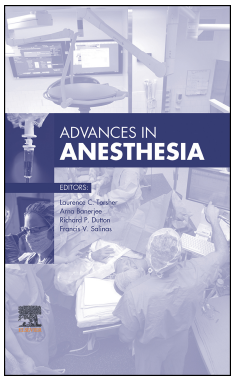

An Executed Plan to Combat COVID-19 in the United States

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An Executed Plan to Combat COVID-19 in the United States

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The author has nothing to disclose.

Abstract:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019. High SARS-CoV-2 transmissibility has led to a world-wide pandemic of acute respiratory disease, devastating populations by overwhelming health systems and causing many deaths. To date, this coronavirus is responsible for greater than 90 million cases in the United States and over one million confirmed deaths. When this virus came to the United States, testing was unorganized, no effective treatments were known, and no vaccines had been discovered. A plan to correct these deficiencies through cooperative science and efficient clinical trials was implemented to combat this novel virus. This plan developed efficient and inexpensive tests, highly effective medicines to treat and prevent disease progression, and vaccines to immunize the population

Key Points

Keywords:

- COVID-19
- SARS-CoV-2
- Pandemic

- COVID screening
- convalescent plasma
- Monoclonal antibodies
- Anti-virals
- Vaccine

An Executed Plan to Combat COVID-19 in the United States

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible coronavirus that emerged in late 2019. (1) High SARS-CoV-2 transmissibility has led to a world-wide pandemic of acute respiratory disease, devastating populations by overwhelming health systems and causing many deaths. To date, this coronavirus is responsible for greater than 90 million cases in the United States and over one million confirmed deaths. (2)

As a coronavirus, SARS-CoV-2 belongs to a group of diverse viruses infecting many different animals including domesticated cattle and sheep, as well as avian mammals including bats. (3) New coronaviruses often lead to upper respiratory infections when they infect humans, infections that more recently have caused a wide clinical spectrum from mild to severe disease and death. Before the advent of these coronaviruses, these viruses were not thought to be pathogenic in humans, in fact, prior to 2002, most coronaviruses were believed to be quite mild and caused little or no disease in humans. SARS-CoV-2, a novel coronavirus responsible for COVID-19, is related to other pathogenic coronaviruses including SARS-CoV and MERS-CoV. (3) While these other coronaviruses sparked a world-wide concern early due to rapid transmissibility and

initial fatalities, both SARS-CoV and MERS-CoV were relatively quickly contained preventing a world-wide pandemic situation seen with SARS-CoV-2.

SARS-CoV-2 arose from Wuhan, China at the end of 2019 and, though attempts at containment were initiated, quickly spread to every country in the world. (4) The virus has proven to be highly contagious between humans, and with the timing of its emergence during the lunar New Year, travel of families to and from Wuhan made containment logistically difficult. (5) SARS-CoV-2 has caused to the largest viral pandemic in our lifetime and is a true threat to world-wide public health.

The emergence of SARS-CoV-2 in the United States, as in other countries, was met with great angst. The hospitals and health care systems were quickly overwhelmed, and no treatments were known. People were dying and there was little that could be done other than supportive care. The SARS-CoV-2 pandemic highlighted the need for and importance of clinical trials to identify diagnostics, therapeutics and vaccines that would combat this virus, as well as novel methods to get these therapies produced and trialed quickly but safely.

This review is written from the perspective of an anesthesiologist/clinical trialist who helped execute a plan at a large midwestern university to bring old and novel therapies for trial to help treat suffering people, and vaccines to trial with a goal of preventing severe disease and slowing viral transmission. To understand how and why this plan

was needed, a firm understanding of the virus itself, as well as its impact on society are also very important.

Viral Emergence and Effects on Society

Emergence:

Prior to 2002, coronaviruses were thought to not cause serious infections in humans. In fact, coronaviruses were better studied in mammals other than humans as human pathogenic coronaviruses were not seen. The emergence of SARS-CoV in a southern China animal market altered that theory. Pathogenic in humans, SARS-CoV caused respiratory illness in more than 8,000 people and caused 774 deaths worldwide. (6,7) Still another coronavirus, MERS-CoV, emerged in 2012. This virus was responsible for Middle East respiratory syndrome and led to respiratory infections in approximately 2428 individuals and killing 838. (8) SARS-CoV and MERS-CoV had bat origins before jumping to new mammalian hosts. SARS-CoV invaded the Himalayan civet. In the Middle East, MERS-CoV jumped to the dromedary camel in the Middle East. Both of these coronaviruses subsequently infected humans, with deadly consequences. (9,10) SARS-CoV-2 may have also originated in bats as bats have a similar coronavirus relative, a coronavirus known as RaTG13. (11) SARS-CoV-2 and RaTG13 have similar S-proteins with about 98% amino acid sequence homology, only dramatically differing

in the sequence of the S-gene coding for the Spike protein. (11) Following initial SARS-CoV-2 cases that dated to December 8, 2019, in Wuhan, the World Health Organization (WHO) was notified of a pneumonia outbreak of unidentified cause by the Wuhan Municipal Health Organization. (12) By mid-January, 2020, Chinese scientists had identified the pathogen as a betacoronavirus with a single-stranded RNA genome. By January 12, 2020, the complete viral RNA genome sequence was made publicly available via the GISAID database. (12, 13) On January 30, the WHO declared SARS-CoV-2 a public health emergency with international potential. Unable to contain the virus in China, and with a large international travel population, the highly transmissible virus was determined to be a threat in all countries. According to Johns Hopkins University, by August, 2020, 216 countries and regions from all six continents had reported more than 20 million cases of SARS-Cov_2 (now called COVID-19), overwhelming local health resources and leading to high mortality. (14)

COVID-19 in the Community

COVID-19 has had a tremendous impact on all communities in the United States, but a disproportionate effect on communities of color. The COVID-19 pandemic has highlighted several health inequities within our health system. For instance, non-Hispanic Black or African American people and Hispanic people are both more than twice as likely to be hospitalized due to COVID-19 than non-Hispanic white people.

(14,15) Further, Non-Hispanic Native Americans or Alaska Native people are 3.1 times more likely to be hospitalized due to COVID-19 than non-Hispanic white people. Racial and socio-economic factors play a role in these inequities. These factors likely contribute to these inequities including, co-morbid conditions, access to healthcare, location, and type of housing. Historically, Black, Hispanic, Native American, and Asian American people are at higher risk of developing co-morbid conditions including diabetes mellitus, reactive airway disease, and hypertension. These co-morbid conditions may predispose a person to developing severe disease from COVID-19. (15,16) It is thought that type of work can also predispose a person to developing COVID-19 as having a job that's considered essential which cannot be performed remotely, or involves public interaction, can increase one's exposure to symptomatic and asymptomatic individuals and thus increase the risk of contracting COVID-19. (17) People in racial and ethnic diversity groups might be more likely to live in multigenerational homes, (18) crowded conditions and densely populated urban areas. Living under the same roof or in groups can make the viral mitigating factor of social distancing difficult. Some diverse populations might also have higher rates of uninsured people causing disparities in healthcare access. (18) Finally, stresses of racism itself can lead to early aging in diverse groups and could contribute to the development of severe disease from COVID-19. (19)

Within our communities, COVID-19 disproportionately affects people living in nursing homes. Often, this population has a high number of elderly, frail adults who are more likely to have a multitude of co-morbid conditions. (20) An inability to properly social distance, a lack of adequate ventilation at the beginning of the pandemic, and the higher likelihood of this population having a multitude of chronic conditions all contributed to the waves of COVID-related deaths we saw in nursing homes at different times during the pandemic. Without question, the nursing home population suffered tremendous death counts because of swift transmission of COVID-19 within this environment and an inability to adequately institute viral mitigating methods, especially early in the pandemic.

COVID-19 and Pregnancy:

While COVID-19 was deadly in the community, it was also thought that SARS-CoV-2 might also influence growing populations. Pregnant people infected with the SARS-CoV-2 could be asymptomatic or symptomatic. COVID-19 symptomatic pregnant people were found to be at increased risk for developing severe sequelae of COVID-19 compared with nonpregnant reproductive-aged females. (21) Most evidence suggested that pregnant women did not have different symptoms from the virus than non-pregnant individuals, but if they developed severe disease, the clinical course was

heightened and worsened. (21) Pregnant women symptomatic with COVID with severe disease were at increased risks for intensive care unit [ICU] admission, need for mechanical ventilation and ventilatory support, and death compared with nonpregnant females of the same age. (21)

Understanding the SARS-CoV-2 Virus

Understanding how to best attack and mitigate this virus requires an understanding of the molecular make-up of this novel coronavirus. Further, an understanding of how the virus binds and invades host cells is imperative to finding neutralizing targets.

Viral Make-up:

Coronaviruses in general, are spherical, enveloped viruses containing a helical symmetry nucleocapsid and a single-stranded RNA genome. (22, 23) SARS-CoV-2 shares approximately 80% genome sequence identity with SARS-CoV. (23) Like other coronaviruses, the SARS-CoV-2 RNA genome contains six functional open reading frames. These ORFs are arranged in order from 5' to 3': replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M) and nucleocapsid (N). (23) Another seven ORFs are scattered throughout the viral genome and likely code for accessory proteins. While many of these genes are similar to counterparts in other coronaviruses, (23) the S-gene, coding for the Spike protein, diverges and has a very different sequence. (24) As with attempts at therapeutics to neutralize previous coronaviruses, the spike protein

of SARS-CoV-2 has become the target for preventative and therapeutic options. (25) The S protein of SARS-CoV-2 is a class I fusion protein that protects its fusion domain. This protein keeps the binding domain hidden and inactive until the virus finds a host cell with an appropriate receptor, and targets that cell for infection. (25) The fusion protein is then enzymatically cleaved, revealing a hook-shaped structure that is necessary for viral binding and incorporation into the membrane of the target cell. (26, 27) It is suspected that nucleotide polymorphisms and mutations within the Spike protein alter viral transmissibility and virus-host cell interactions. (26) The speed and efficiency of these mutations is remarkable.

SARS-CoV-2 can rapidly mutate and alter its conformation in order to adapt to its current environment. (28) Mutations in the SARS-CoV-2 genome have allowed for changes in disease severity, viral transmission, and host immunity evasion. As such, containment attempts of SARS-CoV-2 have involved world-wide population vaccination coverage and physical mitigation methods (social distancing, hand washing, mask wearing) to control the spread of the virus. History will be able to determine the extent to which these methods were able to control viral transmission.

COVID-19 Receptor:

In humans, SARS-CoV-2 enters cells using the same receptor as SARS-CoV, a receptor known as angiotensin-converting enzyme 2 (ACE2). ACE2 is a type I membrane

glycoprotein responsible for the conversion of angiotensin II into angiotensin 1. This receptor is highly expressed in the lungs, nose, heart, intestine, and kidneys of humans. (29) The SARS-CoV-2 S-protein receptor binding domain (RBD), a 211 amino acid sequence, has been the target for neutralizing antibodies, both host and manufactured. By neutralizing the SARS-CoV-2 RBD, it is felt that one could effectively block the virus and prevent worsening of symptoms and progression of disease. Though similar to that of SARS-CoV, the RBD of SARS-CoV-2 functionally facilitates a much stronger interaction and tighter bond between itself and the host cell. (29)

SARS-CoV-2 Pathogenesis

There is a wide range of SARS-CoV-2 clinical pathologies in humans, spanning from asymptomatic infection to mild symptoms to severe respiratory failure and death. Asymptomatic infections have been well documented. (30,31) One review performed prior to the introduction of COVID-19 vaccination estimated that 33 percent of people with SARS-CoV-2 infection never develop symptoms. (31) Asymptomatic infections were a confounder early in the pandemic because it had been believed that only symptomatic individuals had viral loads high enough to transmit infection. This theory proved false, and as the pandemic thrived, asymptomatic spread of disease was a major contributor to community positivity rates. (31)

The original COVID-19 and several subsequent variants would bind to airway epithelial cells via the ACE2 receptor in the upper respiratory tract. Replicating and releasing from the upper airway cells, SARS-CoV-2 migrated into the lower airways. Here, SARS-CoV-2 would enter lung alveolar epithelial cells. (32) The rapid replication of SARS-CoV-2 in the alveoli triggers a strong immune response that precipitates a cytokine storm syndrome which ultimately leads to acute respiratory distress syndrome and respiratory failure, a principal cause of death in COVID-19 patients. (32) Though far from universal, older patients (>60 years) and those with serious pre-existing diseases (particularly obesity, pulmonary complications, and diabetes) have demonstrated a greater risk of progressing to severe COVID-19 disease and death.

All ages and genders are susceptible to SARS-CoV-2 infection. The median age of COVID-19 infection is around 50 years old, and clinical manifestations can differ dramatically with age. To date, 78% of hospitalizations and approximately 75% of all deaths related to COVID have occurred in the over 65 demographic. (33) Older men with co-morbidities tend to develop severe disease, whereas most young people and children have only mild symptoms (non-pneumonia or mild pneumonia), if they have any symptoms at all. When infected with COVID-19, the most common symptoms are fever, fatigue, and dry cough, with sputum production, headache, hemoptysis, diarrhea, anorexia, sore throat, chest pain, chills. Nausea and vomiting are somewhat

less common symptoms. (34) In the age of delta and omicron, a sore throat and persistent cough have been commonly described. (35) Self-reported olfactory and taste disorders were also reported by patients in Italy, and subsequently described world-wide. (36) Viral Incubation period was about 3-5 days as most people showed signs of viral infection on day 6-10 after exposure. Symptoms of dyspnea and pneumonia developed within a median time of 8 days from illness onset. (37)

The degree of SARS-CoV-2 contagiousness may be due to certain virologic features of SARS-CoV-2. Human-to-human transmission can occur through respiratory droplets containing SARS-CoV-2 particles and can spread by speech, coughing, or sneezing. (38) The recipient mucous membranes of the eyes, nose, or mouth are viral portals. Additionally, transmission can occur by direct contact with contaminated surfaces as the virus is able to survive outside of the host for at least several hours. (38,39, 40) Typically, respiratory viruses have the highest transmission rate from human to human while the infected person has viral symptoms. It is believed that the virus is at its peak during this time. COVID-19 is also thought to be most transmissible during the symptomatic period as well. (41) However, the possibility of viral transmission from an asymptomatic individual (44) exists as there is clear evidence of asymptomatic or presymptomatic spread of SARS-CoV-2 demonstrated by colonization and replication in a human throat during the initial infection. (42, 43) In fact, it is possible that

unknown and asymptomatic infections might account for approximately 80% of transmitted cases because of this high viral transmissibility during the asymptomatic period. (44) Asymptomatic period infectiousness, aerosolized particles, and viral survivability outside of the host likely contributes greatly to the rapid global spread of the virus. Public health mitigation interventions to reduce transmission have proven successful in China and several other countries, such as South Korea.

SARS-CoV-2 Diagnosis:

The COVID-19 pandemic has highlighted the imperative nature of prompt and accurate viral diagnosis. Currently, polymerase chain reaction (PCR) genomic amplification methods represent the gold standard. (45) PCR testing, while accurate is very expensive due to the complexity of the process and the required technical elements. Requirements for testing include PCR reaction reagents, expensive analysis machines, and experienced laboratory technicians. (45) Early in the pandemic, important supplies including nasopharyngeal swabs used for sample collection were in short supply greatly limiting the ability to test symptomatic people adequately and efficiently. An inability to adequately test patients in the first days of the pandemic likely contributed to the rapid transmission of the virus as people could not be isolated and contact tracing was not possible. Further complicating the testing deficiencies in the United States, PCR reaction reagents were lacking, and supply did not meet

demand. Eventually, as the supply of collection equipment and of PCR reagents production was increased, efficient testing was generally made widely available. Mass testing centers were set up throughout the country so that people could quickly be screened for COVID if they had contact with a known COVID patient or if they had symptoms of COVID.

PCR testing in developing countries has been particularly challenging. Economic disparities in developing and underdeveloped countries prevent effective mass PCR testing programs. As such, other methods are required to screen symptomatic people. Rapid antigen and antibody tests and immunoenzymatic serological tests represent became inexpensive to produce and easy to use. Rapid testing has become the most widely used technique for monitoring the spread of SARS-CoV-2 infection. These low-cost point-of-care tests enabled for effective viral surveillance systems. Still, in other countries where COVID tests (either Rapid or PCR tests) were in short supply other systems needed to be developed to screen patients. In the absence of diagnostic testing, it became necessary to consider the patient's symptoms, contact history, and also the patient's medical history. The integration of all of these elements provides a solid foundation for adequately diagnosing COVID-19 infection and effectively managing and containing the COVID-19 pandemic. (49)

Accurate and early COVID-19 diagnosis is crucial for controlling its spread. Testing 36-72 hours following a known exposure is recommended, especially if one is symptomatic and is at high risk for becoming symptomatic. False negative results can also arise when nasopharyngeal or oral swabs are improperly used by collection technicians, therefore multiple detection methods should be available to confirm a COVID-19 diagnosis. One such alternative method was chest CT. COVID-19 patients demonstrated typical CT features including bilateral multilobar ground-glass opacities with a peripheral or posterior distribution. Thus, it has been suggested that CT scanning combined with repeated swab tests should be used for individuals with high clinical suspicion of COVID-19 but are testing negative for the virus. (50)

With an understanding of SARS-CoV-2 from a molecular and pathogenic standpoint, and diagnostics and mitigation methods in place, the next frontier to conquer was therapeutics. Patients hospitalized were being treated symptomatically, although no therapeutics had been developed to combat the virus. The collaboration of private and public sectors was brought together in an effort known as WARP SPEED. The goal of WARP SPEED was to have these sectors work together to rapidly develop and trial therapeutics.

Operation Warp Speed

In response to the COVID-19 pandemic, the US government quickly activated Operation Warp Speed (OWS). OWS created a partnership between the Departments of Health and Human Services (HHS) and Defense (DOD) with the goal of accelerating the development of a COVID-19 vaccine. (57) This partnership decided the best strategy would be to employ different private companies to utilize different vaccine platforms to generate expeditious delivery of safe and effective vaccines. These companies also took steps, such as starting large-scale manufacturing during clinical trials and combining clinical trial phases or running them concurrently. Clinical trials gathered data on safety and efficacy, with more participants in each successive phase (e.g., phase 3 has more participants than phase 2). (57,58) Common to each of these companies was the compressed timelines. To meet OWS timelines, some vaccine companies relied on data from other vaccines using the same platforms, where available, or conducted certain animal studies at the same time as clinical trials. However, as is done in a non-pandemic environment, all vaccine companies gathered initial safety and antibody response data with a small number of participants before proceeding into large-scale human studies (e.g., phase 3 clinical trials). (57,58) There was no compromise of safety and effectiveness in the development of these vaccines.

Anti-COVID-19 Therapeutics:

When COVID-19 first came to the United States, we were ill prepared to treat the disease. Since then, convalescent plasma, monoclonal antibodies, intravenous antivirals, and oral antivirals have become common place for early intervention. The only late intervention therapy that has proven useful once a person is mechanically ventilated is steroid use.

Chloroquine and hydroxychloroquine

Though not a WARP SPEED initiative, anecdotal evidence from around the world suggested a potential use for the drugs, chloroquine and hydroxychloroquine. These antimalarial and immune altering medicines were thought to elicit a therapeutic benefit by inhibition of cellular entry by the virus. (59) These medicines are known to inhibit the glycosylation of cellular receptors and prevent, at least partially, virus–host receptor binding. Further, they can increase the endosomal pH to inhibit membrane fusion. (52) It is currently accepted that these medicines provide no clinical benefit to effectively treat patients with COVID-19. (51, 52) Even though some studies showed they can inhibit SARS-CoV-2 infection in vitro, clinically this has not proven to occur. Further, two clinical studies demonstrated no association with death rates in patients receiving chloroquine or hydroxychloroquine compared with those not receiving the drug. (52) On 15 June 2020, owing to the side effects observed in clinical trials, the US

Food and Drug Administration (FDA) revoked the emergency use authorization (EUA) for chloroquine and hydroxychloroquine as a treatment of COVID-19. (53)

Passive Immunity and Convalescent Plasma

Without effective treatments available to combat COVID-19, a group of scientists and physicians looked to a historical therapy that had been used to treat previous viral disease including measles and Ebola. This therapy relied on having donors who had recovered from COVID-19 and were likely to have levels of neutralizing antibodies in their plasma. Collecting this plasma and transfusing it into a sick person would potentially provide therapeutic benefit to the sick patient. (60)

Under the guidance of the Mayo Clinic Expanded Access Program, convalescent plasma could be collected and administered to hospitalized patients. The program was expanded to nearly 2900 hospitals in every state and several countries, and over 70,000 patients were transfused one or two units of plasma. While never designed to be a randomized clinical trial, the Mayo EAP became the largest trial testing the safety of convalescent plasma transfusions. (54) Several randomized controlled trials, including the large RECOVERY trial in the UK, (55) failed to establish survival benefit to transfused patients versus controls. Others have pointed to trial deficiencies to explain the lack of effect. First, convalescent plasma was usually administered to patients when they had already developed severe disease while hospitalized. Second, plasma

antibody titers were not adequately measured. More recently, high titer convalescent plasma was used to treat outpatients with COVID-19 in a randomized controlled trial. (56) Using convalescent plasma in this manner reduced hospitalizations by 50%. As convalescent plasma will be readily available and inexpensive to collect in any area where a COVID spike is occurring, the WHO should re-evaluate its position and allow convalescent plasma to be used in countries where monoclonal antibodies are not in plentiful supply.

Passive Immunity and Monoclonal Antibodies

Arising from the work done with convalescent plasma, and with knowledge from past experiences with viruses including Ebola, monoclonal antibodies were developed as therapeutic options for the treatment and potential prophylaxis for Covid-19. (61) Like convalescent plasma, monoclonal antibodies are a form of passive immunity where laboratory generated antibodies are targeted to specific epitopes on the virus. In the case of SARS-CoV-2, multiple antibodies were created to bind the viral spike protein to prevent viral binding and invasion of the host cell. In contrast with convalescent plasma, which consists of many antibodies contained in plasma, monoclonal antibodies are directed toward specific targets and selected specifically for their ability to neutralize the virus. (62, 63)

Bamlanivimab (Eli Lily) was the first monoclonal antibody given an emergency use authorization (EUA) by the FDA in November 2020. This single monoclonal antibody was approved to treat mild COVID-19 disease in outpatients who were at risk of developing severe disease and being hospitalized. Specifically, Bamlanivimab (Bam) is a neutralizing human IgG1κ monoclonal antibody. (64) In a clinical trial known as the BLAZE-1 trial in patients with mild-to-moderate COVID-19, this monoclonal antibody showed an effect of on reducing viral loads in treated patients versus untreated control patients. More important than the viral load reduction, however, was that the treated patients showed less emergency room visits and reduced hospitalizations compared to untreated control patients. (65) In the highest risk of developing severe disease group of patients (body mass index>35 or aged>65 years), there was a sharp contrast between the treated and untreated with treated patients showing pronounced reductions in emergency room visits and hospitalizations. (65)

Unfortunately, because of the rapid rate in which SARS-CoV-2 can mutate its spike protein, this monoclonal antibody became less effective as variants began to circulate. The large number of COVID-19 variants developed resistance to Bamlanivimab neutralization. This led the FDA and CDC to revoke the EUA for this monoclonal antibody as it was no longer useful in treatment against COVID-19 variants beta and gamma. (66) Bamlanivimab did demonstrate effectiveness against the Delta variant

when combined with a second monoclonal (Etesevimab) and its use was briefly resumed, but stopped again as it had little efficacy versus the Omicron variant. (64)

A second authorized monoclonal therapeutic consisted of the 2 monoclonal antibodies casirivimab and imdevimab and was developed by Regeneron. Like the Lily monoclonal, this novel monoclonal antibody cocktail had directed activity versus the SARS-CoV-2 spike protein and was distributed under the name REGEN-COV. This cocktail was trialed as both an infusion and a subcutaneous injection. (67) In November, 2020 the FDA issued an EUA for REGN-COV to be used in at risk patients with COVID-19 who currently had mild-moderate symptoms. In clinical trials, more than 4000 outpatients with mild-to-moderate COVID-19 and at least one risk factor for severe disease were randomized to receive either REGEN-COV or placebo. (68) The REGEN-COV treatment groups showed sharp reductions in COVID-19–related hospitalization or all-cause death, and a more rapid resolution of COVID-19 symptoms compared to placebo. The EUA was then amended to include use as a post-exposure prophylactic treatment as treated individuals demonstrated a profound decrease in developing symptomatic COVID-19 compared to untreated controls in clinical trial. In a post-exposure prophylaxis arm of the clinical trial, 1505 participants who lived in a household with a patient infected with COVID-19 but were asymptomatic and had a negative SARS-CoV-2 test. The treatment group demonstrated an 81% risk reduction in the development of PCR-confirmed COVID-19 infection through 29 days. (69) This

would be particularly important for nursing home patients, and others who live in close quarters with a person infected with COVID-19, as well as for COVID-19 negative immunocompromised people with a strong exposure to COVID.

Like the Lily monoclonal antibody, REGEN-COV proved less effective against arising COVID-19 variants and the EUA was eventually withdrawn for this treatment. Other monoclonal antibodies have been granted emergency use authorization by the FDA for use against various COVID-19 variants. One such monoclonal, sotrovimab was created from an antibody identified in 2003 in a survivor of severe acute respiratory syndrome (SARS). (70) This monoclonal, developed by Glaxo Smith Kline, showed initial effectiveness against Delta and Omicron variants in the COMET-ICE clinical trial. This trial, like others, demonstrated effectiveness in outpatients with COVID-19 experiencing mild-moderate symptoms, but who were at risk of developing severe disease. Ultimately, the SARS-CoV-2 variants evaded this monoclonal, and the emergency use authorization was withdrawn. Finally, Astra Zeneca has developed a 2 monoclonal antibody cocktail called Evusheld that is being trialed as a prophylaxis drug for immune compromised patients who are either resistant to a COVID-19 vaccine, or unable to mount a robust antibody response to the vaccine. It is thought that this passive immunity can provide up to 6 months of protection against developing symptomatic COVID-19. (71)

Dexamethasone

Treatments for patients with severe disease from COVID-19 requiring high flow oxygen or mechanical ventilation have been a challenge. Neither convalescent plasma nor monoclonal antibodies demonstrated any survival benefit to patients with this late stage COVID. Further, neither treatment demonstrated a reduction in days on the ventilator or days hospitalized once the virus had caused severe disease. With treatments limited, physicians looked to dexamethasone, a corticosteroid that suppresses the immune response. It was felt that by suppressing the cytokine storm, dexamethasone could provide benefit. (72) A large clinical trial known as the RECOVERY trial in the United Kingdom demonstrated that when patients with severe COVID were treated with dexamethasone (6 mg once daily for up to 10 days), they saw a reduction in 28-day mortality. No benefit was observed in patients not requiring oxygen. This trial changed clinical practice which had dictated that corticosteroids were contraindicated and re-wrote the clinical treatment guidelines so that COVID-19 patients requiring supplemental oxygen could be administered dexamethasone. (73)

Anti-virals:

Until recently, Remdesivir had been the only antiviral medicine approved by the FDA to be used in COVID-19 patients. Two large randomized clinical trials have been performed using Remdesivir, a study carried out by the VA and the ACTT-1 Trial. The

ACTT-1 trial found that IV Remdesivir provided no survival benefit, but patients who recovered from COVID were able to be discharged earlier from the hospital. (74) The VA study also showed no survival benefit, but demonstrated that patients stayed in the hospital longer than untreated patients. It has been speculated that the VA study did not follow the same protocol as the ACTT-1 trial, specifically that patients stayed in the hospital for the entire length of their Remdesivir course. (75) In ACTT-1, patients were discharged from the trial when they met the trial endpoint, no further infusions were administered. This detail may have not been properly communicated and could explain the longer hospital stays observed in the VA trial.

An oral anti-viral medicine known as Paxlovid has just come to market. This oral pill is administered to a person with very early COVID-19 twice per day for 5 days. Paxlovid has many medication interactions with other medicines, so its use is limited to people with no contraindications. (75) The clinical trial investigating Paxlovid in outpatients with risk factors for developing severe disease, demonstrated a marked reduction in hospitalizations (70%) compared to untreated control patients, and no deaths. More recently, several patients have found themselves with rebound COVID infections following their 5-day course of Paxlovid. This is a phenomenon that requires further study. (76, 77)

Active Immunity and Vaccines

As soon as COVID-19 was declared a public health emergency and classified as a pandemic, an intense world-wide effort was undertaken to develop safe and effective COVID-19 vaccines. Vaccines generated were from several different “classes” however their general principle was all the same – to force the human immune system to generate anti-COVID antibodies in the absence of active infection. The antibodies, then, would serve to survey and protect a person should they contract the virus. The development of the vaccines had to be expeditious, and the clinical trials had to be incredibly efficient in order to deliver these too naive populations within a finite timeline.

mRNA Vaccines

Scientists have developed a new type of vaccine that uses a genetic molecule called messenger RNA (mRNA) that codes for a portion of the virus rather than a part of an actual bacteria or virus. These novel vaccines had been developed for other viruses but had never been manufactured on a large scale. Their premise is to use mRNA, a genetic molecule necessary for protein production. In essence, these vaccines are delivering a genetic blueprint to human cells to make a protein designated by the mRNA and then the body develops antibodies against the produced protein. Once cells finish making a protein, the mRNA quickly breaks down by cellular enzymes. mRNA from vaccines does not enter a cell’s nucleus and does not alter native DNA.

Pfizer Vaccine

This mRNA vaccine was the first COVID-19 vaccine to receive emergency use authorization. Like other mRNA vaccines, it has an mRNA message to build the spike protein. This mRNA is enclosed within a lipid envelope. A US clinical trial involving 43,548 participants, funded by BioNTech and Pfizer, demonstrated that this vaccine (2 doses, 3 weeks apart) was very effective in preventing symptomatic COVID-19. In fact, 95% of individuals administered the vaccine did not contract COVID. (78) A clinical trial was also performed in Israel with 1.2 million people and the efficacy of the vaccine was confirmed. (80) Side effects were minimal, usually consisting of fatigue, arm soreness, headache, and/or fever. A rare but significant side effect following vaccination can include myocarditis (inflammation of the heart muscle) and pericarditis (inflammation of the tissue surrounding the heart). (79) These side effects are more likely following the second dose of the vaccine and occur more commonly in teenage males. As immunity begins to wane somewhere around 5-6 months post dose two, it is recommended that a third shot be administered as a booster dose. This has shown in studies to restore immunity back to the high efficacy level seen after the second shot. Especially in people with compromised immune systems, a fourth shot is recommended as a second booster as well.

More recently, the Pfizer mRNA vaccine has been approved for children ages 5 to 11 years old. The side effect profile is like that in adults, although the incidence of myocarditis appears much lower. Overall efficacy is similar to what is seen in adults, despite the dose being approximately 1/3 the dose that the adult receives. Again, waning immunity after 5-6 months has prompted to recommendation to issue a booster shot at that time. (81)

Moderna mRNA Vaccine

The second COVID-19 vaccine to be granted emergency use authorization by the FDA and CDC also used an mRNA platform and came from Moderna. Though it has a very similar makeup to the Pfizer vaccine, they differ in the amount of mRNA (30 mcg Pfizer vs 100 mcg Moderna) and the lipid envelope is manufactured using slightly different lipids. In a US clinical phase 2/3 trial involving over 30,000 individuals, only eleven developed symptomatic COVID-19 following 2 doses 1 month apart. (82) The vaccine had a 94% efficacy rate and was 100% effective against severe illness, hospitalization, and death from COVID-19. (82) The trial population included more than 7000 people over the age of 65, and another 5000 who were 65 or younger, but had co-morbidities that placed them in a group most at risk of developing severe COVID. Paramount to the success of these clinical trials to be completed in the contracted timeline was the need to ensure that all ages, genders, and diversity of communities were appropriately

represented and reflected the US population. Though the Moderna was believed to have longer lasting immunity compared to the Pfizer vaccine, antibody levels did begin to wane after approximately 6 months and a booster was recommended.

The Moderna vaccine clinical trial for children, ages 11-17 had been completed in the US, but no EUA was initially authorized because of concerns of myocarditis and pericarditis, in especially teenage males. Data has now been collected world-wide and Moderna was deemed safe and effective in a unanimous vote by the FDA advisory committee on June 14, 2020. Overall, the incidence of myocarditis in this age group is very rare, but a warning has been attached to the vaccine label listing this as a potential adverse effect, especially after the second shot. (79)

Johnson and Johnson adenoviral vaccine

The J&J/Janssen COVID-19 vaccine contains a piece of a modified SARS-CoV-2 viral DNA coding for the COVID Spike protein. The vector adenovirus, which typically causes the common cold, has been modified so that it cannot reproduce itself and thus is non-pathogenic. This adenovirus vector serves as a shuttle to move the spike protein DNA into cells where the cells then utilize their own machinery to synthesize the spikes. (83) The body then generates antibodies to these spike proteins to produce both acute and lasting immune responses to deter symptomatic COVID. The Johnson and Johnson vaccine had a lower efficacy rate for preventing symptomatic COVID when compared

to the mRNA vaccines (74-78%) but this was billed as a one shot vaccine. (83) Real life data from this vaccine uncovered a rare but significant side effect where people developed blood clots with diminished platelet counts, and the FDA has now limited its use in favor of the mRNA vaccines. Further waning immunity after 5-6 months suggested the need for a booster to restore immunity levels and the FDA and CDC issued a statement that receiving an mRNA booster would be recommended.

Conclusions

While there is plenty of room for criticism around the response by the US to the COVID-19 pandemic, much was accomplished in a relatively short period of time. Beginning with the genomic sequence published by the Chinese prior to COVID invading every country on earth, the US had already begun to understand and characterize the pathogen. In relatively quick fashion, the response included an understanding of the virus itself, development of testing to be able to provide early and efficient viral diagnoses, development of effective treatments to help prevent people from dying from severe COVID, development of treatments to help prevent disease progression and hospitalizations, development of treatments to act as prophylaxis agents in close contacts, and finally the development of safe and effective vaccines to provide the world population immunity to protect them from severe disease, hospitalization, and death from COVID-19. The work continues, however, as

evolving COVID-19 variants continue to find ways to evade immunity. Potential areas for future research include development of newer vaccines with variant mRNA "blueprints" to better fight off infection and newer treatments, both anti-viral medications and monoclonal antibodies to continue to treat people sick from COVID-19. Highlighted in this plan and in the future directions is the need to continue to be able to perform large and efficient clinical trials accurately representing the population of the US so that we can build the best treatments, build the best preventatives, and develop the best vaccines to keep all people, young and old, safe, and virus-free.

Clinics Care Points

References

1. Holmes EC, Goldstein SA, Rasmussen AL, Robertson DL, Crits-Christoph A, Wertheim JO, Anthony SJ, Barclay WS, Boni MF, Doherty PC, Farrar J, Geoghegan JL, Jiang X, Leibowitz JL, Neil SJD, Skern T, Weiss SR, Worobey M, Andersen KG, Garry RF, Rambaut A. The origins of SARS-CoV-2: A critical review. Cell. 2021 Sep 16;184(19):4848-4856. doi: 10.1016/j.cell.2021.08.017. Epub 2021 Aug 19. PMID: 34480864; PMCID: PMC8373617.
2. https://www.cdc.gov/coronavirus/2019-ncov/index.html
3. Sharun K, Dhama K, Pawde AM, Gortázar C, Tiwari R, Bonilla-Aldana DK, Rodriguez-Morales AJ, de la Fuente J, Michalak I, Attia YA. SARS-CoV-2 in animals: potential for unknown reservoir hosts and public health implications. Vet Q. 2021 Dec;41(1):181-201. doi: 10.1080/01652176.2021.1921311. PMID: 33892621; PMCID: PMC8128218.
4. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ. Coronavirus Disease 2019-COVID-19. Clin Microbiol Rev. 2020 Jun 24;33(4):e00028-20. doi: 10.1128/CMR.00028-20. PMID: 32580969; PMCID: PMC7405836.
5. Sharma A, Ahmad Farouk I, Lal SK. COVID-19: A Review on the Novel Coronavirus Disease Evolution, Transmission, Detection, Control and Prevention. Viruses. 2021 Jan 29;13(2):202. doi: 10.3390/v13020202. PMID: 33572857; PMCID: PMC7911532.

6. Drosten, C., Günther, S., Preiser, W., Werf, S., Brodt, H. R., Becker, S., et al. (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *Engl. J. Med.* 348, 1967–1976. doi: 10.1056/NEJMoa030747.

7. Lau, L. T., Fung, Y. W. W., Wong, F. P. F., Lin, S. S. W., Wang, C. R., Li, H. L., et al. (2003). A real-time PCR for SARS-coronavirus incorporating target gene pre-amplification. *Biochem. Biophys. Res. Commun.* 312, 1290–1296. doi: 10.1016/j.bbrc.2003.11.064.

8. Zaki, A. M., Van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D., and Fouchier, R. A. (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med.* 367, 1814–1820. doi: 10.1056/NEJMoa1211721.

9. Song, H. D., Tu, C. C., Zhang, G. W., Wang, S. Y., Zheng, K., Lei, L. C., et al. (2005). Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. *Proc. Natl. Acad. Sci. U.S.A.* 102, 2430–2435. doi: 10.1073/pnas.0409608102.

10. Azhar, E. I., El-Kafrawy, S. A., Farraj, S. A., Hassan, A. M., Al-Saeed, M. S., Hashem, A. M., et al. (2014). Evidence for Camel-to-Human Transmission of MERS Coronavirus. *N. Engl. J. Med.* 370, 2499–2505. doi: 10.1056/NEJMoa1401505.

11. Liu K, Pan X, Li L, Yu F, Zheng A, Du P, Han P, Meng Y, Zhang Y, Wu L, Chen Q, Song C, Jia Y, Niu S, Lu D, Qiao C, Chen Z, Ma D, Ma X, Tan S, Zhao X, Qi J, Gao GF, Wang Q. Binding and molecular basis of the bat coronavirus RaTG13 virus to ACE2 in humans and other species. Cell. 2021 Jun 24;184(13):3438-3451.e10. doi:

10.1016/j.cell.2021.05.031. Epub 2021 May 24. PMID: 34139177; PMCID: PMC8142884.

12. Allam Z. The First 50 days of COVID-19: A Detailed Chronological Timeline and Extensive Review of Literature Documenting the Pandemic. Surveying the Covid-19 Pandemic and its Implications. 2020:1–7. doi: 10.1016/B978-0-12-824313-8.00001-2. Epub 2020 Jul 24. PMCID: PMC7378494.
13. Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Front Microbiol. 2020 Jul 22;11:1800. doi: 10.3389/fmicb.2020.01800. PMID: 32793182; PMCID: PMC7387429.
14. Rahman S, Montero MTV, Rowe K, Kirton R, Kunik F Jr. Epidemiology, pathogenesis, clinical presentations, diagnosis and treatment of COVID-19: a review of current evidence. Expert Rev Clin Pharmacol. 2021 May;14(5):601-621. doi: 10.1080/17512433.2021.1902303. Epub 2021 May 3. PMID: 33705239; PMCID: PMC8095162.
15. Bambra C, Riordan R, Ford J, Matthews F. The COVID-19 pandemic and health inequalities. J Epidemiol Community Health. 2020 Nov;74(11):964-968. doi: 10.1136/jech-2020-214401. Epub 2020 Jun 13. PMID: 32535550; PMCID: PMC7298201.
16. Zhang JY, Shang T, Ahn D, Chen K, Coté G, Espinoza J, Mendez CE, Spanakis EK, Thompson B, Wallia A, Wisk LE, Kerr D, Klonoff DC. How to Best Protect People With Diabetes From the Impact of SARS-CoV-2: Report of the International COVID-19 and Diabetes Summit. J Diabetes Sci Technol. 2021 Mar;15(2):478-514. doi:

10.1177/1932296820978399. Epub 2021 Jan 21. PMID: 33476193; PMCID: PMC7925443.

17. The Lancet. The plight of essential workers during the COVID-19 pandemic. Lancet. 2020 May 23;395(10237):1587. doi: 10.1016/S0140-6736(20)31200-9. PMID: 32446399; PMCID: PMC7241973.

18. Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, Agha M, Agha R. The socio-economic implications of the coronavirus pandemic (COVID-19): A review. Int J Surg. 2020 Jun;78:185-193. doi: 10.1016/j.ijsu.2020.04.018. Epub 2020 Apr 17. PMID: 32305533; PMCID: PMC7162753.

19. Devakumar D, Shannon G, Bhopal SS, Abubakar I. Racism and discrimination in COVID-19 responses. Lancet. 2020 Apr 11;395(10231):1194. doi: 10.1016/S0140-6736(20)30792-3. Epub 2020 Apr 1. PMID: 32246915; PMCID: PMC7146645.

20. Dykgraaf SH, Matenge S, Desborough J, Sturgiss E, Dut G, Roberts L, McMillan A, Kidd M. Protecting Nursing Homes and Long-Term Care Facilities From COVID-19: A Rapid Review of International Evidence. J Am Med Dir Assoc. 2021 Oct;22(10):1969-1988. doi: 10.1016/j.jamda.2021.07.027. Epub 2021 Aug 3. PMID: 34428466; PMCID: PMC8328566.

21. Di Mascio D, Buca D, Berghella V, Khalil A, Rizzo G, Odibo A, Saccone G, Galindo A, Liberati M, D'Antonio F. Counseling in maternal-fetal medicine: SARS-CoV-2 infection in pregnancy. Ultrasound Obstet Gynecol. 2021 May;57(5):687-697. doi: 10.1002/uog.23628. PMID: 33724545; PMCID: PMC8251147.

22. Pal M, Berhanu G, Desalegn C, Kandi V. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. Cureus. 2020 Mar 26;12(3):e7423. doi: 10.7759/cureus.7423. PMID: 32337143; PMCID: PMC7182166.

23. Das A, Ahmed R, Akhtar S, Begum K, Banu S. An overview of basic molecular biology of SARS-CoV-2 and current COVID-19 prevention strategies. Gene Rep. 2021 Jun;23:101122. doi: 10.1016/j.genrep.2021.101122. Epub 2021 Apr 1. PMID: 33821222; PMCID: PMC8012276.

24. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV--a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009 Mar;7(3):226-36. doi: 10.1038/nrmicro2090. Epub 2009 Feb 9. PMID: 19198616; PMCID: PMC2750777.

25. Zhu C, He G, Yin Q, Zeng L, Ye X, Shi Y, Xu W. Molecular biology of the SARs-CoV-2 spike protein: A review of current knowledge. J Med Virol. 2021 Oct;93(10):5729-5741. doi: 10.1002/jmv.27132. Epub 2021 Jun 14. PMID: 34125455; PMCID: PMC8427004.

26. Li, L., and Petrovsky, N. (2016). Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert Rev. Vaccines* 15, 313–329. doi: 10.1586/14760584.2016.1124762.

27. Peacock TP, Goldhill DH, Zhou J, Baillon L, Frise R, Swann OC, Kugathasan R, Penn R, Brown JC, Sanchez-David RY, Braga L, Williamson MK, Hassard JA, Staller E, Hanley B, Osborn M, Giacca M, Davidson AD, Matthews DA, Barclay WS. The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. Nat

Microbiol. 2021 Jul;6(7):899-909. doi: 10.1038/s41564-021-00908-w. Epub 2021 Apr 27. PMID: 33907312.

28. Guruprasad L. Human SARS CoV-2 spike protein mutations. Proteins. 2021 May;89(5):569-576. doi: 10.1002/prot.26042. Epub 2021 Jan 17. PMID: 33423311; PMCID: PMC8014176.

29. Yan, R., Zhang, Y., Li, Y., Xia, L., Gou, Y., and Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 360, 1444–1448.

30. Oran DP, Topol EJ. Prevalence of Asymptomatic SARS-CoV-2 Infection : A Narrative Review. Ann Intern Med. 2020 Sep 1;173(5):362-367. doi: 10.7326/M20-3012. Epub 2020 Jun 3. PMID: 32491919; PMCID: PMC7281624.

31. Oran DP, Topol EJ. The Proportion of SARS-CoV-2 Infections That Are Asymptomatic : A Systematic Review. Ann Intern Med. 2021 May;174(5):655-662. doi: 10.7326/M20-6976. Epub 2021 Jan 22. PMID: 33481642; PMCID: PMC7839426.

32. Du L, Bouzidi MS, Gala A, Deiter F, Billaud JN, Yeung ST, Dabral P, Jin J, Simmons G, Dossani Z, Niki T, Ndhlovu LC, Greenland JR, Pillai SK. Human Galectin-9 Potently Enhances SARS-CoV-2 Replication and Inflammation in Airway Epithelial Cells. bioRxiv [Preprint]. 2022 May 16:2022.03.18.484956. doi: 10.1101/2022.03.18.484956. PMID: 35378763; PMCID: PMC8978940.

33. https://www.cdc.gov/coronavirus/2019-ncov/covid-data/investigations-discovery/hospitalization-death-by-age.html.

34. Alimohamadi Y, Sepandi M, Taghdir M, Hosamirudsari H. Determine the most common clinical symptoms in COVID-19 patients: a systematic review and meta-

analysis. J Prev Med Hyg. 2020 Oct 6;61(3):E304-E312. doi: 10.15167/2421-4248/jpmh2020.61.3.1530. PMID: 33150219; PMCID: PMC7595075.

35. https://www.deseret.com/coronavirus/2022/3/11/22972900/new-covid-variant-delta-omicron-symptoms-coronavirus.

36. Giacomelli A, Pezzati L, Conti F, Bernacchia D, Siano M, Oreni L. Self-reported olfactory and taste disorders in SARS-CoV-2 patients: a cross-sectional study [published online ahead of print, 2020 Mar 26]. Clin Infect Dis. 2020;71:889-90.

37. Elias C, Sekri A, Leblanc P, Cucherat M, Vanhems P. The incubation period of COVID-19: A meta-analysis. Int J Infect Dis. 2021 Mar;104:708-710. doi: 10.1016/j.ijid.2021.01.069. Epub 2021 Feb 3. PMID: 33548553; PMCID: PMC7857041.

38. Doremalen, N., Bushmaker, T., Morris, D., Holbrook, M., Gamble, A., Williamson, B., et al. (2020). Aerosol and surface stability of HCoV-19 (SARS-CoV-2) compared to SARS-CoV-1. *Engl. J. Med.* 382, 1564–1567. doi: 10.1056/NEJMc2004973.

39. Fan, J., Liu, X., Pan, W., Douglas, M. W., and Bao, S. (2020). Epidemiology of coronavirus disease in gansu province, China, 2020. *Emerg. Infect. Dis.* 26, 1257–1265. doi: 10.3201/eid2606.20025.

40. Xiang Ong, S. W., Tan, Y. K., Chia, P. W., Lee, T. H., Ng, O. K., Wong, M. S. Y., et al. (2020). Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. *JAMA* 323, 1610–1612. doi: 10.1001/jama.2020.3227.

41. Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/S0140-6736(20)30566-3.

42. Arons, M. M., Hatfield, K. M., Reddy, S. C., Kimball, A., James, A., Jacobs, J. R., et al. (2020). Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. *N. Engl. J. Med.* 382, 2081–2090. doi: 10.1056/NEJMoa2008457.

43. Pan, Y., Zhang, D., Yang, P., Poon, L. L. M., and Wang, Q. (2020). Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect. Dis.* 20, 411–412. doi: 10.1016/S1473-3099(20)30113-4.

44. Bai, Y., Yao, L., Wei, T., Tian, F., Jin, D., Chen, L., et al. (2020). Presumed asymptomatic carrier transmission of COVID-19. *J. Am. Med. Assoc.* 323, 1406–1407. doi: 10.1001/jama.2020.2565.

45. Murad D, Chandrasekaran S, Pillai A, Garner OB, Denny CT. SARS-CoV-2 Infection Detection by PCR and Serologic Testing in Clinical Practice. J Clin Microbiol. 2021 Jun 18;59(7):e0043121. doi: 10.1128/JCM.00431-21. Epub 2021 Jun 18. PMID: 33903168; PMCID: PMC8218740.

46. Safiabadi Tali SH, LeBlanc JJ, Sadiq Z, Oyewunmi OD, Camargo C, Nikpour B, Armanfard N, Sagan SM, Jahanshahi-Anbuhi S. Tools and Techniques for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)/COVID-19 Detection. Clin Microbiol Rev. 2021 May 12;34(3):e00228-20. doi: 10.1128/CMR.00228-20. PMID: 33980687; PMCID: PMC8142517.

47. https://www.washingtonpost.com/investigations/2020/04/18/timeline-coronavirus-testing/

48. Adeel M, Farooq T, Shakoor N, Ahmar S, Fiaz S, White JC, Gardea-Torresdey JL, Mora-Poblete F, Rui Y. COVID-19 and Nanoscience in the Developing World: Rapid Detection and Remediation in Wastewater. Nanomaterials (Basel). 2021 Apr 12;11(4):991. doi: 10.3390/nano11040991. PMID: 33921482; PMCID: PMC8069490.

49. Kabir MA, Ahmed R, Iqbal SMA, Chowdhury R, Paulmurugan R, Demirci U, Asghar W. Diagnosis for COVID-19: current status and future prospects. Expert Rev Mol Diagn. 2021 Mar;21(3):269-288. doi: 10.1080/14737159.2021.1894930. Epub 2021 Mar 7. PMID: 33621145; PMCID: PMC7938658.

50. Jalaber C, Lapotre T, Morcet-Delattre T, Ribet F, Jouneau S, Lederlin M. Chest CT in COVID-19 pneumonia: A review of current knowledge. Diagn Interv Imaging. 2020 Jul-Aug;101(7-8):431-437. doi: 10.1016/j.diii.2020.06.001. Epub 2020 Jun 11. PMID: 32571748; PMCID: PMC7287482.

51. Gupta A, Malviya A. Chloroquine and hydroxychloroquine for COVID-19: time to close the chapter. *Postgraduate Medical Journal* 2021;**97:**676-677.

52. Singh B, Ryan H, Kredo T, Chaplin M, Fletcher T. Chloroquine or hydroxychloroquine for prevention and treatment of COVID-19. Cochrane Database of Systematic Reviews 2021, Issue 2. Art. No.: CD013587. DOI: 10.1002/14651858.CD013587.pub2. Accessed 14 June 2022.

53. https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital-setting-or.

54. Joyner MJ, Bruno KA, Klassen SA, Kunze KL, Johnson PW, Lesser ER, Wiggins CC, Senefeld JW, Klompas AM, Hodge DO, Shepherd JRA, Rea RF, Whelan ER, Clayburn AJ, Spiegel MR, Baker SE, Larson KF, Ripoll JG, Andersen KJ, Buras MR, Vogt MNP, Herasevich V, Dennis JJ, Regimbal RJ, Bauer PR, Blair JE, van Buskirk CM, Winters JL, Stubbs JR, van Helmond N, Butterfield BP, Sexton MA, Diaz Soto JC, Paneth NS, Verdun NC, Marks P, Casadevall A, Fairweather D, Carter RE, Wright RS. Safety Update: COVID-19 Convalescent Plasma in 20,000 Hospitalized Patients. Mayo Clin Proc. 2020 Sep;95(9):1888-1897. doi: 10.1016/j.mayocp.2020.06.028. Epub 2020 Jul 19. PMID: 32861333; PMCID: PMC7368917.

55. RECOVERY Collaborative Group. Convalescent plasma in patients admitted to hospital with COVID-19 (RECOVERY): a randomised controlled, open-label, platform trial. Lancet. 2021 May 29;397(10289):2049-2059. doi: 10.1016/S0140-6736(21)00897-7. Epub 2021 May 14. PMID: 34000257; PMCID: PMC8121538.

56. Sullivan DJ, Gebo KA, Shoham S, Bloch EM, Lau B, Shenoy AG, Mosnaim GS, Gniadek TJ, Fukuta Y, Patel B, Heath SL, Levine AC, Meisenberg BR, Spivak ES, Anjan S, Huaman MA, Blair JE, Currier JS, Paxton JH, Gerber JM, Petrini JR, Broderick PB, Rausch W, Cordisco ME, Hammel J, Greenblatt B, Cluzet VC, Cruser D, Oei K, Abinante M, Hammitt LL, Sutcliffe CG, Forthal DN, Zand MS, Cachay ER, Raval JS, Kassaye SG, Foster EC, Roth M, Marshall CE, Yarava A, Lane K, McBee NA, Gawad AL, Karlen N, Singh A, Ford DE, Jabs DA, Appel LJ, Shade DM, Ehrhardt S, Baksh SN, Laeyendecker O, Pekosz A, Klein SL, Casadevall A, Tobian AAR, Hanley DF. Randomized Controlled Trial of Early Outpatient COVID-19 Treatment with High-

Titer Convalescent Plasma. medRxiv [Preprint]. 2021 Dec 21:2021.12.10.21267485. doi: 10.1101/2021.12.10.21267485. PMID: 34981068; PMCID: PMC8722611.

57. Lancet Commission on COVID-19 Vaccines and Therapeutics Task Force Members. Operation Warp Speed: implications for global vaccine security. Lancet Glob Health. 2021 Jul;9(7):e1017-e1021. doi: 10.1016/S2214-109X(21)00140-6. Epub 2021 Mar 26. PMID: 33780663; PMCID: PMC7997645.

58. McDougle L. Ensuring Safety of Operation Warp Speed Vaccines for COVID-19. J Natl Med Assoc. 2020 Oct;112(5):446-447. doi: 10.1016/j.jnma.2020.10.003. PMID: 33292930; PMCID: PMC7718775.

59. Hernandez AV, Roman YM, Pasupuleti V, Barboza JJ, White CM. Hydroxychloroquine or Chloroquine for Treatment or Prophylaxis of COVID-19: A Living Systematic Review. Ann Intern Med. 2020 Aug 18;173(4):287-296. doi: 10.7326/M20-2496. Epub 2020 May 27. PMID: 32459529.

60. Brown BL, McCullough J. Treatment for emerging viruses: Convalescent plasma and COVID-19. Transfus Apher Sci. 2020 Jun;59(3):102790. doi: 10.1016/j.transci.2020.102790. Epub 2020 Apr 20. PMID: 32345485; PMCID: PMC7194745.

61. Nelson PN, Reynolds GM, Waldron EE, Ward E, Giannopoulos K, Murray PG. Monoclonal antibodies. Mol Pathol. 2000 Jun;53(3):111-7. doi: 10.1136/mp.53.3.111. PMID: 10897328; PMCID: PMC1186915.

62. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV--a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009 Mar;7(3):226-36.

doi: 10.1038/nrmicro2090. Epub 2009 Feb 9. PMID: 19198616; PMCID: PMC2750777.

63. Hussain A, Hasan A, Nejadi Babadaei MM, Bloukh SH, Chowdhury MEH, Sharifi M, Haghighat S, Falahati M. Targeting SARS-CoV2 Spike Protein Receptor Binding Domain by Therapeutic Antibodies. Biomed Pharmacother. 2020 Oct;130:110559. doi: 10.1016/j.biopha.2020.110559. Epub 2020 Aug 1. PMID: 32768882; PMCID: PMC7395593.

64. Nathan R, Shawa I, De La Torre I, Pustizzi JM, Haustrup N, Patel DR, Huhn G. A Narrative Review of the Clinical Practicalities of Bamlanivimab and Etesevimab Antibody Therapies for SARS-CoV-2. Infect Dis Ther. 2021 Dec;10(4):1933-1947. doi: 10.1007/s40121-021-00515-6. Epub 2021 Aug 10. PMID: 34374951; PMCID: PMC8353431.

65. Gottlieb RL, Nirula A, Chen P, Boscia J, Heller B, Morris J, Huhn G, Cardona J, Mocherla B, Stosor V, Shawa I, Kumar P, Adams AC, Van Naarden J, Custer KL, Durante M, Oakley G, Schade AE, Holzer TR, Ebert PJ, Higgs RE, Kallewaard NL, Sabo J, Patel DR, Klekotka P, Shen L, Skovronsky DM. Effect of Bamlanivimab as Monotherapy or in Combination With Etesevimab on Viral Load in Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. JAMA. 2021 Feb 16;325(7):632-644. doi: 10.1001/jama.2021.0202. PMID: 33475701; PMCID: PMC7821080.

66. Hemmer CJ, Löbermann M, Reisinger EC. COVID-19: Epidemiologie und Mutationen : Ein Update [COVID-19: epidemiology and mutations : An update]. Radiologe. 2021

Oct;61(10):880-887. German. doi: 10.1007/s00117-021-00909-0. Epub 2021 Sep 20. PMID: 34542699; PMCID: PMC8450702.

67. Kreuzberger N, Hirsch C, Chai KL, Tomlinson E, Khosravi Z, Popp M, Neidhardt M, Piechotta V, Salomon S, Valk SJ, Monsef I, Schmaderer C, Wood EM, So-Osman C, Roberts DJ, McQuilten Z, Estcourt LJ, Skoetz N. SARS-CoV-2-neutralising monoclonal antibodies for treatment of COVID-19. Cochrane Database Syst Rev. 2021 Sep 2;9(9):CD013825. doi: 10.1002/14651858.CD013825.pub2. PMID: 34473343; PMCID: PMC8411904.

68. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, Xiao J, Hooper AT, Hamilton JD, Musser BJ, Rofail D, Hussein M, Im J, Atmodjo DY, Perry C, Pan C, Mahmood A, Hosain R, Davis JD, Turner KC, Baum A, Kyratsous CA, Kim Y, Cook A, Kampman W, Roque-Guerrero L, Acloque G, Aazami H, Cannon K, Simón-Campos JA, Bocchini JA, Kowal B, DiCioccio AT, Soo Y, Geba GP, Stahl N, Lipsich L, Braunstein N, Herman G, Yancopoulos GD; Trial Investigators. REGEN-COV Antibody Combination and Outcomes in Outpatients with Covid-19. N Engl J Med. 2021 Dec 2;385(23):e81. doi: 10.1056/NEJMoa2108163. Epub 2021 Sep 29. PMID: 34587383; PMCID: PMC8522800.

69. O'Brien MP, Forleo-Neto E, Musser BJ, Isa F, Chan KC, Sarkar N, Bar KJ, Barnabas RV, Barouch DH, Cohen MS, Hurt CB, Burwen DR, Marovich MA, Hou P, Heirman I, Davis JD, Turner KC, Ramesh D, Mahmood A, Hooper AT, Hamilton JD, Kim Y, Purcell LA, Baum A, Kyratsous CA, Krainson J, Perez-Perez R, Mohseni R, Kowal B, DiCioccio AT, Stahl N, Lipsich L, Braunstein N, Herman G, Yancopoulos GD, Weinreich DM; Covid-

19 Phase 3 Prevention Trial Team. Subcutaneous REGEN-COV Antibody Combination to Prevent Covid-19. N Engl J Med. 2021 Sep 23;385(13):1184-1195. doi: 10.1056/NEJMoa2109682. Epub 2021 Aug 4. PMID: 34347950; PMCID: PMC8362593.

70. Gupta A, Gonzalez-Rojas Y, Juarez E, Crespo Casal M, Moya J, Rodrigues Falci D, Sarkis E, Solis J, Zheng H, Scott N, Cathcart AL, Parra S, Sager JE, Austin D, Peppercorn A, Alexander E, Yeh WW, Brinson C, Aldinger M, Shapiro AE; COMET-ICE Investigators. Effect of Sotrovimab on Hospitalization or Death Among High-risk Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. JAMA. 2022 Apr 5;327(13):1236-1246. doi: 10.1001/jama.2022.2832. PMID: 35285853; PMCID: PMC8922199.

71. Levin MJ, Ustianowski A, De Wit S, Launay O, Avila M, Templeton A, Yuan Y, Seegobin S, Ellery A, Levinson DJ, Ambery P, Arends RH, Beavon R, Dey K, Garbes P, Kelly EJ, Koh GCKW, Near KA, Padilla KW, Psachoulia K, Sharbaugh A, Streicher K, Pangalos MN, Esser MT; PROVENT Study Group. Intramuscular AZD7442 (Tixagevimab-Cilgavimab) for Prevention of Covid-19. N Engl J Med. 2022 Jun 9;386(23):2188-2200. doi: 10.1056/NEJMoa2116620. Epub 2022 Apr 20. PMID: 35443106; PMCID: PMC9069994.

72. Maláska J, Stašek J, Duška F, Balík M, Máca J, Hruda J, Vymazal T, Klementová O, Zatloukal J, Gabrhelík T, Novotný P, Demlová R, Kubátová J, Vinklerová J, Svobodník A, Kratochvíl M, Klučka J, Gál R, Singer M; REMED Study Group. Effect of dexamethasone in patients with ARDS and COVID-19 - prospective, multi-centre,

open-label, parallel-group, randomised controlled trial (REMED trial): A structured summary of a study protocol for a randomised controlled trial. Trials. 2021 Mar 1;22(1):172. doi: 10.1186/s13063-021-05116-9. PMID: 33648568; PMCID: PMC7917377.

73. RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, Elmahi E, Prudon B, Green C, Felton T, Chadwick D, Rege K, Fegan C, Chappell LC, Faust SN, Jaki T, Jeffery K, Montgomery A, Rowan K, Juszczak E, Baillie JK, Haynes R, Landray MJ. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021 Feb 25;384(8):693-704. doi: 10.1056/NEJMoa2021436. Epub 2020 Jul 17. PMID: 32678530; PMCID: PMC7383595.

74. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC; ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med. 2020 Nov 5;383(19):1813-1826. doi: 10.1056/NEJMoa2007764. Epub 2020 Oct 8. PMID: 32445440; PMCID: PMC7262788.

75. Ohl ME, Miller DR, Lund BC, Kobayashi T, Richardson Miell K, Beck BF, Alexander B, Crothers K, Vaughan Sarrazin MS. Association of Remdesivir Treatment With Survival

and Length of Hospital Stay Among US Veterans Hospitalized With COVID-19. JAMA Netw Open. 2021 Jul 1;4(7):e2114741. doi: 10.1001/jamanetworkopen.2021.14741. PMID: 34264329; PMCID: PMC8283561.

76. Drożdżal S, Rosik J, Lechowicz K, Machaj F, Szostak B, Przybyciński J, Lorzadeh S, Kotfis K, Ghavami S, Łos MJ. An update on drugs with therapeutic potential for SARS-CoV-2 (COVID-19) treatment. Drug Resist Updat. 2021 Dec;59:100794. doi: 10.1016/j.drup.2021.100794. Epub 2021 Dec 9. PMID: 34991982; PMCID: PMC8654464.

77. Singh AK, Singh A, Singh R, Misra A. An updated practical guideline on use of molnupiravir and comparison with agents having emergency use authorization for treatment of COVID-19. Diabetes Metab Syndr. 2022 Feb;16(2):102396. doi: 10.1016/j.dsx.2022.102396. Epub 2022 Jan 13. PMID: 35051686; PMCID: PMC8755553.

78. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW Jr, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020 Dec 31;383(27):2603-2615. doi: 10.1056/NEJMoa2034577. Epub 2020 Dec 10. PMID: 33301246; PMCID: PMC7745181.

79. Singh B, Kaur P, Cedeno L, Brahimi T, Patel P, Virk H, Shamoon F, Bikkina M. COVID-19 mRNA Vaccine and Myocarditis. Eur J Case Rep Intern Med. 2021 Jun

14;8(7):002681. doi: 10.12890/2021_002681. PMID: 34268277; PMCID: PMC8276934.

80. Bar-On YM, Goldberg Y, Mandel M, Bodenheimer O, Freedman L, Kalkstein N, Mizrahi B, Alroy-Preis S, Ash N, Milo R, Huppert A. Protection of BNT162b2 Vaccine Booster against Covid-19 in Israel. N Engl J Med. 2021 Oct 7;385(15):1393-1400. doi: 10.1056/NEJMoa2114255. Epub 2021 Sep 15. PMID: 34525275; PMCID: PMC8461568.

81. Woodworth KR, Moulia D, Collins JP, Hadler SC, Jones JM, Reddy SC, Chamberland M, Campos-Outcalt D, Morgan RL, Brooks O, Talbot HK, Lee GM, Bell BP, Daley MF, Mbaeyi S, Dooling K, Oliver SE. The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Pfizer-BioNTech COVID-19 Vaccine in Children Aged 5-11 Years - United States, November 2021. MMWR Morb Mortal Wkly Rep. 2021 Nov 12;70(45):1579-1583. doi: 10.15585/mmwr.mm7045e1. PMID: 34758012; PMCID: PMC8580204.

82. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGettigan J, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T; COVE Study Group. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med. 2021 Feb 4;384(5):403-

416. doi: 10.1056/NEJMoa2035389. Epub 2020 Dec 30. PMID: 33378609; PMCID: PMC7787219.

83. Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, Stoop J, Tete S, Van Damme W, Leroux-Roels I, Berghmans PJ, Kimmel M, Van Damme P, de Hoon J, Smith W, Stephenson KE, De Rosa SC, Cohen KW, McElrath MJ, Cormier E, Scheper G, Barouch DH, Hendriks J, Struyf F, Douoguih M, Van Hoof J, Schuitemaker H. Interim Results of a Phase 1-2a Trial of Ad26.COV2.S Covid-19 Vaccine. N Engl J Med. 2021 May 13;384(19):1824-1835. doi: 10.1056/NEJMoa2034201. Epub 2021 Jan 13. PMID: 33440088; PMCID: PMC7821985.