Insights into the evolutionary and prophylactic analysis of SARS-CoV-2: A review

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ABSTRACT

In late 2019, following the emergence of a β-originated SARS-CoV-2, phylogenetic and evolutionary approaches have been demonstrated to strengthen the diagnostic and prophylactic stratagem of COVID-19 at an unprecedented level. Despite its clinical prominence, the SARS-CoV-2 gene set remains largely irrefutable by impeding the dissection of COVID-19 biology. However, many pieces of molecular and serological evidence have predicted that SARS-CoV-2 related viruses carry their roots from bats and pangolins of South East Asia. Analysis of viral genome predicts that point mutations at a rate of 10^{-4} nucleotides per base in the receptor-binding domain allow the emergence of new SARS-CoV-2 genomic variants at regular intervals. Research in the evolution of molecular pathways involved in emergence of pandemic is critical for the development of therapeutics and vaccines as well as the prevention of future zoonosis. By determining the phyletic lineages of the SARS-CoV-2 genomic variants and those of the conserved regions in the accessory and spike proteins of all the SARS-related coronaviruses, a universal vaccine against all human coronaviruses could be formulated which would revolutionize the field of medicine. This review highlighted the current development and future prospects of antiviral drugs, inhibitors, mesenchymal stem cells, passive immunization, targeted immune therapy and CRISPR-Cas-based prophylactic and therapeutic strategies against SARS-CoV-2. However, further investigations on Covid-19 pathogenesis is required for the successful fabrication of successful antivirals.

1. Introduction

Since the pernicious upsurge of Severe Acute Respiratory Syndrome (SARS) in 2003, an interspecies coronavirus outbreak in 2012; the Middle East respiratory syndrome (MERS), and a current pandemic of 2019 due to novel severe acute respiratory syndrome coronavirus (SARS-CoV-2), coronaviruses from subfamily *Coronavirinae*, family *Coronoviridae*, realm *Riboviria* and order *Nidovirales* (Alexander et al., 2020) have become an emerging public health concern (World health organization (WHO), 2021; World Health Organization (WHO), 2020) of the 21st century. This sudden outbreak of novel coronavirus strain, i.e. SAR-CoV2 in Wuhan, China has once again brought this family of viruses into the limelight as it has occasioned countless deaths and affected voluminous people round the globe (Chan et al., 2020). Given the potential risk of demonstrating a slow mutation rate of 10^{-4} nucleotides per second (Su et al., 2016), it has become an outrageous microorganism for future generations. Therefore, this catastrophe urges scientific

community to bring a revolution in research to fathom the genomic organization, phylogenesis, and prophylaxis of this deadly family. Coronaviruses (CoVs) are commonly associated with respiratory and gastrointestinal tract disorders and represent a myriad of four viral genera: α, β, γ, and δ coronaviruses. Among them, six CoVs have been discovered, including HCoV−OC43, HCoV-229E, HCoV-HKU1, HCoV-NL6 and SARS-CoV (Skariyachan et al., 2019) that can elicit both excessive inflammatory responses and cytopathogenic effects within the infected host.

SARS related viruses i.e. SARS-CoV-2 fall under the category of enveloped virus family and carry spherical and pleomorphic virions with a diameter of 80−120 nm. These viruses have the prevalent genome amongst all known RNA viruses, with 32–43 % GC and 62 % AU-rich content (Barcena et al., 2009; Woo et al., 2010). Primary organization of the viral genome is similar to that of order *Nidovirales*. Their genome encompasses non-segmented RNA having 3′ poly-A tail and 5′ cap structure, involved in translation of replicase polyproteins

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(Du et al., 2009). DNA nano ball sequencing predicts that the transcriptome of SARS-CoV-2 conceals its complexity in numerous discontinuous transcription events that involve the synthesis of nested sub-genomic RNAs. Moreover, the classical genomic and 09 sub-genomic RNAs in SARS-virus generate transcripts of mysterious open reading frames (ORFs) through the phenomenon of fusion, deletion or frameshift (Kim et al., 2020). Usually, CoV genomes are composed of six ORFs, starting from the 5′ end of the genome. Approximately two-third (~20 kb) of the viral RNA encrypts two large open reading frames (ORF1a and ORF1b) that can encode up to 16 non-structural proteins (NSP) that allow genomic replication of the virus, sub-genomic mRNA synthesis (Enjuanes et al., 2016), and two polyproteins (pp1a and pp1ab) (Changchuan, 2020). Furthermore, the genomic organization of SARS-CoV-2 has an unpredictable number of canonical open reading frames that usually encode accessory proteins such as 3a, 6, 7a, 7b, 8 and 10 that are not involved in viral replication but in pathogenesis (Michel et al., 2020). Moreover, by the application of ribosome-profiling techniques, Finkel et al. (2021) have precisely identified 23 unannotated viral ORFs from the genome of SARS-CoV-2 that may perform as regulatory units for the balanced production of various viral proteins. Moreover, the quantification expression of canonical viral ORFs serves as a prerequisite for deciphering the virus-host interactions and functional analysis of viral proteins.

A conserved repetitive sequence of UUUAAAC is positioned around the genome positions of 12–14,000 bp in alpha and beta coronaviruses for the particular phenomenon of ribosomal slippage to initiate the transcription of ORF 1ab (Brian and Baric, 2005). However, the SARS-CoV-2 genome contains 41 sites for RNA modification with the most common motif of AAGAA (Kim et al., 2020). These RNA modifications may impart a momentous role to the persistence of virus inside the host and immune evasion in infected tissues due to the unresponsiveness of innate immune system to RNAs with nucleoside alteration (Karikó et al., 2005). The ORF 1ab gene encodes various products, i.e. papain-like proteases (Plpro), main proteases (Mpro), helicases, methyltransferases, RNA dependent RNA polymerase (RdRp) and numerous innate immune antagonists (Perlman and Netland, 2009). The 3′ ORF region of the genome encodes a series of structural proteins that includes type I glycoprotein, Spike-S (~150 kDa) protein forms peplomers on the upper surface of virion to give it a crown-like appearance, the membrane-M (~25−30 kDa) protein that has a cytoplasmic tail and a short N-terminal ectodomain, a hydrophobic envelope-E (~8−12 kDa) and nucleocapsid proteins (N-proteins) (Magiorkinis et al., 2004).

These nucleocapsid proteins can bind with viral mRNA to generate ribonucleoprotein complex. It also divulges RNA chaperone activity during *in vitro* analysis (Zuniga et al., 2007). Besides the presence of leader sequence and untranslated regions on the 5′ and 3′ end of genome, several stem-loop structures are essential for replication of RNA, transcription, and cellular and viral protein interaction (Yang and Leibowitz, 2015). Furthermore, the presence of Transcriptional regulatory (TR) sequences at the start of each accessory gene allocates the significant process of replication. According to *in vitro* biochemical analysis, these intergenic TR sequences interact with N-proteins at the flanking site via unpaired adeno-dinucleotide sequence in a stem-loop structure of transcriptional regulatory sequence (Yang et al., 2021). The genomic organization of coronaviruses is given in Fig. 1. This review highlights the novelty in genomic organization and plausible prophylactic approaches for coronavirus that has arisen with a higher fatality rate, more vague epidemiological characteristics, paucity of licensed vaccines and most importantly, its circulation in humans with both sporadic and epidemic features.

2. Evolutionary and phylogenetic analysis of COVID-19

Since there exists an evolutionary relationship between the genomes of MERS-CoV, SARS-related coronaviruses, it has been inferred that SARS-CoV-2 is an instant relative of Bat SARS-CoV and distant relative of MERS-CoV (Malik et al., 2020). By tracing the similarities to the protein level, no substitution in the amino acid sequence is perceived in NSP7, NSP13, matrix, and envelope as well as in accessory proteins, p6 and 8B. Whereas, underlying differences are observed in NSP2, NSP3, receptor binding domains, and spike proteins of SARS-CoV-2 and thus creating distinct features in host tropism and transmission mechanisms compared to SARS-CoV (Chen, 2020). Generally, spike protein is sub-divided into functional domains, i.e. S1 domain (poisonous domain) is involved in binding with host receptor and S2 domain allows cell membrane fusion (Oudit et al., 2009). In SARS-CoV-2, these aforementioned domains of spike protein share 68 % similarity with bat-SL-CoVZC45 (GenBank Accession no.MG772933) and 93 % similarity with bat-SL-CoVZXC21 (GenBank Acc. no. MG772934.1). With the help of the maximum likelihood method, it has been revealed that the two strains mentioned above share 100 % bootstrap support with current SARS-CoV-2 (Guo et al., 2020). Similarly, there exists 96 % identity of the prevailing SARS-CoV-2 with the bat isolated RaTG13, found in *Rhinolophus affinis* depicting its origin from the bat (Wan et al., 2020). However, the S1 domain of SARS-CoV-2 carries conserved amino acid regions with SARS-CoV, which indicates that both viruses use the same receptor to infect *Homo sapiens* (Lu et al., 2020).

Moreover, the length of SARS-CoV-2 S-protein carry 1282 amino acids which are relatively extensive than the other two viruses, i.e. SARS-CoV (1255 amino acids) and Bat-SL-CoV (1246 amino acids). In addition, at 5′ end, Pb1ab is a first open reading frame in the whole genome that encodes NSPs with 7096 amino acids in SARS-CoV-2, 7073 amino acids in SARS-CoV, and 7078 amino acids in MERS-CoV (Lu et al., 2020). However, in contrast to SAR-CoV, S-protein of this unique coronavirus carries 3 little insertions at N-terminal and 4 different modifications in the receptor-binding domain (Zhou et al., 2020). In
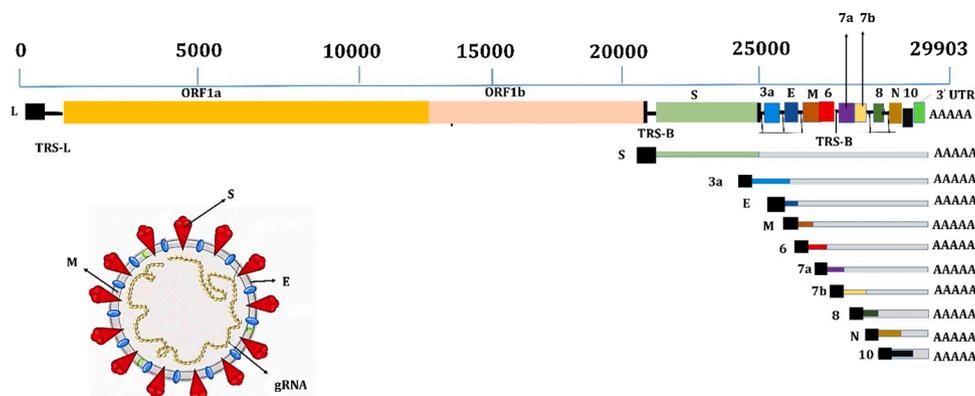

Fig. 1. Schematic representation of SARS-CoV-2 genomic organization, canonical sub genomic mRNAs in grey color and the virion structure (modified from Kim et al., 2020).

addition, SARS-CoV-2 carries a single intact ORF on gene 8, which promote the notion of its origin from bat (Ren et al., 2020). Therefore, it can be stated that these genomic differences have played a momentous character in the sudden outbreak of COVID-19 and evade the world with more fatality rate than SARS (2003) and MERS (2012).

However, Wacharapluesadee et al. (2021) have given the molecular and serological evidence regarding SARS-CoV-2 that such viruses are actively circulating in bats and pangolins of South East Asia. At the whole genomic level, Malaysian Pangolin CoV is 90.55 % and 91.02 % similar to Bat CoV RaTG13 and SARS-CoV-2, respectively. Therefore, it is considered as the SARS-CoV-2's second-closest relative behind RaTG13. Interestingly, several Pangolin-CoV genes shared more amino acid sequence similarity with SARS-CoV-2 genes than they showed with genes of RaTG13. Among Pangolin-CoV and SARS-CoV-2, 05 essential amino acids in RBD are identical. However, the phylogenetic analysis grounded upon N-protein revealed that the 02 amino acid sites (38 P and 268Q) common in SARS-CoV, RaTG13, and pangolin CoV had been mutated to 38S and 268A in SARS-CoV-2 (Zhang et al., 2020). These witnessed amino acid alterations in N-protein would open new horizons in evolution of more sensitive immunogens for serological detection of SARS-CoV-2.

2.1. Epidemiological exploration of SARS-CoV-2 genomic variant

The evolutionary rate of SARS-CoV-2 from December 2019 to October 2021 remained at a constant rate of~ 2 mutations per month. However, the functional outcomes of spike mutations are expanding speedily, and repository of this knowledge entails the analysis of rapid change in amino acid frequencies, linked with unusual epidemiological characters. During the course of SARS-CoV-2 progression in *Homo sapiens*, a D614 G missense mutation within the receptor-binding motif of the virus arose and became a primary circulating variant (S-614 G) in different European countries, including Turkey and Iran. In this course, Zhou et al. (2021) have reported that the S-614 G variant exhibited an increased attachment to human host cell surface receptor angiotensin-converting enzyme 2 (ACE-II) with enhanced replication and transmissibility in human airway epithelial cultures and hamster/ferret models of SARS-CoV-2 infections. This greater infectivity can be attributed to higher viral shedding and increased fusion possibility of the viral membrane with targeted cell membrane by changing the receptor-binding site's conformation (Sallam et al., 2021). In this regard, *in silico* docking studies indicate that the resultant mutant spike protein carries a significant numeral of hydrogen bonds with TMPRSS2 at the cleavage site than wild type. This variation increases the interactivity of TMPRSS2 with the S2 domain, thus facilitating the viral entry inside the host cell. Such discoveries can lead to success in the prognosis of infections caused by SARS-CoV-2 (Raghav et al., 2020).

Moreover, an investigation of Indian SARS-CoV-2 disclose the predominance of D614 G mutation in spike protein, thus forecasting an increased interaction between TMPRSS2 and viral infectivity (Raghav et al., 2020). In addition, reported mutations in the S genes with the utmost recurrent ones includes D936Y/H, P1263 L, and L5F (Korber et al., 2020; Lokman et al., 2020). Following the advent of D614 G, another amino acid substitution within the receptor-binding region, N439 K, appeared with an increased frequency in multiple lineages in Scotland. However, the initial lineage with N439 K named as B.1.141 extinct quickly, and another pedigree that autonomously acquired N439 K (B.1258) evolved and disseminated broadly in Europe. S protein with N439 K mutation exhibited an increased binding affinity with the hACE2 receptor. The viruses with such mutation demonstrate comparable *in vitro* replication fitness and induce infection with identical clinical consequences in contrast to wild-type strain. All N439 K variants have resulted from the similar mutations at nucleotide level: C-to-A transversion at third codon. The variant has appeared to bring deleterious effects in ≥34 countries, including Brazil, Nigeria, and the United States of America (Thomson et al., 2021).

Another RBM amino acid change, Y453 F related to an augmented ACE2-binding affinity received significant consideration after its documentation in sequences linked with infections in *Homo sapiens* (humans) and *Neovision vision* (minks); particularly one lineage recognized in Denmark and primarily entitled as 'cluster 5' (now B.1.1.298) (Lassaunière et al., 2020). According to the reports of 5th November 2020, 214 population infested with mink related SARS-CoV-2 were all carrying the mutation Y453 F. The B.1.1.298 pedigree also has Δ69−70, an amino-terminal domain (NTD) deletion that has arisen after numerous intervals around the worldwide population of SARS-CoV-2, including the second N439 K lineage B.1.258. Δ69−70 is prophesied to modify the configuration of an open NTD loop and has been linked with enlarged infectivity (Harvey et al., 2021). Correspondingly, current studies propose that in the United Kingdom, SARS-CoV-2 variations occur at N501Y in spike protein along with 23 separate mutations on spike; 17 of which appeared to be linked with those viral proteins that give specific characters to the virus (Conti et al. 2021).

Moreover, Naveca et al. (2021) reported that distribution of lineage B.1.195 was the primary culprit in driving the first exponential growth phase that was later supplanted by descent B.1.1.28 in between May and June 2020. The succeeding wave accords with an advent of variant of concern (VOC) P.1 that emerged from an indigenous B.1.1.28 clade in late November 2020 and swapped the parental lineage in <2 months. This study has attempted to reveal the phylogenetic relationship of emerging SARS-CoV-2 virulent strains on behalf of spike protein analysis (Fig. 2).

2.1.1. Variations outside the spike protein

Of several noteworthy mutations, the pervasive C241U mutation in 5′UTR of viral genome has still not been associated with any particular condition. Normally, it is seen as neutral yet it permits the virus to gain a minor level of acclimation to its host's metabolism, with better resistance to viperin activity. Furthermore, it is present in the viral genome's 5′ UTR leader, hence it is crucial for translational regulation and host specificity (Tidu et al., 2020). Since then, there are countless instances of blooms that came as a result of this transformation. For instance, an intriguing chain of genetic changes started with an early mutation, G11083U (protein Nsp6, L37 F), that is now widely dispersed globally and attributed to distinct clades in India (Banerjee et al., 2020). Similar mutation, G1440A (G392D, protein Nsp2), accompanied by G2891A (A876 T, ubiquitin-like domain of protein Nsp3), has now been detected in various countries (Liu et al., 2020). Thus, showing a discrepancy between Orf7a and Orf7b translation.

Similarly, the Orf8 zone of SARS-related coronaviruses is exceedingly varied. It fluctuates during epidemics, demonstrating that it is under continual selection pressure, occasionally creating two peptides, Orf8a and Orf8b (Chen et al., 2020a, 2020b). Furthermore, the evolution of replicase protein, Nsp12, is of tremendous importance. Initially in the pandemic, C14408U (P314 L) mutation at the end of a zinc finger in Nsp12 has been accompanied by further sequential changes inside the 5′ end C241U mutation. This mutation has emerged in several branches of the viral evolutionary tree. It considerably impacted the activity of the replicase, since this mutation has now been followed by "blooms" of novel lineages of the virus, showing that an altered replication mechanism was mutagenic (Cluzel et al., 2020). In addendum to the genetic cellular interactions of virus, there reside substantial polymorphisms in humans (e.g. Lewis, HLA, etc.) which are found in most of the populations, whereas others are confined to particular ethnic groups, and these variations offer additional limitations on viral evolution (Luo et al., 2021). Now that the pandemic has been in force for more than two years, fresh interpretations should be employed to explore the mutations that take place continuously, particularly in view of the fact that vaccination is gaining attraction.
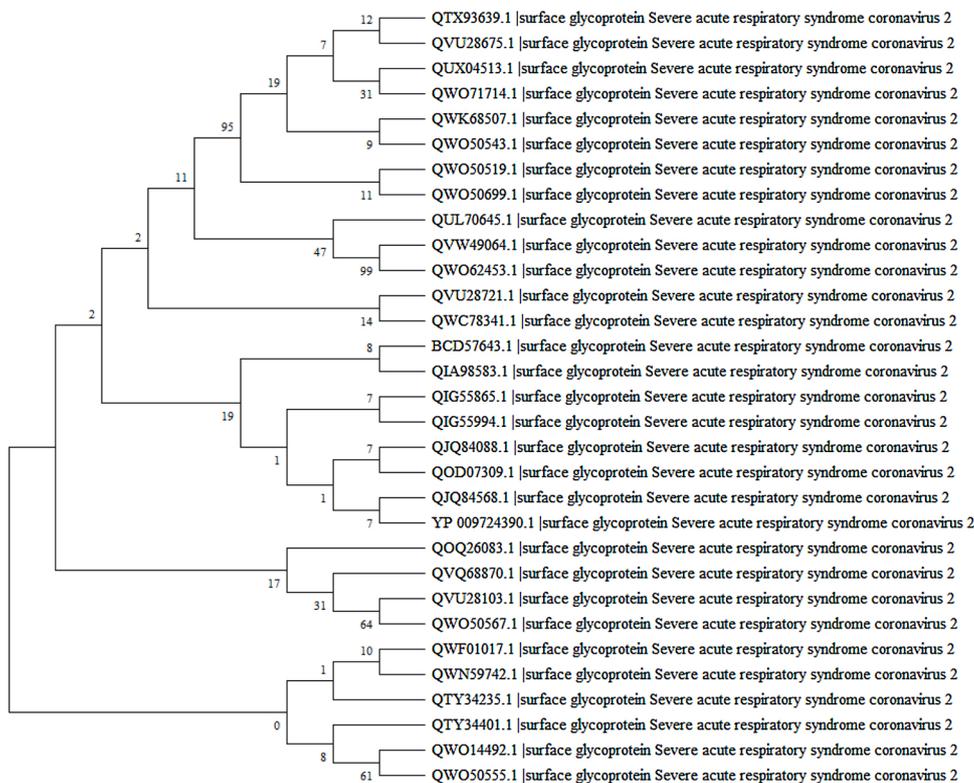
Fig. 2. The evolutionary history of Surface glycoprotein of SARS-CoV-2 from USA, Iran, Taiwan, New Zealand, Peru, Egypt, Pakistan, Ghana, Libya, Australia, United Kingdom, China, Thailand, Japan, Hong Kong, Brazil, India was inferred by using the Maximum Likelihood method and General Time Reversible model. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. This analysis involved 24 nucleotide sequences. Evolutionary analyses were conducted in MEGA X.

3. Clinical and pathological properties of COVID-19

When SARS-CoV-2 enters human body both innate and adaptive immune response become functional to prevent its infection. Macrophages are mostly affected by CoV and then these macrophages represent SARS-CoV-2 antigens to T lymphocytes. This process results in T cell activation followed by differentiation to induce T cell subset (Th 17) associated cytokine production. This whole process results in massive production of cytokines in body to amplify immune response. If virus persist in host these cytokines are continually secreted, and it has a negative effect on natural killer cells (NK) and effects CD8 T cell activation (Li et al., 2020a, 2020b). However, these CD8 T cells are very effective in clearing CoV from body by producing an effective mediator response. Attachment of SARS-CoV-2 through S protein on depetidyl peptodase (DPP4R) receptor on the host results in breaking of membrane and genomic RNA of virus appears in cytoplasm and an immune response against dsRNA is partially generated during SARS-CoV-2 replication.

Proinflammatory cytokines are released by toll-like receptors (TLR-3) that have been sensitized by dsRNA to induce inflammation (Akira et al., 2006). TLR3 is activated in response to dsRNA sensitization and cascades of signaling pathways activation (IRFs and NFB activation, respectively) to produce type I IFNs and proinflammatory cytokines, initiating the inflammatory response (Maloir et al., 2018). Type I IFNs stimulate the synthesis of antiviral proteins and hence perform a critical role in the uninfected cells protection. Thus, the host immune system contributes significantly to the body's defence against this virus. However, accessory proteins of SARS-CoV-2 may occasionally interfere with TLR-3 signaling and bind to the CoV's dsRNA, preventing TLR-3 activation during the replication phase and therefore infecting the host immune system. When TLR-4 identifies viral S proteins via the MyD88-dependent signaling pathway, pro-inflammatory cytokines are triggered. Numerous immune mediators are produced in response to virus-cell contact, and large amounts of cytokines (IL1, IL6, IL8, IL21, TNF, andMCP1) and chemokines are also produced in SARS-CoV-2 infected host cells (Li et al., 2020a, 2020b). Thus, both the innate and

adaptive immune responses function in coordination to protect the host from this viral attack and to develop memory cells through successful activation of the adaptive immune system in order to provide a long-term response that protects the host from subsequent viral attack.

However, the neurotropism of SARS-CoV-2 and the etiology of its neurological manifestations, and postmortem histological findings in patients' central nervous systems who died from COVID-19, have received far less attention than the virus's well-known pulmonary tropism and respiratory complications. After hypoxic encephalopathy, the most often identified abnormalities in brain tissue are ischemic and hemorrhagic lesions, as well as reactive astrogliosis and micro gliosis. These findings do not appear to be the result of SARS-CoV-2 infection; rather, systemic inflammation and coagulopathy caused by COVID-19 appear to be the source (Maiese et al., 2021). However, further study is necessary to validate this hypothesis and to identify any other alterations in neural tissue that may occur. A standardized technique for doing autopsy on these instances should include a brain examination of individuals who died with COVID-19. Fig. 3 provides a comprehensive overview of the immune system's response to SARS-CoV-2.

According to the current data, approximately, one-third of SARS-CoV-2 infections are asymptomatic. Longitudinal studies indicate that the majority of persons who have a positive PCR test but no symptoms at the time of testing remain asymptomatic. Adapting COVID-19 control approaches to account for the prevalence and transmission risk of asymptomatic SARS-CoV-2 infection should be considered (Oran and Topol, 2021). According to Sah et al. (2021), elders seemed to have a significantly lower proportion of asymptomaticity (95 %) than children (46.7 percent). Early findings on COVID-19 indicated that children were mostly relieved of severe symptoms, with just 2–6% of children admitted to an intensive care unit. Nonetheless, since mid-April 2020, clusters of paediatric instances of severe systemic hyper inflammation and shock have been linked to COVID-19. This illness, termed SARS-CoV-2-associated multisystem inflammatory syndrome (MIS) in children, exhibits striking similarities to Kawasaki disease. Patients with a multisystem inflammatory syndrome linked with SARS-CoV-2 typically experienced a prolonged fever, gastrointestinal symptoms, a
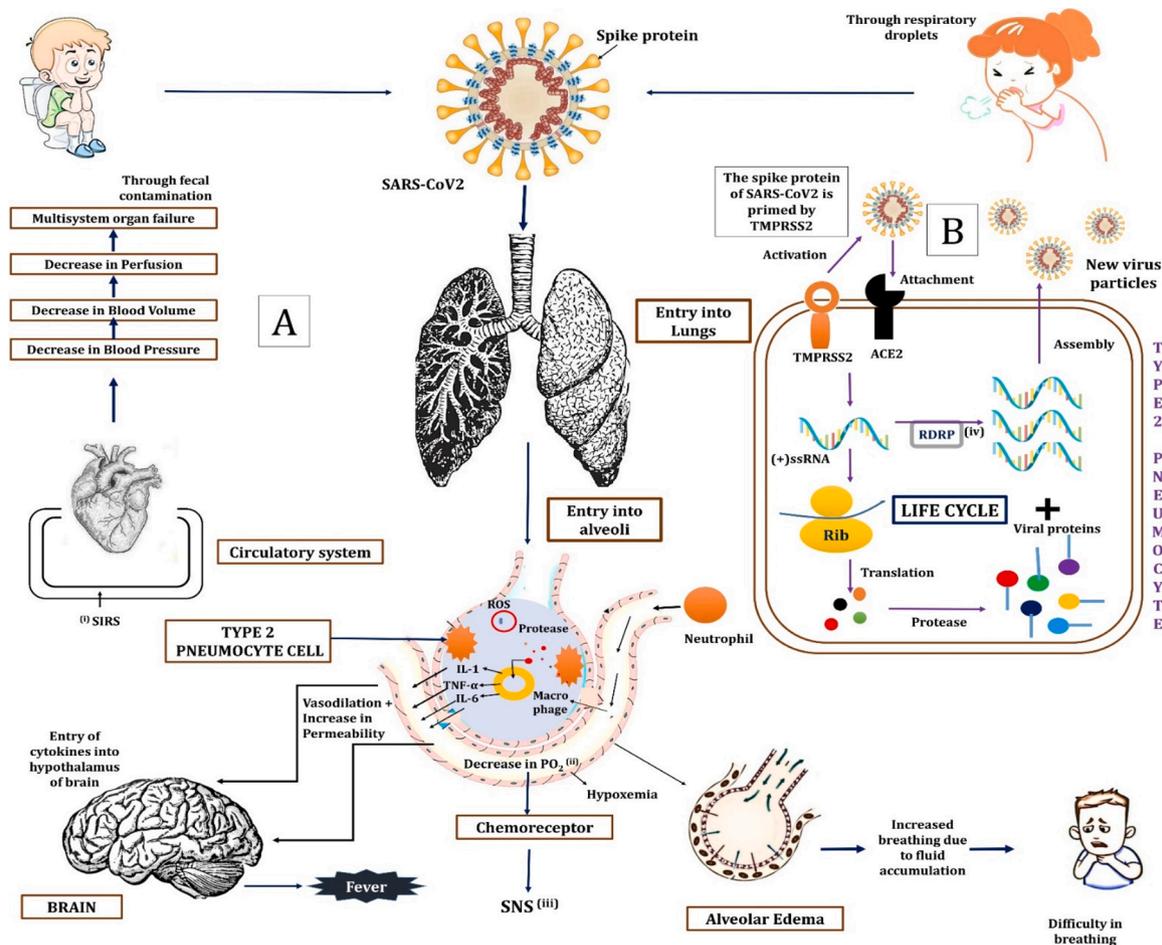
Fig. 3. A schematic representation of and immune response of patient to SARS-CoV-2. **(A)** Through fecal contamination and respiratory droplets of infected person, virus is targeting lungs of other healthy person. After the entry of virus inside the alveoli of lungs, macrophages get activated to release cytokines i.e. IL1, IL6 and TNF-α. The produced cytokines will start to infiltrate into the pulmonary capillary surrounding the alveoli. From there, by increase in permeability and vasodilation of blood capillary, cytokines will influence Nervous system, Circulatory system and Respiratory system. By affecting hypothalamus of brain, the condition of pyrexia or fever may prevail and by affecting circulatory system, the problem of Multi system organ failure may appear. Critically ill COVID-19 patients certainly meets clinical Systemic inflammatory response syndrome (SIRS) criteria that is further supported by markedly elevated levels of pro-inflammatory cytokines. However, after attacking the chemoreceptors in sympathetic nervous system, respiratory rate of patient may increase drastically. Due to the infiltration of cytokines, the phenomenon of alveolar edema may increase breathing rate and the patient face the problem of asthma too. **(B)** After using ACE2 receptor to get entry inside the host and utilizing RNA dependent RNA polymerase, the inserted RNA make multiple copies of viral genome and the produced viral proteins will assemble to give rise new viral particles.

[i] **SIRS** = Systemic inflammatory response Syndrome.

[ii] **PO$_2$** = Partial Pressure of Oxygen.

[iii] **SNS** = Sympathetic nervous system.

[iv] **RDRP** = RNA dependent RNA polymerase.

polymorphic rash, conjunctivitis, and mucosal abnormalities. Additionally, a subgroup of these patients (20–100 % of them) suffered hypotension and shock as a consequence of either acute myocardial dysfunction or systemic hyper-inflammation/vasodilation. Additionally, coronary artery dilatation or aneurysms have been somewhat recorded in 6–24 % of instances, while arrhythmias have been documented in 7–60 %. Acute phase management requires cardiac support, immunomodulation, and anticoagulation. Due to the possibility of progression of cardiac manifestations and the unclear prognosis, these patient needs long-term systematic follow-up (Sperotto et al., 2021).

Furthermore, many investigations have demonstrated that re-positive SARS-CoV-2 RT-PCR testing is prevalent in recovered COVID-19 patients. Re-positive tests were discovered in 2.4–69.2 percent of COVID-19 patients discharged from the hospital and lasted for 1–38 days after discharge, depending on the population size, patient age, and specimen type. Numerous possible explanations for re-positive SARS-CoV-2 tests in recovered COVID-19 patients are being considered at the

moment, such as false-negative and false-positive RT-PCR tests; reactivation; and re-infection with SARS-CoV-2, however the mechanism underlying these re-positive cases remains unknown. Preventing re-positive testing in discharged patients is crucial for preventing the spread of the pandemic. Reducing the percentage of false-negative tests prior to discharge is important. Additionally, specimens from many body areas should be acquired before discharge to identify SARS-CoV-2 viral RNA. Additional research should be conducted to develop novel assays that target a vital region of the RNA genome in order to improve their sensitivity and specificity (Dao et al., 2021).

4. Prophylactic approaches of COVID-19

The degree to which transmutations effect the antigenic phenotype of SARS-CoV-2 will allow variants to evade immunity convened by innate infection or vaccination needs to be established. Nevertheless, there exists an emerging indication regarding the altered antigenic

phenotype of SARS-CoV-2. These strains are circulating and disturbing immune recognition to the notch that demands instant consideration. A current mutation rate in the structural proteins of SARS-CoV-2 are reflected as a chief obstacle in the deterrence and control of COVID-19. In spite of these inter- and intra-individual variation, the mutations that significantly decrease antibody binding typically occur at few spots of the receptor-binding motif. In this regard, the D614 G, S477 N, and E484 K variants of the S protein are thought to cause a threat to immune system or amplified ACE2 binding by the virus, thus affecting COVID-19 vaccine development and antibody treatment (Koyama et al., 2020). Particularly, E484 is a region in the RBD where mutations typically influence the binding and neutralization of antibodies. Here neutralization through plasma antibodies is reduced by 10 folds that is evidenced in the emerging 20 H/501Y.V2 and 20 J/501Y.V3 SARS-CoV-2 lineages (Greaney et al., 2021a). This phenomenon can be attributed to the fact that E484 is frequently targeted by antibodies that utilize heavy-chain germ line genes, common among anti-SARS-CoV-2 RBD antibodies, IGHV3−53, and IGHV3−66 (Greaney et al., 2021b). In order to find an everlasting remedy for toxicities initiated by this virus, the need of an hour is to formulate antiviral drugs and vaccines not only for COVID-19 but also for the maladies that afflicted populaces in 2003 (SARS) and 2012 (MERS). Fig. 4 and Table 1 illustrates the treatment strategies highlighted for the infections of SARS-CoV-2. An effective vaccine against all these viral strains will be an imperative milestone in precluding the future outbreaks of these harmful viruses.

4.1. Universal vaccines

Since there is a close relationship between the receptor-binding domains, potential immune evasion mechanism (Shokri et al., 2020), and an extended incubation period (average 2–11 days) of SARS-CoV, MERS-CoV and SARS-CoV-2 (Lessler et al., 2009; World Health Organization (WHO), 2020). It is a prerequisite for modern times to foster the development of universal vaccines that could vanish these epidemics and pandemics for centuries. The key protein targets of SARS-CoV-2 are 3CLpro, PLpro, RdRp, S protein, ACE2, and AT2. The distinctive spike

(S) protein should be the principal target for vaccination and therapeutic antibody production (Du et al., 2009). Numerous SARS S-protein-specific neutralizing antibodies have been testified (Berry et al., 2010). These neutralizing antibodies have a solid aptitude for epitopes within the receptor-binding domain (RBD) that also bind to angiotensin–converting enzyme 2 receptor (Brink et al., 2005). Existing evidence suggests that repeated SARS-CoV-2 infection is associated with elevated neutralising antibodies and persistent memory responses after infection (Abdel-Moneim et al., 2021). However, *in vivo* analysis designates that the receptor-binding domain scrambles one of the significant neutralizing epitope clusters (Cao et al., 2010). By taking the concept of universal vaccines to another level, Chiuppesi et al. (2020) have developed a multi-antigenic SARS-CoV-2 vaccine candidate by employing a synthetic pox-virus platform of Modified Vaccinia Ankara vector (MVA) to formulate vaccine against infectious diseases (i.e. COVID-19) and malignancies. After immunization of mice with the vaccine platform, co-expressing the S and N-antigens of SARS-CoV-2, the model organism exhibited antigen-specific humoral and cellular immunity with effective neutralizing antibodies. These vaccines have been reported to encourage a vigorous humoral and cellular immune response to S and N-antigens rather than the strategies that solely involve S-antigen. However, the estimation of protective efficiency of twofold recombinant sMVA-CoV2 vaccine vectors in animal models is the subsequent step towards an advanced research into clinical evaluation.

In this regard, the development of human neutralizing antibodies against SARS may open new horizons for effective prophylaxis (Zhu et al., 2007). Studies have been directed to define broad-spectrum antibodies that work in neutralizing multiple viral strains (Rockx et al., 2010). Hence, analogous structures amongst the S proteins, nucleocapsid proteins, and other accessory proteins can be considered as a cradle of potential universal vaccine. Recently, Bradfute et al. (2020) have reported that IgG against integral S protein or S1 subunit of protein is recognized as a neutralizing antibody against SARS and COVID-19. Once we familiarize our immune system with the universal vaccine, we would develop immunity against all the strains that share the same fragment. Thus, this strategy would potentially avert forthcoming
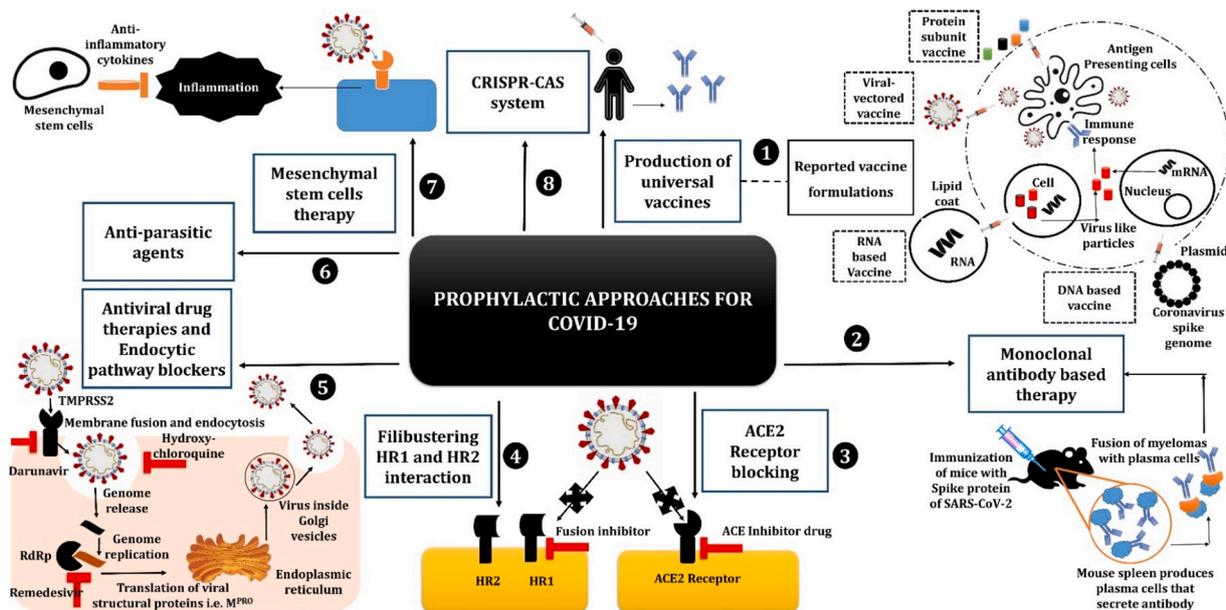

Fig. 4. Schematic representation for prophylactic approaches for COVID-19. (1) The production of universal vaccine incorporates the concept of immunity from all strains of coronaviruses and the reported vaccine formulations involve Protein subunit vaccine, Viral vectored vaccine, RNA based vaccine and DNA based vaccines that involves Antigen presenting cells and the immune reponse in the form of antibodies after certain dosage to the cell. (2) Formation of monoclonal antibodies involve the hybridoma technique to treat the malady of COVID-19. (3), (4) ACE2 receptor blockers and Fusion inhibitors impede the entry of virus inside the host cell. (5) Various antiviral drugs and endocytic blockers like Darunavir that inhibits the priming of SARS-CoV-2, hydroxyl-chloroquine and various endocytic pathway blockers that inhibits the endocytosis of virus, remdesivir inhibits the activity of RdRp thus inhibiting the replication of viral genome. (6) Mesenchymal stem cells produce anti-inflammatory cytokines impede the inflammation caused by viral infections.

Table 1

Prophylactic approaches to treat SARS-CoV-2 and its potential targets.

Prophylactic approach	Potential target	References
Universal Vaccine	Viral analogue Spike and Accessory proteins	(Du et al., 2009; Rockx et al., 2010)
ACE2 receptor blocking	Renin-angiotensin system (RAS),Type I Ang II receptor	(Glowacka et al., 2010; Wu, 2020)
Monoclonal antibody	Spike proteins of virus	(Zhai et al., 2020)
Filibustering HR1 and HR2 receptor	S2 domain of Spike protein, HR1 and HR2 receptor	(Zhu et al., 2004;Xia et al., 2020a,2020b)
Use of Inhibitors	Various interactions sites of SARS-CoV-2	(Wang et al., 2020a, 2020b)
Antiviral drug therapies	RNA dependent polymerase (RdRp),Type II trans membrane serine protease (TMPRSS2),Viral glycoproteins, Endoplasmic reticulum-Golgi intermediate compartment (ERGIC)	(Savarino et al., 2003; Sheahan et al., 2017; Hoffmann et al., 2020),(Hu et al., 2020)
Blocking viral endocytic pathway	Protein kinases 1 (AAK1), cyclin G-associated kinase	(Richardson et al., 2020)
Anti-parasitic agents	Reticence of IMPα1/ β1 mediated nuclear import of viral proteins	(Caly et al., 2020)
Targeted immunotherapy	Dendritic cells, B, T cells, release of cytokines and chemokines	(Li et al., 2020a, 2020b)
Mesenchymal Stem Cells	Amplified number of peripheral lymphocytes, the decline in the C-reactive protein, and waning of over-activated cytokine-secreting immune cells (CXCR3+CD4+ T cells, CXCR3+CD8+ T cells, and CXCR3 + NK cells) and CD14+CD11c + CD11bmid regulatory dendritic cell (DC)	(Leng et al., 2020; Liang et al., 2020)
CRISPR-CAS based study	Application of Cas13a based assay to inhibit replication of SARS-related coronaviruses	(Abbott et al., 2020)

eruptions of other coronaviruses.

4.1.1. Reported vaccine formulations for SARS-CoV-2

The fast development of SARS-CoV-2 vaccine is a universal urgency. Harder et al. (2021) have documented that vaccine efficiency among symptomatic and asymptomatic illnesses was 80–90 %. Vaccinated individuals are less prone towards the infection and transmission of virus. In this regard, Fougeroux et al. (2021) have established two capsid-like particles (CLP) based vaccines displaying RBD of SARS-CoV-2 spike protein. These RBD-CLP vaccines exhibited a dynamic potential to incite elevated intensities of the serum anti-spike antibodies by binding ACE2 receptors with Nano-molar affinity rather than the soluble RBD vaccines. This reported RBD-CLP vaccine is a favorable candidate in precluding the noxious havoc of COVID-19. Following are the vaccine formulations that are undergoing clinical trials:

4.1.1.1. Protein subunit vaccine. These vaccines (inoculation) are formulated through recombinant antigenic proteins or synthetic peptides, but they possess little immunogenicity and demand adjuvants to generate operative immune responses after vaccination. In this regard, by utilizing Molecular clamp technology and adjuvant system AS03, a perfusion-stabilized S protein subunit vaccine has been formulated that is going through clinical trials (Tu et al., 2020). Similarly, Richmond et al. (2021) have stated phase 1 clinical prosecutions of trimeric form of spike protein combined with two different adjuvants. The vaccine has reported a high neutralizing antibody response with a Th-1 based cellular immune response and an acceptable safety profile. In addition,

Mazumder et al. (2020) have described the development of triple antigen VLP vaccine candidate, PRAK-0302, in a chiefly categorized *S. cerevisiae* based D-Crypt™ platform. The vaccine exhibited the potential to induce specific neutralizing antibodies in the model organism with elevated lymphocyte proliferation and IFN-γ- levels. Such statistics underpin the clinical progress and testing of designed vaccine in humans.

Similarly, Tian et al. (2021) have reported the procurement of SARS-CoV-2 subunit vaccine (NVX-CoV2373) from the full-length spike protein that remains stable in the perfusion form. This formulation is known to exist in 27.2 nm thermostable particles that bind with extraordinary affinity to hACE2, and elicit an immune response of multi-functional $CD4^+$ and $CD8^+$ T-cells and antigen-specific multi-center B cells in mice. In baboons, the vaccine provoked an elevated immune response with a higher titer of anti-S antibodies and block the binding of S-protein with hACE2. These outcomes sustain the unending clinical phase 1/2 trials for the security and immunogenicity of NVX-CoV2373.

4.1.1.2. Viral vector vaccines. By cloning the optimized full-length gene of spike protein in Ad5-nCoV (Adenovirus- type 5) vector that expressed recombinant S protein of SARS-CoV-2, a four-time increase in spike protein and RBD neutralizing antibodies has been reported in the clinical trials (Zhu et al., 2020a, 2020b). Similarly, Bos et al. (2020) have stated the Ad26 vector-based vaccine that encrypts perfusion stabilized SARS-CoV-2 spike immunogen with a wild type signal peptide. After immunization of mice with this formulation, neutralizing humoral and cellular immune response was reported. This optimized Ad26 vector-based vaccine for SARS-CoV-2 is presently being appraised in phase I of clinical trials.

4.1.1.3. mRNA vaccine. Having an edge of versatility along with the quick fabrication of vaccines for SARS-CoV-2, three mRNA vaccine formulations have made their way to the clinical trials with the two-dose immunization regime. In spite of the high efficiency of COVID-19 mRNA vaccinations, outbreaks do occur. Duerr et al. (2021) had compared the genomes of SARS-CoV-2 from 76 breakthrough cases to those from unvaccinated controls after immunization with BNT162b2 (Pfizer/BioNTech), mRNA-1273 (Moderna), or JNJ78436735 (Janssen). Resultantly, there was no correlation between the type of immunization received and the genomic among breakthroughs. The statistics indicate that among vaccinated individuals, vaccine breakthrough was not common with variant of concern Alpha and variant of interest Iota. This demonstrates the affectivity of mRNA COVID-19 vaccines against various variants. However, N-terminal domain and receptor binding domain, linked to immune evasion appeared to be more prevalent in the vaccine group. Nonetheless, diminishing immunoglobulin reaction in recuperating patients after infection of SARS-CoV-2 and re-infection of humans have increased prevalent apprehension about a conceivable extent of SARS-CoV-2 vaccine security. In this regard, Huang et al. (2021a, 2021b) have reported the development of a nucleoside modified mRNA vaccine encapsulated in lipid encoding viral RBD. A single dose of mRNA-RBD to hACE2 transgenic mice elicits both vigorous neutralizing antibody and cellular immune response. Moreover, after immunization of mice with mRNA-RBD vaccines, the higher levels of neutralizing antibodies were sustained for approximately 6.5 months and confirmed long-term protection from SARS-CoV-2 infections.

4.1.1.4. DNA vaccines. Among the techniques available for developing vaccines, DNA and mRNA-based vaccination is a promising substitute to traditional strategies. The S-protein has been used as the antigen of all DNA vaccines currently being verified in clinical trials. In this regard, a plasmid pGX9501 has been fabricated to encrypt spike protein of SARS-CoV-2. The developed INO-4800 vaccine induced both cellular and humoral immune response along with cross-reactive responses against

SARS-CoV that was experienced within few days ensuing a single immunization of mice and guinea pigs (Smith et al., 2020).

4.2. Other therapeutic approaches

4.2.1. Monoclonal antibody

Provoking an immune response against SARS-CoV-2 directly could be another potential stratagem for the treatment of this lethal virus. Viral infections can be prohibited by hindering the ability of the virus to recognize, infiltrate, or concurrence to the host cells (Richard et al., 2016; Shanmugaraj et al., 2020). In this regard, 47D11 has been reported as the first SARS-CoV-2 neutralizing human monoclonal antibody that has been vetted from readily accessible hybridoma libraries of genetically modified mice immunized with SARS-CoV and MERS-CoV spike proteins (Wang et al., 2020a, 2020b). Various *in vitro* experimentations have revealed that 47D11 carries a dynamic ability to neutralize SARS-CoV and SARS-CoV-2 infections with IC_{50} values of 0.19 and 0.57 μg mL^{-1}, respectively. This indicates the importance of antibodies from SARS-survivors in combating SARS-CoV-2 infections (Chen et al. 2021). For humanized monoclonal antibody production of 47D11, the cDNAs encoding chimeric variable domains of heavy and light chains of 47D11 H2L2 mAb have been cloned into expression plasmids (Ptrioz) having heavy IgG1 and Ig kappa light chain constant regions of human. Because of having interleukin-2 signal sequences in the plasmids, efficient secretion of antibodies was made possible to treat the SARS-CoV-2 infections (Marghani, 2020).

Large-scale production of functional monoclonal antibodies (mAbs) aiming the spike proteins of SARS-CoV-2 by Regeneron Company would be a remarkable phase in crippling this viral infection entirely (Zhai et al., 2020). For now, it is claimed by Regeneron Company that they have sequestered numerous virus-neutralizing or virus-clearing antibodies from mice and testified patient's recovery from COVID-19 infection. Given that vaccinations are the most efficient strategy to prevent COVID-19, certain subpopulations may probably benefit from neutralizing monoclonal antibodies (mAbs) like bamlanivimab and etesevimab following exposure to coronavirus 2 causing severe acute respiratory syndrome. (SARS-CoV-2). In contrast to vaccine-induced immunity, which builds over time, neutralizing mAb injection is a speedy and passive immunotherapy that has the ability to minimize disease progression, emergency department visits, hospitalization, and mortality. While bamlanivimab is allowed for usage in the event of an emergency in a range of countries. Additionally, countries are increasingly relying on bamlanivimab and other authorised monoclonal antibodies in light of the evolving variant landscape (Nathan et al., 2021).

4.2.2. ACE2 receptor blocking

The coronavirus spike protein and the cognate host cell receptor (ACE2) are considered operative and suitable objectives for intrusions, as ACE2 is a powerful negative regulator of renin-angiotensin system (RAS). This system is involved in evoking inflammatory lung diseases and plays a part in regulating blood pressure along with body fluids (Glowacka et al., 2010). The ACE2 enzyme has a dynamic ability to catalyze the degradation course of angiotensin II to angiotensin-(1–7). However, it is a delicate phenomenon to engender an equilibrium amid angiotensin II and angiotensin-(1–7), since angiotensin II has an ability can bind to AT1 receptor for vasoconstriction and angiotensin-(1–7) can reduce vasodilation after attaching to AT2 (Imai et al., 2005). The spike (S) protein of SARS-CoV and SARS-CoV-2 intermingles in comparable fashion with ACE2 receptor, and by introducing recombinant ACE2 proteins to lungs tissue, we can find a cure or at least decrease lung injuries in patients of COVID-19. This strategy was applied on mice during SARS in 2005 and cured mice from acute lung injury (Imai et al., 2005).

In addition, lung injury in COVID-19 patients can also be alleviated by aggregating the level of ACE2 in lungs tissues, making use of a therapeutic vector to overcome ACE2 paucity induced by the virus.

ACE/ACE2 functions can also be poised by ACE inhibitor, i.e. Lisinopril. Bequeathing with some studies, type I Ang II receptor blocker, i.e. losartan, can also be tested for relieving lung injury caused by COVID-19 (Wu, 2020). Since, natural products are risk-free and easily accessible to treat certain viral infections. In molecular docking study of SARS-CoV-2 spike protein with its hACE2 receptor, Basu et al. (2020) have selected five phytochemicals that reside in the subclass of flavonoid and anthraquinone. Amongst them, phytochemical hesperidin can attach with ACE2 and RBD/ACE2 complex non-competitively to impart anti-viral activity in SARS-CoV-2 infections by destabilizing spike protein binding with human ACE2 receptor. Recently, through computational analysis, Jena et al. (2021) have reported that catechins and curcumin demonstrates stout binding affinity to the viral S protein, host receptor ACE2 and complex (RBD/ACE2 complex). Curcumin unswervingly binds with the Receptor Binding domain (RBD) of viral S protein whereas, catechin binds with amino acid residues present near the RBD site of S proteins. Thus, the protein-protein interaction studies of these two polyphenols have predicted their efficiency in obstructing the development of the S-protein ACE2 complex. The partial antagonist of AT1 and AT2, i.e. L-163,491 may also diminish lung injury caused by coronaviruses (Bracken et al., 2000)), but its effects on COVID-19 still needs to be determined.

According to the GISAID database (October 2021), the phenomenon of RBD mutations in population correlates positively with enhanced binding affinity to ACE2. (Zahradník et al., 2021) suggested that RBD of SARS-CoV-2 may competitively block the ACE2 receptor binding site. Nevertheless, this requires the use of an RBD with a picomolar affinity for ACE2. In this context, a yeast display method was introduced that uses carboxy- and amino-terminal fusions of exceedingly brilliant fluorescent moieties to report expression at extraordinarily low levels and permits selection down to picomolar concentrations. Additionally, it was discovered that the high-affinity variant RBD-62 may be employed in vitro to prevent SARS-CoV-2 infection as well as α, β and γ variants. When administered before or after infection, RBD-62 dramatically decreased clinical illness in a hamster model of SARS-CoV-2 infection.

4.2.3. Filibustering HR1 and HR2 interaction

An additional imperative approach to obstruct the fusion of SARS-CoV-2 with the host cell membrane encompasses the blockage of HR1 and HR2 interface in the course of the viral access to the host (Xia et al., 2020a). These fusion proteins, HR1 and HR2, play a distinct role in fusion with the host membrane. Following the binding of RBD in the S1 subunit of S protein to the ACE2 receptor on the target cell, heptad region the 1 and 2 domains in S2 subunit undergo interaction to form six-helix bundle fusion core with each other. Thus, bringing viral and cellular membranes in juxtaposition for fusion and infection. Recently, Xia et al. (2020b) have pinpointed several mutated amino acids in the HR1 domain of SARS-CoV-2, thus allowing an enhanced interaction with HR2.

On the other hand, the SARS-CoV functionality can be blocked by employing the synthetic peptides from HR-2 regions and binding with an enhanced affinity to a peptide from the HR-1 region (Zhu et al., 2004). As SARS-CoV-2 has the exact fusion mechanism with SARS-CoV, this strategy can bring revolution in its way. Recently, pan-coronavirus fusion inhibitor EK1 has been appeared to be effective against five HR-1 regions of human coronaviruses (HCoVs), including MERS-CoV, and 3 SARS-related CoVs. An intranasal incorporation of this peptide in a dose-dependent manner can constrain SARS-CoV-2 mediated membrane fusion and carries therapeutic and prophylactic potential against COVID-19. Similarly, lipo-peptide from EK1, i.e. EK1C4 is more effective against the membrane fusion process with a concentration of 1.3 and 15.8 nM. This EK1C4 peptide affix to one of the three hydrophobic grooves of the HR1 trimer through its EK1 moiety and also anchors to one of the other grooves of the HR1 trimer through its cholesterol counterpart. In this regard, several hydrogen bonds establish between HR1 and the helical regions of EK1C4, despite interaction with

the HR2 domain for membrane fusion. This strategy thus impedes the membrane fusion of SARS-CoV-2 and carries a dynamic potential for advanced therapeutic techniques (Xia et al., 2020b).

4.2.4. Use of inhibitors

Numerous strategies are being considered to combat contagions caused by SARS-CoV-2, that includes an application of inhibitors for viral and host proteases along with host-directed therapies. Recently, scientists have proposed the therapeutic use of α-interferon in the form of aerosols (5 M units two times a day), lopinavir/ritonavir, chloroquine phosphate, ribavirin, and abidor as antiviral medications (Wang et al., 2020a, 2020b). However, ongoing clinical trials and studies advocate the administration of Remdesivir (GS-5734) in the patient as it carries prophylactic activity against coronaviruses (Al-Tawfiq et al., 2020; Cascella et al., 2020). According to the GISAID database (October 2021), the prevalence of RBD mutations in the population is significantly associated with enhanced binding affinity to ACE2. (Zahradník et al., 2021) demonstrated the in vitro examination of their high-affinity variant RBD-62, which may be employed to prevent SARS-CoV-2 infection, and α, β, and γ variants. When delivered before or after infection, RBD-62 dramatically decreased clinical disease in a hamster model of SARS-CoV-2 infection.

4.2.5. Antiviral drug therapies

Antiviral strategies to inhibit the sepsis caused by SARS-CoV-2 and the pathogenic outcomes of COVID-19 are a prerequisite to the pandemic-free century. For this reason, the antiviral therapies need to target not merely the viral replication but also bound the erythrogenic responses of the host. Being a part of the cap 'n' collar basic leucine zipper family of the transcription factors and erythroid-derived nuclear factor, NRF2 is currently recognized as a vital regulator of the inflammatory responses. Also, the element plays a significant role as a transcriptional repressor of the inflammatory genes in murine macrophages (Thimmulappa et al., 2006). However, Olagnier et al. (2020) have described that the expression of NRF2 dependent genes is repressed in biopsies from patients of COVID-19. Still, the treatment of cells with NRF2 antagonist, 4-octyl-itaconate (4-OI), and the scientifically accepted dimethyl fumarate (DMF) obstructs the replication of SARS-CoV-2 across the cell lines and induce IFN-dependent antiviral program. This strategy has also been observed in halting the pathogenesis of certain other viruses like Herpes Simplex Virus type-1 and-2, Vaccinia virus, and Zika virus through a type I interferon (IFN)-independent channel. Similarly, RNA-dependent RNA polymerase machinery allows SARS-CoV-2 to replicate inside the host cell and encode its viral 2′-O-methyltransferase, which catalyzes the methylation of the 5′-end cap to impede degradation of exoribonuclease. Recent studies have revealed drugs including antivirals, alkaloids, cardiac glycosides, and anti-cancers to inhibit the 2′-O-methyltransferase pathway to weaken SARS-CoV-2 infections and fabricate innovative therapeutic approaches (Paramasivam, 2020).

Recent clinical trials and studies recommend that Remdesivir (GS-5734) administration has the possibity to treat COVID-19 patients (Cascella et al., 2020). Remdesivir (GS-5734) is a phosphoramidate prodrug of 1′-cyano-substituted adenosine analog that demonstrates far-reaching antiviral potential against the RNA viruses (Siegel et al., 2017)). This wide-spectrum antiviral medication can restrain the activity of RNA-dependent polymerase (RdRp) (Sheahan et al., 2017). Previous studies have designated that nucleotide analogs generally reveal low efficacy against coronavirus due to the virus's exonuclease enzyme. Nevertheless, Remdesivir (RDV) was operative against SARS-CoV, MERS-CoV, and bat CoV strains (Kupferschmidt and Cohen, 2020), due to the presence of additional three nucleotides that proscribe expurgation by 3′-5′ exonucleases. Remdesivir as RDV-TP (Remdesivir-triphosphate) competes with natural counterpart ATP and integrates more competently than adenosine triphosphate (Gordon et al., 2020). Once Remdesivir is added into the nascent viral RNA chains, it will continue to extend three more nucleotides down to stop the strand formation. Recently, Biering et al. (2021) had studied seven antiviral compounds that were not previously reported to have activity against SARS-CoV-2. From these compounds it emerged that human RAD51 inhibitor, frequently known as B02 exhibits synergy with remdesivir. Biering et al. (2021) has evaluated seven antiviral drugs previously unknown to have action against SARS-CoV-2. From these compounds, it was determined that a human RAD51 inhibitor, commonly referred to as B02, synergizes with remdesivir.

Hence, the promising mechanism of action is overdue RNA chain termination. However, the venerable outcomes were achieved in the first sufferer of SARS-CoV-2 in the United States after seven days of intravenous administration of Remdesivir and preceding bilateral lower-lobe rales were vanished on day 8, making the drug operative for the treatment of the SARS-CoV-2 infections (Holshue et al., 2020). However, ongoing clinical trials and studies advocate the administration of Remdesivir (GS-5734) in the patient as it carries prophylactic activity against coronaviruses (Al-Tawfiq et al., 2020; Cascella et al., 2020). However, the drug is not yet licensed or approved anywhere globally because of its ability to augment liver enzyme production and ultimately recompense the liver (Ed, 2020). Recently, Hattori et al. (2021) have characterized compounds GRL-1720 and 5 h, carrying indoline and indole moiety to target the main protease (M^{pro}) of SARS-CoV-2 by making covalent bond and polar interactions with multiple active site amino acid residues of the main protease. The outcomes of VeroE6-cell-mediated assays with RNA-qPCR and immune-cytochemistry revealed the EC50 values of both compounds to be 15 ± 4 and 4.2 ± 0.7 μM for GRL-1720 and 5 h, respectively. However, further clinical studies have revealed that 5 h can synergize with Remdesivir to combat SARS-CoV-2.

Similarly, favipiravir (an inhibitor of the same enzyme used by Remdesivir) is in its infancy for clinical trials to treat COVID-19 infections (Delang et al., 2018). In the cells, favipiravir gets transformed into favipiravir-RTP (an activated phophoribosylated form), acting as a substrate by viral RdRp, ensuing the inhibition of RNA polymerase activity and subsequently stops viral replication (Furuta et al., 2017). Therefore, favipiravir may be a promising antiviral medication for SARS-CoV-2 than lopinavir/ritonavir (Dong et al., 2020). Moreover, darunavir is a second-generation HIV-I protease inhibitor and was effective against COVID-19 infections (Dong et al., 2020). SARS-CoV-2 utilizes type II transmembrane serine protease (TMPRSS2) to enter target cells. Inhibitor of TMPRSS2 (darunavir) may block the viral entry and thus organize a treatment option. Imatinib- BCR ABL kinase carries antiviral activity against coronavirus as it can constrain the binding of capsids with endosomal sheath to prevent its replication (Hoffmann et al., 2020). However, all these antiviral drugs have specific side effects too, and all are residing in line with clinical trials.

In recent times scientists have revealed Chloroquine- an approved malarial drug, as an effective medication for pneumonia caused by COVID-19 (Touret and Lamballerie, 2020). The proposed mechanisms of Chloroquine proved to be effective include the first mechanism encompasses the pH-dependent viral replication and viral glycoprotein along with host cell glycosylation prevention (Savarino et al., 2003). In addition, the second mechanism may target the obstruction of viral assembly in the Endoplasmic Reticulum-Golgi intermediate compartment (ERGIC) like structures (Hu et al., 2020). Furthermore, it is also plausible that chloroquine might induce certain effects in the host instead of unswerving viral action by mitigating the expression of pro inflammatory factors and receptors that can incite acute respiratory distress syndrome, which is primarily charged for coronavirus related mortality (Zhu et al., 2020a, 2020b). Therefore, to make chloroquine effective against COVID-19, research is required for its actual mode of action, Moreover, the exact dosage required by different age groups of people under different conditions of disease severity need to be studied properly.

Nevertheless, recent developments in *in silico* studies have made it

conceivable to practically segregate billions of complexes for their possible anti-viral and biological activity. This advancement has led Huang et al. (2021a, 2021b) to apply a biological-activity-based modeling (BABM) approach to foresee 311 compounds having high potential activity against SARS-CoV-2. Of these, 32 % compounds exhibited antiviral activity in a cell culture live virus assay by acting as viral entry inhibitors or autophagy modulators. In nutshell, these compounds cumulatively carry the potential to be further exploited into anti-SARS-CoV-2 therapies. Treatments for HIV and HCV have shown that a combination of antiviral medicines with diverse modes of action is the most effective (Simonis et al., 2021). This strategy is necessary in this scenario to avoid the emergence of antiviral resistance during prolonged therapy. Combining RDV with additional antiviral or immunomodulatory agents, on the other hand, may be a successful method for optimizing treatment results.

4.2.6. Blocking endocytic pathway

COVID-19 infections can also be prohibited by blocking the endocytic pathway exploited by viruses to gain entry inside a cell (Yang and Shen, 2020). This endocytic pathway is regulated through protein kinases 1 (AAK1). Thus, a disruption of kinase1 may intervene the viral passage into the cell and intrude with the intracellular assembly of virus particles. Nevertheless, the inhibitors used to bind the AAK1 enzyme appeared to have drastic effects on infected population. Moreover, cyclin G-associated kinase (another regulator of endocytosis) can be blocked by Janus kinase inhibitor baricitinib and can effectively treat SARS-CoV-2 patients (Richardson et al., 2020). However, the inhibitor has not yet been acknowledged for clinical manifestations.

4.2.7. Anti-parasitic agents

Another FDA-approved anti-parasitic agent-Ivermectin could be a promising treatment for COVID-19 infections. *In vitro* studies designate that a single dose of this drug can control viral replication within $24-48$ hours and lessen viral RNA to ~5000-fold in 48 h when administered to Vero–hSLAM cells infected with COVID-19. This drug works against broad spectrum viruses such as influenza, Zika, HIV, Dengue virus and can execute the COVID-19 infected cells within two days (Caly et al., 2020). The precise mechanism of Ivermectin against the virus is still anonymous, but it is hypothesized that it is due to the reticence of IMPα1/β1 mediated nuclear import of the viral proteins (Yang et al., 2020).

4.2.8. Targeted immunotherapy

Another practical approach to treat COVID-19 is the usage of targeted immunotherapy. Targeted immunotherapy is considered a paramount substitute for viral strains with narrow treatment windows and has developed resistance against multiple drugs. In order to regulate inflammatory response for SARS treatment, glucocorticoid was used in the year 2003. Apart from the viral pathogenicity, the body's inflammatory reaction also plays a critical role in SARS-induced lung injury cases (Li et al., 2020a, 2020b). For this reason, in pneumococcal cases of SARS-CoV-2, it is imperative to have a control mechanism of cytokine production and inflammatory response, as they are liable for the accretion of cells and fluids.

4.2.8.1. Mesenchymal stem cells (MSCs).
Recently, Mesenchymal stem cells (MSCs) of clinical-grade are employed to treat pneumonia instigated by SARS-CoV-2 intravenously. This treatment authorized the recovery of patients who exhibited less severe symptoms (Leng et al., 2020; Liang et al., 2020). Improvements in COVID-19 patients after MSC injection is due to various immunomodulatory effects (Atlur et al., 2020). The improvement in COVID-19 patients is regarded to the stout anti-inflammatory activity of MSCs. Such developments were embraced by an amplified numeral of peripheral lymphocytes, the decrease in the C-reactive protein, and waning of overactivated cytokine-secreting

immune cells (CXCR3 + CD4 + T cells, CXCR3 + CD8 + T cells, and CXCR3 + NK cells) by 3–6 days from the blood (Leng et al., 2020). Moreover, a consortium of CD14 + CD11c + CD11bmid regulatory dendritic cell (DC) population has been augmented after MSC treatment. In this way, MSC infusion might improve the body's immune response by stimulating B, T, and dendritic cells, which will play a vital role in treating patients from COVID-19.

4.2.9. CRISPR Technology as a novel treatment strategy for COVID-19

In addition to the traditional antiviral approaches entailed above, new therapeutic methodologies have recently been projected as a potential therapeutic alternative to the endless mayhem of COVID-19. Abbot et al. (2020) have recently developed CRISPR-Cas13 based assays to develop PAC-MAN technology by using different crRNAs to degrade RNA of multiple strains of SARS-CoV-2 and live influenza A virus in human epithelial cells. Strikingly the computational investigation exposed that six crRNAs could effectively target >90 % of all coronaviruses. From this study, it has been deduced that PAC-MAN technology can effectively target the replication mechanisms of SARS-CoV-2 strains, but the study needs to pass through biosafety clinical trials.

Similarly, Patchsung et al. (2020) have reported the clinically validated highly sensitive enzymatic reporter locking assay (SHERLOCK) by the biocatalyst Cas13a from *Leptotrichia wadei* along with reverse-transcription loop dependent isothermal amplification (RT-LAMP) for the discovery of SARS-CoV-2. The reported assay is proven acquiescent to multiplexed detection strategies in a single-lateral flow strip with internal check of ribonuclease contamination. This detection strategy has the potential to facilitate the SARS-CoV-2 diagnosis in sites with inadequate capitals.

5. Conclusion and future perspective

The sudden advent of the SARS-CoV-2 pandemic has brought widespread fright and has endangered the global community. The phylogenetic analysis indicate that the virus has bat and pangolin as their immediate hosts. However, as per the information available, it can be specified that SARS-CoV2 is either a result of zoonotic outbreak or an accidental release of a laboratory strain. It's critical to solve this problem, as it mainly affects the risk/benefit stability of our interactions with that of ecosystems, intensive breeding of wild and domestic animals, and scientific policy and biosafety rules. In any case, considering the evolution of the molecular pathways participated in the genesis of pandemic viruses is important for evolving vaccination and treatment methods to avert future zoonotic diseases

In addition, the rising of analogous strains of SARS-CoV-2, circulating around the globe with consistent point mutations in different human beings making this virus challenging to treat. Therefore, designing a suitable therapeutic agent against various strains of SARS-CoV-2 needs to contemplate certain factors. Instead of various therapeutic approaches, i.e. antiviral drugs, MSCs etc., we must design a universal vaccine against similar and conserved proteins of SARS-CoV, MERS CoV SARS-CoV-2, and all other human coronaviruses to prevent its future outbreak. In this case, conserved S proteins and other accessory and RBD proteins are a potent source to develop its broad-spectrum vaccine. Once vaccines became available, administration was hampered by low initial supply, inefficient vaccine delivery, and widespread vaccine reluctance (Creech et al., 2021). These impediments hampered the capacity to vaccinate a sufficiently large proportion of the population to achieve some form of population immunity. Apart from the United States, low- and middle-income countries have failed to secure even the bare minimum of vaccination doses.

The COVID-19 vaccine development is on its way, but other emergent strains, both existing and yet to emerge, pose a threat to vaccine effectiveness. For instance, another highly virulent SARS-CoV-2 variant (SouthAfricaV501.V2 clade), for example, has been documented in

South Africa and, like B.1.1.7, it seems to be spreading faster than other strains. This lineage has now become the prevailing strain, and it, too, has been mutated in several areas of the viral spike protein, like a group of Brazilian (B 1.1.28) variants that are now dominant in Amazonas state (Oosterhout et al., 2021). The fear is that mutation-induced variation will result in vaccine-resistant strains. Vaccine escaped mutants may be favoured during long-term infections in patients with weakened immune systems and longer transmission chains. Such conditions increase the input of new mutations as well as the time it takes for natural selection to act on this novel variation. Moreover, the increased persistence of mutations in major areas of the spike protein in the vaccine breakthrough group is of concern and necessitates further investigation. Therefore, in order to achieve higher case numbers and the inclusion of different VOCs and VOIs, genomic surveillance of vaccine breakthrough cases should really be conducted on a large scale throughout the world.

Sometimes, SARS-CoV-2 infected individuals remain asymptomatic due to inadequate longitudinal follow up. Even in the context of vaccination, protracted efforts at pandemic control may be required if proactive strategies to detect asymptomatic infections, such as quick contact tracing, are not in place. Along with universal vaccines, miRNA-based therapeutic approaches need to be concerned as miRNAs have the potential to evolve fast and target new mRNA transcripts. Recently, El-Nabi et al. (2020) have hypothesized an approach concerning miRNAs to target ORF and UTRs of SARS-CoV-2 to make a targeted delivery system possible. But this hypothesis has not yet undergone clinical trials. Despite targeting a single gene, a combinatorial strategy to target multiple genes of broad-ranged SARS-CoV-2 strains may effectively combat the pandemic, and it can also prevent viral resistance against miRNA.

Since the CRISPR-based antiviral therapeutics hold a great potential to treat COVID-19, the rationale for successful antiviral CRISPR therapies needs to be done. However, it can only be achieved through an in vitro study. The existence of pre-clinical and clinical data to manifest the safety of the technology is still insufficient. Moreover, the delivery of CRISPR components into patients is major hurdle that obstructs the clinical applications of this technique in current pandemic. In nutshell, the need of an hour is to develop a safe and effective therapeutic strategy to deal with the havoc of COVID-19 that can only be achieved by the appropriate experimental analysis of current techniques.

Compliance with ethical Standards/Ethical statement

The authors declare that they have no conflict of interest. The authors assure the integrity and quality of work. It is also stated that there is no plagiarism in this work and all points taken from other authors are well cited in the text. This study is completely independent and impartial. This article does not contain any studies conducted on human or animal subjects. This work received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Declaration of Competing Interest

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