*Review*

# SARS-CoV-2/COVID-19: Viral Genomics, Epidemiology, Vaccines, and Therapeutic Interventions

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**Abstract:** The COVID-19 pandemic is due to infection caused by the novel SARS-CoV-2 that impacts the lower respiratory tract. The spectrum of symptoms ranges from asymptomatic infections to mild respiratory symptoms to the lethal form of COVID-19 which is associated with severe pneumonia, acute respiratory distress and fatality. At present, the global case fatality rate of COVID-19 laboratory confirmed cases is ~4.7% ranging from ~0.3-0.4% in Chile and Israel to ~10.8% in Italy. To address this global crisis, up-to-date information on the viral genomics and transcriptomics is crucial for understanding the origins and global dispersal of the virus, providing insight into viral pathogenicity, transmission and epidemiology, and enabling strategies for therapeutic interventions, drug discovery and vaccine development. Therefore, this review provides a comprehensive overview of COVID-19 epidemiology, genomic etiology, findings from recent transcriptomic map analysis, viral-human protein interactions, molecular diagnostics, and the current status of vaccine and novel therapeutic intervention development. Moreover, we provide an extensive list of resources that will help the scientific community access numerous types of databases related to SARS-CoV-2 OMICs and approaches to therapeutics related to COVID-19 treatment.

**Keywords:** SARS-CoV-2; COVID-19; Coronavirus; Pandemic; Viral Genomics

## 1. Introduction

In December 2019, several cases of a new respiratory illness were described in Wuhan, Hubei Province, China. By January 2020, it was confirmed that these infections were caused by a novel coronavirus which was subsequently named SARS-CoV-2, while the disease it caused COVID-19 [1-3]. This novel coronavirus is closely related to the previously described SARS-CoV identified in the 2002-2003 outbreak [4]. The World Health Organization (WHO) recently declared the ongoing SARS-CoV-2 outbreak as a pandemic [1]. To contain the spread of the virus, we are witnessing the implementation of strict measures unprecedented in modern times. Major cities and entire nations have been placed under lockdown with restrictions on travel and gatherings as well as closure of schools and businesses. These measures, along with the closure of international borders and restrictions on international travel have had significant economic impact, resulting in a sharp decline in major financial indices and prompting fears of a global recession.

As the number of confirmed infections and fatalities continue to increase daily, it is crucial to further our understanding of the virus transmission patterns and epidemiology. Despite only ~3 months into the outbreak, there is a wealth of information emerging on the virus genomic makeup & evolution, and its transcriptomic mapping, including virus-human protein interactions. Such information is urgently needed for the identification of therapeutic targets for intervention and vaccine development, in addition to informing preventive policies and patient care decisions. The primary purpose of this review is to provide an update on the epidemiology, modes of transmission, a summary of the genomics and transcriptomics of SARS-CoV-2, as well as therapeutic interventions in the absence of a vaccine. Furthermore, we examine how the viral genomics and molecular epidemiology informs therapeutic and vaccine development as well as public health strategies. We have also compiled a resource table outlining the numerous databases related to SARS-CoV-2 whole genome sequencing, transcriptomic map, strain tracing, SARS-CoV-2-human protein-protein interactions, and clinical trials for repurposed drugs and vaccines (Table).

**Table: SARS-CoV-2 Resources Related to Genomics, Transcriptomics and Phenotypes**

| Category | Data Type | Database |
|---|---|---|
| SARS-CoV-2 Genome Sequencing Data | DNA Sequencing Data | https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/ |
| SARS-CoV-2 Transcriptomic Map | RNA Sequencing Data | Open Science Framework: accession number doi:10.17605/OSF.IO/8F6N9 |
| SARS-CoV-2 and Human Protein Interactions | Mass Spectrometry Raw Data | http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD018117 |
| List of Clinical Trials | Clinical Trial Related Information | https://clinicaltrials.gov/ct2/home |

## 2. Epidemiology and Transmission of SARS-CoV-2

To date (March 29, 2020), over 480,000 laboratory-confirmed cases of COVID-19 have been reported worldwide with more than ~31,000 deaths in more than 175 countries [5]. In most countries, increases in the number of confirmed cases are following an exponential growth trajectory during the early and peak stages of the outbreak. At present, the global case fatality rate of COVID-19 laboratory confirmed cases is ~4.7% ranging from ~0.3-0.4% in Chile and Israel to ~10.8% in Italy [6]. Whilst it is difficult to compare case fatality rates between countries when they are at different stages of the outbreak, variations are most likely due to the scope of population testing, the age structure and health status of the population, and the health systems within each country [6]. Clinical characteristics of SARS-CoV-2 patients from China [7,8], South Korea [9], and the United States [6] have recently been reported with fever, dry cough and shortness of breath being the most common clinical presentations. Although the outbreak is evolving, the current global data suggests that the number of cases is doubling every four days, with ~20% of confirmed COVID-19 patients requiring hospitalization (median hospital stay of 12 days), and 25% of hospitalized patients (~5% of all cases) needing intensive critical care [6,7,9]. The severity and outcome of the disease seem to be highly correlated with the late age of onset where more severe forms of COVID-19 were observed for adults ≥55 years [6,7,9]. Additionally, an age-dependent fatality rate has been demonstrated with the lowest risk observed among those under the age of 19 (0-0.1%) and 20-54 years (0.1-0.8%); however, the risk of mortality increases incrementally, affecting 1.4-4.9% in the 55-74-year age group, 4.3-10.5% among those aged 75-84 years, with the highest fatality rate of 10.4-27.3% in those aged ≥85 years [6,7,9,10]. Individuals with underlying health issues, such as cardiovascular disorders, diabetes, liver & kidney disease, malignant tumours, or a suppressed immune system, seem to have the severe form of the disease and increased fatality rate [6,7,9-11].

SARS-CoV-2 has a natural origin [12] and is primarily transmitted via inhalation of droplets expelled when an infected patient coughs. Fomite-mediated transmission is another important source of transmission when hands which have touched surfaces contaminated by droplets are used to touch the face, eyes, or nose. Current modeling of SARS-CoV-2 spread estimates a basic reproduction number ($R_0$) of 2.2 [13] (see **Box)**. This reported $R_0$ is higher than seasonal influenza, indicating the potential for sustained human-to-human transmission within a population unless strict containment and public health measures are implemented and sustained. As a new coronavirus, there is currently insufficient data to reach a consensus on the potential for seasonality of SARS-CoV-2 transmission. However, two major factors that may have an influence on seasonality are changes in environmental parameters and human behavior [14]. Specifically, outdoor (e.g., temperature, humidity, sunlight/vitamin D status) and indoor environmental factors (e.g., temperature, humidity, air change rate, etc.) influence both virus transmission parameters (e.g. virus viability, airborne aerosolization, droplet spray and direct contact) and host defenses (e.g., airway antiviral immune defense and efficiency of nasal and bronchial mucociliary clearance). Although the seasonality has not been confirmed for SARS-CoV-2, there are now accumulating evidence that high temperature and humidity might play a significant role in transmission [15,16].

To evaluate the stability of SARS-CoV-2 in aerosols and on different surfaces [17], a series of well-controlled experiments revealed that the virus remained viable in aerosols throughout the duration of the experiment (3 hours; median half-time of 1.1-1.2 hours) [17]. SARS-CoV-2 was found to be most stable on plastic and stainless steel with viable virus detected up to 72 hours post-application and no

viable virus was found on copper or cardboard after 4 and 24 hours, respectively [17]. In this experimental model, SARS-CoV-2 exhibited similar stability to SARS-CoV. Therefore, the differences

**Box: SARS CoV-2 Related Definitions**

- **SARS-CoV-2:** Severe acute respiratory syndrome coronavirus 2
- **COVID-19 or Covid-19:** Corona virus disease, 2019. COVID-19 is the official name of the disease manifested by the SARS-CoV-2.
- **$R_0$:** Reproduction number that defines the number of secondary cases that will be produced by a single infectious index case in a population that is fully susceptible to the disease. For example, a $R_0$ of 2 means that, on average, one primary index case would infect two other people generating two secondary cases. Continuous horizontal (human-to-human) transmission will occur if $R_0$ is above the critical threshold of one.
- **Fomite Transmission:** A fomite is any inanimate object (i.e. surface) when contaminated with or exposed to infectious agent, can serve as a source to transmit the agent into a new host.
- **Non-Pharmacological Interventions (NPIs):** NPIs are evidence based non-invasive, mostly policy/regulation driven interventions on human health. NPIs (i.e. Physical ["Social"] distancing) can be very effective to contain viral shedding.

in the epidemiological trends of the 2002-2003 SARS-CoV outbreak and the ongoing SARS-CoV-2 pandemic are more likely due to other factors such as high viral loads in the upper respiratory tract and the potential for individuals infected with SARS-CoV-2 to shed and transmit the virus whilst asymptomatic [17-19]. Overall, the findings indicate that continued aerosol and fomite transmission (see **Box**) of SARS-CoV-2 is highly plausible as the virus remains viable and infectious in droplets for numerous hours and on surfaces for up to three days [17]. This has now raised the concern that airborne transmission might be occurring [17], though epidemiological evidence rules out the relevance of such transmission to disease spread [20,21].

## 3. Genomics of SARS CoV-2

SARS-CoV-2 is a β-coronavirus similar to the viruses that cause SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome). Human coronaviruses are not new and have been identified in the population since the late 1960s, causing mild symptoms similar to common colds [22]. Of the seven strains known, four infect the upper respiratory tract and cause mild symptoms, while three are associated with the lower respiratory tract, causing severe disease, including SARS-CoV, MERS-CoV, and now SARS-CoV-2. Like other coronaviruses, SARS-CoV-2 is an enveloped, single-stranded, positive-sense RNA virus with a non-segmented genome ~30 kb in size [12,23] (**Figure 1**). The viral genome codes for 16 non-structural proteins (Nsps) required for virus replication and pathogenesis, four structural proteins, including envelope (E), membrane (M), nucleocapsid (N), and spike (S) glycoprotein important for virus subtyping & response to vaccines, and nine other accessory factors [23,24] (**Figure 1**). The first SARS-CoV-2 genome was published on 24th January 2020, just a few weeks into the outbreak [25] and exhibited genomic and phylogenetic similarity to SARS-CoV, particularly in the S gene and receptor-binding domain (RBD), indicating the capability of direct human-to-human transmission. The genomic sequence of SARS-CoV-2 shows that although it is 75-80% identical to SARS-CoV [4,12], it is even more closely related to several bat coronaviruses, in particular the Bat SARS-related coronavirus SARSr-CoV RaTG13 [25]. Phylogenetic

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analyses of SARS-CoV-2 genomes have identified bats as the primary reservoir of SARS-like coronaviruses [26] displaying high sequence similarity (96.2%) between BatCoV and SARS-CoV-2

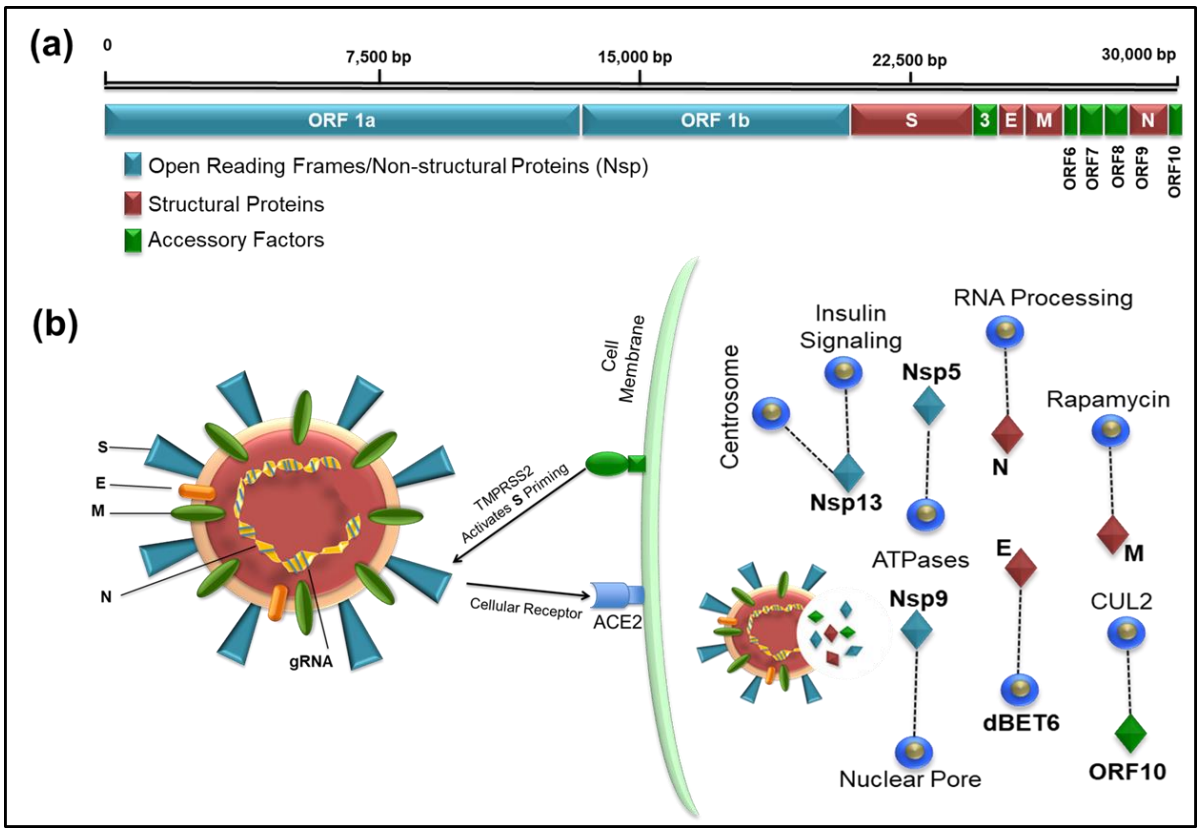


**Figure 1.** (**a**) Illustration of the full-length genome of SARS-CoV-2 showing the location of open reading frames 1a and 1b encoding the Non-structural proteins, Nsp (blue), structural proteins (brown), and accessory factors (green). The numbers on top refer to the genomic RNA. (**b**) Schematic representation of the SARS-CoV-2 virus particle and its interaction with its host cellular receptor, ACE2. The infection pathway is shown where after docking of the virus particle on cell surface, the TMPRRSS2 cellular protease activates the viral protein S allowing entry of SARS-CoV-2 into human cells. The protein coded by the viral genes and some of the notable interactions (dashed line) with other host proteins are shown that can potentially be targeted by drugs (blue circles).

genomes. [27] Sequence analysis of the viral spike protein further suggests new mutations in its RBD determine not only the host range but also the cellular tropism of the virus [2,28-30]. Interestingly, a similar observation was made in viruses from pangolin SARSr-CoVs, one of the putative intermediate host species that may have been used by SARS-CoV-2 for its species jump into humans [31]. A few months prior to the emergence of SARS-CoV-2, the Pangolin-CoV whole genome was sequenced from a dead Malayan Pangolin (*Manis javanica*) that showed 91.02% and 90.55% identical genome sequences to SARS-CoV-2 and BatCoV RaTG13, respectively [32]. The sequence analysis also revealed that the S1 protein of Pangolin-CoV was much more closely related to SARS-CoV-2 than to RaTG13. Whilst these findings suggest Pangolin species as a reservoir of coronaviruses, the analysis does not prove the potential of Pangolin as the intermediate host of SARS-CoV-2.

Sequences of SARS-CoV-2 have now been reported from many parts of the world and this data has proved useful in tracking the global spread of the virus [33] (see Table for resources related to genomics). For example, a recent analysis of 103 SARS-CoV-2 genomes has identified two major

subtypes (designated L and S) that are well-defined by two different single nucleotide polymorphisms (SNPs) [31]. This rapid evolution of SARS-CoV-2 is not surprising as coronaviruses habour error-prone RNA-dependent RNA polymerases which make the occurrence of mutations and recombination events rather frequent [34-37]. Within Wuhan, China, the L type was found in ~70% of cases and was observed to be the more aggressive and contagious form compared to the original S type [31]. The virus has further mutated and expanded into numerous strains. The geographical diversity of different strains may help correlate COVID-19-related severity, mortality rate, and treatment options. Genomic epidemiology of SARS-CoV-2 should also shed light on the origins of regional outbreaks, global dispersal, and epidemiological history of the virus [38]. More importantly, in case of an inability to diagnose infections empirically due to the speed of epidemics or lack of test kits, such as the case with COVID-19, genomic epidemiology could be used to estimate virus rate of replication in the population as well as burden of infection, allowing healthcare professionals to make urgent policy decisions appropriately. Thus, there is ongoing work geared towards mapping the spread of different SARS-CoV-2 strains across the world.

## 4. Transcriptomic Map and SARS CoV-2-Human Protein-Protein Interactions to Identify Drug Targets

The transcriptome profile of SARS-CoV-2 isolated from COVID-19 patients has recently been constructed using both "long read DNA/RNA (Nanopore) sequencing" and "sequencing by synthesis" techniques [23] (see **Table** for SARS-CoV-2 sequencing and OMICs related resources). Direct RNA sequencing (without requiring reverse transcription) has further allowed detection of RNA modifications on the genomic RNA (**Table**). By combining both sequencing and RNA modification data, scientists in South Korea have identified 41 potential RNA modification sites that could be important for virus replication and its associated pathogenesis [23]. Thus, transcriptomic insights should further provide a better understanding of the viral life cycle and its virulence.

After cell entry, the virus RNA transcript produces nonstructure proteins (Nsp1 through Nsp16) using two open reading frames (ORF1a and 1b, **Figure B**). A recent study on protein-protein interaction mapping using mass spectrometry identified 332 SARS-CoV-2-human protein interactions, including 69 interactions that can be targeted by existing FDA-approved drugs [24] (**Table**). We observed one interesting similarity in both the transcriptomic and proteomic studies, where the last reading frame (ORF10) expression was extremely low. Although the transcriptomic study questioned the annotation of ORF10 due to extremely low RNA expression, the proteomic analysis identified strong interaction of ORF10 with CUL2 complex, an E3 ubiquitin-protein ligase complex that mediates ubiquitination of target proteins [23,24]. This suggests that the virus may be able to subvert this complex and use it for degradation of host restriction factors that limit virus replication, making it a good target for drug development against the virus.

In humans, the *ACE2* gene encodes the angiotensin-converting enzyme-2. Evidence from recent studies suggests that ACE2 is the host receptor for the novel SARS-CoV-2 similar to SARS-CoV [39,40]. The binding of SARS-CoV-2 to the ACE2 receptor (via the S protein) [40] is 10-20 folds higher compared to SARS-CoV, which may be one of the reasons for the higher human-to-human transmission of SARS-CoV-2. The binding between SARS-CoV-2 and ACE2 has been confirmed by multiple recent independent studies [24,39]. ACE2 is found in the lower respiratory tract of humans on epithelial cells lining the lung alveoli and bronchioles as well as the endothelial cells and myocytes

of pulmonary blood vessels, partly explaining the severe respiratory syndrome associated with these viruses [41]. ACE2 is also found on the enterocytes in the small intestines, that may further explain the gastrointestinal symptoms associated with the viral infection as well as its detection in faeces [42]. In a recent study, it has been shown that the *ACE2* gene displays single nucleotide polymorphims with differential allele frequency accross the globe [43]. The allele frequency for the host gene was also shown to be different between males and females.

The viral spike (S) protein is responsible for viral entry into susceptible cells by interacting with the ACE2 receptor[25]. This process requires "priming" of the S protein by the host transmembrane serine protease 2 (*TMPRSS2*) which cleaves the S protein into two functional subunits: S1 and S2. The S1 subunit then is able to interact with the ACE2 receptor, while the S2 subunit facilitates viral fusion with the host cell membrane, allowing virus entry into the target cell [39] (**Figure A**). The current knowledge of the cellular infection pathway involving ACE2 and TMPRSS2 thus provide good candidates for therapeutics, such as antibodies that can interfere with virus attachment and fusion with target cells (such as protease inhibitors).

## 5. Molecular Diagnosis of COVID-19

As the COVID-19 pandemic continues to spread rapidly, there is a growing demand for rapid point-of-care testing of the virus. The current gold standard for diagnosing COVID-19 depends upon detection of the viral genetic material (RNA) in a nasopharyngeal swab or sputum sample. While this technique is sensitive and can detect the virus earlier in the infection, it requires polymerase chain reaction (PCR), a technology that amplifies the amount of genetic material to detectable levels and takes several hours to perform [44]. In recent weeks, rapid molecular tests using automated platforms have received fast-track approvals from regulatory authorities. These are high throughput automated tests with a turnaround time of 45-60 minutes.

To detect newer mutated strains, it is essential to apply next generation sequencing to identify the viral genome with specific mutations. Currently, 'sequencing by synthesis' technique (by Illumina Inc.) and 'long read sequencing' (by Oxford Nanopore Technology) are being used to identify viral genomes at single-base resolution levels [12,23,31]. Nanopore sequencing technology might have an advantage over other sequencing platforms due to its small and compact size, allowing flexibility of conducting DNA/RNA sequencing in the field in remote locations that lack full-fledged accredited reference laboratories.

Besides targeting the genome, another diagnostic approach for SARS-CoV-2 aims at detecting antibodies produced by the patient's immune system against the virus. Several such "antibody" tests have been reported over the past few weeks for SARS-CoV-2 [45]. Although the antibody associated tests are faster, their use for diagnosis is limited by the fact that it usually takes several days and up to two weeks after an infection takes place for antibodies to be detectable. Therefore, antibody-based testing is not a reliable method to diagnose COVID-19; however, they may be useful for population-based testing to estimate the proportion of the population with immunity and identifying susceptible individuals. Such information may also be useful for public health measures, including return-to-work protocols and social segregation of susceptible individuals. A third type of testing relies on detecting viral proteins (antigens) [46] likely to be useful since they do not depend on a detectable rise in patient-produced antibodies. Globally, several companies are working on developing such

rapid antigen-antibody-based and CRISPR-Cas12 based assays which have received rapid emergency use authorization by respective regulatory agencies [47].

A comprehensive list of SARS-CoV-2 diagnostic assays (both molecular and immunological) that have been commercialized and those under developments globally can be found at: https://www.finddx.org/covid-19/pipeline/.

## 6. Vaccine Development for SARS-CoV-2

No vaccines currently exist (March 29th, 2020- date of manuscript submission) to prevent SARS-CoV-2 infection. However, many efforts are in progress, including the classical inactivated and attenuated vaccines, the subunit and viral vector-based vaccines, as well as the newer DNA- and RNA-based vaccines. Each approach has its own advantages and disadvantages and all approaches are being developed simultaneously to come up with an effective vaccine.

Among the four structured proteins, the spike protein is considered the most promising for vaccine development since i) it is common to different coronaviruses encountered, and ii) is exposed to an individual's immune system, allowing the body to make an immune response against it and remember it for future protection. Furthermore, such a vaccine can prevent infection since it would inhibit virus entry into susceptible cells. Due to their previous experience with vaccine development for SARS in 2003 (against SARS-CoV), scientists have had a head start in using the S protein for vaccine development and one such vaccine has already entered human clinical trials, while others are on their way.

So far, the first vaccine to go into clinical trials is the mRNA-1273 vaccine. It is a novel RNA-based vaccine which uses part of the genetic code of the spike protein embedded in special lipid-based nanoparticles for injection into the body [48]. It has been developed by the biotech firm Moderna Therapeutics who was already working on SARS-CoV and MERS-CoV vaccines which were adapted to SARS-CoV-2. The RNA part of the vaccine instructs cells to express the S protein to elicit the immune response. After having shown potential in animal testing, the first Phase I clinical trial of this vaccine started on March 16, 2020 in collaboration with the NIH on 45 healthy individuals between the ages of 18-55 years. Several other mRNA-based vaccines (e.g. by CureVac, BNT162 by BioNTech & Prifzer) are in different stages of development. The BioNTech mRNA vaccine (Mainz, Germany) encapsulates the nucleic acid in special 80 nm ionizable, glycol-lipid nanoparticles. Clinical testing is expected to start in April, 2020 [48].

Another vaccine that is ready for clinical trials in China has been developed by CanSino Biologics (Tianjin, China), the company that also has developed and marked a vaccine for Ebola. The vaccine based on their adenovirus vaccine platform, is named Ad5-nCoV. It is entering phase I clinical trials in healthy individuals between 18-60 years of age in Wuhan, China. According to the Chinese Registry of Clinical Trials: (http://www.chictr.org.cn/showproj.aspx?proj=51154)

The Precision Vaccines Program (PVP) at Boston Children's Hospital is developing a novel vaccine designed especially for the older population who are at the greatest risk of developing severe complications (https://discoveries.childrenshospital.org/coronavirus-vaccine/). The study is funded by the US National Institutes of Health and the vaccine is testing efficacy of different adjuvants (small molecules added to vaccines to boost immune response) along with the S protein. Since older adults have weakened immune systems, the goal is to generate a robust, longer lasting, and broader immune

response against the S protein in the older aged population. Towards this end, the vaccine is currently being tested using age-specific human *in vitro* systems to expedite the process.

Other than these, there has been an acceleration in developing other novel vaccine approaches and therapeutic interventions to combat viral infection [48]. Some of these approaches in advanced stages of development and/or testing are summarized below.

***Contemporary and Novel Approaches to Vaccine Development***

- ***INO-4800:*** a DNA-based vaccine by Inovio Pharmaceuticals, a Pennsylvania Biotech company. Phase I clinical trials are expected to start in April in the US followed by China and South Korea. Funding from Bill and Melinda Gates Foundation is accelerating its testing and scale-up for intradermal delivery using electroporation along with collaboration with the Beijing Advaccine Biotechnology.
- ***Codagenix Biotech:*** Is developing a computationally designed and re-coded live-attenuated SARS-CoV-2 vaccine in which sequence of the target gene of interest has been changed by swapping its optimized codons with non-optimized ones. Codagenix is collaborating with Serum Institute of India to scale-up manufacturing of this vaccine.
- ***Clover (Sichuan) Biopharmaceuticals:*** Is developing a recombinant SARS-CoV-2 S protein trimer subunit vaccine and is in preclinical testing with GlaxoSmithKline's Pandemic Adjuvant Technology.
- ***Sanofi Pharmaceuticals:*** Is proceeding with preclinical and clinical trials using a recombinant vaccine of undisclosed SARS-CoV-2 protein(s) expressed in baculovirus system to mount immune response.
- ***Johnson & Johnson (Jansen):*** In partnership with Biomedical Advanced Research and Development Authority (BARDA) is also developing single-dose, intranasal, recombinant adenovirus-based vaccine by expressing an undisclosed viral protein to stimulate the immune system using human retinal cells. The vaccine is currently being tested in animals.
- ***Altimmune, Inc.:*** Is currently testing a one-dose, intranasal, replication-defective, adenovirus-vector-based vaccine incorporating the SARS-CoV-2 S protein. The vaccine is currently being tested in animals.
- ***Novavax:*** Is developing a nanoparticle-based vaccine that displays the S protein with saponin-based Matrix (M) protein adjuvant. This vaccine stimulates entry of antigen-presenting cell into the injection site and enhances antigen presentation in local lymph nodes to boost the immune response.

***Other Experimental Therapeutic Interventions***

- **AbCellera:** This Canadian biotech has discovered >500 unique antibodies from sera of a convalescent COVID-19 patient and in partnership with Eli Lilly is developing purely human IgG1 mAbs-based treatments for coronavirus infection.
- **Alnylam Pharmaceuticals** has developed technology for delivering aerosolized siRNAs against SARS-CoV2 to deliver directly to lungs which is being tested both *in vitro* and *in vivo*.
- **Apeiron Biologics** is employing recombinant protein strategy by designing recombinant ACE2 enzyme (APN01) allowing it to bind to virus particles in circulation thus making them refractory for entering into the cells.

- **InflaRx and Beijing Defengrei Biotechnology** are using human IgG1 mAbs against complement factor 5a. Antibodies from both companies have been approved for clinical trials in China.
- **NanoViricides** are being created in another approach in which the S protein is chemically attached to "virucidal nanomicelles". Testing is in progress in culture only.

An extensive list of vaccines under developments, their current status, and other therapeutic interventions can be found at: https://www.nature.com/articles/d41587-020-00005-z.

## 7. Drug Repurposing for COVID-19

Given the need to find effective treatment for symptomatic patients, the approach of repurposing old drugs with antiviral properties and agents approved or under investigation for other viral infections has been adopted. In the abscence of a vaccine, the World Health Organization recently launched the SOLIDARITY trial which is an international clinical trial to address this challenge. The drugs included in this trial are lopinavir and ritonavir, lopinavir and ritonavir plus interferon beta as well as chloroquine, and remdesivir. The role of existing antiretroviral drugs and pathways are as follows:

- **Lopinavir-Ritonavir combination (Kaletra)**: This is an FDA-approved drug for HIV treatment. Lopinavir is a protease inhibitor that inhibits a late step in viral replication, while ritonavir helps boost the activity of lopinavir by inhibiting CYP3A liver enzymes that slows down the rate at which lopinavir is broken down in the liver. Findings from *in vitro* work indicates its potential for COVID-19 treatment. In China and South Korea, Lopinavir-Ritonavir has been used either on its own or in combination with either alpha interferon (China) or chloroquine/hydroxychloroquine (South Korea) for COVID-19 treatment with some success. However, new data from China reported in the New England Journal of Medicine on Wednesday 18th March 2020, casts doubt on the beneficial effect in seriously ill COVID-19 patients [49].
- **Favipiravir (Favilavir or Avigan):** This is an RNA-dependent RNA polymerase inhibitor developed by Fujifilm Toyama Chemical's in Japan that is safe and has been effective in other viral infections, including influenza. It has now been found to be useful against SARS-CoV-2 as tested in clinical trials in Wuhan and Shenzhen involving 340 patients. In addition to speeding up viral clearance from the body from 11 to 4 days, it ameliorates lung injury.
- **Chloroquine/Hydroxychloroquine:** Chloroquine is an inexpensive drug for the treatment of malaria and features on the WHO list of essential medicines. It is also used as an anti-inflammatory agent for the treatment of autoimmune diseases. Chloroquine is thought to inhibit virus replication by increasing endosomal pH as many viruses such as Ebola and Marburg that require the acidic environment of the endosome for successful replication. However, a recent study showed that the anti-inflammatory effects of chloroquine are mediated by upregulation of the cyclin-dependent kinase inhibitor, p21 [50]. *In vitro* studies have shown its potent antiviral effect against the SARS-CoV-2 [51]. A multicenter clinical trial in China has reported efficacy with amelioration of exacerbation of pneumonia and acceptable safety margin with use of chloroquine for treatment of COVID-19 [11]. Hydroxychloroquine is an analogue of chloroquine which is more stable with better clinical safety profile and has anti-SARS-CoV-2 activity. It has been shown to quicken recovery and clearance of the virus in COVID-19 patients and used successfully

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in combination with the macrolide antibiotic azithromycin [52]. Larger studies with controlled design are needed before conclusive recommendations for chloroquine in the treatment of COVID-19 can be made.

- **Remdesivir:** Remdesivir is a nucleotide analogue with broad spectrum antiviral activity against many RNA viruses. Like Favipiravir, it blocks RNA-dependent RNA polymerase, an enzyme that replicates the viral genome, inhibiting an early step in virus replication, compared to protease inhibitors that target the late steps of virus replication. This is an investigational drug developed by Gilead Sciences for the treatment of Ebola, MERS-CoV, and SARS-CoV2, and other RNA viruses. Currently, it is not approved for any of the diseases.

  **SNG001**: SNG001 is an inhaled experimental drug developed by the UK biotech firm Synairgen. The ability to inhale the drug will allow the patients to "self-administer" it by using a small hand-held nebulizer. It was developed for the severe lung disease chronic-obstructive pulmonary disorder (COPD), but due to the current COVID-19 crisis, it has been fast-tracked for use in a 100-patient clinical trial that will be starting soon.
- **Tocilizumab:** Tocilizumab was developed by the Tokyo-based Chugai and Roche of Basel, Switzerland. It is an immunosuppressive drug (antibody) against IL-6 receptor that blocks IL-6 production during the cytokine storm observed in severely ill COVID-19 patients.
- **Kinases:** p21-activated protein kinases (PAKs) are cytosolic serine/threonine protein kinases downstream of small (p21) GTPases, including members of the Cdc42 and Rac families. Multiple studies have shown that the major pathogenic kinase in this group, PAK1, plays a major role in the entry, replication and spread of several important viruses, including influenza and HIV [53,54]. Coronaviruses exploit macropinocytosis to gain entry into cells and this process has been shown to be dependent on PAK1 activity [55,56]. Targeting of PAK1 to prevent micropinocytosis has been implicated for therapeutic intervention [57]. This strongly suggests that PAK1-inhibitors could be valuable for the treatment of COVID-19 infection. PAK-1 inhibitors include caffeic acid and its ester, propolis, ketorolac and triptolide. Unfortunately, all these have problems with solubility and cell penetration. However, newer PAK-1 inhibitors, such as 15K (the 1,2,3-triazolyl ester of ketorolac, that is 500 times more potent at inhibiting PAK1 than the parent compound [58], minnelide (in which a hydroxyl group of triptolide is phosphorylated, boosting its water-solubility over 3000 times [59], and frondoside A [60] are much more potent and may be of value in suppressing the effects of this virus.

## 8. Non-Pharmacological Interventions

At present, there are no vaccines or specific pharmacological interventions available to contain the horizontal transmission of SARS-CoV-2. Moreover, effective COVID-19-specific pharmaceutical interventions and vaccines are not expected to be available for 3-12 months [3]. Therefore, the most effective public health response to the ongoing outbreak is to follow non-pharmacological interventions (NPI) such as early case identification and isolation, vigilant contact tracing of potential secondary cases, travel restrictions & bans, stringent contact reductions, physical ("social") distancing, improved hygiene and continuous hand wash. Such an approach requires closure of non-essential public spaces, services and facilities, a transition to digital learning modalities for educational institutions, and self-isolation/work from home initiatives for businesses. Modelling estimates indicate that integrated NPIs are likely to achieve the strongest and most rapid effect, if

implemented early in the outbreak [61]. These NPIs are interim measures as the quest for better understanding of the viral genomics continues and the information garnered unlocks the doors for development of effective therapeutic interventions and vaccines.

## 9. Future Directions for COVID-19 Research

The global efforts to contain the COVID-19 pandemic are primarily aimed to reduce the number of infected persons, minimize the excessive burden on healthcare systems, and reduce the social and economic impact of the pandemic. These efforts will provide the much-needed respite during the period required for the development, testing and approval of an effective vaccine. Until vaccines are available, it is likely that non-pharmacological interventions will remain the primary line of defense to contain this pandemic. Therefore, accurate and up-to-date data on the daily number of new cases and the case characteristics can inform modeling of future projections of new cases and planning for anticipated healthcare capacity. Timely and accurate national data on hospital bed and intensive care capacity along with daily census is essential for such planning. COVID-19 will have a significant global impact on the social, cultural and economic infrastructures that are envisaged to be long lasting and may take many years to recover. Healthcare systems should consider integrating effective regulatory measures to tackle future pandemics. This crucial lesson was learnt by countries which experienced the previous SARS-CoV outbreak and informed the response to this pandemic in Hong Kong, Singapore, and Taiwan, for example. Genomic characterization will have implications related to pathogenicity, transmissibility and response to therapy of the viral isolates for local and global populations. The understanding of the genetic makeup of the viral strains is also critical for drug discovery and designing of effective vaccines. To better prepare for the next global pandemic, application of artificial intelligence (AI) should be evaluated to track infections before the outbreak happens. Bluedot Inc., a Canadian AI company for infectious diseases, flagged unusual infection related activity in Wuhan, China and reported the spread nine days before WHO officially declared the outbreak [62]. In this era of emerging viral infections, the global community must work together and deploy the very best of its technological resources to address the current pandemic and ensure preparedness for future outbreaks.

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