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Review

COVID-19: A review of newly formed viral clades, pathophysiology, therapeutic strategies and current vaccination tasks

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ABSTRACT

Today, the world population is facing an existential threat by an invisible enemy known as severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) or COVID-19. It is highly contagious and has infected a larger fraction of human population across the globe on various routes of transmission. The detailed knowledge of the SARS-CoV-2 structure and clinical aspects offers an important insight into the evolution of infection, disease progression and helps in executing the different therapies effectively. Herein, we have discussed in detail about the genome structure of SARS-CoV-2 and its role in the proteomic rational spread of different muted species and pathogenesis in infecting the host cells. The mechanisms behind the viral outbreak and its immune response, the availability of existing diagnostics techniques, the treatment efficacy of repurposed drugs and the emerging vaccine trials for the SARS-CoV-2 outbreak also have been highlighted. Furthermore, the possible antiviral effects of various herbal products and their extracted molecules in inhibiting SARS-CoV-2 replication and cellular entry are also reported. Finally, we conclude our opinion on current challenges involved in the drug development, bulk production of drug/vaccines and their storage requirements, logistical procedures and limitations related to dosage trials for larger population.

1. Introduction

Coronavirus (CoV) is a major pathogen predominantly affecting the human respiratory system [1]. The Latin etymology of the term corona hails from its crown form [2]. The CoVs belong to the coronavirinae subfamily and are categorized into four genera based on genomic architecture and evolutionary relationships: (i) alphacoronavirus (αCoV), (ii) betacoronavirus (βCoV), (iii) gammacoronavirus (γCoV) and (iv) deltacoronavirus (δCoV) [3]. Among the six human-infecting CoVs, 229E and NL63 come under the α-CoV genus, whereas, HKU1, OC43, Severe Acute Respiratory Syndrome-related CoV (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are classified under the β-CoV genus. Each genus is then subdivided into 4 lineage subgroups: A, B, C, and D [4]. In the end of 2019, many patients had been severely affected with pneumonia by the causative pathogen known as SARS-CoV-2 in Wuhan, China. Upon this taxonomy background, phylogeny, and existing practice, the CoV Research Group of the International Committee on Virus Taxonomy officially announced that this virus is related to extreme SARS-CoV and called it as 'SARS-CoV-2', which falls under the B type lineage [5]. The spherical/pleomorphic-shaped SARS-CoV-2 is about 80–120 nm in size and is classified under the order of *Nidovirales*. The coiled nucleocapsid of SARS-CoV-2 is formed by complexing a single-stranded positive-sense genomic RNA (+ssRNA) with a protective helical nucleocapsid (N) protein shell. Further, it is encased by three fundamental proteins: spike (S), matrix (M) membrane and a highly hydrophobic small envelope (E) protein [6], as shown in Fig. 1. Most of the CoVs family cause pathogenic enzootic infections that affect humans, birds and many animals, and often chronically instigate enteric, respiratory, and neuronal diseases [7–11]. The World Health Organization (WHO) has announced SARS-CoV-2 as most highly pathogenic human CoVs when compared to SARS-CoV and MERS-CoV [12]. By 17th October 2021, the total number of infected cases of SARS-CoV-2 worldwide was reported to be as 241,170,384 including 4,910,066 deaths, in major countries such as the United State

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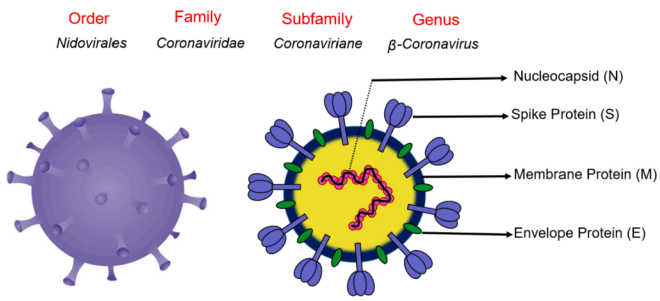

Fig. 1. Diagrammatic depictions reveal the SARS-CoV-2 virion structure and morphology.

of America, India, Brazil, United Kingdom, Russia, Turkey, France, Iran, Argentina, and Spain. It has spread to >216 countries worldwide and declared as 'pandemic' [13], as shown in Fig. 2. The major symptoms of SARS-CoV-2 infection include cough, loss of appetite, high fever, headache, breath shortness, vomiting, dyspnea, sore throat, rhinorrhea, diarrhea and abdominal pain. The infected patients also displayed increased numbers in leukocytes, plasma pro-inflammatory cytokines and abnormal respiratory findings [14–16]. A communicable disease, SARS-CoV-2 is spreading from human to human through two main routes: virion-laden in respiratory fluid droplets (less than 1–2000 μm) arising from infected coughs or sneezes, and also from coming in contact with virus-contaminated surfaces (e.g. skin-to-skin and touching infected objects and surfaces). The infection is likely to spread in crowds and in areas where people are within 1–2 m, [17–19] as shown in Fig. 3. Another possible transmission mode is through infected fecal matter due to the virus's ability to survive in stool samples for 4 days [20]. The disease severity in patients with SARS-CoV-2 is based on chronic conditions, including coronary heart disease, diabetes and hypertension [21]. The incubation time duration is estimated to be between 4 and 14 days before the onset of the disease. Recent studies suggest that certain patients with SARS-CoV-2 display damage to other organs such as the brain (encephalitis), kidney, heart and eye (conjunctivitis) [22]. The SARS-CoV-2 can live for extended periods on the exterior of sterile sponges, aluminum and sterile surgical gloves, which increases the risk of transmission through contact. These components can help as a defense matrix for the SARS-CoV-2 virions, making therapies for sanitization inefficient or exploited for enhanced inactivation [23,24]. The enveloped virus of SARS-CoV-2 inserts its nucleic acid into host epithelial cells and keeps its lipid bilayer intact throughout pathways for its infectivity. Besides, the enveloped virus has a dynamic protein capsid that satisfactorily confines the elastically-strained genomic coil, sustains osmotic pressure in the surroundings and readily disassemble to release their genome into the host cell [25]. Physical therapies such as desiccation, heating, UV irradiation and chemical decontamination with different agents (e.g. alcohols, oxidants, acids and few specialized surfactants) can induce the rupture in SARS-CoV-2 envelope and capsid. They can be highly successful in lowering or even stopping the virus's

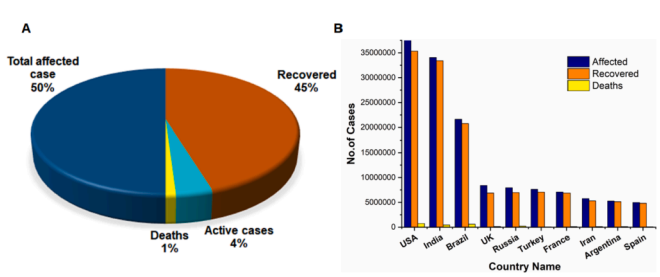

Fig. 2. Statistical reports of the SARS-CoV-2 pandemic. (A) Worldwide report of SARS-CoV-2 infection and (B) topmost leading countries with SARS-CoV-2 infection as on October 17, 2021.

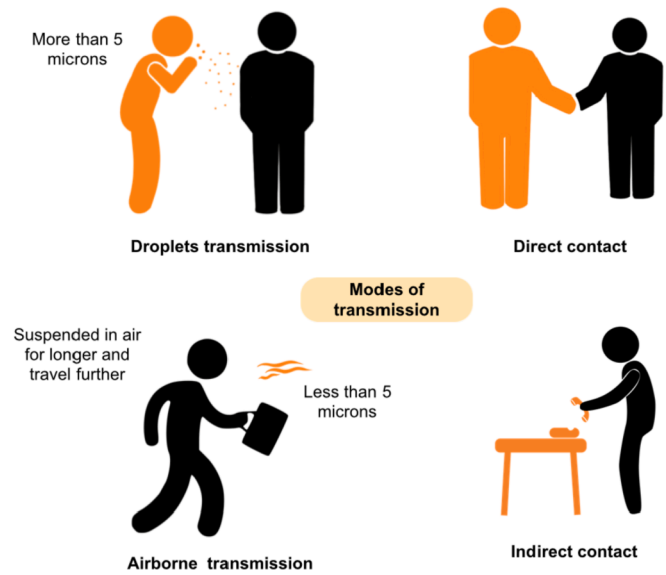

Fig. 3. Key routes of human-human transmission of SARS-CoV-2.

propagation and transmission [26–30]. It is worth noting that the severity of pathogenic SARS-CoV-2 was profoundly underestimated by society at the outset of the viral outbreak. As a result, SARS-CoV-2 has caused tremendous social harm, brought global fear, medium mortality rates and high transmission than other deadly viral outbreaks. Hence, the current state of affairs may be different, and have shown modified methodologies to deal with these kind of viruses. By using various molecular medicine based tools and techniques, the research communities from various countries have developed host-based and virus-based targets/vaccines to fight SARS-CoV-2 infections. In this review, we have comprehensively summarized the physiochemical features, mutation, clades, and infectivity of SARS-CoV-2. Further, the key performances of the innate immune system towards the viral infection, possible therapeutic targets with activity rely on host/virus and diagnostics methods have been discussed in detail. The recent progress in the development of drugs, approved vaccines in multiple phases of clinical trials across the countries and possibilities of herbal production have also been highlighted. The discussion on vaccine hesitancy, list of approved vaccines and global vaccine distribution plans (Covax) has been incorporated into this review to provide a complete information about the SARS-CoV-2.

2. Genome and structure of SARS-CoV-2

2.1. SARS-CoV-2 genome structure

A SARS-CoV-2 genome +ssRNA composed of 29,891 nucleotides, 11 protein-coding genes, 38% of the guanine-cytosine (GC) content and encodes 9860 amino acids (aa). The sequenced SARS-CoV-2 has 95% of the same genomic structure as SARS-CoV, identified in a cave from Yunnan Province, China, in 2013 [31]. The 5′-3′ gene lineup noted as 5′-replicase (open reading frame (ORF1/ab))-structural proteins [S, E, M and N] −3′ untranslated regions (UTRs) poly (A) tail, is shown in Fig. 4. The length of 3′ and 5′-UTRs contains 358 and 265 nucleotides, respectively [32]. At the 3′ end, the six accessory genes (3a, 6, 7a, 7b, 8, and 9a) are scattered among the structural genes, in which some genes play a vital role in SARS-CoV-2 pathogenesis. At 5′ terminal, a region of approximately 20 kb coincides with two ORFs (ORF1a and ORF1b), in which ORF1a formulates a huge polyprotein (pp1ab, about 790 kDa). Then, two proteases such as 3C-like protease or main protease (3CLpro) and papain-like protease (PLpro) cleave the pp1ab and playing a key role in maturing them into non-structural proteins (nsps). The dimeric 3CLpro consists of two parts, namely intracellular N-terminal catalytic

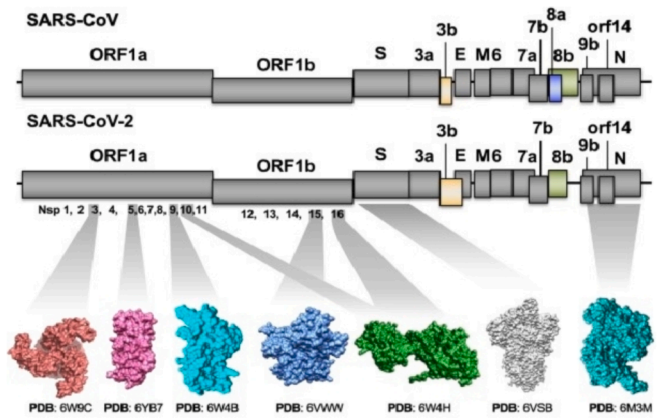

Fig. 4. Genomic distribution profile of SARS-CoV and SARS-CoV-2. (Adapted and reprinted with permission from ref. [34]), copyrights © 2020 Cell press.

and the extracellular C-terminal domains in each of their monomers. The similarity of 3CLpro sequence between SARS-CoV and newly emerged SARS-CoV-2 was noticed to be 96%. However, the minimal differences noted were only observed on their surface proteins [33–36]. PLpro enzymes consist of 4 distinct domains: a single SARS-unique (SUD), an ADP-ribose-1d-phosphatase (ADRP), a transmembrane (TM), and a fold-like ubiquitin (UB1) domain [37]. The genomic RNA consists of a well-organized ribosomal frameshift signal, in which both heptanucleotide "slip site" (slippery sequence) and RNA pseudoknot structure are arranged one after another. The slippery sequence occasionally causes a frameshift at the end of ORF1a and consecutively translate them into a joint ORF1a and 1b polyprotein [38,39]. Notably, SARS-CoV-2 pp1ab encompasses 14 exact proteolytic cleavage sites for PLpro and 3CLpro. While PLpro can cleave the N-terminus of amino acid (aa) sequences at three sites, namely 181–182, 818–819 and 2763–2764 aa, 3CLpro cleaves C-terminus of aa sequence at 11 different sites. After completion of cleavage process, the formed 15 nsps play a pivotal role in host cell infection and RNA synthesis. Besides viral proteins, the disparate cellular proteins of the host such as poly(A)-binding protein, mitochondrial aconitase, heterogeneous nuclear ribonucleoprotein A1 and polypyrimidine-track-binding (PTB) protein are also interacting with the critical cis-acting elements of SARS-CoV-2 replication [40,41].

2.2. Structural and accessory proteins of SARS-CoV-2

The SARS-CoV-2 structural proteins, named as S, E, M, and N proteins are produced by ORF-2, ORF-4, ORF-5 and ORF-9, respectively (Table 1) [62]. These structural proteins share a huge sequence closeness to the same protein that present in SARS-CoV and MERS-CoV [63]. The exterior of the SARS-CoV-2 virion is guarded by the trimeric S glycoprotein (180/90-kDa) shaped as club-like projections. It has a diverging sequence with similarity of <75% to other SARS-related CoVs [64]. S protein is frequently formed by two subunits S1 and S2, which are crucial for viral entry as they bind to angiotensin-converting enzyme 2 (ACE2) receptors on the host cell plasma membrane surface [65]. The S1 subunit receptor-binding domains (RBD) undergoes transient hinge-like conformational motions (receptor-inaccessible states/receptor-accessible) with the host-cell ACE2 receptor [66,67]. The attraction between the S protein RBD (394 glutamine residues) and ACE2 receptor (31 lysine residues) creates a "Van der Waals" forces that reinforce a binding between viral-host cell [68–70].

The most bountiful structural constituent of the viral envelope is the M glycoprotein produced by the M gene, which contains 222 aa (23 kDa). It extends the membrane bilayer three times, flees a concise NH_2-terminal domain on the lateral side of the virus, and a long COOH terminus (cytoplasmic domain) on indoors of the virion [71]. It delineates the shape of the viral envelope by forming the homotypic interaction

Table 1
The functions of structural and assessory proteins in SARS-CoV-2.

Protein	Genes coding	Functions	References
Spike (S) protein	ORF-2	Viral entry, binding with host receptor and membrane fusion, main component for giving the virus crown like structure.	[42–45]
Envelope (E) protein	ORF-6	Virion entry, viral mutation and viral production	[46]
Membrane (M) glycoprotein	ORF-7	Leaving a short NH_2 and a long COOH terminus on outside and indoor of virus (cytoplasmic domain), attach with other structural proteins and determine the shape of viral envelope.	[47,48]
Nucleocapsid (N) protein	ORF-8a	Viral assembly, viral replication cycle and viral-host cellular infection	[49,50]
pp1a	ORF-1a	Express the virus exploit a slippery sequence as 5′-UUUAAAC-3′, contain the nsps 1–11	[51]
pp1ab	ORF-1b	Express the virus exploit a slippery sequence as 5′-UUUAAAC-3′, contain the nsps 1–16	[51]
7a	ORF-7a	Increases the expression of JNK, NF-κβ, and p38 MAP kinase. It involves in cell cycle arrest, inhibits host translation and stimulate apoptosis.	[52]
3a	ORF-3a	Increases the expression of JNK, IL-8, NF-κβ, and RANTES. It involves in cell cycle arrest, ion-channel activity and stimulate apoptosis.	[52]
3b	ORF-3b	Stimulate type-I IFN production, and prevents cell signaling, cell cycle arrest and induces apoptosis.	[52]
Region of nsp1coding	nsp1	Antiviral host response and trigger mRNA degradation	[50,51]
Unknown	nsp2	Unknown function and often bind to prohibiting proteins	[51]
Papain-like protease	nsp3	Involves in viral polyprotein cleavage. It Interacts with N protein and can block the host immune response.	[52]
Transmembrane domain	nsp4	DMV formation and combines with nsp3 and nsp6. It also involves in proliferation of cellular membrane and more important for DMVs structure.	[52,53]
3CLpro	nsp5	Involves in viral polyprotein cleavage.	[53]
Transmembrane domain	nsp6	DMV formation and complex with nsp3 and nsp4.	[54]
Unknown	nsp7	Primase, hexadecameric complex with nsp8	[52,55]
Primase	nsp8	Primase activity, hexadecameric complex with nsp7	[55,56]
Unknown	nsp9	Activity of RNA/DNA binding	[57]

(continued on next page)

Table 1 (*continued*)

Protein	Genes coding	Functions	References
Unknown	nsp10	complex with nsp14, proliferation of cellular membrane and maintaining the activity of 2′-O-MTase activity	[52,58]
Unknown	nsp11	short peptide at ORF-1a end	[54]
RNA dependent RNA polymerase	nsp12	Viral replication	[54,59]
Superfamily 1- helicase	nsp13	Helicase, viral replication, virulence and tropism affection	[50,60]
3′–5′- Exonuclease	nsp14	Exonuclease 3′–5′ activity and viral replication	[50,60]
N7-methyltransferase Nidoviral endoribonuclease specific for U	nsp15	Viral replication and poly (U)-specific endoribonuclease	[61]
S-adenosylmethinonine dependent nbose 2′ - O-methyltransferase	nsp16	Viral replication, 2'-O-ribose methyltransferase inhibition and IFN antagonism	[60,61]

JNK-c - Jun N-terminal kinases, **DMV**-Double-membrane vesicle, **NF-κB**-Nuclear factor-κB, **IL-8**- Interleukin 8, **RANTES**-Regulated on Activation, Normal T Cell Expressed and Secreted, **IFN**-Interferon.

between them. It often interacts with the envelope E protein present in the host cell membrane budding compartment, produces the viral envelope and accountable for constructing virus-like particles (VLPs) [72,73]. During the assembly and budding process, M protein is performing a dominant aspect in retaining the S protein by its interaction. It interacts with N protein, assists in RNA packaging and functions in viral immunoevasion [74,75]. The envelope (E) protein is a smallest single-pass type III transmembrane protein (8 kDa), consists of approximately 76–109 aa that are prevailing in tiny amounts on the viral envelope [76], as shown in Fig. 5. The E protein is an exceedingly perpetuate protein across β-CoV as only 3 variants have been raised until now. Its homopentamer facet supports virus assembly, budding, envelope formation and pathogenesis [77–79]. It also serves as viroporins that self-assemble into the host membrane, emerging pentameric protein-lipid pores to participate in ion transport and the apoptosis induction by persuading the pore formation of the host cell membrane [80]. While an extensive amount of E protein is let out during the viral replication process, only a minimal amount is unified into the viral envelope. However, much of the protein is found at the site of intercellular trafficking [81].

The N protein (50–60 kDa) are phosphoproteins that interact with viral genetic material in a beads-on-a-string pattern, producing the helically symmetric nucleocapsid (Fig. 6) [83]. During the replication and transcription process, the N-terminal domain (NTD) of N protein interacts with nsp3 of replicase-transcriptase complex (RTC) to tether with genomic RNA, regulating the viral RNA synthesis and altering the metabolism in infected host [84,85]. After translation, a helical

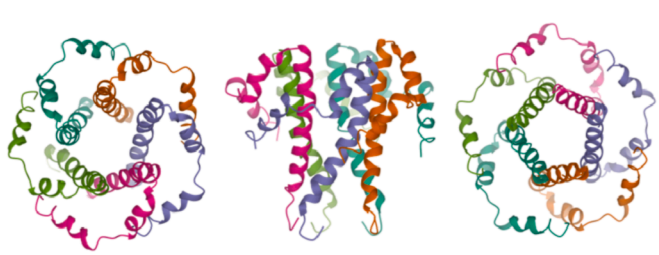

Fig. 5. The structure of E protein pentameric ion channel predicted by nuclear magnetic resonance (NMR) spectroscopy (Adapted, redrawn and reprinted with permission from ref. [82], copyrights © 2018 Elsevier).

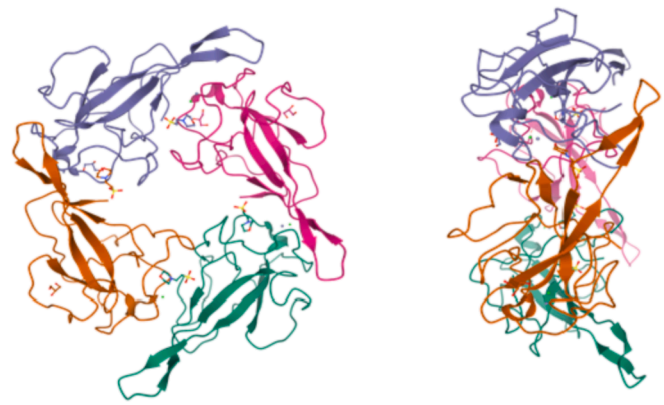

Fig. 6. The Structure of N protein predicted by NMR (Adapted, redrawn and reprinted with permission from ref. [87], copyrights © 2020 Elsevier).

ribonucleocapsid (RNP) plays a prominent role in virion assembly [86].

In addition to these four proteins, SARS-CoV-2 encodes at least six or more accessory proteins (3a, 6, 7a, 7b, 8b, and 9b) that are produced by ORF-3a, ORF-6, ORF-7a, ORF-7b, ORF-8 and ORF-10, respectively. Notably, all of them are translated from the subgenomic (sg) RNA [88]. The ORF-3a is an ion channel protein, triggers pattern recognition receptor (PRRs) superfamily members by interacting with apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC)-TNF-receptor-associated factor (TRAF3)-dependent ubiquitination. This contributes to viral spreading and infection [89]. Using a yeast two-hybrid system, ORF-6 associates with nsp8 and promoting RNA polymerase activity [90,91]. Besides, ORF-7a, a type I transmembrane protein situated within the endoplasmic reticulum (ER) and Golgi network, is linked to the trafficking of the protein. Both ORF-6 and ORF-7 show protruding performance in SARS-CoV-2 pathogenesis [92]. While SARS-CoV has two ORF-8 proteins (ORF-8a and ORF-8b), SARS-CoV-2 has only a single ORF-8 protein with 366 nucleotides that encodes a protein with 121 aa [93]. A recent report has shown that ORF-8 interacts with ORF-6 and N protein, whereby it serves as a possible type I IFN signaling pathway inhibitors. IFN is the chief component of the host innate immune system protecting the host from the viral infection [94]. ORF-10 is the tiny accessory protein that consists of a 38-residue peptide with the highest number of immunogenic epitopes that could alter SARS-CoV-2 pathogenicity [95].

3. Mutation and clades of SARS-CoV-2

3.1. Mutations of SARS-CoV-2

Insights into the entire genome sequence of reference (Wuhan genome) and mutated SARS-CoV-2 species will succor in prospective vaccine and drug development. In general, RNA viruses, except for *Nidoviruses*, are susceptible to random mutations due to the scarcity of exonuclease proofreading activity of the virus-encoded RNA polymerases [96]. The first complete SARS-CoV-2 genome sequence was available on January 5, 2020, in the National Center for Biotechnology Information (NCBI) Genbank, and thousands of genomes have been sequenced till date [97]. The Global Initiative on Sharing All Influenza Data (GISAID) has made available those genomic data shared from all over the world in its database. There are currently about 4,383,873 million SARS-CoV-2 genome sequences (as of 16th October 2021) available on the GISAID data base (https://www.gisaid.org/hcov19-variants/). It has been useful to detect the viral mutations and track the virus movement around the world. SARS-CoV-2 mutations are caused due to errors like base substitution, insertion or deletion during the replication of genome [98], causing changes in viral characteristics and virulence. Hence they need to be studied for designing new

vaccines, antiviral drugs and diagnostic assays as they could be used to determine changes in the viral immune escape, drug resistance and pathogenesis associated mechanisms. The first SARS-CoV-2 genome has about 80% sequence congruence with that of SARS-CoV, and it has undergone more than 10,000 documented single mutations announced on January 5, 2020 [99,100]. Nevertheless, noted with SARS-CoV, SARS-CoV-2 S glycoprotein has 725 mutations over its 1255 residues, and their sequence closeness is only 76%. Among 725 mutations, 89 were on the RBD, which has 194 aa residues, proposing that the RBD is a concern to higher mutations [101]. RBD pivotal aa (5 out of 6) were disparate between SARS-CoV and newly emerged SARS-CoV-2, hence, it has a robust binding affinity to ACE2 than SARS-CoV [102]. The existence of S protein with plenty of mutations suggests the infectivity alteration of SARS-CoV-2 subtypes. It revealed that the existing mutations trigger the SARS-CoV-2 infectivity alteration and predict the future infection tendency. A recent report by Koyama et al. described that 10,022 SARS-CoV-2 samples were acquired from 68 countries, including the USA, UK, Australia and Netherland. Among them, 5775 distinct genome variants were noted, which comprises a total number of 2969 missense mutations, 142 non-coding deletions, 484 mutations in the non-coding regions, 1965 synonymous mutations, 66 non-coding insertions, 11 frameshift deletions, 36 stop-gained variants, 100 in-frame deletions and 2 in-frame insertions. It demonstrates the diverse in genomic variants involving structural transmission with the prospect of several debuts into the population [103]. One more bulletin from Mercatelli et al. divulged that the acquired 48,635 SARS-CoV-2 samples worldwide have a total of 3,53,341 mutations than the Wuhan reference genome (NC_045512.2). Notably, 48,379 samples consist of at least one mutation and an average of 7.23 mutations per sample. It is worth noting that few samples had more than 15 mutation occurrences. Notably, C > T transition, A > G transition and G > T transversions were estimated to be 55.1, 14.8 and 12%, respectively. Among them, A>G transition is most widespread in Europe, Africa and the America while G>T transversion is the second leading occurrence in Oceania and Asia [104]. One mutation event in the gene encoding of spike protein at the 614th position in the amino aspartic acid (D) is substituted by acid glycine (G), which is most widespread and named as D614G. The D614G was first noticed in Germany and China, eventually spread to all of Europe, Canada, USA, Australia and India [105]. Spike proteins are made of three smaller peptides arranged in open and closed orientations. When more peptides are in open orientations, they are easily accessible for binding of host proteins, as shown in Fig. 7. The D614G mutation incites a more transmissible form of SARS-CoV-2 by changing a single amino acid in the viral RNA code, which relaxes the connection between peptides and results in open conformations to increase the chances of infection [106].

3.2. Clades of SARS-CoV-2

A clade is a name for a group of a virus with genetic variations. It is also named as subtypes, genotypes or groups that all arise from a common ancestor [107]. SARS-CoV-2 is itself a clade inside the family *Coronaviridae* and the genus *betacoronavirus*. SARS-CoV-2 genome has changed by numerous mutations in the past few months, as they moved across the globe. A recent report displayed 11 major mutation occurrences, which are defined in five major clades according to its respective amino acid mutations: D392 (ORF1ab, G392D), S_{84} (ORF8, L84S), L_{378} (ORF1ab, V_{378}I), V251 (ORF3a, G251V) and G_{614} (S, D_{614}G). The percentage of individual clades is as follows: clade G_{614} (71.14%), S_{84} (10.85%), V_{251} (7.66%), D_{392} (1.03%) and L_{378} (1.70%) of all the sequenced viral genome.

3.2.1. Clade D614G

The clade G_{614} has been widespread globally with the non-coding variant 241C > T along with 3037C > T and ORF1ab P_{4715}L [108]. The most prevalent mutation of transversion altered $23{,}403^{rd}$ nucleotide

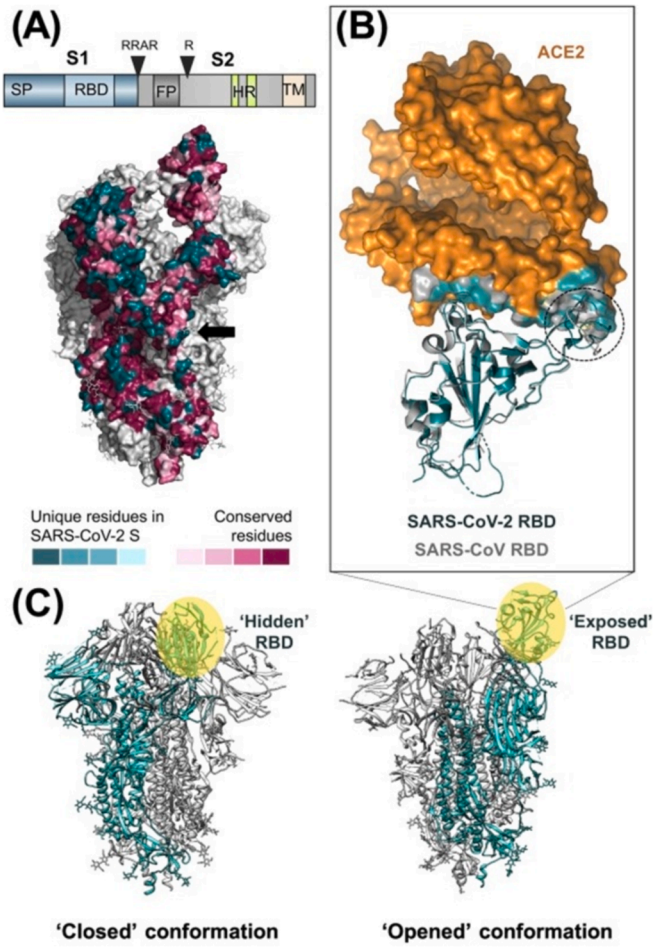

Fig. 7. (A-B) Structural representation of the smaller peptides in a spike protein RBD domain occurs as 'open' or 'closed' orientations (C) (Adapted and reprinted with permission from ref. [34], copyrights © 2020 Cell press).

adenosine into guanosine (A_{23403}G) in the spike protein of SARS-CoV-2 genome G-clade, which is widespread in Europe, Oceania, South America and Africa. The clade G and its two derivative GH and GR having four mutations, namely C_{3037}T, C_{241}T, A_{2340}3G and C_{14408}T. The ORF3a further characterizes GH's derived Q_{57}H mutation and GR having an N gene trinucleotide mutation, named RG_{203}KR mutation [109]. The G clade and its offspring (GH and GR) are most familiar worldwide and accounting for 74% among the sequenced SARS-CoV-2 genome [110]. Phylogenetic studies displayed that the mutations in ORF8:L_{84}S and ORF3a: G_{251}V regions of SARS-CoV-2 are associated with the formation of new clades S and V, respectively. The nsp6: L_{37}F mutation is also occasionally observed in combination with the above mentioned clades. While G and GR clades notably exist in Europe, S and GH clades have been mostly widespread across the globe [111,112]. The clade L was the first SARS-CoV-2 type (reference genome NC_045512.2) to appear in Wuhan in 2019 December. It mutated into clade S in the following month, and it gave rise to clade V around mid-January 2020. The clade G also appeared around the same period. By the end of February 2020, clade G gave rise to GR and GH clades and started to spread across the globe. Notably, L and V clades have gradually disappeared over the same time. Similarly, the clade S is also declining but still could be seen in US and Spain [104]. Apart from these clades, few infrequent mutations are also seen, and grouped under clade O. The characteristics of each clade have been given in the table along with their names based on different nomenclature systems [Table 2]. Despite these mutations, SARS-CoV-2 has limited variability; hence the virus structure remains the same even with different variants. The average

Table 2
Year-Letter Nomenclature names clades that persists for at least few months with important SARS-CoV-2 geographic spread.

GISAID Clade	Year Number	PANGOLIN Lineage	Genomic Coordinate	Effect on Protein sequence	Class	Effect on protein	References
L	19A	B	NC_045512.2 Wuhan reference genome				[97]
S	19B	A	C8782T	S76S	Silent SNP	nsp 4	[104]
			T28144C	L84S	aa-changing SNP	ORF8 protein	
V	19A	B.2	G11083T	L37F	aa-changing SNP	nsp 6 (transmembrane protein)	[137]
			G26144T	G251V	aa-changing SNP	ORF3a protein	
G	20A	B.1	A23403G	D614G	aa-changing SNP	Spike protein	[138]
			C14408T	P314L	aa-changing SNP	nsp12, post-ribosomal frameshift (RNA-dependent RNA polymerase)	
			C3037T	F106F	Silent SNP	nsp 3 (predicted phosphoesterase)	
			C241T	–	5' UTR SNP	5' Untranslated Region	
GH	20C	B.1.*	G25563T	Q57H	aa-changing SNP	ORF3a protein	[137]
			In addition to the 4 mutations of clade G				
GR	20B	B.1.1	GGG28881AAC	RG203KR	aa-changing SNP triplet	Nucleocapsid protein	[139]
			In addition to the 4 mutations of clade G				

pairwise difference between any two genomes is 9.6 single nucleotide polymorphisms (SNPs), which show that SARS-CoV-2 has attained only a moderate genetic diversity [113]. This also means that vaccines developed or currently under development have a high chance of succeeding the spread of the virus. There are two commonly used nomenclature systems called Year-Letter nomenclature and PANGOLIN (Phylogenetic Assignment of Named Global Outbreak LINeages) nomenclature. Year-Letter nomenclature names clades that persist for at least several months with a significant geographic spread. Their frequency should exceed 20% in a global sample with a variation in at-least 2 positions compared to its parent clade. The clades such as 19A, 19B, 20A, 20B, and 20C are the currently named clades in this system (https://nextstrain.org/blog/2020-06-02-SARSCoV2-clade-naming). PANGOLIN lineages offer elaborated outbreak cluster information, whereas other two nomenclatures served a huge scale entire view of clade trends [114].

3.2.2. B.1.1.7 lineage in UK

Given the fact that the replication of SARS-CoV-2 to produce more of their copies inside the host cells results in mistakes and generates an erroneous reproduction of its genetic material termed as mutation [109]. The genetic variant assessment in SARS-CoV-2 provides an expanding knowledge on new clade or lineage formation and adaptation to contain its outbreak. Similar like other RNA viruses getting mutated over a time, the fundamental aspects of viral biology reveal different mutations of SARS-CoV-2. The recent estimates specify that the nucleotide mutations are acquired by circulating SARS-CoV-2 lineages at a rate of about 1–2 mutations per month [115]. In this context, the SARS-CoV-2 Genomics UK (COG-UK) consortium surveillance dataset identified and reported a distinct phylogenetic cluster to the WHO on December 14, 2020. After the genomic sequencing, it is termed as VUI 202012/01 (Variant Under Investigation) or "B.1.1.7 lineage" [116]. The B.1.1.7 refers to a mutation in the SARS-CoV-2 RNA that renders the virus more contagious up to 70% with a vast number of genetic variations in the S protein. The B.1.1.7 RNA differs from oldest RNA by 23 mutations with at least 8 lying in the RNA portion corresponding to the S protein [117]. Notably, two deletions differ in new B.1.1.7 when compared to previously documented mutations and is significantly reducing the sensitivity of human antibodies against SARS-CoV-2. The 23 specific deletions/insertion of the reference Wuhan SARS-CoV-2 virus altered into the B.1.1.7 S variant, includes 8 spike protein mutations associated with high infectivity. These deletions may also be accompanying with diverse mutations in the CoV S protein binding region, including those stated in farmed mink infections and mutations that have been exposed to play a crucial role in the virus's ability to evade human immune systems. A truncated ORF8 gene is also present in B.1.1.7 and the deletions in this region is associated with a decreased severity of the disease as per previous observations [118]. As a result, it is most prominant to determine the functional effects of these mutations and deletions, especially the combination that is present in B.1.1.7. As the number of cases and regions that documented the infections with B.1.1.7 is rising with each passing day, a detailed investigation on number of mutations, the cause for this particular variant and the different biological assets of mutation candidates is urgently needed [119]. Two earliest sampled genomes belonging to the B.1.1.7 lineage were obtained in Kent on 20-Sept-2020 and Greater London on 21-Sept-2020. Since then, the infections of B.1.1.7 are on the rise in the UK and it has 1623 genomes as of 15 December 2020. Of all these, 519 samples from Greater London, 555 in Kent, 545 in other parts of the United Kingdom (including Scotland and Wales) and 4 in other nations have shown B.1.1.7 infections. Based on epidemiological and genetic studies, several research teams have concluded that B.1.1.7 is spreading more efficiently than other strains [117]. One of the most notable modifications in VUI-202012/01 seems to be N501Y, a transition from asparagine (N) to tyrosine (Y) at amino-acid site of 501. This is due to its location within the RBD of the spike glycoprotein, more precisely within the RBM, a component of the RBD used to bind with human ACE2. The RBD mutations can affect the recognition of antibodies and the specificity of ACE2-binding. It may also contribute to the virus becoming more contagious [120]. It is highly likely that N501Y affects the receptor binding affinity of the spike protein. As a result, this mutation alone or in combination with a deletion in the N-terminal domain at 69/70 provides high transmissibility for the virus. Separately, another variant having the same N501Y change was also detected in South Africa [116]. Though it has a separate lineage from the UK variant, it also showed higher transmissibility for the virus.

The N501Y change was also detected simultaneously in Australia and US (June–July 2019) and Brazil (April 2019). Hence, there was no clarity whether it was imported from UK. As 501 position is in RBD, this mutation significantly reduces the neutralizing ability of antibodies and leads to high transmissibility [121]. Various studies on different monoclonal antibodies showed that one antibody (LYCoV016) exhibited decreased efficacy against SARS-CoV-2 variants having mutation at 501 position. Presently, there are no neutralization data for N501Y mutation obtained from polyclonal sera of natural infections. Recently, Dr. Shibo Jiang, Fudan University, Shanghai, reported the SARS-CoV-2 with 501Y mutation linked with high infectivity and virulence in mouse models. It displayed that N501Y mutation triggers to increase the SARS-CoV-2 binding affinity with the mouse ACE2 receptor and also increase virulence [122].

3.2.3. B.1.351 variant

It is also known as 501Y.V2 in the GH clade, emerged in late 2020 in Eastern Cape, South Africa, and found to be high transmissibility [123].

B.1.351 contains 9 spike mutations (in addition to D614G) in various domains such as NTD (e.g. a cluster of mutations at 242-244del and R246I), RBD (three mutations at K417N, E484K and N501Y) and one mutation (A701V) near the furin cleavage site [124]. The E484K mutation plays a crucial role in the loss of neutralizing activity of some monoclonal antibodies as well as most convalescent and vaccine sera against variant B.1.351. There is a growing concern that these new variants could impair the efficacy of current mAb therapies and vaccines as these mutations reside either in the antigenic supersite of NTD or in the ACE2-binding site (also known as the receptor-binding motif (RBM), which is a major target of potent virus-neutralizing antibodies) [123,125]. The receptor-binding domain mutations are largely driven by E484K, provide tighter ACE2 binding and widespread escape from monoclonal antibody neutralization. Further, K417N and N501Y mutations act together against some important antibody classes [125]. As a result, the newly emerging strains have picked up multiple changes (e.g. deletions and substitutions) in the spike protein. For instance, the identified B.1.351 variant has acquired mutations in the ACE2 interaction surface of the RBD that led to increased transmissibility [126]. Thus, it has rapidly expanded and became the dominant strain in the regions where they were first identified, and posing a serious challenge to spread across the globe.

3.2.4. B.1.427/B.1.429 variant

The new variant of concern (VOC) named as CAL.20C (B.1.427/B.1.429) was originally detected in California and is currently spreading throughout the US and other countries. The variant has five mutations as S13I, W152C and L452R in S protein and I4205V and D1183Y in the ORF1a. It has two mutations (S13I and W152C) in the NTD while having L452R mutation in the RBD [127]. As of March 26, 2021, the sequenced genomes reported in GISAID for the B.1.427 and B.1.429 lineages are 4292 and 10,934, respectively. B.1.429 is less susceptible to neutralizing antibodies and may be linked to worse outcomes of disease [128]. In fact, two recent vaccine trials have shown lower protective efficacy against B.1.351 in South Africa. As the above said spike mutations are specifically targeting the neutralizing antibodies, there is a growing concern about possible immune escape, which could compromise vaccine efficacy and increase the risk of reinfection [129,130].

3.2.5. B.1. 526 variant

B.1.526 variant has unique set of spike mutations and mostly scattered in the Northeast of the USA. Nearly all of the newly identified B.1.526 variants have a set of common mutations in the spike protein such as L5F, T95I, D253G, E484K, D614G and A701V [131]. The B.1.526 variant showed the ability to neutralize antibodies and making current SARS-CoV-2 treatments less effective when tested against 4 monoclonal antibodies, 10 convalescent plasma and 10 vaccine sera. Notably, the decrease in neutralizing activities were found to be 7.7 and 3.4-fold for convalescent plasma and vaccine sera against E484K variant, respectively. Recently, several versions of B.1.526 have emerged with different mutations on the spike protein's receptor-binding domain. While some versions have E484K mutation similar to B.1.351 and P1, other versions have S477N mutation on the spike protein instead of E484K mutation [132]. As a result, it increases the affinity for the ACE2 receptor. Though these versions have both D614G and A701V mutations, L5F, T95I and D253G mutations are not observed in previously reported variants. It has been reported that the B.1.526 variant with S477N mutation had neutralizing titers similar to SARS-CoV-2 with D614G mutation. The B.1.526 variant with E484K mutation shows decrease in neutralizing activity against convalescent sera by 3.8-fold [124].

3.2.6. P.1 Lineage and Double mutant' SARS-CoV-2 variant

The P.1 or Brazilian variant, also known as 20 J/501Y·V3, is one of the SARS-CoV-2 variant emerged from Brazil. It was first identified by the National Institute of Infectious Diseases (NIID), Japan, on 6 January 2021 in four people who had arrived in Tokyo after visiting Amazonas, Brazil. The genome sequencing revealed that the emergence of Lineage P.1 contains 10-lineage-defining amino acid mutations in the virus spike protein (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y and T1207I) when compared to its immediate ancestor (B.1.1.28). Three key mutations present in P.1, N501Y, K417T and E484K, are observed only in the spike protein RBD [133]. In addition, it has been understood that E484K helps the virus to evade the antibodies generated by previous infections and make it less vulnerable to antibody drugs. The P.1 (the shortened form of B.1.1.28.1) has mutation at the 484 position of the spike protein that might decrease the susceptibility of the virus to immunization through vaccination or prior infection [134].

Recently, the Indian SARS-CoV-2 Consortium on Genomics (INSACOG) identified a new "double mutant" variant of the SARS-CoV-2, wherein two mutations, including E484Q and L452R rise together in the same virus [135]. These mutations may confer immune escape and increased infectivity for the virus. The E484Q mutation is known to be similar to E484K-a mutation seen in the B.1.351 (South Africa) and P.1 (Brazil) variants, which have emerged independently several times. However, the L452R mutation (which is also found in the "double mutation") was first identified as part of B.1.427/B.1.429 lineage in the US, hence, it is also called as the "California variant" [136].

4. Mode of entry, infection and replication of SARS-CoV-2

4.1. Mode of entry and infection of SARS-CoV-2

As mentioned earlier, SARS-CoV-2 easily enters the host body through droplet infection and makes its way into the respiratory tract. Homotrimers transmembrane S glycoprotein made by 1273 amino acids protruding the exterior of SARS-CoV-2 facilitates viral entry into host epithelial cells [140–142]. SARS-CoV-2 S glycoprotein is a type I viral fusion protein that require protease cleavage for its activation and subsequent fusion. Subsequently, two subunits (S1 and S2) are participating in viral fusion with the host cell membrane [143], as shown in Fig. 8. While the S1 receptor subunit consists of three domains, namely a single peptide, an extracellular N-terminal domain (14–305 aa) and RBD (319–541 aa), only the single peptide is used for tethering SARS-CoV-2 to host cells. The S2 subunit consists of a well-maintained fusion peptide (788–806 aa) and double heptad repeats (HR1 (912–984 aa) and HR2 (1163–1213 aa)) followed by a transmembrane region (1214–1237 aa) and a cytoplasm domain (1238–1273 aa) [144]. The S2 subunits interact with ACE2 receptor of host cells and initiate the viral-host cell membrane fusion process. Both S protein domains (S1 and S2) are separated from each other through a flexible loop comprising a cleavage spot accessible to host cell proteases [145]. Two-step proteolytic cleavages are occurring for the activation of S protein: 1) the cleavage at the site between S1 and S2 and 2) the activation cleavage at the site of S2 [146]. The S protein of SARS virus is activated by endosomal host proteases, namely transmembrane protease and serine 2 (TMPRSS2) [147]. While

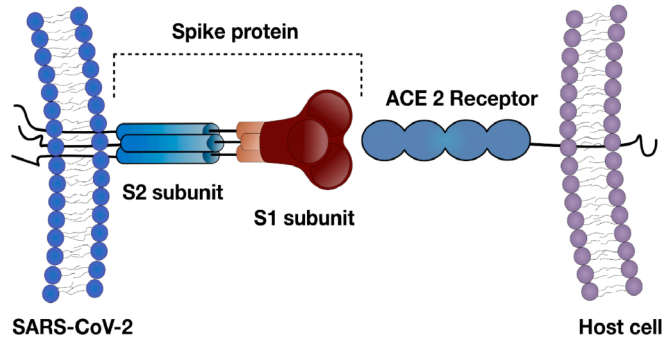

Fig. 8. The cellular interaction between the host cell ACE2 and SARS-CoV-2 S protein.

the S2 cleavage position in both viruses is identical, the cleavage site in S1/S2 differs. The S1/S2 cleavage site of SARS-CoV-2 is Arginine (Arg) 815, whereas it is Arg 797 for SARS-CoV [148,149]. The different host proteases such as trypsin, cathepsin L, furin, TMPRSS-4, TMPRSS-2 and human airway trypsin-like protease (HAT) are required depending upon the cell types for the cleavage of S protein [150,151]. ACE2 is an integral membrane glycoprotein known to have the highest expression in organs such as kidneys, lungs and heart [152]. The ACE2 is strongly expressed in cells such as kidney proximal tubule cells, absorptive enterocytes in the ileum and colon, bladder urothelial cells, cholangiocytes, myocardial cells, lungs AT2 (type II alveolar) cells, upper esophagus and stratified epithelial cells [153–155]. ACE2 has three domains, namely N-terminal signal peptide, C-terminal collectrin-like domain (CLD) and HEXXH zinc-binding metalloprotease motif bound peptidase domain (PD). The C-terminal domain consists of a ferredoxin-like fold "Neck" domain engaged with small extracellular domain, the single transmembrane hydrophobic helix and an intracellular segment [156]. The S1 domain of SARS-CoV-2 is attached to a helix 1 (Lys31 and Tyr 41) and b5 region (Lys353) of the PD domain of ACE2. Subsequently, the cleavage occurs at C-terminal (aa 697 to 716) by the activity of TMPRSS2 that enhances the S-protein-driven viral entry, as shown in Fig. 8. The ACE2 binding affinity with SARS-CoV-2 S protein is higher (~15–40 nM) than SARS-CoV S protein [157,158]. Also, the expression of ACE2 protein at the lung alveolar epithelial cell exterior permits the respiratory tract infection by SARS-CoV-2. Recent reports displayed that men have overexpression of ACE2 in lung alveolar cells than females, and ACE2 expression was higher in Asian people than Caucasian and African American people [159,160]. The renin-angiotensin system (RAS) has a major role in regulating blood pressure, electrolytes and fluid balance in the human body. ACE2 forms angiotensin (1-7) by interacting and cleaving angiotensin II, and the resulting complex of ACE2/angiotensin-(1-7)/MAX axis counteracts the negative impact of RAS. This complex causes inflammation, hypercoagulation, major adverse cardiovascular event, insulin resistance, endothelial dysfunction and respiratory problems [161–163]. SARS-CoV-2 is entering into the cells of central nervous system, gastrointestinal tract and respiratory tract including the pancreas via the ACE2 receptor, and thus, causing adverse tissue damages [164]. Recent reports displayed that the SARS-CoV-2 causes acute pancreatitis, which induces the self-digestion of pancreas, secretion deficiency and formation of large endocytic vacuoles in acinar cells [165,166]. After the viral attachment, SARS-CoV-2 enters into host cells through endocytosis, thereafter enters into endosomes, and finally, the membrane fusion occurs between viral and lysosomal membranes [167].

4.2. Replication and progeny assembly of SARS-CoV-2

The viral genome is discharged into the host cell cytosol, wherein its replicase gene (ORF1a and ORF1ab) is translated to formulate replicase pp1a and pp1ab. The translated polyprotein cleaves and gets transformed to 16 nsps by PL-pro and 3CLpro [168–170]. PL-pro cleaves nsp1, nsp2 and nsp3 from the polyprotein N terminal, whereas 3-CL pro cleaves the remaining nsps from the C terminal. These non-structural proteins then carry the viral replication and transcription processes. The viral proteins such as nsp3, nsp4 and nsp6 subunits, are altered in the endoplasmic reticulum (ER) to direct the formation of viral replication organelles (ROs) [171,172]. The viral genome replication takes place in the DMV of these viral ROs. This helps in immune evasion as they protect the viral RNA from innate immune responses [173]. The RNA-dependent RNA polymerase (RdRp), also called as nsp12, forms either RNA replicase-transcriptase complex (RTC) or RNA polymerase complex with nsp7-nsp8 heterodimer and nsp8 as cofactors in DMVs [174]. Then, both nsp7 and nsp8 proteins increase the poor RNA processivity by lowering the dissociation rate of nsp12 and RNA. The nsp8 protein also acts as a primase enzyme synthesizing short oligonucleotides primers for subsequent extension by the nsp12. The helicase enzyme (nsp13) unwinds the double-strand RNA for nsp12 polymerase [55]. The RTC complex plays a major role in replication process as it generates negative sense genomic RNAs, which would act as a template for positive-sense genomic RNA (gRNA) and subgenomic RNA (sgRNA) (by discontinuous replication) [175]. The nascent RNA strands synthesized are then proofread for misincorporated nucleotides by nsp14 exonuclease enzymes. The genomic and sgRNAs are then polyadenylated at their 3′ end and capped at their 5′ end as it protects them from the host antiviral response and degradation by cellular exonucleases [176]. Then, the capping process is performed by a GTPase, nsp13, nsp16-2'-*O*-methyltransferase/nsp10 complex and nsp14-N7-methyltransferase. While the sgRNA is translated into respective structure in ER, the full-length positive-sense genomic RNA released from DMVs combines with N proteins and forms a helical ribonucleoprotein (RNP) complex in the host cell cytoplasm [177]. Later, the structural proteins (S, M and E) and ribonucleoprotein (RNP) complexes (in encapsulated budding form) are separately entering into the endoplasmic Reticulum-Golgi intermediate compartment (ERGIC), a budding compartment. The RNP interacts initially with M protein to form inner structures and generates the basic viral structure by interacting further with S and E proteins. The matured viral budding is released later from the Golgi apparatus. After completing the viral replication cycle, the assembled SARS-CoV-2 progeny is released from host cells via exocytosis [178–180], as shown in Fig. 9.

5. Host-responses to SARS-CoV-2 infection

The international spread of SARS-CoV-2 is correlated with the host immunological naivety, accessibility of social dynamics, global communication and subdued innate immune responses [181]. The innate immunity is the preliminary virus removal system in the human body that induces adaptive immunity through the secretion of chemokines and cytokines [182]. The first line of innate immunity is activated by the binding of viral S protein with alveolar lung cells, which causes the activation of Pattern Recognition Receptors (PRRS), a local immune response that induces co-stimulatory signals for T lymphocytes (adaptive immune cells) [183].

Previous reports displayed that the lung epithelial cells, macrophages, and dendritic cells are bearing PRRs subfamily proteins such as nucleotide-binding oligomerization domain (NOD)-like receptor, endosomal and extracellular and toll-like receptors (TLRs) and cytosolic RIG-I like receptors on their cell surface. They are activated by the internalized single or double-strand viral RNAs. The activated PRR receptor proteins subsequently stimulate cytokine secretion, including tumor necrosis factor-alpha (TNF-a), type I/III IFNs, IL-6, Interleukin-18 and IL-1. Among them, type I/III IFNs are prominent cytokines for inducing the defense mechanism against the virus [184]. The secreted cytokines carry the primary adaptive immune response in target cells against the virus [185]. The second line of adaptive immune response is performed by T and B-cell responses. While CD8+ and CD4+ T cells are involved in triggering the production of antibody against the virus and killing the virus-infected host cells, respectively, the plasma cells (produced from B-cells secreted antibody) inhibit the viral infection and activate the alveolar macrophages to engulf apoptotic cells and neutralized viruses [186]. These kinds of adaptive immune responses can stop viral load into host cells and enable the recovery of patients from SARS-CoV-2 infection with minimal lung damage [187], as shown in Fig. 10.

Notably, severely affected patients with SARS-CoV-2 validate remarkably impaired IFN-I cytokine production than mild or moderately infected patients [188]. The increased production of cytokines in severely infected SARS-CoV-2 patients such as IL-2, TNFα, IL-6, IL-10, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory proteins (MIP1α), interferon-γ-inducible protein 10 (IP-10) and granulocyte colony-stimulating factor (G-CSF), leads to severe lung and even systemic pathology [Table 3]. The high-level of cytokine production causes increased vascular permeability, plasma leakage and

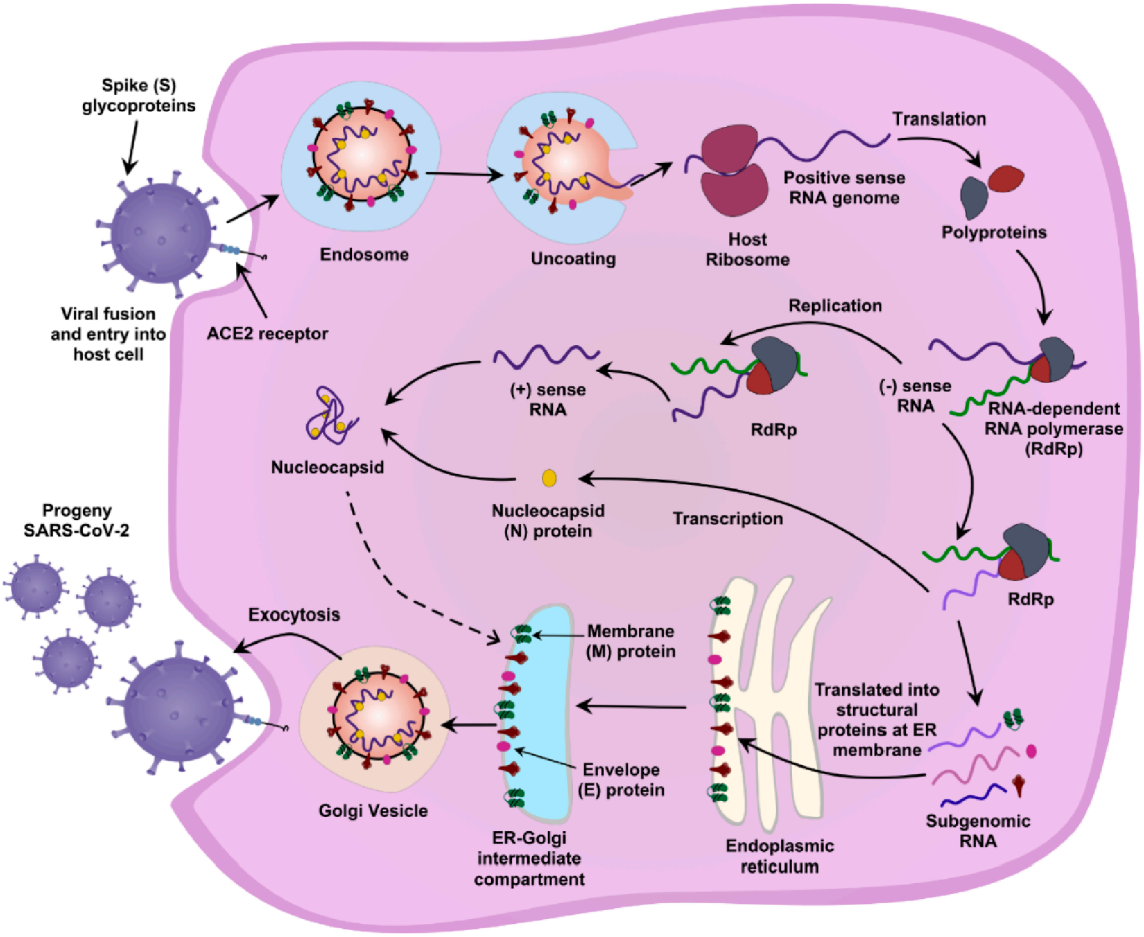

Fig. 9. Scheme reveals the SARS-CoV-2 life cycle in host epithelial cells. The cellular interaction between ACE2 receptor and S-protein facilitates SARS-CoV-2 entry into host cells. After completing the endocytosis and uncoating process, the SARS-CoV-2 components can be reused to formulate new viruses by host cells. Lastly, the new progeny viruses are released from host cells by exocytosis process.

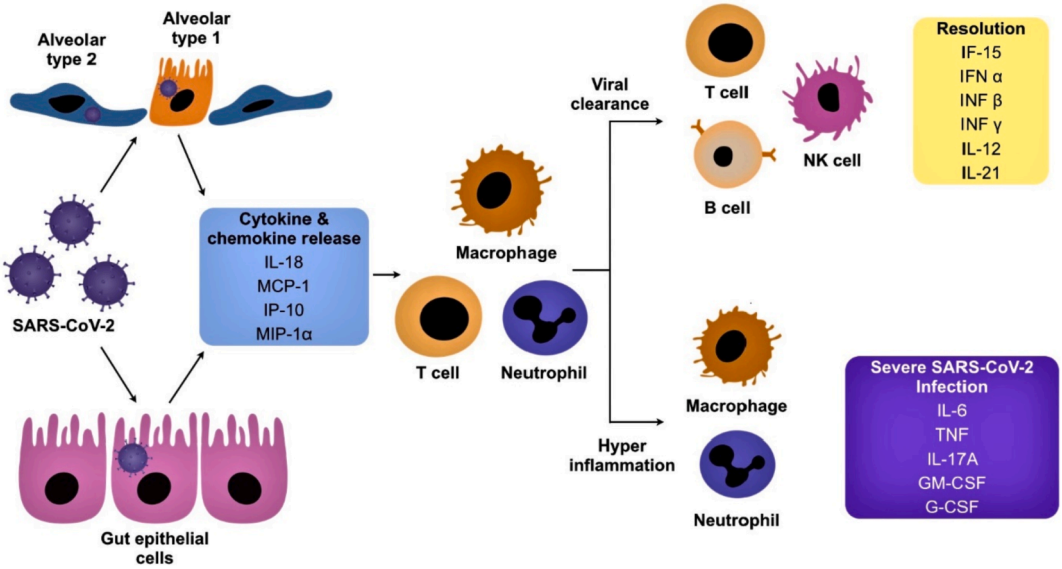

Fig. 10. Overview of innate immune response and interferon signaling between the recovery and severely affected patients by SARS-CoV-2.

accumulation into the alveolar cavity. These kinds of events cause pneumonia, tissue damage in vital organs, respiratory failure and even multiple organ failure [189,190]. The pro-inflammatory phase is followed by the immune suppression stage, which is characterized by a reduction in peripheral lymphocyte count. Lymphopenia is commonly seen in severe cases as they have reduced lymphocytes counts such as

Table 3
Abnormal secretion of the blood parameters and inflammatory cytokines in SARS-CoV-2 infection.

S. No	Biomarker	Function	Ref.
1	Counts of predominant immune cells such as $CD4^+$, $CD8^+$ and NK cell	The decreased level of CD4+, CD8+ and NK cell in peripheral blood mononuclear cell (PBMC) associated with severity of SARS-CoV-2	[210,211]
2	MCP-3, IP-10 and IL-1Ra	Among 48 cytokines, MCP-3, IP-10 and IL-1Ra were closely linked with SARS-CoV-2 disease severity and outcome.	[212]
3	Serum amyloid A (SAA)	The elevated level of SAA was identified in 80% SARS-CoV-2 patients and utilized as an auxiliary index for diagnosis	[213]
4	Platelet count	High platelet-to-lymphocyte and thrombocytopenia (TC) ratio was correlated with poor outcome and increased TC was associated with the incidence of myocardial injury in SARS-CoV-2	[214,215]
5	C-reactive protein (CRP)	Expressed as a biomarker at early stage of disease progression, CRP level was positively linked with disease severity, lung lesions and risk of acute myocardial injury.	[216–218]
6	GM-CSF	The increased level of $CD14^+$, $CD16^+$, GM-CSF^+, monocytes and GM-CSF+IFN- γ+ T cells are correlated with disease severity.	[219,220]
7	IL-2, IFN-γ, IL-6, IL-8 and IL-10	Correlated with the severity of SARS-CoV-2 [IL-6, IL-8 and IL-10]. The increased levels of IL-2 and IFN-γ were correlated with the higher risk of respiratory failure.	[221–223]
8	IL-18	The occurrence of IL-18 was to be main in antibodies producing B cell that is most important in the recovery.	[224]
9	Lymphocyte count	Lymphocyte count is correlated with disease severity.	[225]
10	IL-4	Some reports displayed IL-4 was found to have potential mediator effect or correlated with impaired lung lesions.	[226]

$CD8^+$ T cells, B cells, CD4+ T cells and natural killer (NK) cells [191]. Thus, the adaptive immune response cannot be effectively initiated due to the reduction and dysfunction of these lymphocytes. Hence, the degree of lymphopenia could be used to predict the prognosis even at an early stage. The uncontrolled viral infection, inefficient viral clearance and weak antibody production stimulate the activation of more macrophages and resulting in severe cytokine storms that lead to death [192]. The patients with underlying medical conditions like chronic kidney disease, asthma, serious heart conditions, diabetes, severe obesity and immunocompromised (due to cancer treatment, smoking, bone marrow or organ transplant and prolonged use of corticosteroids) are severely affected by SARS-CoV-2 infection and worst treatment outcomes [193].

5.1. Brain injury

The brain glial cells and neurons express ACE2 receptors on their cell surface, which are a potential target for cellular infection by SARS-CoV-2. The SARS-CoV-2 can pass from the general circulation into the cerebral circulation by rupturing the endothelial lining and infect the neurons. It also leads to bleeding within the cerebral tissue [194]. About 88% of severe SARS-CoV-2 patients had impaired consciousness with hyposmia and acute cerebrovascular diseases at the early stages [195].

5.2. Acute cardiac injury

Acute cardiac injury is a major complication in SARS-CoV-2 infection caused by various mechanisms such as direct viral infection, cytokine storm, respiratory dysfunction and hypoxemia [196], as shown Fig. 11. The elevated myocardial injury biomarkers (e.g., higher cardiac troponin and creatine kinase) were often seen in patients treated in intensive care units (ICU), showing its severity [197]. The elevated levels of troponin T, C-reactive protein and N-terminal pro-brain natriuretic peptide are linking myocardial injury to the severity of inflammation and ventricular dysfunction induced mortality [198]. The meta-analysis, a quantitative, formal and epidemiological study, revealed that the mortality rate of patients without myocardial injury (11.2%) was lower than patients with myocardial injury (67.1%) [199]. The cytokine storm and increased D-dimer level directly activates coagulopathy in severe cases (20–30%) of SARS-CoV-2 infection [200,201]. The coagulation pathway is activated by multiple mechanisms. For instance, the SARS-CoV-2 binding with alveolar ACE2 receptors induces inflammation and reduces the pulmonary vasoconstriction, which causes the depletion of blood oxygen (O_2) level. The reduced O_2 level is described as a hypoxia condition. The vascular response to hypoxia is controlled by hypoxia-inducible transcription factors (HIF), facilitated by thrombus formation, leading to blood clots [202]. In other words, anti-phospholipid antibodies (produced from immune cells) cause severe tissue damage due to coagulation [203]. Besides, the destruction of alveolar and capillary endothelial cells causes more inflammation and blood clots. Further, the increased rate of inflammatory cytokines, including TNF-α, IL-6 and IL-8, can cause venous thromboembolism (VTE) by activating the blood coagulation. The abnormal blot coagulation is associated with poor treatment outcomes and reduced survival rate in SARS-CoV-2 infected patients [204].

5.3. Acute renal injury

Recent reports have found that the lymphocyte infiltration and severe acute tubular necrosis in kidney tubules are caused by SARS-CoV-2 infection. The detection of a cluster of virus-like particles with distinctive spike proteins in podocytes and the tubular epithelium of the kidney under electron microscopy revealed the direct viral infection of the kidney in SARS-CoV-2 infected patients [205]. Various biological factors such as systemic hypoxia, possible drug or hyperventilation-relevant rhabdomyolysis and abnormal coagulation contribute to acute renal injury. The optical microscopic analysis also displayed the diffuse proximal tubule injury with Frank necrosis, non-isometric vacuolar degeneration and brush border loss. Also, the hemosiderin granules and pigmented casts are observed in acute renal injury, which has obstructing capillaries lumen without fibrinoid material or platelet and prominent erythrocyte aggregates [206]. In a recent report, a clinical investigation on kidney injury of SARS-CoV-2 patients showed high serum creatinine, new-onset proteinuria, the infiltration of lymphocytes like CD68+ macrophage into tubulointerstitium and the enhanced deposition of complement, C5b-9 on the tubules [207]. Further, it showed the direct viral infection on kidney organoids for shedding viral progeny, which further confirms its ability to cause acute renal failure in humans [208].

5.4. Rhabdomyolysis

The patients with SARS-CoV-2 were reported to have developed rhabdomyolysis with symptoms of muscle pain and weakness. It could have resulted from the direct viral infection as severe immune response to the viral infection causes cytokine storm and muscle tissue damage. Further, the circulating viral toxins could directly destroy muscle cell membranes [209].

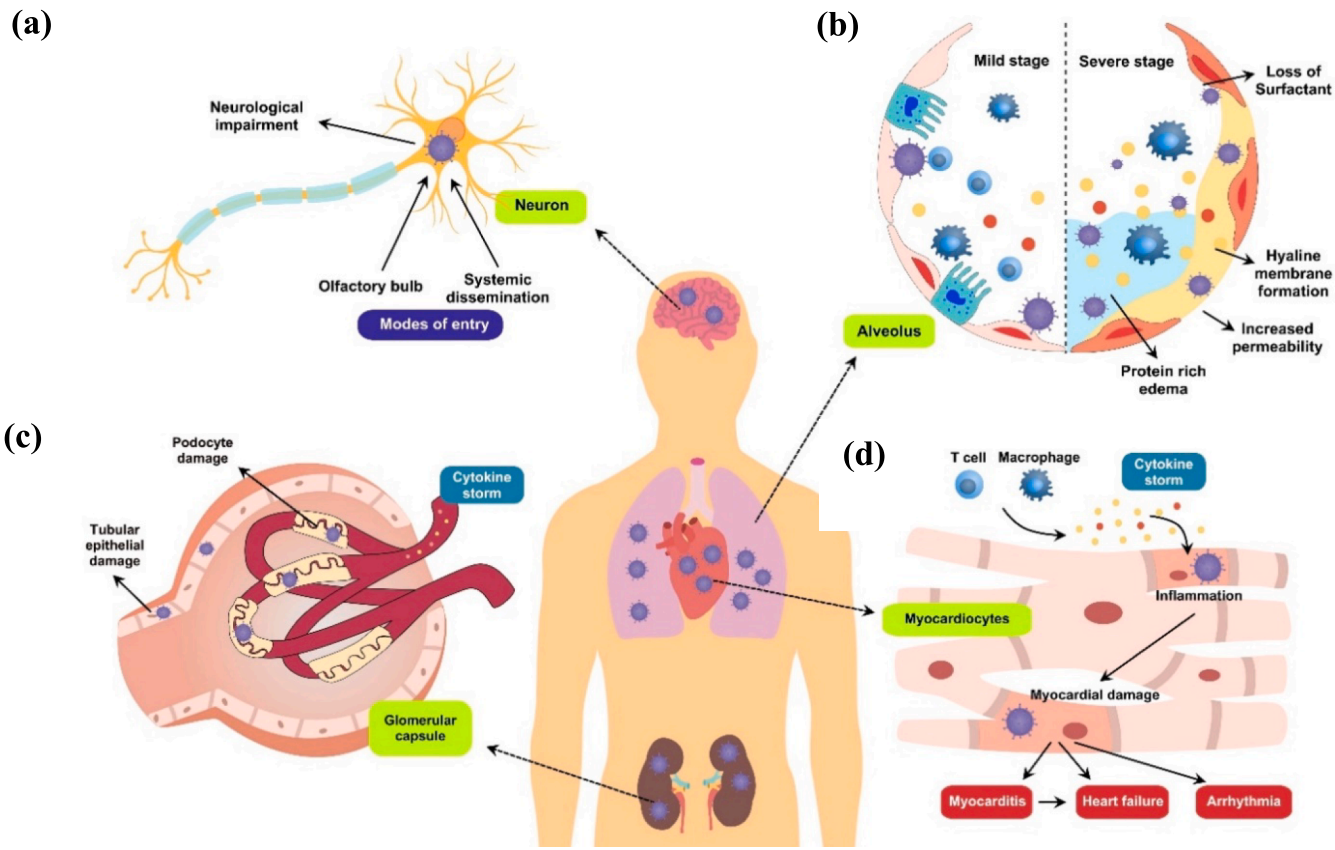

Fig. 11. Host responses during the viral infection. SARS-CoV-2 infects ACE2 expressing target cells in (a) brain, (b) lung, (c) kidney and (d) heart.

5.5. *Neurological manifestations*

Neurological manifestations of SARS-CoV-2 are neurological and other extra respiratory symptoms that occur in patients as a direct result of the virus's neuroinvasive features or as an indirect outcome of downstream multi-organ dysfunction and abnormal biochemistry.

5.5.1. *General neurological symptoms*

The virus causes neuropsychiatric problems in some patients, including altered consciousness and encephalopathy. More serious neurological consequences such as cerebrovascular accidents and seizures, were uncommon, however occurring in only 3 and 0.5% of cases, respectively. Neurological problems were shown to be more common in those who had "severe" SARS-CoV-2 infections [227,228]. A recent report discovered that the majority of neurological manifestations occurred early in the disease, which could be a strong indicator of future clinical worsening. Severe neurological diseases such as bleeding of cerebral vein thrombosis, ischemic stroke etc. were more important and potentially enduring neurological sequelae by SARS-CoV-2 in patients that lead to a significant proportion of death [229,230].

5.5.2. *Stroke*

The percentage of thromboembolic complications in patients with SARS-CoV-2 revealed the incidence of ischemic stroke to be 1.6% and 2.5%, respectively. Beyond regular cardiovascular and metabolic comorbidities and those associated to a prolonged stay in intensive care amenities, there are obviously added risk factors that predispose individuals with SARS-CoV-2 to develop thromboembolic stroke [231,232]. The SARS-COV-2 patients with thrombo-inflammatory conditions showed increase in concentration levels of platelet (62%), interleukin-6 (IL-6) (100%), D-dimer (100%) and fibrinogen (94%). They offer an association between inflammation and consequent coagulopathy induced by IL-6 and fibrinogen [233]. An inflammatory state is 'on' when the alveoli is damaged that triggers the release of inflammatory cytokines such as IL-6. The downstream effects can be classified into two parts: (i) the release of procoagulant factors, and (ii) the damage to the capillary endothelium that leads to a dysregulation of its antithrombotic capabilities. Thus, both of these factors lead to the formation of microvascular thrombosis, which has the ability to embolize the entire body [233].

5.5.3. *Guillain-Barre syndrome (GBS)*

GBS is a significant neurological complication associated with SARS-CoV-2 infection. Both the onset of GBS symptoms and the SARS-CoV-2-specific respiratory symptoms are exceedingly different. A mild temperature, apparent lower limb weakness, upper limb weakness, loss of deep tendon reflexes and a variety of sensory abnormalities, are all signs of GBS [234]. GBS has been linked to recent inoculation with a variety of bacteria, which could explain the disease's clinical heterogeneity. Several mechanisms have been hypothesized to explain the role of viruses in causing an acute areflexic state in GBS. The antibodies against surface glycoproteins are most likely produced in response to a pathogen that also interacts to native protein structures found on the surface of neurones, resulting in the clinical characteristics seen in GBS [235].

6. SARS-CoV-2 presence in sewage and public health concerns

In recent years, the investigation of waterborne infections is considered as an important tool to know more about the localized infections and disease origins [236]. It helps us in controlling viral infections and devising a better preventive strategy. Similar investigations show the evidence of presence of SARSCoV-2 in surface/ground water, sewage stream lines and contaminated drinking water [237–239]. SARS-CoV-2 has been proposed as a waterborne virus that enters and

contaminates water sources via infected faeces [240]. Although the fecal-oral transmission of SARS-CoV-2 has not been confirmed yet, a detailed investigation is necessary to determine the impact of water and related sanitation initiatives in the spreading of COVID-19 by this route. The investigation on the presence of SARS-CoV-2 in faeces and its persistence in the environment suggests that it could stay for a longer time in the water and pollute the environment [241]. While the respiratory droplet infection by close contact is the predominant and most common mode of SARS-CoV-2 transmission, the possibility of fomite-based vertical and faeco-oral transmission can indeed be ruled out given the growing body of evidence [242]. However, SARS-CoV-2 needs to be explored for its ability to sustain in water, transmissibility through water and potential to infect humans. When compared to non-enveloped viruses, the enveloped viruses such as coronaviruses (CoVs) have various structural and survival features to stay alive for longer time in water [243,244]. Hence, wastewater management is seen to be a viable method for tracking the spread of CoVs in a particular locality, i.e. viral clusters. The monitoring of critical reservoir on a regular basis will aid in detecting increasing viral concentrations or indicators, which might be used as early warning signs of an epidemic [245,246]. Such reports can reveal the actual virus burden in communities, thus allowing proper control measures to be implemented to prevent further spread of SARS-CoV-2. Furthermore, when handling the stools of SARS-CoV-2 positive individuals, stringent personal hygiene (hand hygiene) and preventive measures must be followed. The appropriate disinfection strategies have to be followed for waste and sewage water coming from healthcare settings, aged homes, quarantine facilities and containment areas. Based on this, a detailed investigation to study the involvement of the fecal contamination route in SARS-CoV-2 transmission must be urgently performed to investigate the role of environmental parameters in keeping them alive and aiding them further transmission. To gain further information about the relation between SARS-CoV-2 RNA concentrations in faeces samples, disease severity and gastrointestinal symptoms, a complete investigation of enteric involvement and viral shedding in the faeces is urgently required.

7. Diagnostic methods for SARS-CoV-2 infection

The high specificity and accurate clinical diagnostic methods for SARS-CoV-2 infection are desirable for dealing with the earlier diagnosis and appropriate treatment (Fig. 12 and Table 4). The symptoms observed from SARS-CoV-2 affected patients are non-specific and could associate with various other respiratory infections [247]. As per the initial report by Guan et al., the patients' data collected from china showed that the most common symptom was fever in 43.8% of patients and 88.7% patients had developed fever after hospitalization. The other symptoms, cough and diarrhea, were observed in 67.8 and 3.8% patients, respectively [248]. In advanced molecular biotechnology, the nucleic acid detection method based on Polymerase Chain Reaction (PCR) has been referred as the gold standard for detecting the virus [249]. Conversely, computed tomography (CT) scan could also be used to examine any abnormalities present in the lungs, and hence, the diagnosis of the virus at earlier stage is possible [250–252]. Besides, molecular tests employing non-PCR based methods and novel emerging diagnostic methods (e.g., isothermal amplification, protein testing, Point of Care (POC) detection and SHERLOCK) are also under development. Following this, we have briefly reviewed and discussed various diagnostic methods used for the detection of SARS-CoV-2.

Table 4
Various emerging diagnosis techniques for SARS-CoV-2 infection.

Platform	Techniques	Clinical sample	Ref.
CRISPR	RPA	Serum	[295]
CRISPR	RT-RPA	Nasopharyngeal swabs	[290]
LAMP	LAMP	Throat swabs	[296]
RPA	RPA	Fecal and Nasal Swabs	[297]
NASBA	Real Time- NASBA	Nasal Swabs	[298]
RCA	RCA	Serum	[299]
RT-LAMP	LAMP	Nasopharyngeal aspirates	[300]
ELISA	ELISA	Serum	[301]
SIMOA	Digital ELISA	Serum	[302]
Biobarcode Assay	Immunoassay assisted by DNA	Serum	[303]
MCLIA	Enzyme assisted immunoassay	Serum	[275]
Field Effect Transistor (FET)	FET	Nasopharyngeal swab	[294]

CRISPR-Clustered Regularly Interspaced Short Palindromic Repeats, **RPA**-Recombinase Polymerase Amplification, **RT- RPA** -Real Time-Recombinase Polymerase Amplification, **RCA**-Rolling Circle Amplification, **NASBA**-Nucleic Acid Sequence-based Amplification.

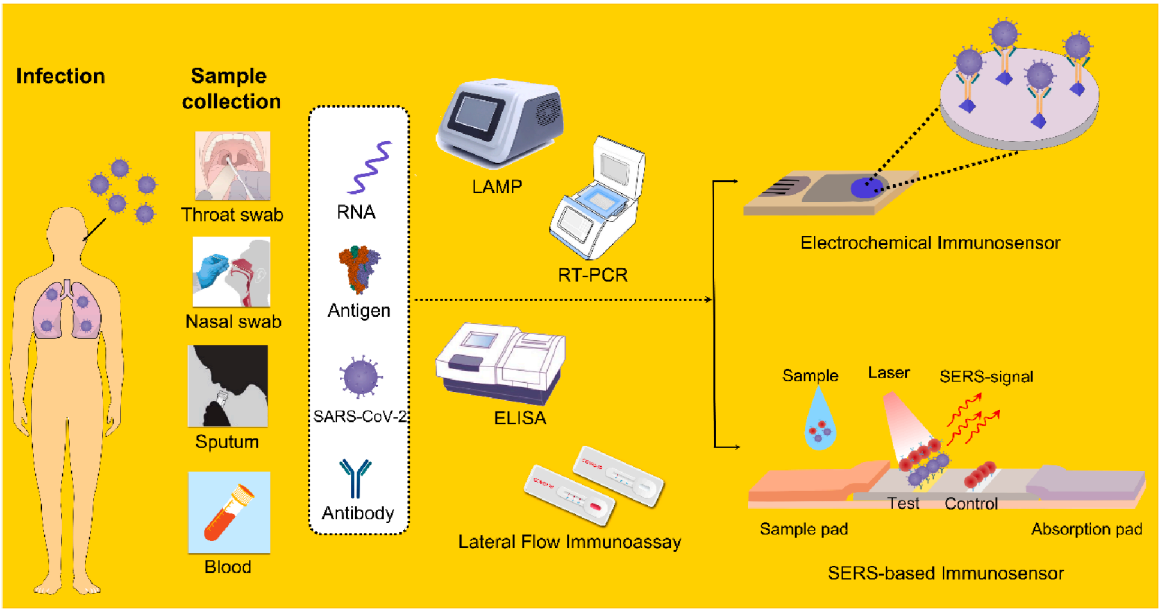

Fig. 12. Various diagnostic methods for SARS-CoV-2 identification in the given biological samples.

Table 5
Mode of action and target of repurposed drugs for SARS-CoV-2 treatment.

S. No	Drug name	Target	Mechanism of action	Adverse effect	Status	Ref.
1	Chloroquine and Hydroxychloroquine	ACE2 viral cell entry Lysosome	Glycosylate inhibition of host cell receptors to block viral entry, acidification of endosomal and proteolytic process. Immunomodulatory effects through inhibition of cytokine production, autophagy and lysosomal activity in host cells.	Overdose may present with respiratory arrest, cardiac arrest and hypokalemia and patients may also be given epinephrine	Phase 4 Completed	[348,349]
2	Lopinavir/Ritonavir	Enzyme 3CL protease	Inhibition of 3CL protease. Resists viral cell entry	Risk to Pediatric Patients	Phase 4 Active, Not Recruiting	[350]
3	Ribavirin	Nucleotide analogue remdesivi (RDV) Inhibits HVC polymerase	RNA dependent RNA polymerase inhibitor Viral RNA synthesis and mRNA capping inhibitor	Hepatotoxicity and hypersensitivity	Phase 4 Active, Not Recruiting	[351,352]
4	Remdesivir	RNA polymerase	RNA dependent RNA polymerase inhibitor. Block viral replication	Over dose - remdesivir are not readily available.	Phase 4 Not yet recruiting	[353]
5	Favipiravir	RNA-dependent RNA polymerase (RdRp)	RNA polymerase inhibitor which prevents RNA replication and transcription	Not recommended, if the pregnancy is confirmed or suspected	Phase 4 Not Yet Recruiting	[354]
6	Tocilizumab	Cytokine storm	Inhibition IL-6 receptor. Block cytokine storm reduction Monoclonal antibody	Over dose - neutropenia	Phase 4 Not yet recruiting	[355,356]
7	Sarilumab	interleukin-6 inhibitor monoclonal antibodies	Inhibition of IL-6 Receptor	Decrease in neutophil count and a reversible decrease in fibrinogen	Phase 4 Completed	[357]
8	Fingolimod	Enzyme dihydroorotate dehydrogenase	inactivating IL-6/STAT3 pathway	Cardiac effects (bradycardia and heart block)	Phase 4 Completed	[358]
9	Canakinumab	interleukin-1 (IL-1)	monoclonal antibody which acts against IL-1-β	Adverse reaction Influenza	Phase 3 Active, not Recruiting	[359]
10	Anakinra	interleukin-1 (IL-1)	monoclonal antibody which acts against IL-1 Receptor	Rheumatoid arthritis AEs (incidence > 10%)	Phase 4 Completed	[360]
11	Gimsilumab	granulocyte macrophage-colony stimulating factor (GM-CSF)	monoclonal antibody which acts against GM-CSF	Not available	Phase 2 Completed	[361]
12	Heparin	Vero E6 cells	Anticoagulant and anti-inflammatory	Platelet counts usually do not fall until between days 5 and 12	Phase 4 Active, not Recruiting	[362–364]
13	Baricitinib	JAK1/2	Antiviral activity Clathrin-mediated endocytosis inhibitor Janus kinases 1 and 2 (JAK1/2 inhibitor	Many adverse reactions	Phase 4 Active, not Recruiting	[365,366]
14	Ruxolitinib	JAK1/2	Inhibition of Janus kinase (JAK)	Myelosuppression, including leukopenia, anemia, and thrombocytopenia.	Phase 4 Completed	[367]
15	Umifenovir	Spike protein /ACE2 Cell membrane	Host cell membrane fusion, DNA and RNA inhibitor	Pathological changes	Phase 4 Completed	[368]
16	Pirfenidone	Spike protein/ACE2	Inhibition of collagen synthesis, down-regulates profibrotic cytokines and decreases fibroblast proliferation Inhibition of TNF – α and IL – 1 β	Photosensitivity rash and gastrointestinal symptoms.	Phase 4 Completed	[369]
17	Camostat Mesilate	Protease inhibitor	TMPRSS2 inhibitor which prevents the viral replication Block viral mutation	Rash, pruritus, nausea, abnormal laboratory test values, and diarrhea	Phase 4 Withdrawn	[370]
18	Darunavir/Cobicistat	Protease	Viral entry inhibitor Inhibits HIV replication	Jaundice (13%), ocular icterus (15%), and nausea (12%).	Phase 4 Active, not Recruiting/ Phase 4 Active, not Recruiting	[370]
19	Aerosolized interferon -α	cytokines	Stimulate antiviral immunity	–	–	[371]
20	Oseltamivir	Neuraminidase	Viral replication inhibitor	No adverse reactions	Phase 4 Completed	[372]
21	Baloxivir marboxil	Viral endonuclease	Viral multiplication inhibitor	–	–	[373]
22	The SARS-Cov-2 specific protease drug candidate	Protease inhibitor	Prevent the viral infectivity Inhibitor of ACE2, S protein and TMPRSS2 serine protease	–	–	[374,375]
23	SARS-Cov-2 specific antibodies	Antibody	Bind with viral cell, and inhibit the viral entry and improve immune system	–	–	[372,376]

Table 6
Developed nasal drugs, its mechanism and current status.

Product Name	Developer	Mechanism	Current Status	Ref
Nitric Oxide Nasal Spray (NONS)	SaNOtize	Reduces Viral RNA generation and fusion of S protein	Phase-III completed	[378]
Nebulizer suspension powder	APEPTICO	By restoring endothelial-epithelial barrier of pulmonary alveoli	Phase II	[379]
Inhaled mRNA antibodies	Neurimmune and Ethris	Encoded with mRNA and helps in neutralizing the anti SARS-CoV-2 antibodies	Phase I	[380]
Alvesco	Covis Pharma	Restrains viral replication	Phase III	[381]
Neuroactive nasal spray	VistaGen Therapeutics	By reducing anxiety through enabling synaptic pathways	Phase III	[382]
Interferon beta for nebulization	Synairgen	Enhances pulmonary antiviral defenses	Phase II	[383]
Leukine sargramostim nebulizer	Partner therapeutics	Treating acute hypoxemia	Phase II	[384]

7.1. PCR based methods

PCR is an enzymatic method separating two DNA strands to produce multiple gene copies, and primer marks the location of the gene segment present in DNA strands. Further, DNA polymerase starts formulating a new DNA strand by adding nucleotides, forming two identical DNA copy from a short RNA segment [219]. This method is mainly used to obtain more DNA copies from minimal quantity of biological samples to make it adequate for laboratory study. In general, the viral genomic RNA is transferred into cDNA using reverse transcription [253,254]. Then, PCR is carried out and the virus is detected using specialized methods. Among them, the sequencing and gel visualization techniques are conventional for the detection of CoVs. The designing of kits generally involves two main steps [255].

- Aligning of sequence and designing the primer
- Optimization of assay and testing

Corman et al. disclosed various SARS-CoV-2 genomes to identify sets of primers and probe through which they have identified three regions having conserved sequence, namely RdRP gene in the ORF1 ab region, E gene and N gene. Notably, the attempts to detect RdRP gene and E gene have shown high sensitivity as the technical limitations for the detection are 3.6 and 3.2 copies per reaction, respectively. However, N-gene resulted in 8.3 copies per reaction, and hence, exhibited poor sensitivity [256]. The assay has been made as a dual-target system with 2 sets of primers, wherein one set detects various coronaviruses and another set of primers specifically detects SARS-CoV-2 [255]. After designing suitable probes and primers, the assay conditions (e.g., incubation times, temperature and reagent conditions) were optimized prior to PCR testing. Though RT-PCR can be executed through one step or two-step assays, combining the reverse transcription and PCR amplification in a single step provides rapid and reproducible results. Despite such advantages, the difficulties in optimizing the reverse transcription process and PCR amplification (mostly due to simultaneous occurrence) result in lower amplification than the desired target. Since the reaction is performed sequentially in two distinct tubes, two-step assay is highly sensitive than one-step assay. However, it is time consuming and slow that results in low number of tests per unit time.

RT-PCR is analytically specific but not reliable due to its high false-negative rate [257]. Unfortunately, the positivity rate with RT-PCR is only 30–50% for laboratory confirmed SARS-CoV-2 patients, particularly in the early stage of the infection and if the samples were collected from upper respiratory tract [258]. It was shown in another report that 3% people confirmed for infection in chest computed tomography (CT) were given false-negative report after RT-PCR testing [259]. Later they were confirmed for viral infection in repeated swab tests. Further, it can amplify spurious nucleic acid contaminations and can provide false-positive results. Hence, a single negative-result from RT-PCR does not rule out SARS-CoV-2 infection in clinical diagnosis.

Table 7
Plant based SARS-CoV-2 vaccine candidates.

Vaccine	Plant Host	Vaccine Antigen	Trial status/ID	References
CoVLP	Transient expression in N. benthamiana	SARS-CoV-2 S protein/ Viral like particle based vaccine	Phase 3 NCT04636697	[435]
KBP-201	Transient expression in N. benthamiana	SARS-CoV-2 RBD-based vaccine	Phase 1/2 NCT04473690	[436]
IBIO-200	Transient expression in N. benthamiana	SARS-CoV-2 Viral like particle based vaccine	Pre-clinical study	[437]
IBIO-201	Transient expression in N. benthamiana	SARS-CoV-2 Spike-based sub-unit vaccine	Pre-clinical study	[437]
IBIO-202	Transient expression in N. benthamiana	SARS-CoV-2 nucleocapsid protein-based sub-unit vaccine	Pre-clinical study	[437]
Recombinant SARS-CoV-2 and RBD protein	Transient expression in N. benthamiana	RBD	Research	[438]
Recombinant SARS-CoV-2 and N protein	Transient expression in N. benthamiana	N protein	Research	[438]
N/M protein based vaccine	Transient expression in N. benthamiana	Recombinant SARS-CoV N and M protein	Research	[439]
Baiya SARS-CoV-2 Vax 1	Transient expression in N. benthamiana	Protein subunit vaccine/ SARS-CoV N and M protein	Pre-clinical study	[440]
Nucleocapsid SARS-CoV	Transient expression in N. benthamiana	SARS-CoV nucleocapsid protein	Pre-clinical study	[441]
SARS-CoV S1 protein fusion	Transient expression in N. benthamiana	SARS-CoV S1-GFP fusion protein	Research	[442]
SARS-CoV S1 protein based vaccine	Stable expression in tomato	SARS-CoV S1 protein	Pre-clinical study	[443]

RBD - receptor binding domain (RBD), GFP - green fluorescent protein.

7.2. Computed Tomography (CT) scan

CT of the chest uses X-ray equipment to examine the abnormalities and helps in diagnosing the cause of unknown cough, fever, shortness of breath and other respiratory symptoms [251,252]. The high-resolution CT test could be utilized for earlier diagnosis and to understand the SARS-CoV-2 infection severity in patients. Bernheim et al. observed

Table 8
Overview of the vaccine production for SARS-CoV-2 treatment. The major companies around the world are participating in developing a vaccine for SARS-CoV-2 infection [472].

Vaccine Name	Characteristics of vaccine	Development status	Details of clinical trials	Developer	Trial ID
NVX-CoV2373	Prefusion protein nanoparticle vaccine	Not actively recruiting Phase 3	Phase 1–130 participants Phase 2b- 2665 healthy, 240 HIV positive participants Doses - 5 μg or 25 μg Intramuscular injection	Novavax	NCT04368988
mRNA-1273	LNP encapsulated mRNA based vaccine encoding S Protein	Active and not recuiting Phase 3	Phase 1 trial (NCT04283461) – 120 participants Phase 2 trial (NCT04405076) - 600 participants Phase 3 (NCT04470427) Dose levels - 50, 100, 200 and 250 μg. Intramuscular injection	Moderna Kaise Permanente Washington Health Research Institute	NCT04470427
COVAX19	Protein sub unit Recombinant of covid-19 S protein with adjuvant of Advax-SM	Completed Phase 1	40 participants Dose – 2 μg and saline Intramuscular injection	Vaxine Pty/Medytox	NCT04453852
V591	Measles virus vector	Completed Phase 1	90 participants Dose – 2 different doses Intramuscular injection	Institute Pasteur	NCT04497298
CVnCoV, CureVac	mRNA based vaccine	Active and not recruiting Phase 1	280 participants Doses – 2, 4, 6, 8, and 12 μg Intramuscular injection	Curevac, CEPI	NCT04449276
BCG Vaccine	live-attenuated strain derived from *Mycobacterium bovis*	Recruiting Phase 4	700 participants Dose - single dose in 0.1 mL saline Intradermal injection	Andrew Dinardo Texas A&M University	NCT04348370
VPM1002	Further development of BCG	Active and not recruiting Phase 3	1200 participants Dose- 1 μg Intradermal injection	University Health Network, Toronto Vakzine Projekt Management GmbH	NCT04439045
INO-4800	DNA plasmid encoded S protein delivered by electroporation	Active and not recruiting Phase 3	120 participants Dose – 1.0 mg Intradermal injection	Inovio Pharmaceuticals	NCT04336410
BNT162	mRNA based vaccine	Recruiting Phase 3	Phase 1/2–200 participants Phase 2/3–32,000 participants Dose - 10 to 100 μg Intramuscular injection	Pfizer, BioNTech Multiple study sites in Europe and North America	NCT04368728
BNT162, BioNTech	BioNTech mRNA vaccine	Recruiting Phase 3	200 participants Dose – 1 to 100 μg (escalating levels of dose) Intramuscular injection	Pharmaceuticals GmbH + Pfizer Inc. BioNTech RNA Pharmaceuticals GmbH	NCT04380701
CoronaVac	Inactivated SARS-CoV-2	Active and not recruiting Phase 2	Phase 1–144 participants Phase 2–600 participants Doses – 3 or 6 μg / 0.5 mL Intramuscular injection	Sinovac Research & Development Co., Ltd	NCT04352608
Ad5-nCoV	Adenovirus Type 5 Vector/Non replicating Viral Vector that express S protein	Completed Phase 1	Phase 1–108 participants Dose – 3 different doses Low - 5E10 vp Ad5-nCoV middle - 1E11 vp Ad5-nCoV high - 1.5E11vp Ad5-nCoV Intramuscular injection	CanSino Biologics Inc.	NCT04313127
RBD-Dimer	Adjuvanted recombinant protein/ protein subunit	Active and not recruiting Phase 1	50 participants Dose – 25 and 50 μg / 0.5 mL Intramuscular injection	Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd.	NCT04445194 NCT04437875

(*continued on next page*)

Table 8 (*continued*)

Vaccine Name	Characteristics of vaccine	Development status	Details of clinical trials	Developer	Trial ID
Gam-COVID-Vac Lyo, Gamaleya	Non replicating viral vector/adenoviral based vaccine combined (rAd26-S + rAd5-S) expressing S protein	Completed Phase 3	Phase 1–138 participants Dose: 2 dose levels Intramuscular injection	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation	
EpiVacCorona	Peptide antigens based vaccine	Active and not recruiting Phase 2	100 participants Dose – a dose of 1 mL Intramuscular injection	Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology "Vector"	NCT04527575
DNA plasmid vaccine - ZyCoV-D	Electroporation to deliver S protein	Active and not recruiting Phase 2	1048 participants Dose – 3 doses Intramuscular injection	Cadila Healthcare Limited	NCT04336410
BBV152	Whole virion based inactivated vaccine	Active and not recruiting Phase 2	755 participants Dose – 0.5 mL Intramuscular injection	Bharat Biotech International Limited	NCT04471519 CTRI/2020/07/026300
LV-SMENP-DC	Lentiviral vector with DCs modification express the synthetic minigene based on domains of selected viral proteins; administered with low antigen specific cytotoxic t lymphocytes	Recruiting Phase 2	100 participants 5E6 and 1E10 LV- DC Subcutaneous injection	Shenzhen Geno-Immune Medical Institute	NCT04276896
AZD1222	ChAdOx1 vector that expresses S protein	Active and not recruiting Phase 3	32,459 participants Dose – 2 Intramuscular injection of 5E10 viral particles Intramuscular injection	AstraZeneca/ Oxford University	NCT04516746
ARCT-021	LNP-encapsulated self-replicating mRNA that encodes the prefusion S protein	Completed Phase 2	106 participants 1 dose in 0.5 mL Intramuscular injection	Arcturus Therapeutics, Inc.	NCT04480957
Inactivated SARS-CoV-2 vaccine	Covid-19 inactivated vaccine	Enrolling by invitation No longer being studied Phase 2	471 participants Dose - 50–150 μg/0.5 mL Intramuscular injection	Institute of Medical Biology, Chinese Academy of Medical Sciences	NCT04470609

virus-positive CT scan reports for 56% of patients in their early stages of disease within 0 to 2 days [250]. The CT images of the SARS-CoV-2 infected cases show bilateral occurrence of uneven ground-glass opacities, which can coalesce into the spherical shaped randomly distributed deep-clustered lesions along the periphery of the lung [260]. In addition to this, an irregular paving pattern (Irregular shaped paved stone pattern) was also observed along with an increase in consolidation in the lungs [261]. It is worth noting that the ground-glass opacities are prominent in the initial period of 0 to 4 days after symptom onset. However, the drawback of CT scan is its specificity for SARS-CoV-2 infection (~ 25%) as its inability to distinguish SARS-CoV-2 infection from normal pneumonia virus affects its specificity [262].

7.3. Isothermal amplification based method

The isothermal amplification techniques includes recombinase loop-mediated isothermal amplification (LAMP), polymerase amplification and helicase-dependent amplification [263]. LAMP retains some fundamental advantages such as exclusion of a thermal cycler, amplification at a constant temperature, faster test results and potentially a larger diagnostic capacity, while maintaining similar sensitivity and specificity, thus making it more suitable than the RT-PCR for monitoring a pandemic. The LAMP technique amplifies DNA under isothermal conditions, wherein the amplification is directly related to the turbidity caused by the production of magnesium pyrophosphate. It is extensively utilized for SARS-CoV-2 detection [264–267]. RT LAMP reaction performed at 60–65 °C resulting in DNA polymerase with strand displacement activity to initiate the DNA synthesis. A primer set used for a typical LAMP assay consists of a four constructed primers (two inner and two outers), which can attach with six disparate sequences in the target genome [268]. The inner primes are termed as a backward inner primer (BIP) and forward inner primer (FIP). Each primer having two unique sense and antisense sequences is matching to the target DNA. The mechanism of LAMP involves three stages: (1) starting material production (2) cycling amplification and elongation and (3) recycling. As the LAMP uses a more number of primers, it is considered highly specific. Notably, the results are easily distinguished by naked eye by visualizing a color change (pH-sensitive dye addition), turbidity change (the reaction of a by-product) and fluorescence coming out of the sample (double-stranded DNA binds to the added fluorescent dye). The above mentioned changes reveal the amplification of DNA in the samples. Further, the procedure is simple, cheaper and faster, and hence, handling of a large number of samples is possible without any difficulty [269]. The reaction time is less than 1 h and the detection limit is nearly 75 copies/μL. Overall, this procedure is simple to follow, easy for visualizing the detection, has less background noise and doesn't need a thermocycler. Optimizing the primers and reaction conditions are the challenges in the LAMP technique.

7.4. Microarray-based method

The microarray detection method is a rapid and high throughput technique in which coronavirus RNA produces labeled cDNA with probes through reverse transcription [219]. This cDNA is loaded into the well and gets hybridized with the microarray fixed solid oligonucleotides. Further, free DNAs are removed by washing steps and SARS-CoV-2 RNA detection is performed by a specific probe detection. Guo et al. displayed a 24 single nucleotide polymorphism mutations predicting among the SARS-CoV S gene by microarray technique [270]. Since CoVs outbreak can happen in large numbers, the assays with an ability to detect an extensive viral range are being developed. In this regard, Luna et al. have attempted to increase the low-density oligonucleotide array to detect the entire CoVs genus whose sensitivity is comparable to RT-PCR technique [271]. Hardick et al. assessed a portable and microarray chip-based Mobile Analysis Platform (MAP) and near-POC diagnostic platform to detect the virus [272].

7.5. Protein testing method

SARS-CoV-2 antibodies or antigens produced in response to infection

Table 9
Recently developed intranasal vaccines and their respective current stages.

Vaccine Name	Type	Developer	Details of clinical trials	Ref
ChAdOx1 nCoV-19	Adenovirus vector-based vaccines	University of Oxford in collaboration with AstraZeneca	Phase I trial	[476]
ChAd-SARS-CoV-2-S	Adenovirus vector-based vaccines	Washington University school of medicine in St. Louis, USA	Phase I trial	[477,478]
Ad5-nCoV and ZF2001 vaccines	Adenovirus vector-based vaccines	CanSino Biologics Inc., China	Phase II trial	[479]
AdCOVID	Adenovirus vector-based vaccines	USA-based Altimmune	Phase I trial	[480]
SARS-COV2-M2sr	M2SR (single replication) vaccines	The University of Wisconsin-Madison in collaboration with FluGen and Bharat Biotech	To initiate Preclinical trial	[481–483]
DelNS1-nCoV-RBD LAIV	Live-attenuated vaccines	Beijing Wantai Biological Pharmacy Enterprise, USA	Phase I trial	[484,485]
Mv-014-212	Live-attenuated vaccines	Meissa Vaccines, Inc., USA	Phase I trial	[486]
CROWNase	Subunit vaccines	Illinois Institute of Technology, Chicago, USA	Preclinical trial	[487]
CovOMV	Subunit vaccines	Intravacc, Netherlands	Preclinical trial	[488]
STINGa-liposomes	Nanoparticle-based subunit vaccines	AuraVax Therapeutics, USA	Preclinical trial	[489]

can be used for the diagnosis. However, the viral protein detection is difficult due to variation in viral load during the infection period. Wang To et al. reported that the SARS-CoV-2 viral load in infected person is decreased as a function of time [273]. The antibodies generated after the infection may provide additional time for the secondary detection of SARS-CoV-2. Further, the antibody test also utilized for close observation of virus. Due to antibody cross-reactivity produced for SARS-CoV-2 with other CoVs, developing serological tests with accuracy is challenging. When Lv et al. investigated cross-reactivity for spike protein of SARS-CoV and SARS-CoV-2 in 15 patients, it showed high cross-reactivity [274]. Long et al. demonstrated the detection of SARS-CoV-2-specific antibodies by magnetic chemiluminescence enzyme immunoassay (MCLIA) [275]. Though there was no cross-binding with S1 subunit of SARS-CoV S antigen, some cross-reactivity was noted with SARS-CoV N antigens when it was added with the serum sample of SARS-CoV-2 patients.

7.6. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

Recently developed CRISPR has taken molecular diagnosis to the next level due to its benefits such as speed, precision, specificity, strength, efficiency, versatility, portable and inexpensive [276]. The CRISPR system enables researcher to alter gene function by making changes in genomic sequence. It closely resembles the pair of molecular scissors that can precisely cut the DNA strands. This system has a family of DNA sequences found in archae and bacteria, and is composed of two major parts: (i) Cas endonuclease and (ii) guide RNA. The former is responsible to break the target genomic site, whereas the latter is used to identify and lead the Cas endonuclease to the target [277–279]. The CRISPR system is further divided into two main classes and six types. The first class is composed of Type I, III and IV while the second class is consisting of Type II, V and VI. In first class, Cas 3 nuclease and Cas 10 nuclease of Type I and III are used to cut DNA and RNA, respectively. In the second class, type II and type V systems use Cas 9 endonuclease and Cas 12 nuclease to cut the DNA, respectively. Cas 13 nuclease is used in Type VI of second class to make cuts in target RNA. Notably, the class II

Table 10
List of approved vaccines across the globe [498].

Vaccine name	Vaccine type	Number of countries approved (Nos.)	Developer	Ongoing trials in different number of countries
mRNA-1273	RNA	76	Moderna	30 trials in 2 countries
BNT162b2	RNA	101	Pfizer/BioNtech	37 trials in 2 countries
TAK-919	RNA	1	Takeda (Moderna formulation)	2 trials in 1 country
ZyCoV-D	DNA	1	Zydus Cadila	5 trials in 1 country
Ad26.COV2·S	Non Replicating Viral Vector	71	Janssen (Johnson & Johnson)	13 trials in 2 countries
AZD1222	Non Replicating Viral Vector	123	Oxford/ AstraZeneca	44 trials in 2 countries
Covishield	Non Replicating Viral Vector	46	Serum Institute of India (Oxford/ AstraZeneca formulation)	2 trials in 1 country
Ad5-nCoV	Non Replicating Viral Vector	9	CanSino	11 trials in 6 countries
Sputnik Light	Non Replicating Viral Vector	18	Gamaleya	4 trials in 2 countries
Sputnik V	Non Replicating Viral Vector	72	Gamaleya	21 trials in 7 countries
BBIBP-CorV (Vero Cells)	Inactivated	66	Sinopharm (Beijing)	11 trials in 9 countries
CoronaVac	Inactivated	40	Sinovac	22 trials in 1 country
Inactivated (Vero Cells)	Inactivated	2	Sinopharm (Wuhan)	8 trials in 5 countries
COVID-19 Inactivated Vaccine	Inactivated	11	Shifa Pharmed Industrial Co	4 trials in 1 country
QazVac	Inactivated	2	Kazakhstan RIBSP	3 trials in 1 country
SARS-CoV-2 Vaccine (Vero Cells)	Inactivated	1	Minhai Biotechnology Co	5 trials in 1 country
KoviVac	Inactivated	1	Chumakov Center	2 trials in 1 country
Covaxin	Inactivated	9	Bharat Biotech	7 trials in 1 country
ZF2001	Protein subunit	3	Anhui Zhifei Longcom	7 trials in 5 countries
CIGB-66	Protein subunit	4	Center for Genetic Engineering and Biotechnology (CIGB)	5 trials in 1 country
EpiVacCorona	Protein subunit	2	FBRI	3 trials in 1 country
MVC-COV1901	Protein subunit	1	Medigen	7 trials in 2 countries
COVAX-19	Protein subunit	1	Vaxine/ CinnaGen Co.	4 trials in 2 countries

system is generally adopted to diagnose the disease and used for genomic manipulation [280–285]. For instance, multiplex diagnostic system was developed by Kellner et al., wherein the nucleic acid pre-amplification is incorporated with CRISPR/Cas to identify the targeted nucleic acid sequences [286]. DNA endonuclease targeted CRISPR trans reporter (DETECTR) is CRISPR based inexpensive diagnostic method, mostly relying on Cas 12 to detect the infection within ~30 mins [283]. Another technique called specific high sensitivity enzymatic reporter unlocking (SHERLOCK) can detect the sequence of nucleic acids in clinical samples [286]. Both these techniques are comparable to PCR in the performance wise, however they are cheaper than PCR. Recenlty, these techniques, -SHERLOCK and DETECTR, are approved and commercially available for the detection of SARS CoV-2 [283,286].

7.7. Specific High Sensitivity Enzymatic Reporter Unlocking (SHERLOCK)

SHERLOCK is a nucleic acid detection approach, wherein the viral RNA sensing is carried out by Cas13a ribonuclease enzyme [287]. In this technique, the viral RNA is converted to cDNA by reverse transcription, followed by isothermal amplification of cDNA using Reverse Polymerase Amplification (RPA) [288]. The Cas13a forms a complex with RNA guide sequence and gets attached with the amplified RNA. When it binds with the target, Cas13a gets activated and produces a fluorescent signal by breaking and releasing the surrounding fluorophore quencher probes. It is highly sensitive and specific as it can detect a single molecule in 1 μL sample volume of DNA and RNA targets [287]. It is also reported that the scaling up of preamplification content can detect even a single-molecule in large sample volumes [289]. In terms of specificity, two similar viruses can easily be distinguished from one another (e.g., dengue and zika virus) [290]. The reason is that Cas13 doesn't get activated when there are more than one mismatches in crRNA target duplex. In the detection of cancer-associated mutations, human genotyping and single base distinction, the specificity can also be increased by introducing mismatchs into the crRNA. Another promising feature of SHERLOCK is rapid detection. However, the SHERLOCK technique is limited due to multi-step nucleic acid amplification that affects the target quantification [287].

7.8. Point of care diagnostics

Pont of care diagnostics is the method of detection in which the sensing is carried out without any need for laboratory or centralized facilities. Among these techniques, the lateral flow assays are made of paper-based membrane strip with two lines marked on it [291]. The first line is filled with gold nanoparticle (AuNPs)-antibody conjugates and another line is filled by capture antibodies. When the blood and urine samples from SARS-CoV-2 infected person is dropped onto strips, the antibodies in the samples spread over the strip. The SARS-CoV-2 antibody present in the sample forms a colourful complex by interacting with AuNPs-antibody conjugates and capturing antibody that can be visualized by naked eye. In the previous report, the lateral flow assay is combined with RT-LAMP to detect MERS-CoV effectively. Another such point of care diagnosis method is the microfluidic device, wherein palm-sized chips consisting of micrometer range channels and reaction chambers are used for the detection of viruses. On the chip, the liquid samples are made to mix with antibody specific reagents and separated via electrokinetic force, vacuum, capillary action, etc. [292]. The advantages of microfluidic devices are compactness, rapid detection, portability and miniaturization. The previous report has demonstrated these microfluidics devices' use as an attachment with mobile to detect sexually transmitted disease antibodies by sequentially moving reagent pre-stored in cassette [293].

7.9. Field Effect Transistor (FET) based biosensor

Seo et al. have demonstrated FET-based biosensor to detect SARS-CoV-2 virus using graphene-based sensing layer [294]. They prepared a FET based biosensing device with the dimension of $100 \times 100\ \mu m^2$ (L × W). Initially, a layer of poly (methyl methacrylate) (PMMA) over graphene whole supported on Copper (Cu) foil was spin-coated. The Cu foil was later etched by CE-100 (a copper etchant) and the obtained PMMA/graphene film was transported to SiO_2/Si substrate. After dried at room temperature, the PMMA layer was removed using acetone and the remaining graphene layer was washed with isopropyl alcohol and dried under N_2 atmosphere. Later, the photolithography followed by reactive ion etching was used to pattern the graphene layers. Before detection, the prepared device is functionalized with 1-pyrene butyric acid N-hydroxysuccinimide ester (PBASE) in methanol for 1 h. Among four structural proteins of the SARS-CoV-2 virus (i.e., spike, envelope, matrix and nucleocapsid), the spike protein was selected because it is a major transmembrane protein, highly immunogenic and shows better specificity due to amino acid sequence diversity among the family of coronavirus [290]. The electrical measurement using current and voltage before and after the attachment of the antibody was carried out. The slope variation indicates the SARS-CoV-2 spike antibody occurrence. The viral antigen detected by FET based biosensor is 1 fg/mL, i.e., the limit of detection. They have also carried out the real-time cultured SARS-CoV-2 detection from clinical samples. Interestingly, the device also showed no cross-reactivity with MERS-CoV antigen.

8. Therapeutic targets for SARS-CoV-2

The unfortunate pandemic of SARS-CoV-2 in early 2020 has caused a challenge to all researchers to find the potential therapeutic agents for the treatment. However, there was no reliable vaccines or drugs available for either treating or controlling SARS-COV-2 virus. Hence, the scientific communities were actively involved in the examination of approved/existing drugs for other diseases for drug repurposing efforts for the treatment of SARS-COV-2. In the initial period of pandemic, the drugs utilized in clinical trials were based on empiric data, which were actually developed for other viruses or parasites [304]. The antiviral therapies were developed to induce direct effect on SARS-CoV-2 either by blocking the viral entry to host cells or controlling the viral enzymes having significant contribution for genome replication (Table 5) [305]. Alternatively, other therapeutic agents were also developed with the aim of boosting the innate immunity towards viruses or hindering the inflammatory response that cause lung injury [306]. Besides, the acquired plasma or hyper immunoglobulin from recovered patients of SARS-CoV-2 infection was also tried to treat SARS-CoV-2 infections. The studies have shown that it can effectively prevent the viral replication and spread. Owing to continued efforts in the past 12–18 months, 23 vaccines have been approved by WHO as of 14th October 2021 for emergency use across the globe. Further, about 300 vaccines are either in different stages of clinical trials or in development stages. Hence, this section discusses the availability of therapies for SARS-CoV-2 infection, their advantages and limitations, as shown in Fig. 13.

8.1. Therapeutic agents to target RNA-dependent RNA polymerase (RdRp)

The SARS-CoV-2 replication and transcription is strongly depending on RdRp to produce new RNA. The RdRp consists of a 500–600 residue catalytic module consists of fingers, palm and thumb domains [307]. The mutation induction in RdRp amino acid residue causes a complete loss or lowering of RNA polymerase activity, modifies the metal cofactor requirements [308]. A class of nucleoside analogs (NAs) and small molecular drugs are utilized as RdRp inhibitors that metabolize intracellularly into their active ribonucleoside 5′-triphosphate (RTP) form and get incorporated into the nascent viral RNA. They can cause chain

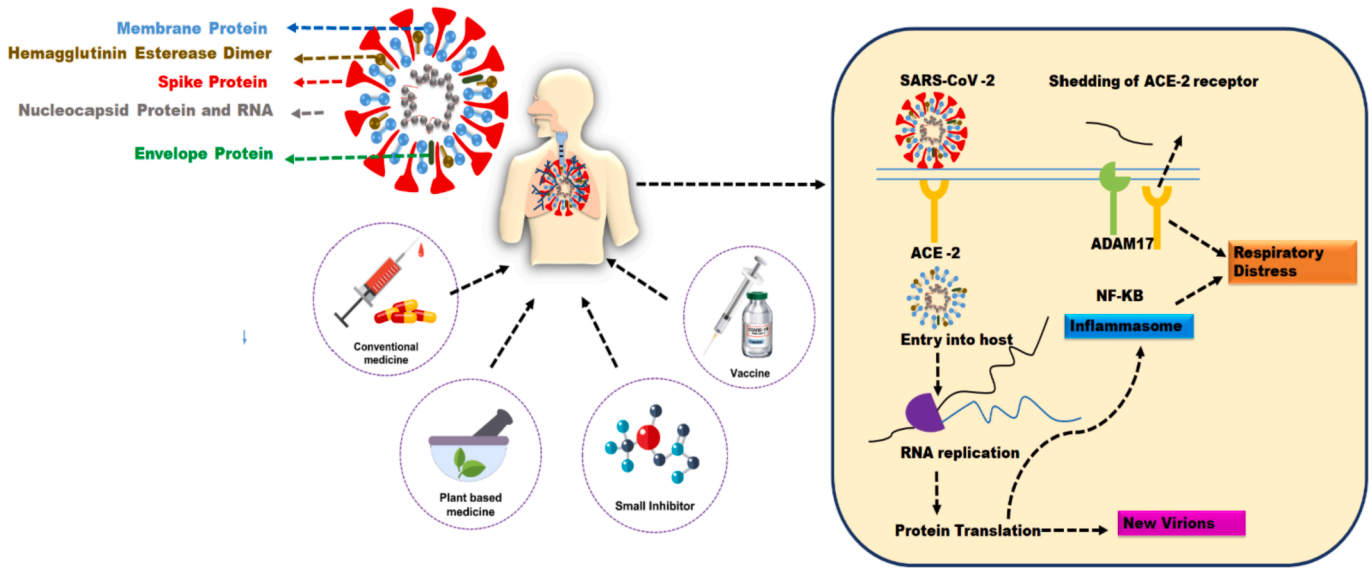

Fig. 13. Disparate treatment options for treating SARS-CoV-2 infection.

termination and induce deleterious mutation in the viral RNA without any impact on other proteins in host cells. Conventionally, the viral RdRp is a low-fidelity enzyme due to a lack of proofreading functions. Recently, it has been demonstrated that the FDA approved HIV and HBV drug tenofovir-diphosphate (TFV-DP), a nucleotide inhibitor, effectively suppresses the SARS-CoV-2 RdRp activity [309].

8.1.1. Remdesivir

Remdesivir (RDV) is a most prominent monophosphate nucleotide analog consists of a 1′-cyano modification sugar (formally known as GS-5734) that displays the broad-spectrum of antiviral effects against numerous RNA viruses, including MERS-CoV and SARS-CoV [310]. RDV has structural resemblance with adenosine and had a half-life of about 1 h in plasma. It gets incorporated into nascent viral RNA and quickly turned into intermediate monophosphate and nucleoside metabolites [311]. The inactive form of monophosphate converts active adenosine triphosphate and inhibits the viral RNA polymerases. The nucleoside triphosphate is behaving as an adenosine triphosphate (ATP) analog ended with a natural ATP substrate to inhibit selective RdRp inside the virally infected cells [312]. RDV was originally developed to inhibit the spread of Ebola virus, however it was unsuccessful in clinical trials. Therefore, it was allowed immediately to enter into clinical trials for SARS-CoV-2 infection [313]. The antiviral activity of RDV (IC_{50} value) was found to be 0.99 uM in E6 cell model of SARS-CoV-2 [314]. The human trial of RDV with adults and pediatric SARS-CoV-2 patients (~12 years old and weighing 40 kg or more) in 5 and 10 days' courses showed that only the 5 days course of RDV demonstrated a statistically significant improvement when compared to the 10 days course and conventional standard of care [315,316]. Further, the efficiency evaluation of RDV with SARS-CoV-2 patients through randomized placebo-controlled trial demonstrated that RDV was superior to placebo in shortening the recovery time from the viral infection. Based on the clinical trials that clearly evaluated the clinical efficiency of RDV inpatients with SARS-CoV-2 (mild-to-severe infection), the U.S. Food and Drug Administration (U.S. FDA) has approved RDV for the treatment of adults and pediatric hospitalized patients with SARS-CoV-2 (over age 12 years and weighing at least 40 kgs or more). It was also recommended for the treatment of severely suspected or laboratory confirmed infection of SARS-CoV-2 and patient with severity being described as $SpO_2 \leq 94\%$ on room air with additional requirement of oxygen, mechanical ventilation and extracorporeal membrane oxygenation [317,318]. However, later it was proved that there is no evidence of RVD improving the survival and outcomes of therapies. Based on this, WHO has insisted and issued a conditional recommendation against the use of RDV as per November 20, 2020 report [319,320].

8.1.2. Favipiravir

Favipiravir (FPV) is a modified pyrazine analog (also known as 6-fluoro-3-hydroxy-2-pyrazine carboxamide, T-705). It was actually approved for antiviral resistant influenza in Japan. It could successfully inhibit the replication of A and B influenzas and avian influenza. The cellular enzymes convert inactive FPV to an active form of favipiravir-ribofuranosyl-5′-triphosphate (FPV-RTP) by ribosylation phosphorylation. As it is recognized as a substrate by RdRp, it prevents the elongation of RNA strand and viral proliferation by inhibiting viral replication and transcription [321–323]. The interesting fact that FPV shows excellent antiviral effects against other RNA viruses (e.g. bunyaviruses, filoviruses and arenaviruses), all of which are identified to cause fatal hemorrhagic fever. This unique antiviral profile makes FPV as a prominent potential drug for specific untreatable RNA viral disease. Both the purine nucleosides and FPV has a conflict for the same binding site, hence, the existence of purine nucleoside analogs as an antiviral agent that could prominantly inhibit FPV [324–326]. The EC_{50} value was noted to be 61.88 μM/L for FPV towards Vero E6 cells infected with SARS-CoV-2. The FPV bioavailability is very high at about 98% in human. Its metabolites are mostly renally cleared, and its half-life is calculated to be 2 to 5.5 h [327]. The clinical trials were executed for FPV towards SARS-CoV-2 in China and Italy in Mid-2020 as both the countries were severely affected in that time period. The FPV doses were started with 1600 mg/day (twice) for a few days, followed by 600 mg twice a day for the next 9 days. It acts as a mutagen and has shown 3-fold increase in total mutation than control [328,329]. Recently, the repeat dose toxicity studies in rats, monkeys and dogs showed the notable adverse effects of oral favipravir on hematopoietic system such as the reduction of red blood cell (RBC) production and increase of the liver function parameters (e.g., alanine aminotransferase, total bilirubin, aspartate aminotransferase, alkaline phosphatase and increased vacuolization in hepatocytes). Besides, testis toxicity was also noted. Hence, the administration of favipiravir should not be recommended for women suspected or confirmed for pregnancy [330].

8.1.3. Sofosbuvir (2'-F, Me-UTP)

Sofosbuvir (SFV) is a pyrimidine nucleoside analog with a hydrophobic masked phosphate group used for SARS-CoV-2 treatment due to

its potential advantages such as low toxicity and high stability of the active molecule [331]. The hydrophobic masked phosphate group of SFV enables it to uptake into the infected cells, and then, it gets changed into its active triphosphate form by cellular enzymes [332]. The activated drug (2'-F, Me-UTP) attaches with RdRp active site using its fluoro and methyl groups at 2' position and prevents RNA chain extension. Furthermore, it performs as a RNA polymerase inhibitor by competing with natural ribonucleotides. Further, it has lower incorporation activity into SARS-CoV-2 polymerase than Uridine-5′-triphosphate (UTP) [333]. It is worth noting that SFV as a single agent, has mild toxicity. The common adverse effects of SFV are headache and fatigue. Currently, the Food and Drug Administration (FDA) Label warns of a risk of symptomatic bradycardia such as low blood pressure, shortness of breath, chest discomfort and pulmonary edema when Epclusa (sofosbuvir (400 mg)/velpatasvir (10 mg)) is used in combination with amiodarone [334].

8.2. Therapeutic agents to target viral proteases

The viral replication depends on the proteolytic cleavage of either one or several viral polyproteins encoded by the virus genetic information. These proteolytic processes are essential for the functions of polyproteins and cleavage of the host protein, facilitating infection, and for the cellular entry and viral replication of SARS-CoV-2. Therefore, viral proteases are a major target in the development of SARS-CoV-2 drug. The diverse range of repurposed drugs and their targets are mentioned in Fig. 14.

8.2.1. Ivermectin

Ivermectin (IVM) is a familiar anti-helmintic agent used as an antiviral agent against flaviviruses such as Japanese encephalitis, dengue fever, chikungunya and tick-borne encephalitis [335,336]. It is worth noting that there are no approved indications by FDA for the above-mentioned applications. However, its anti-inflammatory properties make it ideal for veterinary use for diseases caused by parasitic worms such as onchocerciasis and intestinal strongyloidiasis [337,338]. In SARS-CoV-2 infection, the nuclear transport of viral protein is essential for viral replication. IVM targets importin α/β1 heterodimer mediated transport of viral proteins by dissociating IMPa/b1 heterodimer. It also prevents the transport of viral protein into the nucleus [339]. Hence, IVM causes hyperpolarization by triggering gamma amino butyric acid (GABA)-gated-Cl^- channels and paralyses the infesting organism [340]. Besides, IVM affects the host response immunomodulation by increasing IL-6 and C-reactive protein levels and activating the neutrophils [341]. It has been reported that 5 μM of IVM could curtail SARS-CoV-2 RNA up to 5000-fold at 48 h without any toxicity. The IC_{50} value of IVM was calculated to be ~2 μM towards SARS-CoV-2 [342]. Notably, the over dosage (higher than recommended dose) with orally administered formulation found to be lethal in mice models with death preceded by significant ataxia, ptosis, bradypnea, emesis, tremors, decreased activity and mydriasis [341,342].

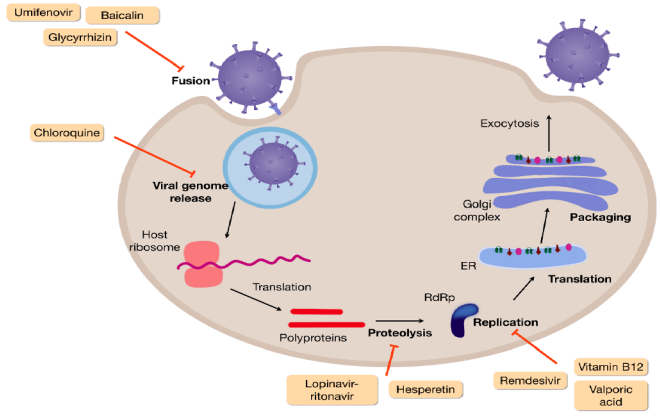

Fig. 14. Schematic illustration showing the role of different repurposed drugs in targeting SARS-CoV-2.

8.2.2. Lopinavir/ritonavir

Ritonavir (RV) is a small peptide molecule developed by Abbott Laboratories in 1996 that can inhibit HIV protease. Then, an enhanced second-generation human immunodeficiency virus 1 (HIV) protease inhibitor, known as lopinavir (LPV) was developed [343]. Retropepsin is a HIV-1 aspartyl protease enzyme liable for cleaving the structural viral Gag polyprotein, which performs a crucial role in HIV viral life cycle [344]. Peptidomimetic LPV drug consists of a hydroxyl ethylene scaffold that mimics the normal peptide linkage cleaved by HIV protease, resulting in the inhibition of HIV protease [345]. It causes the production of immature and non-infectious virus particles and proteolysis of Gag protein. In other words, LPV delivered alone causes low human bioavailability of ~25%, but in the case of co-administration with ritonavir, it improves LPVs bioavailability and reduces the drug metabolism [343]. The previously published Lancet report discloses that the amalgamations of lopinavir, interferon, ritonavir, and ribavirin have found to be very effective in targeting SARS-CoV-2 infection at the earlier stage. The combinational antiviral therapy divulged superior effects and shortened hospital duration in both combination and control groups and the duration was calculated to be 7 and 12 days ($p = 0.001$), respectively [346]. Further, the oral Kaletra solution is highly concentrated (contains approximately 42% ethanol) and posing a potential risk of overdose in children and infants. The common side effects of overdose in infants are cardiogenic shock, complete AV block, cardiomyopathy, lactic acidosis and acute renal failure. Further, the drug-drug interactions present in the formulations may also induce adverse side effects. The other effects observed are hepatotoxicity, pancreatitis and allergic reactions/hypersensitivity [347].

8.3. Nasal spray

Nasal drugs /spray have been developed for disinfection and treatment for SARS-CoV-2. To the obvious fact that the viral loads are expelled out of nasal cavities from infected patients, disinfecting nasal cavities can help to decrease the risk of infection in uninfected person as well as to reduce the viral load in infected person [377]. Few of developed products are given in Table 6 along with their working mechanism.

8.4. Therapeutic agents for blocking the virus–cell membrane fusion

8.4.1. Recombinant human angiotensin-converting enzyme 2 (rhACE2)

The rhACE2 is also known as APN01, can block the SARS-CoV-2 entry into the host cell by interfering with the interaction between S protein and the ACE2 receptor of host cells [385]. A recent report stated that SARS-CoV-2 infected human kidney and blood vessel organoids treated with rhACE2 could block early entery of SARS-CoV-2 infection in host cells by a factor of 1000–5000 times [386]. The rhACE2 could preclude further ACE2 receptor activation, thereby perpetuating pulmonary vascular integrity and inhibiting acute respiratory distress syndrome (ARDS) [387].

8.4.2. Hydroxychloroquine and chloroquine

Hydroxychloroquine (HCQ) and chloroquine (CQ) are long-standing oral drugs specifically used to treat malaria and chronic inflammation. These two drugs have unique properties such as lipophilic weak bases and superior diffusion into organelles membrane (e.g. cell membranes, lysosomes, endosomes and Golgi vesicles). Both drugs become protonated, trapped in the organelles and upsurging the pH in the cell interior [388]. It is postulated that the raise in endosomal pH prevents the viral particles fusion and their cellular entry [389]. Also, they are known to interfere in the glycosylation of ACE2. This can make spike protein-

ACE2 binding less efficient and impedes the virus's entry into the cells [390]. HCQ and CQ can prevent the antigen processing, T-cell activation, TLR-cyclic GMP-AMP Synthase (cGAS), CD154 expression and down-regulate the pro-inflammatory genes [391]. The EC_{50} values for HCQ and CQ towards SARS-CoV-2 were estimated to be 6.14 and 23.90 μM, respectively [392]. However, both HCQ and QC trigger severe side effects, including neuropsychiatric effects, hypoglycemia, retinopathy and QTc prolongation [393,394].

8.5. Peptidomimetics inhibitors

8.5.1. Therapeutic efficiency of peptidomimetics inhibitors for SARS-CoV-2

Peptidomimetics are compounds with pharmacophore as an essential element that mimics natural compounds such as peptides and proteins to interact with biological targets. This alternative can overcome the limitations of naturally available peptides, i.e., limited stability against proteolysis and poor bioavailability. Notably, they improve the receptor selectivity on the one hand while lowering the degradation rate by peptidases on the other hand. These special properties make them potential candidates for targeting SARS-CoV-2 [395]. Previous investigations demonstrate the potential of peptidomimetics in developing peptide-based therapeutics. Yan et al. have shown the structural image of human ACE2 with the SARS-CoV-2 RBD using cryo-electron microscopy. Notably, RBD was bound to ACE2 extracellular peptidase domain [396]. In an another investigation, a detailed study on targeting membrane fusion revealed that S2 site of the spike (S) protein facilitates the fusion, which includes the fusion peptide (FP), heptad region, namely HR1 and HR2, transmembrane site and a cytoplasmic tail. While S2 creates the hydrophobic α helix interface due to HR1 and HR2 sites' interaction following the receptor binding process [397], the membrane fusion begins as soon as the hydrophobic FB gets inserted into the host cell. Similarly, M. Hoffmann et al. reported that SARS-CoV-2 entry into the human cell is mainly dependant on ACE2 and TMPRSS2 [137]. This type of study and other research reports could provide a fundamental outline for the development of therapeutics.

8.5.2. Therapeutic efficiency of inhibitors for HR-1 site of SARS CoV-2 as target

Recently, Xia et al. carried out a study related to derivatives of SARS-CoV-2 HR2 peptides [398]. They prepared HR1 and HR2 based peptides called as 2019-nCoV-HR1P and 2019-nCoV-HR2P, respectively. The derivatives had been designed in combination with pan-CoV fusion inhibitor (EK1 peptide) and their biological properties were studied [399]. A similar peptide derived from HR2 site was successfully used against SARS-CoV to inhibit the fusion process. It inhibited the process of binding of HR1 region with HR2 region and prevented the pore formation. While the authors reported that 2019-nCoV-HR2P and EK1 displayed inhibitory activity with IC_{50} of 0.18 and 0.19 μM, respectively. Interestingly, 2019-nCoV-HR1P did not show any such effects up to 40 μM of concentration. Additionally, 2019-to-HR2P and EK1 showed prominent inhibitory effect on SARS-CoV-2 infection in ACE2 expressed 293 T cells with IC_{50} values of 0.98 and 2.38 μM, respectively [400].

As peptide-based therapeutics have half-life timeless in in-vivo, the lipid conjugation can increase the antiviral potency and improve the pharmacokinetics. To get more detailed information, Xia et al. developed a series of lipopeptides to 'C' end of EK1 by connecting the cholesterol through polyethylene glycol (PEG) and/or an amino acid (GSG) [401]. This design (also termed as EK1C4) is very effective against pseudotyped SARS-CoV-2 and S-mediated cell–cell fusion of SARS CoV-2 when compared to EK1 therapy. The IC_{50} values were estimated to be 1.3 and 15.8 nM for pseudotyped SARS-CoV-2 and S-mediated cell–cell fusion of SARS CoV-2, respectively. It also demonstrates a good inhibitory effect against SARS CoV and MERS CoV. In vivo studies showed better prevention against HCoV-OC43 infection in mice models. The investigations on pre and post-infection showed that EK1C4 could effectively inhibit SARS-CoV-2 activity. Similarly, Zhu et al. developed a lipopeptide fusion inhibitor based on HR2 sequence called as IPB02, which displayed a good response in preventing SARS-CoV-2 infection [402]. The inhibition effect was determined by an assay of dual split protein with an IC_{50} of 0.025 μM whereas single-cycle infection assay exhibited the IC_{50} value of 0.08 μM.

8.5.3. Inhibitors for RBD of spike (S) protein as target

Peptide-based inhibitors can prevent the SARS-CoV-2 initiation to host cell entry by binding to ACE2 receptor through RBD of S protein. A report by Zhang et al. shows ACE2 peptidase domain (PD) α1 helix as an essential component for attaching the SARS-CoV-2-RBD using molecular dynamic simulations [403]. The authors had prepared a natural 23-mer peptide from the human ACE2 (hACE2) α1 helix (called as SBP1) and investigated the peptide-protein binding processes using kinetic binding assays. It showed that SBP1 binding with SARS-CoV-2 was strong with a dissociation constant (KD) of 47 nm and equivalent with that of full-length hACE2. Then, the biotinylated SBP1 prevents the contact between host cell receptor (ACE2) and RBD domain of SARS-CoV-2, and inhibiting the viral entry into host cells. However, a real-time investigation is needed to prove its efficiency against SARS-CoV-2. Recently Han et al. have screened compounds that could mimic ACE2 using computer modelling [404]. The XRD crystal structure investigations revealed that 15 amino acids from the ACE2 receptor can bind with the RBD of viral S protein during the cellular entry into host cells [405]. This report also suggests four peptides with this region were considered and involved with two α-helices extracted from PD of ACE2. Among four peptides, two have shown stable structure and interacted strongly with RBD of SARS-CoV-2. From all these studies and findings, it has been understood that at least one peptide could bound to the spike protein and block the interaction of SARS-CoV-2 with ACE2. Through computational findings, Barh et al. state that many anti-S protein peptides can inhibit the viral entry [404]. The interaction of spike protein of SARS-CoV-2 with peptides was identified by HPEPDOCK protein-peptide docking server. After extensive investigations, researchers have identified 10 peptides that could be effectively used against SARS-CoV-2. Similarly, Huang et al. have designed many peptides through computational approaches and showed their interaction with SARS-CoV-2 [406]. Researchers have also remade the peptide designs using the structural bioinformatics and logo analyses to enhance the binding affinity to RBD of SARS-CoV-2 [404]. Thus, these computational approaches in combination with in-vitro and in-vivo experiments could lead to the design of appropriate therapeutics [407].

8.6. Small molecules

8.6.1. Therapeutic efficiency of inhibitors for TMPRSS2 as host target

Nafamostat, a serine protease inhibitor used for disseminated intravascular coagulation (DIC), has an ability to prevent SARS-CoV-2 host cell entry by preventing the pathway through TMPRSS2 [408]. By performing dual split protein (DSP) reporter assay using spike protein of SARS-CoV-2, Yamamoto et al. have shown that nafamostat inhibits the fusion activity and blocks the SARS CoV-2 replication in pulmonary Calu-3 cells with EC_{50} of 10 nM after pretreatment [409]. The same group similarly used DSP to identify nafamostat as an inhibitor for MERS' fusion spike protein. It effectively impacted the terminating viral replication up to 100-fold at a minimal concentration of 1 nM. The viral activity decreased by 300-fold when added during the infection suggesting good antiviral activity. Nafamostat showed inhibitory effect towards TMPRSS2 serine proteases to prevent SARS-CoV-2 S protein-mediated cell fusion in the range of 10–1000 nM concentration. From previous reports and outcomes, it has been understood that camostat-mediated viral entry inhibition never surpassed 65% even at high drug concentration of upto 100 M. It revealed 35% of the SARS-CoV-2 used endosomal cathepsins for cell entry [410]. Camostat could also inhibit SARS-CoV-2 by 50 to 60% in TMPRSS2+ cell lines with no side effects, and the inhibition efficacy is increased to 100% by the addition of E64d

[147]. Through animal studies, it was found that camostat mesylate controlled the viral entry by 10-fold in calu 3 airway epithelial cells and interestingly increased the mice's survival rate [411,412]. Camostat is known to inhibit an in-vivo infection caused by a virus that utilizes TMPRSS2, and could be a suitable component of a drug combination against SARS-CoV-2.

8.6.2. Therapeutic efficiency of inhibitors for Cat/BL as host target

Oxocarbazate inhibitor CID23631927 has revealed better anti-viral effect and selectivity towards CatL of SARS-CoV [413,414]. It is noteworthy that oxocarbazate inhibited CatL with IC_{50} and inhibitor constant (K_i) value of 0.4 and 0.29 nM, respectively. The CID23631927 has successfully inhibited SARS-CoV entry in human embryonic kidney 293 T cells, and there was no toxicity up to the concentration of 100 μM observed in human aortic endothelial cells [415]. Based on this, oxocarbazate inhibitor can be a potential therapeutic agent for treating SARS-CoV-2.

8.7. Plant-based molecules for targeting SARS-CoV-2 proteins

Most of the plants have the potential to mitigate the new SARS-CoV-2 infection. Numerous traditional herbal remedies also inhibited SARS-CoV-2 infection in a healthy person and enhanced patient health state with severe or mild symptoms [416]. For example, Runfeng et al. reported an herbal mixture described as Lianhuaqingwen that consists of a mineral medicine (gypsum and menthol) and a mixture of 11 medicinal species [416]. It showed strong anti-inflammatory and inhibitory effects towards Vero E6 cells infected with SARS-CoV-2. The herbal mixture also restricted SARS-CoV-2 replication, chemokine (C—C motif) ligand 2/monocyte chemoattractant protein-1 (CCL-2/MCP-1) and pro-inflammatory cytokines such as IL-6, C–X–C motif chemokine 10/interferon-γ-inducible protein 10 (CXCL-10/IP-10) and TNF-α in a dose-dependent manner. The IC_{50} value of lianhuaqingwen was noted to be 411.2 μg/mL against SARS-CoV-2 [416,417]. By virtually screening 83 compounds from traditional medicines, Lung et al. identified that theaflavin, an antioxidant polyphenol, showed inhibitory activity against SARS-CoV-2 RdRp [418]. Similarly, Zhang et al. virtually screened 115 traditional medicines and highlighted 13 medicinal plants consisting of naturally occurring polyphenolic compounds such as kaempferol and quercetin, which are of considerable interest in the treatment of SARS-CoV-2 [419]. Recent reports showed that phytochemicals such as 3,5,7,3′,4′,5′-hexahydroxy flavanone-3-O-β-D-glucopyranoside, amaranthin, methyl rosmarinate, licoleafol, calceolarioside B, myricetin3-O-β-D-glucopyranoside, myricitrin, (2*S*)-eriodictyol 7-O-(6″-O-galloyl)-β-D-glucopyranoside and 5,7,3′,4′-tetrahydroxy-2′-(3,3-dimethylallyl) isoflavone could be potential anti-viral agents against SARS-CoV-2 [420].

The plant-based chemical components have high potential to inhibit the viral proteins effectively and block the replication/transcription complexes. The major targets of SARS-CoV-2 are PLpro, 3CLpro, RdRP and S protein. PLpro enzyme provides a significant contribution in the SARS-CoV-2 replication and maturation processes [421]. Similarly, 3CLpro enzyme plays a crucial role in SARS-CoV-2 life cycle by directly triggering the nsps maturation [422]. The essential viral enzyme 3CLpro monomer consists of three domains, termed as domain I (8-101aa residues), domain II (102–184 aa residues) and domain III (201–303 aa residues), and a long loop (185–200 aa residues) that attaches domains II and III. The 3CLpro active site has a Cys-His catalytic dyad (Cys145 and His41) that placed in the space between domains I and II. Hence, a detailed investigation on the 3CLpro structure and its catalytic mechanism might provide more information for the anti-SARS-CoV-2 drug development. The RdRp, also named nsp12 is a conserved protein needs to form SARS-CoV-2 replication/transcription complex by catalyzing the RNA replication from a RNA template [174]. Therefore, nsp-12 has high potential for use as prominent therapeutic target. In other words, the S protein cleavage activation and its structural integrity performs a prominent role in SARS-CoV-2 virulence and invasion. Notably, the blocking of viral entry into host cells by targeting either S proteins or the host cell surface receptor is valuable for developing the suitable therapeutic strategies. Thus, these four structural and functional proteins make attractive targets for SARS-CoV-2 drug development [55].

Recently, the molecule-protein docking was carried out between different molecules of plants and reported targets of SARS-COV-2. The use of plant-derived phytochemicals with proven antiviral activities such as quercetins, flavonoids and gallates are effectively able to inhibit the proliferation of SARS-CoV-2 [423]. More specifically, it is believed that the plants with high phenolic compounds such as *Satureja hortensis*, Zataria multiflora, *Rosmarinus officinalis*, Thymus spp., *Eucalyptus caesia*, Mentha spp., and Artemisia kermanensis could be very effective in controlling SARS-CoV-2 infections [424,425]. Recently, an aqueous extract of *Senna alata* (a large genus of flowering plants in the sub family of Caeslpinioideae and family of Fabaceae) was demonstrated to show better relief from the virus symptoms in SARS-CoV-2 patients. Currently, it has been used in the treatment of malaria, flu, fever and other medical conditions [426]. Similarly, Nicotiana benthamiana has a significant place in plant based vaccine preparation. It is a SARS-CoV-2 RBD based vaccine and has shown good ability to induce positive SARS-CoV-2-specific immunity when formulated with CpG adjuvant in pre-clinical trials (status trial phase I/II, NCT04473690) via intramuscular immunization [427,428]. It is being developed by British American Tobacco company through Kentucky BioProcessing unit (KBP, biotech subsidiary in the US). Besides, Medicago Inc. (Quebec City, QC, Canada) is also developing virus like particles (VLPs) (NCT04636697) to combat SARS-COV-2 using N. benthamiana [429]. They have successfully developed VLPs having structural similarity with actual corona virus using Covid-19 spike protein and lipid membrane of Nicotiana benthamiana plant. As it is lacking nucleic acid, it is obviously noninfectious. The clinical study with volunteer people has shown an enhanced ability to induce the antibiotic response in human [430,431]. Further, the VLPs developed with influenza virus have also demonstrated good biocompatibility, safety and efficacy in animal models and human clinical trials [432]. The vaccine developed from tomato and low nicotine tobacco plants have also shown stable expression of S protein (S1) against SARS. Notably, it exhibited a significant increase in amount of SARS-CoV-specific antibodies after immunization in mice model. It can be concluded that the plant based vaccines developed so for have shown promising results in pre-clinical trials [433,434]. Hence, any continued efforts in this direction might result in plant based safe vaccines for SARS-CoV-2 in near future (Table 7).

8.8. Stem cell therapy for COVID-19

Stem cells (SCs) have the ability to differentiate into a variety of diverse cell types in the body. Self-regenerative and differentiation ability of certain types stem cells (e.g. mesenchymal stromal cells (MSCs)) plays an important role in stimulating the regeneration of alveolar epithelial type II cells by secreting vascular endothelial and hepatocyte growth factors [444,445]. Alternatively, various kinds of chemokines present at the site of Inflammation can also attract MSCs and its secretion of immunoregulatory cytokines to modify the functioning of various immunocytes such as dendritic cells, NK cells, T cells, B cells, macrophages and neutrophils [446]. Notably, the factors such as the transforming growth factor β, prostaglandin E2, Indoleamine 2,3-dioxygenase and human leukocyte antigen isoform have been recognized as the major effectors in the above-mentioned processes [447]. Thus, MSCs may provide a therapeutic alternative for patients with severe or critical COVID-19 symptoms either by repairing the lung damage or inhibiting the over-activated inflammatory response and influencing the progression of pulmonary fibrosis. The MSC therapy has been shown to minimise pulmonary lesions and limit the inflammatory response generated by influenza virus infection in both human and animal models [448,449]. The efficacy and safety of MSC treatment in patients with

acute respiratory distress syndrome (ARDS) have also been studied. The intravenous transfusion of MSCs in moderate or severe COVID-19 patients was proven to be safe and well tolerated in recent phase-1 clinical trials [451]. For the first time, the intravenous MSC therapy has improved the clinical outcome of COVID-19 patients while displaying good immunological tolerance in critically ill patients [451]. Similarly, menstrual blood-derived MSCs (MB-MSCs) were used in a clinical trial for severe patients, and it was discovered that MSC transplantation could help in treating COVID-19, particularly in ICU patients. In a recent study, the infusion of umbilical cord MSCs (UC-MSCs) into COVID-19 patients with moderate or severe disease was found to be safe in phase 2 and 3 studies with 96-week follow-up course [452]. When the influence of high dose of MSCs (upto 200×10^6 cells) and exosomes produced from allogeneic MSCs were tested for therapy efficacy, the treatment was found to be well tolerated and showed prospective improvement in some clinical measures [453]. Further, COVID-19 patients have also been treated using stem cells other than MSCs such as human embryonic stem cell-derived immunity- and matrix-regulatory cells (hESC-IMRCs) [450].

8.9. Molecular docking against 3CLpro, PLpro and RdRp

The docking score was recently assessed between 3CLpro crystalline structure (5R7Y) and medicinal plant compounds by Glide XP protocol. The top three scoring compounds are rutin, rocymosin-B and verbascoside, which are mostly extracted from various medicinal plants such as *Glycyrrhiza glabra*, *Allium myrianthum, Anastatica hierochuntica* and *Marrubium vulgare*. These compounds are binding to Cys-His catalytic dyad (Cys145 and His41) of 3CLpro. The plant compounds are also binding with 3CLpro amino acids' active site via hydrogen bonding and polar contributions [453,454]. The phytochemical, verbascoside was bound to the crystal structure of the PLpro (PDB ID: 6W9C) active site [455]. The extracted luteolin-7-rutinoside from *Cynara scolymus* is an active compound, easily interacts with PLpro amino acid residues by hydrogen bonds, π–π stacking and negatively charged interactions. A phytochemical component of *Cichorium intybus, Olea europaea* and *Marrubium vulgare* called as caftaric acid that displayed an inhibitory efficacy against the crystal structure of RdRp with cofactors (PDB ID: 6M71). Notably, it has the docking score of −10.664 kcal mol^{-1} and interacts with RdRp amino acid residue by π–π stacking interactions and polar interactions. Another compound, named fenugreekine isolated from *Trigonella foenum graecum*, possessed a docking score of −9.894 kcal mol^{-1}. It interacts with RdRP active site via Van der Waals interactions [456]. Similarly, hesperidin extracted from *Citrus aurantium L* provides a significant contribution in targeting the binding boundary between ACE2 and S protein by laying on the RBD surface middle shallow pit. By superimposing ACE2-RBD complex to hesperidin-RBD complex, hesperidin may inhibit the interaction of ACE2 with RBD. Hence, the plant derived components such as luteolin 7-rutinoside B, verbascoside, rocymosin rutin, caftaric acid, fenugreekine and cyanidin 3-(6″-malonylglucoside) have shown promising potential for further drug development.

In a recent report, the antiviral potential of PF-00835231 for the inhibition of SARS-CoV-2 was investigated. The study revealed that it is the active component of the first-in-class 3CLpro-targeting regimen in clinical trials performed with 3D in vitro models of the human airway epithelium [457]. Similarly, three real-time reverse transcription-PCR (RT-PCR) assays targeting the RdRp/helicase (Hel), spike (S) and nucleocapsid (N) genes of SARS-CoV-2 have also been reported. Among these assays, the COVID-19-RdRp/Hel assay exhibited lower limit of detection in in-vitro (1.8, 50% tissue culture infective doses [$TCID_{50}$]/mL with genomic RNA and 11.2 RNA copies/reaction with in vitro RNA transcripts). Notably, the RdRp-P2 assay cross-reacted with SARS-CoV in cell culture experiments. Thus, the highly sensitive and specific COVID-19-RdRp/Hel assay might help us in improving the laboratory diagnosis of COVID-19 [458]. Further molecular dynamic simulation reports may substantiate the on-going investigations on anti-SARS-CoV-2 therapeutic agent's development from the natural origin [455].

9. Development of SARS-CoV-2 vaccines and ongoing clinical trials

Vaccines are described as substances of biological preparation contain weakened or killed agents resembling to disease-causing microorganisms or their surface protein/toxin. It can trigger the production of disease specific antibodies without prompting the disease by specifically acting as an antigen. As a consequence, it enhances the immunity against one or more viral diseases. In recent years, vaccine technology has significantly evolved for developing various kinds of vaccines ranging from cell-cultured vaccines, DNA and RNA vaccines and recombinant protein vaccines to licensed vectored vaccines for controlling and curing various deadly viral diseases [459]. Specifically, the SARS-CoV-2 spreading has created huge stress on the global population, both financially and emotionally. In normal circumstances, the average time to bring a vaccine to the international market is more than 10 years [460]. However, WHO had reduced this time duration to a matter of few months during the SARS-CoV-2 pandemic by providing the guidance to accelerate all clinical trials in short periods and simplifying the logistical procedure. Due to coordinated efforts from various governing bodies and countries across the globe, we are successful in getting many WHO approved vaccines for the treatment of SARS-CoV-2 in the end of 2020. However, the developed vaccines don't ensure safety and effectiveness against different variants of SARS-CoV-2. Further, the applicability of developed vaccines for the wider human population is also under investigation. Hence, a large scale screening on different phases of various vaccines are being performed extensively to develop a reliable, safer and effective SARS-CoV-2 vaccines for people of different age groups ranging from elders, middle age and kids to pregnant woman. In this section, vaccines prepared from different viruses (whole) or virus parts and their status in clinical trials are highlighted briefly [461].

9.1. Vaccines administered via intramuscular routes

9.1.1. Viral like particles (VLPs)

VLPs are protein mimicking structures having similar size, shape and morphology of real virus. As they lack nucleic acid/genetic property, they are noninfectious in nature. They can undergo self-assembly with different types of proteins and form chimeras, known as cVLPs. The immune system detects VLPs in the same way as real viruses, thereby by inducing immune responses [462,463]. Upon entry into the body, they trigger B- and T-cell immune responses by simulating the antigen presenting cells via antigen presenting cell mediated activation of B- and T-cells. They are also involved in developing CD8+ cytotoxic T-cell mediated destroying of pathogen cells. One such example for licensed vaccines based on this approach is the use of human papillomavirus. At present, many SARS-CoV-2 vaccine candidates developed using VLPs are in different stages of clinical evaluation, as shown in Table 8 [464].

9.1.2. Protein-based vaccines

The purified recombinant proteins from different etiologic agents are most prominent candidates under the investigation for vaccines. The protein-based vaccines are formulated by using the harmless protein fragment or protein shells that mimic the SARS-CoV-2 virus to stimulate the human immune response [459]. Notably, there are several examples for recombinant protein-based vaccines being used in humans, for example, hepatitis B vaccine (HBV). As discussed in the previous sections, S protein is playing an important role in host cell receptor binding and cell membrane fusion, thus, S protein based SARS-CoV-2 vaccines might improve antibody production and virus neutralization efficacy. As a result, S protein is mostly used as a recombinant protein subunit in many cell-based systems to induce the protein expression [465]. This

approach has shown better protection in immunized animal cells in in-vivo experiments. However, there is a clear risk of generation of polarized (T_H2 over T_H1) immune response, which is usually prevented by the use of suitable adjuvants. In this category, a saponin based matrix-M adjuvant vaccine known as Novavax has been recently reported, which has demonstrated 89% of efficacy against SARS-COV-2 in clinical trials conducted in UK [466]. It should be noted that there are many protein subunit-based vaccines in the development stage and clinical trials, however none of them are authorized for the use [https://jamanetwork.com/journals/jama/fullarticle/2777059].

9.1.3. Viral-vector vaccines

Viral vector vaccines use replication-deficient viruses to express the genetic sequence of the antigen in host cells. The genetically modified viruses such as adenovirus and measles are used as vectors to produce SARS-CoV-2 proteins in the human body because it reduces their virulence and cannot cause disease. It is divided into two types of vectors: one type is still replicated within human cells, and another one is disabled to divide due to the absence of key genes. These types of genetically modified vectors stimulate antibody production in the human body [467]. Currently, two viral vector vaccines, namely adenovirus serotype 26 vector vaccine (Ad26.CoV2.S) and chimpanzee adenovirus vector (ChAdOX), have shown promising results and considered as potential candidates for the treatment of SARS-CoV-2 [468].

9.1.4. Nucleic-acid vaccines

Nucleic acids such as DNA and RNA are inserted into human cells as a genetic instruction, which can induce an immune response [467]. DNA-based vaccines are non-replicating, non-infectious and provide long term immunogenicity to the host. Further, they are stable, less expensive, prepared in short time duration and easily getting degraded in host models. However, it has poor immunogenicity when used in humans [466]. Due to this, RNA-based vaccines are preferred over DNA-based vaccines. Often, mRNA based vaccines are directly injected into the host cell and allowed for translation in the cytoplasm. Currently, two types of mRNA based vaccines are established, namely self-amplifying mRNA and non-amplifying mRNA based vaccines. The self-amplifying mRNA-based vaccine technology has the capability to ramp-up vaccine production to meet the increase in demand for vaccines [469–471].

9.1.5. Inactivated vaccine

They are generally produced either by completely killing or inactivating the pathogen. When they are injected into the host, they induce protective antigen against epitopes that are present on the surface of real virus. However, these vaccines tend to produce the weaker immune response, thus it requires the adjuvants to provide an effective immune response [472,473]. While the inactivated polio vaccine is the better example for the completely killed (whole) pathogen, the tetanus and diphtheria vaccination is the example for subunit formulation [474]. Sinopharm and Sinovac are among the manufacturers farthest along in the development of this type of vaccine. They have also successfully completed phase 3 clinical trials for their vaccines and obtained international authorization for emergency use [466].

9.2. Intranasal vaccination

Nasal vaccination is considered to be a reliable and influencing method to prevent SARS-CoV-2 infection as the viral invasion mainly occurs via nasal mucosa. Among the available vaccines, adenovirus vector-based vaccines are considered as reliable vectors of antigens to hosts owing to its potential to induce both adaptive and innate responses [476]. They are safer, less expensive and produced in higher quantities to meet the demands [475]. Alternatively, SARS-COV-2-M2sr categorized under M2SR is another type of vaccine which is produced upon deletion of M2 gene that activates the immune response (innate and cellular) in the host [481–483]. The advantage of such vaccine is that it delivers multiple antigen targets to the immune system without producing progeny virus. Furthermore, DelNS1-nCoV-RBD LAIV categorized under live attenuated vaccine is another type of vaccine comprises of H1N1, H3N2 and B with genetic segments of S protein of SARS CoV-2, hence mimicking the infection to induce the immune response [484,485]. Mv-014-212 vaccine candidate developed by meissa vaccines is also undergoing phase 1 clinical trials. An inhaled therapy, named as CROWNase, developed by Illinois Institute of Technology, Chicago, USA is currently in preclinical trial. The S protein of SARS-CoV-2 makes the virus to interact with the hACE2 and causes infection. The S protein has human derived molecule coating which helps in overcoming the immune system of human being which leads to spread of infection easily. CROWNase works by removing the human derived molecule coating [486]. Similarly, CovMOV developed by Intravacc embeds viral S antigen in bacterial outer membrane vesicles and is in the preclinical trial [488]. AuraVax therapeutics has also proposed a liposomal stimulator of interferon genes or STING agonist to use as an adjuvant in vaccine for SARS-CoV-2 [489]. Notably, it triggers mucosal immune response and provides better protection against the virus. The list of aforementioned vaccines along with their current state is given in Table 9.

10. Challenges in vaccines development and commercialization

The introduction of the vaccine into the human body is described as vaccination that protects the body from specific infection by developing the resistance and strengthening the immune system against pathogens [490]. It is considered as a harmless and effective route to control or kill pathogens by triggering the immune cells to produce antibodies that react against the exposed disease. The immunization process saves several millions of people from more than 20 life-threatening diseases and makes them live longer and healthier by building resistance against specific diseases [491]. Up to 180 vaccines including inactivated or live-virus vaccines, vectored vaccines, recombinant protein vaccines, DNA and RNA vaccines have been developed against SARS-CoV-2. Most vaccine designs target the receptor binding proteins and membrane fusion process because of their key responsibilities in SARS-CoV-2 infection and pathogenesis. These events are mainly depending on the large viral surface protein, namely S protein. Specifically, most vaccines target S proteins or its domain protein, including RBD, to avert its attachment to the host cell surface protein to neutralize the SARS-CoV-2 virus. Interestingly, the greatness of the vaccines towards the SARS-CoV-2 S protein is somewhat different when compared to other vaccines. After confirming their activity and safety profiles in in-vitro experiments, the vaccines are being tested on human volunteers in a phased manner in clinical trials by increasing the size of volunteer population to investigate their safety in human population. Later, they are being administered to health care workers, older adults and people with underlying disease conditions such as diabetes and heart disease [492]. It could also induce life-threatening allergies for people who are in treatment for chemotherapy and chronic illnesses as it severely affects the immune system. Interestingly, it is very safe and effective in people with underlying conditions such as liver or kidney disease, asthma, diabetes and hypertension. The vaccine ingredients may stabilize and control these diseases [493]. However, these impacts may differ for every vaccine.

The major ingredients of vaccines such as weakened or killed antigen, adjuvants, preservatives and stabilizers play a crucial role in encountering the virus and boosting the host immune response. For instance, the added preservatives ensure a vaccine stays effective, and stabilizers are protecting the vaccine during storage and transportation [494]. After satisfying the potential benefit of immunization, the approval for commercial use of vaccines will be obtained from study investigators, regulatory agencies and overseeing ethical committees. The time duration from preclinical trial to manufacturing in large scale

often takes over a decade. However, the clinical trials of SARS-CoV-2 vaccines are performed in parallel for different phases to complete them in a short period. Common challenges faced by newly developed SARS-CoV-2 vaccines are listed in the following sections.

10.1. Ensuring the safety and effectiveness of vaccines

Most vaccines for SARS-CoV-2 disease have been developed in a short time span (within 10–16 months) in order to control the rate of infection and casualties around the globe. Hence, the concerns over the safety, effectiveness and side effects of newly developed vaccines need to be addressed in detail [495]. For example, the Strategic Advisory Group of Experts (SAGE) announced that the developed Pfizer-BioNTech COVID-19 mRNA vaccine is safe and effective to people. As of 14th October 2021, 23 vaccines have been approved for emergency use across the globe, as shown in Table 10. However, the vaccination is not recommended for some specific populations and it may be either due to lack of supply or limited data and contraindications [496]. Further, a certain fraction of population who had received the COVID 19 vaccine developed severe headache, abdominal pain, leg pain, shortness of breath and severe type of blood clot called cerebral venous sinus thrombosis (or CVST) combined with low levels of blood platelets (thrombocytopenia) [497]. As a result, the regular medical assessments and post-approval clinical studies are urgently needed to confirm their safety and effectiveness.

10.2. Long-term protection

Given the fact that the long-time protection of vaccine from specific disease is the main thing in preventing the infections, their ability of long-term protection should be fully investigated for newly developed SARS-CoV-2 vaccines. At present, most SARS-CoV-2 vaccines are given to people in two dose regimens. As reinfection of cases raise concerns over the immunity after vaccination, the additional studies need to be performed urgently to give further direction for the people who recuperate from SARS-CoV-2 disease in order to suppress the subsequent wave of infections. A detailed investigation on reinfections (even after vaccination) and the ability of vaccines against new mutations will shed more light on long-term protection of vaccines against SARS-CoV-2 [499].

10.3. Storage and transportation of vaccines

The storage and transportation mechanisms are also pose great challenges for the commercialization of vaccines as the existing installations are not able to cope up with the demands caused by larger vaccination schedules across the globe. The prepared vaccines are packed carefully in glass vials due to its ability to withstand extreme temperatures for safe cold vaccine storage and transport globally [500]. When a vaccine is too hot or cold, it becomes less effective or even inactive. If stored at the incorrect temperature, the vaccines can be ruined or unsafe for use. Most vaccines require refrigerated storage at temperature in the range between 2 and 8 °C [501]. Some vaccines even require ultra-cold temperature from −20 to −70 °C [502]. For frozen vaccines, the storage temperature is maintained at 2–8 °C. Since the regular refrigerators cannot consistently maintain these low temperatures, specialized medical refrigerators are required for these precious products. The messenger RNA (mRNA) based vaccines (mRNA-1273-Moderna and BNT162b2-Pfizer-BioNTech) are developed using the strands of mRNA held together within lipid particles. The prepared vaccines are vulnerable to degradation at room temperature and need doses to be frozen for transportation, then thawed for the use [503]. Therefore, the concerns regarding the storage temperature could slow down the rollout of SARS-CoV-2 vaccines. Similarly, the mRNA-1273 vaccine is stored at temperature in the range between −25 and −15 °C. As it is stable and active at −20 °C, it can be stored in standard −20 °C freezers meant for hospitals and pharmacies [504]. The Indian vaccines developed can be stored at a temperature of −20 °C, hence they can be stored at normal hospital refrigerators. However, the efficacy and other temperature related data is yet to be declared officially. For instance, Pfizer-BioNTech announced that BNT162b2 vaccine displayed 90% efficacy when stored at −70 °C. Similarly, the Sputnik V vaccine liquid form must be stored at −18 °C or below to maintain its stated 92% efficacy [505].

10.4. Mass production of plastic syringes, vials and needles

Up to 275 vaccines are being developed worldwide till date and 23 of them are being currently used in different countries. As most of the countries are facing frequent waves of infections and there is no sign for the end of Covid-19 pandemic, the need for vaccines along with syringes, needles and plastic vials for effective administration and storage of vaccines is growing with each passing day. Based on the available data, the USA has ordered about 850 million of syringes and needles for their two doses of vaccination [506]. Hence, the requirement across the globe has to be taken care while a series of vaccines are being rolled out. In addition, further developments are required on the design and mechanical integrity of glass vials for efficient storage and transport of vaccines. For instance, the parameters such as mechanical integrity, resistant to breakage, chemical stability, mechanical durability, thermal stability and compact designs/dimensions are not only playing a key role in maintaining the activity of vaccines but also preventing the loss of doses during the transportation and handling of vaccines. It is worth noting that they should be stable in the temperature range between −196 °C to 121 °C while having the chemical stability to handle liquids with pH range of 3–14 [507].

10.5. Vaccine hesitancy

Worldwide acceptance of vaccine is necessary to prevent further spread of SARS CoV-2 and improving the immune system. Vaccine hesitancy is predominant in upper middle/high-income countries (UMIC) than low and middle-income countries (LMIC). As per the recent report in LMIC (Asia, Africa and South America) and UMIC (Russia and US) with 44,260 participants, the willingness of people to take vaccine were found to be 80.3, 30.4 and 64.6% for LMIC, Russia and US, respectively. Even though large-scale production and availability of vaccine is limited in LMIC, the willingness to take vaccine seems to be comparatively higher. The personal interest for protection against SARS CoV-2 is the main reason for such huge difference between LMIC and high income countries. However, the hesitance towards vaccine is the fear of having side effects post vaccination. It is worth to mention that apart from fearful thoughts, misleading information from untrusted web sources could be the another reason of vaccine hesitancy [508]. The probable solution is seeking advice from the health expert even for very common doubts and not trusting any untrusted web-sources. It also seems to be the common practice of assuming the same side effects of post vaccination of one with other vaccinated individual. However, it is important to know that the side effects may vary among different individuals. The willingness to take the vaccine with trust is the key determinant in the successful vaccination campaign [509].

10.6. Global vaccine distribution plans (Covax)

COVID-19 vaccines global access (COVAX) acts as a pillar of the Access to COVID-19 Tools (ACT) Accelerator. ACT accelerator collaboration is done globally for the development, production and equitable access to SARS CoV-2 diagnostic kit, treatment and vaccines. Gavi, WHO and Coalition for Epidemic Preparedness Innovations (CEPI) in collaboration with COVAX and UNICEF (Key Delivery Partner) uphold the responsibility for the development, manufacturing of vaccine and equitable access for every country [510,511]. WHO had estimated that

US$1.7 billion was required in 2020 for SARS CoV-2 response in addition to government need for their respective national action plan and strategic preparedness and response plans (SPRP). The SPRP 2021 was approximated to be US$ 1.96 billion which will allow WHO to continuously support countries in terms of reducing transmission, countering misinformation, morbidity rates, equitable access and protecting the riskier groups. CEPI also focuses on accelerating the development of vaccines against emerging infectious threats and enabling equitable access to people. Similarly, UNICEF procures 2 billion doses of vaccine annually for approximately 100 countries. Further, Gavi, UNICEF and WHO are also heavily investing on reliable infrastructure, technical support and delivery of vaccines [512–514].

11. Future outlooks

Currently, SARS-CoV-2 is spreading rapidly as the third severely contagious human disease and has caused serious threat globally. Beyond doubts, it has now been widely accepted that the prevention of public gatherings and following quarantine strategies and standard operating procedures is the way to move forward for controlling SARS-CoV-2 infections. As it has coexisted with people for a long time, it has developed a niche in human beings. As a result, continuous monitoring of the gene alterations in new SARS-CoV-2 infections is required to promptly recognize any deletion or insertion in the genomic sequence. It will shed more light not only on the phenotypic related disease severity but also the development of new vaccines and medicines with required modifications in the structure. As the originating source of SARS-CoV-2 and the mode of cross-species infection is yet to be discovered, a detailed investigation needs to be done on the origination and transmission pathways of SARS-CoV-2 via the extra-respiratory and respiratory routes for further understanding on the rapid spreading of the virus. Further, the investigations on the molecular mechanisms of viral-host interactions and pathogenesis along with the molecular modulation of the host immune response during SARS-CoV-2 infection is urgently needed to determine the outcomes of medical therapies. These details will also help in the identification of specific biomarkers to describe the immune correlation of the disease. We believe that any support comes from theoretical studies can substantially improve the understanding in the above mentioned investigations and positively contribute to the development of different medical therapies.

Recently, various diagnostic methodologies have emerged for effective identification, monitoring and control of SARS-CoV-2 infections. However, most of the detection techniques are still relying on PCR and antibody based techniques that help in identifying and isolating the virus strains. Notably, other detection techniques such as RT-LAMP, CRISPR, LAMP, electrochemical sensing etc. are currently under development, and further experiments on sensitivity and repeatability are urgently needed. Further, the above mentioned methods have the capability to handle only the nasopharyngeal samples that limit their potential in the detection of SARS-CoV-2. As a result, any breakthrough in directly using blood and oral samples in the upcoming technologies may improve the reliability, sensitivity and reproducibility for SARS-CoV-2 detection. Various vaccines and therapeutic agents are being developed to fight against the SARS-CoV-2. They are most widely used to overcome the dysfunctional immune responses and suppress the viral infection to synergize and block pathologies at several steps. However, continuously evolving SARS-CoV-2 and mutating itself into different forms and safety concerns related to vaccines available in the markets demands alternative vaccines with good safety profiles. Hence, a rapid development of therapeutic agents/vaccines especially for targeting 3CLpro, RdRp and PLpro in combination with high-throughput and virtual screening tools could help us in developing effective and safer anti-SARS-CoV-2 therapy. Finally, a long-term efficient vaccine development against SARS-CoV-2 is also one of the important task as it provides an active acquired immunity to the host. At the same time, the correlation between the disease severity and immune dysfunction in patients with SARS-CoV-2, should serve as a note of caution in vaccine development and evaluation. At present, it seems that we are in the middle of never ending war against SARS-CoV-2.

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