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REVIEW

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Problems associated with antiviral drugs and vaccines development for COVID-19: approach to intervention using expression vectors via GPI anchor

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ABSTRACT

The outbreak of a novel coronavirus responsible for the severe acquired respiratory syndrome: SARS-CoV-2, also known as coronavirus disease 2019: COVID-19, represents a pandemic threat that has been declared a public health emergency of international concern. The CoV spike (S) glycoprotein is a key target for diagnostic, development of antibodies, entry inhibitors, and vaccines. COVID-19 also recognizes angiotensin-converting enzyme 2 (ACE2) as its host receptor binding to viral S protein. Several antiviral drugs and vaccines have been evaluated for the treatment and prevention of the infection by the virus. To facilitate medical countermeasure development, the problems associated with antiviral drugs and vaccines development for containing the spread of COVID-19 are discussed. There is an urgent need to study deeply on the structure, mutations, and function of COVID-19 as well as its pathophysiology from a large population. Construction of expression vectors for any protein targeting to the cell plasma membrane via the glycosyl-phosphatidylinositol, GPI, anchor for studying intermolecular interactions, as described in Ref. # 62 (Nguyen, K. V., Naviaux, R. K., Nyhan, W. L. Lesch-Nyhan disease: I. Construction of expression vectors for hypoxanthine-guanine phosphoribosyltransferase (HGprt) enzyme and amyloid precursor protein (APP). *Nucleosides Nucleotides Nucleic Acids* 2020, 39, 905-922), between the S protein of COVID-19 as well as its variants and ACE2 could be useful in antiviral drugs and vaccines development.

1. Introduction

The spike (S) glycoprotein (or just S protein or "spike protein") is the familiar spike that studs the surface of the coronavirus, giving it the appearance of a crown to electron microscopy, hence "corona" (Latin: crown). Coronavirus have been known to medicine for some time,[1] but it

is only very recently that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as coronavirus disease 2019: COVID-19 virus, new and dangerous to humans, was identified. The disease name COVID-19 was recommended by the World Health Organization (WHO) and the SARS-CoV-2 was the scientific name of the new strain of coronavirus recommended by the International Committee on Taxonomy of Viruses. The S protein of COVID-19 plays the most important roles in viral attachment, fusion and entry, and it serves as a target for diagnostic, development of antibodies, entry inhibitors, and vaccines. The S protein mediates viral entry into host cells by first binding to host receptor through the receptor-binding domain (RBD) in the S1 subunit of S protein and then fusing the viral and host membranes through the S2 subunit of S protein.[2] COVID-19 emerged in December 2019 likely from the Huanan seafood market in Wuhan, China,[2] but it was likely circulating unnoticed around the Wuhan area for at most two months before the first human cases of COVID-19 were described in Wuhan in late-December 2019. COVID-19 has been shown to be closest related (with ∼ 88% genome sequence identity) to two bat-derived SARS-like CoVs (bat-SL-CoVZC45 and bat-SL-CoVZXC21), with ∼ 79% overall sequence identity to SARS-CoV and ∼50% to the middle-east respiratory syndrome CoV (MERS-CoV). SARS-CoV and SARS-CoV-2 are betacoronaviruses of lineage B, and MERS-CoV is the first betacoronavirus belonging to lineage C that is known to infect humans. The sequencing results were used for CoV species classification and the high genome sequence identities between SARS-CoV, MERS-CoV and COVID-19 suggest an evolving similarly in virological properties.[2–6] Civets are the intermediate host of SARS-CoV, whereas dromedary camels are the intermediate hosts of MERS-CoV. However, the intermediate hosts of COVID-19 have not been determined. At present, the prevailing viewpoints suggest Malayan pangolins and turtles.[2–6] Considering the relatedness of COVID-19 to SARS-CoV, some drugs and preclinical vaccines against SARS-CoV could probably be used to treat this virus.[2–6] At the present time, any mention of the number of cases, affected countries, and case/fatality ratio will be outdated at print time. There is then an urgent need to develop antiviral drugs as well as specific vaccines for containing the spread of COVID-19. For such a purpose, several research works have been performed. This article summarizes the problems associated with antiviral drugs and vaccines development for COVID-19, and concludes by a need to study deeply on the structure, mutations, and function of COVID-19 as well as its pathophysiology from a large population in which the construction of expression vectors via the glycosyl-phosphatidylinositol, GPI, anchor allowing to studying intermolecular interactions between the S protein of COVID-19 as well as its variants and ACE2 could be useful in antiviral drugs and vaccines development.

2. Antiviral drugs and vaccines development for covid-19

2.1. COVID-19 overview

2.1.1. Biological features

Coronaviruses (CoV) are enveloped, single-stranded, 5′-capped, positive-strand RNA viruses of the order Nidovirales, with the genome sizes ranging from 26 to 32 kilobases.[2] They have been identified across a range of avian and mammalian hosts, but did not attract much attention until November 2002, with the emergence of severe acute respiratory syndrome CoV (SARS-CoV) from Guangdong in southern China,[2–6] resulting in 8,096 confirmed cases of infection and 774 deaths across 27 countries (with a case-fatality, CFR, of about 10%). In September 2012, a second human pathogen: middle-east respiratory syndrome CoV (MERS-CoV), emerged in Saudi Arabia,[2–6] causing 2494 confirmed cases of infection with 858 deaths across 27 countries (CFR of about 34.4%). COVID-19 raised intense concerns not only within China but also internationally. According to recent data from the WHO, Centers for Disease Control and Prevention (CDC), and reports to the WHO from various countries and their allies agencies, as of July 22, 2020, a total of 14,562,550 confirmed cases of COVID-19 were reported, including 607,781 deaths, in China and at least 216 other countries and/or territories (CFR of about 4.2%).[2–6] In a report from the WHO (https://www.who.int/emergencies/diseases/novel-corona-virus-2019/situation-reports), Europe and the Americas became the locations of the most serious epidemics, instead of China, which had previously been the location for the most serious outbreaks. In China, people with overseas exposures have become the high-risk population, as with Wuhan-related exposures. COVID-19 is highly contagious, and the speed of the spread and the infectivity of COVID-19 dramatically exceeded those of MERS-CoV and SARS-CoV. The basic reproduction number (R0) reflects the rate of disease transmission. Recent data revealed an R0 for COVID-19 of 2.56, indicating that one patient could transmit the disease to 2,56 other people. Male sex and older age (whose immune systems get weaker with age) are two significant risk factors. Generally, the males generate mild immune responses and females mount stronger innate and adaptive immune responses and are relatively resistant to virus infections, which explain why males and females showed different response patterns after infection of viruses. This gender-dependent has a biological explanation: estrogen can stimulate an immune response, whereas testosterone can blunt it. In addition, many immune-related genes are on the X chromosome, of which women have two copies and men have only one. These differences may help explain why far more women than men are afflicted with auto-immune disease, which occurs when a robust immune response attacks the

body's healthy tissue. Health workers are one of the high-risk groups. Diabetes could be one of the risk factor for progression to severe/critical outcomes. Human-human transmission is considered a major transmission mode of COVID-19. The driving transmission of COVID-19 contains droplet transmission, contact transmission and aerosol transmission.[2–6] The RNA viral includes at least six open reading frames (ORFs). The first ORF (ORF1a/b) comprises approximately 2/3 of the genome and encodes replicase proteins, and the remaining ORFs mainly encode four structural proteins: spike (S), membrane (M), envelope (E) and nucleocapsid [N]. The N protein is important for the virus capsid and modulates the initial innate immune response by inhibiting type I interferon (IFN) production. The M protein and the E protein are involved in virus morphogenesis, assembly and budding. The S protein mediates virus entry into cells. The genome organization of COVID-19, SARS-CoV and MERS-CoV are determined and the major distinctions between COVID-19 and SARS-CoV are in open reading frame-3b (orf3b), spike and open reading frame-8 (orf8), especially in spike S1 and orf8.[2–6]

2.1.2. Clinical symptoms and virulence

COVID-19, SARS-CoV, and MERS-CoV show several similarities regarding their symptoms, which include fever, cough, myalgia, fatigue and lower respiratory signs. However, the symptoms vary with the state of the illness and in the process of disease progression. Notably, 60% of patients suffering from SARS-CoV have watery diarrhea in addition to the abovementioned symptoms with the characteristics that there is a representative biphasic clinical course. Patients who suffer from MERS-CoV have symptoms that include fever, cough (predominantly dry), malaise, myalgia, nausea, vomiting, diarrhea, headache and even renal failure. Not surprisingly, the symptoms of MERS-CoV resemble SARS-CoV, but the clinical course is unpredictable and changeable. More than half of the MERS-CoV patients are reported to develop acute renal damage at an average time of approximately ten days after the onset of symptoms; additionally, the majority of the cases require renal replacement therapy. The majority of patients with COVID-19 infections presents with fever (98%), cough (76%), and myalgia or fatigue (44%). It has been reported that 55% of patients can present with dyspnea, which develops a median of eight days after the onset of initial symptoms. In light of the studies from all over the country, the symptoms suggest that the target cell is likely present in the lower respiratory tract, as patients who are infected with COVID-19 seldom have conspicuous upper respiratory symptoms such as sneezing or sore throat. The autopsy reports of new coronavirus pneumonia (NCP) patients indicate that the disease mainly causes distal airway inflammatory reactions and alveolar

damage, which is coincidental with the abovementioned symptoms. SARS-CoV and MERS-CoV recognize different receptor. SARS-CoV recognizes angiotensin-converting enzyme 2 (ACE2) as its receptor, whereas MERS-CoV recognizes dipeptidyl peptidase 4 (DPP4) as its receptor.[2–6] Similar to SARS-CoV, COVID-19 also recognizes ACE2 as its host receptor binding to viral S protein.[2–6] Both ACE2 and DPP4 are recognized via the C-terminal domain in S1 (S1-CTD) as a RBD. ACE2 binds to the COVID-19 S ectodomain with 15 nM affinity, which is approximately 10- to 20-fold higher affinity than ACE2 binding to to SARS-CoVS.[2–6] As previously mentioned, S1 is one of the major distinctions between COVID-19 and SARS-CoV. These realizations lead one to wonder how these viruses can recognize the same receptor of the host cell despite their inherent differences. The RBD of SARS-CoV contains two subdomains: a core and an extended loop. The core is constructed of a five-stranded anti-parallel β sheet (β1 to β4 and β7) and three short connecting α helices (αA to αC). The extended loop subdomain is positioned to one side of the core, and a two-stranded β sheet (β5 and β6) forms a gently concave outer surface, the base of which cradles the N-terminal helix of ACE2. Although only 9 out of 13 glycans in the S1 subunit are conserved among COVID-19 S and SARS-CoV S, their overall structures are similar, and the most notable difference is the position of the RBDs in their respective down conformations: tightly against the N-terminal domain (NTD) in SARS-CoV and angled closer to the central cavity of the trimer in COVID-19. In contrast to SARS-CoV and other SARSr-CoVs, there is a four amino acid residue insertion at the S1/S2 boundary of COVID-19 S that results in the presence of a furin cleavage site. The S1-S2 site cleaved during biosynthesis is not necessary for S-mediated entry, but it may contribute to the high affinity of COVID-19 S for human ACE2.[2–6] Expression of the ACE2 receptor is found in many tissues, including lung, heart, kidney, liver, endothelium, intestine, oral mucosa and even testis. ACE2 is reported to improve acute lung injury, suppress hypertension and cardiac dysfunction, reduce glomerular and biliary fibrosis, and stimulate brown adipose tissue. All these factors could be targets for COVID-19 to damage human health. The lungs are the main target organs of COVID-19. According to recent research, ACE2 is expressed in 0.64% of all human lung cells, and the majority of them are type II alveolar cells (AT2) (average 83%). Consistent with these findings, COVID-19 presents as lesions involving mainly destruction of the distal alveoli. Other lung cells, such as type I alveolar cells (AT1), endothelial cells, airway epithelial cells, fibroblasts and macrophages, were also reported to express ACE2. Though their ratio is low and variable among individuals, they may also be the targets of COVID-19. Severe acute respiratory syndrome from COVID-19 also causes gastrointestinal

symptoms, and approximately 3% of patients develop diarrheal symptoms. According to several recent studies, acute kidney injury (AKI) has been reported in over 20% of patients who suffered from COVID-19 in China and the U.S. These outcomes may be attributed to ACE2 because ACE2 is expressed in intestine and kidney. Notably, ACE2 is also expressed via endothelial cells, and other major clinical events commonly observed in COVID-19 patients including high blood pressure, thrombosis, pulmonary embolism, cerebrovascular and neurologic disorders.[2–6]

2.1.3. Laboratory diagnosis

Laboratory diagnosis plays a leading role in the early detection of infected individuals, which enable an early discovery of the source of infection and interruption of epidemic transmission. The viral RNA, considered one of the gold standards of detection, can be found in the upper respiratory tract (URT) (collection of specimens via the oropharyngeal (OP) or nasopharyngeal (NP) swabs), lower respiratory tract (LRT) (collection of specimens via sputum, tracheal aspirate (TA), bronchoalveolar lavage (BAL) fluid, pleural fluid), stool, blood, saliva, and urine of patients who are infected with SARS-CoV, MERS-CoV, and COVID-19. Currently, nucleic acid tests (NAT) are widely considered the optimal method for diagnosis, since specific primers and standard operation procedure have been established during sequencing of the total genome of the coronavirus. In general, real-time polymerase chain reaction (RT-PCR) is thought to be the preferred and most widely used NAT method. The ORF1a, ORF1b, S gene, and N gene, in addition to the M gene and 3′ untranslated region (UTR), are all gene target of RT-PCR assays, which can have high sensitivity. The RT-PCR methods for SARS-CoV, MERS-CoV and COVID-19 varied in genome target, sequence, assay use, etc. Most of the in-house assays, as well as commercial kits, can detect two or three regions of the virus genome. However, there are many knowledge gaps and limitations to overcome, including the difficulties of obtaining testing kits due to the global shortage, the requirements of having access to sophisticated equipment, and the management of false negatives that need to be retested. At present, many NAT kits have been developed for COVID-19, especially RT-PCR. However, according to previous studies, the currently available RT-PCR kits are variable and offer sensitivities ranging between 45 and 60%. Otherwise, medical imaging technology that is commonly used to diagnose SARS-CoV, MERS-CoV and COVID-19 including chest X-ray (CXR), computed tomography (CT) and high resolution computed tomography (HRCT) also plays a vital role in the diagnosis.[2–6]

2.2. Antiviral drugs for COVID-19

Given the above findings, it is critical to define the RBD in COVID-19 S protein as the most likely target for the development of virus attachment inhibitors, neutralizing antibodies, and vaccines.[2–6] One problem is that COVID-19 is a new pathogen posing a global threat and so presents new challenges both in primary prevention, where a vaccine is required, and in secondary prevention, where a therapeutic compound (ideally, "in a pill") is required to treat patients who are infected. The rapid global emergence of COVID-19 outlines the importance of and immediate need for antivirals. Potential broad-spectrum targets include viral gene products that are widely conserved and do not exist in the host cell, or that are structurally and functionally different enough from cellular homologous to achieve selective inhibition. For RNA viruses, the RNA-dependent RNA polymerase (RdRp) presents an optimal target due to its crucial role in RNA synthesis, lack of host homolog and high sequence and structural conservation. The RdRP remains the target of choice for the treatment of several viral diseases, including chronic liver disease cause by hepatitis C virus infection. Due to the urgent need for effective treatments, there has been increased interest in re-purposing currently available drugs for immediate use. Recent compassionate clinical trials of remdesivir (Veklury, produce by Gilead Sciences Inc. and administered intravenously) (Clinical-Trials.gov: NCT04257656, NCT04252664, NCT04280705, etc.) have been conducted.[7,8] Remdesivir works against coronaviruses closely related to COVID-19 in animal models, as well as against the related MERS-CoV, including in non-human primates (NHPs). Remdesivir was also tested for treatment of ebolavirus infections in humans (and found to be less successful than other treatments[7]); therefore, safety data exist for this therapeutic agent, which should accelerate the process of clinical testing against COVID-19. Remdesivir's mechanism of action as a nucleotide analog, specifically an adenosine analogue, is not clear but it likely that it inserts into viral RNA chains, causing their premature termination.[7,8] Remdesivir targets then the reproduction of the virus by blocking the function of RdRp of the virus.[7,8] During the 2020 COVID-19 pandemic, given the preliminary results about remdesivir, the U.S. Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) on May 1, 2020 to permit the use of remdesivir for the treatment in adults and children hospitalized with suspected or laboratory-confirmed COVID-19.[8] Remdesivir must be administrated intravenously, which represents a limitation to its use. Remdesivir has also received full or conditional approval in several other countries since that time.[9] But updated guidelines from the WHO in November 2020 include a conditional recommendation against the use of remdevir for the treatment of COVOD-19 after a clinical study, called the Solidarity Trial, which is

substantially larger and includes 2,570 patients receiving remdesivir and 6,331 patients who are getting the usual standard of care and found no significant benefit for recovery or survival.[10] The WHO's conditional recommendation does not actually conclude that remdesivir has no benefit but rather there is not enough evidence to conclude that the drug reduces mortality.[10] Recently, a report from Beigel et al.[11] shows that remdesivir provides moderate clinical benefit in the treatment of patients with COVID-19. Lack of a decisive verdict on remdesivir is a slight distinction, but it could be enough for some doctors to continue using the treatment.[11,12] Remdesivir can cause gastrointestinal symptoms (e.g., nausea), elevated transaminase levels as well as an increase in prothrombin time, and hypersensitivity reactions. Liver function test and prothrombin time should be obtained in all patients before remdesivir is administered and during treatment as clinically indicated. However, given high mortality despite the use of remdesivir, it is clear that treatment with an antiviral drug alone is not likely to be sufficient for all patients. Current strategies are evaluating remdesivir in combination with modifiers of the immune response (e.g., the Janus kinase, JAK, inhibitor baricitinib in Adaptive COVID-19 Treatment Trial 2, ACTT-2; and interferon beta-1a in ACTT-3). The data showed that baricitinib plus remdesivir was superior to remdesivir alone in reducing recovering time and accelerating improvement in clinical status among patients with COVID-19, notably among those receiving high-flow oxygen of noninvasive ventilation. The combination was associated with fewer serious adverse events such as hyperglycemia, anemia, decreased lymphocyte count, and acute kidney injury.[13] However, it is important to note herein that DNA and RNA are so closely related that it can be difficult to make a drug that affects only one type of polymerase. Furthermore, we should be aware of the impact from the overuse of such antiviral agent on the viruses that have long lived in harmony with the human body and now play a role in regulation of human health.[14] Recently, a combination of the two licensed human immunodeficiency virus (HIV) inhibitors, lopinavir and ritonavir (produced by AbbVie as Kaletra and Aluvia, respectively), is also being tested in clinical trials (e.g., ClinicalTrials.gov: NCT04264858, etc.).[7] Lopinavir is a bona fide protease inhibitor, whereas ritonavir was initially designed as protease inhibitor but was found to boost the half-life of lopinavir by inhibiting cytochrome P450. The combination was compassionately used as treatment for SARS-CoV in 2003–2004 and showed some promise. Effectiveness of the combination was limited in mice but appreciable in NHP models of MERS-CoV. The mechanism of action of lopinavir is not clear, but it likely inhibits one or more coronavirus proteases. At present, there is no strong evidence for the efficacy of

lopinavir/ritonavir combination in the treatment of COVID-19, and there is insufficient evidence to recommend the use of these drugs for COVID-19 outside of research studies.[15,16] Other treatment options with ongoing or planned clinical trials include dosing recombinant human ACE2 to neutralize the virus and prevent lung damage (ClinicalTrials.gov: NCT04287686) and using the antiviral arbidol (Umifenovir), a fusion inhibitor.[7] The findings showed that arbidol significantly contributes to clinical improvements including peripheral oxygen saturation, requiring intensive care unit (ICU) admissions, duration of hospitalization, but further studies on arbidol against COVID-19 using a larger sample size and multicenter design are still needed.[7,17] Another interesting option is the use of convalescent serum as treatment[7]; clinical trials to test this are ongoing in China (ClinicalTrials.gov: NCT04264858), and compassionate use of this strategy has recently started in the United States, U.S. (e.g., at Mount Sinal Medical Center, NY). In such a case, treatment involves then administering antibodies from patients who have had COVID-19, as well as man-made ones such as monoclonal antibodies[7,18–20] have been helpful in both decreasing the mortality and whether people die from this disease, as well as decreasing their length of stay in the hospital. Both of theses therapeutics, if given early, can help neutralize infection, helping prevent more serious outcomes. The FDA issued the EUA to permit the use intravenously of monoclonal antibodies bamlanivimab, etesevimab, casirivimab, and imdevimab that target different parts of the SARS-CoV-2 spike for the treatment of COVID-19. These antibodies treatments, which are still expensive, are continuing to be studied extensively. However, there have been concerns that some antibodies treatments may not work as well if the virus has mutated.[21–23] However, so far it is unclear to what extent the new variants evade the antibody treatments. Further research is necessary to determine that. Similarly, polyclonal human immunoglobulin G (IgG) derived from transgenic cows could be used, because this strategy has been successful for MERS-CoV in animal models and has been tested for safety in clinical trials (ClinicalTrials.gov: NCT02788188).[7,23,24] Recent compassionate clinical trials of the anti-malaria drug chloroquine or hydroxychloroquine have been also conducted.[7,25] The data showed no potential benefits of these drugs for the treatment of COVID-19, and long-term and high-dose of these drugs can cause serious cardiac adverse events.[26,27] Finally, chloroquine and hydroxychloroquine are not approved via the FDA for the treatment of COVID-19. A variety of therapeutic approaches including novel antivirals, modifiers of the immune response or other intrinsic pathways, and combination approach are needed to continue to improve outcomes in patients with COVID-19.

2.3. Vaccine production for COVID-19

2.3.1. Vaccine production overview

Fighting the spread of the viruses once they are inside the cells (host) is not a good way because it would be harmful to the cells. The best way to contain the spread of the viruses is to block their entry into the cells. For such a purpose, vaccine production against the virus is the specific way of blocking. There are different ways to produce a vaccine[7] such as:

1- Weakened (live attenuated) and inactivated (killed) viruses: by using weakened viruses, they reproduce very poorly (fewer than 20 times) once inside the body and do not cause disease (viruses usually cause disease by reproducing themselves many times (thousands of times) in the body). The vaccines for measles, mumps, German measles (rubella), rotavirus, oral polio (not use in U.S.), chickenpox (varicella), and influenza (intranasal version) vaccines are made this way. The advantage of "weakened" vaccines is that one or two doses provide immunity life-long. The limitation of this approach is that these vaccines usually cannot be given to people with weakened immune systems (like people with cancer or acquired immuno-deficiency syndrome, AIDS); by using inactivated (or killed) viruses with a chemical, they cannot possibly reproduce themselves to cause disease. The inactivated polio, hepatitis A, influenza (shot), and rabies vaccines are made this way. There are two benefits to this approach: (a) the vaccine cannot cause even a mild form of the disease that it prevents; (b) the vaccine can be given to people with weakened immune systems. However, the limitation of this approach is that it typically requires several doses to achieve immunity. China's Sinovac (developed by the Chinese company Sinovac Biotech) and India's Covaxin (developed by the Indian company Bharat Biotech) are a coronavirus inactivated vaccines and they can be stored in a standard refrigerator at 2 °C–8 °C.

2-Protein (part of the virus): using this strategy, just one part of the virus is removed and used as a vaccine (protein-based vaccine). The hepatitis B, one shingles vaccine (Shingrix®), and the human papillomavirus (HPV) vaccines are made this way. The vaccine is composed of a protein that resides on the surface of the virus. This strategy can be used when an immune response to one part of the virus is responsible for protection against disease. These vaccines can be given to people with weakened immunity and appear to induce long-lived immunity after two doses. Novavax and a Sanofi-GlaxoSmithKline partnership are protein-based vaccines, which involve injecting a protein found on the surface of the coronavirus (S protein) directly into the body. These vaccines can be stored at regular refrigerator temperature (2 °C–8 °C) making them easier to ship than some other leading candidates.

3- mRNA (Messenger Ribonucleic Acid): here, instead of proteins are used for immunize, an mRNA vaccine provides a synthetic mRNA of the virus that codes for S protein, which the host body then uses to produce the viral proteins itself. The biggest advantage of the mRNA vaccine is that they can bypass the hassle of producing pure viral proteins, sometimes saving months or years to standardize and ramp up the mass production. The mRNA vaccines basically mimic natural infection of the virus, but they contain only a short synthetic version of the mRNA viral, which encodes only the antigen protein. Since the mRNA used in vaccination cannot become part of the person's chromosomes, they are safe to use. By using this strategy, biotechnology firms such as Pfizer-BioNTech and Moderna Inc. (co-developed by Moderna Inc. and the Vaccine Research Center at the National Institutes of Health) announced recently their success for vaccine production against the COVID-19. However, this strategy is subjected to the problem of the storage of the mRNA for the delivery of the vaccines because the mRNA is not stable (need to be stored at $-70\,°C$ i.e., $-94\,°F$). Such mRNA vaccine needs then to be stored in dry ice i.e., solid CO_2. Solid CO_2 is obtained from a sublimation process to convert gas CO_2 into solid CO_2. This process is expensive. Moderna Inc. has recently performed some "modifications" for stabilizing the mRNA (mRNA encapsulated in lipid nanoparticles)[7] and so that this mRNA vaccine could be stored at $-20\,°C$ i.e., $4\,°F$ up to 6 months. The mRNA vaccine from Pfizer-BioNTech should be stored at $-70\,°C$ i.e., $-94\,°F$. In anyway, up to present, these both mRNA vaccines still need to be stored in dry ice for delivery. This causes a problem for the distribution of these vaccines, especially in developing countries.

4-Adenoviruses used as vector for delivering the gene of interest (called a viral vector vaccine): adenoviruses have long been a popular viral vector for gene therapy. There is currently no adenovirus vaccine for the general public. The vaccine is not approved for use outside of the military, as it has not been tested in studied in the general population or on people with weakened immune systems. Recently, Oxford-AstraZeneca and Johnson & Johnson, introduce a coronavirus gene that encodes for S protein to the body using genetically engineered and weakened version of a common-cold virus (known as an adenovirus) from chimpanzees (Oxford-AstraZeneca), and from humans (Johnson & Johnson). It has been modified to look more like coronavirus-although it cannot cause illness. Russia's Sputnik V vaccine candidate uses a similar technology. Both the Oxford-AstraZeneca and Johnson & Johnson vaccines are considered vital for lower-income countries and those in hot climates because they are cheaper, easier to transport and can be stored for long periods at normal refrigerator temperatures ($2\,°C$-$8\,°C$).

All of these platforms have advantages and disadvantages, and it is not possible to predict which strategy will be faster or more successful. The use

of weakened or inactivated viruses (1) or protein (2) is time-consuming because it needs the production in large amount of the virus (1) following by the isolation of the protein (2). The use of the mRNA (3) is not time-consuming and safer than the weakened viral (1) or protein (2) based vaccines because it does not carry the risk of the injected virus becoming active (1), or a protein contamination during the isolation process of the viral protein (2) but it is subjected to the stabilizing problem of mRNA and needs to be stored in dry ice for delivery.

The development of vaccines for human use can take years, especially when novel technologies are used that have not been extensively tested for safety or scaled up for mass production. Why does this take so long? There are two important steps that are typically needed before bringing a vaccine into clinical trials. First, the vaccine is tested in appropriate animal models to see whether it is protective. Based on the published studies, animal models of SARS-CoV and MERS-CoV include civet cats, camelidaes, monkeys, mice, hamsters, ferrets, rabbits and other potential hosts. Mouse model has been widely used for many different viral investigations. It has been considered as the best small animal model for hepatitis B virus (HBV), hepatitis C virus (HCV), Zika virus, and among others. Due to its low cost, small size, easy operation and high reproducibility, mouse model is suitable for large-scale studies of viruses not only for the pathogenesis but also for antivirals. Importantly, mouse can be easily manipulated at the genetic level for precision research. For instance, a lot of genetically mutated mice are available for studies in anti-viral immunity, viral pathogenesis and viral infection and transmission restriction. However, animal models for COVID-19 might be difficult to develop. The virus does not grow in wild-type mice and only induced mild disease in transgenic animals expressing human ACE2 (hACE2). Other potential animal models include ferrets and NHPs. Even in the absence of an animal model that replicates human disease, it is possible to evaluate the vaccine because serum from vaccinated animals can be tested *in vitro* neutralization assays. Second, vaccines need to be tested for toxicity in animals, e.g., rabbits. Usually, viral challenge is not part of this process, because only the safety of the vaccine will be evaluated. This testing, which has to be performed in a manner compliant with Good Laboratory Practice (GLP), typically takes 3–6 months to complete. For some vaccine platforms, parts of the safety testing might be skipped if there is already sufficient data available for similar vaccines made in the same production process. Doing this for the first time can be tedious and time-consuming. Vaccines for human use are produced in processes that comply with current Good Manufacturing Practice (cGMP) to ensure constant quality and safety of vaccines. This requires dedicated facilities, trained personnel, proper documentation, and raw material that were produced to be

of cGMP quality. These processes have to be designed or amended to fit COVID-19 vaccines. Typically, clinical development of vaccines starts with small phase I trials to evaluate the safety of vaccine candidates in humans. These are followed by phase II trials (formulation and doses are established to initially prove efficiency) and finally by phase III trials, in which the efficiency and safety of a vaccine need to be demonstrated in a large cohort. However, in an extraordinary situation like the current one, this scheme might be compressed and an accelerated regulatory approval pathway might be developed. If efficacy is shown, a vaccine might be licensed by regulatory agencies. Another important point is that production capacity to produce sufficient amounts of cGMP-quality vaccine needs to be available. For vaccines based on existing vaccine platforms, e.g., inactivated or live attenuated vaccines, this can be relatively easily achieved, because existing infrastructure can be used. For vaccines based on novel technologies, e.g., mRNA, this capacity needs to be built, and this typically takes time. Finally, it takes time to distribute vaccines and administer them. To vaccinate a large proportion of the population would likely take months. Given that the population is currently naïve to COVID-19, it is highly likely that more than one dose of the vaccine will be needed. Prime-boost vaccination regimens are typically used in such cases, and the two vaccinations are usually spaced 3–4 weeks apart. It is likely that protective immunity will be achieved only 1–2 weeks after the second vaccination. This therefore adds another 1–2 months to the timeline.

2.3.2. Problems associated with vaccines for COVID-19

Whichever the method used for the vaccine production, it is important to note herein that there are still many problems concerning the evaluation of the efficiency and safety of the COVID-19 vaccine that need to be solved:

1. -Real efficiency of the vaccine against COVID-19: for such a purpose, no one can predict anything about that because:

   -production of antibodies against COVID-19 varies from "person to person" depending on the status of their immune system. A stronger immune system will produce more antibodies against COVID-19. What are the minimum levels of antibodies needed for the protection against COVID-19? People who had been vaccinated could still transmit the COVID-19 to other people? It is important to note herein that the vaccines prevent illness, but may not infection. Preventing the illness from infection and the infection due to the transmission of the COVID-19 are two different issues. COVID-19 vaccines are being authorized based on how well they keep you from getting sick, needing hospitalization and

dying. Scientists do not know yet how effective the vaccines are at preventing the coronavirus from infecting you to begin with, or at keeping you from passing it on to others;

-severity of the COVID-19 from infected patients varies from "person to person" depending on the status of their immune system (varies from asymptomatic, mild, and severe, and the proportion of infections that do not lead to symptoms is higher in younger individuals). Why? In fact, this is a complicated issue because it depends on the virulence of the virus. Virulence is the ability of a pathogen to cause damage to a host. This is a function of different factors such as the route of entry into the body, the effects of host defense mechanisms, and intrinsic characteristics of the pathogen called virulence factors. Virus virulence factors allow it to replicate, modify host defenses, and spread within the host, and they are toxic to the host. They determine whether infection occurs and how severe the resulting viral disease symptoms are. For now, there are only suggestions and no valid answers. In addition, the children are less affected by COVID-19. Why?;

-How long the produced antibodies will still be "effective" for protection against COVID-19? For now, there is no valid report regarding this issue from a large population;

-Is there a protection against COVID-19 for a second infection with COVID-19? This issue depends on the presence of the "memory B and T cells." For now, there is no valid report regarding this issue from a large population;

2-Real safety of the vaccine against COVID-19 such as no important side effects observed: for such a purpose, it is too soon now to say something about the safety of the vaccine because this issue needs a follow-up study for many months and even many years after vaccination from a large population;

3-Mutation rate of COVID-19: COVID-19 (as well as SARS-CoV's family), is a RNA virus in which the mutation rate is very high and there will be then different "mutated COVID-19 viruses" over time leading to the presence of new strains i.e. new variants characterized by their differing isoforms of surface proteins (difference in S protein i.e. in mRNA too). Depending on the location and the nature of the mutation, this event could affect the effectiveness of the vaccine.

3. Variants of covid-19 and structural and functional implications of the mutations

3.1. Variants of COVID-19

Tracking mutations in the spike gene has been therefore the primary focus to date because of its relevance to vaccine and antibody-based therapy

strategies currently under development. Such interventions take months to years to develop. For the sake of efficiency, contemporary should be factored in during development to ensure that the interventions will be effective against circulating variants when they are eventually developed. Mutations in COVID-19 are common: mutations are expected and are a natural part of evolution. As the COVID-19 outbreak continue to evolve and the scientific evidence rapidly expands, the information provided in this document is only current as of the date of respective literature searches. It is important to note that the majority of the included papers were preprints (not yet peer-reviewed). Up to present, over 4,000 mutations have been detected in the S protein alone, according to the COVID-19 Genomic United Kingdom (COG-U.K.) Consortium, but only a very small minority are likely to be important and to change the virus in an appreciable way.[21,28] Mutations that make viruses more infectious do not necessary make them more dangerous resulting in more severe disease.[21,28] The focus on mutations is a common way to prevent the spread of the virus. Single amino acid changes are worth monitoring because they can be phenotypically relevant. Among coronaviruses, point mutations have been demonstrated to confer resistance to neutralizing antibodies in MERS-CoV and SARS-CoV.[29,30] In the HIV envelope, single amino acid changes are known to alter host species susceptibility, increase expression levels, change the viral phenotype from tier 2 to tier 1, cause an overall change in neutralization sensitivity, and confer complete or newly complete resistance to classes of neutralizing antibodies.[30] Here are some COVID-19 variants that emerged recently are concerning, most notably:

1. - B.1: D614G variant

The COVID-19 arrived in the U.K. from over 1,000 separated incidents, it also shows that a variant with the mutation G614 has completely replaced the previous D614: D614G, associated with the B.1 lineage of COVID-19 in which G614-bearing viruses show significantly higher infectious titers *in vitro* than their D614 counterparts suggesting that the G614 form might be transmitted more readily because of an intrinsic fitness advantage.[30,31] This variant seeded large outbreaks in Europe in early 2020 and subsequently dominated the outbreaks in the Americas, thereby largely replacing previously circulating lineages.[30,31] This rapid rise has led to the suggestion that this variant is more transmissible.[31] The spike D614G amino acid change is caused by an A-to G nucleotide mutation at position 23,403 in the Wuhan reference strain.[2] This mutation D614G is almost always accompanied by three other mutations: a C-to-T mutation in the 5′ UTR (position 241 relative to the Wuhan reference sequence[2]), a

silent C-to-T mutation at the position 3,037, and a C-to-T mutation at position 14,408 that results in an amino acid change in RdRP P323L. The haplotype comprising these four genetically linked mutations is now the globally dominant form. Prior to March 1, 2020, it was found in 10% of 997 global sequences; between March 1 and March 31, 2020, it represented 67% of 14,951 sequences, and between April 1 and May 18, 2020 (last data point available in May 29, 2020 sample), it represented 78% of 12,194 sequences. The transition from D614 to G614 occurred asynchronously in different regions throughout the world, beginning in Europe, following by North America and Oceania and then Asia.[30] The G614 variant increased in frequency even in region where D614 was the clearly dominant form of a well-established local epidemic when G614 entered the population. Examples of this scenario include Wales, Nottingham, and Spain; Snohomish country and King country; and New South Wales, China, Japan, Hong Kong, and Thailand. The increase in G614 often continued after national stay-at-home orders were implemented and, in some cases, beyond the 2-week maximum incubation period.[30] Regarding the origins of the D614G four-base haplotype, it was reported that the earliest examples of sequences carrying parts of the four mutation haplotype that characterizes the D614G were found in China and Germany in late January 2020, and they carried three of the four mutations that lacking only the RdRp P323L substitution. This may be an ancestral form of the D614G. One early Wuhan sequence and one early Thai sequence had the D614G change but not the other three mutations; these may have arisen independently. The earliest sequence that carried all four mutations was sampled in Italy on February 20, 2020. Within days, this haplotype was sampled in many countries in Europe.[30] For the D614G change in the S protein,[30,31] aspartic acid, Asp (D) is a hydrophilic polar amino acid carrying negative charge at neutral pH. Glycine, Gly (G), being one of the common amino acids, does not have a side chain. Glycine is unique as it contains the hydrogen as its side chain (rather than a carbon as is the case for all other amino acids). This means that there is much more conformational flexibility in glycine and as a result of this, it can be reside in parts of protein structures that are forbidden to all other amino acids (e.g., tight turns in structures). The uniqueness of glycine also means that it can play a distinct functional role, such as using its backbone (without a side chain) to bind to phosphates. This means that if one sees a conserved glycine changing to any other amino acid, the change could have a drastic impact on function. Only glycine can function to bind to the phosphates of the ATP molecule using its main chain. Generally, glycine is often found at the surface of proteins within loop-or coil regions, providing high flexibility to the polypeptide chain at these locations. This suggests that it is rather hydrophilic.

Substitution of a polar and negative charge Asp (D) 614 to a non-polar, rather hydrophilic, and neutral Gly (G) 614 residue in the S protein could affect the anionic environment and thus affect the spatial arrangement needed for interactions between S protein and ACE2 receptor.

2. - Spanish variant: 20A.EU1 and 20A.EU2 variants with A222V and S477N mutations, respectively

Later, another variant of COVID-19: 20 A.EU1 (with mutation A222V in the S protein, in which a change from alanine, A, to valine, V, at amino acid site 222) that emerged in early summer of 2020, presumably in Spain, and subsequently spread to multiple locations in Europe. This variant, 20 A.EU1, and a second variant 20 A.EU2 with mutation S477N in the S protein (a change from serine, S, to asparagine, N, at amino acid site 477) account for the majority of recent sequences in Europe.[32] Regarding the 20 A.EU1 (with mutation A222V in the S protein, in which a change from alanine, A, to valine, V, at amino acid site 222) and the 20 A.EU2 with mutation S477N in the S protein (a change from serine, S, to asparagine, N, at amino acid site 477) account for the majority of recent sequences in Europe[32]: both alanine, Ala, (A), and valine, V, (V) are hydrophobic, non-polar, and with aliphatic side chains. Being hydrophobic, both alanine (A) and valine (V) prefer to be buried in protein hydrophobic cores. Alanine is probably the dullest amino acid. The side chains of alanine and valine are very non-reactive, and are thus rarely directly involved in protein function like catalysis, but they can play a role in substrate recognition or specifically, particularly in interactions with other non-reactive atoms such as carbon and can be involved in binding/recognition ligands such as lipids. Substitution of alanine (A) by valine (V) from A222V mutation in the S protein should not affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor; serine, Ser, (S) is a polar, and neutral amino acid. The hydroxyl group is fairly reactive, being able to form hydrogen bonds with a variety of polar substrates. Serine can be reside both within the interior of a protein, or on the protein surface. Its small size means that it is relatively common within tight turns on the protein surface, where it is possible for the serine side chain hydroxyl oxygen to form a hydrogen bond with the protein backbone. Substitution of serine (S) by asparagine (N), an amide side chain and classified as a polar (at physiologic pH), neutral aliphatic amino acid (prefers generally to be on the surface of proteins, exposed to an aqueous environment) from S477N mutation in the S protein should not affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor.

3. - B.1.1.7: U.K. variant with N501Y mutation

Recently, there is the presence of another variant of COVID-19: Variant of Conserve (VOC-202012/01),[33] previously known as the first Variant Under Investigation in December 2020: VUI-202012/01 with a year, month, and number,[34] and also known as N501Y.V1 strain or lineage B.1.1.7.[28,33–36] The variant was first detected in Kent (county of South East of United Kingdom, U.K.) in October 2020 from a sample taken the previous month, and it quickly began to spread by mid-December 2020 and is under investigation in December 2020. Then, this variant was designed as VUI-202012/01 on detection and on review re-designed as VOC-202012/01. This VOC-202012/01 variant is defined by 23 mutations: 13 non-synonymous mutations, 4 deletions, and 6 synonymous mutations (i.e., there are 17 mutations that change proteins and six that do not).[37] One of the most important changes in VOC-202012/01 seems to be N501Y.[28,33–36] It is correlated with a significant increase in the rate of COVID-19 infection in U.K; this increase is thought to be at least partly because of change N501Y (a change from asparagine, N, to tyrosine, Y at amino acid site 501) inside, which is needed for binding to ACE2 in human cells.[5] Mutations in the S protein's RBD can change antibody recognition and ACE2 binding specificity.[21,28] Furthermore, it can lead to the virus becoming more infectious.[21,28] Indeed, in a report published by Public Health England on 21 December 2020, Chand et al.[33] conclude that it is highly likely that N501Y affects the receptor binding affinity of the spike protein and it is possible that this mutation alone or in combination with the deletion at 69/70 (a deletion of the amino acids in positions 69–70) in the N terminal domain (NTD)[2] of the S protein is enhancing the transmissibility of the virus. The 69–70del has, however, been discovered "in viruses that eluded the immune response in some immunocompromised patients, and has also been found in association with other RBD changes."[33] Chand et al.[33] also concluded that "it is possible that variants at this position affect the efficacy of neutralization of virus," but noted that there is currently no neutralization data on N501Y available from polyclonal sera from natural infection. This B.1.1.7 variant is believed to be 70% more contagious than the current strain circulating in US, and may be responsible for as many as 60% of cases in U.K. Recently, it was reported that people infected with this variant are up to 64% more likely to dye than those with other strains.[38,39] This analysis suggests that B.1.1.7 variant is not only more transmissible than preexisting COVID-19 variants, but may also cause more severe illness.[38,39] The N501Y change has also been detected elsewhere: in Brazil in April, in Australia in June-July, in the U.S. in July, in Japan, Viet Nam, Canada, Lebanon, France, Spain, Sweden,

and Germany in December, and it is not yet clear if it arose spontaneously in the U.K., or was imported.[40] According to Centers for Disease Control and Prevention (CDC), the B.1.1.7 variant could become the dominant strain in the U.S. by March 2021. Although the variant was first detected in Kent, it may never know where it originated. Discovery in the U.K. may merely reflect that the U.K. does more sequencing than many other countries. It has been suggested that the variant may have originated in a chronically infected immunocompromised person, giving the virus a long time to replicate and evolve.[36,41,42] Regarding the N501Y mutation in the S protein,[28,33–42] asparagine, Asn, (N), has an amide side chain and is classified as a polar (at physiologic pH), neutral aliphatic amino acid. Being polar asparagine prefers generally to be on the surface of proteins, exposed to an aqueous environment. Tyrosine, Tyr, (Y), has a phenolic, ionizable side chain (pH-dependent ionization) and the –OH group of tyrosine is also able both to donate and accept a hydrogen bond so that the aromatic tyrosine is often called amphipathic due to its ability to have both polar and non-polar character. It is then possible for tyrosine to play a dual role, with part of the side chain being buried in protein hydrophobic cores and another being exposed to an aqueous environment, at the surface of proteins. The aromatic side chain can also mean that tyrosine is involved in stacking interactions with other aromatic side chain. In addition, tyrosine contains a reactive hydroxyl group, thus making it much more likely to be involved in interactions with non-carbon atoms. Such a mutation could affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor.

4. - B.1.351: South Africa variant with N501Y, K417N, and E484K mutations

Recently, another variant of COVID-19 that also appears to be contagious is the N501Y.V2 strain (also known as B.1.351 lineage), which is currently spreading in South Africa.[21,22,42] This variant was first detected in the Nelson Mandela Bay metropolitan area of the Eastern Cape province of South Africa and reported by the country's health department on 18 December 2020.[43] Researchers and officials reported that the prevalence of the variant was higher among young people with no underlying health conditions, and by comparison with other variants, it is more frequently resulting in serious illness in those cases.[44,45] The South African health department also indicated that the variant might be driving the second wave of the COVID-19 pandemic in the country due to the variant spreading at a more rapid pace than other earlier variants of the virus.[43,44] Scientists noted that the variant contains several mutations (three RBD

mutations and five additional NTD mutations) in that allows it to attach more easily to human cells because of three mutations in the S protein's RBD of the virus: N501Y (has also detected in U.K.),[28,33–42] K417N (a change from lysine, K, to asparagine, N, at amino acid site 417), and E484K (a change from glutamic acid, E, to lysine, K, at amino acid site 484).[21,22,42–47] Two mutations found in 501.V2: K417N and E484K are not found in VOC-201012/01, also 501.V2 does not have the 69–70del mutation found in VOC-201012/01.[48,49] On 28 December, the variant had been detected in two individuals in Switzerland[50] and in one individual in Finland.[51] On 29 December, the strain had been detected in a visitor from South Africa to Japan,[52] and in one overseas traveler in Queensland, Australia.[53] On 30 December, the variant has been detected in Zambia.[54] For K417N mutation: lysine, Lys, (K), is a polar, and positive charge amino acid. It frequently plays an important role in structure of proteins. It can be considered to be somewhat amphipathic as the part of the side chain nearest to the backbone is long, carbon-containing hydrophobic, whereas the end of the side chain is positively charged. For this reason, one can find lysine where part of the side chain is buried and only the charged portion is on the outside of the protein. However, this is by no means always the case and generally lysine prefers to be on the outside of proteins. Lysine is also frequently involved in salt-bridges. Due to the positively-charged amino group on the side chain of lysine, it is sometimes involved in forming hydrogen bonds with negatively-charged non-protein atoms (e.g., anions or carboxylate groups). For these raisons, lysine is quite frequent in protein active or binding sites. Substitution of lysine (K) by asparagine, Asn, (N), an amide side chain classified as a polar (at physiologic pH), neutral aliphatic amino acid in S protein could affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor. Regarding the E484K mutation: glutamic acid, Glu, (E), is a polar and negative charge amino acid. Being charged and polar, glutamic acid generally prefers to be on the surface of proteins, exposed to an aqueous environment. The negative-charge means that it can interact with positively-charged non-protein atoms, such as cations like zinc. Glutamic acid is frequently involved in salt-bridges, and it is quite frequently involved in protein active or binding sites. In certain cases, it can perform a role in the catalytic site of proteins such as proteases or lipases. Substitution of glutamic acid (E) by lysine, Lys, (K), a polar and positively-charged amino acid in S protein could affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor.

5.   - B. 1.1.248: Brazilian P.1 variant with N501Y, E484K, and K417T mutations

Recently, the Brazilian variant, called P.1 (also known as N501Y.V3 strain or B.1.1.248 lineage) similar to the South African variant was also detected.[55] This Brazilian P.1 variant was detected on 9 January 2021 by the Japanese authorities notified the WHO after detecting it in airport tests from four travelers from Brazil. WHO is currently working with both Japan and Brazil to evaluate the Brazilian P.1 variant, determining if this variant is more severe, has higher transmission, or if it could be detriment to current therapies, diagnostics or vaccines for the disease. Its mutations include the N501Y mutation, which it has in common with the variants reported by South Africa and the U.K., the E484K and the K417T. Regarding the K417T mutation, substitution of lysine (K) by threonine, Thr, (T), an amide side chain classified as a polar (at physiologic pH), neutral aliphatic amino acid in S protein could affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor.

6. - B.1.427/1.429: CAL.20C variant with L452R mutation

Another new COVID-19 variant: L452R (substitution of leucine, Leu (L) by arginine, Arg (R), at amino acid 452 in the S protein's RBD of the virus), was discovered recently in Los Angeles, California, U.S.A. (also known as B.1.427 or B.1.429 lineage, defined by five mutations: ORF1a: I4205V, ORF1b: D1183Y, S: S13I, W152C, L452R), and designated as CAL.20C,[56] of which the L452R was of particular concern. This new variant of COVID-19 was first identified in Denmark in March of 2020 and it showed up in Los Angeles, California, U.S.A., as early as May 2020. This variant affects the S protein of the virus, so there is a chance that the currently-developed vaccines will be less effective against it. Indeed, leucine, Leu (L), is a hydrophobic, non-polar amino acid. Being hydrophobic, leucine prefers to be buried in protein hydrophobic cores. It also shows a preference for being within alpha helices more so than in beta strands. The leucine side chain is very non-reactive and is thus rarely directly involved in protein functions like catalysis, although it can play a role in substrate recognition. In particular, it can be involved in binding/recognition of hydrophobic ligands such as lipids. Leucine can be substituted by other hydrophobic, particularly aliphatic, amino acids. Arginine, Arg (R) is a positively-charged, polar amino acid. Arginine generally prefers to be on the surface of the protein. The positive charge means that it can interact with negatively-charged non-protein atoms (e.g., anions or carboxylate groups). Arginine contains a complex guanidinium group on its side chain that has the geometry and charge distribution that is ideal for binding negatively-charged groups on phosphates (it is able to form multiple

hydrogen bounds). Arginine is also frequently involved in salt-bridges where it pairs with a negatively charged aspartate or glutamate to create stabilizing hydrogen bonds that can be important for protein stability. Arginine is quite frequent in protein active or binding sites. Substitution of leucine, Leu (L) by arginine, Arg (R) in S protein can thus be disastrous and could affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor.

7. - B.1.525 variant with E484K, Q677H, and F888L mutations

B.1.525 lineage with three mutations of "biological significance": E484K, Q677H (substitution of glutamine, Gln, (Q), by histidine, His, (H), at the position 677), and F888L (substitution of phenylalanine, Phe, (F), by leucine, Leu, (L), at the position 888) has been detected in 13 countries, according to the available sequence data from the Global Initiative on Sharing Avian Influenza Data (GISAID), such as U.S., Canada, Denmark, U.K., France Belgium, Spain, Nigeria, Ghana, Australia, Jordan, Finland, and Singapore.[57–59] Countries differ widely in their ability to track the emergence of variants, and it is possible the variant is in more places that have yet to notice it. The fact that the B.1.525 variant (or B.1.525 lineage) has been found in so many countries indicates that it has been around for some time. For the Q677H mutation: glutamine, Gln, (Q), is a hydrophilic polar and neutral amino acid. Glutamine is quite frequently involved in protein active or binding sites. The polar side chain is good for interactions with other polar or charged atoms. Glutamine can be substituted by other polar amino acids. Histidine, His (H), is a hydrophilic polar amino acid. Histidine has a pKa near to that of physiological pH, meaning that it is relatively easy to move protons on and off of the side chain i.e., changing the side chain from neutral to positive charge. This flexibility has two effects. The first is ambiguity about whether it prefers to be buried in the protein core or exposed to solvent. The second is that it is an ideal residue for protein functional centers. The side chain has a pKa of approximately 6.5, which means that only about 10% of molecule will be protonated. The precise pKa depends on local environment. Histidine is the most common amino acid in protein active or binding sites. It is very common in metal binding sites (e.g., zinc). Substitution of glutamine, Gln, (Q) by histidine, His (H), in S protein could not affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor. Regarding the F888L mutation: phenylalanine, Phe, (F), is a hydrophobic, non-polar amino acid. The phenylalanine side chain is fairly non-reactive, and is thus rarely directly involved in protein function, although it can play a role in substrate recognition. The aromatic side chain can also mean that

phenylalanine is involved in stacking interactions with other aromatic side chains. Phenylalanine can be substituted with other aromatic or hydrophobic amino acids. Substitution of phenylalanine, Phe, (F), by leucine, Leu, (L), a hydrophobic, non-polar amino acid, in S protein could not affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor.

8. - B.1.526 variant with E484K, S477N, and D614G mutations

Recently, a new COVID-19 variant dubbed B.1.526 with the most common sets of the S protein mutations: L5F, T95I, D253G, E484K, S477N, D614G, and A701V were also reported.[60,61] There are two main branches of this lineage, one having E484K and the other including S477N, both located within the S protein's RBD of the virus. The E484K mutation has also been detected in South Africa: B.1.351 lineage or 501.V2 strain, and in Brazil: B.1.1.248 lineage (P.1) or N501Y.V3 strain, and the S477N mutation has also been detected in Europe from Spanish variant: 20 A.EU2 strain. In this lineage, there is also the D614G variant in the S protein, which was spread in different regions throughout the world, beginning in Europe, following by North America and Oceania and then Asia.[29] This B.1.526 variant was first identified by the researchers at Columbia University Vagelos College of Physicians and Surgeons (VP&S, a graduate medical school of Columbia University) in samples collected in New York in November 2020 and by mid-February 2021, it represented about 12% of cases. This variant was also described in research published online by researchers at the California Institute of Technology (Caltech) while analyzing viral genetic sequences stored in the GISAID database.[60,61] Experts are expressing concern that the B.1.526 variant in New York could be even more worrisome than the one in California i.e., the CAL.20C variant with the L452R mutation. The E484K virus mutation, which is present in all the three variants (B.1.351, the variant first identified in South Africa; B.1.1.248 or P.1, the variant identified in Brazil; B.1.526, the variant identified in New York) helps the virus dodge the vaccines and contributes to weaken the body's immune response to the virus.[61] The S477N mutation located near the binding site of multiple of antibodies[61] may affect how tightly the virus binds to human cells, and has been implicated to increase viral infectivity through enhanced interactions with ACE2.[61] The D614G mutation was suggested more transmissible.[30,31] The A701V mutation located adjacent to the S2' cleavage site and is shared with variant B.1.351 of South Africa.[61] The D253G mutation has been reported as an escape mutation from antibodies against the N-terminal domain.[60] The overall pattern of mutations in this lineage suggests that it arose in part in response to

selective pressure from antibodies, and it appears that the frequency of lineage B.1.526 has increased rapidly in New York.[60,61] For the D253G mutation, as mentioned above for D614G variant, substitution of a polar and negative charge Asp (D) 253 to a non-polar, rather hydrophilic, and neutral Gly (G) 253 residue in the S protein could affect the anionic environment and thus affect the spatial arrangement needed for interactions between S protein and ACE2 receptor. For the A701V mutation, as mentioned above for A222V mutation of 20A.EU1 variant, substitution of alanine (A) by valine (V) from A701V mutation in the S protein should not affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor.

9. –B.1.617 "Double mutant" COVID-19 variant found in India with E484Q and L452R mutations

The Indian SARS-CoV-2 Consortium on Genomics (INSACOG) has recently identified a B.1.617 lineage "double mutant" COVID-19 variant with E484Q and L452R mutations. The L452R mutation has also been detected in the California strain: CAL.20C variant. The government said that an analysis of the samples collected from India's western Maharashtra state showed an increase in the fraction of samples with the E484Q and L452R mutations compared with December 2020. There may be a separate lineage developing in India with the E484Q and L452R coming together. This Indian "double mutant" variant was recently found in a patient from the San Francisco Bay Area via the school's Clinical Virology Laboratory. This is the first described case with this variant in the U.S. Public Health England (PHE) has recently identified 77 cases of this variant in the U.K. and designated it a Variant Under Investigation (VUI). Regarding the E484Q mutation, substitution of glutamic acid, Glu (E) by glutamine, Gln, (Q), a hydrophilic polar and neutral amino acid in S protein could affect its role in protein structure and function needed for interactions between S protein and ACE2 receptor. In addition, a COVID "triple mutant" has been recently identified in patient samples collected from four states: Maharashtra, Delhi, West Bengal and Chhattisgarh in India, and defined as the B.1.618 lineage. This is because three COVID variants have merged to form a new, possibly deadlier variant. The triple mutant has been classified as a "variant of interest".

3.2. Structural and functional implications of the mutations

The putative importance of structural and functions implication of mutations is based on three distinct sets of observations. First, is the prediction structurally and functionally of consequences of mutations on the protein

in question for its specific role in the disease followed by the confirmation of the prediction by performing experimental *in vitro* work. Second, is the confirmation of the predictions by performing experimental *in vivo* work in animal models (if it exists). Third, analysis of the frequency of the variants over time in a large population in order to know whether or not the variants later will be dominants. Here, although there is no evidence that the mutations cause more severe illness, the prediction of consequences of missense mutations on the structure and function of the S proteins for interaction with its ACE2 receptor is still needed. Indeed, the mutations in the S protein of the COVID-19 as well as its variants could affect the affinity between S protein and its ACE2 receptor for entry into the cells (host) that could have an impact on the transmissibility of the virus and results in be more or less infectious. In general, for the prediction of consequence of missense mutations, the charged amino acids are easy to assign while the polarity is not always straightforward to assign. It is also important to note herein that even with a mutation that took place in the S protein, which is not known to play a direct role in receptor binding or membrane fusion for COVID-19 but such a mutation can sometimes mediate long-range effects on protein conformation or stability. It is then necessary to confirm experimentally the effects of mutations. Regarding this issue, recently, Korber et al.[30] reported an *in vitro* experimental approach to obtain an evidence of increased fitness of the D614G mutation using pseudovirus models for infection (using the recombinant D614 and G614 variants, lentiviruses, and vesicular stomatitis viruses (VSV) resulting in D614 and G614 variants-bearing viruses: lentiviral and VSV pseudotypes) in established cell lines HEK 293 T stably expressing the ACE2 receptor. They also tested whether the D614G variations would be similarly neutralized by a polyclonal antibody obtained from convalescent sera of six San Diego, California, U.S.A, residents, likely infected in early to mid-March 2020, when D614 and G614 were circulating, demonstrate equivalent or better neutralization of a G614-bearing pseudovirus compared with D614-bearing pseudovirus (pseudovirus neutralization assay). Although they do not know with which virus each of these individuals were infected, these initial data suggest that, despite increased fitness in cell culture, G614-bearing virions are not intrinsically more resistant to neutralization by convalescent sera. However, there is currently no scientific consensus on the effect of the D614G mutation on COVID-19 infectivity and transmissibility, and there is some skepticism that it could produce a meaningful effect at the population level given that COVID-19 is already highly transmissible and rapidly spreading.[30] The effect of the D614G replacement has been characterized *in vitro* with pseudovirus models and *in vivo* in animal models, but this may not accurately recapitulate the effect of variants on virus

transmissibility within the human population.[31] Therefore, experimental evidence should be complemented with large-scale population studies that can detect meaningful changes in human-to human transmission while laboratory experiments can identify changes in virus biology, their extrapolation to identify population level effects on transmission requires caution.[31] The data are heavily skewed toward hospitalized cases, and therefore more severe disease, and so it is not possible to evaluate small differences in virulence that may be present in milder or asymptomatic infections. This is especially problematic for evaluating effects that may be confounded by age, as the proportion of infections that do not lead to symptoms is higher in younger individuals.[31] The pseudovirus models approach has also used by other authors for testing whether the S protein with A222V mutation of the 20 A.EU1 variant[32] had an obvious functional effect on spike's ability to mediate viral entry, and finally found no evidence of increased transmissibility of the V222 variant.[31]

Experimentally, the pseudovirus models approach as described in Ref.[30,32] is laborious and could be subjected to artifact, especially lentiviruses and vesicular stomatitis viruses (VSV) (instead of coronaviruses) used for infection in established cell lines HEK 293 T stably expressing the ACE2 receptor. Herein, a simpler one by using expression vectors as described in Ref.,[62] especially the one with the glycosyl-phosphatidylinositol, GPI, anchor, can be used as a model for the construction of expression vectors for any protein targeting to the cell plasma membrane for studying intermolecular interactions and could be therefore useful for studying intermolecular interactions between S protein of coronavirus and ACE2 receptor. Here is the outline of this approach: the entire coding sequence (CDS) of both the S protein obtained from the coronavirus variant in question and a more established variant that was circulating earlier in the pandemic: original strain of COVID-19 for example, is used for the construction of expression vectors. The site-directed mutagenesis technique[62] can be used for the construction of expression vectors with the S protein from any coronavirus variants. Cell lines expressing the ACE2 receptor are used for test such as HEK 293 cells, Vero cells, or Hela cells for example.[37–39] For cells cultured purpose, it is recommended that the selected cell lines expressing the ACE2 receptor should be also used for transfection. In the present study, HEK 293 cells are selected as the ones expressing ACE2 receptor and these cell lines are also used for transfection. First, perform the transfection in HEK293 cells by the expression vectors followed by verification of the expression of the S protein targeting to the cell plasma membrane. Transfection in HEK 293 cells by the expression vectors without the CDS of S protein of coronavirus are used as negative controls. Then, put the transfected HEK 293 cells in with the cell lines expressing the ACE2 receptor (HEK 293 cells) and see if there are

molecular interactions between S protein of the coronavirus and ACE2 receptor. Afterwards, introduce antibodies from the blood of someone who has survived the coronavirus or had a vaccine. Ordinarily, antibodies should bind to the coronavirus via its S protein, which interferes with its ability to interacting with ACE2 receptor. We can see therefore a difference in the capacity of the antibodies to interfere with the molecular interactions between S protein of the coronavirus and ACE2 receptor. Then, by performing these assays, we can confirm the effects of mutations of the coronavirus variant via evaluating the efficiency of intermolecular interactions between S protein of coronavirus and ACE2 receptor and check the efficiency of the vaccine against the new coronavirus variant via evaluating the capacity of the antibodies to interfere with the molecular interactions between S protein of the coronavirus variant and ACE2 receptor. In sum, by using such expression vectors which mimic the structure of the surface spike (S) glycoprotein of the virus, we can get an answer with direct evidence related to a specific question regarding the affinity of molecular interaction between S protein of the coronavirus as well as its variants and ACE2 receptor, and also check the status of the antibodies whether or not they are capable to neutralizing the virus. Furthermore, this approach could be useful in antiviral drugs and vaccines development.

Lack of effective screening and containment, likely undermining local efforts to keep COVID-19 cases low, may explain the variant's success despite travel restrictions and quarantine requirements across Europe to containing the spread of this novel COVID-19 variant, 20 A.EU1, that emerged in Spain in early summer 2020, and subsequently spread to multiple locations in Europe.[32] These results demonstrate how genomic surveillance is critical to understanding how travel can impact COVID-19 transmission, and thus for informing future containment strategies as travel resumes.[32] The COVID-19 spike gene has accumulated mutations within the RBD[2] and the NTD[2] of the S protein. These domains are major targets of antibody response elicited by the vaccines. It is true that the vast majority of vaccines in development target the S protein of COVID-19. If it changes beyond recognition due to mutations, the vaccines may be unable to induce the necessary immune response within people. However, the proteins that coat the shell of COVID-19 would need to undergo significant genetic transformations to render the vaccines redundant-something that, at this stage, does not appears to have happened.

4. Status of the current vaccines

Development of vaccines to prevent the COVID-19 has occurred with unprecedented speed.[63] Up to present, some COVID-19 vaccines based on

different platforms have been authorized for emergency use.[64–72] Recently, however, mutations in COVID-19 arise naturally through viral replication leading to the emerging of new variants of COVID-19. This raises questions about whether the current vaccines will be effective against all of them. To date, three COVID-19 variants of public health importance have been identified: lineage B.1.1.7 originated in U.K. (of note, the B.1.1.7 lineage with the N501Y mutation circulating in the U.K., however, has now evolved to include the E484K mutation in U.K.[73]); lineage B. 1.351 originated in South Africa; and lineage B. 1.1.248 (or lineage P.1) originated in Brazil. These three variants have been termed variants of concern (VOCs). As mentioned above, these VOCs have many mutations, including some in the RBD of the S protein, encoded by the S gene. The RBD mutations of interest in the S gene include the following amino acid substitutions: N501Y, K417N/T and E484K, in which such substitutions predictably could affect interactions between S protein and ACE2 receptor for entry into the cells (host) (see 3.1. Variants of COVID-19). The N501Y and E484K mutations are then found in all three VOCs. All three mutations in the S protein's RBD are of particular concern since they potentially reduce antibody neutralization and increase affinity for ACE2 receptor, and have been associated with evidence of increased transmissibility, severity, and/or possible evasion with potential implications for reinfection and vaccine effectiveness:

1. - It was reported that the B.1.1.7 variant is not only more transmissible than preexisting COVID-19 variants, but may also cause more severe illness.[38,39] The first official record of a reinfection case with the B.1.1.7 lineage was also recently published.[74] The rate of transmission of new mutations is then concerning. Indeed, when transmissibility is higher for a VOC, the VOC can lead to a rapid increase in cases, putting a strain on health care resources;

2. - The E484K mutation has been associated with potential immune escape in which, for the first time, a reinfection case with this E484K variant was also detected.[75] This finding of the E484K mutation, in an episode of COVID-19 reinfection might have major implications for public health policies, surveillance and immunization strategies;

3. - Sabino et al.[76] suggested that the P.1 variant identified in Manaus, Brazil might have higher transmissibility than preexisting lineages. They noted a high frequency (42%, 13/31) of the P.1 lineage among samples sequenced from a cluster of COVID-19 cases in Manaus in December 2020, but it was absent in 26 publicly - available genome surveillance samples collected in Manaus from March to November 2020. Additionally, the P.1 variant has the N501Y mutation, which is found

in the B.1.1.7 and B. 1.351 variants that has been associated with increased transmissibility.[38,39,76] The authors note that contact tracing and outbreak investigation data are needed to better understand relative transmissibility of this lineage. No research on the impact of the P.1 variant on disease severity was identified;

4. - Wang et al.[77] found that the B.1.351 variant showed resistance to neutralization by convalescent plasma (~11–13 fold) and vaccine sera (~6.5 – 8.6 fold), and that this was likely due to the E484K mutation, which is also present in P.1.[77] They hypothesized that similar resistance to neutralizing plasma would be found in the P.1 lineage. Similarly, Jangra et al.[78] reported that polyclonal sera from vaccinated individuals and those previously infected with previous strains of COVID-19 had reduced neutralizing activity against the E484K mutation that is present in P.1. Additionally, they suggested that vaccinated individuals might be less protected against P.1, compared to the previous strain of COVID-19.[78] Specifically, their *in vitro* study found that serum neutralization efficiency from individuals who received the Pfizer-BioNTech vaccine was lower against a COVID-19 strain that had the E484K mutation, compared to the previous strain of COVID-19. Human sera with high neutralization antibody titers against the previous strain of COVID-19 were still able to neutralize the E484K COVID-19 strain.[78] However, neutralization efficiency of donor sera with low or moderate immunoglobulin G (IgG) against the S protein had neutralization values similar to negative control samples against the E484K strain. This suggests that to enhance protection against newly emerging COVId-19 variants, the highest vaccine-induced titers possible are needed. Since COVID-19 variants with the E484K mutation might be better at evading antibodies from the plasma of recovered COVID-19 patients infected with earlier strains,[78] the P.1 variant, containing this mutation, could increase the risk of re-infection or infection in vaccinated individuals.[79] Some reports support the possibility of reinfection with P.1 such as Naveka et al.[80] describes the first confirmed case of reinfection with the P.1 lineage in a 29 years old female from Amazonas, Brazil, who was previously infected with a B.1 lineage virus (i.e. with D614G variant). The patient (with no history of immunosuppression) was originally infected in March 16, 2020 with symptoms of myalgia, cough, sore throat, nausea, and back pain. After being exposed to a positive case on December 19, 2020, the patient exhibited the second symptomatic COVID-19 episode on December 27, 2020. Genomic sequencing confirmed that the infections were from two different COVID-19 lineages in each COVID-19 episode: a B.1 lineage in the initial infection and a P.1 lineage at reinfection. Urgent studies are

necessary to determine whether reinfection with newly emerging lineages harboring the mutation E484K is a widespread phenomenon or is limited to a few sporadic cases;

5. - The variants are now spreading globally at exponential rates in which the D614G has subsequently become globally dominant. The repeated, independent evolution of spike position 501 in 501Y.V1 (B.1.1.7 variant), 501Y.V2 (B.1.351variant) and 501Y.V3 (P.1 variant), strongly argues for a selective advantage, likely enhances transmissibility, of these new variants. Wibmer et al.[81] have shown that the 501Y.V2 lineage, containing nine spike substitutions, and rapidly emerging in South Africa during the second half of 2020, is resistant to neutralizing antibodies found in 48% of individuals infected with previously circulating lineages. These data, showing a 13-fold reduction in mean titer, are corroborated by VSV-pseudotyped and live virus (live-virus neutralization assay testing was performed by a microneutralization focus-forming assay in Vero E6 cells at the African Health Research Institute, South Africa) assays showing an 11-to 33-fold and 6-to 204-fold reduction in mean titer (including complete knock out) relative to the original lineage, respectively. The 501Y.V3 lineage has similar changes including 417T and 484K (in RBD) as well as 18F and 20N (in NTD), thus also having strong potential for high levels of neutralization resistance. The independent emergence and subsequent selection of 501Y lineages with key substitutions conferring neutralization resistance strongly argues for selection by neutralization antibodies as the dominant driver for SARS-CoV-2 spike diversification and makes these lineages of considerable public health concern. This suggests that, despite the many people who have already been infected with SARS-CoV-2 globally and are presumed to have accumulated some level of immunity, new variants such as 501Y.V2 may pose a substantial reinfection risk. While higher titers of neutralizing antibodies are common in hospitalized individuals, most people infected with SARS-CoV-2 develop low-to-moderate neutralization titers. Therefore, the data herein suggest that most individuals infected with previous SARS-CoV-2 lineages will have greatly reduced neutralization activity against 501Y.V2. This dramatic effect on plasma neutralization can be explained by the dominance of RBD-directed neutralization antibodies, supported by studies showing reduced plasma neutralization titers mediated by the E484K change alone.[81] Notably, the K417N change also has a crucial role in viral escape, effectively abrogating neutralization by a well-defined, multidonor class of VH3–53/66 germline-restricted public antibodies that comprise some of the most common and potent neutralizing antibodies to SARS-CoV-2.[81] The marked loss of neutralization against 501Y.V2 pseudovirus

compared to the RBD-only chimeric pseudovirus demonstrates the important role that substitutions in the NTD play in mediating immune escape. For 501Y.V2, this resistance to neutralization is likely mediated by a three-amino-acid deletion that completely disrupts a dominant public antibody response to the N5-loop supersite. This deletion predominates among 501Y.V2 variants and occurs either alone or with an R246I substitution that is also important for neutralization by several NTD-directed neutralizing antibodies. Altogether, these data highlight the need for increased, ongoing genomic surveillance during the COVID-19 pandemic. Many therapeutic strategies currently under development have been derived. The overwhelming majority of monoclonal antibodies already on the path to licensure target residues K417 or E484 and are therefore likely to be futile against 501Y.V2. In addition, emerging variants may limit the use of recently identified neutralizing antibodies that target the NTD N5-loop supersite. Some of these monoclonal antibodies have already been granted the EUA in the United States (Regeneron Pharmaceuticals and Eli Lilly and Company), including antibodies ineffective against 501Y.V2 such as REGN10933 and LY-CoV555). These data also have implications for the effectiveness of COVID-19 vaccines, largely based on immune responses to the original S protein. Indeed, sera from the Moderna Inc. and Pfizer-BioNTech vaccines show significantly reduced neutralization of 501Y.V2[77,81];

6. - Although the mRNA COVID-19 vaccines from Pfizer-BioNTech[64] and Moderna Inc.[65] have modest neutralizing antibody activity after the first dose, they produce a greater increase in neutralization activity after the second dose than that produced by the ChAdOx1 nCoV-19 (Oxford-AstraZeneca)[67] and heterologous Sputnik V (adenovirus-26 followed by adenovirus-5 vector)[68] COVID-19 vaccines. Neutralizing activity of the two mRNA vaccines against the B.1.351 variant has also been observed to be lower, by a factor of 8.6 (mRNA-1273 vaccine of Moderna Inc.)[65] or 6.5 (BNT-162b2 vaccine of Pfizer-BioNTech)[64] on pseudovirus neutralization assay, than activity against the D614G virus, whereas no difference was evident against the B.1.1.7 variant.[77,81,82] Results of a recent interim analysis of the NVX-CoV2373 nanoparticle spike protein COVID-19 vaccine (Nonavax),[71] described in a press release, have not been published. However, reports suggest that the vaccine may have lower efficacy against the B.1.351 variant than against the original virus or the B.1.1.7 variant.[82] Recently, a two-dose regimen of the ChAdOx1 nCoV-19 vaccine (Oxford-AstraZeneca)[67] did not show protective against mild-to-moderate COVID-19 due to the B.1.351 variant.[82] As a precaution, Pfizer-BioNTech[64] and Moderna Inc.[65]

have begun developing a new form of their mRNA vaccines that could be used as a booster shot against the B.1.351 variant. In the same way, due to the less protective found against the B.1.351 variant, the redesign of inactivated COVID-19 vaccine from Sinovac Biotech and China National Pharmaceutical Group (Sinopharm) that requires the cultivating and inactivating the virus is also underway. Recently, the U.S. FDA granted Johnson & Johnson[72] the EUA in February 2021. Johnson & Johnson 's[72] candidate is the third vaccine (after the Pfizer-BioNTech[64] and Moderna Inc.[65] vaccines) being granted the EUA and the first one requiring only one dose, while both Pfizer-BioNTech[64] and Moderna Inc.[65] vaccines are administered at two doses, given several weeks apart. Furthermore, it can be refrigerated between $2\,°C$-$8\,°C$ for three months, making transportation and storage far less of a challenge, and it is cheaper, easier to produce and appears to have a milder set of side effects (of note, some cases of blood clots, especially the cerebral venous sinus thrombosis with low level of blood platelets, observed in people who received the Johnson & Johnson[72] and Oxford-AstraZeneca[67] vaccines have been recently reported). The development of a new form of the vaccine from Johnson & Johnson[72] against the various variants of the coronavirus such as the B.1.351 strain found in South Africa is also underway. Although the degree of attenuation that compromises an effective neutralizing antibody response *in vivo* is unknown, the pseudovirus and live-virus neutralization assay experiments, however, provide evidence of reduced or abrogated vaccine-induced antibody neutralization against the B.1.351 variant.[81,82] Comparison of the RBD triple mutant (containing only K417N, E484K, and N501Y) and the B.1.351variant in the pseudovirus neutralization assay suggest that much, though not all, of the vaccine-elicited neutralization is directed to the RBD.[82] A similar loss of neutralizing activity against the B.1.351 variant in antibodies induced by natural infection after the first wave of the COVID-19 outbreak has been reported.[82] Although efforts to develop second-generation COVID-19 vaccines targeted against B.1.351 and P.1 variants are underway, the only COVID-19 vaccines likely to be available for most of 2021 have been formulated against the original virus. While that sounds worrisome, there is reason to be hopeful. Vaccinated people exposed to a more resistant variant still appear to be protected against serious illness. People who are vaccinated should still wear masks in public and comply with public health guidelines.

The speed with which the new variants of COVID-19 became the dominant form globally suggests then a need for continued vigilance because

some of the new mutations are worrisome in that they increase the ease of the virus entry in cells and may help evade antibodies so that new vaccines which completely mimic such new mutations should be engineered. In the meantime, it is a reminder that we need to continue to take precautions: wear masks, socially distance, avoid crowds, keep washing our hands well and often, travel restrictions and quarantine requirements. The focus on mutations is a common way to prevent the spread of the virus. The more people infected, the more likely that we will see new variants. In anyway, scientists had predicted that the COVID-19 would evolve and might acquire new mutations that would thwart vaccines. So long as the authorized vaccines continue to work against the variants, the challenge will be to inoculate as many as people as possible and to prevent the COVID-19 from evolving into more impervious forms. Since then, the fact that there is production of antibodies against COVID-19 (after vaccination) does not guarantee that there will be a "perfect" protection against COVID-19 variants over time. Taking into account for high mutation rate of the COVID-19, it is therefore not possible to have a "definitive vaccine" against COVID-19 and its variants. As an example, we have new "flu vaccine" every year against influenza viruses (influenza virus is also a RNA virus) due to new variants. Regarding this issue, as for information, there were 500 million people worldwide (about one-third of the world's population at the time-in four successive waves) infected by the Spanish flu (influenza A virus subtype H1N1) occurred in 1918–1920 with 50 million deaths (675,000 deaths in USA) making it one of the deadliest pandemic in human history. Unlike today, there were no effective vaccines or antivirals, drugs that treat the flu. By the summer of 1919, the flu pandemic came to an end, as those that were infected either died or developed immunity. Over time, those who contracted the virus developed immunity, and life returned to normality by the early 1920s. It is not clear exactly how or where the 1918 influenza outbreak began, but, at some point, the novel H1N1 virus passed from birds to humans. Reports at the time suggest the virus became less lethal as the pandemic carried on in waves. Almost 90 years later, in 2008, researchers announced they had discovered what made the flu so deadly: a group of three genes enabled the virus to weaken a victim's bronchial tubes and lungs and clear the way for bacterial pneumonia.[83–85] But the strain of the flu did not just disappear. The influenza virus continuously mutated, passing through humans, pigs and other mammals. Descendants of the 1918 H1N1 virus make up the influenza viruses we are fighting today. The pandemic-level virus morphed into just another seasonal flu. Since 1918, there have been several other influenza pandemics, although none as deadly.[86,87] As for information, it has required 50 years to get the polio vaccine and actually, there is no vaccine against HIV

(responsible for AIDS), no "definitive vaccine" against *Plasmodium Falciparum* (responsible for malaria that has afflicted humans for thousands of years). In general, it requires averagely 16 years to get a vaccine.

5. Conclusion

The rapid spread of the variants of COVID-19 underscores the importance of a coordinated and systematic sequencing effort to detect, track, and analyze emerging COVID-19 variants. In many countries, we do not know which variants are circulating now since little recent sequence data are available, and it is only through multi-country genomic surveillance that it has been possible to detect and tract the variants. If any mutations are found to increase the transmissibility of the virus, previous effective infection control measures might no longer be sufficient. Along similar lines, it is imperative to understand whether novel variants impact the severity of the disease. So far, we have no evidence for any such effect.[30,45] Also, animal models are the fundamental tools to investigate the viral pathogenesis, to develop vaccines and antiviral drugs. A rapid generation of mouse-adapted (MA) viral strains or mice carrying human receptor is a good option for urgent and effective animal studies. Furthermore, development of humanized animal model might provide a direct infection of coronavirus to human tissue. There is then an urgent need to study deeply on the structure, mutations, and function of COVID-19 as well as its pathophysiology from a large population such as the "long COVID" complications,[88] post-traumatic stress disorder (PTSD),[89,90] and metabolic dysregulation[91,92] observed in patients with COVID-19. Regarding diabetic patients, diabetes could be one of the risk factor for severity and mortality in COVID-19 infected people.[2–6,91] Recently, Nair et al.[93] described the production of "stem-cell-derived β cells" (SC-β cells) that are similar to pancreatic β cells and respond to fluctuating glucose levels by increasing or decreasing secretion of insulin, as appropriate. To test whether they might be therapeutically useful, the researchers transplanted human embryonic stem cell-derived SC-β cells into mice genetically engineered to display type 1 diabetes-like symptoms. After 2 weeks, the SC-β cells were producing significant amounts of insulin in response to glucose and prevented the mice from developing dangerously high blood glucose levels. Although the process will need to be adapted for large-scale manufacturing, and further tests must be conducted to determine if SC-β cells can be a long-term replacement for β cells in people, this dramatically improved process for making large amounts of β cells is a promising step toward developing therapeutic stem cell therapies. SC-β cells technology can lead to advances in treating diabetes and in artificial organ development, especially if ways to protect newly transplanted β cells from the autoimmune

attack are developed. Additionally, SC-β cells offer a valuable new resource for investigating beta cell biology and disease modeling, as well as opportunities for drug screening and testing novel potential therapies. In addition, the expression vectors as described in Ref.,[62] especially the one with the glycosyl-phosphatidylinositol, GPI, anchor, can be used as a model for the construction of expression vectors for any protein targeting to the cell plasma membrane for studying intermolecular interactions and could be therefore useful in antiviral drugs and vaccines development as well as for studying the effects of mutations of COVID-19 (studying intermolecular interactions between the S protein of COVID-19 as well as its variants and ACE2). So long as the authorized vaccines continue to work against the variants, the challenge will be to inoculate as many as people as possible and to prevent the COVID-19 from evolving into more impervious forms. The scientific community needs to get ahead of this emerging problem and investigate vaccine approach known to reduce the potential for viral escape. In the meantime, be vigilant: wear a face mask, practice social distancing by staying 6 feet apart, avoid crowds, keep washing our hands well and often, travel restrictions and quarantine requirements are still needed for a long period of time (with or without vaccines) up to $\geq$70% of protection in the general population to assure there is herd immunity: the point at which the virus can no longer find new hosts to infect. It is a new disease, so nobody knows the precise level, and new variants of the virus could push the number higher. It is the best way to contain the spread of COVID-19 and so that there will be no more COVID-19 as well as its variants via herd immunity.

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