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Review article

SARS-CoV-2 spike protein mutation variants: Recent advances in theragnostic and vaccines development

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ABSTRACT

Background: COVID-19, caused by SARS-CoV-2 and linked to over 6.5 million deaths, has severely disrupted global healthcare systems with long-lasting impacts. Developing effective vaccines and treatments requires a deep understanding of host-virus interactions. Designing new tactics to reduce the consequences of the developing variations of concern (VOC) requires an understanding of the evolution of the virus and the effects of genetic variation on host immune evasion and vaccination efficacy. New SARS-CoV-2 variants, commonly classified as variants of interest (VOI), have been identified based on factors such as their ability to spread, the severity of illness they cause, and their potential to evade detection and immune responses. Due to its special ability to attach to host receptors, generally preserved nature, high immunogenicity in inducing neutralizing antibodies, and suitability as a target of T-cell responses, the spike protein is the focus of most vaccines currently being developed and/or used. Emerging SARS-CoV-2 strains, however, are showing variations in the spike protein, which may influence the effectiveness of vaccines and antibody-based treatments. In addition to strengthening viral immune evasion mechanisms. Currently, there is a lot of interest in how mutations in the spike protein affect immunity and vaccination, and how well the available vaccines protect against newly emerging variants. This review covers the effects of SARS-CoV-2 spike protein mutations on immune evasion and vaccine-induced immunity as well as future directions that may help with studies concentrating on developing efficient and novel vaccines and/or immunotherapy strategies that take viral evolution into account.

Introduction

1. Introduction about the SARS-CoV-2

The World Health Organization (WHO) officially ended the Public Health Emergency of International Concern (PHEIC) for COVID-19 on May 5, 2023. Despite this, the virus has continued to spread. By 2024, there was still no consensus among experts on whether COVID-19 should still be considered a pandemic. This uncertainty stems from the fact that the beginning and end of pandemics are not clearly defined and vary depending on the criteria used. As of April 1, 2025, COVID-19 had resulted in 7,057,132 confirmed deaths, with estimates placing the true death toll

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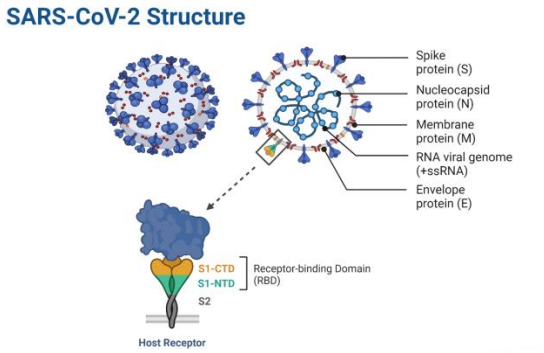
between 18.2 million and 33.5 million [1, 2, 3, 4, 5]. (SARS-CoV-2) is a part of the Coronaviridae family of beta coronaviruses. Single-stranded positive ribonucleic acid (RNA) viruses make up the family [6]. They're zoonotic viruses that can spread from animal to human. When for the first time this happens, it's called a spillover event. The COVID-19 virus was discovered to be strongly linked to the coronaviruses seen in bats. SARS-CoV-2 sequence homology has also been discovered in Malayan pangolin coronaviruses. SARS-CoV-2 has yet to be linked to a zoonotic source. Because SARS-CoV-2 lacks the polybasic cleavage site and mutations in the spike (S) protein that the bat and pangolin coronaviruses do, the theory of undetectable post-spillover human-to-human transmission suggests that the virus may have acquired these genomic characteristics before the pandemic began.

1.1 Structure, Etiology and pathogenesis

The virus comprises single-stranded positive-sense RNA (+ssRNA) having a 5' -cap structure and 3' -UTR poly-A tail (~30 kb) [7]. The SARS-CoV-2 genome is fewer than 30 kb in length and includes 14 open reading frames (ORFs), which encode accessory proteins along with structural proteins like an envelope (E), spike (S), membrane/matrix (M), and nucleocapsid (N) and non-structural proteins (NSPs) for viral replication and assembly processes [8]. About 65% of the viral genome is contained in the first ORF, which produces the polyproteins pp1ab (nsp1–16) or pp1a (nsp1-11). Six of these nsps (NSP3, NSP9, NSP10, NSP12, NSP15, and NSP16) are essential for viral replication Click or tap here to enter text.. (ACE2) Angiotensin-converting enzyme 2 receptors are found on the surfaces of host cells, and the S protein, a transmembrane protein, helps the viral envelope to bind to these receptors. The spike protein consists of subunits that are involved in receptor binding and cell membrane fusion, (S1) and (S2), respectively [9]. The N protein is engaged in RNA replication, immune evasion, and virion formation by attaching itself to the viral genome . One of the highly prevalent and well-conserved proteins in the virion structure is the M protein. Through interactions with accessory proteins 3a and 7a, and accessory protein N, this protein facilitates viral particle formation and budding. The SARS-CoV-2 structure's smallest element, the E protein, promotes the synthesis, growth, and release of virions. The receptor-binding domain (RBD) of the spike protein is the part of the coronavirus genome that is the most complex. Six RBD amino acids are necessary for coronaviruses to bind to the ACE2 receptor and infect cells (Fig 1). The ACE2 protein is prevalent in the gastrointestinal system, lungs, heart, kidneys, and liver as well as in many other body tissues of many mammals [10].

Figure 1. This figure depicts the SARS-CoV-2 outer structure with its essential structural (S, N, M, and E) protein and the RBD



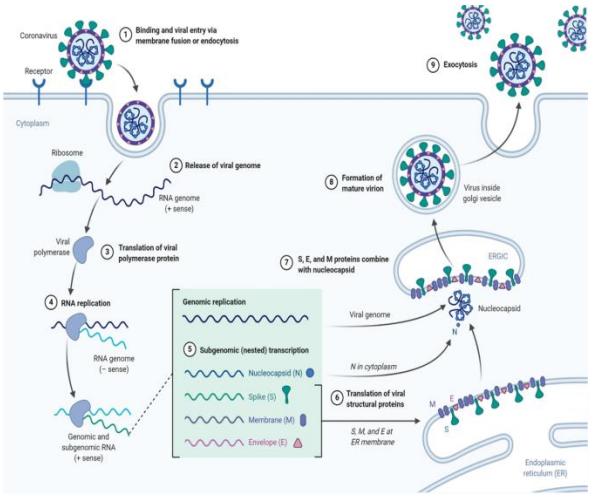
The (COVID-19) viral spike (S) protein binds to angiotensin-converting enzyme 2 (ACE2) receptors, which are the functional receptors for the virus and are abundantly expressed in lung epithelial cells. Aside from acting as a receptor for SARS-CoV-2 in the lungs, ACE2 has a variety of physiological activities, the majority of which entail defense against lung injury. By eliminating a single amino acid from the C-terminal end of the peptide endothelial site, ACE2 breaks down the octapeptide Ang II to produce the heptapeptide Ang1–7. The coronavirus S protein is proteolytically broken by the host proteases trypsin and furin at the sites situated at the boundary between the S1 and S2 sites upon attachment to ACE2 receptors, and the virus is typically consumed through a process known as endocytosis. It has been discovered that after entering the cytoplasm, the virus performs a unique three-step membrane fusion method that requires

1. First is receptor-binding and induced conformational changes in Spike glycoprotein.
2. Second is the proteolysis of cathepsin L through intracellular proteases.
3. Tertiary is the initiation of membrane fusion mechanisms within endosomes.

The viral nucleocapsid is subsequently uncoated by proteasomes, which typically hydrolyze exogenous proteins but can also break down exogenous proteins like the SARS nucleocapsid protein. The virus is finally released into the cytoplasm by the endosome.

The virus's genetic material, which is a single-stranded positive-sense RNA, is then released into the host's cytoplasm. The replication and transcription procedures are then carried out, with the replication/ transcription complex (RTC) acting as a mediator. The viral genome encodes the RTC, which is comprised of non-structural proteins (nsp). The RTC is thought to cause double-membrane formations in the host's cytoplasm. The genome is subsequently translated to make replicase proteins from ORF 1a/b after the positive sense RNA. These proteins use the genome as a template to make full-length negative sense RNA, which can subsequently be used to make the next full-length genome. The endoplasmic reticulum produces structural viral proteins M, S, and E, which are then transported to the endoplasmic reticulum-Golgi intermediate compartment. Nucleocapsids are generated in the cytoplasm by the encapsulation of replicated genomes by N protein, and they then unite within the membrane to form new virions. Finally, after packaging, fresh virions are transported from infected cells to smooth-walled cell membrane vesicles, where they are secreted by a process known as exocytosis. Meanwhile, because SARS-CoV-2 infection causes inflammatory responses in lung epithelial cells, the stress of viral generation on the endoplasmic reticulum for the formation of new virion finally leads to cell death. SARS-CoV-2 infection activates caspase-8, causing cell death and the production of inflammatory cytokines in lung epithelial cells.

Figure 2. The pathogenesis\life cycle of the SARS-CoV-2 . **https://doi.org/10.3390/ijms21165932**



2. Vaccine Development

The S protein may be a good aim for CoV vaccine development because it is crucial for virus-cell receptor binding and virus-cell membrane fusion. Studies have demonstrated that in SARS patients who have recovered, antibodies produced against the S protein are immunodominant and long-lasting. Additionally, several studies have shown that the anti-S antibody has protective benefits in both humans and animals and can neutralize MERS-CoV and SARS-CoV. Additionally, several S protein-based vaccines against MERS-CoV and SARS-CoV have been shown in preclinical models to obtain potent immune responses and protective effects. These results provide credence to the idea that the CoV S protein is an ideal vaccine target for eliciting immunological defense and neutralizing antibodies. In addition to the S protein, other structural proteins have also been researched as possible vaccine targets. Because N protein is not visible on the CoV surface, N protein-based vaccinations typically do not result in the production of neutralizing antibodies [11]. However, compared to the S protein, the N protein has the benefit of being more conserved among CoV species, making it a possible target for a T-cell-inducing, all-encompassing CoV vaccine [12]. On the other hand, animals that have received an M protein-based vaccine can produce a high titer of antibodies. For M protein-based vaccines, however, there hasn't been any evidence of protective immunity or neutralizing antibodies in preclinical settings. Last but not least, there have only been a small number of CoV E protein-based vaccination experiments published so far, and none of them showed the generation of neutralizing antibodies or protective immunity. In vitro, SARS-CoV infection of human cell lines is promoted by the full-length S protein utilized in the SARS-CoV vaccination, claims a study. These results raise concerns about the safety of S protein-based SARS-CoV and MERS-CoV immunizations. Designing vaccines that only contain significant neutralizing epitopes, such as the S1 subunit or the RBD domain of the S protein, is one possible solution to the antibody-dependent enhancement (ADE) issue. This tactic can lessen the non-neutralizing antibodies that CoV vaccinations induce, which will lessen the ADE effect. Research also found that following a viral challenge, vaccination with the SARS-CoV virus-like particle (VLP) vaccine causes eosinophilic immunopathology in the lung [13].

2.1 Major SARS-CoV2 Variants

The emergence of SARS-CoV2 Variants - The coronavirus exonuclease enzyme reduces

replication errors by 15-20 folds in vitro, leading to an in vivo viral mutation rate that is over ten times lower than that of influenza. Recombination, however, takes place when many mutations affect the same host, increasing their diversity and mutation rate. Recombination among circulating SARS-CoV-2 variants is still occurring to some extent, despite it being difficult to identify due to the similarity of most sequences. SARS-CoV-2 most likely developed as a result of recombination between several SARS-related coronaviruses. The SARS-CoV-2 spike protein, a 1,273 amino acid trimeric glycoprotein, is in charge of aiding virus entry into host cells. Each spike monomer's S1 attachment domain (residues 1-686) and S2 fusion domain (687-1,273) are extensively exposed. The S1 receptor-binding domain (RBD; residues 306–534) can be found in two states: open (up) and closed (down). When in the up position, it attaches to the human angiotensin-converting enzyme 2 (ACE2) receptor, which is required for access to the vast majority, if not all, cells [14]. As SARS-CoV-2 continues to evolve, its variants are reclassified in the U.S. based on their characteristics and how widespread they are. The current categories include Variants Being Monitored (VBM), Variants of Concern (VOC), Variants of Interest (VOI), and Variants of High Consequence (VOHC). As of January 2023, no variants fall under the VOI or VOHC classifications. To streamline efforts across agencies, the U.S. Department of Health and Human Services (HHS) formed the SARS-CoV-2 Interagency Group (SIG), which includes the CDC, NIH, FDA, BARDA, and DoD. This group focuses on the swift assessment of new variants and tracks how they may affect vital tools such as vaccines, treatments, and diagnostic tests [14].

2.2 Molecular Details & Mutations

Mutation of the S1 Subunit comprising the RBD

Given the importance of the RBD in ACE2 recognition and binding, it seems reasonable that changes in the amino acid sequence of the RBD can greatly influence S binding affinity for ACE2 and, eventually, SARS-CoV-2 infectivity. While there are mutations all across this region, the majority of them are on the surface of S, allowing for direct interactions with potential ligands [15].

A different idea for the positive selection of mutations inside the SARS-RBD CoV-2 has enhanced resistance to post-vaccinated sera. Nearly all RBD mutations offer resistance, according to studies employing monoclonal antibodies made by SARS-CoV-2 vaccine recipients [16]. Given that the majority of the antibodies generated against SARS-CoV-2 target the RBD and are neutralizing antibodies, this is not surprising.

S1 Subunit N-Terminal Domain (NTD) Mutations

The positive selection of variants containing mutations in the NTD of the SARS-CoV-2 spike protein is influenced by several variables, including broad common mutations throughout the NTD subdomain. 35% of SARS-CoV-2 antibodies target the NTD, although only around one-third of these antibodies have a neutralizing effect. Further studies have revealed a "supersite" to which practically all of the neutralizing antibodies that target NTD attach. It is noteworthy that antibodies to the NTD area can inhibit cell-cell fusion, suggesting that the NTD may contribute to the creation of syncytium [17]. Resistance to neutralizing antibodies, particularly those found in post-vaccination sera, may have a significant impact on the positive selection for SARS-CoV-2 [18]. Further research found that the main driver of this rise is cell-cell fusion enhancement, with little to no impact on the neutralization of NTD-neutralizing antibodies. The viral burden increased for those with variations bearing the T95I mutation, especially in the presence of 142, according to the examination of the information for qPCR cycling thresholds obtained from SARS-CoV-2-infected patients. Other mutant residues, even if they are not in the supersite region, may influence the topology of the supersite and impact the ability of post-vaccinated sera to neutralize SARS-CoV-2, according to structural modeling, which also revealed topological changes that T95I may produce in the NTD "supersite" (Fig 3).

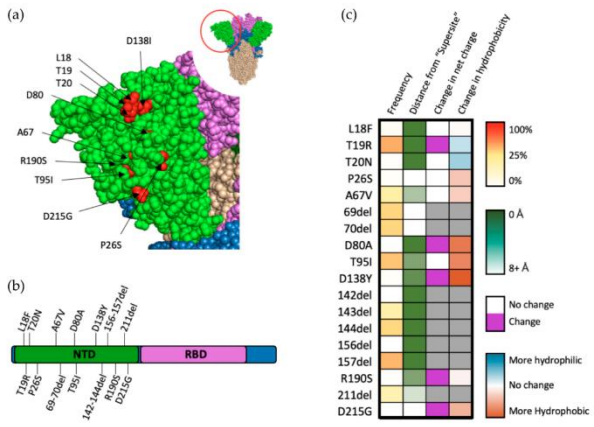


Figure 3. Alterations to the SARS-CoV-2 spike protein's NTD. (**a**) A structural representation of the substitutions (red) in the SARS-CoV-2 spike protein's NTD (green). (**b**) A visual representation of the spike protein's NTD mutations. (**c**) Recurrence, residue separation from the NTD "supersite", charge modification at physiological pH, and hydrophobicity modification at pH 7. https://doi.org/10.3390/v14030640

S2 Subunit Mutations

The processes by which mutations within the S2 subunit modify SARS-CoV-2 infectivity may take many different forms because of the S2 subunit's five functionally distinct subdomains. Studies have found that SARS-CoV-2 only has four often-seen mutations in the S2 region: T716I, D950N, S982A, and D1118H [19]. Two of these four mutations (D950N and S982A) are found in the HR1 domain, suggesting that modifications in this region may be particularly prone to inducing positive selection. Both mutations drastically alter the charge or hydrophobicity at these sites, which increases the likelihood that this positive selection will alter the way HR1 and HR2 are connected. Structural investigations of the region between the heptad repeat domains (where D1118H occurs) reveal that the heptad repeat domains can interact with the target cell membrane when the S2 domain is relocated after fusion. The S982A substitution enhances the presentation of the "up" RBD state by eliminating the interaction with T547, which stabilizes the "down" RBD state [20]. This shift in RBD status is partially counteracted by the matching A570D mutation discovered in the Alpha SARS-CoV-2 strain. We have demonstrated that by forming an interprotomer hydrogen bond with N856, the D570 residue can restore the connection that stabilizes this "down" confirmation. When the D1118H mutation occurs, the three histidine residues (one from each monomeric S) form a histidine triad inside the S trimer, retaining the overall structure of the trimeric S complex [20]. It was claimed that this impact would compensate for any local destabilizations caused by concomitant mutations like T716I, even though the role this stabilization plays is still not supported by any actual evidence [21] (Fig 4).

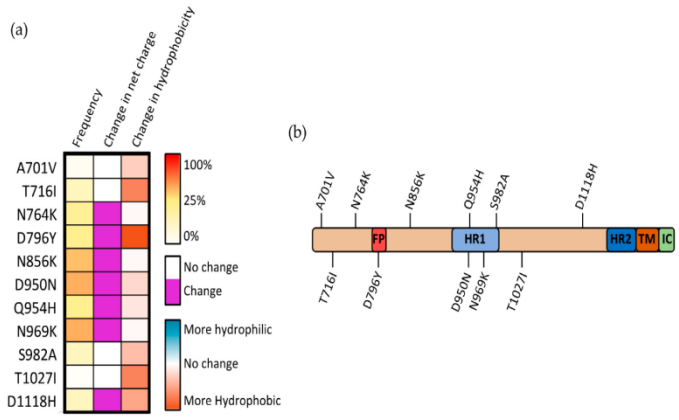


Figure 4. Alterations in the S2 component of the SARS-CoV-2 spike protein. (**a**) For mutations occurring inside the S2 subunit, frequency, modification in charge at physiological pH, and alteration in hydrophobicity. (**b**) A graphic illustration of the S2 subunit's mutations. https://doi.org/10.3390/v14030640

3. Currently Circulating SARS-CoV-2 Variants (Structure, pathogenesis, and epidemiology of different variants)

a. Alpha

Structure:

17 mutations were observed out of which 8 were in the spike protein that regulates the entry and attachment of the virus into the human body. Among these 3 they conceivably affect the functioning of the virus. Mutation N501Y is major in the receptor binding domain and it also increases the capacity of virus binding with human angiotensin-converting enzyme 2(ACE-2). Another mutation P681H remains beside the furin cleavage site in the spike protein which is very important for infection and transmission. The deletion ΔH69/ΔV70 in spike has taken place in many lineages of SARS-CoV-2 that are responsible for immune escape in immunocompromised people, thus increasing the viral infectivity [22].

Pathogenesis:

RBD is present on the spike protein that recognizes ACE2 as its binding receptor. N501 in RBD interconnects with the remains of hACE2 which results in the formation of a hydrogen bond with Y41 of hACE2 to make the virus-binding hotspot K353 at the RBD-hACE2 interface3 more stable. Due to N501Y mutation increase in RBD binding affinity to hACE211 takes place which results in the introduction of the aromatic ring of Y501 into a cavity between Y41 and K353 of

hACE2. Thus, this leads to greater viral transmission [23].

Epidemiology:

In late December 2020, there was a sudden increase in the number of cases which lasted for more than a year. This was first reported in the United Kingdom(UK). This variant was further found in another 45 countries out of which,17 were European. According to the reports, due to this variant, the mortality rate increased by 65% and the transmissibility rate by 70% as this variant was considered to have higher transmissibility. This variant has a very high number of mutations among which one increases the affinity to bind with the ACE2 receptors. Thus, these findings raised an alarm to face the new variant that hastened the pandemic's spread [24],[25].

b. Beta Structure:

A total of 9 mutations were observed in the spike protein in addition to D614G, which includes a cluster of other mutations in the N-terminal domain (NTD), the next 3 mutations in the receptor binding domain (RBD) which are E484K, N501Y, and K417N, and 1 mutation close to the furin cleavage which is A701V.

Pathogenesis:

E484K, N501Y, and K417N form new inter-protein connections that boost the binding process and thus, increase the infectivity by altering the internal structural dynamics. The presence of E484K and K417N along with N501Y has increased the binding affinity to human ACE2. Many of the mutations are present in the ACE-2 binding site and antigenic supersite of NTD 16, and NTD 17 which is a major target of powerful virus-neutralizing antibodies the efficacy of many vaccines was of major concern.

Epidemiology:

This variant was first detected in South Africa's Eastern Cape Province in December,20220 which rapidly spread over the continent and replaced the other SARS-CoV-2 lineages circulating there. The transmissibility of this variant was 50% more than the previous variants circulating in South Africa. E484K, N501Y, and K417N mutations are connected to immune evasion, decrease in neutralization, and increased transmissibility which increases the spreading of this variant [26].

c. Gamma Structure:

This variant consists of 10 mutations in the spike protein in addition to D614G, in which the receptor-binding domain (RBD) consists of mutations in N501Y, K417T, and E484K, N-terminal domain (NTD) consists of mutations in P26S, L18F, R190S, D138Y, and T20N. The mutation present near the furin cleavage site is H655Y. N501Y, K417T interacts with the human ACE2.

Pathogenesis:

The K417T mutation present in spike protein is responsible for less sensitivity towards neutralizing antibodies and increased transmissibility. E484K substitution in amino acid sequence alters antibody recognition which increases immune system invasion potential. N501Y increases the binding affinity with human ACE-2 and inserts more concentrations in nasal and pharynx regions thus, increasing the transmission rate.

Epidemiology:

This variant first emerged in Manaus, Brazil IN November 2020. The variant is 1.7–2.4 times more transferable than the other circulating variants and can enter the antibody even after vaccination and infection [27]. Gamma has also been linked to reinfections and breakthrough infections in those who have received vaccinations. It also showed a drop in anti-RBD antibody neutralization.

d. Delta Structure:

This variant consists of 8 mutations in the spike protein. Out of these two mutations were found at the RBD. The receptor binding domain (RBD) contains L452R and T478K, whereas the N-terminal domain (NTD) has T19R, G142D, and del157/158 (RBD). Threonine at position 478 becomes Lysine(T478K) and Leucine at position 452 becomes Arginine (L452R). The N501Y which was present in the other variants was absent in this case.

Pathogenesis:

The P681R mutation present in the spike protein makes the process of cleavage easier and thus, increases the viral cell-to-cell interaction. According to the reports, the mutation L452R can increase the binding power for the host entry receptor ACE2 which makes a transmission, viral replication, and fitness easier [28]. The T478K mutation consisting of threonine(a non-charged

amino acid) and a lysine(positive amino acid) can change the electrostatic surface of the protein and thus, enhance the steric hindrance. This can increase the ability of RBD to bind with ACE2 and improve the capability to invade inside the host cell.

Epidemiology:

This variant was first observed in India in October 2020. It is characterized by high infectivity and is 60% more transmissible than the alpha variant. It is 1,260 times greater in viral load than the previous variant. It is highly communicable and the symptoms cannot be easily noticed. The exhaled viral concentration is also high which makes the exposure easier. Thus, these outcomes due to mutation lead to a rapid increase in the spread of this variant.

e. Omicron

Structure:

In the omicron variant, the mutations were seen basically at the spike protein where 30 amino acid substitutions, 3 deletions, and 1 insertion were observed when compared to the original SARS-CoV-2. Among these, the 15 amino acid substitutions were found at the receptor binding domain known as RBD and the other 3 substitutions were found at the cleavage site at S1/S2 junction. The N-terminal domain also known as NTD contains 11 mutations that regulate the moistening of plasma-neutralizing activity in affected patients. More effective binding with the human ACE-2 entry receptor is seen with the RBD mutations.

Pathogenesis:

The usage of transmembrane serine protease 2 (TMPRSS2) becomes less ineffective as it is inadequate in spike cleavage. The ineffective spike cleavage and TMPRSS2 utilization result in greatly decreased viral replication in the lungs and drastically diminish virus pathogenicity because SARS-CoV2 accesses lung cells largely through the TMPRSS2-mediated plasma membrane entry route. Omicron cannot be neutralized by antibodies due to the high number of mutations as it alters the pathogen's capability for entering the cell, replication, and pathogenesis [29].

Epidemiology:

In November 2021, there was a rapid increase seen in COVID-19 cases in South Africa because of omicron. This quickly spread to the neighboring countries Botswana, Namibia, Zimbabwe, Swaziland, and Mozambique. Increased mutations that were found on the S protein were the cause of invasion, escape from neutralizing antibodies, and increased transmissibility for the already immunized individuals. Due to high mutation, it was expected that PCR could not be easily used but, it was recognized by S-gene dropout or S-gene target failure. Sub-lineage omicron variants such as BA.1, BA 1.1, and BA.2 were found. The difference lay in the regions of the spike gene that were connected to glycosylation, receptor binding, and resistance to monoclonal antibodies.

Table 1. Variants of concern and their spike mutations

WHO name	Lineage + additional mutations	Name	Spike proteins mutations	First Detected	Country of detection	Date of designation	Concerns
Alpha	B.1.1.7	VOC-202012/01	N501Y, D614G, P681H	Sep 2020	UK	18 Dec 2020	Increased transmissibility, severity
Beta	B.1.351	501 Y.V2	K417N, E484K, N501Y, D614G	Oct 2020	South Africa	18 Dec 2020	Increased transmissibility, severity, and possible reduction of vaccine effectiveness
Gamma	P.1	VOC-202101/02	K417T, E484K, N501Y, D614G	Jan 2021	Brazil	11 Jan 2021	Increased transmissibility, severity, and possible reduction of vaccine effectiveness
Delta	B.1.617.2	VOC-21APR-02	L452R, D614G, P681R	Dec 2020	India	11 May 2021	Highly transmissible, severe, possible reduction of vaccine effectiveness

4. Impacts of SARS-COV-2 Variants and Mutations on Immunity

SARS-CoV is a positive single-stranded RNA betacoronavirus that causes severe respiratory tract infections. The incubation period observed is between 0-14 days. The first stage is marked by the onset of upper respiratory tract infection (URTI) which includes ageusia, rhinitis, and anosmia along with lower respiratory infection (LRTI) which includes thoracic pain, fever, and cough. The second stage is marked by continued LRTI after which immediate consultation and hospitalization are required. Here, abnormal blood parameters can be observed. In the third stage from 9-12 days after the onset of infection sudden decline due to pulmonary (macro and micro) embolism and cytokine storm syndrome leads to acute respiratory distress syndrome which is marked as stage four and finally death [30].

Innate Immune Response

SARS-COV-2 enters the human host through the ACE-2 receptors. After entering the host

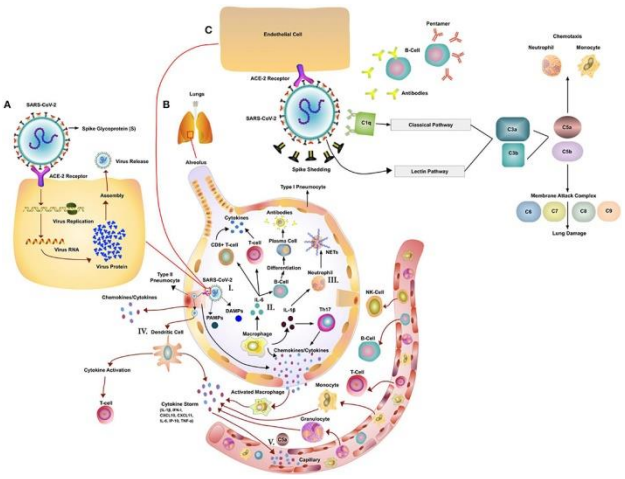
it starts replicating and thus, transmitting the infection to other cells of the body. Due to transmission pyroptosis is induced in the infected cells and the release of Damage-associated molecular patterns also known as DAMPs takes place. These DAMPs are identified by pattern recognition receptors also known as PRRs which are present in alveolar macrophages, endothelial cells, and epithelial cells of the lungs. which results in the activation of the production of chemokines and cytokines [31]. The recognition of viral RNAs by Toll-like receptors (TLRs) and a few sensors of viral infections RIG- I and MDA-5 leads to the synthesis of type I IFN (interferon) among which IFN-α and IFN-β act as protective interferon against any viral infection. Due to these response transcriptions of the NLRP3 gene (NLR family pyrin domain containing 3) and induction of many cellular responses like an aggregation of the protein, ROS production and Calcium influx takes place which activates different inflammasome complexes mainly the NLRP3 inflammasomes. These inflammasomes contribute to the activation of caspase-1-dependent cleavage which further activates gasdermin-D (pore-creating protein) mediated pyroptosis and proinflammatory cytokines (IL-1β and IL-18) [32]. Moreover, an increase in the amount of enzyme Lactate dehydrogenase has also been observed. Type-I IFN response is susceptible to SARS-CoV-2 is the reason why less severe or no symptoms are seen because DAMPs are recognized as soon as type-I interferons are released causing viral inhibition. When the IFN-I production is delayed as in the case of people with weak immune systems monocytes, neutrophils, and macrophages are activated leading to the release of proinflammatory cytokines from these cells which further results in the damaging of alveoli inside the lungs causing the severe acute respiratory syndrome. This causes the blood pressure level to decrease and thus multiple organ failure takes place [33]. Decreased quality of Natural killer or NK cells is observed in PBMC (Peripheral blood mononuclear cells). Normally, NK cells are not present in lung cells, but when SARS-CoV-2 infection occurs chemokines facilitate these cells to migrate from peripheral blood to the lungs [34].

Adaptive Immune Response

It involves the B lymphocytes and T lymphocytes that respond to cell-mediated and humoral immune responses. As the virus enters the host's body the viral peptides are given to the T cell receptor (TCR) of cytotoxic T (Tc or CD8 T cells) cells through the MHC-I of the nucleated cells which leads to the death of these cells. The helper T cells (Th or CD4 T cells) are presented with the proteins released by these viruses by the MHC II molecule which causes the release of IL-2 and IL-6 molecules which leads to the formation of plasma and memory B cells by multiplication of the virus-specific B lymphocyte. To neutralize the virus the plasma B cells secrete IgA, IgM, and IgG antibodies These antibody response takes up to 19 days from the onset of SARS-CoV-2 to show its symptoms that are followed by the production of certain antibodies in the blood serum as a result of infection or immunization [35]. The IgM antibody peaked at two to five weeks and declined further from three to five weeks post symptom depending on the blood group of the individual. The IgG peaked at three to the seventh week from the onset of symptoms and remained continued for up to the eighth week. Neutralizing antibodies were detected within seven to fifteen days from the arrival of the disease which kept on increasing till 14-22 days before increasing and further decreasing [36].

Decreases in CD4 and CD8 T cells were drastic but during mild infections, the level was slightly higher or normal [37]. Decreased level of granzyme B along with an increase in expression of NKG2A in cytotoxic T and NK cells is seen. The NKG2A+ cytotoxic lymphocytes declined which suggested the hindered cytotoxic lymphocyte function and increased the progression of SARS-CoV-2 in the initial stages [38].

Figure 5. Potential Immune Response/Pathogenesis of SARS-Cov-2. (A) Replication cycle of SARS-CoV-2: Spike (S) protein of the SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) present on the lung's epithelial cell (B) The innate and adaptive immune responses to COVID-19 virus (C) Effects of CoV-mediated complement activation [39].



5. Vaccine Efficacy Against SARS-CoV2 Variants (Vaccine types, Evidence from pre-clinical, clinical, and current vaccination programs)

When a natural infection arises, the human immune system often develops an immunological defense against a specific pathogen. The body is ideally constantly protected from the linked disorders by this immune response. While memory cells are produced, persist for a long time, and then become active when the same type of antigenic material is reintroduced into the body later, antibodies and cytotoxic cells fight the disease-related pathogen. This phenomenon gives rise to the immunization principle [40]. Since Edward Jenner developed the first vaccine, Variolae vaccinae, for smallpox in 1798 [41], vaccinations have been successfully utilized to prevent a variety of infectious diseases. By starting the innate immune response, vaccines trigger an adaptive immunological response that is unique to an antigen. They trigger humoral immunity by encouraging B lymphocyte cells to make certain antibodies, and cell-mediated immunity by activating highly specific subsets of T lymphocytes. After the infection has been destroyed, the adaptive immune system then creates immunological memory.

In contrast to disease-sterilizing immunity, COVID-19 vaccinations primarily promote disease-preventing/attenuating immunity [42]. The production and use of vaccinations is a very challenging, long, and tedious procedure. Additionally, it requires an evaluation of vaccine effectiveness, safety, and use with or without significant side effects. The most popular approach to battling the COVID-19 pandemic to date is the use of spike proteins, and more especially RBD-based vaccine design [43].

To produce vaccines that are safe and effective against the disease, it is necessary to perform animal tests that also set the amount and timing of injections. The basis of biological and immunological studies in vaccine development is small animals, particularly rats. Rats, mice, guinea pigs, rabbits, and other animals can typically be used as animal models to assess the immunogenicity, tolerability, and safety of potential vaccines. Similar biological consequences following vaccination might not occur, though, because these animals and humans are different species. Because innate and adaptive immune responses in non-human primates (NHPs) and humans are comparable, investigations of NHPs are useful for understanding and illuminating human immune responses. Clinical trials are essential for developing vaccines, in addition to preclinical trials (animal experiments). Phase I trials evaluate vaccine safety, dose, and tolerance; Phase II and III trials examine vaccine effectiveness and side effects. The high error rate of virus RNA-dependent RNA polymerase (RdRp) and the presence of a highly variable receptor-binding domain in the spike (S) protein, however, have an impact on the effectiveness of vaccines [10].

Vaccine-induced immunity

Active immunity or acquired immunity refers to the immunological response that the body produces after receiving a vaccination. The immune system is engaged throughout this procedure. To differentiate into helper T cells, CD4+ T cells need the antigen peptide (AP)-MHC (major histocompatibility complex) class II molecular complex (Th cells). When CD8+ T cells differentiate into cytotoxic T lymphocytes, they are dependent on the AP-MHC class I molecular complex (CTL). The cells assist in the activation of B cells, which then create antibodies. Following antigen activation, B and T cells develop matching memory cells to defend the body against the same infection for a prolonged period. The major seven platforms used in the development of COVID-19 vaccines can be divided into three categories based

on the type of antigen. Inactivated vaccines (inactivated SARS-CoV-2), virus-like particles (VLP) vaccines (viral particles without nucleic acid), and subunit vaccines (S protein or receptor-binding domain (RBD) created in vitro) are all examples of the first strategy, which is based on the protein synthesized in vitro. The second paradigm is based on the antigen gene expressed in vivo and includes mRNA vaccines, DNA vaccines, and viral vector vaccines (using replication-defective designed viruses carrying the mRNA of S protein or RBD) (RNA sequences of S protein or RBD). The live-attenuated vaccine is the third delivery method. To shield recipients from viral invasion, these vaccinations can produce antibodies that can neutralize viruses. Additionally, several mRNAs and viral vector vaccines can stimulate Th1 cell responses and long-lasting responses in the human germinal center [16], which offer more effective protection. Additionally, COVID-19 vaccination-induced memory cells have a significant impact on vaccine immunity.

6. Challenges & Future Scope

The SARS-CoV-2 genetic changes would pose the greatest threat to the prevention and control of the pandemic because they could jeopardize ongoing attempts to develop new treatments, tests, and vaccines. Through a variety of ways, including altered interaction with immune regulatory genes, epitope loss [36], evading T-cell killing, and low affinity to neutralizing antibodies, genetic changes in VOC assist the virus to elude immunity. However, due to structural changes that boosted the RBD's accessibility and binding affinity to ACE2, the circulating VOC displayed higher transmissibility, infectivity, and immune evasion. Regrettably, research has revealed that the SARS-CoV-2 S protein is less stable than the SARS-CoV S protein and that the S2 fusion subunit is more conserved than the S1 subunit. To solve this issue, it is advised to combine mAbs that can recognize different epitopes. Recombined antibodies intriguingly demonstrated effective neutralization of S protein variations [42]. This suggests that combining vaccinations made from several domains of the spike protein may be more efficient. To create effective vaccines and/or antibody-based treatments, however, prediction of mutational pathways, mapping of immunogenic epitopes in the S protein, and structure-based investigation of Ab-epitope binding should be the top priorities. The application of tiny proteins like nanobodies might also be a novel strategy. Despite increasing efforts, there are still obstacles preventing the development of several types of effective vaccinations against SARS-CoV-2 infection. These issues are the restrictions and shortcomings of the various COVID-19 vaccine formulations [43].

7. Conclusion

Although inactivated vaccines are effective at boosting immunity, mass production of them is challenging. Live attenuated vaccines are time-consuming to produce and have safety issues as well. Additionally, some proteins, such as the spike protein, are challenging to express, which limits the availability of recombinant vaccines. RBD is a highly immunogenic protein, but because it is so tiny, it is also susceptible to antigenic drift. Contrarily, vector-based vaccinations may be rendered ineffective by pre-existing immunity, necessitating the use of animal strains or medically uncommon strains. While RNA vaccines are highly immunogenic, DNA vaccines have limited immunogenicity. However, RNA vaccines do not provide adequate mucosal immunity and require specific storage facilities due to their instability. Therefore, to ultimately stop the COVID-19 pandemic, vaccinations and immunotherapy must be developed while taking these limitations into account.

Conflict of interest

None.

Data availability

Data available on request / reasonable request.

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Authors contribution

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References

1- Rigby J, Satija B. WHO declares end to COVID global health emergency. Reuters: London, UK. 2023.
2- Mathieu E, Ritchie H, Rodés-Guirao L, Appel C, Gavrilov D, Giattino C, Hasell J, Macdonald B, Dattani S, Beltekian D, Ortiz-Ospina E. Coronavirus (COVID-19) vaccinations. Our World in Data. 2020.
3- Ducharme J. Experts Can't Agree If We're Still in a Pandemic. TIME. Retrieved. 2024.
4- Colarossi J. Is COVID-19 Still a Pandemic?. The Brink. Boston University. Archived from the original on. 2024.
5- Charters E, Heitman K. How epidemics end. Centaurus. 2021;63(1):210-24.
6- https://doi.org/10.1111/1600-0498.12370
7- Klein S, Cortese M, Winter SL, Wachsmuth-Melm M, Neufeldt CJ, Cerikan B, Stanifer ML, Boulant S, Bartenschlager R, Chlanda P. SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography. Nature communications. 2020;11(1):5885. https://doi.org/10.1038/s41467-020-19619-7
8- Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. Journal of medical virology. 2020;92(4):418-23. https://doi.org/10.1002/jmv.25681
9- Abduljalil JM, Abduljalil BM. Epidemiology, genome, and clinical features of the pandemic SARS-CoV-2: a recent view. New microbes and new infections. 2020;35:100672. https://doi.org/10.1016/j.nmni.2020.100672
10- Khailany RA, Safdar M, Ozaslan M. Genomic characterization of a novel SARS-CoV-2. Gene reports. 2020 ;19:100682. https://doi.org/10.1016/j.genrep.2020.100682
11- Devaux CA, Rolain JM, Raoult D. ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. Journal of Microbiology, Immunology and Infection. 2020 ;53(3):425-35. https://doi.org/10.1016/j.jmii.2020.04.015
12- Wang N, Shang J, Jiang S, Du L. Subunit vaccines against emerging pathogenic human coronaviruses. Frontiers in microbiology. 2020 ;11:298. https://doi.org/10.3389/fmicb.2020.00298
13- Triveri A, Serapian SA, Marchetti F, Doria F, Pavoni S, Cinquini F, Moroni E, Rasola A, Frigerio F, Colombo G. SARS-CoV-2 spike protein mutations and escape from antibodies: a computational model of epitope loss in variants of concern. Journal of Chemical Information and Modeling. 2021 ;61(9):4687-700. https://doi.org/10.1021/acs.jcim.1c00857
14- Krammer F. SARS-CoV-2 vaccines in development. Nature. 2020 ;586(7830):516-27. https://doi.org/10.1038/s41586-020-2798-3
15- Tulimilli SV, Dallavalasa S, Basavaraju CG, Kumar Rao V, Chikkahonnaiah P, Madhunapantula SV, Veeranna RP. Variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and vaccine effectiveness. Vaccines. 2022 ;10(10):1751.
16- Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KH, Dingens AS, Navarro MJ, Bowen JE, Tortorici MA, Walls AC, King NP. Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. cell. 2020 ;182(5):1295-310. 10.1016/j.cell.2020.08.012
17- Emam M, Oweda M, Antunes A, El-Hadidi M. Positive selection as a key player for SARS-CoV-2 pathogenicity: Insights into ORF1ab, S and E genes. Virus research. 2021 ;302:198472.

https://doi.org/10.1016/j.virusres.2021.198472

18- Kemp SA, Collier DA, Datir RP, Ferreira IA, Gayed S, Jahun A, Hosmillo M, Rees-Spear C, Mlcochova P, Lumb IU, Roberts DJ. SARS-CoV-2 evolution during treatment of chronic infection. Nature. 2021 ;592(7853):277-82. https://doi.org/10.1038/s41586-021-03291-y

19- Kumthip K, Khamrin P, Yodmeeklin A, Ushijima H, Maneekarn N. Molecular detection and characterization of SARS-CoV-2 in wastewater in Thailand during 2020–2022. Journal of Infection and Public Health. 2023 ;16(11):1884-90. https://doi.org/10.1016/j.jiph.2023.09.011

20- Magazine N, Zhang T, Wu Y, McGee MC, Veggiani G, Huang W. Mutations and evolution of the SARS-CoV-2 spike protein. Viruses. 2022 ;14(3):640. https://doi.org/10.1016/j.jiph.2023.09.011

21- Sinha A, Sangeet S, Roy S. Evolution of Sequence and Structure of SARS-CoV-2 Spike Protein: A Dynamic Perspective. ACS omega. 2023 ;8(26):23283-304. https://doi.org/10.1021/acsomega.3c00944

22- Pérez-Lago L, Sola Campoy PJ, Buenestado-Serrano S, Estévez A, de la Cueva Technician VM, López MG, Tecnichian MH, Suárez-González J, Alcalá L, Comas I, González-Candelas F. Epidemiological, clinical and genomic snapshot of the first 100 B. 1.1. 7 SARS-CoV-2 cases in Madrid. Journal of travel medicine. 2021 ;28(4):taab044. https://doi.org/10.1093/jtm/taab044

23- Kim Y, Kim EJ, Lee SW, Kwon D. Review of the early reports of the epidemiological characteristics of the B. 1.1. 7 variant of SARS-CoV-2 and its spread worldwide. Osong public health and research perspectives. 2021 ;12(3):139. 10.24171/j.phrp.2021.0037

24- Choi JY, Smith DM. SARS-CoV-2 variants of concern. Yonsei medical journal. 2021 ;62(11):961. 10.3349/ymj.2021.62.11.961

25- Hadj Hassine I. Covid-19 vaccines and variants of concern: a review. Reviews in medical virology. 2022 ;32(4):e2313. https://doi.org/10.1002/rmv.2313

26- Zhan Y, Yin H, Yin JY. B. 1.617. 2 (Delta) Variant of SARS-CoV-2: features, transmission and potential strategies. International journal of biological sciences. 2022 ;18(5):1844. 10.7150/ijbs.66881

27- Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Molecular cell. 2020 ;78(4):779-84. 10.1016/j.molcel.2020.04.022

28- Upreti S, Samant M. A review on immunological responses to SARS-CoV-2 and various COVID-19 vaccine regimens. Pharmaceutical Research. 2022 ;39(9):2119-34. https://doi.org/10.1007/s11095-022-03323-w

29- Melenotte C, Silvin A, Goubet AG, Lahmar I, Dubuisson A, Zumla A, Raoult D, Merad M, Gachot B, Hénon C, Solary E. Immune responses during COVID-19 infection. Oncoimmunology. 2020 ;9(1):1807836. https://doi.org/10.1080/2162402X.2020.1807836

30- Mishra KP, Singh AK, Singh SB. Hyperinflammation and immune response generation in COVID-19. Neuroimmunomodulation. 2021 ;27(2):80-6. https://doi.org/10.1159/000513198

31- Brodin P. Immune determinants of COVID-19 disease presentation and severity. Nature medicine. 2021 ;27(1):28-33. https://doi.org/10.1038/s41591-020-01202-8

32- Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF. The trinity of COVID-19: immunity, inflammation and intervention. Nature Reviews Immunology. 2020 ;20(6):363-74. https://doi.org/10.1038/s41577-020-0311-8

33- Upreti S, Samant M. A review on immunological responses to SARS-CoV-2 and various COVID-19 vaccine regimens. Pharmaceutical Research. 2022 ;39(9):2119-34. https://doi.org/10.1007/s11095-022-03323-w

34- Ahmed F, Jo DH, Lee SH. Can natural killer cells be a principal player in anti-SARS-CoV-2 immunity?. Frontiers in Immunology. 2020 ;11:586765. https://doi.org/10.3389/fimmu.2020.586765

35- Post N, Eddy D, Huntley C, Van Schalkwyk MC, Shrotri M, Leeman D, Rigby S, Williams SV, Bermingham WH, Kellam P, Maher J. Antibody response to SARS-CoV-2 infection in humans: A systematic review. PloS one. 2020 ;15(12):e0244126. https://doi.org/10.1371/journal.pone.0244126

36- Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, Xu Y, Tian Z. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cellular & molecular immunology. 2020 ;17(5):533-5. https://doi.org/10.1038/s41423-020-0402-2

37- Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nature medicine. 2020 ;26(4):450-2. https://doi.org/10.1038/s41591-020-0820-9

38- Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. Journal of medical virology. 2020 ;92(4):418-23. https://doi.org/10.1002/jmv.25681

39- Abduljalil JM, Abduljalil BM. Epidemiology, genome, and clinical features of the pandemic SARS-CoV-2: a recent view. New microbes and new infections. 2020 ;35:100672. https://doi.org/10.1016/j.nmni.2020.100672

40- Parsamanesh N, Pezeshgi A, Hemmati M, Jameshorani M, Saboory E. Neurological manifestations of coronavirus infections: role of angiotensin-converting enzyme 2 in COVID-19. International Journal of Neuroscience. 2022 ;132(9):917-24. https://doi.org/10.1080/00207454.2020.1849193

41- Naqvi AA, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, Atif SM, Hariprasad G, Hasan GM, Hassan MI. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2020 ;1866(10):165878. https://doi.org/10.1080/00207454.2020.1849193

42- Samavati L, Uhal BD. ACE2, much more than just a receptor for SARS-COV-2. Frontiers in cellular and infection microbiology. 2020 ;10:317. https://doi.org/10.3389/fcimb.2020.00317

43- van Hemert MJ, van den Worm SH, Knoops K, Mommaas AM, Gorbalenya AE, Snijder EJ. SARS-coronavirus replication/transcription complexes are membrane-protected and need a host factor for activity in vitro. PLoS pathogens. 2008 ;4(5):e1000054. https://doi.org/10.1371/journal.ppat.1000054

44- Masters PS. The molecular biology of coronaviruses. Advances in virus research. 2006 ;66:193-292. https://doi.org/10.1016/S0065-3527(06)66005-3

Bora J, Deep A, Dashmana A, Lata S, Thapliyal S, Talukdar N, Deshwal RK, Rustagi S, Malik S. SARS-CoV-2 spike protein mutation variants: Recent advances in theragnostic and vaccines development. Microbes Infect Dis 2025; 6(3): 1060-1072.