Editorial

Covid-19 pandemic: following the science

By

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ABSTRACT

Almost as soon as the novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered, scientists worldwide started probing its biology, while others developed diagnostic tests or vaccines to control it. However, studies are still on-going on the biology, management and prevention of Corona Virus Disease 2019 (COVID-19). This paper examined the role of Science in this regard. By following the science we now know that the structure and genomic sequence of SARS-CoV-2 is the key to understanding its mode of transmission and infection. It is also critical in developing novel plans for the management or prevention of the disease. Thus in this regard current evidence indicates the virus requires binding of SARS-CoV-2 Spike (S) glycoprotein to angiotensin-converting enzyme 2 (ACE2) receptor for entry into host cell. Thus the COVID-19 pandemic has elicited scientific groups worldwide to evolve unusual technologies for the identification of COVID-19 infection or to readjust existing identification systems conforming to the characteristics of the new SARS-CoV-2 virus. The innovative methods for detection of the virus such as RT-PCR-based molecular tests, rapid antigen or antibody tests and immunoenzymatic serological tests were described in the paper. The paper shed light on Science of the therapeutics used for management of COVID-19 based on the following: inhibition of virus entry, inhibition of virus replication, immunomodulatory agents and immunoglobulin therapy. The paper showed that by exploiting different technologies, scientists were able develop anti-COVID-19 candidate vaccines which target the entire SARS-CoV-2 molecules or pieces of molecules exhibited on surface of the virus. During their replication in host cells SARS-CoV-2 can alter their genome sequence and this process is referred to as mutations. The paper discussed the current upsurge in several mutations and variants of SARS-CoV-2.

Key words: Pandemic, coronavirus, transmission, replication, immunity

INTRODUCTION

The word plague has been a recurring word in the history of pandemics. The word plague originates from Doric Greek word *plaga* (strike, blow) and has often been used in ancient times as a general term for an epidemic disease causing a high rate of mortality, or a description of any sudden outbreak of a disastrous evil or affliction (Huremović, 2019). The best-known plagues were recorded in the bible where the Egyptians were visited by ten plagues in order to free the Israelites, held in captivity by the Pharaoh, the ruler of Egypt. In the Christian world the occurrence of plague in biblical times is believed to be a "Divine punishment for sins" or as heralding the "End of Days" (that is, the end of the world).

It is of note that these accounts, whether true or not, have been rooted in responses of the world to pandemics as no remarkable attention has been given to these phenomena. This absence of attention is a serious issue, as outbreaks in

pandemics can be considered as some of the greatest catastrophes in human history (Huremović, 2019). As evident in history, pandemic outbreaks have not only led to deaths to millions but have also brought about innovations and headways in Sciences and Technology (Huremović, 2019).

The World has been gripped by a pandemic over the first half of 2020. Specifically, in December of 2019, some residents of Wuhan in China developed pneumonia of unknown cause (Lu et al., 2020). Subsequent deep sequencing analysis of samples from lower respiratory tract of these individuals in January 2020 identified the causative agent as a novel virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Huang et al., 2020). The disease caused by SARS-CoV-2 was named on February 11th, 2020 as Corona Virus Disease 2019 (COVID-19) by the Chief Executive of the World Health Organization (WHO), Dr. Tedros Adhanom Ghebreyesus and by March 11th, 2020, WHO declared the disease a pandemic due to its spread to 114 countries, with 118,000 recorded cases and more than 4000 deaths (Di Gennaro, 2020). Available records as of 1 February 2021, indicate there are over 100 million people globally from more than 210 countries with confirmed SARS-CoV-2 infection and also more than 2 million deaths from the disease (Wang et al., 2021). This disease is caused by an RNA virus, with a quintessential crown-like aspect due to the glycoprotein spikes on its envelope as observed under an electron microscope (Di Gennaro, 2020).

Coronavirus induced epidemic has been reported in the past to cause a notable global health menace. The province of Guangdong in China had an outbreak of coronaviruses (CoVs) with Severe Acute Respiratory Syndrome (SARS)-CoV in November, 2019 and also an outbreak of the Middle East Respiratory Syndrome (MERS)-CoV was reported in September 2012 in Saudi Arabia (Shereen et al., 2020). Previous studies have revealed the presence of four genera of CoVs and these are α-coronavirus, β-coronavirus, δ-coronavirus, and γ-coronavirus. The α-coronaviruses and β-coronaviruses are most likely present in rodents and bats, while δ-coronavirus and γ-coronavirus are probably present in avian species. (Di Gennaro, 2020). Besides SARS- CoV-2, SARS-CoV and MERS-CoV that cause more fatality humans can be infected by several other coronaviruses such as HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43 that are associated with mild respiratory disease (WHO, 2020). SARS-CoV-2, SARS and MERS are all beta coronaviruses whose outbreaks are believed to be related to interactions between humans and animals (Zhu et al., 2020). The new coronavirus 2019-nCoV (SARS-CoV-2) may be transferred through the airway, by direct contact, and may be through patients' excreta which may contain the life virus (Zhang et al., 2020).

As the Covid-19 pandemic evolved, innovative technological applications and initiatives were deployed to meet challenges that emerged in the health sector, educational sector and other sectors of human endeavors. However, the devastation occasioned by the emergence of SARS-CoV-2 makes the disease caused by this virus a foe to mankind. However it's a "foe" that we cannot see, and that we do not know much about. So how do we confront such a foe? The only course of action is to analyse it in terms of what it needs, what it perpetrates in order to remain alive, and what are its weaknesses. Mankind has always been deployed Science and Technology as a powerful weapon to fight epidemics, and the world is making full use of this weapon to secure the battle against COVID-19. Almost as soon as SARS-CoV-2 was discovered, scientists worldwide started probing its biology, while others developed diagnostic tests or vaccines to control it. However, studies are still on-going on the biology, control and prevention of COVID-19. This paper aims to follow the Science in this regard.

SARS-CoV-2: KNOW THE VIRUS BEHIND THE COVID-19 PANDEMIC

Coronaviruses are made of a large genome, ranging from 26 to 32 kilobases in sizes (Woo et al., 2010). The structure of coronavirus is illustrated in Figure 1. Corona is a Latin word for crown which depicts the spike-like protrusions on its surface. Structurally the coronavirus consist

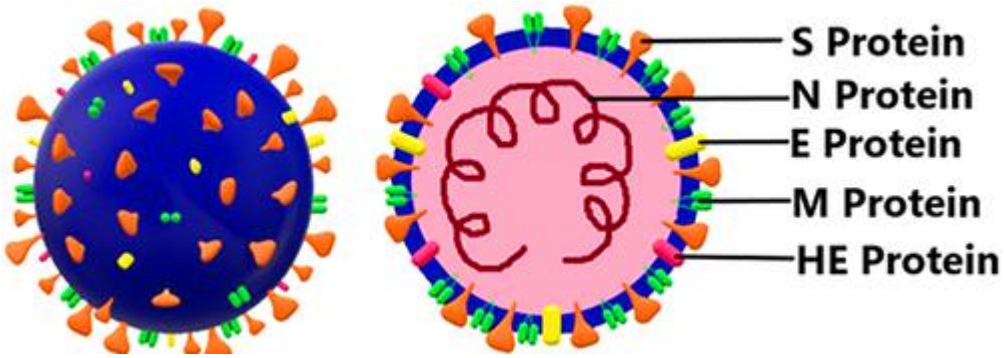

Figure 1. Structure of SARS-CoV-2-Detailed 3D model of SARS-CoV-2 virus along with its cross-section showing all the different proteins present in it, namely the spike (S) protein, membrane (M) protein, envelope (E) protein, nucleocapsid (N) protein, and Hemagglutinin-esterase (HE) protein. Source: Harrison et al. (2020).

of the following:

Trimeric spike (S) protein
This protein recognizes angiotensin converting enzyme 2 (ACE2) receptors attached to the membrane of the host cell found in the lungs, kidneys heart, intestines, esophagus and other organs. Proteases found in the host's cells such as serine 2, cathepsins, trypsin, and furin cause cleavage of the spike protein, that enables the fusion of the virus inside the host cell, and this is mediated by S2 protein of the virus (Millet and Whittaker, 2015).

Envelope (E) proteins
These are the tiniest and are found mainly in the endoplasmic reticulum (ER) and the Golgi apparatus, where they direct the fitting together and release of virus from the host cell. Consequently, they are involved in viral replication.

Membrane (M) proteins
They are glycoproteins found on the surface of the virus and are the most abundant proteins of the virus. They consist of the N-terminal domain on the outside of the virus, three transmembrane domains, and the C-terminal domain inside the viral membrane (EA and Jones, 2019). In addition to controlling the assembly of various components of the virus, it is also involved in the formation and gives shape to the virus envelope (Neuman et al., 2011).

Nucleocapsid (N) protein
This is attached to the positive-sense single stranded RNA (+ssRNA) of the virus. It breaks down the resistance mechanism and deregulate the cell cycle of the host cell and also aid in the assembly of the virus by working alongside with other structural proteins. The viral genome is packaged into capsids by this component in order to protect it (McBride et al., 2014).

Hemagglutinin-esterase (HE) protein
This is a glycoprotein, which helps in the attachment and destruction of sialic acid receptors to the surface of the host cell. The viral envelope is located under the surface proteins. It is composed of a fatty bilayer that breaks down on contact with soap and water. Inside the viral envelope is a capsid that surrounds the genetic material of the virus. Hence it is advisable to periodically wash the hands with soap and water in order to ensure safety. There are two major lineages of SARS-CoV-2. The L lineage is a common strain with a higher rate of mutations and is more severe and aggressive than S, the lesser common strain. These strains were formed due to single nucleotide polymorphisms (SNPs) at positions 8782 and 28 144 in the viral RNA, which are believed to be necessary for replication and pathogenesis of the virus (Kumar et al., 2021).

HOW IS SARS-CoV-2 TRANSMITTED?

SARS-CoV-2 is highly infectious and can be directly transmitted when an individual inhales the respiratory droplets of an infected person or indirectly transmitted on contact with objects used or touched by an infected person (Kumar et

al., 2021). The Proposed SARS-CoV-2 transmission routes are shown in Figure 2. Transmission of the virus through droplets from infected persons is one of the major transmission routes. Other possible routes can come from direct contact with an infected individual which may occur among family members in a household. The possible transmission of the virus through door handles has also been postulated although this route may be less frequent as compared to droplet or contact-driven transmission. The precursor SARS-CoV epidemic has been shown to involve airborne and fecal–oral human-to-human transmission but this mode is yet to be established in the current pandemic. In Figure 2 solid arrows are indicative of confirmed routes of transmission with decrease in arrow width indicating the relative importance of each transmission route. Possible transmission routes which are yet to be confirmed are represented with dashed lines. Areas where the virus have been detected in infected persons are indicated with SARS-CoV-2 symbol.

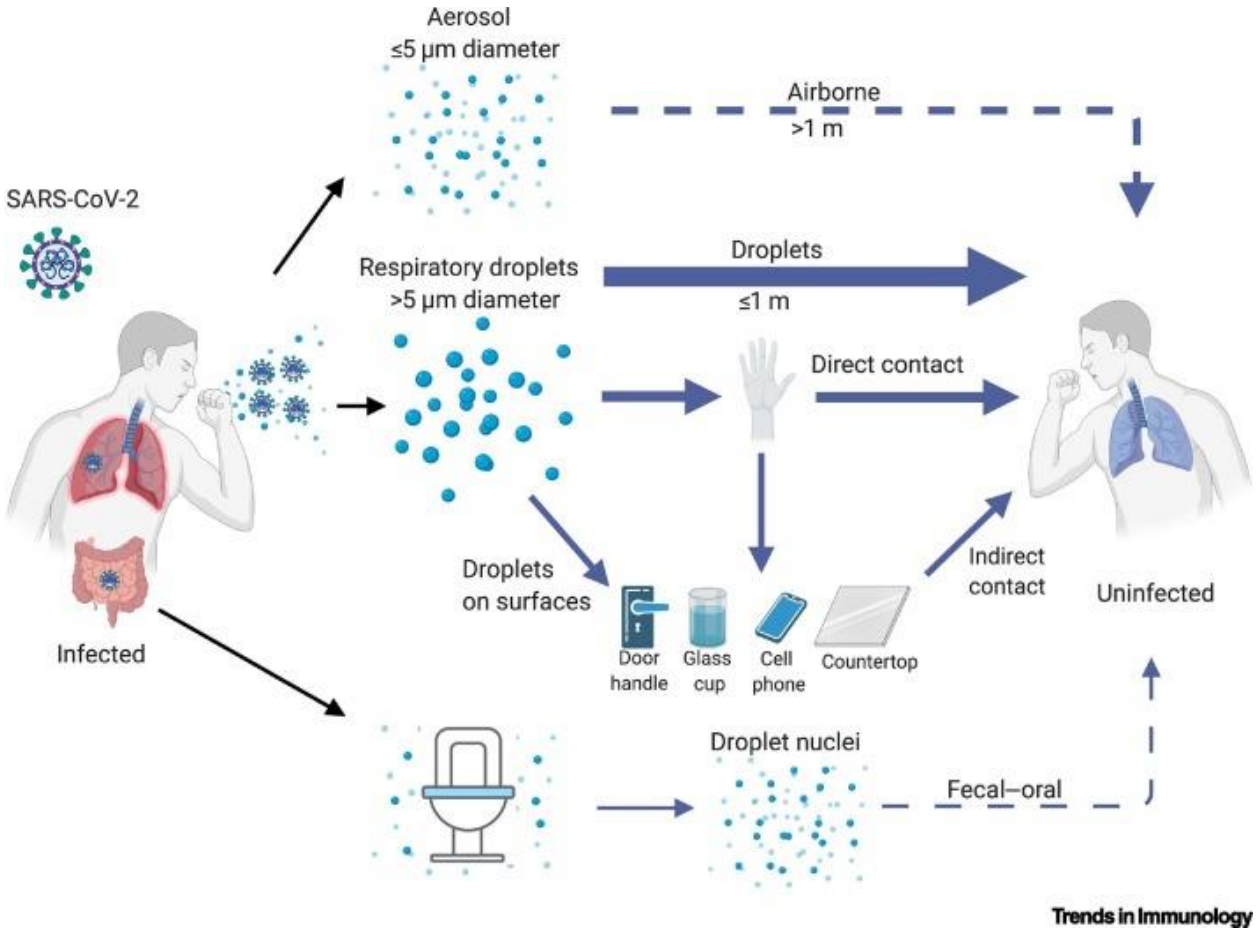

Figure 2. Possible routes of transmission of SARS-CoV-2. Source: Harrison et al. (2020).

MODE OF ENTRY AND REPLICATION OF SARS-CoV-2

The virus can gain entry into the body through the nose, eyes, or mouth. The spike protein attaches specifically to the ACE2 receptors present on the type 2 pneumocytes in the alveoli of the lungs. The collapsing pressure and the surface tension in alveoli are reduced by surfactants produced by type 2 pneumocytes (Bombardini and Picano, 2020; Mirastschijski et al., 2020). The virus enters into the host cell after binding of the ACE2 receptor (Figure 3). Entry of the virus is facilitated by host cell proteases that cleave the spike protein of the virus. The mechanism of entry involves direct cell entrance by membrane fusion or by endocytosis (Wang et

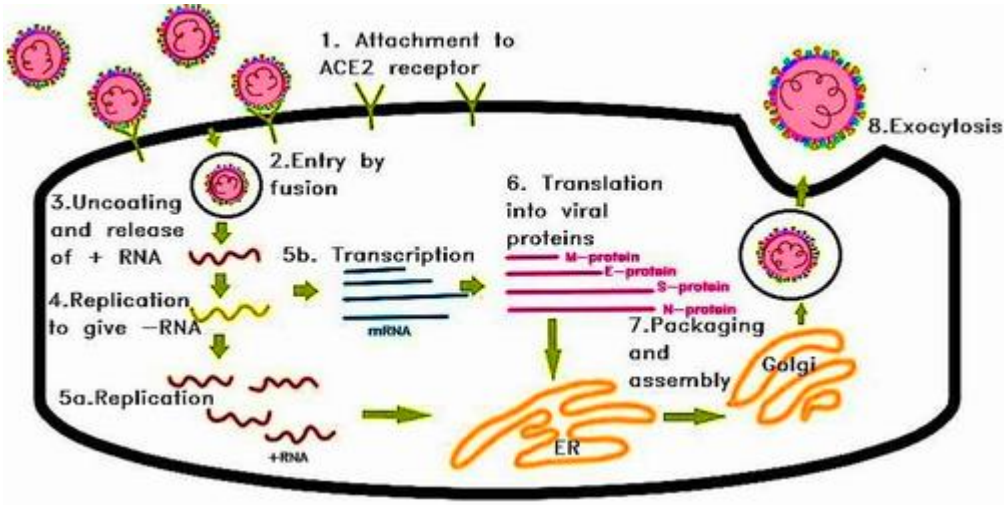

Figure 3. Stages in the replication of SARS-CoV-2 inside the host cell.
Source: Harrison et al. (2020).

al., 2008). When inside the host cell, the SARS-CoV-2 releases its positive-sense RNA within the host cell cytoplasm and is translated into polyproteins, pp1a and pp1ab which assist in the replication and transcription of the viral RNA. This production of positive-sense RNA by RNA-dependent RNA polymerase enzyme gives a negative-sense RNA. The negative-sense RNA can be transcribed or replicated to give positive-sense RNAs (incorporated in the viral genome). Transcribed mRNAs can be translated to manufacture the membrane, spike, envelope, and nucleocapsid proteins which are carried by host cell ER to the Golgi apparatus, where they are assorted into vesicles and assembled close to the host cell membrane (Nakagawa et al., 2016). The new viruses that are formed leave the host cell by exocytosis to infect other cells. This process results in death of the host cell (Figure 3).

STAGES OF COVID-19 INFECTION

There are three stages involved: asymptomatic stage, upper Airway Infection stage and acute respiratory distress syndrome (ARDS) stage. These stages are illustrated in Figure 4.

Asymptomatic stage

After transmission of the SARS-CoV-2 through respiratory aerosols it binds to the nasal epithelial cells in the upper respiratory tract. Entry of the virus into host cells is by binding to the receptor ACE-2, which is abundant in epithelial cells of the nose (Wan et al., 2020; Hoffmann et al., 2020). The virus is replicated and propagated locally and there is infection of ciliated cells in the conducting airways in the process (Sims et al., 2005). This stage which lasts for a couple of days also generates an immune response which is a limited. The individuals are extremely contagious at this stage despite the low viral load and the virus can be spotted via nasal swab testing.

Upper airway infection stage

In this phase, the virus migrates from the nasal epithelium to the upper respiratory system through the conducting airways. At this stage the disease presents with symptoms of malaise, fever and dry cough because of the involvement of the upper airways. The immune response is greater than in the first phase and involves the release of C-X-C motif chemokine ligand 10 (CXCL-10) and interferons (IFN-β and IFN-λ) from the virus-infected cells (Tang et al., 2005). Because of the immense immune response which is sufficient to contain the spread of infection, majority of patients do not progress beyond this phase.

Acute respiratory distress syndrome (ARDS) and hypoxia stage

A few infected patients progress to the acute respiratory distress syndrome (ARDS) and hypoxia stage and this is associated with severe symptoms. The virus seizes and goes into the type

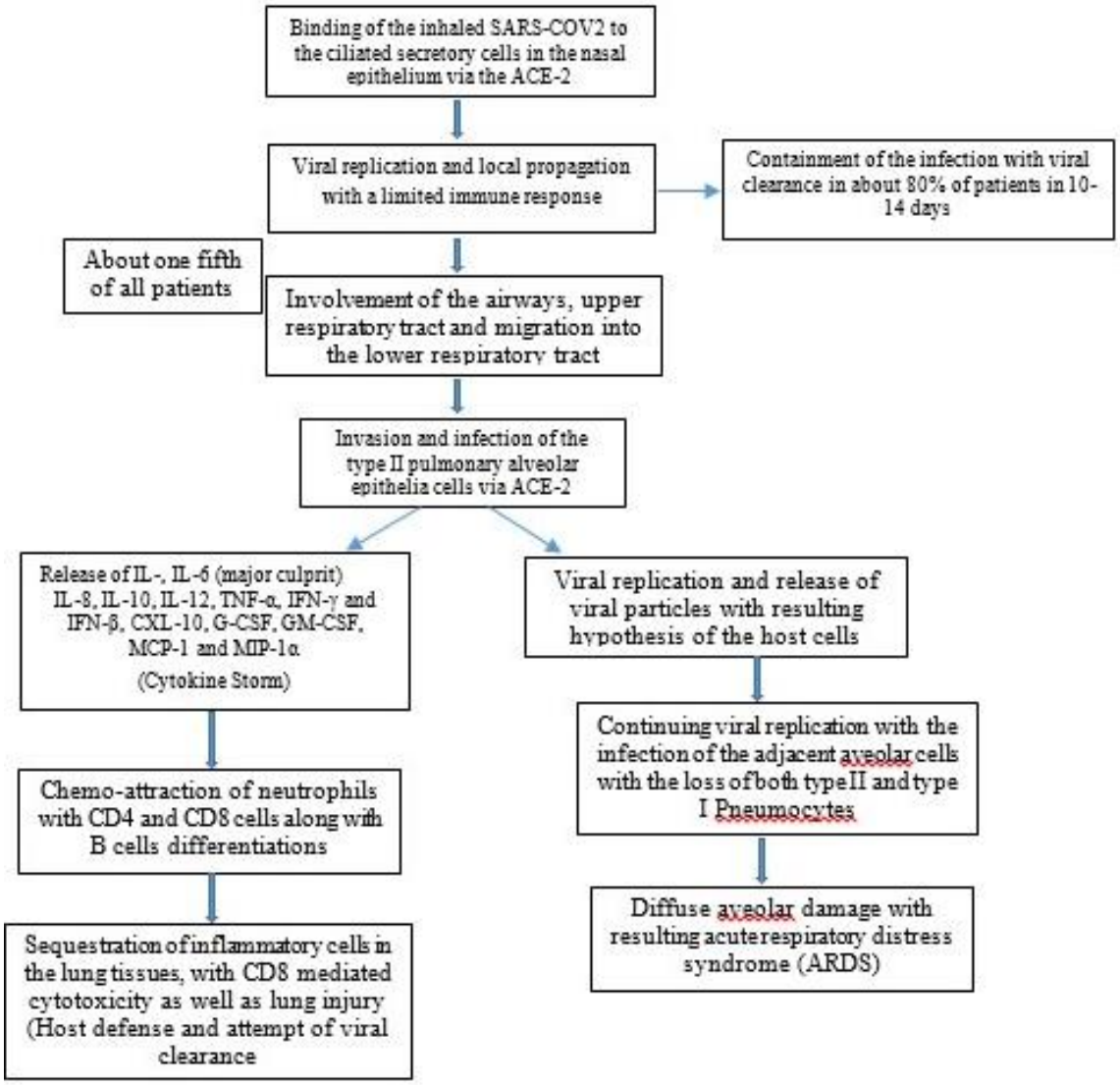

Figure 4. Stages in COVID-19 infection.
Source: Paracher (2021).

2 epithelial cells of the alveolus once again through the host receptor ACE-2 and undergoes replication to form more viral nucleocapsids. The virus-filled pneumocytes generate a lot of different cytokines and inflammatory markers such as interleukins (IL-1, IL-6, IL-8, IL-120 and IL-12), monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor-α (TNF-α), IFN-λ and IFN-β, CXCL- 10 and macrophage inflammatory protein-1α (MIP-1α). This process is known as 'cytokine storm'. In response to this, neutrophils, CD4 helper T cells and CD8 cytotoxic T cells are attracted to the infection and are sequestered in the lung tissue. These cells are in the helm of fighting off the virus, but in the process they bring about inflammation and lung damage. The host cell go through apoptosis with the release of new viral particles, which in turn infect the adjacent type 2 alveolar epithelial cells in the same way. Due to the continuing injury caused by the sequestered inflammatory cells and viral replication which cause loss of both type 1 and type 2 pneumocytes, there is diffuse alveolar damage which eventually ends in an acute respiratory distress syndrome (Cascella et al., 2020; Xu et al., 2020).

INNOVATIVE METHODS FOR THE DETECTION OF COVID-19: NO HIDING PLACE FOR THE VIRUS

Knowledge of the molecular and structural characteristics of SARS-CoV-2 has assisted

researchers world-wide in developing various diagnostic solutions for a correct diagnosis of COVID-19 (Tromberg et al., 2020; Guglielmi, 2020). Some of these solutions by research groups worldwide involve novel technologies or are re-adaptation of existing diagnostic systems according to the characteristics of the new SARS-CoV-2 virus. Among these solutions, the most frequently used and authenticated methodologies are RT-PCR-based molecular tests, immunoenzymatic serological tests and rapid antigen or antibody tests. Any of these types of diagnostic tests can be deployed at a specific time of infection.

RT-PCR-Based molecular tests

Real Time Polymerase Chain Reaction (RT-PCR)-based molecular methods is the flag-ship of standard techniques used worldwide for conformational diagnosis of COVID-19 infection because of its rapid identification, high sensitivity and specificity (Mahendiratta et al., 2020). The total sequencing of the SARS-CoV-2 genome (Wu et al., 2020) has allowed researchers all over the world to device molecular primers and probes precise to SARS-CoV-2 RNA sequences. This has enabled them to differentiate the diagnosis of COVID-19 infections from other ailments such as seasonal flu or bacterial infections with similar symptoms with COVID-19 (da Silva et al., 2020; Li et al., 2020). The virus can be detected directly by determining the viral genome or the antigen in these samples. The test involves purifying the viral genome from the samples and amplifying it with primers specific to the SARS-CoV-2 virus. Identification of the virus is done using the genomic portions of SARS-CoV-19 encoding for the RdRP gene (RNA-dependent RNA polymerase gene), for proteins comprising the spike molecules (S gene) and nucleocapsid (N gene), for proteins of the envelope (E gene), for the membrane, etc.

RT-PCR-based molecular tests are widely used because of comparatively low costs of the entire viral RNA extraction, reverse transcription and amplification procedure, and the accessibility of RT-PCR thermal cyclers in private laboratories, hospital laboratories and research institutes (Dorlass et al., 2020). The technique is also easy to execute and requires no highly trained personnel (Neilan et al., 2020). In addition, RT-PCR kits guarantees outstanding reproducibility and standardization of the data obtained which are less swayed by operator bias (Smyrlaki et al., 2020). The RT-PCR test is subject to a number of limitations that can lead to false-negative or false-positive outcomes. One of these limitations of RT-PCR is its low responsiveness in correctly diagnosing samples with low viral load, including swabs taken incorrectly or obtained from asymptomatic or paucisymptomatic persons (Pan et al., 2020). Other limitations include the influence of impurities and interferers contained in the sample or introduced by the operator which have the ability of inhibiting the reaction (Pan et al., 2020). The execution time of the analysis, which can take up to 24 h to obtain a result in non-automated systems is also a limitation of the method.

Rapid antigen or antibody tests

Scientific companies have developed various rapid tests to detect viral antigens or anti-SARS-CoV-2 human antibodies in nasal, salivary or oropharyngeal swabs and blood samples. These tests are lower in cost, have simple procedures that does not need highly trained personnel (Pilarowski, 2021) as compared to RT-PCR tests and direct testing at the point-of-care and the quickness in getting a result (30 min).The principles of these test are based on the direct detection of viral proteins known as rapid antigen tests or human antibodies against SARS-CoV-2 antigens known as rapid antibody tests. As for the rapid antigen tests, the identification of COVID-19-positive persons is by the detection of SARS-CoV-2 Spike proteins or nucleocapsid which serves as the viral antigens in swabs obtained from the upper airways of the subject with supposed infection. This test is stem from the bond between antigens and antibodies. Similarly, a rapid antibody test is based on the same principle. In this test human immunoglobulin G (IgG) or human immunoglobulin A (IgA) and human immunoglobulin M (IgM) against SARS-CoV-2 antigens immobilized in the test lines bind to antigens of these antibodies present in the virus. Further processes in the test results in a colorimetric reaction which shows the

positivity of the sample.

It is noteworthy that these tests yield qualitative results which assist to prove if the individual is positive or not for COVID-19 infection without evaluating the viral load. The rapid antibody tests can be used to show whether an individual is carrying anti-SARS-CoV-2 antibodies; but cannot show whether the individual has an active SARS-CoV-2 infection or already rectified disease (Mak et al., 2020; Lanser et al., 2020). The rapid antigen and rapid antibody tests have some limitations such as low specificity and sensitivity with a value of 99.5 and 56.2% respectively (Dinnes et al., 2020).

In general, rapid antigen and rapid antibody tests are notably used as screening strategies for large portions of the population (Augustine et al., 2020; Canetti et al., 2020); however, they cannot be used for confirmation of COVID-19. Therefore, RT-PCR analyses should always be carried out for confirmation of infection.

Immunoenzymatic serology test

This is based on the principle of indirect Enzyme-Linked Immunosorbent Assay (ELISA). Human immunoglobulins, proteins, antigens and other peptides can be detected and quantitated with the use of ELISA and this involves the binding of the target protein and a specific antibody which develops into a detectable signal (Alhajj and Farhana, 2020). This technique gives rise to highly precise and sensitive results within a short time (Alhajj and Farhana, 2020). Briefly, these tests involve immobilized viral antigens that are bound after recognition by anti-SARS-CoV-2 antibodies present in the serum of supposed infected persons. Further processes are undertaken which results in a colorimetric reaction easily detectable by optical densitometry (or fluorescence or chemiluminescence) whose intensity is an indication of the quantity of IgM, IgG or IgA anti-body presents in the sample.

Alternatively the test can involve immobilized antibodies against SARS-CoV-2 antigens which are capable of binding antigens contained in the serum samples of patients. Further processes of positive samples results in a colorimetric reaction. ELISAs are very important in tracking COVID-19 infection as they are used for the detection of IgM and IgG antibodies specific for SARS-CoV-2 antigens (Tre-hardy et al., 2021). This can be undertaken by taking the blood, plasma, or serum of the patient and testing it for IgG and IgM antibodies which are proteins induced due to immune response to viral antigen. The IgM antibodies are detectable after 7 days of infection and remain in the blood for another 14 days of infection. Conversely the IgG antibodies appear during the 2nd week of infection and remains in the blood to provide long-term immunity (Jacofsky et al., 2020). Detection of the antibodies as a diagnosis technique may not be advantageous as the period of their presence is when the patient is in the recovery phase (Figure 5). The patient might have transmitted the virus to others during this time. So a false-positive result can be obtained after the patient recovers by detecting long-term antibodies in the blood. SARS-CoV-2 ELISAs are common choices in the rapid screening and exact quantitation of viral proteins (antigens) or human antibodies.

Imaging

Chest X-Rays as well as CT scans can be used as supplementary tests in addition with RT-PCR tests. These tests can allow for assessment of the progression and damage due to COVID-19. CT scans can be applied to establish the presence of damaged lungs, inflammation, and pneumonia which are common symptoms of COVID-19 (Meng et al., 2020). CT scan images for suspected COVID-19 cases can be interpreted with artificial intelligence (Jin et al., 2020). In addition, clots in the pulmonary circulatory system can be identified by a technique called a CT angiogram (Al-Ajlan et al., 2017).

Specific High-Sensitivity Enzymatic Reporter unLOCKing (SHERLOCK)

This is another method of detecting COVID-19. This test involves the use of a sample from the upper part of the respiratory system of a suspected COVID-19 patient. The RNAs are isolated from the sample and amplified and reporter genes added. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas13 is then introduced in addition to

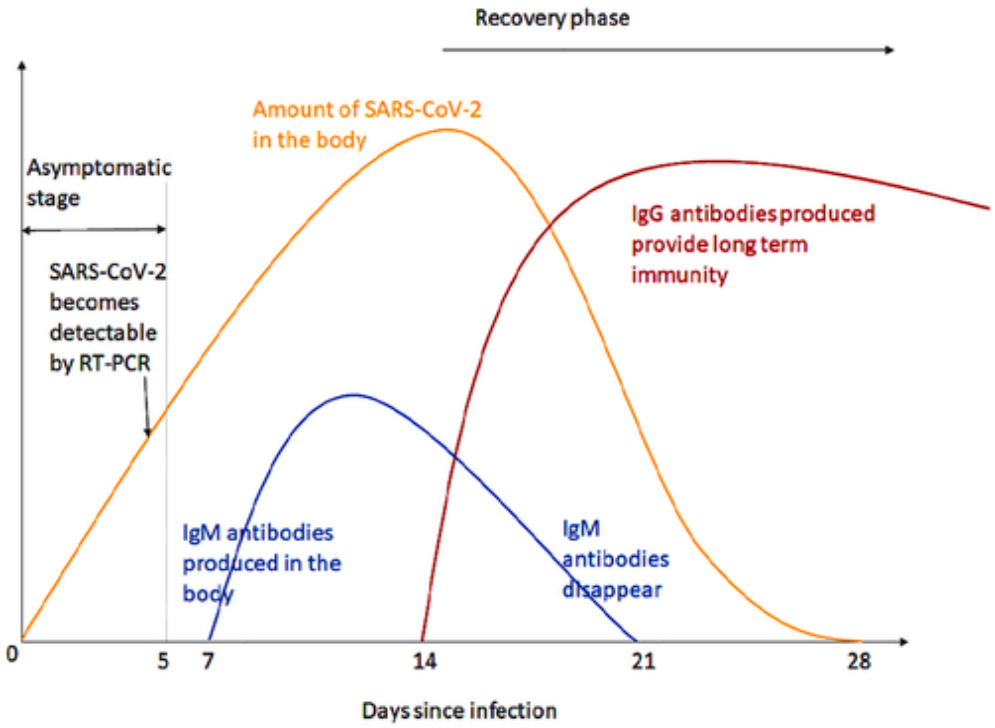

Figure 5. Timeline of the COVID-19 infection until recovery, along with the antibodies produced. It can be noted from here that antibody testing can diagnose a patient in his recovery period. Source: Kumar et al. (2021).

a guide RNA that is expected to pick out the viral RNA for cleaving. This cleaving generates a signal if the virus RNA is present. A lateral flow assay device is used for the test. The presence of two bands indicate presence of viral RNA, while only one is obtained if the reporter gene is not cleaved and this occurs when viral RNA is absent. This technique has been successfully used to detect very small amounts of pathogens (Xiang et al., 2020). In general, within a short period of time, scientists have been able to develop several methods for diagnosing COVID-19 infection. However, an accurate diagnosis of COVID-19, would require taking into account, the test to adopt, the patient's medical records, the time of exposure to SARS-CoV-2, the type of specimen selected and analyzed and how to explain the result. Evaluation of all of these elements, will help for the accurate detection of COVID-19 infection and successfully control the COVID-19 pandemic.

THERAPEUTIC INTERVENTIONS: ANY HOPE FOR MANKIND?

Science is yet to provide any proven effective therapies for COVID-19 or antivirals against SARS- CoV-2, but some treatments have been beneficial in the management of the disease. The potential therapeutics against SARS- CoV-2 are summarized below.

Inhibition of virus entry

Drugs that interfere with entry of the virus may be a potential treatment for COVID-19. Drugs such as umifenovir (arbidol) are used for treatment of influenza and other respiratory viral infections are useful in this regard. It can target the interaction between the S protein and ACE2 and inhibit entry of the virus. However, clinical studies indicate that umifenovir might not improve mild to moderate COVID-19 infection (Li et al., 2020; Lian et al., 2020). Camostat mesylate is another potential drug for treatment of SARS-CoV-2 infection. This drug is used for the treatment of pancreatitis and postoperative reflux oesopha but has recently been shown to block the entry of SARS- CoV-2 into human lung cells (Hu et al., 2021). However sufficient clinical data to support its efficacy is still lacking. Chloroquine and hydroxychloroquine have also shown promise in the treatment of COVID-19 as they interfere with the entry of SARS- CoV-2.

Chloroquine and hydroxychloroquine are commonly used for treatment of malaria and other diseases such as rheumatoid arthritis. The basis of their action against COVID-19 is predicated on the inhibition of the glycosylation of cellular receptors and interference with virus–host receptor binding. Other mechanisms involved in their action relate to increase in endosomal pH and inhibition of membrane fusion. Never the less the use of these drugs for the treatment of COVID-19 is a bone of contention among scientists as there are no concrete evidence of their efficacy (Rosenberg et al., 2020; Geleris et al., 2020).

Inhibition of virus replication

Some drugs can inhibit replication of COVID-19 virus. These drugs include remdesivir, favilavir, ribavirin, lopinavir and ritonavir. Lopinavir and ritonavir inhibit replication of 3-Chymotrypsin-Like Cysteine Protease (3CLpro), while the others target RNA-Dependent RNA Polymerase (RdRp) (Wang et al., 2020). The viral 3CLpro enzyme controls coronavirus replication and is essential for its life cycle (Tahir ul Qamar et al., 2020). On the other hand RdRp, is a highly versatile enzyme that assists in RNA synthesis by catalyzing the RNA-template-dependent formation of phosphodiester bonds. Available evidence indicates that remdesivir is active against SARS- CoV-2 (Hu et al., 2021). As a result during the peak of COVID-19 infection in the US, the FDA had to issue an emergency order authorizing the use of remdesivir for the management of patients hospitalized for severe COVID-19. On the other hand Favilavir, which was developed in Japan as an ant-influenza drug, was approved as therapy for COVID-19 in Russia, China and India. Studies show that lopinavir and ritonavir have in vitro inhibitory activity against SARS- CoV and MERS- CoV (Hu et al., 2021). Studies also show that both drugs were more effective when they are administered to patients concurrently with other drugs, such as ribavirin and interferon beta-1b (Hu et al., 2021).

Immunomodulatory agents

A strong immune response can be triggered by SARS-CoV-2 and as earlier stated this may cause cytokine storm syndrome (Hu et al., 2021) This situation can be potentially exploited in the management of COVID-19 with administration of anti-inflammatory and immunosuppressant drugs such as dexamethasone that inhibit the excessive inflammatory response. Eculizumab is a specific monoclonal antibody that hinders the proinflammatory complement protein C5. Preliminary findings showed that Eculizumab can induce a drop of inflammatory markers and C-reactive protein levels, suggesting its potential for the treatment of severe COVID-19 (Diurno et al., 2020). The interferon response is one of the vital innate immunity defenses against virus invasion because it induces the expression of diverse interferon- stimulated genes, which can interfere with virus replication. Prior studies showed that SARS-CoV-2 is sensitive to type I interferons than SARS- CoV, suggesting the possible efficacy of type I interferons in the early treatment of COVID-19 (Hu et al., 2021). The efficacy of different therapies involving interferons singly or in combination with other agents is currently under clinical trials in several countries (Hu et al., 2021).

Immunoglobulin therapy

The use of convalescent plasma as a treatment for COVID-19 has been explored in many countries. Preliminary findings were encouraging as it ameliorated clinical standing after the therapy (Hu et al., 2021). However, this treatment may cause adverse effects such as antibody- mediated enhancement of infection, transfusion- associated acute lung injury and allergic transfusion reactions. Monoclonal antibody therapy is a type of immunotherapy that is effective for the treatment of some viral infections. Monoclonal antibodies are man-made proteins that act like human antibodies in the immune system. Recent studies have shown that specific monoclonal antibodies can neutralize SARS- CoV-2 infection in vitro and in vivo (Wang et al., 2020; Hu et al., 2021). Monoclonal antibody therapy presents the prospect for the cure and prevention of COVID-19. The neutralizing epitopes of these monoclonal antibodies also provide important enlightenment. However, the high cost, low bioavaibility and limited capacity of manufacturing have restricted the use of monoclonal antibody therapy.

VACCINATION-A MAJOR TOOL AGAINST COVID-19 IN OUR TOOL BOX

Over 150 vaccine designs have been officially proposed as at today (Forni and Mantovani, 2021). Almost fifty of these are already undergone human trials and some have already been approved for use. By exploiting different technologies, these anti-COVID-19 candidate vaccines are attacking the entire SARS-CoV-2 molecules or pieces of molecules expressed on surface of the virus. These candidate vaccines have been grouped on the basis of the technological principles utilized to obtain a defensive immune response. However, the effectiveness or time span of the produced defense or the cover of the vaccine depends on the features that make it distinctive.

The emergence of these candidate vaccines in record time underscores human scientific creativity which is unprecedented in the history of vaccine development. It is pertinent to state that despite the competition by vaccine manufactures with each other with their associated practical secrets, their formulation proceeds from basic scientific research that is premised on open cooperation between various laboratories in the world. Most pioneers of innovative vaccine have focused their endeavors on activating an immune response against the Spike protein (Figure 6) due to the several and freely available rudimentary research data on the mechanisms of SARS-CoV-2 infection. The data that emerged from various trials and academic work indicate that vaccines designed on the basis of nucleic acids encoding for the Spike protein, carried by liposomes or adenoviruses, can evoke an effective protective response (Forni and Mantovani, 2021). The development of these vaccines based on previously unproven technologies has significantly contributed in curbing a pandemic that has caused more than 4 million deaths (Cohen, 2020). On the basis of their mechanism of action coronavirus vaccines can be grouped into five types

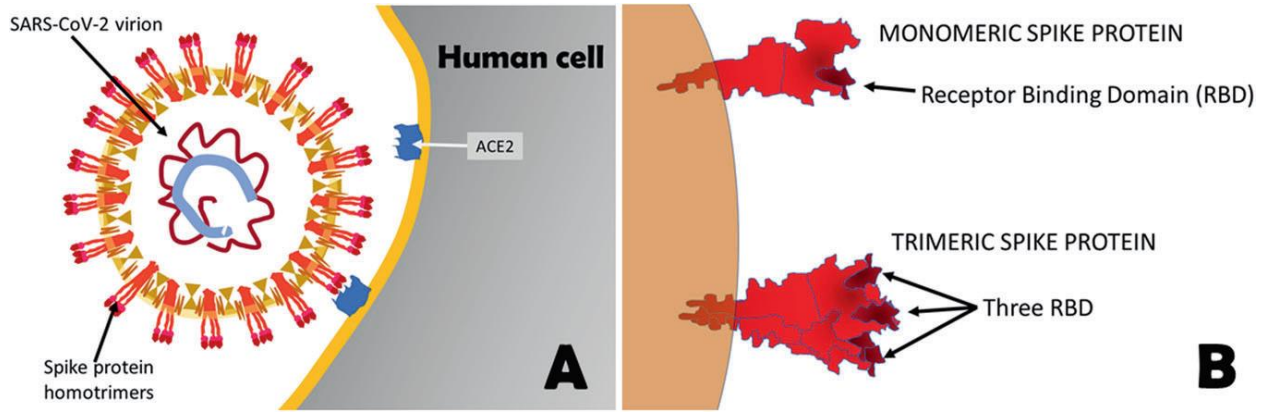

Figure 6. Spike as a target for vaccine development. A depicts the binding mechanism of coronavirus to the human cell, B depicts the different types of spike proteins and their receptor binding domains which is involved in the binding mechanism.
Source: Forni and Mantovani (2021).

TYPES OF CORONAVIRUS VACCINES

Viral vector vaccines for COVID-19

In this technology unrelated harmless viruses are used as vectors to convey into the cell's DNA coding for the Spike proteins of SARS-CoV-2. This mechanism exploits the vector virus's great capacity to infect and convey the mRNA into the human cells (Figure 7). The University of Oxford/AstraZeneca vaccine uses this technology to protect against COVID-19. When administered, genetic material in the vector virus induces the human cells to produce a specific SARS-CoV-2 protein, which is recognized by our immune system and a response is triggered (Strizova et al., 2021). This response builds immune memory which enables the body to fight off the virus in future. These virus-based vaccines are administered by intramuscular injection. However, it is noteworthy that efforts aiming at nasal administration by inhalation are

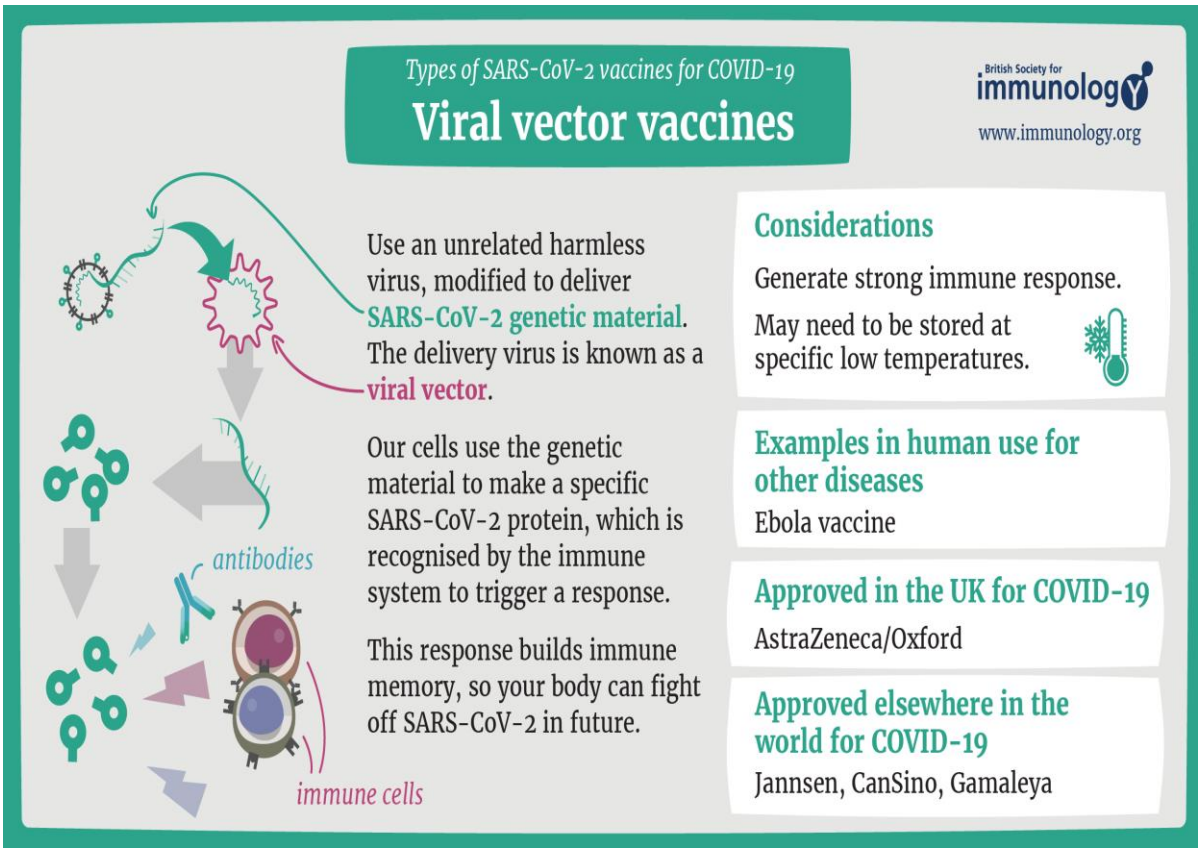

Figure 7. Mechanism of action of viral vector vaccine for COVID-19 (downloaded from website of the British Society for Immunology).

ongoing and if successful the candidate vaccine could inhibit the ability of coronavirus entering the body as it will be neutralized by mucosal immunity induced in the nose.

Genetic vaccines for COVID-19

The Pfizer/BioNTech and Moderna COVID-19 vaccines use nucleic acids to generate immunity against COVID-19. A portion of genetic material (RNA or DNA) of the SARS-CoV-2 virus present in the vaccine allows the body to code for a particular viral protein (Figure 8). This protein is recognized by the immune system and activates a specific response which builds immune memory, so that the body can repel SARS-CoV-2 in future (Forni and Mantovani, 2021).

Inactivated vaccines for COVID-19

One of the oldest technologies used for development of vaccines is based on killed microorganisms (inactivated vaccines). This technology has been exploited by producing vaccine which carries the killed SARS-CoV-2 virus, which is recognized by the immune system to activate a response without giving rise to COVID-19 illness (Figure 9). This response protects the body from COVID-19 by building enough immunity so the body can fight off SARS-CoV-2 in future. Inactivated vaccines effect has more stability than live attenuated vaccines never the less their effect is enhanced with an adjuvant. The immune response generated is directed either against the Spike protein or many other SARS-CoV-2 antigens (Forni and Mantovani, 2021). Inactivated Vaccines have been traditionally effective against polio, rabies and hepatitis A, and showed promising antibody titers against SARS-CoV-2 compared to other vaccine types (Raman et al., 2021). An example of an IV is CoronaVac developed by Sinovac in China and is currently in use in several countries.

Attenuated vaccines for COVID-19

The earliest vaccines in history are based on a living microbe that has been weakened so it cannot give rise to disease. This mechanism has

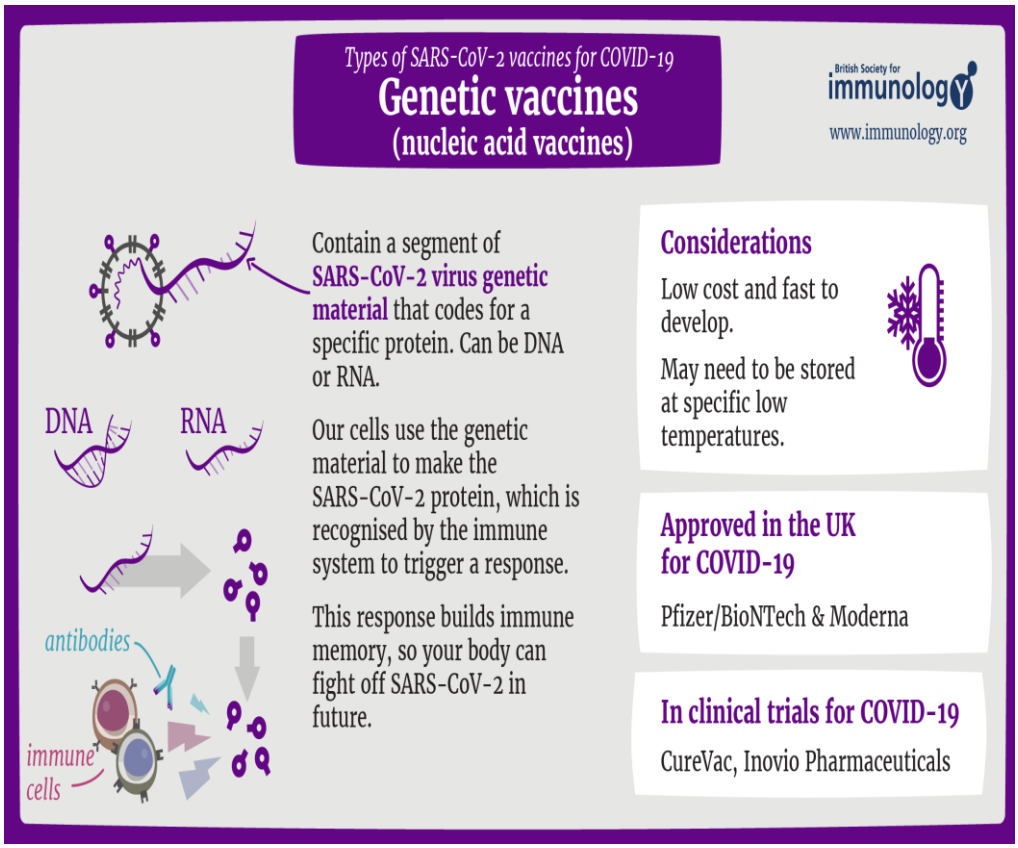

Figure 8. Mechanism of action of genetic vaccine for COVID-19 (downloaded from website of the British Society for Immunology).

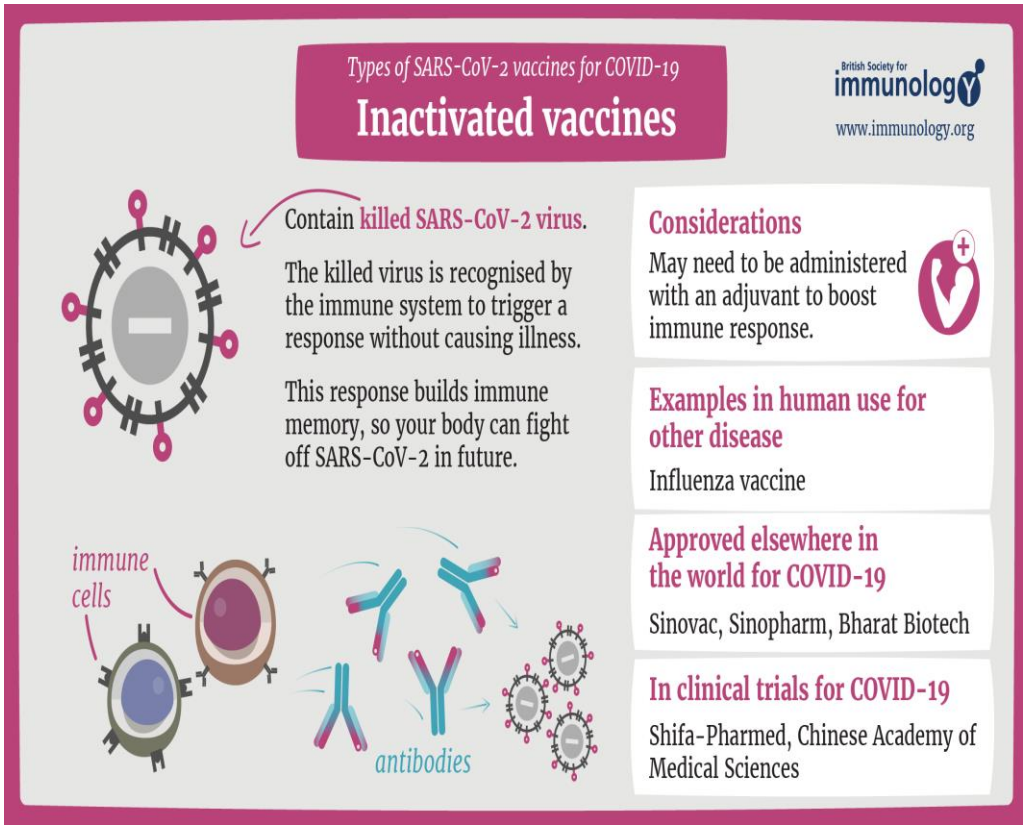

Figure 9. Mechanism of action of inactivated vaccines for COVID-19 (downloaded from website of the British Society for Immunology).

been exploited for development of anti-COVID vaccines (Figure 10). The strategy is to develop a vaccine which contains the weakened SARS-CoV-2 virus. The weakened virus is recognized by the immune system which triggers a response without giving rise to COVID-19 illness (Forni and Mantovani, 2021). This response raises immune memory that fights off SARS-CoV-2 in future. Attenuated viruses retain the ability to cause limited disease when they replicate in vivo. They are also very effective in energizing the immune system by inducing a strong and continuous immune memory that is effective in preventing infection. Attenuated vaccines have been used for the protection of people in many nations who suffered disabling and fatal diseases.

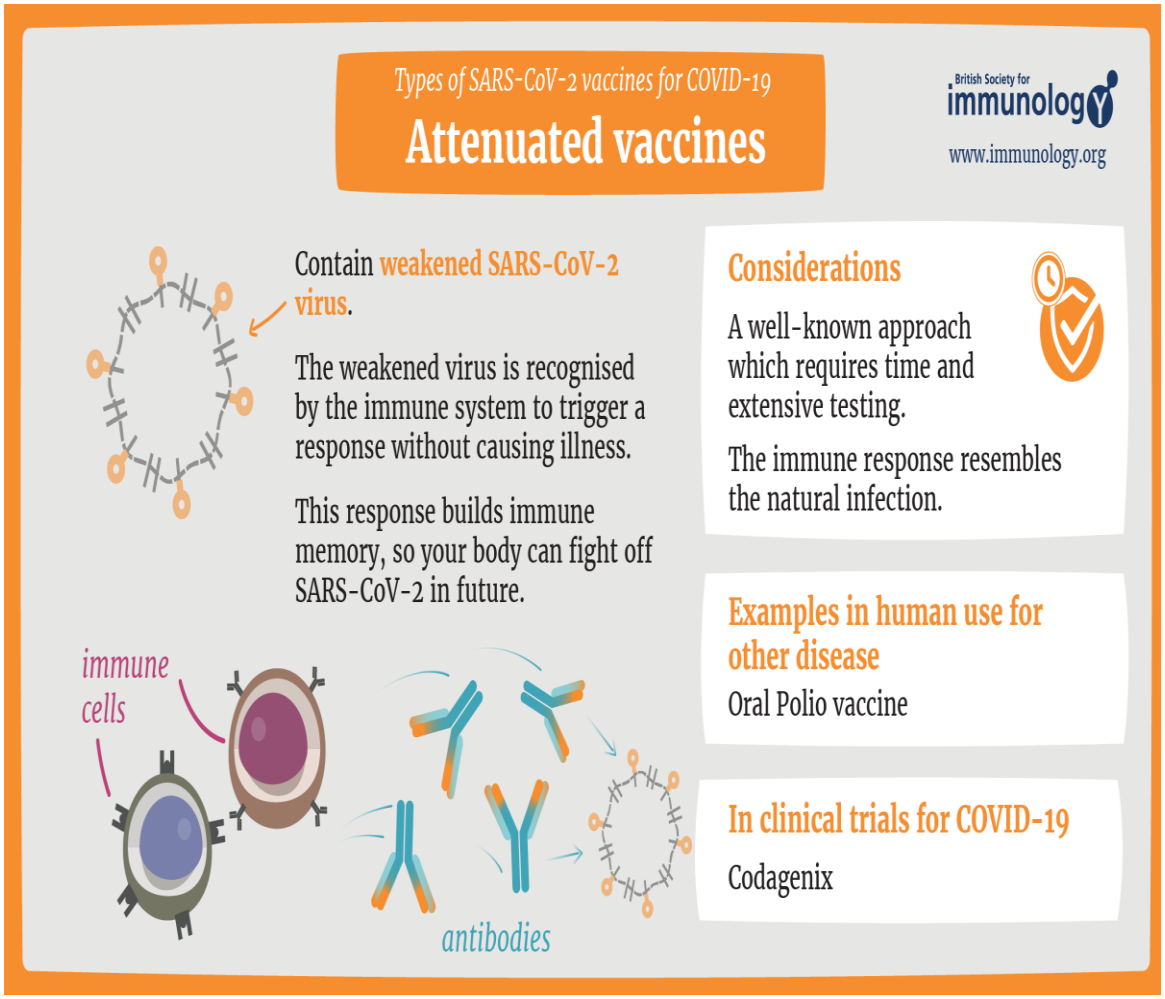

Figure 10. Mechanism of action of Attenuated vaccines for COVID-19 (downloaded from website of the British Society for Immunology).

Protein vaccines for COVID-19

This form of vaccine carries proteins from the SARS-CoV-2 virus. These proteins can be purified from the microbes but in general, they are produced in vitro through the recombinant DNA technology (Figure 11). These proteins are recognized easily by the immune system to activate or trigger a response and this enables the body to build immune memory, to hold back SARS-CoV-2 in future (Forni and Mantovani, 2021). The mechanism of action of the various vaccine outlined in this review involves targeting of the spike proteins or its fragments or other proteins such as the nucleoprotein (N). As stated earlier, the large trimeric clusters of the Spike protein that are on the surface of the virus play a crucial role in the attachment of the SARS-CoV-2 to human cells. A

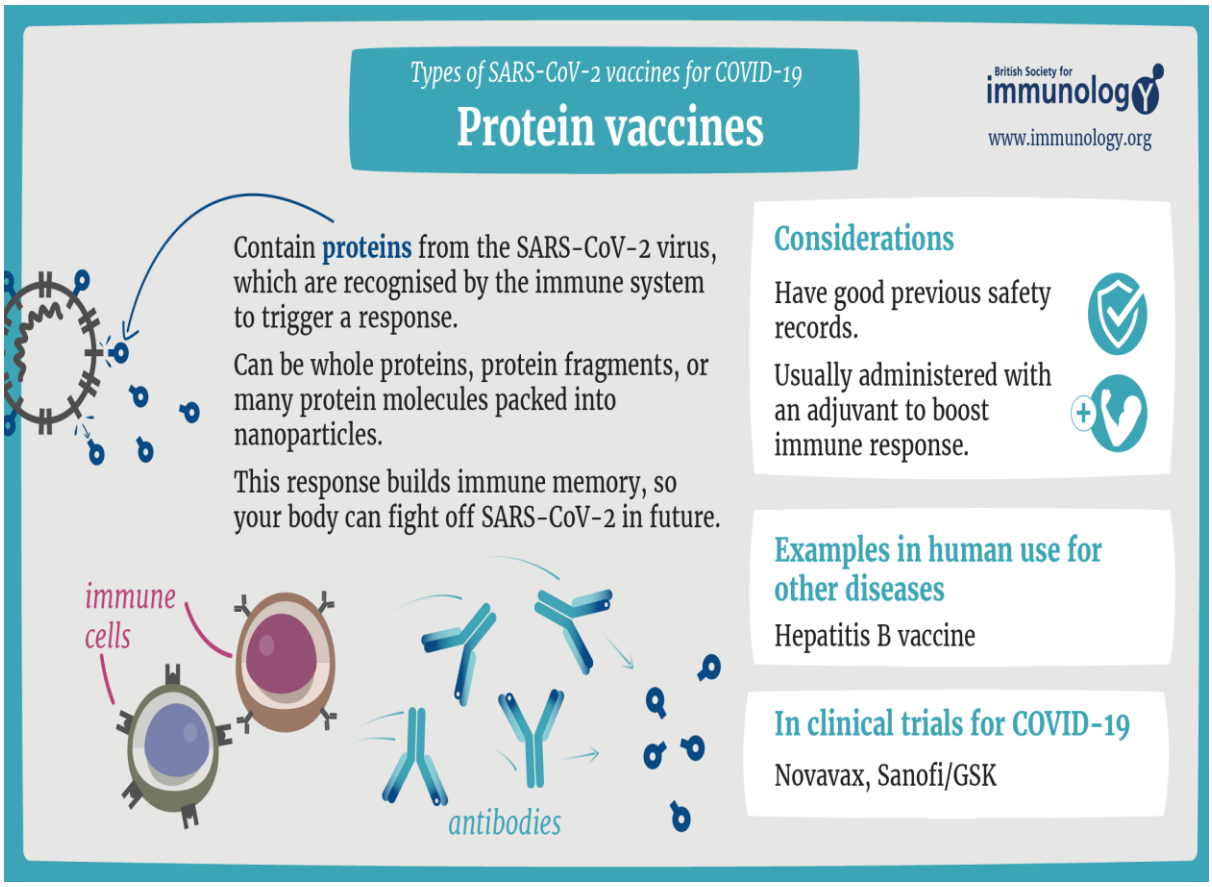

Figure 11. Mechanism of action of Protein vaccines for COVID-19 (downloaded from website of the British Society for Immunology).

Protein based vaccine has been developed by Novavax, and designated as NVX-CoV2373 (a nanoparticle based recombinant pre-fusion S protein). This vaccine displays a success rate of 96% against SARS CoV-2 (Raman et al., 2021). It is noteworthy that NVX-CoV2373 reportedly generates higher amounts of antibodies against SARS CoV-2 virus in contrast to other types of vaccines (that is, mRNA vaccines and viral vector-based vaccines) (Raman et al., 2021).

EMERGENCE OF SARS-CoV-2 VARIANTS: SHOULD WE BE CONCERNED?

During their replication in host cells SARS-CoV-2 can make alterations to their genome sequence and this process is referred to as mutations. A group of coronaviruses that have the same peculiar mutations is called a variant. In recent times there has been an upsurge in a number of mutations and variants of SARS-CoV-2. SARS-CoV-2 variants having a single D614G mutation in the spike (S) glycoprotein first started appearing in the beginning of March 2020, and has been on the rise since June of 2020 (Raman et al., 2021), perhaps owing to enhanced viral capability and transmissibility (Plante et al., 2021). The appearance of new SARS-CoV-2 variants is a matter of concern because of mutations in the genes coding for the spike protein. Such mutations could affect the structure of the protein and hence adjusting the interaction of the spike protein with the human ACE2 receptor, adjusting immune response, or compromising the success of treatments by monoclonal antibodies (Raman et al., 2021). The World Health Organization has categorized B.1.617.2 (Delta Variant) as a "variant of concern." This variant was first identified in Maharashtra, India in early October 2020. Studies show that due to the mutations this variant has been conferred with stronger binding prospect to the human ACE2 receptor and more capacity to avoid hosts' immune systems as compared to

other variants (Starr et al., 2021). The B.1.617.2 variant has so far spread to almost 92 countries as of late June 2021. Available studies indicate that Delta is at least 40 and at most 60% more contagious than the Alpha (U.K./B.1.1.7) variant and possibly one of the utmost transmissible variant as of now (Raman et al., 2021). The Delta variant has brought an enormous second wave of infections in India and cases due to this variant are on the rise world-wide (Raman et al., 2021).

A recent study indicates that the effectiveness of vaccination with BNT162b2 after two doses of the vaccine reduced to 87.9% with the Delta variant from 93.4% with the Alpha variant, while that of Oxford-AstraZeneca vaccine ChAdOx1 was reduced to 59.8% from 66.1% respectively (Abdool Karim and de Oliveira, 2021). Available data also indicate that Pfizer-BioNTech and the Oxford-AstraZeneca vaccine were 96 and 92% efficacious, respectively, at averting hospitalization from the Delta variant (Raman et al., 2021). Africa is projected to be a major epicenter for COVID-19 in a few months from now and the surge in new cases is driven by the Delta variant (Steinhauser and Parkinson, 2021). The low vaccination rate in the continent is worrisome as there would be no benefits of lower hospitalization from the delta variant infection. A new form of Delta known as 'Delta plus' has also emerged with additional mutation in its K417N gene which may contribute to immune escape (Raman et al., 2021). However the transmissibility and effect on vaccine efficacy of delta plus is currently uncertain.

CONCLUSION AND FUTURE OUTLOOKS

The rapid spread of SARS-CoV-2 which causes COVID-19 the highly infectious disease has presented a serious threat to health worldwide. The SARS- CoV-2 outbreak has been on for about two years now, and it is possible that this new virus will have a place in humans or remain with us for some time. This review provides an understanding on SARS-CoV-2 the virus responsible for COVID-19 pandemic in terms of its structure, replication, transmission, diagnosis and management but the origin of the virus is yet to be confirmed which may hold the key to preventing similar future outbreaks. It is encouraging that the numerous research efforts on the SARS-CoV-2 have resulted in the advancement of vaccines and other biotherapeutics at an unequalled pace. COVID-19 has indeed spurred the scientists and governments around the world to have a common purpose. In spite of the fact this pandemic has given rise to several SARS-CoV-2 variants, most of the available vaccines have demonstrated effectiveness against them, notwithstanding the reduced efficacy. In this regard, continued genomic monitoring of SARS- CoV-2 in new occurrences is required worldwide, in order to quickly recognize any mutation that may evolve in phenotypic changes of the virus.

The future seems to be encouraging due to the remarkable strides made in COVID-19 vaccine design and development. In view of all the innovations and breakthroughs observed during this pandemic what would we say is the legacy of Science after the end of the pandemic? Definitely the verdict would be that Science has delivered. However there are still challenges ahead. One of the challenges for the world is to make vaccines available to people of all levels of the public, as well as those from developing countries, so that the spread of SARS-CoV-2 infections can be finally contained. Another challenge is the issue of infection observed in some vaccinated individuals which is worrisome. Arising from this is the need to know the duration of immunity generated by vaccination. Is there a need for booster doses on regular intervals of time? In this regard the vaccines already in use would require long time monitoring in order to determine their duration of immunity. A further challenge is the need to characterize different SARS-CoV-2 variants in order to identify the transmission rate and subsequently identify target sites for effective development of therapies for its containment.

CONFLICT OF INTEREST

The author has no declared any conflict of interest.

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