 days of enrollment compared with those who received SOC (n = 4,321) (21.6% vs. 24.6%; RR 0.83; 95% CI, 0.74–0.92, P < 0.001). The survival benefit was greatest among participants who required invasive mechanical ventilation at randomization: 29.0% of participants in the dexamethasone group died within 28 days of enrollment compared with 40.7% of those in the control arm (RR 0.65; 95% CI, 0.51–0.82, P < 0.001). Among patients who required supplemental oxygen but not mechanical ventilation at enrollment, 21.5% in the dexamethasone arm died within 28 days of enrollment compared with 25.0% of those in the control arm (RR 0.80; 95% CI, 0.70–0.92, P = 0.002). No survival benefit was seen among participants who did not require oxygen therapy at enrollment; 17.0% of dexamethasone participants died within 28 days of enrollment compared with 13.4% in the control arm (RR 1.22; 95% CI, 0.93–1.61, P = 0.14). Interpretation of these results is limited by several factors: full analysis of the trial is ongoing; the results of key secondary endpoints, potential adverse events, and efficacy in key subgroups have not been reported; there were not standardized or objective criteria for oxygen supplementation; and the age distribution of patients differed by respiratory status at the time of randomization (patients who received mechanical ventilation were more likely to be <70 years of age). • Small retrospective cohort studies and case series have yielded conflicting results regarding corticosteroids, with some suggesting benefits associated with short courses of corticosteroids and others showing potential harm. • Conversely, several publications from China including a meta-analysis of 15 studies (which included studies for treatment of COVID-19, SARS, or MERS)24 and a retrospective review of critically ill patients with COVID-19 suggest an increased risk of multi-organ dysfunction and no benefit in (to possibly increased risk of) mortality with use of corticosteroids.

Interferon Alfa and Interferon Beta			
Interferon Alfa	• IFN alfa-2b: Leukemia, melanoma, lymphoma, condylomata acuminata, Kaposi sarcoma, hepatitis B, hepatitis C • IFN alfa-1b is not available in the United States.	• Elicits antiviral, antiproliferative, and immunomodulatory activities on numerous cell types	**For COVID-19:** • An open-label, Phase 2 clinical trial randomized 127 participants (median age 52 years) 2:1 to combination antiviral therapy or LPV/r. In the combination antiviral therapy group, the treatment regimen differed by time from symptom onset to hospital admission. Participants admitted within 7 days of symptom onset (n = 76) were randomized to triple drug therapy (IFN beta-1b 8 million units SQ every other day for up to 7 days total, LPV/r, and ribavirin); those admitted ≥7 days after symptom onset (n = 51) were randomized to double therapy (LPV/r and ribavirin) because of concerns regarding potential inflammatory effects of IFN. All participants in the control group received LPV/r alone regardless of time from symptom onset to hospitalization. The study participants were patients in Hong Kong with confirmed SARS-CoV-2 infection who were hospitalized regardless of disease severity until they had two negative nasopharyngeal swabs. The median time to a negative SARS-CoV-2 PCR on a nasopharyngeal swab (the primary endpoint) was shorter for the combination group than for the control group (7 days vs. 12 days, P = 0.001). The combination group had more rapid clinical improvement as assessed by NEWS2 and SOFA score and a shorter hospital stay (9 days for combination group vs. 14.5 days for control group, P = 0.016). There was no difference in oxygen use between the groups. The antiviral and clinical effect was more pronounced in the patients hospitalized within 7 days of symptom onset suggesting that IFN beta-1b with or without ribavirin was the critical component of the combination therapy. The study provides no information about the effect of IFN beta-1b administered >7 days after symptom onset. • Not Peer Reviewed. In a retrospective cohort study of 77 adults with moderate COVID-19 in China, those who used nebulized IFN alfa-2b with or without umifenovir (Arbidol) achieved viral clearance in the upper respiratory track faster and had lower systemic inflammation than those who used only umifenovir. However, results are difficult to interpret because participants in the IFN alfa-2b group were substantially younger than those in the umifenovir only group (mean age 40 years vs. 65 years) and had fewer comorbidities (15% vs. 54%) at study entry. The nebulized formulation of IFN alfa-2b is not FDA approved for use in the United States.
Interferon Beta	• Multiple sclerosis (IFN beta-1a, IFN beta-1b)	Elicits antiviral, antiproliferative, and immunomodulatory activities on numerous cell types (T cell, B cell, and cytokine function) • Among IFN subtypes, IFN beta-1b shows greatest *in vitro* inhibition of MERS-CoV. • *In vitro* activity against MERS-CoV in lung cells.	

Interleukin-1 Inhibitor			
Anakinra	• Rheumatoid arthritis • Cryopyrin-associated periodic syndromes, specifically neonatal-onset multisystem inflammatory disease35 • IV formulation is not approved for use in the United States	• Competitively inhibits IL-1 binding to the IL-1 type I receptor	**For COVID-19:** • A case-control study compared outcomes in 52 consecutive patients with COVID-19 treated with anakinra to outcomes in 44 historical controls. The patients in both groups were admitted to the same hospital system in Paris, France. Cases were consecutive admissions from March 24 to April 6, 2020, with laboratory-confirmed SARS-CoV-2 infection or lung infiltrates on chest imaging typical of COVID-19, and either significant hypoxia (SpO2 ≤93% with ≥6L/min O2) or worsening hypoxia (SpO2 ≤93% with >3L/min O2 and a loss of ≥3% of O2 saturation on room air in the previous 24 hours). Historic controls were patients fulfilling the same eligibility criteria and admitted to the hospital from March 18 to March 24, 2020. SOC for both groups entailed use of HCQ, AZM, and parenteral beta-lactam antibiotics. Anakinra was dosed SQ as 100 mg twice daily for 72 hours, followed by anakinra 100 mg daily for 7 days. Clinical characteristics were similar between the groups, except that the case patients had a lower mean BMI (25.5 kg/m2 for cases vs. 29.0 kg/m2 for controls), longer duration of symptoms (8.4 days for cases vs. 6.2 days for controls), and a higher frequency of HCQ use (90% for cases vs. 61% for controls) and AZM use (49% for cases vs. 34% for controls). The primary outcome of either admission to the ICU for mechanical ventilation or death occurred among 13 cases (25%) and 32 controls (73%) (HR 0.22; 95% CI, 0.11–0.41). However, within the first 2 days of follow up, in the control group, 6 patients (14%) had died and 19 patients (43%) had reached the composite primary outcome, which further limited intragroup comparisons and specifically analyses of time to event. CRP levels decreased by Day 4 among those receiving anakinra. Thromboembolic events occurred in 10 patients (19%) in the anakinra group and 5 patients (11%) in the control group. The clinical implications of these findings are uncertain, due to limitations in the study design related to unmeasured confounding combined with the very high early event rate among the retrospective controls. • A single-center case series reported on open-label use of anakinra in 9 hospitalized patients with COVID-19, presenting with 4–12 days of symptoms, requiring oxygen ≤6 L/min, and serum CRP ≥50 mg/L. Anakinra was administered SQ, 100 mg every 12 hours for 3 days followed by 100 mg daily for up to 7 more days. Two patients also received HCQ plus AZM; the other 7 patients received no specific additional treatments. Anakinra was discontinued in one patient who progressed to acute respiratory failure after the first dose of the drug. Good clinical outcomes were observed in the other eight patients as assessed by oxygen flow, decline in CRP, and no progression in infiltrates on serial CT scans. Three patients had elevated liver transaminase levels. Results are difficult to interpret because of the low number of patients in the case series, the short follow-up, and the absence of a comparison group. • A single-center, retrospective, cohort study in Italy compared outcomes in 29 patients following open-label anakinra use with outcomes in 16 historical controls. All patients had COVID-19 with moderate to severe ARDS requiring noninvasive ventilation, and evidence of hyperinflammation. High-dose IV anakinra 5 mg/kg twice daily was administered for a median of 9 days, followed by SQ administration (anakinra 100 mg twice daily) for 3 days to avoid inflammatory relapses. Both the anakinra and control (standard treatment) groups received HCQ and LPV/r. In the high-dose anakinra group, reductions in CRP levels were noted following anakinra initiation. The 21-day survival rate was 90% in the anakinra group and 56% in the control group (P = 0.009); however, the patients in the anakinra group were younger (median age of 62 years in anakinra group vs. 70 years in control group), and fewer patients had chronic kidney disease. High-dose anakinra was discontinued in 7 patients (24%) due to AEs (bacteremia in 4 patients, elevated liver enzymes in 3 patients); however, retrospective assessment showed that these events occurred with similar frequency in the control group. An additional group of 7 patients received low-dose SQ anakinra (100 mg twice daily); however, treatment in this group was stopped after 7 days because of lack of clinical or anti-inflammatory effects.

Interleukin-6 Inhibitors **Elevations in IL-6 levels may be an important mediator when severe systemic inflammatory responses occur in some patients with COVID-19; IL-6 inhibition may reduce these effects.**			
Sarilumab	• Rheumatoid arthritis	• Human recombinant monoclonal antibody • IL-6 receptor antagonist	**For COVID-19:** • Press Release: In a Phase 2/3 clinical trial (ClinicalTrials.gov Identifier NCT04315298), hospitalized COVID-19 patients were randomized (2:2:1) to receive sarilumab 400 mg, sarilumab 200 mg, or placebo. Preliminary data were released after an IDMC recommended discontinuing the 200-mg arm and restricting future enrollment to critically patients only. Of the first 457 participants enrolled, 145 were randomized to sarilumab 400 mg, 136 to sarilumab 200 mg, and 77 to placebo. At study entry, 28% of the patients had severe illness, 49% had critical illness, and 23% had multisystem organ -21% in the sarilumab 400 mg group, sarilumab 200 mg group, and placebo group, respectively (primary outcome of the Phase 2 trial). At the time of data analysis, of the 226 critical patients, the proportion of patients who had died or were on a ventilator was lower in the sarilumab 400 mg group (28%) than in the sarilumab 200 mg group (46%) and in the placebo group (55%). Comparing mortality alone, the proportion of patients who died was also lower in the sarilumab 400 mg group (23%) than in the sarilumab 200 mg group (36%) and in the placebo group (27%). In contrast to the positive trend in outcomes among the patients with critical illness, the press release cited “negative trends” for most outcomes in patients with severe illness who received sarilumab.
Siltuximab	• Multicentric Castleman disease	• Human-mouse chimeric monoclonal antibody • IL-6 antagonist	**For COVID-19:** • Not Peer Reviewed. In a single-center observational study of 21 patients with COVID-19 who developed pneumonia/ARDS and received treatment with IV siltuximab, some patients experienced decreased CRP levels (16 of 21 patients) and improved clinical condition (7 of 21 patients) following siltuximab treatment. Other patients experienced no clinically relevant change in condition (9 of 21 patients) or worsening condition (5 of 21 patients). Among the 5 patients with worsening condition, there was 1 death and 1 cerebrovascular event (median follow-up of 8 days).
Tocilizumab	• Cytokine release syndrome (induced by CAR T-cell therapy) • Rheumatoid arthritis • Giant cell arteritis • Polyarticular juvenile idiopathic arthritis • Systemic juvenile idiopathic arthritis	• Recombinant humanized monoclonal antibody • IL-6 receptor antagonist	**For COVID-19:** • Press Release: Early results were reported for the CORIMUNO-TOCI trial (ClinicalTrials.gov Identifier NCT04331808), an open-label, randomized trial of hospitalized patients with COVID-19 (n = 129) at 7 sites in France. The patients, who had moderate or severe disease at study entry, were randomized to receive tocilizumab plus SOC (n = 65) or SOC alone (n = 64). The dosing strategy was tocilizumab 8 mg/kg on Day 1; if there was no response (i.e., no decrease of oxygen requirement), a second infusion was repeated on Day 3. In this preliminary report, the proportion of participants who died or needed ventilation (noninvasive or mechanical) was lower in the tocilizumab group than in the SOC alone group. Detailed results of the trial have not been reported. • 63 hospitalized adult patients were enrolled in a prospective open-label study of tocilizumab for severe COVID-19. All patients received off-label ARV PIs. Patients received either tocilizumab IV (8 mg/kg) or SQ (324 mg); within 24 hours, a second dose was administered to 52 of the 63 patients. Following tocilizumab, fevers resolved in all but one patient, and CRP, ferritin, and D-dimer levels declined. The PaO2/FiO2 ratio increased between admission (152 +/-53 mm Hg) and Day 7 (284 +/-116 mm Hg). No moderate or severe AEs attributable to tocilizumab were

				reported. Overall mortality was 11% (7 deaths among the 63 patients). No details were provided regarding the rate of secondary infections after tocilizumab use. The authors report an association of reduced mortality with earlier use of tocilizumab, but provide no details regarding a comparison group or specify an a-priori comparison, which limits interpretation of this result. • An uncontrolled, retrospective cohort study of 21 hospitalized COVID-19 patients who received tocilizumab reported improvement in oxygenation and systemic inflammation. At study entry, among the 21 patients (mean age 56 years; range 25 to 88 years), 17 had severe disease and 4 had critical disease. All patients were febrile, had abnormal chest CT findings, and required oxygen supplementation (2 required mechanical ventilation). Mean CRP level was 75 mg/L, mean IL-6 expression level was 153 pg/mL, mean D-dimer level was 0.80 µg/mL, and mean lymphocyte percentage was 15.5%. Eighteen patients were given tocilizumab IV infusion once, and within 12 hours, 3 patients received a second infusion for indication of fever. Following tocilizumab administration, fevers normalized, lymphocyte percentages improved, and CRP levels declined. By Day 5, oxygen requirements were reduced in 15 of 20 participants (75%). There were no serious AEs attributed to tocilizumab, and no concurrent bacterial, fungal, or viral infections were observed during the treatment. The interpretability of this retrospective case series is limited due to its small sample size and lack of control group. • Additional data supporting the use of tocilizumab for COVID-19 include a small retrospective cohort study, a case series, and a case-control study
Kinase Inhibitors: Bruton's Tyrosine Kinase Inhibitors				
Acalabrutinib	• Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) • Mantle cell lymphoma (MCL)	• Second-generation oral BTK inhibitor • Inhibits BTK signaling of the B-cell antigen receptor and cytokine receptor pathways • Potential modulation of signaling that promotes inflammation and cytokine storm	**For COVID-19:** • Data regarding acalabrutinib are limited to a retrospective case series in 19 patients with severe COVID-19. However, data interpretation to discern any clinical benefit is limited by the study's small sample size and lack of a control group.	
Ibrutinib	• Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) • Mantle cell lymphoma (MCL) • Marginal zone lymphoma (MZL) • Waldenström macroglobulinemia (WM) • Chronic graft-*versus*-host disease (cGVHD) in stem cell transplant recipients	• First-generation oral BTK inhibitor • Inhibits BTK signaling of the B-cell antigen receptor and cytokine receptor pathways • Potential modulation of signaling that promotes inflammation and cytokine storm	**For COVID-19:** • Data regarding ibrutinib are limited to an uncontrolled, retrospective case series of 6 patient with COVID-19 who were receiving ibrutinib for a condition other than COVID-19. However, evaluation of the data for any clinical benefit is limited by the study's small sample size and lack of control group.	

Zanubrutinib	• Mantle cell lymphoma (MCL)	• Second-generation oral BTK inhibitor • Inhibits BTK signaling of the B-cell antigen receptor and cytokine receptor pathways • Potential modulation of signaling that promotes inflammation and cytokine storm	• No clinical data for COVID-19, SARS, or MERS
Baricitinib	• Rheumatoid arthritis	• JAK inhibitor selective for JAK1, JAK2, and TYK2, relative to JAK3 • Theoretical direct antiviral activity through inhibition of kinases (AAK1 and cyclin G-associated kinase) that regulate viral endocytosis in pulmonary AT2 epithelial cells, which may prevent SARS-CoV-2 entry into and infection of susceptible cells. • Dose-dependent inhibition of IL-6 induced STAT3 phosphorylation	**For COVID-19:** • Not Peer Reviewed. A small, nonrandomized study of 12 patients with moderate COVID-19 pneumonia compared therapy with baricitinib and LPV/r with SOC alone (i.e., combination LPV/r and HCQ).58 Baricitinib and LPV/r therapy demonstrated a statistically significant time to improvement in clinical and respiratory symptoms and reduction in measured CRP.
Ruxolitinib	• Myelofibrosis • Polycythemia vera • Steroid-refractory acute graft-*versus*host disease	• JAK inhibitor selective for JAK1 and JAK2 • Theoretical antiviral properties through inhibition of AAK1 which may prevent viral entry into and infection of pulmonary AT2 alveolar epithelial cells • Inhibition of IL-6 via JAK1/JAK2 pathway inhibition	**For COVID-19:** • A small, prospective, single-blind randomized controlled Phase 2 trial in patients with COVID-19 in China compared ruxolitinib 5 mg PO twice daily (n = 20) to placebo (vitamin C 100 mg; n = 21), both given in combination with SOC therapy. The median age of the patients was 63 years. There were no significant demographic differences between the two arms. Treatment with ruxolitinib was associated with a nonsignificant reduction in median time to clinical improvement (12 days for ruxolitinib vs. 15 days for placebo; P = 0.15), defined as a 2-point improvement on a 7-category ordinal scale or hospital discharge. There was no difference between the groups in the median time to discharge (17 days for ruxolitinib vs. 16 days for placebo; P = 0.94). More patients in the ruxolitinib group than in the placebo group had radiographic improvement on CT scans of the chest at Day 14 (90% for ruxolitinib vs. 61.9% for placebo; P = 0.05), and a shorter time to recovery from initial lymphopenia when present (5 days for ruxolitinib vs. 8 days for placebo; P = 0.03). The use of ruxolitinib was not associated with an increased risk of AEs or mortality (no deaths in the ruxolitinib group vs. 3 deaths [14% of patients] in the control group). Despite the theoretical antiviral properties of JAK inhibitors, there was no significant difference in time to viral clearance among patients who had detectable viral loads at randomization to ruxolitinib (n = 8) or placebo (n = 9). Limitations of this study include the small sample size, the exclusion of ventilated patients at study entry, and the frequent concomitant use (by 70% of patients) of antivirals and steroids. • A small retrospective single-arm study in Germany reported no safety concerns in 14 patients with severe COVID-19 who received a brief course of ruxolitinib therapy (median 9 days).

Tofacitinib	• Rheumatoid arthritis • Psoriatic arthritis • Ulcerative colitis	• JAK inhibitor selective for JAK1 and JAK3 with modest activity against JAK2 • Blocks signaling from gammachain cytokines (IL-2, IL-4) and gp 130 proteins (IL-6, IL-11, IFNs) • Shown to decrease levels of IL-6 in rheumatoid arthritis	• No clinical data for COVID-19, SARS, or MERS

Key: AAK1 = Adaptor-associated kinase 1; ADE = antibody-dependent enhancement; AE = adverse event; ARDS = acute respiratory distress syndrome; ARV = antiretroviral; AT2 = alveolar type 2; AZM = azithromycin; BTK = Bruton's tyrosine kinase; CAR = chimeric antigen receptor; CRP = C-reactive protein; CI = confidence interval; CT = computerized tomography; EHR = electronic health record; EIND = Emergency Investigational New Drug Application; FDA = Food and Drug Administration; GAK = cyclin G-associated kinase; HCQ = hydroxychloroquine; HR = hazard ratio; HSR = hypersensitivity reaction; ICU = intensive care unit; IDMC = independent data monitoring committee; IFN = interferon; IL = interleukin; IND = Investigational New Drug application; IV = intravenous; IVIG = intravenous immune globulin; LPV/r = lopinavir/ritonavir; JAK = Janus kinase inhibitor; MERS = Middle East respiratory syndrome; MERS-CoV = Middle East respiratory syndrome coronavirus; MSC = mesenchymal stem cells; NEWS2 = National Early Warning Score 2; OR = odds ratio; PCR = polymerase chain reaction; PI = protease inhibitor; RR = age-adjusted rate ratio; SARS = severe acute respiratory syndrome; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SOC = standard of care; SOFA = sequential organ failure assessment; SQ = subcutaneous; STAT3 = signal transducer and activator of transcription 3; TACO = transfusion-associated circulatory overload, TRALI = transfusion-related acute lung injury.

Credit/Source: NIAID-RML [15]. Available at: https://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/table-2a-potential-antiviral-agents-clinical-data/. Information presented in this table may include data from pre-prints or non-peer reviewed articles.

Table 2. Potential antiviral agents under evaluation for treatment of COVID-19: clinical data to date. (*Last updated July 30, 2020*).

Drug Name	FDA-Approved Indications	Preclinical Data/Mechanism of Action	Clinical Data to Date (Find clinical trials on ClinicalTrials.gov)
Azithromycin **Note:** Most studies of COVID-19 use AZM with HCQ.	• Mycobacterial (nontuberculous) infection • STIs and various bacterial infections	• Induction of IFN-stimulated genes, attenuating viral replication • Enhanced neutrophil activation • Attenuation of inflammatory cytokines (IL-6 and IL-8) in epithelial cells and inhibition of fibroblast growth factor in airway smooth muscle cells	AZM has primarily been studied for the treatment of COVID-19 in combination with HCQ. The RECOVERY trial includes an AZM monotherapy arm, which is currently enrolling. • Please see the description of the combination therapy study results in the Hydroxychloroquine Plus Azithromycin section below and in Hydroxychloroquine Plus Azithromycin.
Chloroquine	• Malaria • Extra-intestinal amebiasis	• Increases endosomal pH, inhibiting fusion of SARS-CoV-2 and the host cell membranes • Inhibits glycosylation of the cellular ACE2 receptor, which may interfere with binding of SARS-CoV to the cell receptor • May block the transport of SARS-CoV-2 from early endosomes to endolysosomes *in vitro*, which may be required to release the viral genome • Immunomodulatory effects	**High-Dose vs. Low-Dose CQ:** • A randomized, double-blind, Phase 2b study compared 2 different CQ regimens, CQ 600 mg twice daily for 10 days (high dose) vs. CQ 450 mg twice daily for 1 day followed by 450 mg for 4 days (low dose), in hospitalized adults with suspected cases of severe COVID-19 (respiratory rate >24 breaths/min, heart rate >125 bpm, oxygen saturation <90%, and/or shock). All patients received ceftriaxone plus AZM; 89.6% of patients received oseltamivir. Of note, both AZM and oseltamivir can increase the QTc interval. • The primary outcome for this analysis was mortality at 13 days after treatment initiation. The planned study sample size was 440 participants, which was sufficient to show a reduction in mortality by 50% with high-dose CQ. The study was stopped by the study's DSMB after 81 patients were enrolled. *Results:* • 41 and 40 patients were randomized into the high-dose and lowdose CQ arms, respectively. • The overall fatality rate was 27.2%. • Mortality by Day 13 was higher in the high-dose arm than in the lowdose arm (death occurred in 16 of 41 patients [39%] vs. in 6 of 40 patients [15%], respectively; P = 0.03). This difference was no longer significant when controlled by age (OR 2.8; 95% CI, 0.9–8.5). • Overall, QTcF >500 ms occurred more frequently in the high-dose arm (18.9% of patients) than in the low-dose arm (11.1% of patients). Among those with confirmed COVID-19, QTcF >500 ms was also more frequent in the high-dose arm (24.1% of patients) than in the low-dose arm (3.6% of patients). • 2 patients in the high-dose arm experienced ventricular tachycardia before death. *Limitations:* • More older patients and more patients with history of heart disease were randomized to the high-dose arm than to the low-dose arm. *Interpretation:* • Despite the small number of patients enrolled, this study raises concerns about an increased risk of mortality when high-dose CQ (600 mg twice daily) is administered in combination with AZM and oseltamivir.

			CQ vs. LPV/r: • In a small, randomized controlled trial in China, 22 hospitalized patients with COVID-19 (none critically ill) were randomized to receive oral CQ 500 mg twice daily or LPV/r 400 mg/100 mg twice daily for 10 days. Patients with a history of heart disease (chronic disease and a history of arrhythmia), or kidney, liver, or hematologic diseases were excluded from participation. The primary study outcome was a negative SARS-CoV-2 PCR test result at Days 10 and 14. Secondary outcomes included improvement of lung CT scan at Days 10 and 14, discharge at Day 14, and clinical recovery at Day 10, as well as safety (which was determined by evaluating study drug-related AEs). *Results:* • Ten patients received CQ and 12 patients received LPV/r. At baseline, patients had good SpO2 levels (97% to 98%). • Compared to the LPV/r-treated patients, the CQ-treated patients had a shorter duration from symptom onset to initiation of treatment (2.5 days vs. 6.5 days, P < 0.001). • Though not statistically significant, patients in the chloroquine arm were younger (median age 41.5 years vs. 53.0 years; P = 0.09). Few patients had comorbidities. • At Day 10, 90% of the CQ-treated patients and 75% of the LPV/rtreated patients had a negative SARS-CoV-2 PCR test result. At Day 14, the percentages for the CQ-treated patients and the LPV/r-treated patients were 100% and 91.2%, respectively. • At Day 10, 20% of the CQ-treated patients and 8.3% of the LPV/r-treated patients had CT scan improvement. At Day 14, the percentages for the CQ-treated patients and the LPV/r-treated patients were 100% and 75%, respectively. • At Day 14, 100% of the CQ-treated patients and 50% of the LPV/rtreated patients were discharged from the hospital. • The risk ratios of these outcome data cross 1, and the results were not statistically significant. • Both drugs were generally well tolerated. *Limitations:* • The trial sample size was very small, and the participants were fairly young. • The CQ-treated patients were younger and had fewer symptoms prior to treatment initiation; these variables could have affected the study protocol-defined outcomes. • Patients who had chronic comorbidities and who were critically ill were excluded from the study. *Interpretation:* • In this small randomized controlled trial, CQ and LPV/r showed similar efficacy in treating COVID-19.
Hydroxy-chloroquine	• Lupus erythematosus • Malaria • Rheumatoid arthritis	• Increases the endosomal pH, inhibiting fusion of SARS-CoV-2 and the host cell membranes4 • May block the transport of SARS-CoV-2 from early endosomes to endolysosomes *in vitro*, which may be required to release the viral genome • Immunomodulatory effects	**New York Department of Health Study on HCQ With or Without AZM:** • A retrospective, multicenter, observational study in New York evaluated the use of HCQ with and without AZM in a random sample of 1,438 inpatients with COVID-19. Patients were categorized into 4 treatment groups: HCQ plus AZM, HCQ alone, AZM alone, or neither drug. The primary outcome measure was in-hospital mortality, and the secondary outcome measure was cardiac arrest and arrhythmia or QT prolongation on an ECG. *Results:* • Patients in the 3 treatment groups had more severe disease at baseline than those who received neither drug.

• In adjusted analyses, patients who received 1 of the 3 treatment regimens did not show a decreased in-hospital mortality rate when compared with those who received neither drug.
• Patients who received HCQ plus AZM had a greater risk of cardiac arrest than patients who received neither drug (OR 2.13; 95% CI, 1.12–4.05).
Limitations:
• Despite the large size of this study, it suffers from the inherent limitations of an observational study. These include residual confounding from confounding variables that were unrecognized and/or unavailable for analysis.
Interpretation:
• Despite the limitations discussed above, these findings suggest that although HCQ and AZM are not associated with an increased risk of inhospital death, the combination of HCQ and AZM may be associated with an increased risk of cardiac arrest.

Observational Study of HCQ at a Large Medical Center in New York City:
• This observational study evaluated 1,376 consecutive adults with COVID-19 who were admitted to a large New York City hospital (after excluding 70 patients who died or who were transferred within 24 hours after presenting to the emergency department). The study assessed the time from study baseline (24 hours after patients arrived at the emergency department) to intubation or death based on whether the patient received HCQ at baseline or during follow-up. Patients who received HCQ were prescribed a twice-daily dose of HCQ 600 mg on the first day and 400 mg daily for 4 additional days; this was based on the clinical guidance of the hospital.
Results:
• 811 patients (58.5%) received HCQ and 565 (41.1%) did not.
• Patients who received HCQ were older and more likely to have hypertension (49.1% vs. 6.7%) and to be on systemic steroids (26.6% vs. 10.1%) than those who did not receive HCQ.
• Patients who received HCQ were more likely to receive concomitant AZM (59.9% vs. 22.5%) and/or other antibiotics (74.5% vs. 54.0%) than those who did not receive HCQ.
• Patients who received HCQ had higher levels of inflammatory markers.
• HCQ-treated patients had more severe hypoxia, with a lower PaO2/FiO2 ratio at baseline than patients who did not receive HCQ (median of 233 mm Hg vs. 360 mm Hg).
• Most patients (85.9%) received HCQ within 48 hours of presentation.
• Using propensity scores to adjust for major predictors of respiratory failure and inverse probability weighting, the study demonstrated that HCQ use was not associated with intubation or death (HR 1.04; 95% CI, 0.82–1.32).
• There was also no association between concomitant use of AZM and the composite endpoint of intubation or death (HR 1.03; 95% CI, 0.81–1.31).
Limitations:
• Despite the large size of this study, it suffers from the inherent limitations of an observational study. These include residual confounding from confounding variables that were unrecognized and/or unavailable for analysis.
Interpretation:
• The use of HCQ for treatment of COVID-19 was not associated with harm or benefit in a large observational study.

Retrospective Observational Cohort from the United States Veterans Health Administration

This study has not been peer reviewed

• An observational, retrospective cohort study analyzed data from patients with confirmed COVID-19 who were hospitalized at the United States Veterans Health Administration medical centers between March 9–April 11, 2020. Patients were categorized as having received either HCQ, HCQ plus AZM, or no HCQ. Doses and duration of HCQ or AZM use were not specified. All patients also received standard supportive management for COVID-19. The primary endpoints were death and the need for mechanical ventilation. Associations between treatment and outcomes were determined using propensity score adjustment, including demographic data, comorbidity data, and clinical data (including predictors of COVID-19 disease severity). Patients were included in the analysis if BMI, vital signs, and discharge disposition were noted in their medical records.

Results:

• 368 patients were eligible for analysis. These patients were categorized into 3 treatment groups: HCQ (n = 97), HCQ plus AZM (n = 113), or no HCQ (n = 158). The median ages for the patients in each group were 70, 68, and 69 years, respectively. All patients were male.

• 70 patients died; 35 of those who died (50%) were not receiving mechanical ventilation.

• No difference was observed between the groups in the risk of mechanical ventilation.

• The risk of death from any cause was higher in the HCQ group than in the no HCQ group (adjusted HR 2.61; 95% CI, 1.10–6.17; P = 0.03). The no HCQ group and the HCQ plus AZM group had similar risks of death from any cause (adjusted HR 1.14; 95% CI, 0.56–2.32, P = 0.72).

• There was no between-group difference in the risk of death after ventilation.

Limitations:

• The patient population was entirely male.

• The dose and duration of administration for HCQ and AZM were not included in the report. Patients were included if they received a single dose of either or both drugs.

• Propensity score adjustment was used to account for differences between the groups; however, the possibility of residual confounding cannot be excluded, as patients who were more ill may have been more likely to receive HCQ.

• No imaging data were presented; the severity of chest X-ray findings could predict worse outcomes.

• The use of other antiviral or immunomodulatory agents were not reported.

• The reason for the high mortality rate among patients who did not receive mechanical ventilation is not clear, especially as most of these patients appear to have had mild or moderate disease at admission.

Interpretation:

• This study showed no beneficial effect of HCQ plus AZM for the treatment of COVID-19 and a possible association between the use of HCQ and an increased risk of mortality; however, residual confounding may have affected the study results.

Randomized, Controlled Trial of HCQ vs. SOC for Mild or Moderate COVID-19:

• This multicenter, randomized, open-label trial compared HCQ 1,200 mg once daily for 3 days followed by HCQ 800 mg once daily for the rest of the treatment duration (2 weeks for patients with mild or moderate COVID-19 [99% of the patients] and 3 weeks for 2 patients with severe disease) and SOC.

• The primary outcome was a negative PCR test result within 28 days. Secondary outcomes were alleviation of symptoms (resolution of fever, SpO2 >94% on room air, resolution of respiratory symptoms), improvement in markers of inflammation (including CRP levels), and improvement of lung lesions on a chest X-ray within 28 days.

Results:

• 75 patients were enrolled in each study arm. Patients were randomized at a mean of 16.6 days after symptom onset.

• The HCQ arm and the SOC arm had similar negative PCR conversion rates within 28 days (85.4% of participants vs. 81.3% of participants, respectively) and similar times to negative PCR conversion (median of 8 days vs. 7 days, respectively).

• There was no difference in the probability of symptom alleviation between the groups in the intention-to-treat analysis.

• AEs occurred in 30% of the participants in the HCQ arm (most commonly diarrhea) and in 9% of the participants in the SOC arm.

Limitations:

• It is unclear how the overall rate of symptom alleviation was calculated.

• The duration of HCQ use (2 weeks) was longer than in most other observational cohort studies or clinical trials for the treatment of COVID-19.

• The study did not reach the target sample size.

Interpretation:

• This study demonstrated no difference in viral clearance between HCQ and SOC.

Observational Cohort of HCQ vs. No HCQ:

• This observational, retrospective cohort study analyzed data for adult patients who were hospitalized for COVID-19 pneumonia at 4 French tertiary care centers over a 2-week period (March 17–31, 2020). Patients aged 18–80 years were eligible if they had PCR-confirmed SARS-CoV-2 infection and required oxygen by mask or nasal cannula. Exclusion criteria included HCQ initiation before hospitalization, receipt of another experimental COVID-19 treatment within 48 hours, organ failure that required immediate admission to the ICU or continuous care unit, admission with ARDS that required noninvasive ventilation with continuous positive airway pressure or mechanical ventilation, discharge from the ICU to standard care, or if a decision was made to limit or stop active treatments prescribed at admission. Patients in 1 treatment arm received a daily dose of HCQ 600 mg within 48 hours of admission; patients in the other arm did not receive HCQ during the same period. The decision to use HCQ to treat a patient was based on local medical consensus and prescriber opinion and was reportedly independent of patient characteristics. Patients were followed from baseline until death, loss to follow-up, or the end of the follow-up period on April 24, 2020. The primary outcome was survival without transfer to the ICU at Day 21. An inverse probability of treatment weighting approach was used to "emulate" randomization.

Results: • Of the 181 patients who were eligible for the analysis, 84 participants received HCQ within 48 hours, 8 received HCQ beyond 48 hours, and 89 did not receive HCQ. • Comorbidities were less common in the HCQ group; overall initial COVID-19 severity was well balanced across the treatment arms. • In the HCQ group, 18% of the patients received concomitant AZM and 52% of the patients received amoxicillin/clavulanic acid. • In the inverse probability of treatment weighted analysis, there was no difference in survival rates without ICU transfer at Day 21 between the HCQ group (76% of participants) and the non-HCQ group (75% of participants). Similarly, there was no difference between the groups in the secondary outcomes of survival rate and survival rate without ARDS at Day 21. • Among the 84 patients who received HCQ within 48 hours, 8 patients (10%) experienced ECG changes that required treatment discontinuation at a median of 4 days from the start of dosing, including 7 patients with a QTc that prolonged >60 ms and 1 patient with new onset, first-degree AV block. None of these patients received AZM. *Limitations:* • This was a retrospective, nonrandomized study. *Interpretation:* • In this retrospective study, there was no difference in the rates of clinically important outcomes between patients who received HCQ within 48 hours of hospital admission and those who did not.			
A Case Series of HCQ vs. Control: • In a case series from France, 26 hospitalized adults with either asymptomatic SARS-CoV-2 infection or upper or lower respiratory tract infection received HCQ 200 mg 3 times daily for 10 days. These patients were compared to 16 control individuals (i.e., those who refused treatment, did not meet eligibility criteria, or were from a different clinic). *Results:* • 6 patients in the HCQ group were excluded from the analysis for the following reasons: • 1 patient died, • 3 patients were transferred to the ICU, • 1 patient stopped the study drug due to nausea, and • 1 patient withdrew from the study. • 6 patients also received AZM. • By Day 6, NP PCRs were negative in 14 of 20 HCQ-treated patients (70%) and 2 of 16 controls (12.5%). • Among the HCQ patients, 8 of 14 (57.1%) who received only HCQ and 6 of 6 (100%) who received HCQ and AZM had negative NP PCRs by Day 6. • Clinical outcomes were not reported for all patients. *Limitations:* • The sample size of the series is small. • The criteria for enrollment of cases and controls is unclear. • Asymptomatic individuals were enrolled. • Exclusion of 6 HCQ-treated patients includes 1 death and 3 ICU transfers. • No clinical outcomes were reported; thus, the clinical significance of a negative PCR is unknown. • The reason for the addition of AZM for some patients is unclear. *Interpretation:* • Methodologic problems with this case series limit the ability to draw conclusions regarding the efficacy of HCQ with or without AZM.			

Hydroxy-chloroquine Plus Azithromycin	See the Azithromycin and Hydroxychloroquine sections above.	See the Azithromycin and Hydroxychloroquine sections above.	**Case Series of HCQ Plus AZM:** • In a case series of 80 hospitalized patients with COVID-19 (including 6 patients from a previous study),14 patients were treated with HCQ 200 mg 3 times daily for 10 days plus AZM 500 mg once daily for 1 day followed by AZM 250 mg once daily for 4 days. Mean time from symptom onset to treatment was about 5 days. The outcomes that were evaluated included the need for oxygen therapy or ICU transfer after ≥3 days of therapy, SARS-CoV-2 level as determined by PCR, SARS-CoV-2 culture (in a subset of patients; a convenience sample), and length of stay in the infectious diseases ward. *Clinical Results:* • 1 patient died (1.2%), 3 required ICU transfer (3.8%), and 12 required oxygen therapy (15%). • 65 patients (81.2%) were discharged to their homes or transferred to other units for continuing treatment; 14 patients (17.4%) remained hospitalized at the time the study results were published. *Laboratory Results:* • SARS-CoV-2 NP PCR was negative in 83% of patients by Day 7 and in 93% of patients by Day 8. • In the subset of patients who had respiratory sample viral cultures performed at Day 5, results were negative for 97.5% of the samples. *Limitations:* • The trial lacked a control group, which is particularly important because many people with mild disease improve in the absence of treatment. • The trial lacked complete or longer-term follow-up. *Interpretation:* • The multiple issues with trial design and the lack of a control group limit the usefulness of this study for informing recommendations. **Small Prospective Case Series of HCQ Plus AZM:** • A prospective case series from France assessed 11 consecutive hospitalized patients with COVID-19. *Results:* • 8 of the 11 patients had significant comorbid conditions: obesity (n = 2), solid cancer (n = 3), hematological cancer (n = 2), and HIV infection (n = 1). • 10 of 11 patients were receiving supplemental oxygen at treatment initiation. • All patients were treated with HCQ 600 mg once daily for 10 days and AZM 500 mg once daily for 1 day followed by AZM 250 mg once daily for 4 days. • Within 5 days, the condition of 3 patients worsened, including 1 patient who died and 2 patients who were transferred to the ICU. • HCQ was discontinued in 1 patient due to QTc prolongation. • Qualitative NP PCR remained positive at Days 5 and 6 after treatment initiation in 8 of 10 patients. *Limitations:* • This is a case series that included a small number of patients. *Interpretation:* • In this small case series, most patients who received HCQ plus AZM did not have rapid viral clearance.

			Case Series of Changes in QTc Interval in Patients Who Received HCQ Plus AZM: • A case series in the United States reported changes in QTc interval in 84 patients with COVID-19 who received the combination of HCQ (400 mg twice daily for 1 day, followed by 200 mg twice daily for 4 days) and AZM (500 mg once daily for 5 days). *Results:* • 84 patients were enrolled; 74% were male, with a mean age of 63 ± 15 years. 65% had HTN, mean serum creatinine was 1.4 mg/dL at baseline, 13% required vasopressors, and 11% had CAD. • Some participants were receiving concomitant drugs that had the potential to prolong the QTc interval; 11% of participants were receiving neuropsychiatric drugs and 8% of participants were receiving levofloxacin, LPV/r, or tacrolimus. • 4 patients died, without arrhythmia. • The mean baseline QTc was 435 ± 24 ms and the mean maximum QTc was 463 ± 32 ms. • The mean time to maximum QTc was 3.6 ± 1.6 days. ECG follow-up was done for a mean of 4.3 days. • 9 patients (11%) developed QTc >500 ms; the QTc increased by 40 to 60 ms and >60 ms in 18% and 12% of patients, respectively. *Limitations:* • This was a descriptive case series. *Interpretation:* • This case series demonstrated that HCQ plus AZM can prolong QTc and that the use of this combination warrants careful monitoring.
HIV Protease Inhibitors Note: LPV/r and DRV/c have been studied in patients with COVID-19	HIV infection	• No data on *in vitro* activity of LPV/r against SARS-CoV-2 • Possible inhibition of SARS-CoV-2 protease 3CLpro • *In vitro* data does not support the use of DRV/c for the treatment of COVID-19	**LPV/r Pharmacokinetics in Patients With COVID-19:** • In a case series, 8 patients with COVID-19 were treated with LPV/r 400 mg/100 mg orally twice daily and had plasma trough levels of LPV drawn and assayed by liquid chromatography-tandem mass spectrometry. *Results:* • The median plasma LPV concentration was 13.6 µg/mL. • After correcting for protein binding, trough levels would need to be approximately 60-fold to 120-fold higher to achieve the *in vitro* EC50 for SARS-CoV-2. *Limitations:* • Only the trough levels of LPV were quantified. • No data are available on effective LPV concentrations for SARS-CoV-2 *in vivo*. *Interpretation:* • The plasma drug concentrations that were achieved using typical doses of LPV/r are far below the levels that may be needed to inhibit SARS-CoV-2. **Randomized Controlled Trial of LPV/r vs. SOC:** • In a clinical trial that randomized 199 patients to receive LPV/r 400 mg/100 mg PO twice daily for 14 days or SOC, patients who were randomized to the LPV/r arm did not have a shorter time to clinical improvement. *Results:* • There was a lower, but not statistically significant, mortality rate for the LPV/r group (19.2%) than for the SOC group (25.0%), and a shorter ICU stay for those in the LPV/r group than those in the SOC group (6 days vs. 11 days; difference of -5 days; 95% CI, -9 to 0 days).

		• The duration of hospital stays and time to clearance of viral RNA from respiratory tract samples did not differ between the LPV/r and SOC arms. • Nausea, vomiting, and diarrhea were all more frequent in the LPV/rtreated group. • The study was powered only to show a fairly large effect. *Limitations:* • The study was not blinded, which may have affected the assessments of clinical improvement. • The study was underpowered to show small effects. *Interpretation:* • A moderate-sized, randomized trial failed to find a virologic or clinical benefit of LPV/r over SOC.
		LPV/r Plus IFN Beta-1b Plus Ribavirin for COVID-19: • Also see Interferons for a description of this trial and its results. • An open-label, Phase 2 clinical trial randomized 127 participants with COVID-19 2:1 to receive either a 14-day course of a combination therapy that included IFN beta-1b 8 million international units administered subcutaneously on alternating days (1–3 doses, depending on time from symptom onset) plus LPV/r 400 mg/100 mg orally every 12 hours and ribavirin 400 mg orally every 12 hours, or a 14-day course of LPV/r 400 mg/100 mg every 12 hours alone. • In the combination therapy group, those who were admitted <7 days after symptom onset (n = 52) received triple-drug therapy; however, IFN beta-1b was not included in the regimen for those who were admitted ≥7 days after symptom onset (n = 34) because of concerns regarding its potential for inflammatory effects. The study population consisted of patients who were hospitalized in Hong Kong; the median age was 52 years and the median time from symptom onset to enrollment was 5 days. Only 12% to 14% of participants were on supplemental oxygen, and only 1 participant was mechanically ventilated. *Results:* • Patients in the combination therapy group showed faster viral clearance and more rapid clinical improvement than those in the control group. *Limitations:* • Participants in both arms received LPV/r, so it is impossible to determine whether LPV/r contributed to the observed treatment effects. However, the possibility that LPV/r may have contributed to the effectiveness of the combination therapy also cannot be ruled out. • The positive clinical impact of the combination therapy was limited to those who were hospitalized <7 days from symptom onset. • Most participants in this study had mild illness, and only slightly more than 10% were on supplemental oxygen. For this reason, the study has limited applicability to hospitalized patients in the United States. *Interpretation:* • This study neither supports nor refutes the use of LPV/r with or without ribavirin in patients with COVID-19. See the Interferons section for further discussion.
		LPV/r vs. Umifenovir vs. SOC • In a trial of 86 hospitalized patients with mild-to-moderate COVID-19, 34 patients were randomized to receive LPV/r, 35 patients received the broad-spectrum antiviral umifenovir (trade name Arbidol; not available in the United States), and 17 patients received SOC.

			Results (Comparison of LPV/r to SOC): • The time to a negative SARS-CoV-2 nucleic acid pharyngeal swab was similar for patients receiving LPV/r (mean 9 days [SD ± 5.0]) and for those receiving SOC (mean 9.3 days [SD ± 5.2]). • Progression to severe illness occurred among 6 patients (18%) in the LPV/r arm and 2 patients (12%) who received SOC. • 2 patients became critically ill; both were randomized to receive LPV/r. *Limitations:* • The trial had a small sample size. • The study was not blinded. • The effectiveness of umifenovir in treating COVID-19 is unknown. Interpretation: • The small sample size of this trial limits its usefulness. **LPV/r vs. CQ:** • A small randomized study in China compared LPV/r to CQ. Please refer to the Chloroquine section above for the study description.
Remdesivir (GS-5734)	• Not approved by FDA	• Binds to the viral RNA-dependent RNA polymerase, inhibiting viral replication through premature termination of RNA transcription • Has demonstrated *in vitro* activity against SARS-CoV-24 • In a rhesus macaque model of SARS-CoV-2 infection, RDV treatment was initiated soon after inoculation; RDVtreated animals had lower lung virus levels and less lung damage than the control animals.	**Multinational Randomized Controlled Trial of RDV vs. Placebo in Hospitalized Patients:** • ACTT is an NIH-sponsored, multinational, randomized, doubleblind placebo-controlled trial in hospitalized adults with COVID-19. Participants were randomized 1:1 to receive IV RDV or placebo for 10 days. The primary study endpoint was time to clinical recovery, which was defined as either discharge from the hospital or hospitalization for infection control purposes only. Severity of illness at baseline and at Day 15 was assessed using an ordinal scale: 1. Not hospitalized, no limitations 2. Not hospitalized, with limitations 3. Hospitalized, no active medical problems 4. Hospitalized, not on oxygen 5. Hospitalized, on oxygen 6. Hospitalized, on high-flow oxygen or noninvasive mechanical ventilation 7. Hospitalized, on mechanical ventilation or ECMO 8. Death *Study Population:* • The study population consisted of hospitalized patients aged ≥18 years with laboratory-confirmed SARS-CoV-2 infection. Patients were enrolled if they met at least 1 of the following conditions: • The patient had pulmonary infiltrates, as determined by radiographic imaging, • SpO2 was ≤94% on room air, • The patient required supplemental oxygen, • The patient was on mechanical ventilation, or • The patient was on ECMO. • The study excluded individuals who had ALT or AST levels >5 times the ULN, those who had an eGFR <30 mL/min, and those who were pregnant or breastfeeding. *Preliminary Results:* • Of 1,063 enrolled participants, 1,059 had preliminary results available for analysis (n = 538 for the RDV group; n = 521 for the placebo group). • The mean age was 58.9 years; 64.3% of participants were male, 53.2% were white, and 79.8% were enrolled in North America. • 52.1% of participants had 2 or more comorbidities; 37% were obese (mean BMI 30.6 kg/m2) • The median time from symptom onset to randomization was 9 days (IQR 6–12 days). • At the time of the preliminary analysis, 391 RDV recipients and 340 placebo recipients had completed the study through Day 29, recovered, or died.

			• 8 RDV recipients and 9 placebo recipients terminated the study prior to Day 29. • At the time of this preliminary analysis, 132 RDV recipients and 169 placebo recipients had not recovered and had not completed the Day 29 follow-up visit. • RDV significantly reduced time to recovery compared to placebo (median time to recovery 11 days vs. 15 days, respectively; recovery rate ratio 1.32; 95% CI, 1.12–1.55; P < 0.001). • Clinical improvement based on the ordinal scale was significantly higher in patients who received RDV than in those who received placebo at Day 15 (OR 1.50; 95% CI, 1.18–1.91, P < 0.001). • The benefit of RDV on reducing time to recovery was clearest in the subgroup of hospitalized patients who required supplemental oxygenation at study enrollment (ordinal scale 5, n = 421; recovery rate ratio 1.47; 95% CI, 1.17–1.84). In a post-hoc analysis of 14-day survival, remdesivir appeared to confer a survival benefit in this subgroup (HR 0.22; 95% CI, 0.08–0.58). • In patients who required high-flow oxygen or noninvasive ventilation at study enrollment (ordinal scale 6, n = 197), there was no observed difference between the remdesivir and placebo groups in time to recovery (recovery rate ratio 1.20; 95% CI, 0.79–1.81). In a post-hoc analysis of 14-day survival, there was no evidence that remdesivir had an impact on the mortality rate in this subgroup (HR 1.12; 95% CI, 0.53–2.38). • Among the patients who were on mechanical ventilation or ECMO at enrollment (ordinal scale 7, n = 272), there was no observed difference between the RDV and placebo groups in time to recovery (recovery rate ratio 0.95; 95% CI, 0.64–1.42). In a post-hoc analysis of 14-day survival, there was no evidence that remdesivir had an impact on the mortality rate in this subgroup (HR 1.06; 95% CI, 0.59–1.92). • Among the patients who were classified as having mild to moderate disease at enrollment, there was no difference in the median time to recovery between the RDV and placebo groups (n = 119; recovery rate ratio 1.09; 95% CI, 0.73–1.62). Mild to moderate disease was defined as SpO2 >94% and respiratory rate <24 breaths/min without supplemental oxygen. • The mortality estimate by Day 14 was lower in the RDV arm than in the placebo arm (7.1% vs. 11.9%, respectively), but the difference was not statistically significant (HR 0.70; 95% CI, 0.47–1.04). • The use of RDV was associated with shorter time to recovery regardless of the duration of symptoms prior to randomization (≤10 days vs. >10 days). • The percentages of participants with serious AEs were similar in the RDV and placebo groups (21.1% vs. 27.0%, respectively). • Transaminase elevations occurred in 4.1% of RDV recipients and 5.9% of placebo recipients. *Limitations:* • At the time of publication, the full dataset was not available for analysis. *Interpretation:* • In patients with severe COVID-19, RDV reduced the time to clinical recovery. The benefit of RDV was most apparent in hospitalized patients who required only supplemental oxygen. There was no observed benefit of RDV in those who were on high-flow oxygen, noninvasive ventilation, mechanically ventilation or ECMO, but the study was not powered to detect differences in subgroups. There was no observed benefit of RDV in patients with mild or moderate COVID-19, but the number of participants in these categories was relatively small.

Multinational Randomized Trial of Different Durations of RDV Treatment in Hospitalized Patients: • This was a manufacturer-sponsored, multinational, randomized, open-label trial in hospitalized adolescents and adults with COVID-19. Participants were randomized 1:1 to receive either 5 days or 10 days of IV RDV. The primary study endpoint was clinical status at Day 14, which was assessed using a 7-point ordinal scale: 1. Death 2. Hospitalized, on invasive mechanical ventilation or ECMO 3. Hospitalized, on noninvasive ventilation or high-flow oxygen devices 4. Hospitalized, requiring low-flow supplemental oxygen 5. Hospitalized, not requiring supplemental oxygen, but requiring ongoing medical care for COVID-19 or for other reasons 6. Hospitalized, not requiring supplemental oxygen or ongoing medical care (other than the care that was specified in the protocol for RDV administration) 7. Not hospitalized *Study Population:* • The study enrolled hospitalized patients aged ≥12 years with RT-PCRconfirmed SARS-CoV-2 infection and radiographic evidence of pulmonary infiltrates. Patients in this study had either SpO2 ≤94% on room air or were receiving supplemental oxygen. The study excluded patients who were receiving mechanical ventilation or ECMO or who had multiorgan failure, an ALT or AST level >5 times ULN, or an estimated CrCl <50 mL/min. Patients were also excluded if they had received an agent with putative anti-SARS-CoV-2 activity within 24 hours of starting treatment in the trial. *Results:* • Of 402 randomized participants, 397 began 5 days (n = 200) or 10 days (n = 197) of RDV treatment. • In the 5-day group, the median age was 61 years; 60% of participants were male, and 71% were white. In the 10-day group, the median age was 62 years; 68% of participants were male, and 70% were white. The frequency of coexisting conditions was similar in both groups. • The median time from symptom onset to first dose of RDV was 8 days in the 5-day group and 9 days in the 10-day group. The median duration of hospitalization before the first RDV dose was 2 days in both groups. • At baseline, patients in the 10-day group had worse clinical status (based on the ordinal scale distribution) than those in the 5-day group (P = 0.02). • A few patients were on mechanical ventilation: 4 patients (2%) were assigned to the 5-day group, and 9 patients (5%) were assigned to the 10-day group. Although mechanical ventilation was an exclusion criterion for enrollment, some patients were intubated between screening and treatment initiation; others were protocol deviations. • 172 participants (86%) in the 5-day group completed a median of 5 days of treatment, and 86 (44%) in the 10-day group completed a median 9 days of treatment. • 65% of patients in the 5-day group and 54% of those in the 10-day group had a 2-point improvement in clinical status on the ordinal scale. • After adjusting for imbalances in the baseline clinical status, the Day 14 distribution in clinical status on the ordinal scale was similar in the 5-day and 10-day groups (P = 0.14) • The time to clinical improvement of at least 2 levels on the ordinal scale (median day of 50% cumulative incidence) was similar in the 5-day and 10-day groups (10 days vs. 11 days, respectively).			

• The median durations of hospitalization among patients who were discharged on or before Day 14 were similar in the 5-day group (7 days; IQR 6–10 days) and 10-day group (8 days; IQR 5–10 days). • By Day 14, 120 patients (60%) in the 5-day group had been discharged and 16 patients (8%) had died; in the 10-day group, 103 patients (52%) had been discharged and 21 patients (11%) had died. • Serious AEs were more common in the 10-day group (35%) than in the 5-day group (21%); 4% of patients in the 5-day group and 10% of patients in the 10-day group stopped treatment because of AEs. *Limitations:* • This was an open-label trial without a placebo control group, so the clinical benefit of RDV could not be assessed. • There were baseline imbalances in the clinical statuses of participants in the 5-day and 10-day groups. At the start of the study, more patients in the 10-day group than in the 5-day group were receiving noninvasive ventilation or high-flow oxygen (30% vs. 24%, respectively), and fewer patients in the 10-day group than in the 5-day group were not receiving supplemental oxygen (11% vs. 17%, respectively). *Interpretation:* • In hospitalized patients with COVID-19 who were not on mechanical ventilation or ECMO, RDV treatment for 5 or 10 days had similar clinical benefit. Because this trial only evaluated a few patients who were on mechanical ventilation, the appropriate duration of RDV treatment for critically ill patients is still unclear.			
Randomized Controlled Trial of RDV vs. Placebo for Severe COVID-19 in China: • This was a multicenter, double-blind, randomized, placebo-controlled trial that evaluated patients with severe COVID-19 in China. Patients were randomized 2:1 to receive IV RDV or normal saline placebo for 10 days. Concomitant use of LPV/r, corticosteroids, and interferons was allowed. The primary study endpoint was time to clinical improvement, defined as improvement on an ordinal scale or discharged alive from the hospital, whichever came first. The planned sample size was 453 patients. • The study enrolled hospitalized adults with laboratory-confirmed COVID-19 whose time from symptom onset to randomization was <12 days, whose O2 saturation was ≤94% on room air or whose PaO2/FiO2 was <300 mmHg, and who had radiographically confirmed pneumonia. *Results:* • Between February 6–March 12, 2020, 237 hospitalized patients were enrolled and randomized to receive RDV (n = 158) or placebo (n = 79). The study was stopped before target enrollment was reached due to control of the COVID-19 outbreak in China. • The participants' median age was 65 years; 56% of the participants in the RDV arm and 65% in the placebo arm were male. • There were more patients with HTN, DM, or CAD in the RDV arm than in the placebo arm. • At Day 1, 83% of the patients required supplemental oxygen by nasal cannula or mask; only 1 patient required mechanical ventilation or ECMO.			

			• The median time from symptom onset to randomization was 9 days in the RDV group and 10 days in the placebo group. • 65% of participants in the RDV group and 68% of participants in the placebo group received corticosteroids. • 28% of participants in the RDV group and 29% of participants in the placebo group received LPV/r. • 29% of participants in the RDV arm and 38% of participants in the placebo arm received IFN alfa-2b. *Study Endpoints:* • There was no difference in the time to clinical improvement between the RDV and placebo groups (a median of 21 days vs. 23 days, respectively; HR 1.23; 95% CI, 0.87–1.75). • For patients who started RDV or placebo within 10 days of symptom onset, faster time to clinical improvement was seen in the RDV arm than in the placebo arm (median of 18 days vs. 23 days, respectively; HR 1.52; 95% CI, 0.95–2.43); however, this was not statistically significant. • The 28-day mortality rate was similar for the 2 study arms (14% of participants in the RDV arm vs. 13% in the placebo arm). • There was no difference between the groups in SARS-CoV-2 viral load at baseline, and the rate of decline over time was similar between the 2 groups. • The number of participants who experienced AEs was similar between the 2 groups (66% of participants in the RDV arm vs. 64% in the placebo arm). • More participants in the RDV arm discontinued therapy due to AEs (12% of participants in the RDV arm vs. 5% in the placebo arm). *Limitations:* • The study was terminated early; as a result, the sample size did not have sufficient power to detect differences in clinical outcomes. • The use of concomitant medications (i.e., corticosteroids, LPV/r, IFNs) may have obscured the effects of RDV. *Interpretation:* • There was no difference in time to clinical improvement, 28-day mortality, or rate of viral clearance between RDV-treated and placebo-treated patients. **Uncontrolled Case Series from RDV Compassionate Use Program** • In an uncontrolled case series of 53 hospitalized patients with COVID-19, most patients needed less oxygen support after receiving compassionate use RDV. There was no comparison group, however, so it is not possible to assess whether the improvement was the result of using RDV.

Key: 3CLpro = 3-chymotrypsin-like protease; ACE2 = angiotensin-converting enzyme 2; ACTT = Adaptive COVID-19 Treatment Trial; AE = adverse effect or adverse event; ALT = alanine transaminase; ARDS = acute respiratory distress syndrome; AST = aspartate transaminase; AV = atrioventricular; AZM = azithromycin; BMI = body mass index; CAD = coronary artery disease; CI = confidence interval; CQ = chloroquine; CrCl = creatinine clearance; CRP = C-reactive protein; CT = computed tomography; DM = diabetes mellitus; DRV/c = darunavir/cobicistat; DSMB = data safety monitoring board; EC50 = half-maximal effective concentration; ECG = electrocardiogram; ECMO = extracorporeal membrane oxygenation; eGFR = estimated glomerular filtration rate; FDA = Food and Drug Administration; HCQ = hydroxychloroquine; HIV = human immunodeficiency virus; HR = hazard ratio; HTN = hypertension; ICU = intensive care unit; IFN = interferon; IL = interleukin; IQR = interquartile range; IV = intravenous; LPV = lopinavir; LPV/r = lopinavir/ritonavir; NIH = National Institutes of Health; NP = nasopharyngeal; OR = odds ratio; PCR = polymerase chain reaction; PO = orally; QTcF = corrected QT interval by Fredericia; RDV = remdesivir; RECOVERY = Randomised Evaluation of COVID-19 Therapy; RT-PCR = reverse transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SD = standard deviation; SOC = standard of care; STI = sexually transmitted infection; ULN = upper limit of normal.
Credit/Source: NIAID-RML [15]. Available at: https://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/table-2a-potential-antiviral-agents-clinical-data/. Information presented in this table may include data from pre-prints or non-peer reviewed articles.

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Vaccines' Candidates Against SARS-CoV-2

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Scientists, health organizations, and pharmaceutical companies are making a large global effort to develop vaccines against SARS-CoV-2, the virus of COVID-19 since the outbreak began. Until now, we have more than 150 candidates. However, 19 vaccine candidates have entered clinical trials in phase 2 and 3 trials (31 July 2020). In this article we aimed to present the platforms for COVID-19 vaccine, the types of vaccines (live, attenuated, inactivated, DNA/RNA, proteins subunits, viral vector), the antigen selection, adjuvants, and we focused on the phase 2/3 trial vaccines at this point (Sinopharm, Coronavac, Moderna, Oxford, Biontech). We searched the data in the main database (PubMed/Medline, Elsevier Science Direct, Scopus, Isi Web of Science, Embase, Excerpta Medica, UptoDate, Lilacs, Novel Coronavirus Resource Directory from Elsevier), in the high-impact international scientific Journals (Scimago Journal and Country Rank - SJR - and Journal Citation Reports - JCR), such as The Lancet, Science, Nature, The New England Journal of Medicine, Physiological Reviews, Journal of the American Medical Association, Plos One, Journal of Clinical Investigation, and in the data from Center for Disease Control (CDC), National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID) and World Health Organization (WHO). We prior selected meta-analysis, systematic reviews, article reviews, and original articles in this order. We reviewed 216 articles and used 106 from March to June 2020, using the terms coronavirus, SARS-CoV-2, novel coronavirus, Wuhan coronavirus, severe acute respiratory syndrome, 2019-nCoV, 2019 novel coronavirus, n-CoV-2, covid, n-SARS-2, COVID-19, corona virus, coronaviruses, vaccine, platform, antigen, subunit, live and attenuated vaccine, RNA vaccine, live vaccine, inactivated vaccine, types of vaccines, adjuvants, replication, viral vector, phase 1-3, trial, with the tools MeSH (Medical Subject Headings), AND, OR, and the characters [,",.; /., to ensure the best review topics. We concluded that although vaccines have shown safety in phase 1 and efficacy in phase 2 and the beginning of phase 3 is starting, the most renowned scientists believe that a vaccine will be available only in the middle of next year.
Keywords: COVID-19. SARS-CoV-2. Types of Vaccines. Phase 3. Immunity.

Introduction

Since the pandemic started on December,2019, the researchers of all over the world are trying to find a vaccine against the SARS-CoV-2. The development of a vaccine normally takes 10 to 15 years. However, because of the COVID-19 pandemic, many centers of research are working together to develop a vaccine within one year. A total of more than 100 different vaccines for SARS-CoV-2 are under development, but a small number of them have reached the stage of development that the vaccines can be tested in humans (Phase 3). Until now, we have some promised phase 3 vaccines.

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This article aims to compile the update of the potential vacines against SARS-CoV-2 as well as the platforms for COVID-19 vaccine development and the types of the vacines.

Biochemical and Molecular Roadmap of SARS-CoV-2 [1]

The terminology for the RNA virus that causes COVID-19, SARS-CoV-2, has been established by the International Committee on Taxonomy of Viruses (ICTV) [2], due to its extensive homology with the 2003 SARS coronavirus. The SARS-CoV-2 coronavirus belongs to the subfamily of Coronavirinae, with a genomic structure of (+)ss-RNA of 30kb in length that includes a 5'-cap structure and 3'-poly-A tail [3]. From the viral RNA, polyprotein 1a/1ab (pp1a/pp1ab) is synthesized in the host to form 16 non-structural proteins (nsps) that organize the replication-transcription complex (RTC) in double membrane vesicles

(DMVs). The nRTC synthesizes a set of minus-strand subgenomic RNAs (sgRNAs) discontinuously [4]. Between open reading frames (ORFs), transcription terminates, and then a subsequent acquisition of a leader RNA occurs. During this process, subgenomic mRNAs need these sgRNAs as the templates [5, 6]. At least six ORFs exist for a typical CoV, including SARS-CoV-2. The first ORFs (ORF1a/b) with over 65% of the whole genome length encode 16 nsps. Of note, two polypeptides (pp1a and pp1ab) come from a -1 frameshift between ORF1a and ORF1b. For the other ORFs on the 35% of the genome close to the 3'-terminus encode at least four main structural proteins, including spike (S), membrane (M), envelope (E), and nucleocapsid (N). All these structural and non-structural proteins are translated from the sgRNAs [4-6]. Currently, more than 200 complete and partial genome sequences of SARS-CoV-2 have been decoded and deposited in the Global Initiative on Sharing All Influenza Data (GISAID) database (https://www.gisaid.org/) [7] and in the National Institutes of Health (NIH) GenBank database (https://www.ncbi.nlm.nih.gov/nuccore/?term=COVID-19) [8].

Phylogenetic analysis showed that SARS-CoV-2 was closely related to two SARS-like coronaviruses present in bats, including bat-SL-CoVZC45 and bat-SL-CoVZXC21, with 88% identity, and showed 79% homology with SARS-CoV, and 50% with MERS-CoV [9]. However, homology modeling disclosed that SARS-CoV-2 had a similar RBD structure to that of SARS-CoV, despite amino acid variation at some key residues [10, 11]. These findings suggest that SARS-CoV-2 emerged from a single animal source within a short period [12]. However, because the sequence similarity between SARS-CoV-2 and its close relatives bat-SL-CoVZC45 and bat-SL-CoVZXC21 is less than 90%, the bat-derived viruses may not be the direct origins of SARS-CoV-2.

Platforms for COVID-19 Vaccine Development [13]

Whole Virion Vaccines (Live Attenuated Virus and Inactivated Virus)

Attenuated Virus Vaccine

Live attenuated vaccines (LAV) are viruses that are rendered replication-incompetent through repeated passage in cell culture, and inactivated vaccines utilise whole pathogen which has typically been killed by exposure to chemicals (e.g. formaldehyde) or heat inactivation [14]. LAV are immunogenic and reproduce the breadth of the humoral and cellular immune protection that would be generated by live viral infection [15, 14] however inactivated vaccines are generally less immunogenic and require more than one dose or an additional adjuvant [16]. Safety issues regarding the generation and subsequent attenuation of the virus, with potential for reactivation in vaccinated individuals, means LAV are not a tenable vaccine strategy for highly pathogenic viruses [14, 17]. This also prevents immunisation of individuals with weakened immune systems who are at further risk of illness if the pathogen reverts [18]. From the perspective of vaccine distribution, LAV are generally kept refrigerated to preserve immunogenicity, which may be problematic in countries that cannot sustain cold chain distribution [16, 18]. LAV for SARS-CoV-1 were tested in pre-clinical trials [19]. There is currently one company, Codagenix, proposing a computationally designed, lab-made SARS-CoV-2 'virus' that is immunogenic but not pathogenic [20]. SinoVac demonstrated safety and immunogenicity of an inactivated SARS-CoV-1 vaccine in a Phase 1 trial [21], and have determined efficacy of a formalininactivated SARS-CoV-2 vaccine in rhesus macaques [22]. Although this vaccine did not demonstrate any ADE-derived pathogenesis, previous whole virus SARS-CoV-1 vaccines trialled in mice induced eosinophil-derived immunopathology upon viral challenge [23], and Th2-driven histopathological changes in the lungs [24].

However, the two doses of Sinovac Biotech's COVID-19 vaccine candidate, dubbed CoronaVac, induced neutralizing antibodies 14 days after vaccination. More than 90% of the 600 healthy volunteers in the phase 2 part of the phase 1/2 study showed that immune response [13]. Now, the Chinese vaccine is in the phase 3 in which Brazil, in a partnership with Butantan Institute and Sinovac Biotech's, a Chinese company, is participating with health volunteers.

Inactivated Virus Vaccine [25]

Multiple SARS-CoV-2 vaccine types are under development, such as DNA- and RNA-based formulations, recombinant subunits containing viral epitopes, adenovirus-based vectors, and purified inactivated virus [26, 27]. Purified inactivated viruses have been traditionally utilized for vaccine development, and such vaccines are secure and efficient for the prevention of diseases caused by viruses such as influenza virus and poliovirus [28, 29]. No antibody-dependent enhancement (ADE) of infection was recognized for the vaccinated macaques despite the observation that a relatively low NAb titer existed within the medium-dose group before infection, giving partial protection. The chance of manifestation of ADE after antibody titers wane could not be ruled out in this study. Although T cell responses elicited by multiple vaccines have been shown to be vital for acute viral clearance, protection from subsequent coronavirus infections is largely mediated by humoral immunity [30-33]. The "cytokine storm" provoked by excessive T cell responses has really been exhibited to accentuate the pathogenesis of COVID-19 [34, 35].

Consequently, T cell responses elicited by any SARS-CoV-2 vaccine(s) would have to be well controlled to withdraw immunopathology. In this context, the safety of PiCoVacc applied in macaques by recording a number of clinical observations was systematically evaluated. Although it is still too early to define the best animal model for studying SARS-CoV-2 infections, it was evidenced by the safety of PiCoVacc in macaques, and no infection intensification or immunopathological exacerbation were observed in this study.

Nucleic Acid: DNA and RNA [36]

Similar to subunit vaccines, specific proteins from the target pathogen are chosen for their immunogenic epitopes, however these proteins are delivered as either plasmid DNA or RNA sequences [37, 38]. Upon vaccination, the host cell manufactures the pathogen protein, which is recognised by the immune system as foreign and generates an immune response [37]. Non-capsulated RNA vaccines are readily removed by the host cell upon injection, so advances in delivery technology, including encapsulation of RNA in liposomes, have been developed to avoid degradation [39].

RNA vaccines have been shown to induce antigen-specific antibody and polyfunctional T-cell responses in phase I clinical trials of cancer vaccines [39], and functional antibodies against Rabies virus glycoprotein [40], however there are currently no licensed RNA vaccines for humans. Although DNA vaccines are immunogenic in small animal models, they show less immunogenicity in human clinical trials and require adjuvants or multiple doses [16, 41]. Four DNA vaccines are available for animal use [39], however there are currently none licensed for humans [42].

There are several nucleic acid vaccines in development for COVID-19 prophylaxis. Nucleic acid vaccines are relatively cheap and rapid to manufacture, with the possibility to mass-produce large-scale GMP product [43].

Replicating Viral Vectors (e.g. Measles) [44]

Replicating viral vector vaccines use a replicating viral vector that has been altered to produce coronavirus proteins in the body. They provide a strong immune response and have long been applied successfully in poultry, using herpesvirus and poxvirus backbones to immunize against Newcastle disease [45] and

infectious bursal disease [46]. In human vaccine development, the attenuated measles virus can be used as a replicating vector [47]. A recent example is a vaccine that is being developed against chikungunya fever [48]. One potential limitation is that previous immunity to the vector may render the vaccine useless in some cases.

Non-replicating Viral Vector (e.g. Adenoviral Vectors and Modified Vaccinia Ankara, MVA) or Recombinant Viral-Vectored Vaccines [13]

Recombinant viral-vectored vaccines utilise the host's innate immunity to generate self-adjuvanted immunogenicity, whilst eliciting a targeted immune response against genetically-encoded pathogen antigens [49]. The viral vector 'backbone' is constructed from a genetically-modified virus [50], examples including adenoviruses, poxviruses, and Vesicular stomatitis virus [50, 51]. This vector typically has insertion sites for gene(s) of the target pathogen, which are expressed intracellularly in the host upon vaccination [52].

Important considerations for development of virus vectored vaccines is the generation of immunity towards the vector, which could hinder the antigen specific response upon a boost vaccination. However reports from preclinical and clinical studies show sufficient protection can be elicited from a single dose [53, 54].

Human adenoviruses (hAds) are a frequently used viral vector, however circulate at high frequency in most populations [55], contributing towards demographically variable yet significant pre-existing immunity that can reduce vaccine efficacy [52]. Vectors constructed from chimpanzee adenovirus (ChAd) were developed to elicit similar or superior immunogenicity as hAd vectors, whilst having significantly reduced seroprevalence and hence neutralising antibodies in most populations [56]. In pre-clinical studies, ChAd vectors have demonstrated up to 100% efficacy with a single vaccination against several emerging pathogens [54, 57]. Clinical trials have established that ChAd vectors also have a good safety profile and immunogenicity for Influenza A [58], ebolavirus [59], and MERS [60].

Adenovirus vectors can be rapidly made to GMP at large scale, and a single vaccination can be sufficient to provide rapid immunity in individuals [61]. This rapid production and distribution pipeline was tested during the 2013-2016 ebolavirus (EBOV) outbreak in Guinea, Liberia, and Sierra Leone, where five viral-vectored vaccines were rapidly escalated to clinical trials [61]. A recombinant vesicular stomatitis virus vector expressing the EBOV glycoprotein (rVSV-ZEBOV) progressed to phase III trials in Guinea and Sierra Leone and provided 100% efficacy across 4,359 individuals vaccinated with a single dose [54]. Following the second ebolavirus outbreak in the Democratic Republic of the Congo (DRC) in 2018, the WHO allowed compassionate use of rVSV-ZEBOV in the DRC, which has now been licensed in the DRC, Burundi, Ghana and Zambia [62]. An Ad26-vectored ebola virus vaccine has also been developed by Janssen and tested extensively in a prime-boost regimen in sub-Saharan Africa for efficacy and immunogenicity [63].

Protein Subunit Vaccines [13]

Protein subunit vaccines include antigenic proteins thought to induce a protective immune response. This vaccine type is produced *in vitro* and circumvents handling highly pathogenic live viruses [14, 64]. Subunit vaccines predominantly elicit a humoral antibody response, and most are administered with an adjuvant, which is a prerequisite to stimulate a strong immune response and generate a higher quality immune memory in humoral and cellular compartments. However, the inclusion of adjuvants can increase the reactogenicity and production costs, which are important considerations [64]. Virus-like particles (VLP)

are a type of subunit vaccine that present many copies of the relevant antigen in a 3D virus-like structure, and may be immunogenic enough to not require the inclusion of adjuvants [64].

Subunit vaccines are an attractive vaccine technology for rapid vaccine development, and multiple institutions worldwide are developing protein subunit-based vaccines. They can be upscaled for mass production at good manufacturing practice (GMP) standards [65], and distribution has less reliance on cold chain systems [16]. However, they can require bespoke manufacturing processes, which can increase cost, and may require specific mammalian cell expression and optimisation [28, 66].

Adjuvant

Also, to live attenuated vaccines and live vector vaccines, adjuvants are demanded to improve the immune response in the development of other types of vaccines. In order to stimulate the development of a SARS-CoV-2 vaccine, the favored adjuvant should be those that have been broadly used in other marketable vaccines, including (1) classic aluminum adjuvant, aluminum adjuvants improve the immune response by helping phagocytosis and reducing the diffusion of antigens from the injection site. It can efficiently stimulate Th2 immune response upon injection [67]; (2) MF59, MF59 is an oil-in-water emulsion composed of Tween 80, sorbitol trioleate, and squalene, and it has already been adopted in flu vaccines in Europe and in the United States. The mechanism of MF59 is to produce a transient immune environment at the injection site, then to recruit immune cells to cause antigen-specific immune responses [68]; (3) Adjuvant system (AS) series adjuvants, which are a series of adjuvants produced by GlaxoSmithKline (GSK), including AS01, AS02, AS03, and AS04. Among them, AS01 is a liposome adjuvant containing 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and saponin QS-21 [69], which has been adopted in malaria vaccines [70]. AS02 is an oil-in-water emulsifier that has MPL and QS-21 [71]. AS03 is an oil-in-water emulsifier containing alpha-tocopherol, squalene, and Tween 80. It has been adopted in influenza vaccines [72]. AS04 is an aluminum adjuvant containing MPL and has been used in a human papillomavirus vaccine and the hepatitis B virus vaccine [73].

Because adjuvants were capable to manage the type of immune response, the optimal adjuvant should be chosen according to the design of the vaccine. In order to provoke a more adequate immune response, a combination of different types of adjuvants could be used to enhance the immune efficacy.

Antigen Selection [74]

Whole Cell Antigens

The whole-cell antigens (WCA) carry all the components of the virus, including proteins, lipids, polysaccharide, nucleic acids, and some other elements. WCA has been utilized for developing whole-cell killed and live-attenuated vaccines [75]. Since the complex structures of WCA, it is inevitable to face more issues in quality control and compatibility evaluation. So far, several companies have successfully isolated the virus of SARS-CoV-2 and began whole-cell killed or live-attenuated vaccine progress. Nevertheless, research on the type of vaccine demands rigorous screening for reaching strains with undoubted low or no pathogenicity [76].

Spike Protein (S Protein)

S protein is currently the most hopeful antigen formulation for SARS-CoV-2 vaccine investigations. Primary, it is surface exposure and thus is capable to be directly identified by the host immune system [77]. Secondary, it mediates the binding with the host cell by attaching to the receptor ACE2, which is imperative for succeeding virus entrance to target cells and

causes subsequent pathogenicity [77, 78]. Lastly, the homolog proteins were already applied for vaccine development against SARS-CoV and MERS-CoV and were proved to be effective [79, 80]. The monomer of S protein from SARS-CoV-2 has 1,273 amino acids, with an approximately 140 kDa. Self-association naturally assembles the S protein into a homo-trimer, typically alike to the first class of membrane fusion protein (Class I viral fusion protein). The S protein includes two subunits (S1 and S2). The S1 subunit can be determined with two domains with the N-terminal domain (NTD) and the C-terminal domain (CTD). The receptor-binding domain (RBD) is placed in the CTD. S2 subunit contains the basic elements needed for membrane fusion, including an internal membrane fusion peptide (FP), two 7-peptide repeats (HR), a membrane-proximal external region (MPER), and a transmembrane domain (TM) [81]. Lately, the structure of the SARS-CoV-2 S trimer in the pre-fusion conformation and the RBD domain in complex with ACE2 has been successfully determined [77, 78], which has contributed to relevant data for vaccine design based on this protein. So far, the potential fragments of S protein for application as antigens in vaccine development include the full-length S protein, the RBD domain, the S1 subunit, NTD, and FP.

The Full-Length S Protein

Full-length proteins are ordered to hold the correct form of the protein, capable of providing more epitopes and presenting higher immunogenicity. Pallesen and colleagues [82] showed that higher titer of neutralizing antibodies in BALB/c mice immunized with recombinant prefusion MERS-CoV S protein. Another study confirmed that S protein produced in baculovirus insect cells was capable to assemble into nanoparticles. Mice immunized with these nanoparticles formulated with alum adjuvant that produced a high titer of neutralizing antibodies [83]. Muthumani and colleagues [84] described that DNA vaccine encoding MERS-CoV S protein was immunogenic in mice, camels, and rhesus macaques. Animals immunized with the DNA vaccine exhibit reduced typical clinical symptoms including pneumonia during the infection. So far, Clover Biopharmaceuticals had declared that they have created a SARS-CoV-2 S protein trimer vaccine (S-Trimer) by using its patented Trimer-Tag© technology, and this vaccine will be produced via a fast mammalian cell-culture based expression system.

RBD

Since the RBD of S protein directly interacts with the ACE2 receptor on host cells, RBD immunization provoked specific antibodies that may obstruct this identification and thus limit the invasion of the virus. Most SARS-CoV-2 subunit vaccines currently under development use RBD as the antigen. Furthermore, the RBD domain was also applied in the development of SARS-CoV and MERS-CoV vaccines. For instance, studies have shown that recombinant RBD is multiple conformational neutralizing epitopes that can cause a high titer of neutralizing antibodies against SARS-CoV [85]. Lan and colleagues [86] described that Rhesus macaques immunized with the recombinant RBD formulated with alum adjuvant could provide neutralizing antibodies, in association with observed mitigation of the clinical symptoms during MERS-CoV infection. Nyon and colleagues [87] also described that hCD26/DPP4 transgenic mice immunized with RBD fused to Fc elicited neutralizing antibodies and were able of protecting against MERS-CoV infection. Moreover, the RBD domain is relatively conserved as associated with the S1 subunit and was described to have multiple conformational neutralizing epitopes [88], making it more proper for vaccine development.

NTD

Similar to RBD, the N-terminal domains (NTD) of S protein from many coronaviruses were related to show carbohydrate receptor-binding

activity. For example, the NTD of spike protein from transmissible gastroenteritis virus (TGEV) was described to attach sialic acid via NTD [89]. The carbohydrate-binding characteristics of IBV M41 strain are also correlated to the NTD of the S protein [90]. So, this domain is also a candidate antigen for vaccine development. One study reported that rNTD of S protein from MERS-CoV led to strong cellular immunity and antigen-specific neutralizing antibodies in mice and was protective against the viral challenge [91]. There is a study that a mAb that attaches to the N-terminal domain (NTD) of the MERS-CoV S1 subunit revealed efficient neutralizing action against the wild-type MERS-CoV strain EMC/2012 [92]. This result revealed that NTD specific antibodies are useful in neutralization. Nevertheless, as the genomes of coronaviruses are extremely variable, it is better to use antibodies targeting different epitopes to avoid the immune evasion of the virus. Although the function of S1-NTD of SARS-CoV-2 has not been clarified, it may also be implicated in the union of certain receptors and can also be a candidate antigen.

S1 Subunit

The S1 subunit, which has both RBD and NTD, is principally involved in the S protein attachment to the host receptor. It is also extensively applied in vaccine development. Wang and colleagues [93] demonstrated that MERS-CoV S1 protein formed with MF59 adjuvant protected hDPP4 transgenic mice against lethal virus challenge, and the protection related well with the neutralizing antibody titer. Adney and colleagues [94] reinforced that immunization with adjuvanted S1 protein decreased and delayed virus shedding in the upper respiratory tract of dromedary camels and complete protection was seen in alpaca against MERS-CoV challenge.

FP

The FP domain of the S2 subunit is involved in the membrane fusion of the virus, which is also a principal step in viral pathogenicity [95]. Hence, it may also serve as a vaccine candidate antigen. Tianjin University has constructed an RBD-FP fusion protein, and a high titer of antibodies was identified in mice immunized with this fusion protein, and the effectiveness is under evaluation.

Nucleocapsid Protein (N Protein)

The N protein is the most abundant protein in coronavirus, and it is normally deeply conserved. N protein has multiple roles including the development of nucleocapsids, signal transduction virus budding, RNA replication, and mRNA transcription [96]. This protein was described to be extremely antigenic, 89% of patients who developed SARS, formed antibodies to this antigen [97]. DNA vaccine encoding SARS-CoV N protein produced strong N-specific humoral and cellular immune responses in vaccinated C57BL/6 mice and was able to significantly decrease the titer of challenging vaccine virus [98]. Till, some other researchers published that the N protein of avian infectious bronchitis virus is related to the induction of CTLs that are associated with a reduction in clinical signs and viral clearance from lungs, proposing that cellular response is essential in N protein-mediated protection [99, 100]. In opposition, another research showed that the N protein immunization did not provide a significant contribution to neutralizing antibody response and provided no protection to infection in hamsters [101]. These results insinuate that there is controversy about whether this protein could be accepted for vaccine development. But, there is no doubt that it can be applied as a marker in diagnostic assays due to its high immunogenicity.

Membrane Protein (M Protein)

M protein is a transmembrane glycoprotein and is implicated in virus attachment, and this protein is the most abundant protein on the surface of SARS-CoV [102]. It was described that immunization with the full length of M protein is capable to evoke efficient neutralizing antibodies in SARS patients

[103]. Immunogenic and structural analysis also showed that the transmembrane domain of the M protein has a T cell epitope cluster that is capable to induce a strong cellular immune response [104]. M protein is also highly conserved in evolution among different species [102], consequently, it may be adopted as a candidate antigen for developing the SARS-CoV-2 vaccine.

Envelope Protein (E Protein)

Compared with S, N, and M protein, E protein is not proper for use as an immunogen, because it consists of 76–109 amino acids in different coronaviruses with channel activity, thus the immunogenicity is restricted. Studies have determined that SARS-CoV E protein is an important virulence factor, and the secretion of inflammatory factors IL-1β, TNF, and IL-6 are significantly decreased after knocking out E protein [105].

Summary Contents

Table 1 summarizes the vaccine platforms developed against SARS-CoV-2, indicating the advantage and disvantage of each one [17]. Table 2 presents the 1/2-3 clinical-trial-phases of the vaccines against COVID-19 by Regulatory Affairs Professionals Society (RAPS) [106], and the Figures 1-8 sumarize the main types being tested range from those containing the whole virus, either in a weakened or inactivated form, or those that contain part of the viral structure, to those that depend on our own cells to produce viral proteins that the immune system can recognise. All of them rely on the same basic principle of mimicking a real viral infection and inducing a protective immune response [76].

Vaccines' Candidates Against SARS-CoV-2

Researchers worldwide are working around fastly to discover a vaccine against SARS-CoV-2, the virus causing the COVID-19 pandemic. Specialists expect that a fast-tracked vaccine development process could speed a successful candidate to market in approximately 12-18 months.

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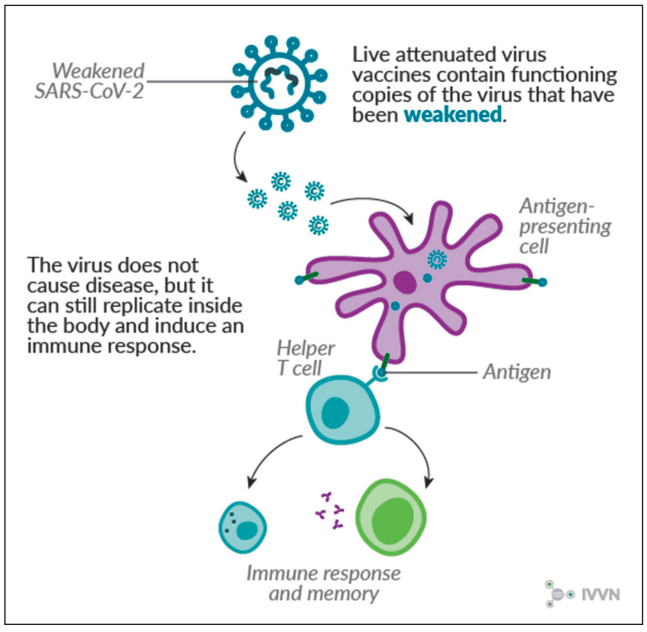
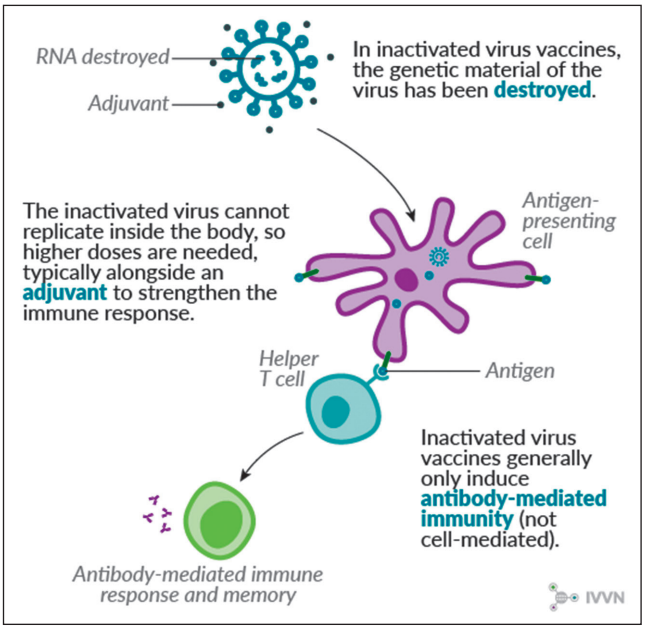
Figure 1. Live attenuated virus vaccine.



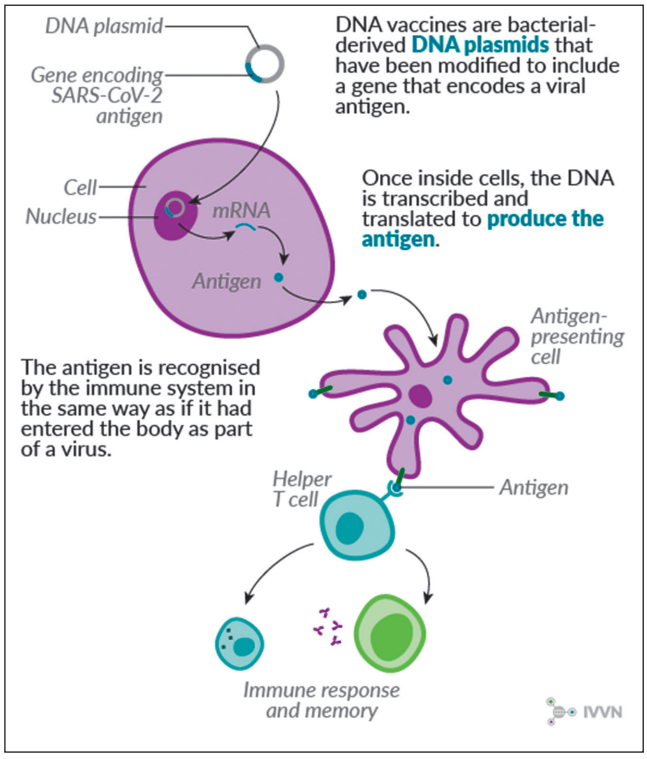
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 2. Inactivated virus vaccine.



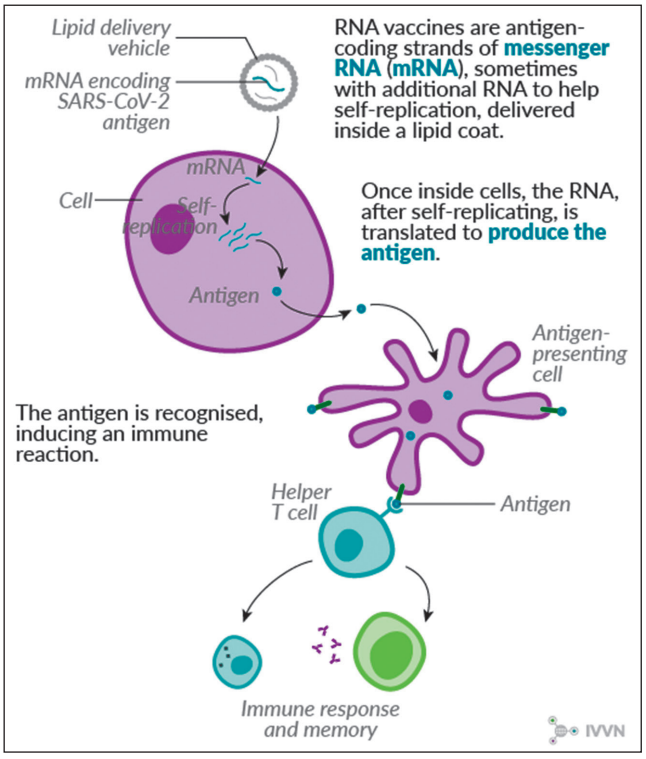
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 3. DNA vaccines.



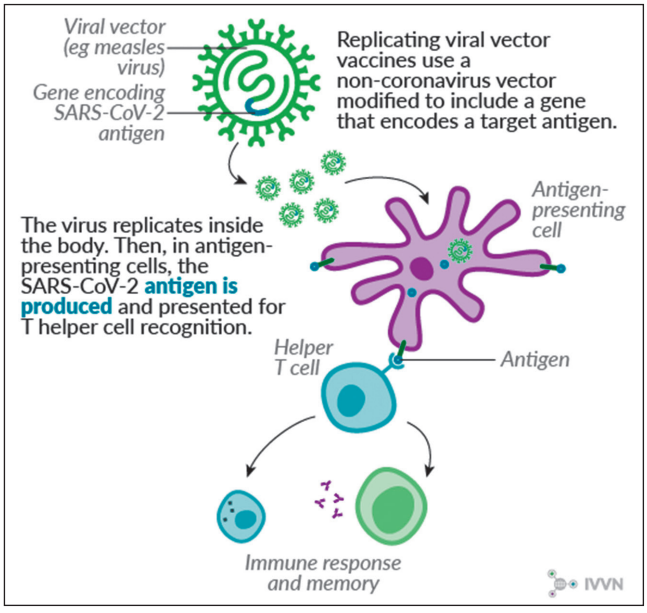
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 4. RNA vaccines.



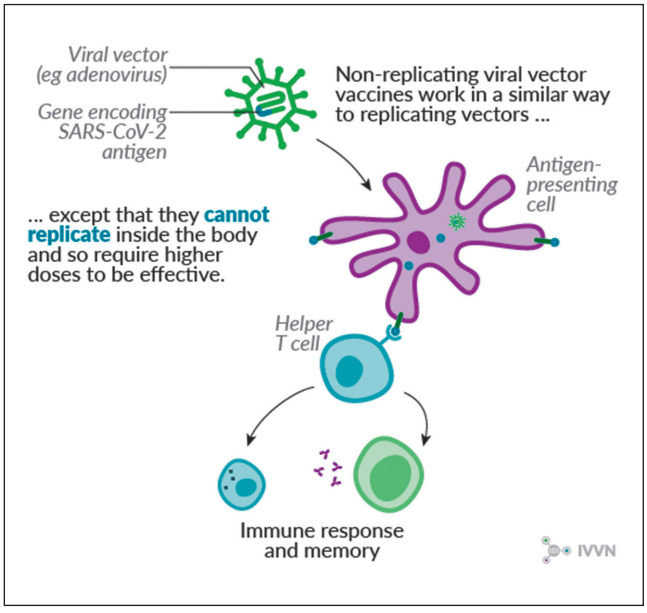
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 5. Viral vector vaccines (replicating).



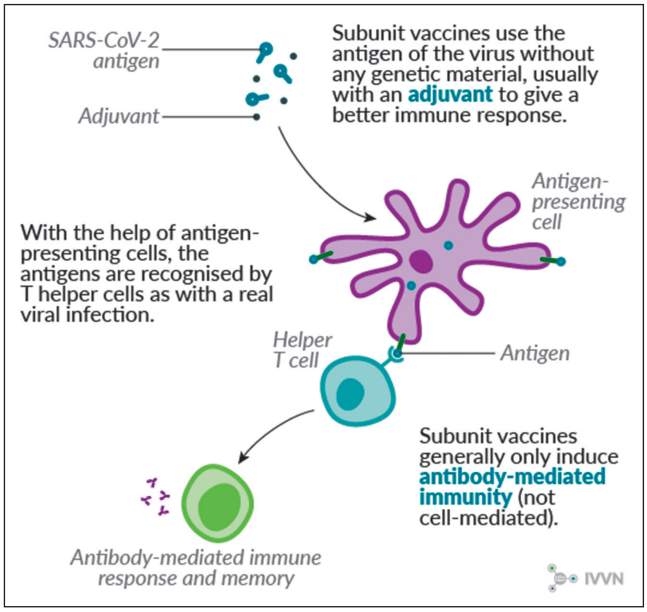
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 6. Viral vector vaccines (non-replicating).



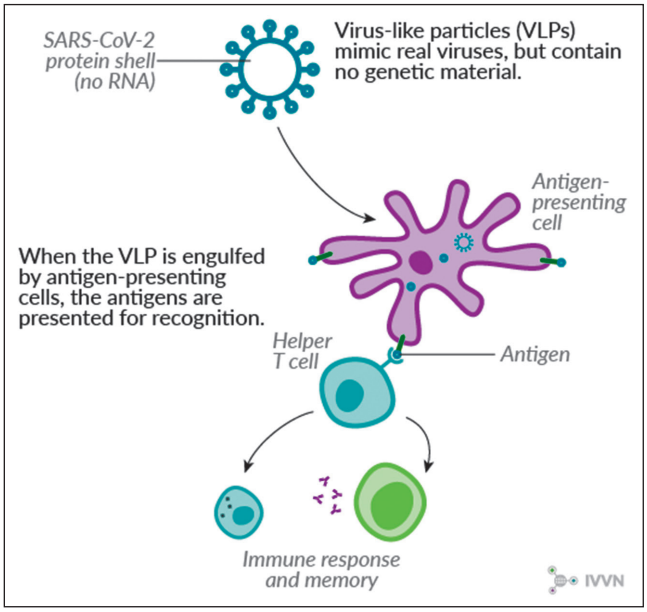
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 7. Viral vector vaccines (non-replicating).



Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 8. Virus-like particles vaccines.



Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Table 1. Selected antigens and vaccine platforms against SARS-CoV-2.

Platform	Target	How It Works	Advantages	Disadvantages	Examples	Group Against COVID-19
RNA vaccines	S protein	They use the RNA to lead the immune system to target the key viral proteins	Easy design. No infectious virus needs to be handled, vaccines are typically immunogenic, rapid production possible.	Safety issues with reactogenicity have been reported.	None	Moderna
DNA vaccines	S protein	They use the DNA to lead the immune system to target the key viral proteins	Easy design. No infectious virus needs to be handled, easy scale up, low production costs, high heat stability, tested in humans for SARS-CoV-1, rapid production possible.	Vaccine needs specific delivery devices to reach good immunogenicity.	None	Inovio
Viral vector-based vaccines	S protein	They use a harmless virus and use it to deliver viral genes to build immunity	Live virus tends to lead stronger immune responses than dead virus or subunit vaccines. Excellent preclinical and clinical data for many emerging viruses, including MERS-CoV.	Vector immunity might negatively affect vaccine effectiveness (depending on the vector chosen). And it is important to choose a truly safe viral vector.	Ebola Veterinary medicine	University of Oxford and Astrazeneca CanSino Biologics Johnson & Johnson
Live attenuated vaccines	Whole virion	It uses a weakened version of the virus	Stimulates a robust immune resonse without serious disease or adverse events. Straightforward process used for several licensed human vaccines, existing infrastructure can be used.	May not be safe for immuno-compromised immune systems. Creating infectious clones for attenuated coronavirus vaccine seeds takes time because of large genome size. Safety testing will need to be extensive.	Measles, Munps and Rubella, Chicken pox	Codagenix Indian Immuno-logicals Inc.
Virus innactivated	Whole virion	It uses the whole virus after it has been killed with heat or chemicals	It is easy to make and safe because the virus is already dead.	This vaccine is not more effective than the live virus. Some previous inactivated virus have made the disease worse. The safety for the novel coronavirus needs to be shown in clinical trials.	Polio	Sinovac Sinopharm (Coronavac)
Subunit	S protein	It uses a pieces of a virus' surface to focus your immune system as a single target.	Focuses the immune response on the most important part of the virus for protection and cannot cause infection.	May not stimulates a strong response, other chemicals may need to be added to boost long-term immunity.	Pertussis Hepatitis B HPV	Novavax AdaptVac

Credit/Source: Adapted from Amand and Kramer [17].

Table 2. Vaccine candidates against COVID-19 in 1/2-3 clinical trial phase.

Candidate	Sponsor	Phase	Institution
Inactivated vaccine	**Wuhan Institute of Biological Pharmaceutical Group (Sinopharm)**	**Phase 3**	**Henan Provincial Center for Disease Control and Products; China National Prevention**
Study Design & Details **Background:** Researchers at Sinopharm and the Wuhan Institute of Virology under the Chinese Academy of Sciences are developing an inactivated COVID-19 vaccine candidate. They have initiated a randomized, double-blind, placebo parallel-controlled Phase 1/2 clinical trial (ChiCTR2000031809) of healthy individuals starting at 6 years old. **Outcomes:** The vaccine has shown a "strong neutralizing antibody response" in Phase 1/2 trials, according to a release from China National Biotec Group. It appeared to be working best at the middle strength when given 28 days apart, as all participants in that dosing schedule developed neutralizing antibodies that can defend a cell from infection. Until now, all 1,120 volunteers in the phase 1/2 trial have received two injections of the vaccine at low, middle or high dosing strengths—or placebo—either 14 days, 21 days or 28 days apart, according to CNBG. The seroconversion rate for the 14-day and 21-day schedule of the mid-dose was 97.6%. At 28 days, it was 100%. The company didn't specify the neutralizing antibody response rates for the low dose or the high one. It also didn't elaborate on the exact levels of immune response, only saying the antibody titers were "high." No serious adverse event was observed. **Status:** A Phase 3 trial is underway conducted in the United Arab Emirates.			
CoronaVac	**Sinovac**	**Phase 3**	**Sinovac Research and Development Co., Ltd.**
Study Design & Details **Background:** CoronaVac (formerly PiCoVacc) is a formalin-inactivated and alum-adjuvanted candidate vaccine. Results from animal studies showed "partial or complete protection in macaques" exposed to SARS-CoV-2, according to a paper published by researchers in the journal Science. **Study Design:** A Phase 1/2 trial enrolled 743 healthy volunteers (18-59 years old) who received two different dosages of the vaccine or placebo. There were 143 participants in Phase 1 (NCT04352608) and 600 participants in Phase 2 (NCT04383574). **Outcomes:** The phase I/II clinical trials were designed as randomized, double-blind and placebo-controlled studies. In total, 743 healthy volunteers, aged from 18 to 59 years old, enrolled in the trials. Of those, 143 volunteers are in phase I and 600 volunteers are in phase II. There have been no severe adverse event reported in either the phase I or phase II trials. The phase II clinical trial results show that the vaccine induces neutralizing antibodies 14 days after the vaccination with a 0,14 day schedule. The neutralizing antibody seroconversion rate is above 90%, indicating a positive immune response. **Status:** Sinovac said a Phase 3 trial in collaboration with Butantan Institute in Brazil is underway, and the company plans to enroll around 9,000 patients in the healthcare industry.			
mRNA-1273	**Moderna**	**Phase 3**	**Kaiser Permanente Washington Health Research Institute**
Study Design & Details **Background:** mRNA-1273 was developed by Moderna based on prior studies of related coronaviruses such as those that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). A Phase 1 trial (NCT04283461) of 105 healthy participants provided the basis for Moderna's investigational new drug application (IND), which was successfully reviewed by the FDA and set the stage for Phase 2 testing. A Phase 2 trial of 600 healthy participants evaluating 25 μg, 100 μg, and 250 μg dose levels of the vaccine was completed, and mRNA-1273 has advanced to a Phase 3 trial (NCT04405076. **Study Design:** A Phase 3 trial of 30,000 participants at high risk for SARS-CoV-2 infection who will receive a 100 μg dose of mRNA-1273 or placebo and then followed for up to 2 years (COVE trial; NCT04470427). **Outcomes:** Phase 1 data published in the New England Journal of Medicine showed mRNA-1273 successfully produced neutralizing antibody titers in 8 participants who received either 25 μg or 100 μg doses. The response was dose dependent in 45 participants across 25 μg, 100 μg, and 250 μg dose levels. In participants with available antibody data, neutralizing antibody titers were on par with what has been in seen in convalescent sera from people who have successfully fought off COVID-19. Results from a challenge in a mouse model showed mRNA-1273 prevented viral replication in the lungs, and neutralizing titers in the mouse model were similar in participants receiving 25 μg or 100 μg doses of the vaccine. Moderna said mRNA-1273 was "generally safe and well tolerated."A study of nonhuman primates challenged with SARS-CoV-2 published in the New England Journal of Medicine had neutralizing activity, and limited inflammation and lung activity after being administered the vaccine. **Status:** On 12 May, the FDA granted Fast Track designation to mRNA-1273. A Phase 3 trial of the vaccine is underway, which is being funded by Operation Warp Speed.			

Bacillus Calmette-Guerin (BCG) live-attenuated vaccine	**University of Melbourne and Murdoch Children's Research Institute; Radboud University Medical Center; Faustman Lab at Massachusetts General Hospital**	**Phase 2/3**	**University of Melbourne and Murdoch Children's Research Institute; Radboud University Medical Center; Faustman Lab at Massachusetts General Hospital**

Study Design & Details

Background: The BCG vaccine is indicated to prevent tuberculosis in those who have a higher risk of the disease. It has been implicated in helping to combat other infections outside TB by boosting the immune system to fight similar infections. In 2017, the World Health Organization (WHO) reported the BCG vaccine may be effective against leprosy and other nontuberculous mycobacteria such as buruli ulcer disease. Other papers have posited the vaccine is effective in preventing acute respiratory tract infections in elderly patients, other respiratory infection and sepsis. A non-peer reviewed paper posted in March 2020 on the preprint server medRxiv has suggested countries with BCG vaccination programs at childhood are faring better in the fight against COVID-19 compared with countries that do not require BCG vaccination. BCG vaccines are being studied in the randomized, controlled, Phase 3 BRACE trial, which aims to recruit 4,170 healthcare workers in hospitals in Australia (NCT04327206). Researchers in The Netherlands launched the randomized, parallel-assignment, phase 3 BCG-CORONA trial on 31 March and plan to enroll 1,500 healthcare workers to receive the BCG vaccine or placebo (NCT04328441). The Faustman Lab is currently evaluating the BCG vaccine's effectiveness in type 1 diabetes and is seeking funding to launch trial to assess whether the vaccine helps prevent COVID-19 in healthcare workers, according to independent reporting from the New York Times.

AZD1222	**The University of Oxford; AstraZeneca; IQVIA**	**Phase 2/3**	**The University of Oxford, the Jenner Institute**

Study Design & Details

Background: The Oxford Vaccine Group at the University of Oxford are developing a new vaccine candidate for COVID-19, a chimpanzee adenovirus vaccine vector called AZD1222 (previously ChAdOx1). The team has previously developed a MERS vaccine. Preclinical data in a paper on the pre-print server bioRxiv that showed a significantly reduced viral load and "humoral and cellular immune response." The vaccine candidate also showed an immune response in mice and pigs, according to information in a pre-print paper.

Study Design: A Phase 1/2 (NCT04324606) single-blinded, multi-center study of 1,090 healthy adult volunteers aged 18-55 years with four treatment arms. Participants in two treatment arms will receive a single dose of AZD1222 or MenACWY, a meningococcal vaccine. A third treatment arm will receive AZD1222 and a booster at 4 weeks. In a fourth arm, participants will receive AZD1222 or MenACWY together with 1 g of paracetamol (acetaminophen) every 6 hours for 24 hours. The trial is active, but not currently recruiting.

Outcomes: Preliminary results from the trial published in The Lancet showed the vaccine candidate had an "acceptable safety profile" with most patients demonstrating an antibody response after one dose and all patients showing a response after two doses.

Status: On 21 May, AstraZeneca announced it has received $1 billion in funding from the Biomedical Advanced Research and Development Authority (BARDA) for "development, production and delivery of the vaccine," beginning in September 2020. The agreement between AstraZeneca and BARDA includes a minimum of 400,000 doses of the vaccine, an upcoming Phase 3 trial of 30,000 participants, and a pediatric trial. On 22 May, Oxford researchers announced that they had begun recruitment for a Phase 2/3 trial of approximately 10,000 healthy adult volunteers to assess how well people across a broad range of ages could be protected from COVID-19. A Phase 3 trial of AZD1222 is being funded by Operation Warp Speed. IQVIA announced they are partnering with AstraZeneca to advance clinical trials for the vaccine Brazil will participate of the Phase III trial in São Paulo.

BNT162	**Pfizer, BioNTech**	**Phase 2/3**	**Multiple study sites in Europe and North America**

Study Design & Details

Background: Pfizer and BioNtech are collaborating BNT162, a series of vaccine candidates for COVID-19. BNT162 was initially four vaccine candidates originally developed by BioNTech, two candidates consisting of nucleoside modified mRNA-based (modRNA), one of uridine containing mRNA-based (uRNA), and the fourth candidate of self-amplifying mRNA-based (saRNA). The companies have selected the modRNA candidate BNT162b2 to move forward in a Phase 2/3 trial.

Study Designs: A Phase 1/2 trial in the US and Germany of 200 healthy participants between aged 18-55 years, with a vaccine dose range of 1 µg to 100 µg is currently recruiting (NCT04380701). A Phase 2/3 trial of about 32,000 healthy participants is active, but not currently recruiting (NCT04368728).

Outcomes: Results of one study of BNT162b1, a modRNA candidate, were reported 1 July on the non-peer-reviewed preprint server medRxiv. Robust immunogenicity was seen after vaccination at all three doses (10 μg, 30 μg and 100 μg). Adverse events were elevated at the highest dose; therefore, participants did not receive a second dose at that level.
Status: Pfizer and BioNTech received FDA Fast Track designation for two of the BNT162 candidates, BNT162b1 and BNT162b2. The companies have published results from a study that suggests one of the BNT162 candidates, BNT162b1, produced a neutralizing antibody response in participants who received the vaccine. However, they selected BNT162b2 as a candidate to advance to a Phase 2/3 safety study "based on the totality of available data from our preclinical and clinical studies, including select immune response and tolerability parameters." A candidate could be ready for regulatory approval as early as December.

Ad5-nCoV	**CanSino Biologics**	**Phase 2**	**Tongji Hospital; Wuhan, China**

Study Design & Details
Background: China's CanSino Biologics has developed a recombinant novel coronavirus vaccine that incorporates the adenovirus type 5 vector (Ad5). Preliminary safety data from a Phase 1 (ChiCTR2000030906; NCT04313127) clinical trial of 108 participants between 18 and 60 years old who will receive low, medium, and high doses of Ad5-nCoV has allowed the company to plan to initiate a Phase 2 trial, according to an announcement. The Phase 2 (ChiCTR2000031781) trial has identical inclusion criteria.
Outcomes: Results from Phase 1 of the trial show a humoral and immunogenic response to the vaccine, according to a paper published in The Lancet. Adverse reactions such as pain (54%), fever (46%), fatigue (44%), headache (39%), and muscle pain (17%) occurred in 83% of patients in the low and medium dose groups and 75% of patients in the high dose group. In Phase 2 of the trial, neutralizing antibodies and specific interferon γ enzyme-linked immunospot assay responses were observed at all dose levels for most participants.
Status: On 25 June, China's Central Military Commission announced the military had been approved to use Ad5-nCoV for a period of 1 year, according to reporting in Reuters.

Adjuvant recombinant vaccine candidate	**Anhui Zhifei Longcom Biopharmaceutical, Institute of Microbiology of the Chinese Academy of Sciences**	**Phase 2**	**-**

Study Design & Details
Background: China's National Medical Products Administration has approved a Phase 1 trial of a COVID-19 vaccine candidate developed by the Anhui Zhifei Longcom Biopharmaceutical and the Institute of Microbiology of the Chinese Academy of Sciences. A Phase 2 trial is underway, with results from Phase 1 expected in September, according to Reuters.

BBIBP-CorV	**Beijing Institute of Biological Pharmaceutical Group (Sinopharm)**	**Phase 1/2**	**Henan Provincial Center for Disease Control and Products; China National Prevention**

Study Design & Details
Background: Sinopharm is developing a second inactivated COVID-19 vaccine candidate, BBIBP-CorV, with the Beijing Institute of Biological Products. BBIBP-CorV is currently being evaluated in a Phase 2 trial (ChiCTR2000032459).
Outcomes: Results from a paper published in the journal Cell appear to show BBIBP-CorV provides "highly efficient protection" against SARS-CoV-2 in rhesus macaques who underwent challenge against the virus.
Status: More than 2,000 vaccines administered between Sinopharm's two inactivated vaccine trials. Both vaccine candidates could be ready for market by the end of the year, according to reporting from Reuters.

GX-19	**Genexine**	**Phase 1/2**	**GenexineGenexine**

Study Design & Details
Background: Genexine, a biotechnology based in South Korea, is testing GX-19, a DNA vaccine candidate for COVID-19. The company has been approved for a Phase 1/2a clinical trial of 190 healthy participants randomized to receive the vaccine or placebo (NCT04445389). The company aims to complete Phase 1 in 3 months before moving to a multinational Phase 2 trial.

Gam-COVID-Vac	**Gamaleya Research Institute, Acellena Contract Drug Research and Development**	**Phase 1/2**	**Various**
Study Design & Details **Background:** The Gamaleya Research Institute in Russia is testing their non-replicating viral vector COVID-19 vaccine candidate, Gam-COVID-Vac, in a Phase 1/2 trial. The trial is expected to recruit about 38 participants to receive the vaccine candidate (NCT04436471) (NCT04437875). **Status:** The institute reportedly plans to test the candidate on a small section of the public in August, which would be the equivalent of a Phase 3 trial.			
Self-amplifying RNA vaccine	**Imperial College London**	**Phase 1/2**	**Imperial College London**
Study Design & Details **Background:** Imperial College London researchers are developing a self-amplifying RNA vaccine for COVID-19. They developed a vaccine candidate within 14 days of receiving the sequence from China. Animal testing is underway. The investigators have received two rounds of funding from the United Kingdom's government – one on 22 April and another on 17 May. **Study Design:** The Phase 1/2 COVAC1 trial will enroll approximately 300 healthy participants between 18 and 75 years old, with an efficacy trial for 6,000 participants planned for October. **Status:** On 7 June, Imperial College London announced it had partnered with Morningside Ventures to establish VacEquity Global Health, an initiative that would help keep costs down for their COVID-19 vaccines down for citizens in the UK and internationally.			
LUNAR-COV19	**Arcturus Therapeutics and Duke-NUS Medical School**	**Phase 1/2**	**Duke-NUS Medical School, Singapore**
Study Design & Details **Background:** Arcturus and Duke-NUS Singapore are partnering to develop a COVID-19 vaccine candidate that uses Arcturus' self-replicating RNA and nanoparticle non-viral delivery system. Pre-clinical data from the company indicates LUNAR-COV19 provides an adaptive cellular (CD8+ cells) and balanced (Th1/Th2) immune response. Arcturus said a Phase 1/2 clinical trials will proceed in Singapore.			
ZyCoV-D	**Zydus Cadila**	**Phase 1/2**	**Zydus Cadila**
Study Design & Details **Background:** India's Zydus Cadila is researching ZyCoV-D, a plasmid DNA vaccine candidates for COVID-19 that targets the viral entry membrane protein of the virus. The company has launched an adaptive Phase 1/2 dose escalation trial and plans to enroll about 1,000 healthy volunteers.			

Credit/Source: RAPS [106].

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