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Medical Intelligence Report

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Topic: Treatments Under Investigation for COVID-19

Overview

There are no specific therapeutics approved by the FDA or other health organizations to treat people with COVID-19 (NIH, 2020). In most cases, treatment includes supportive care to relieve symptoms, but supportive care will not hasten a return to health. There are a number of experimental treatments that have been used for treatment of other illness and are being evaluated for their efficacy against COVID-19.

Technical advances have allowed for a huge increase in the speed at which information is available about the new virus, SARS-CoV-2. The viral genome has been sequenced, laboratories are able to grow the virus for study, and the three-dimensional structure of ten different proteins associated with the virus have been produced. In previous outbreaks, these steps could take months or years to accomplish. Based on previous studies, there are several anti-viral medications that can be tested in patients right away, preliminary vaccines that have been produced and are being tested, and antibody treatments under development.

SARS-CoV-2 is related to the virus that caused the SARS outbreak in 2003 (called SARS-CoV or SARS-CoV-1). Researchers have done extensive research on SARS-CoV, and the information from this research is guiding the development of treatments for COVID-19. For example, researchers have identified the human protein the virus binds in order to infect a cell (Hoffman et al., 2020). The protein, called ACE2, is the same protein used by SARS-CoV. The virus that causes MERS, on the other hand, uses a different human protein to infect cells. The researchers were also able to determine that another human protein, called TMPRSS2, is required to cut the viral protein that interacts with ACE2 before it can bind and infect a cell. There are inhibitors of TMPRSS2 that have been approved in Japan for clinical use in people (e.g. camostat), and this process might be the basis for a treatment that is discussed below.

The three-dimensional structure of one of the proteins on the outside of the virus shell, called a spike protein because it sticks up off the surface, of both SARS-CoV and SARS-CoV-2 have been produced (Wrapp et al., 2020). Comparisons between the two structures showed that the spike proteins have a similar shape, but the surface where the virus binds to its human target (ACE2) has different characteristics in the two proteins. This difference in characteristics leads to 10 to 20-fold stronger binding to ACE2 in the case of SARS-CoV-2 than with SARS-CoV. The authors of the study suggest that this could be one of the differences between the viruses that leads to the increased transmission rate of SARS-CoV-2. Antibodies that prevent (or neutralize) the interaction between the spike protein of SARS-CoV and ACE2 have been developed since the outbreak in 2003, and analysis by the paper's authors suggest that the changes in the surface of the SARS-CoV-2 spike protein will prevent the SARS-CoV based antibodies from working as a treatment for COVID-19. However, knowledge of the structure of the spike protein is expected to help speed the development of vaccine and antibody treatments for COVID-19.

Clinical Trials

There are currently over 70 world-wide clinical trials investigating interventional treatments for COVID-19 listed on the National Institutes of Health database, ClinicTrials.gov. There are a number of other trials that have been reported on the Chinese-based database.

SOLIDARITY Trial

The WHO has also announced the launch of a multi-country clinical trial for potential coronavirus therapies trial called SOLIDARITY (Branswell, 2020). Organization of a centralized trial will also mean that the protocols are similar throughout the world and make it easier to interpret the information collected. The Director-General Tedros Adhanom Ghebreyesus said "Multiple small trials with different methodologies may not give us the clear strong evidence we need about which treatments help to save lives." There is speculation that the announcement means that testing in China was not sufficient to indicate whether potential treatments prevent individuals from developing severe disease or save those with severe disease from death. At the time of the announcement several countries had already joined the trial, including Argentina, Bahrain, Canada, France, Iran, Norway, South Africa, Spain, Switzerland, and Thailand.

The drugs included in the WHO trial are:

- Remdesivir
- Lopinavir and ritonavir
- Lopinavir and ritonavir plus interferon beta
- Chloroquine

The treatments will be compared to the standard of care, which is the regular medical support used to treat patients at this time. SOLIDARITY is designed so that drugs in the study can be updated (added or dropped) as more information becomes available without needing to begin another trial.

Researchers will be reporting information to answer the following questions:

- Do any of these drugs reduce mortality?
- Do any of these drugs reduce the time a patient is in the hospital?
- Are the symptoms of those taking the drugs less severe based on the need for ventilation or treatment in intensive care units?

Individual Clinical Trials

In a discussion of the use of repurposed medication for COVID-19 treatment published on February 27, experts cite 11 treatments were being tested in clinical trials, excluding traditional Chinese medicines and blood-derived products, such as serum from recovered patients and stem cells (Harrison, 2020).

Table 1. List of drugs currently being tested for use with COVID-19

Drug or Cocktail	Originator Company	Status	Number of Trials
ASC09/ritonavir, lopinavir/ritonavir, with or without umifenovir	Ascletis, AbbVie, Pharmstandard	ASC09: experimental Ritonavir & lopinavir: approved (in HIV) Umifenovir: approved for influenza, in Russia and China	At least 3
ASC09/ oseltamivir, ritonavir/ oseltamivir, oseltamivir	Ascletis, Gilead, AbbVie	Approved (as above, oseltamivir approved for influenza in US)	1
Azvudine	Zhengzhou Granlen PharmaTech	Experimental	1
Combinations of baloxavir marboxil/ favipiravir & lopinavir/ ritonavir	Shionogi, Toyama Chemical	Baloxavir marboxil: approved for influenza, US Favipiravir: approved for influenza, Japan, China, Italy	2
Combinations of darunavir/ cobicistat alone or with lopinavir/ ritonavir & thymosin α1	Janssen, Gilead	Darunavir & cobicistat: approved (for HIV1/AIDS) Thymosin α1: approved as an immune booster	2
Remdesivir	Gilead	Approved for Ebola	At least 6
Chloroquine or hydroxychloroquine	Shanghai Zhongxi, Pharmaceutical, Shanghai Ziyuan, Pharmaceutical, Wuhan Wuyao Pharmaceutical	Approved (for malaria, other auto-immune disorders)	At least 10
Methylprednisolone	Generic	Approved (multiple indications)	1
Interferon α-2b or in combination with lopinavir/ritonavir & ribavirin	Biogen, Merck	Approved (multiple indications)	2
Camrelizumab & thymosin	Incyte, Shanghai Hengrui Pharmaceutical	Approved in China	2
Tocilizumab	Chugai Pharmaceutical, Zhejiang Hisun Pharmaceutical,	Approved (multiple indications)	1

	Jiangsu Qyun Bio-Pharmaceutical		

Adapted from Harrison, 2020

Researchers in China have reported the effect of five, FDA-approved anti-viral medications and two experimental drugs on the infection capability of SARS-CoV-2 in cell culture. These drugs were chosen because they would be expected to have an effect on RNA-based viral infections (Wang et al., 2020). The approved medications tested include ribavirin, penciclovir, nitazoxanide, nafamostat, and chloroquine. Remdesivir and favipiravir were the experimental medications.

Cells cultured in the lab were infected with SARS-CoV-2, and the effect of each drug on viral production was evaluated. Three of the drugs (ribavirin, penciclovir, and favipiravir) required a high dose to inhibit viral production, suggesting they would be less effective in treating COVID-19. The cell culture-based results showed that nafamostat, nitazoxanide, remdesivir, and chloroquine were effective in inhibiting infection of the cells by SARS-CoV-2 at levels that may be amenable to use as a drug in humans.

Clinical Trials of Vaccines

The National Institutes of Health announced on March 16, 2020 that they have started enrolling participants in a vaccine trial for COVID-19, which was designed by National Institute of Allergy and Infectious Diseases scientists and collaborators at the biotechnology company Moderna called mRNA-1273 (NIH, 2020). The trial is being conducted in the state of Washington and will include 45 healthy adult volunteers between the ages of 18 and 55 years. Only residents in the Seattle area will be allowed to join the study. Participants will be given differing doses to determine the safety of the vaccine in what is called a Phase 1 safety trial. During the trial, participants will receive two doses of the vaccine through a shot in the upper arm approximately 28 days apart. Participants will be asked to return to the clinic for follow-up visits between vaccinations and for additional visits across the span of a year after the second shot. At intervals within this period, blood samples will be tested to detect and measure the immune response to the experimental vaccine and to monitor the health of the volunteers. The vaccine has been tested in animal models with promising results, but the safety needs to be tested before administering the new drug to a large number of people.

Another phase 1 trial has been registered in the National Institutes of Health database that is located at Tongji Hospital in Wuhan, China. Multiple collaborators developed the potential vaccine that will be tested in 108 healthy participants who are between 18 to 60 years of age.

There are also a number of pre-clinical vaccine candidates, which means they are still being evaluated in laboratory and animal experiments. There are a number of companies who had been working on vaccines to the related SARS and MERS, which may speed up their process. Some of the companies reportedly working on COVID-19 vaccine candidates include Pfizer, BioNTech, Inovio Pharmaceuticals, Novavax, CureVac, Generex Biotechnology, Vaxart, Imperial College London, Medicago, Takis Biotech, Johnson & Johnson and BARDA, and Altimmune.

New COVID-19 Testing Procedures

Testing for COVID-19 has been in the forefront of discussions about the outbreak, leading to some confusion over its role. For individuals, testing allows doctors to plan how to manage an illness based on the diagnosis. For public health officials, testing allows for identification of the number and location of people who are sick. With this information, epidemiologists and other officials can make recommendations for community-wide interventions and predict the course of the outbreak. If there are not adequate numbers of tests available, emphasis shifts from charting the outbreak to treating those with severe symptoms.

In the United States, there is a shortage of some of the components necessary to test all of the people who may have had contact with an infected person now that community spread is occurring. Companies have been working to increase the production of the components, and other companies are working to get their equivalent components approved by the FDA for use (Herper, 2020). The health-supply company Roche announced that they expected to be able to provide test kits sometime in the week of March 16, which will increase the testing capacity from 26,000 a day to over 70,000 tests a day. Reportedly, 400,000 new kits were delivered (Feuer, 2020). There are over 80 other companies working to develop tests, and the estimated time of availability is the first week of April. As the number of testing kits increases, the turnaround time for results is also expected to decrease from 3 to 4 days to within 24 hours. Other companies have designed all-in-one cartridge PCR tests (described below) that can be done on site. For example, on March 21, the FDA issued an emergency use authorization for a diagnostic test developed by Cepheid (Cepheid, 2020). The tests do not require additional components, and results are reported within 45 minutes. The tests are expected to be available on March 30. The press release did not mention the number of the tests they would be able to produce.

Researchers have been working to improve and increase the pace of testing for COVID-19. The current testing uses a process called PCR that can detect pieces of the virus's genetic material (called RNA) in respiratory fluids. PCR procedures are able to detect very small amounts of specific pieces of RNA so that even if there are only a small number of virus particles in the sample, they can be detected. While the PCR tests can detect a very small level of virus in an infected person there are some disadvantages to these types of tests.

Disadvantages of PCR tests

- Can only detect an active infection and not determine if someone was previously infected and recovered
- Require respiratory fluids that can contain active virus putting staff at risk for infection during testing
- Results may vary based on how soon in the infectious process a person is, the efficiency in which the sample was taken, and the amount of time it takes the sample to arrive at the lab (delayed samples must be redone)

Another possible type of test is called a serological test (or sero test), which measures antibodies to the virus present in circulating blood. Serological tests are being developed by several groups. Serological tests are not as effective at detecting current infections as PCR but they are useful because they do not require handling of infectious virus and they can be quickly ramped up to allow for population screening to determine epidemiological information such as the precise rate of infection in an affected area that allows for an accurate determination of the infection fatality rate.

Serological tests employ a part of the outside of the virus to test for the presence of antibodies in an individual's blood. In the case of SARS-CoV-2, a protein on the outside of the virus, called the spike protein, was isolated from the virus's genome. Because the spike protein is accessible on the outside of the virus, human immune cells target these types of proteins with antibodies in order to launch an immune response to infection. Researchers can produce relevant parts of the spike proteins, called the receptor binding domain (or RDB). When blood serum from a person who is or has been infected with the virus is mixed with the receptor binding domain, they interact. Scientists can monitor this process with a test called an ELISA, which detects the bound antibodies and changes color in their presence.

A group of researchers, mainly from Mount Sinai Hospital in New York, released a report on the development and testing of a serological test for COVID-19 (Amanat et al., 2020). Based on their report, the researchers were able to create a test that allows for screening and identification of COVID-19 antibodies in blood plasma and serum as early as 3 days after symptom onset. The test allows for the identification of people who were previously infected or are currently infected. Those who were previously infected can be counted to better understand the spread of the infection, and additionally, it will allow for the screening of healthcare workers who have had the disease and developed immunity, who can then care for infected patients while minimizing the risk of viral spread to colleagues and other patients.

The researchers have made their research, and the methods to replicate it, public so that other laboratories can start to utilize the method to develop tests across the world.

In an interview in the publication *Science*, Florian Krammer from the Icahn School of Medicine at Mount Sinai who is one of the authors of the study remarked that "other labs could easily scale it up to screen a few thousand people a day, and quickly amass more data on the accuracy and specificity of the test" (Vogel, 2020).

Remdesivir

Remdesivir is considered the best candidate for treating COVID-19 because it has been shown to be safe in humans and has documented efficacy against coronaviruses in cell cultures. There are ongoing clinical trials investigating the use of the anti-viral medication remdesivir for treatment of coronaviruses, including COVID-19. Remdesivir is a small molecule that mimics one of the components used during production of the virus's RNA. When remdesivir is incorporated instead of the normal component, a nucleotide called adenosine, production of the virus's RNA is halted (Brown et al., 2019 and Sheahan et al., 2020). Early investigations of remdesivir show that it is able to inhibit the production of new virus particles in several different types of RNA viruses, including MERS, SARS, and Ebola.

There is experimental evidence that remdesivir improves pulmonary function, reduces the amount of virus in the lung, and reduces severe lung pathology in mice with MERS (Sheahan et al., 2020). Cellular experiments suggest that remdesivir has a broad activity to inhibit the function of a wide variety of different coronaviruses, including two strains that have been found to be greatly divergent in RNA sequences (Brown et al., 2019). Phase 2 and 3 studies of the drug for treatment of Ebola are underway, which means that safety of the drug in people has already been established.

A clinical trial has been initiated in Omaha, Nebraska to test the effects of remdesivir in people infected with COVID-19 (NIH, 2020). As of February 25, 2020, the first patient with COVID-19 acquired on the Diamond Princess cruise was given their first dose of remdesivir. Similar trials are also occurring in China. The trials were designed in coordination with the WHO to allow for adaptation to evaluate additional investigative treatments and to enroll participants at other sites in the United States and worldwide.

The study design includes a treatment group who receive 200 milligrams of remdesivir intravenously on the first day of enrollment to the study and another 100 milligrams each day for the duration of hospitalization or up to 10 days total. A second group will receive a placebo treatment so that any effect from remdesivir can be quantified.

Remdesivir is also included in the SOLIDARITY trial initiated by the WHO.

Chloroquine or Hydroxychloroquine

Chloroquine and hydroxychloroquine are derivatives of quinine, the malaria treatment, that have also been found to have inhibitory effects on RNA viruses (Devaux et al., 2020). Hydroxychloroquine was developed as an alternative to chloroquine to reduce the toxic effects that develop at the high dosage levels.

Quinine derivatives have been found to be useful in the treatment of a range of other illness in addition to malaria, such as auto-immune disorders as well as bacterial, fungal, and viral infection. There have also been studies of the effect of quinine derivatives on HIV infection, which indicated that the medications could inhibit the viral replication cycle in cell culture, but the

effect was not robust enough for use as a treatment. There are multiple laboratory experiments of anti-viral activity on a wide array of RNA viruses, but these effects have not always been evident later in patients enrolled in clinical trials. The effects of treatment seem to be very dependent on the concentration of chloroquine used, the duration of treatment, and the clinical team in charge of the trial.

Quinine derivatives are thought to act against viruses by reducing the acidity at the point where the virus is entering the cell, thus preventing infection (Wang et al., 2020). This action of increasing the pH of a compartment in the cell called the endosome, keeps the viruses outer shell from fusing with the cell and releasing the contents. Later in viral production, the changes in the endosome also interfere with the correct modification of the spike proteins, which prevents them from interacting with cellular receptors on new, human cells so they are not infectious.

The immune-modulating effect of quinine derivatives is also thought to contribute to their anti-viral effects.

Effectiveness of Quinine Derivatives on SARS and MERS

During the outbreaks of SARS (in 2003) and MERS (in 2012), quinine derivatives were utilized as an experimental treatment. There was evidence of therapeutic effect with chloroquine in people with SARS-Cov, but the effect was less reproducible in people with MERS.

Cell Culture Investigations

Cell culture experiments of the effect of chloroquine on SARS-CoV-2 infection indicate that the drug does disrupt the viral life-cycle at both the entry to cells and later during virus manufacture. The effect of chloroquine on cells occurred at a low concentration of the drug that the authors felt was achievable using the typical oral dosing and at levels normally considered as safe.

Another published report compared the effect of chloroquine with hydroxychloroquine. Cell culture experiments showed that hydroxychloroquine was more potent at inhibiting SARS-CoV-2 than chloroquine (Yao et al., 2020).

Efficacy in Individuals with COVID-19

A published report of the use of chloroquine in Hunan Province showed promising effects in 100 people with COVID-19 (Gao et al., 2020 and Devaux et al., 2020). The researchers observed a more rapid decline in fever and improvement of lung CT scans, and individuals treated with chloroquine required a shorter time to recover compared with control groups. Importantly, severe adverse reactions were not noted.

The details of this study are sparse, and it was not registered in the clinical trial registries either in China or the world-wide database administered by the National Institutes of Health in the United States (ClinicalTrials.gov). Because of the lack of information on dosing, length of

treatment, methods to measure recovery, etc., it is difficult to ascertain the validity of the claims in the report.

Another important unknown is the severity of the illness of those treated with chloroquine in the report from China. These is some preliminary evidence that different treatments for COVID-19 may be more effective when given early in disease progression to prevent severe symptoms, but there is a reduced effect of the drug in people who already have severe symptoms.

Further study is ongoing, and a search of ClinicalTrials.gov indicated five trials with chloroquine for treatment of COVID -19 and four with hydroxychloroquine. Authors of a review of chloroquine or hydroxychloroquine with COVID-19 reported that there are 23 ongoing trials listed on the Chinese registry of clinical trials.

Side Effects of Chloroquine

The use of chloroquine is associated with several safety challenges. At the levels and dosing period needed for treatment of malaria, there is evidence of toxicity. The levels required for treatment of COVID-19 are obviously still being evaluated.

There is evidence that some of the negative effects of chloroquine are cumulative, which means that the longer people take the drug the more likely they are to develop adverse reactions (Devaux et al., 2020). Also, both chloroquine and hydroxychloroquine have a long half-life in the body (around 50 days), which means that the effects of using the drug will be present for a long time after treatment is stopped. They are also widely distributed in the body, meaning that many different tissues will be exposed during treatment (Schrezenmeier and Dörner, 2020). In studies using hydroxychloroquine in the treatment of rheumatoid arthritis, it has also been found that the amount of the drug available in the blood varies between patients on the same dose, and even in the same patient at different time points. The basis of this difference is not known. Use of chloroquine has also been found to change the blood levels of other drugs. A study of digitoxin (used for heart failure and irregular heartbeat) showed that use of digitoxin with chloroquine led to a fourfold increase in the amount of digitoxin in the blood. It is thought that chloroquine interrupts one of the common the metabolic pathways used by the body to process drugs and remove them from circulation (cytochrome P450).

Adverse reactions previously reported with use of chloroquine:

- Macular retinopathy, retinal damage
- Cardiomyopathy
- Cardiac rhythm disorders
- Gastrointestinal effects, including nausea, vomiting, diarrhea and abdominal discomfort
- Headache
- Skin rash or itching
- Hair loss
- Mood or mental changes

(MedlinePlus, 2020)

Retinopathy is more commonly associated with chloroquine than with hydroxychloroquine, however new studies suggest that hydroxychloroquine-related retinopathy is more common than previously realized (Schrezenmeier and Dörner, 2020). Because both drugs are cleared from the body through the kidneys, individuals with kidney disease or reduced kidney function are more likely to develop retinopathy.

There have also been a number of recent chloroquine-related poisonings in Nigeria after news of a possible effect on COVID-19 (JHU, 2020). The Ministers of Health in Lagos and Nigeria have both released statements warning against the unlicensed use of chloroquine and emphasizing that it has yet to demonstrate efficacy against COVID-19.

An additional case of poisoning with chloroquine was reported in Arizona (Vigdor, 2020). A married couple treated themselves with chloroquine found in a product used for maintenance of fish tanks. Both immediately experienced nausea and vomiting, and the man later died of cardiac arrest from chloroquine poisoning. The woman is reported to be in the hospital in stable condition and was expected to make a full recovery. The woman mentioned that they had heard of chloroquine in the news and realized that they had some available in the form of the tank treatment. The doctor treating the couple was quick to reiterate that

> "This is not going to be a magic pill for us to get us through this. You need to listen to the scientists," he said. "People are panicking and making decisions based on symptoms without being tested."

He also mentioned that 85% of people who get the coronavirus would survive with no specific treatment, and antiviral medications are needed only in patients who had tested positive and are critically ill. Additionally, the use of medications needs to be closely monitored because they can have serious side effects.

In recommendations for the use of chloroquine for the treatment of COVID-19, a number of precautions have been recommended to monitor possible adverse effects of the drug (Gao et al., 2020). The Chinese recommendations suggest regular blood tests to monitor development of anemia, thrombocytopenia or leukopenia (reduction in red and with blood cells), disturbances in the serum electrolyte levels, and dysfunction in the liver or kidneys. It was also suggested that

people being treated with chloroquine receive routine electrocardiography to monitor changes in heart rhythm. Patient interviews are also done on a regular basis to identify visual and mental disturbance or deterioration. The Dutch Center of Disease control stresses the need for stopping treatment at day five of treatment to reduce the risk of side effects, considering the long half-life of the drug, and the need to differentiate between chloroquine phosphate and chloroquine base because the doses are different.

Methylprednisolone

Methylprednisolone, a corticosteroid, functions by relieving inflammation. It can be used to reduce swelling in the lungs in people with pneumonia to help improve breathing. In studies of individuals with severe community-acquired pneumonia and a high inflammatory response, researchers have reported less treatment failure (defined using outcomes such as development of shock, need for invasive mechanical ventilation, and death within 72 hours of treatment) in those who were treated with methylprednisolone (Science News, 2017). There was treatment failure in 13% of those on methylprednisolone while treatment failure occurred in 31% in the placebo group. Patients who received methylprednisolone treatment had a 66% lower odds of treatment failure. Because of the utility of methylprednisolone in previous studies of individuals with severe pneumonia, it has also been used to treat pneumonia from coronavirus outbreaks, including SARS and MERS.

However, there is evidence that methylprednisolone is not as effective in treatment of SARS and MERS, suggesting caution when used for COVID-19.

In previous coronavirus outbreaks, the use of systemic corticosteroids was found to give no benefit to the symptoms, and there were possible harms to the recipient. Therefore, they are not recommended to treat people with suspected COVID-19. In similar studies of influenza, use of systemic corticosteroids led to a higher risk of mortality and secondary infections. There is also evidence that the use of systemic corticosteroids in the treatment of coronaviruses, such as MERS, led to slower clearance of the virus from the lower respiratory tract (WHO Clinical Management of Severe, 2020).

Another study published in 2017 described the long-term outcomes of people who recovered from SARS (Wu et al., 2017). People who had had SARS had differences in some of the metabolites in their blood serum compared to the general population. The largest difference was in the amount of two lipids (phosphatidylinositol and lysophosphatidylinositol), and the change was correlated with treatment with methylprednisolone. The authors propose that these results suggest that high-dose pulses of methylprednisolone might cause long-term systemic damage associated with serum metabolic alterations.

Inhaled Corticosteroid Ciclesonide

A research group studying the MERS virus observed that application of the approved steroid compound ciclesonide to cells infected with MERS blocks replication of the virus with a low level of toxicity to the cells (Matsuyama et al., 2020). Based on their previous experiments, the researchers also tested the effect of ciclesonide on SARS-CoV-2. In cell-based experiments,

application of ciclesonide suppressed viral replication with a similar efficacy as lopinavir (discussed below). The effect of ciclesonide was greater than that of entry inhibitors such as camostat (discussed below). Other forms of steroid treatments that were also tested (cortisone, prednisolone, dexamethasone, and fluticasone) did not suppress viral growth.

Ciclesonide is a well-established inhaled corticosteroid treatment that is safe for use in infants to adults. Based on previous studies, it exerts an anti-inflammatory effect in the lungs. It also has the benefit that as it is given in an inhaled formulation, the majority of the medication remains in the lungs with little effect on other parts of the body as occurs in systemic corticosteroids, such as methylprednisolone.

Studies of the effect of the medication in people with COVID-19 have not yet been reported, but it may be useful to combat both inflammatory response from viral pneumonia symptoms and viral replication.

Interferon-2 beta

Interferon-2 beta (IFNb) has also been shown to have anti-viral activity in cell culture against the virus that causes MERS (Martinez, 2020). Clinical trials in humans have also been started to test the effects against both MERS and COVID-19. In one experiment in an animal model of MERS, IFNb in combination with lopinavir and ritonavir was found to improve pulmonary function, but it did not reduce virus replication or severe lung pathology. In the same study, the effects of IFNb with lopinavir and ritonavir were compared to remdesivir. Remdesivir was found to improve pulmonary function, reduce lung viral loads, and ameliorate severe lung pathology in the animal model. Remdesivir was also found to be effective when used prophylactically (very early in infection) in animal models as well as after symptoms progressed while the INFb combination therapy was not effective when used prophylactically.

Lopinavir and Ritonavir

There are also several other anti-viral drugs that were developed to target RNA-based viruses, including lopinavir and ritonavir. The use of these medications is also being investigated, but there is no evidence from previous studies of an effect on coronaviruses, as has been reported for remdesivir. However, the combination of lopinavir and ritonavir is approved for the treatment of HIV and has been found to be safe for use in people.

Preliminary results from use of lopinavir–ritonavir in China suggest that the combination is not effective for treatment of COVID-19. The results from a clinical trial were recently released describing the outcome of treatment of 199 patients with laboratory-confirmed SARS-CoV-2 infection where 99 received lopinavir with ritonavir and 100 received standard care (Cao et al., 2020). The researchers found that the time to clinical improvement and mortality rate measured 28 days after the beginning of treatment was similar between the two groups. Based on analysis, lopinavir–ritonavir led to a median time to clinical improvement that was shorter by one day than that observed with standard care. Gastrointestinal adverse events were more common in the lopinavir–ritonavir group. However, serious adverse events were more common in the standard-care group.

The authors conclude that in hospitalized adult patients with severe COVID-19, no benefit was observed with lopinavir with ritonavir treatment beyond standard care.

Favipiravir

Favipiravir is an antiviral drug that selectively inhibits the RNA-dependent RNA polymerase of influenza virus that has been approved for use in Japan (marketed as Avigan) for treatment of the flu (Furuta et al., 2013). Previous studies have shown that favipiravir works synergistically in combination with oseltamivir to increase its anti-viral effect against influenza. It has also been shown to block the replication of many other RNA viruses, but in the initial research study, coronaviruses were not mentioned.

A report in the Guardian quotes an official at China's science and technology ministry, Zhang Xinmin, as saying that favipiravir has a high degree of safety and is clearly effective in treatment of COVID-19 (McCurry, 2020). The clinical trial reportedly involved 340 participants. The official stated that people given the medicine tested negative for the virus after a median of four days compared to a median of eleven days for those who received the standard of care but no favipiravir. There was also evidence of an improvement in lung condition based on x-ray scans in 91% of those who received favipiravir compared to improvements in 62% of those who did not receive the drug. Reports in other sources showed that the number of patients treated were 240 in Wuhan and 80 in Shenzhen (NKH World-Japan, 2020 and Keown, 2020).

Researchers in Japan are using favipiravir in people with mild to moderate symptoms to reduce progression of the virus. However, the Japanese researchers have noticed that favipiravir is not as effective for people with severe symptoms. This same effect has been reported with use of lopinavir and ritonavir (McCurry, 2020).

Favipiravir has also been utilized in Ebola virus outbreaks as an emergency treatment. A report of 163 patients diagnosed with Ebola in 2015 in Guinea included 73 who were treated with favipiravir (Stulpin, 2019). In those who were treated with favipiravir, the case fatality rate was lower than that in untreated participants (42.5% and 57.8%, respectively). However, the difference was not found to be statistically significant, which means that the fluctuation in the outcome might be due to random chance rather than a positive effect from favipiravir. There was a statistically significant increase in the survival time in people treated with favipiravir. A second trial of the use of favipiravir for the treatment of Ebola indicated that it may reduce mortality rates among patients with Ebola virus and lower viral loads early in their disease course (Infectious Disease News, 2015). This second study showed that the medication did not have a benefit on mortality late in infection with Ebola when the amount of virus present was large. One of the researchers in the study stated that use of favipiravir reduces the viral load within four days when patients were treated early and had initial low viral load.

Camostat mesylate

In order for the viruses responsible for SARS and COVID-19 to infect a human cell, their spike proteins (described above) must be cut by a type of protein called proteases (Hoffman et al.,

2020). The specific protease used by SARS-CoV and SARS-CoV-2 is called TMPRSS2. Camostat mesylate is a known inhibitor of TMPRSS2 that has been previously used to treat chronic pancreatitis (Zhou et al., 2015).

Cell culture experiments have shown that treatment with camostat mesylate inhibits the infection of cells with SARS-CoV. The studies showed that camostat mesylate is a broad-spectrum antiviral that keeps the virus from entering the cell, thus preventing infection.

Cellular studies of SARS-CoV-2 showed that application of camostat mesylate partially blocked the entry of the virus into cultured cells (Hoffman et al., 2020). The fact that it was not completely blocked suggests that there is a second protease that is involved in cutting the spike protein. When a second inhibitor (E-64d) targeted for the CatB/L protease was applied in combination with camostat mesylate, viral entry was completely inhibited. Additional experiments in cells that were originally harvested from human lungs indicate that camostat mesylate treatment significantly reduced infection of lung-based cells with SARS-CoV-2.

The authors conclude that TMPRSS2 is a host cell factor that is critical for spread SARS-CoV-2 as well as several other clinically relevant viruses, and that camostat mesylate has been shown to be safe for other uses in humans, suggesting that inhibition of TMPRSS2 is not toxic to cells.

Camostat mesylate is available as an oral medication that has previously been used to reduce pancreatic enzyme activity and has been widely used for the treatment of chronic pancreatitis-associated pain in Japan (marketed as Foipan) (Ramsey et al., 2019). Other uses of camostat mesylate include reduction of kidney disease from chronic diabetes (Onbe et al., 1991). The long use of the drug and approval in Japan suggests a reasonable safety profile that would allow for trials to determine its efficacy in COVID-19.

Antibody Treatments

Antibodies are made by immune cells called B cells in response to infection or the presence of other particles that are not recognized as part of the individual. An antibody has a specific area that binds to a specific molecule, and only that specific molecule. Antibodies are produced in response to an infection, and if the infectious entity remains in the environment, long-term production can occur to provide long-term immunity.

Antibodies can also be produced in animals or cells to be used as treatments for disease. If an antibody is produced in cells grown in culture, all the antibodies are the same, and the resulting treatment is called a monoclonal antibody. In animals, multiple cells are responsible for the production of antibodies, and the resulting milieu is called a polyclonal antibody. Animal based treatments are used less often because allergic reactions are more common due to cross reactivity while the cells used to grow monoclonal antibodies in culture are often derived from human sources to reduce the allergic reactions.

When antibodies are made externally and given as a treatment, they are a form of passive immunotherapy. Vaccines are treatments that stimulate the body to produce its own antibodies. Passive immunotherapy provides the antibodies and the body is not required to produce them.

However, this means that when the treatment is ended, the passive effect also ends. Antibodies for passive immunotherapy can be isolated from the blood of the infected patients or it can be manufactured in the laboratory.

Experiments of Antibodies for Treatment of SARS and COVID-19

Experiments have shown that the viruses that cause SARS and COVID-19 both use the same point of entry into cells. The virus responsible for MERS, on the other hand, uses a different part of human cells to gain entry for infection (Shanmugaraj et al., 2020). Because SARS and COVID-19 viruses use the same protein to enter human cells, the so-called spike protein, researchers have been investigating if antibodies designed to treat SARS could work for COVID-19.

In preliminary studies in cell culture, antibodies harvested from patients who had previously recovered from SARS were able to reduce the entry of SARS-CoV-2 into cells, but not completely inhibit infection (Hoffman et al., 2020). Antibodies generated in rabbits that bind to the spike protein of SARs-CoV were also able to inhibit the entry of SARS-CoV-2.

These results suggest that previous infection with SARS may offer some level of protection against infection with COVID-19.

Based on these results, antibodies from SARS infections may have some cross-reactivity, but that there seem to be some important differences that prevent antibodies based on SARS from completely inhibiting COVID-19 infection. There are currently eleven antibodies that have been investigated to inhibit infection with SARS-CoV, and 14 antibodies that have been designed to prevent MERS (Shanmugaraj et al., 2020). However, there are no approved therapies using these methods to date, suggesting that while the process can be accomplished in cell culture, it is more difficult to reproduce in humans.

Several companies have reported that they are working on the development of antibody therapy for COVID-19. Regeneron has reported that they are developing antibodies to SARS-CoV-2 for use as a treatment, and they expect to have hundreds of thousands of doses ready for testing in humans by late summer (Tirrell, 2020). Researchers at Regeneron have previously made the same types of treatments for Ebola, asthma, high cholesterol, rheumatoid arthritis, and cancer. In the report, officials from the company explained that they were doing laboratory testing to determine which two antibodies have the best attributes for a treatment. Their plan is to combine two antibodies to create a cocktail-like treatment. The Ebola treatment is a combination of three antibodies, which the researchers suggest helps to prevent loss of effectiveness in the event of a mutation in the virus. Based on previous research, the antibody treatments are expected to be effective for about a month after injection, and the antibody cocktail can be used both as a prophylactic for protection from illness and a treatment. Initial testing and treatment is expected to be restricted to healthcare workers or people at high-risk to protect them until a vaccine becomes available.

Serum Antibody Treatments

Until specific treatments are available, researchers have also been investigating a long-used technique to isolate the antibodies contained in the blood serum of people who have recovered from illness (Pearce, 2020 and Fox, 2020). The technique has been used since the 1890's and involves giving people at high risk for infection or are sick a dose of blood serum that contains a high level of what are called neutralizing antibodies. Neutralizing antibodies bind to the virus and prevent infection, thereby neutralizing the viral particles. Different people produce different kinds of antibodies at different levels depending on the way their immune system responded to the infection, and therefore some people produce larger amounts or more effective neutralizing antibodies.

The modern technique to obtain serum antibodies involves:

- Screening of recovered individuals to identify those who produce high levels of neutralizing antibodies
- Blood serum donation using apheresis, a process where the blood is processed so that red blood cells are returned to the donor and only serum and plasma are removed
- The blood is screened using normal blood banking processes.
- The serum is processed to remove toxins, medications, or trace illness and concentrate the mixture by removing excess water.

The technique has been utilized for treatment during influenza, measles, and mumps outbreaks in the past (Casadevall and Pirofski, 2020). Recently, physicians used serum from recovered patients to treat the H1N1 influenza outbreak in 2009 and 2010. Studies of the process showed that those treated with the serum had reduced respiratory viral burden, reduced inflammatory responses, and lower mortality. The technique was also recently used to treat Ebola in 2013 in West Africa. A study of the treatment with serum antibodies in Sierra Leone showed that people treated with whole blood from a recovered individual had a longer survival compared to those who received standard treatment.

Most studies suggest that use of serum antibodies is more effective for prophylactic use in those who are at high risk. This effect is also evident with the use of antibody treatments produced by pharmaceutical companies as described above. However, there is a benefit in some cases of people who are already ill. A higher dose is needed for treatment than for prophylaxis.

Researchers at Johns Hopkins Hospital have been working on developing a procedure for local hospitals to produce their own serum antibodies, and hospitals in China used the technique to treat patients during the initial outbreak (Pearce, 2020).

Takeda Pharmaceutical in Japan is developing a related product from the blood from recovered individuals called intravenous immunoglobin, or IVIG. This preparation consists of antibodies of all types purified from the blood plasma of healthy people. Use of purified antibodies requires a smaller volume of liquid during dosing, avoids the possibility of transfer of other infectious agents, and is more efficient (Herper and Feurstein, 2020). Takeda is currently working on a COVID-19 specific IVIG made from the blood of people who have recovered. As with serum

antibody treatment, it is not necessary to determine which antibody works best as with the antibody treatments described above, which could increase the speed for it to be available.

Risks Associated with Serum Antibodies

Generally the use of serum derived antibodies is a stopgap measure until other, more effective preventions or treatments are available, such as vaccines. There is a higher risk associated with serum based antibodies than those produced using laboratory procedures because there is a transfer of blood substances. However, use of blood products is well-established with many screening and processes in place to reduce the risks. The risks directly associated with the use of blood products include inadvertent infection with another infectious disease agent and reactions to serum constituents. Use of antibodies as a treatment can cause extreme inflammatory responses in some people from the sudden increase in antibodies in the blood that mimic the symptoms of an allergic reaction (Casadevall and Pirofski, 2020b).

Other Treatments Being Tested in Clinical Trials

There are several other potential treatments being investigated. They are a mixture of HIV, influenza treatments, and treatments that affect the immune response. There is not specific information available that shows an effect on coronavirus.

ASC09- ASC09, also called TMC-310911, is an experimental drug that was developed for use against HIV. It targets the protease of the virus, which is a protein that is needed to cut the viral proteins into the correct size within the cell during production of new viruses. Based on studies for HIV, ASC09 is thought to have a similar mechanism to darunavir, another drug targeted for HIV protease inhibition (AIDSinfo, 2019). The drug has been evaluated in a fourteen day Phase 2 trial in combination with ritonavir for treatment of HIV and is found to have a good safety profile.

Oseltamivir (Tamiflu)- Oseltamivir is an FDA approved medication marketed as Tamiflu to treat influenza (MedlinePlus, 2018). Oseltamivir is a type of drug called a neuraminidase inhibitor, which prevents the activity of a protein in cells that allows influenza to form correctly and produce infectious virus. The protein neuraminidase modifies on influenza is a spike protein that is on the surface of the virus. If the spike protein is not modified correctly, it interacts with other influenza viruses, causing clumps that cannot infect human cells (Shtyrya et al., 2009).

Azvudine- Azvudine is a reverse transcriptase inhibitor that was designed for treatment of HIV.

Baloxavir marboxil- Baloxavir marboxil is marketed as Xofluza and is an antiviral medication for treatment of influenza A and influenza B. It was approved for medical use in Japan and in the United States in 2018. Baloxavir marboxil is an inhibitor of the endonuclease activity that targets RNA production for the manufacture of new influenza viruses.

Darunavir and Cobicistat- Darunavir and cobicistat are a combination used to treat HIV. Darunavir is a protease inhibitor similar to those described above. Cobicistat, with trade name

Tybost is a licensed drug for use in the treatment of human immunodeficiency virus infection. Its major mechanism of action is through the inhibition of human CYP3A proteins.

Camrelizumab and Thymosin- Camrelizumab (marketed as AiRuiKa) is a programmed cell death 1 (PD-1) inhibitor that received conditional approval in China for the treatment of relapsed or refractory classical Hodgkin lymphoma (Markham and Keam, 2019). One of the most important immune checkpoints developed to limit over-activation of immune cells is PD-1. Thymosin is a hormone secreted from the thymus. Its primary function is to stimulate the production of T cells, which are an important part of the immune system. Thymosin also assists in the development of B cells to plasma cells to produce antibodies.

Tocilizumab- Tocilizumab or atlizumab (marketed as Actemra) is a humanized monoclonal antibody against IL-6 receptor. Immunosuppressive drugs can reduce the inflammatory response. Researchers suggest it might be useful in cases of severe inflammation associated with viral pneumonia.

References

AIDSinfo. TMC-310911. Last Reviewed: January 2, 2019 Accessed on March 20, 2020 at https://aidsinfo.nih.gov/drugs/549/tmc-310911/0/professional

Amanat F, Nguyen T, Chromikova V, Strohmeier S, Stadlbauer D, Javier A, Jiang K, Asthagirir-Arunkumar G, Polanco J, Bermudez-Gonzalez M, Caplivski D, Cheng A, Kedzierska K, Vapalahti O, Hepojoki J, Simon V, Krammer F. A serological assay to detect SARS-CoV-2 seroconversion in humans. medRxiv 2020.03.17.20037713; doi: https://doi.org/10.1101/2020.03.17.20037713

Branswell H. WHO to launch multinational trial to jumpstart search for coronavirus drugs. *STAT*. Published March 18, 2020. Accessed on March 22, 2020 at https://www.statnews.com/2020/03/18/who-to-launch-multinational-trial-to-jumpstart-search-for-coronavirus-drugs/

Brown AJ, Won JJ, Graham RL, et al. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antiviral Res*. 2019;169:104541.

Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19 [published online ahead of print, 2020 Mar 18]. *N Engl J Med.* 2020;10.1056/NEJMoa2001282. doi:10.1056/NEJMoa2001282

Casadevall A, Pirofski LA. The convalescent sera option for containing COVID-19 [published online ahead of print, 2020 Mar 13]. *J Clin Invest*. 2020;138003.

Cepheid. Coronavirus (COVID-19) Update: FDA Issues first Emergency Use Authorization for Point of Care Diagnostic. Published March 21, 2020. Accessed on March 23, 2020 at https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-first-emergency-use-authorization-point-care-diagnostic

Chamoto K, Al-Habsi M, Honjo T. Role of PD-1 in Immunity and Diseases. *Curr Top Microbiol Immunol*. 2017;410:75–97. doi:10.1007/82_2017_67

Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19 [published online ahead of print, 2020 Mar 10]. *J Crit Care*. 2020;S0883-9441(20)30390-7.

Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? [published online ahead of print, 2020 Mar 11]. *Int J Antimicrob Agents*. 2020;105938.

Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*. 2020;14(1):72–73.

Harrison C. Coronavirus puts drug repurposing on the fast track. Published February 27, 2020. *Nature*. Accessed on March 17, 2020 at https://www.nature.com/articles/d41587-020-00003-1

Herper M. Thermo Fisher to produce millions of coronavirus diagnostic tests. Published on March 14, 2020. *The STAT*. Accessed on March 15, 2020 at https://www.statnews.com/2020/03/14/thermo-fisher-to-produce-millions-of-coronavirus-diagnostic-tests/

Herper M, Feuerstein A. How blood plasma from recovered patients could help treat the new coronavirus. *STAT*. Published on March 5, 2020. Accessed on March 24, 2020 at https://www.statnews.com/2020/03/05/how-blood-plasma-from-recovered-patients-could-help-treat-coronavirus/

Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor [published online ahead of print, 2020 Mar 4]. *Cell*. 2020;S0092-8674(20)30229-4.

Feuer, W. Widespread coronavirus testing in US is 'weeks, if not months, out,' says CEO of test manufacturer Roche. *CNBC*. Published Mar 23, 2020. Accessed March 24, 2020 at https://www.cnbc.com/2020/03/23/roche-ceo-widespread-coronavirus-testing-in-us-is-weeks-if-not-months-out.html

Fox M. Drug makers are racing to develop immune therapies for Covid- 19. Will they be ready in time? *STAT*. Published March 19, 2020. Accessed on March 19, 2020 at https://www.statnews.com/2020/03/19/coronavirus-antibody-therapies-will-they-be-ready-in-time/

Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. *Antiviral Res.* 2013;100(2):446–454.

Johns Hopkins University. Daily update on COVID-19. Published March 23, 2020. Accessed on March 23, 2020 at https://myemail.constantcontact.com/COVID-19-Updates---March-23.html?soid=1107826135286&aid=nSXi768j9Q8

Keown, A. FujiFilm Influenza Drug Shows Efficacy in Treating Coronavirus, Chinese Researchers Say. *BioSpace*. Published March 19, 2020. Accessed on March 20, 2020 at https://www.biospace.com/article/fujifilm-influenza-drug-shows-efficacy-in-treating-coronavirus-chinese-researchers-say/

Stulpin C. Survival times improved in favipiravir treated Ebola patients. *Healio*. Published March 29, 2019. Accessed on March 23, 2020 at https://www.healio.com/infectious-disease/emerging-diseases/news/online/%7B36aa58be-6b3b-445b-bf98-2367f159f310%7D/survival-times-improved-in-favipiravir-treated-ebola-patients

Markham A, Keam SJ. Camrelizumab: First Global Approval [published correction appears in Drugs. 2019 Aug 24;:]. *Drugs*. 2019;79(12):1355–1361.

Martinez MA. Compounds with therapeutic potential against novel respiratory 2019 coronavirus [published online ahead of print, 2020 Mar 9]. *Antimicrob Agents Chemother*. 2020;AAC.00399-20.

Matsuyama S, Kawase M, Nao N, Shirato K, Ujike M, Kamitani W, Shimojina M, Fukushi S. The inhaled corticosteroid ciclesonide blocks coronavirus RNA replication by targeting viral NSP15. bioRxiv 2020.03.11.987016; doi: https://doi.org/10.1101/2020.03.11.987016

McCurry J. Japanese flu drug 'clearly effective' in treating coronavirus, says China. *The Guardian*. Published March 18, 2020. Accessed on March 20, 2020 at https://www.theguardian.com/world/2020/mar/18/japanese-flu-drug-clearly-effective-in-treating-coronavirus-says-china

MedlinePlus. Chloroquine. Last Revised June 15, 2017. Accessed on March 20, 2020 at https://medlineplus.gov/druginfo/meds/a682318.html

MedlinePlus. Oseltamivir. Last Revised, January 15, 2018. Accessed on March 20, 2020 at https://medlineplus.gov/druginfo/meds/a699040.html

National Institutes of Health. New Releases. NIH clinical trial of remdesivir to treat COVID-19 begins. Published on February 25, 2020. Accessed on February 27, 2020 at https://www.nih.gov/news-events/news-releases/nih-clinical-trial-remdesivir-treat-covid-19-begins

NHK World-Japan. China: Avigan effective in tackling coronavirus. Published March 17, 2020. Accessed on March 20, 2020 at https://www3.nhk.or.jp/nhkworld/en/news/20200317_48/

Onbe T, Makino H, Kumagai I, Haramoto T, Murakami K, Ota Z. Effect of proteinase inhibitor camostat mesilate on nephrotic syndrome with diabetic nephropathy. *J Diabet Complications*. 1991;5(2-3):167–168.

Pearce K. Antibodies from covid-19 Survivors could be used to Treat patients, protect those At risk. *The HUB*. Published March 13, 2020. Accessed on March 19, 2020 at https://hub.jhu.edu/2020/03/13/covid-19-antibody-sera-arturo-casadevall/

Ramsey ML, Nuttall J, Hart PA; TACTIC Investigative Team. A phase 1/2 trial to evaluate the pharmacokinetics, safety, and efficacy of NI-03 in patients with chronic pancreatitis: study protocol for a randomized controlled trial on the assessment of camostat treatment in chronic pancreatitis (TACTIC). *Trials*. 2019;20(1):501. Published 2019 Aug 14.

Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat Rev Rheumatol*. 2020;16(3):155–166.

Science Daily. Corticosteroid decreases treatment failure for patients with severe community-acquired pneumonia and high inflammatory response. *Science Daily*. Published on February 17,2015. Accessed on March 20, 2020 at www.sciencedaily.com/releases/2015/02/150217114001.htm

Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19*). Asian Pac J Allergy Immunol*. 2020;38(1):10–18.

Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat Commun*. 2020;11(1):222. Published 2020 Jan 10.

Shtyrya YA, Mochalova LV, Bovin NV. Influenza virus neuraminidase: structure and function. *Acta Naturae*. 2009;1(2):26–32.

Tirrell M. Regeneron aims to have coronavirus antibody treatment ready for human testing by early summer. Published March 17, 2020. Accessed on March 19, 2020 at https://www.cnbc.com/2020/03/17/regeneron-aims-to-have-coronavirus-drug-ready-for-testing-early-summer.html

Vigdor N. Man Fatally Poisons Himself While Self-Medicating for Coronavirus, Doctor Says. *The New York Times*. Published March 24, 2020. Accessed on March 24, 2020 at https://www.nytimes.com/2020/03/24/us/chloroquine-poisoning-coronavirus.html

Vogel G. New blood tests for antibodies could show true scale of coronavirus pandemic. *Science*. Published March 19, 2020. Accessed on March 23, 2020 at https://www.sciencemag.org/news/2020/03/new-blood-tests-antibodies-could-show-true-scale-coronavirus-pandemic

Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* 2020;30(3):269–271.

World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected. Published January 28, 2020. Accessed

on February 27, 2020 at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/patient-management

Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. 2020;367(6483):1260–1263.

Wu Q, Zhou L, Sun X, et al. Altered Lipid Metabolism in Recovered SARS Patients Twelve Years after Infection. *Sci Rep*. 2017;7(1):9110. Published 2017 Aug 22.

Yao X, Ye F, Zhang M, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [published online ahead of print, 2020 Mar 9]. *Clin Infect Dis.* 2020;ciaa237.

Zhou Y, Vedantham P, Lu K, et al. Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral Res.* 2015;116:76–84.