Severe Acute Respiratory Syndrome Coronavirus 2: Genomic Observations and Emerging Therapies

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Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of the disease COVID-19, first emerged in late December 2019 in China, and has subsequently become a pandemic with unprecedented clinical impact. The virus appears to more severely affect older individuals and those with co-morbid medical conditions, specifically those with chronic lung disease, obesity, heart failure and diabetes. Fortunately, children appear to be less severely affected, though mortality and severe disease have been reported. In addition, children's role in spreading the disease (potentially through asymptomatic shedding of the virus) remains an important area requiring further investigation. The emergence of SARS-CoV-2 has highlighted the importance of metagenomic next generation sequencing as a tool for pandemic investigation. Though no proven therapeutic options currently exist, ongoing genomic and clinical trial data may help inform the identification and development of both repurposed and novel therapeutic agents for use in this disease.

Keywords: SARS-CoV-2, COVID-19, pandemic

Background

SEVERE ACUTE RESPIRATORY SYNDROME coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019, first emerged in late December 2019 in China,¹ and has subsequently become a pandemic with unprecedented clinical impact. The virus appears to affect older individuals and those with comorbid medical conditions, specifically those with chronic lung disease, obesity,^{2,3} heart failure, and diabetes more severely.⁴ Fortunately, children appear to be less severely affected, though mortality and severe disease have been reported.⁵ In addition, children's role in spreading the disease (potentially through asymptomatic shedding of the virus) remains an important area requiring further investigation. The emergence of SARS-CoV-2 has highlighted the importance of metagenomic next-generation sequencing (NGS) as a tool for pandemic investigation. Though no proven therapeutic options currently exist, ongoing genomic and clinical trial data may help inform the identification and development of both repurposed and novel therapeutic agents for use in this disease.

Genomic Data/Overview

During an outbreak of a novel virus, genome sequences of the virus can provide crucial information and insight, including but not limited to the type of virus, closest virus relative, source, virulence, and pandemic potential, and can

guide the development of both therapeutic and preventive measures. The initial cases of coronavirus disease 2019 (COVID-19) were associated with severe respiratory symptoms and pneumonia and a mortality rate higher than seen with seasonal respiratory viruses. Clinical testing revealed that the initial cases were negative for 22 known respiratory pathogens (18 viruses and 4 bacteria), including influenza viruses, respiratory syncytial virus, and human metapneumovirus.⁶ Efforts to identify the etiology of these unknown cases quickly turned to metagenomic sequencing using NGS in combination with virus cell culture and electron microscopy. Briefly, metagenomic analysis was conducted on RNA that was extracted from bronchoalveolar lavage fluid and a combination of Illumina and Nanopore NGS was used to identify potential causative viruses.⁶ Three nearly full-length genome sequences were obtained from viral culture and patient samples and were found to have typical betacoronavirus genomic organization with 86.9% nucleotide sequence identity to a previously published SARS-like coronavirus (CoV) obtained from a bat (MG772933.1).⁶ Although related at the genome level to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), it was distinct from these human coronaviruses, and thus in January of 2020, the authors termed it 2019-novel coronavirus (2019-nCoV).⁶

In early 2020, the *Coronaviridae* study group of the International Committee on Taxonomy of Viruses met to

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classify this novel coronavirus formally. Based on phylogeny, taxonomy, and established practice, the study group determined that 2019-nCoV formed a sister clade to the prototype SARS-CoV, and designated the novel coronavirus officially as SARS-CoV-2.⁷ On February 11, 2020, the World Health Organization (WHO), following best practices for the naming of new human infectious diseases, which were developed in consultation and collaboration with the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO), named the clinical disease of this outbreak coronavirus disease 2019 (COVID-19).¹

Coronaviruses are enveloped single-stranded positive sense RNA viruses, with large genomes 28–32 kb in size.⁸ The coronavirus genome consists of a leader sequence, ORF1ab encoding proteins for RNA replication, and subgenomic genes for nonstructural proteins and structural proteins.⁸ The coronaviruses are subdivided into 2 genera: *alphacoronavirus* and *betacoronavirus*. Of the now 7 recognized coronaviruses that can infect and cause disease in humans, 2 are grouped in the *alphacoronavirus* genus, and 5 are in the *betacoronavirus* genus. The human coronaviruses hCoV-NL63 and hCoV-229E, which are considered common and possibly seasonal and which generally cause respiratory symptoms associated with the common cold, are in the *alphacoronavirus* genus.⁹ In the *betacoronavirus* genus, there are 2 additional common human coronaviruses, hCoV-OC43 and hCoV-HKU1, which also typically cause milder respiratory symptoms, and 3 zoonotic-transmitted coronaviruses associated with more severe clinical symptoms: SARS-CoV-1, MERS-CoV, and now SARS-CoV-2.⁹ An in-depth genomic comparison revealed that there are 380 amino acid differences between SARS-CoV-1 and SARS-CoV-2.¹⁰ Genomic analysis of hundreds of SARS-CoV-2 genomes has begun in order to identify genetic groups, and at least 3 major viral clades—S, V, and G—have been proposed.^{11,12}

The use of NGS greatly accelerated the identification of the cause of the COVID-19 outbreak in China, and it took only a few weeks to determine that the outbreak was due to a coronavirus that was new to the human population. Continued genomic and epigenomic analysis of SARS-CoV-2 is critical, as it can be used to determine the number of virus strains, how the virus is introduced into new regions, the number of introductions of the virus, if there is community spread of virus, if outbreaks are linked, and how the virus is evolving. Furthermore, as novel vaccines and therapeutic agents are created and tested, knowledge of viral susceptibility or resistance to these interventions will be critical, and genomic analysis will guide and inform these efforts. Currently, only a small number of repurposed and novel agents have been proposed as potential therapeutic options, several of which are discussed below.

Repurposed therapies

Hydroxychloroquine. The aminoquinolone antimalarial agent hydroxychloroquine was one of the first drugs proposed for treatment of SARS-CoV-2,¹³ given evidence of *in vitro* activity against the virus.¹⁴ The antiviral effects of this compound have been recognized for more than 50 years,¹⁵ with *in vitro* efficacy noted for Chikungunya virus,¹⁶ Zika virus,¹⁷ Dengue virus,¹⁸ Ebola virus,¹⁹ human

immunodeficiency virus (HIV),²⁰ and influenza virus,²¹ among others.²² However, despite this promise as an antiviral agent, evidence of clinical efficacy in humans or animal models is notably lacking. Human randomized trials of this drug in the treatment of Chikungunya²³ and Dengue fever²⁴ failed to demonstrate any significant clinical advantages, while trials in HIV have been conflicting and have assessed primarily surrogate outcome markers.^{22,25} Hydroxychloroquine did not demonstrate any benefit in the prevention of influenza infection in a randomized trial,²⁶ while study of the drug in guinea pigs infected with Ebola did not demonstrate any benefit either.¹⁹ Clinical trials with this agent (with or without azithromycin) for the treatment of SARS-CoV-2 have been limited and marked by small sample sizes, a lack of methodological rigor, and use of surrogate endpoints.^{27,28} In addition, reports of adverse cardiac effects (e.g., prolonged QT syndrome), particularly in combination with azithromycin, are also a concern.²⁹ Hence, the reports of *in vitro* activity of hydroxychloroquine against SARS-CoV-2 should be interpreted with caution until the results of large clinical trials are available. Many such studies are underway, with 104 clinical trials assessing hydroxychloroquine currently registered on clinicaltrials.gov at the time of writing of this article (April 17, 2020).

HIV antiretroviral therapy. The use of the antiretroviral agent lopinavir-ritonavir has also been proposed as a possible therapy for SARS-CoV-2, given reports of activity against SARS-CoV.³⁰ *In vitro* efficacy had been previously established,^{30,31} and a non-randomized trial of lopinavir-ritonavir utilizing historical controls and concomitant corticosteroids and ribavirin in 152 adults with SARS-CoV infection had demonstrated significant reductions in the composite outcome of acute respiratory distress syndrome and death at 21 days.³¹ Unfortunately, a trial of 199 adults with COVID-19 randomized to either lopinavir-ritonavir or standard of care did not demonstrate any significant reductions in mortality, time to clinical improvement, or viral RNA detection over time.³² In addition, the drug was discontinued early in this trial in 13.8% of subjects due to adverse events.

Novel therapies

Remdesivir. One of the more promising agents for treatment of SARS-CoV-2 is the parenteral adenosine nucleotide analogue remdesivir,³³ which inhibits the function of the viral RNA-dependent RNA polymerase.³⁴ This drug has demonstrated *in vitro* activity against a large variety of divergent coronaviruses,^{34,35} prophylactic and therapeutic potential in a murine model of SARS-CoV disease,³⁵ and prophylactic activity in a rhesus macaque model of MERS-CoV disease.³⁶ In an uncontrolled report of 53 adults with SARS-CoV-2 infection receiving at least 1 day of a planned 10-day course of remdesivir, 68% demonstrated clinical improvement, with mortality reported in 7 (13%).³⁷ This cohort was severely ill (57% were mechanical ventilated at enrollment, with 8% on extracorporeal membrane oxygenation), though due to the absence of randomization and a control group, definitive conclusions could not be drawn. Additional studies are ongoing, with 6 trials currently recruiting subjects.

NHC. β -D-N4-hydroxycytidine (NHC) is an oral ribonucleoside analog with activity against many RNA viruses.³⁸ Recently, this agent was shown to possess *in vitro* activity against MERS-CoV, SARS-CoV, and SARS-CoV-2.³⁸

Interestingly, the agent possessed increased activity against a murine coronavirus carrying resistance mutations for remdesivir, suggesting a possible role in treatment of remdesivir-resistant SARS-CoV-2 infection. An oral prodrug of NHC (EIDD-2801) has demonstrated good oral bioavailability in ferret and primate models, suggesting oral use may be an option in humans.³⁹ The drug is currently awaiting study in Phase I clinical trials.³⁹

Global Cooperative Research

The WHO's current large 4-arm Solidarity trial is unprecedented in its scope and ambition, and may prove to be a reliable and practical model for real-time study of novel therapies during emerging pandemics.⁴⁰ The trial as planned will take place in more than 70 countries, involve thousands of subjects, and aim to reduce the time required for completion of typical randomized clinical trials by 80%.⁴¹ An overarching component of the trial will be an emphasis on simplicity and feasibility for busy clinicians enrolling subjects. Participating sites need only scan an electronic consent form to the WHO, and the study Web site will randomize the subject to whatever medications are available at the local hospital. No prospective collection of biological specimens will occur, and only high-yield clinical information will be collected (length of hospital stay, mortality, and need for oxygen or mechanical ventilation).⁴⁰ The Solidarity trial will assess many of the treatment modalities discussed above, with the 4 treatment arms consisting of hydroxychloroquine, lopinavir-ritonavir, remdesivir, and lopinavir-ritonavir with interferon beta-1 α compared to standard of care.⁴¹

Conclusion

As the COVID-19 pandemic continues to spread, it is clear that research (bench, translational, and clinical) will be essential to the ultimate control and prevention of the disease. Metagenomic NGS to identify specific viral lineages, origins, and mutations coupled with enhanced efforts at drug discovery and ultimately clinical trials may provide a glimmer of light at the end of an otherwise dark tunnel.

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