Horizons

Volume 5 | Issue 1 Article 4

12-18-2020

A CRISPR Look at COVID-19

Jolie Ching
University of Hawai'i at Mānoa

Shane Nakamura
University of Hawai'i at Mānoa

A CRISPR Look at COVID-19

Jolie Ching and Shane Rene Nakamura

Honors 291 (Sophomore Seminar)
Mentor: Dr. Zoia Stoytcheva

The largest pandemic in recent times—novel coronavirus disease 2019 (COVID-19)—has caused economic shutdowns and social isolation on a global scale. Life as we know it has been placed on hold for the time being. Meanwhile, the scientific community worldwide is trying to understand COVID-19 biology and search for detection methods, prevention strategies, and treatments. Many researchers and biotech companies are turning to CRISPR, a cutting-edge advancement in biotechnology, to aid in developing methods for detection and finding a treatment for the virus. As a result, the need for unified regulation on CRISPR is more apparent than ever.

Introduction

Near the end of 2019, the human race encountered a strain of virus that had never been seen before. Belonging to the family of coronaviruses that cause respiratory illnesses, the novel virus now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has transformed our world. More commonly known to the public as coronavirus 2019, or COVID-19, the virus that was identified to have originated in China has rapidly spread across the world. As businesses are forced to shut down and jobs are lost, the social and economic structure of societies around the globe are being strained to their limits. The public is left with no choice but to socially distance and isolate themselves to avoid transmitting the virus. In countries where the outbreak of the virus has been deemed severe, mandatory lockdowns are in effect and all but essential workers are allowed to leave their homes in hopes of minimizing the infection curve. Meanwhile, scientists are attempting to develop detection methods and treatment options for the virus.

Jolie Ching
I am currently an incoming junior pursuing a Bachelor of Science in Computer Science. This paper was an opportunity for me, as a computer science student, to expand my knowledge into an almost entirely different field. During the research process, I came to the realization that biotechnology is not totally unlike computer science—both of which require logic, precision, and technical skills. I would like to acknowledge Dr. Zoia Stoytcheva for her guidance over the course of two years as well as Dr. Egle Ortega for her valuable feedback.

Shane Rene Nakamura
I am currently an incoming junior pursuing a Bachelor of Science in Biology. My goal is to attend medical school after getting my undergraduate degree and become an orthopedic surgeon in the future. Writing this paper became an opportunity to use what I learned in my honors research class about biotechnology and connect it with a current world crisis that is COVID-19. Due to my personal writing style of being detail-oriented, one of the major challenges of this paper from the start was finding relevant information on the virus but not providing too much of it. With the help and guidance from our teacher and project mentor Zoia Stoytcheva, I was able to revise my work in a way that made the information easier to read.

Horizons, Vol. 5, 2020, pp. 14–19

Timeline of COVID-19 Spread

The spread of COVID-19 can be traced back to December of 2019 when a cluster of pneumonia cases caused by a new strain of coronavirus occurred in Wuhan, China. By January 15th, the United States announced its first confirmed case of COVID-19 from a traveler who just returned from Wuhan, China (Mcnamara, 2020). At the time, around 300 cases of the virus were reported in China ("1st Case of New Coronavirus Detected in US", 2020). Despite these early warnings, many countries did not yet have preventative measures in place and the number of cases continued to rise around the world. By March 1st, a total of 87,137 cases were confirmed globally, with 79,968 cases in China and 7,169 outside. Five days later, the State of Hawai'i reported its first case of COVID-19 ("Hawaii Reports First COVID-19 Case," 2020).

In less than five months, COVID-19 spread across the world to 185 countries, having been declared an official pandemic by the World Health Organization on March 11—just four months after its initial discovery (Johns Hopkins University). At the time of manuscript preparation, April 18, there are 2,330,259 cases confirmed globally: 735,242 in the U.S., and 160,917 total deaths (Roser et al., 2020).

History of Coronaviruses

Coronaviruses are not a new medical phenomenon. First discovered in 1930, coronaviruses are a group of viruses that cause diseases in mammals and birds. They have crown-like spikes on their surface, hence the name "corona." The first signs of this type of virus were shown when domesticated chickens experienced acute respiratory infection caused by infectious bronchitis virus (IBV) (Kahn & McIntosh, 2005). A decade later, two more animal-related coronaviruses were reported: mouse hepatitis virus (MHV) and transmissible gastroenteritis virus (TGEV). In the early 1960s, the first human coronaviruses were found in patients experiencing symptoms characteristic of the common cold. Since then, five more human coronaviruses have been found causing serious respiratory tract infections in their patients: SARS-CoV in 2003, HCoV NL63 in 2004, HKU1 in 2005, MERS-CoV in 2012, and most recently SARS-CoV-2 in 2019 (Human Coronavirus Types, 2020). These coronaviruses fall into four categories known as alpha, beta, gamma, and delta. The first two groupings are derived from bats and commonly infect mammals while the latter two both derive from and infect birds.

COVID-19 is classified as a β-coronavirus as it is suggested to have originated from bats. Studies found that its genome shared a 96.2% sequence match with Bat CoV RaTG13, indicating bats as a common ancestor (Zhang et al., 2020). Despite this evidence, it is unclear whether bats transmitted COVID-19 to humans. Other alternative hosts include turtles, pangolins, and snakes due to the similarity of residual receptors observed in protein sequences alignment and phylogenetic analyses (Guo et al., 2020). However, the source of the virus and its transmission route to humans remains inconclusive.

Transmission of COVID-19

Although scientists do not know how the virus came to infect humans, human-to-human transmission has been extensively researched. COVID-19 is spread primarily through close contact with infected individuals as well as contact with contaminated environmental surfaces and aerosolization (Van Doremalen et al., 2020; Dexter et al., 2020). When an infected person sneezes or coughs, droplets of moisture containing the virus are spread through the air. If someone is nearby—say within six feet—they can potentially inhale those droplets and become infected. Additionally, the virus can survive from 1 to 72 hours on a variety of surfaces including plastic, stainless steel, copper, and cardboard (Van Doremalen et al., 2020). Reports state that virus transmission between healthcare workers and their patients was the most common route of infections (Cai J et al., 2020; Rothan & Byrareddy, 2020).

The cell receptor known as angiotensin-converting enzyme 2 (ACE2) plays a key role in the infection mechanism of COVID-19. ACE2 is a membrane-bound aminopeptidase that is essential to the cardiovascular and immune system. It is a receptor for coronaviruses, particularly of the SARS-CoV variety. COVID-19 infection is triggered by the binding of the spike protein S1 of the virus to ACE2, enabling the attachment of the virus to the cell membrane (Rothan & Byrareddy, 2020). As ACE2 is expressed in the heart and lungs, COVID-19 primarily infects alveolar epithelial cells, resulting in respiratory illnesses (Baig et al., 2020).

Measures to reduce human-to-human transmission of COVID-19 are required to control the current outbreak. While the search for a treatment is ongoing, efforts should be directed to protecting vulnerable populations such as children, healthcare workers, and the elderly. The elderly are especially susceptible to COVID-19 and comprise the majority of early death cases due to weak immune systems (Zheng et al., 2020). Reducing transmission of the virus can be achieved by social distancing and adequate detection methods.

Current Means of Detection for COVID-19

Current means of detection for COVID-19 consist of chest CT (computed tomography) scans, RT-PCR (qPCR), and serological IgG/IgM antibody detection assays.

A chest CT scan is an imaging method that uses x-rays to create cross-sectional pictures of the chest and upper abdo-

men (Ai et al., 2020). The scan takes around 30 seconds to a few minutes to complete. Iodine or Gadolinium-based contrast dyes are delivered to the body before the test in order to provide clearer images (Radiological Society of North America, n.d.). For COVID-19, chest CTs are often used to view the lungs, as signs of the virus appear as pulmonary opacities characteristic of pneumonia—a common result of the virus.

A reverse transcriptase polymerase chain reaction (RT-PCR) assay detects viral RNA, a key structural characteristic of COVID-19. This method requires multiple reagents to perform the test: RNA obtained through cells of bronchoalveolar lavage fluid specimens, sputum, or nasal swabs, primers that help synthesize DNA, reverse transcriptase enzyme, dNTPs which help to expand DNA strands, and the polymerase enzyme (Wang et al., 2020). This process takes around 1 to 2 hours and uses an mRNA starting template instead of DNA for the reverse transcriptase enzyme to produce a complementary single-strand DNA in a process known as reverse transcription. This DNA strand is then amplified via a PCR reaction to confirm the presence of viral RNA.

COVID-19 can also be detected by examining proteins using transmission electron microscopes, which allow the structure of the virus to be observed (Udugama et al., 2020). COVID-19 consists of four major structural proteins: a small integral membrane glycoprotein (M), a nucleocapsid protein (N), a large spike glycoprotein (S), and an envelope (E). The S protein gives the virus its crown-like appearance and enables it to infect other cells. Positive-sense single-stranded RNA genomes are coiled up inside (Fehr and Perlman, 2015).

CRISPR as a Means of Detection for COVID-19

Although current methods of detection for the virus are generally accurate, they are resource-intensive and costly. Methods such as qRT-PCR require specially trained personnel who are familiar with the protocols and instruments used. This limits the opportunity for testing to those who have easy access to facilities that are able to provide these procedures. Accessibility to the reagents and equipment needed to perform the tests also presents an issue, slowing the process of detection. In addition, chest CTs are often costly with the national price ranging from $675 to $8600, the average cost being $1,900 (NewChoiceHealth.com, n.d.). However, with CRISPR technology, faster and cheaper detection methods are being developed.

A cutting-edge advancement in science involves a gene-editing technology known as CRISPR-Cas systems. CRISPR, a moniker for Clustered Regularly Interspersed Short Palindromic Repeats (Jansen R, 2002) was adapted from the genome editing system in bacteria (Hovath P., 2010, Ishino Y. 1987, Jinek M. 2012). It originates from type II CRISPR-Cas systems known to provide bacteria with adaptive immunity to viruses and plasmids (Doudna & Charpentier, 2014). This gives CRISPR the unique property of being able to manipulate genetic material at particular locations in the genome—areas that are specified by a short section of guide RNA. Since its discovery, several variations of the CRISPR-Cas system have been developed (Hsu, 2014).

A detection technique using CRISPR, The Specific High-sensitivity Enzymatic Reporter unLOCKing, dubbed SHERLOCK, method is a Cas13a-based CRISPR system. Cas13a specializes in targeting RNA—not DNA unlike many CRISPR-Cas systems (Yan, et. al, 2019). There are four main steps to SHERLOCK—the first two of which are reagent preparations and sample extractions. The third step involves pre-amplification of the RNA (or DNA with a reverse transcriptase) through a process known as recombinase polymerase amplification (RPA). Compared to the hours-long process of PCR, SHERLOCK can generate results within 3–10 minutes (Kellner et al., 2019). Additionally, RPA does not require specialized technology as it can perform at a single temperature.

Once the RPA process finishes amplifying nucleic acid fragments from COVID-19, they are combined with a Cas13a nuclease, a guide RNA complex that matches the targeted nucleic acid sequence. Short nucleic fragments' that are coupled to a fluorescent reporter and a quencher are also introduced. If the coronavirus sequence is present, Cas13a is activated and the RNA reporter is cleaved, resulting in a fluorescent signal. This signal is an indicator to determine whether the target sequence (that is, the coronavirus) is present in the nucleotides (Gronowski, 2018).

A spin-off of this process, known as SARS-CoV-2 DETECTR, is being investigated by Mammoth Biosciences and the University of California at San Francisco. A notable difference between the two experimental procedures is that the DETECTR method uses Cas12a rather than Cas13a and takes only 45 minutes (Alvarez, 2020).

Research of Treatments for COVID-19

There are currently very limited treatment options for COVID-19. However, with CRISPR, a promising method known as PAC-MAN (Prophylactic Antiviral CRISPR in huMAN cells) is being tested at Stanford University. Despite being in the early stages of development, PAC-MAN has been deemed "capable of inhibiting coronavirus fragment expression in human lung epithelial cells" (Abbott et al., 2020). PAC-MAN's CRISPR approach allows the treatment to degrade key parts of COVID-19's genome, specifically areas that encode the RNA-dependent RNA polymerase (RdRP) and Nucleocapsid proteins which are known to be essential for coronavirus replication and function. The Stanford team chose Cas13d, a protein possessing high catalytic activity in human cells, to target and degrade these regions. The Cas13d system uses 35 CRISPR-associated RNAs (otherwise known as crRNAs) that

contain a 22-nucleotide spacer sequence that is able to lead the Cas13d protein to specific RNA molecules for degradation.

There are, however, some limitations to the research conducted by Stanford University. The PAC-MAN experiment was not conducted on live COVID-19 strains as the researchers were unable to gain access to these strains at the time. Instead, the researchers relied on synthesized fragments of the virus as well as live infections using a H1N1 IAV strain in human lung epithelial cells. Regardless of whether their samples were alive, the research demonstrated that a group of 6 crRNAs can target 91% of sequenced coronaviruses (not just COVID-19) and a group of 22 crRNAs are able to target all 60 sequenced coronaviruses (Abbott et al., 2020). While there are still challenges for the researchers to address, PAC-MAN is proof of the various ways in which CRISPR can be adapted to suit humanity's needs.

Biotechnology in Our World

CRISPR falls under the realm of biotechnology—a field that pertains to "the manipulation (as through genetic engineering) of living organisms or their components to produce useful, usually commercial products" (Biotechnology, 2020). The field can be split into several subsections, including medical, agricultural, food/consumer goods, and environmental.

With biotechnology, many solutions to global issues are now within reach. From developing genetically modified organisms (GMOs) to detecting and treating diseases or even altering human DNA, the possibilities generated by CRISPR are seemingly endless. Biotechnology will eventually come to play a significant role in everyday life. However, with the tremendous potential of biotechnology such as CRISPR also comes the need to be wary of how it is used, who uses it, and how it may impact society and the ecosystem. For this reason, regulations surrounding CRISPR are needed to ensure the world's safety.

Historical Roots of Biotechnology Regulation

The creation of regulations overseeing biotechnology is a relatively new one. 1973's Gordon Conference Letter on Nucleic Acids brought together the field's top researchers to study the hazards of the (then recently discovered) recombinant DNA techniques. These researchers, recognizing the need to address laboratory safety problems in order to proceed with the research without alarming the public and inviting government regulation, drafted the Berg Letter which entailed a plan to reduce potential risks of their experiments (Weiner, 2001).

Two years later, the Asilomar Conference was held to address the safety of genetic engineering. The aim of this event was once again to minimize public interference by demonstrating to the world that scientists could regulate themselves. Immediately after the Asilomar Conference, the National Institute of Health (NIH) Recombinant DNA Advisory Committee (RAC) was established with the task of converting the conference's results into safety guidelines for all recombinant DNA experiments receiving NIH funding (Wivel, 2014). For the next couple of years, scientists on the board would argue about the perceived harshness of the safety guidelines. Several research universities and scientific organizations lobbied the local and national legislation to make the guidelines less restrictive. Eventually, the NIH guidelines were downgraded, coinciding with the commercialization of biotechnology. In fact, by the early 1980s, all 11 signers of the Berg Letter were involved with biotechnology companies (Weiner, 2001). Where does this leave biotechnology regulations today?

Current Regulations regarding Biotechnology:

As of 2020, there are no universally recognized international regulations on gene therapy or genome editing. The Declaration of Helsinki, formed in 1964, is the closest thing we have. Formed as a reaction to the experiments carried out by Germany in World War II, the declaration states various ethical standpoints on human experimentation. However, because it is a non-legally binding document, the Declaration of Helsinki only has power if it is cited in national regulations. The declaration has further undergone a series of revisions since its creation. Finally, in 2006, the U.S. Food and Drug Administration (USFDA) announced it would remove all reference to the Helsinki Declaration in their national regulations (Burgess, 2012). Meanwhile, the European Union still cites the 2000 revision.

Currently, there is no federal legislation that either bans or places restrictions on experiments that manipulate human DNA. While somatic gene therapy (which only affects a human's non-reproductive cells) is not explicitly banned, there exist heavy restrictions placed on the editing of the human lineage via germline therapy—a process that involves the modification of genes. The Dickey-Wicker Amendment of 1995, for example, forbids NIH from funding any research involving the manipulation of human embryos. Furthermore, in 2015, Congress passed a provision stating that the USFDA must approve of any human clinical trials that involve somatic gene therapy (Reardon, 2015). They must further approve of any somatic gene therapy products before they enter the marketplace. Meanwhile, germline therapy is explicitly banned from being sold. The National Institute of Health gives the following reason for banning edits to the human lineage: "The idea of germline gene therapy is controversial. While it could spare future generations in a family from having a particular genetic disorder, it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known. Because people who would be affected by germ-

line gene therapy are not yet born, they can't choose whether to have the treatment. Because of these ethical concerns, the U.S. Government does not allow federal funds to be used for research on germline gene therapy in peoples" (U.S. National Library of Medicine, National Institutes of Health, n.d.). However, it is important to note that privately-funded research on germline therapy is still considered legal.

Other countries such as China and Russia also express similar sentiments—that gene editing would "require national approval" (Normile, 2019) and is "premature at this point" (Grebenshchikova, 2019). However, incidents such as the 2019 creation of CRISPR babies where a Chinese scientist designed HIV-resistant twins are at odds with this claim (Raposo, 2019). Though there is a global consensus that this type of technology should be regulated, no substantial unified action has been taken so far. Given the urgent nature of the pandemic, the world needs regulation regarding CRISPR now more than ever.

Conclusion

The COVID-19 pandemic has revealed the need for efficient detection methods and treatments that can target viruses with no effective vaccines. Some researchers have turned to CRISPR for a solution. Tests such as SHERLOCK and the DETECTR have been found to detect the presence of COVID-19 without sacrificing accuracy. Further, the PAC-MAN experiment has proved that CRISPR can be used to target and degrade virus' genes (although an in vivo approach is still in development).

While there is reason to be cautiously optimistic about the CRISPR approach to treatment and detection, the world must be wary and prepared. In every country's race for a cure also lies the possibility for high risks and exploitation of the urgent nature of the pandemic. Like social distancing, regulations on genetic engineering do not work if those measures are only confined to one place. To protect human lineage from being unethically altered, a global precedent for regulations on genetic engineering must be established.

References

1st Case Of New Coronavirus Detected in U.S. (2020, January 21). National Public Radio. https://www.npr.org/sections/goatsandsoda/2020/01/21/798137962/china-tries-to-control-travel-in-and-out-of-wuhan-to-stem-coronavirus-spread

Abbott, T., Dhamdhere, G., Liu, Y., Lin, X., Goudy, L., Zeng, L., Chemparathy, A., Chmura, S., Heaton, N., Debs, R., Pande, T., Endy, D., Russa, M. L., Lewis, D., & Qi, L. (2020). Development of CRISPR as a prophylactic strategy to combat novel coronavirus and influenza. *BioRxiv*. https://doi.org/10.1101/2020.03.13.991307

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., . . . Xia, L. (2020). Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology*, 200642. doi: 10.1148/radiol.2020200642

Baig, A. M., Khaleeq, A., Ali, U., & Syeda, H. (2020). Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host–Virus Interaction, and Proposed Neurotropic Mechanisms. *ACS Chemistry Neuroscience*, 11(7), 995–998. https://doi.org/10.1021/acschemneuro.0c00122

Biologics Evaluation and Research. (n.d.). Information about Self-Administration of Gene\Therapy. www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/information-about-self-administration-gene-therapy

Biotechnology. (2020, April 20). *Merriam-Webster*. https://www.merriam-webster.com/dictionary/biotechnology

Burgess, L. (2012, November 2). FDA abandons the Declaration of Helsinki. *The South African Journal of Bioethics & Law*. http://www.sajbl.org.za/index.php/sajbl/article/view/222/230

Cai J, Sun W, Huang J, Gamber M, Wu J, He G. (2020). Indirect Virus Transmission in Cluster of COVID-19 Cases. *Emerging Infectious Diseases*. 26(6), 1343–1345. doi: 10.3201/eid2606.200412.

Dexter, F., Parra, M., Brown, J., & Loftus, R. (2020). Perioperative COVID-19 Defense: An Evidence-Based Approach for Optimization of Infection Control and Operating Room Management. *Anesthesia and analgesia*. 131(1), 37–42. doi: 10.1213/ANE.0000000000004829

Doudna, J., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213). https://doi.org/10.1126/science.1258096

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. *Methods in molecular biology*, 1282, 1–23. https://doi.org/10.1007/978-1-4939-2438-7_1

Grebenshchikova, E. (2019). Russia's stance on human genome editing. *Nature*, 575, 596. https://doi.org/10.1038/d41586-019-03617-x

Gronowski, A. (2018). Who or What is SHERLOCK?. *The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine*, 29(3), 201–204. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6247122/

Guo, Y.-R., Cao, Q.-D., Hong, Z.-S., Tan, Y.-Y., Chen, S.-D., Jin, H.-J., Tan, K.-S., Wang, D.-Y., & Yan, Y. (2020). The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—an update on the status. *Military Med Res*, 7(11). https://doi.org/10.1186/s40779-020-00240-0

Hawaii reports first COVID-19 case. (2020, March 6). State of Hawaii Department of Health. https://health.hawaii.gov/prepare/hawaii-reports-first-covid-19-case/

Human Coronavirus Types. (2020, February 15). [Gov]. Center for Disease Control and Prevention. https://www.cdc.gov/coronavirus/types.html

Kahn, J., & McIntosh, K. (2005). History and Recent Advances in Coronavirus Discovery. *The Pediatric Infectious Disease Journal*, 24(11), S223–S227. https://doi.org/10.1097/01.inf.0000188166.17324.60

Kellner, M., Koob, J., Gootenberg, J., Abudayyeh, O., & Zhang, F. (2019). SHERLOCK: Nucleic acid detection with CRISPR nucleases. *NCBI*, 14(10), 2986–3012. https://doi.org/10.1038/s41596-019-0210-2

Mcnamara, A. (2020, January 21). CDC confirms first case of coronavirus in the United States. *CBS News*. https://www.cbsnews.com/news/coronavirus-centers-for-disease-control-first-case-united-states/

NewChoiceHealth.com. (n.d.). "Chest CT Scan Cost and Procedure Comparison." www.newchoicehealth.com/procedures/chest-ct-scan.

Normile, D. (2019, February 28). China tightens its regulation of some human gene editing, labeling it 'high-risk.' *Science*. https://www.sciencemag.org/news/2019/02/china-tightens-its-regulation-some-human-gene-editing-labeling-it-high-risk

Radiological Society of North America. (n.d.). "Patient Safety—Contrast Material." Patient Safety—Contrast Material. www.radiologyinfo.org/en/info.cfm?pg=safety-contrast

Raposo V. L. (2019). The First Chinese Edited Babies: A Leap of Faith in Science. *JBRA assisted reproduction*, *23*(3), 197–199. https://doi.org/10.5935/1518-0557.20190042

Reardon, S. (2015). US Congress moves to block human-embryo editing. *Nature*. doi:10.1038/nature.2015.17858

Roser, M., Ritchie, H., Ortiz-Ospina, E., & Hasell, J. (2020, May 5). Coronavirus Pandemic (COVID-19). Our World in Data. https://ourworldindata.org/coronavirus

Rothan, H., & Byrareddy, S. (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *Science Direct*. 109(102443). https://doi.org/10.1016/j.jaut.2020.102433.

Udugama, B., Kadhiresan, P., Kozlowski, H. N., Malekjahani, A., Osborne, M., Li, V.Y.C., Chan, W.C.W. (2020). Diagnosing COVID-19: The Disease and Tools for Detection. *American Chemical Society Public Health Emergency Collection*. doi: 10.1021/acsnano.0c02624

U.S. National Library of Medicine, National Institutes of Health. (n.d.). "What Are the Ethical Issues Surrounding Gene Therapy?—Genetics Home Reference—NIH." ghr.nlm.nih.gov/primer/therapy/ethics

Van Doremalen, N., Bushmaker, T., Morris, D., Holbrook, M., Gamble, A., Williamson, B., Munster, V. (2020). Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *The New England Journal of Medicine*. doi: 10.1056/NEJMc2004973

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., & Tan, W. (2020). Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *Jama*. doi: 10.1001/jama.2020.3786

Weiner, C. (2001). Drawing the Line in Genetic Engineering: Self-Regulation and Public Participation. *Perspectives in Biology and Medicine*, 44(2), 208–220. https://doi.org/10.1353/pbm.2001.0039

Wivel, N. (2014). Historical Perspectives Pertaining to the NIH Recombinant DNA Advisory Committee. *Human Gene Therapy*, 25(1), 19–24. https://doi.org/10.1089/hum.2013.2524

Xiao-Jie, L., Hui-Ying, X., Zun-Ping, K., Jin-Lian, C., & Li-Juan, J. (2015). CRISPR-Cas9: a new and promising player in gene therapy. *Journal of medical genetics*, 52(5), 289–296. doi:10.1136/jmedgenet-2014-102968

Yan, F., Wang, W., Zhang, J. (2019). CRISPR-Cas12 and Cas13: the lesser known siblings of CRISPR-Cas9. *Cell Biology Toxicology 35*, 489–492. https://doi.org/10.1007/s10565-019-09489-1

Zhang, T., Wu, Q., & Zhang, Z. (2020). Pangolin homology associated with 2019-nCoV. *BioRxiv*. https://doi.org/10.1101/2020.02.19.950253

Zheng, Y.-Y., Ma, Y.-T., Zhang, J.-Y., & Xie, X. (2020). COVID-19 and the cardiovascular system. *Nature Reviews Cardiology 17*, 259–260. https://doi.org/10.1038/s41569-020-0360-5