Chapter 9
COVID-19: Recent Developments in Therapeutic Approaches

Umar Farooq Gohar, Irfana Iqbal, Zinnia Shah, Hamid Mukhtar, and Muhammad Zia-Ul-Haq

Introduction

Coronaviruses (CoVs) are either pleomorphic or spherical particles which envelop a single positive-stranded RNA genome [1] which is the largest of all known RNA viral genomes [2]. The earliest known coronavirus infection was a severe respiratory infection (SRI) of chicken, reported in the mid-1930s, and was named infectious bronchitis virus (IBV). Human coronaviruses (HCoV) were discovered in and after the mid-1960s, when in 1965 Tyrrell and Bynoe described the presence of an enveloped virion with the feature morphology of previously defined IBV. They named it B814 and proceeded to grow it on tissue cultures at which they could not succeed at that time [3]. Two years later, in 1967, Almeida and Tyrrell established the similarities in morphology of B814 and IBV through electron microscopy of fluid from inoculated organ cultures. They found the particles to be enveloped within a membrane coating, to be pleomorphic in shape, and to have multiple crown-like structures attached to their envelope's surface projections [1]. These crown-like structures are the envelope glycoproteins. Subsequent studies reported more viruses (e.g., 229E, OC43) with a similar morphology to IBV and B814, and in 1968 these viruses were grouped under a new name of "coronavirus" (corona means crown-like), reflecting their characteristic halo- or crown-like surface projections. Before the 2000s, HCoVs were long considered as inconsequential pathogens [4]. Only two HCoVs, 229E [5, 6] and OC43 [7], were known since the 1960s to

U. F. Gohar (✉) · Z. Shah · H. Mukhtar
Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan
e-mail: dr.mufgohar@gcu.edu.pk

I. Iqbal · M. Zia-Ul-Haq
Office of Research, Innovation and Commercialization, Lahore College for Women University, Lahore, Pakistan

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occasionally ever cause severe illness in healthy human subjects [2]. Causing only the "common cold," these viruses never surfaced important enough to be explored. In the twenty-first century, however, two highly pathogenic HCoVs, responsible for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), were identified for causing global pandemics in 2002 and 2012, respectively. These pandemics questioned the unexplored coronaviruses and their potential for causing future outbreaks. Genomic sequencing helped identify SARS-CoV [8] and MERS-CoV [9] as new but highly pathogenic human coronaviruses. Recently, a new strain of HCoV emerged as a local outbreak from a cluster of patients initially diagnosed with pneumonia of an unknown etiology. The infection now called COVID-19 steadily spread from a sea food market located in Wuhan, China, in December 2019 and rapidly transitioned into a global outbreak [10]. This is the third highly pathogenic human coronavirus discovered to date and is referred to as 2019-nCoV or SARS-CoV-2 (SC2). The name SARS-CoV-2 reflects its phylogenetic similarity with that of SARS-CoV [11].

Coronaviruses are taxonomically classified under the order *Nidovirales*, family *Coronaviridae*, by the ICTV: International Committee on Taxonomy of Viruses (Table 9.1). The coronavirus family is split into four genera: α-, β-, γ-, and δ-CoVs [2, 13] (Fig. 9.1). The genera α- and β- are found to be infectious toward mammals, while the other two are known to predominantly cause avian infections, but some γ- and δ-CoVs may also infect mammals [1]. There are seven known HCoVs, including SC2. Four of these are also called common HCoVs as people around the world get infected by these very often. The common HCoVs possess low pathogenicity and are responsible for mild infections – as that of "common cold." SARS-CoV, MERS-CoV, and SC2, unlike the common four, are highly pathogenic HCoVs with high mortality rates. *Betacoronaviruses* further have four lineages and SC2 belongs to lineage b of *Betacoronaviruses* – Fig. 9.2 [12]. SC2 is an alarmingly infectious virus which is capable of human-to-human transmission. Despite having a low mortality rate, strict follow-up of WHO safety guidelines is key to preventing its spread and, in worst-case scenario, its evolution into another novel coronavirus.

The evidence of interspecies transmissibility of coronaviruses was not clear to researchers until when a database of coronavirus gene sequences was made available. Table 9.2 shows lists of some coronaviruses and their hosts. Gene sequence

Table 9.1 Taxonomy of SARS-CoV-2

Lineage	b
Genus	*Betacoronavirus*
Subfamily	*Coronavirinae*
Family	*Coronaviridae*
Order	*Nidovirales*
Class	*Pisonivirecetes*
Phylum	*Pisuviricota*
Kingdom	*Orthornavirae*
Realm	*Ribovaria*

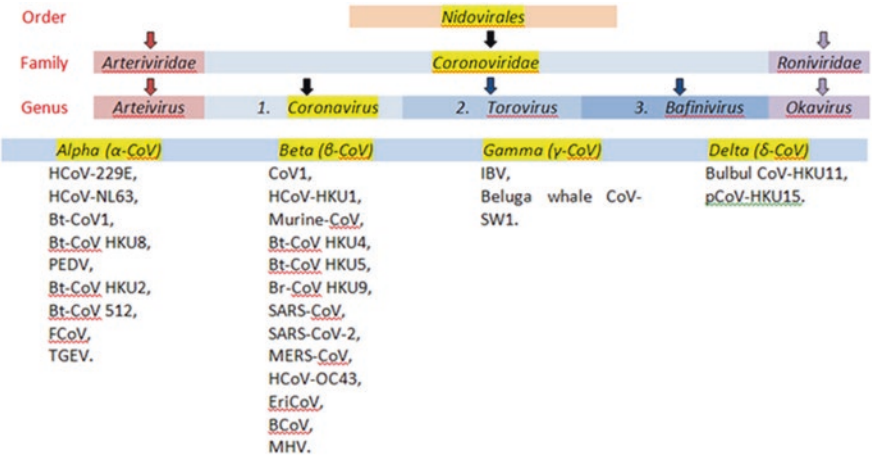

Fig. 9.1 Coronavirus classification. The families *Coronaviridae*, *Arteriviridae*, and *Roniviridae* fall under the order *Nidovirales*. *Coronavirus*, along with the genus *Torovirus*, and a new tentative genus *Bafinivirus* are established under *Coronaviridae* [2]. The genus *Coronavirus* consists of four genera: *alpha*, *beta*, *gamma*, and *delta* coronaviruses. The following coronaviruses for each coronavirus genus (α-, β-, γ-, and δ-CoVs) are shown [1, 12]: human coronaviruses (HCoV) 229E, NL63, HKU1, and OC43; *Miniopterus* bat coronavirus 1 (Bt-CoV1); *Miniopterus* bat coronavirus HKU8; *Porcine epidemic diarrhea virus* (PEDV); *Rhinolophus bat coronavirus HKU2* (Bt-CoV HKU2); *Scotophilus bat coronavirus 512* (Bt-CoV 512); Feline coronavirus (FCoV); transmissible gastroenteritis virus (TGEV); *Betacoronavirus 1* (CoV 1); *Murine coronavirus* (Murine-CoV); *Tylonycteris bat coronavirus HKU4* (Bt-CoV HKU4); *Pipistrellus bat coronavirus HKU5* (Bt-CoV HKU5); *Rousettus bat coronavirus HKU9* (Bt-CoV HKU9); severe acute respiratory syndrome-related coronavirus (SARS-CoV); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Middle East respiratory syndrome-related coronavirus (MERS-CoV); *Hedgehog coronavirus 1* (ERiCoV); bovine coronavirus (BCoV); mouse hepatitis virus (MHV); infectious bronchitis virus (IBV); *Beluga whale coronavirus SW1* (beluga whale CoV-SW1); *Bulbul coronavirus HKU11* (bulbul-CoV HKU11); and *Porcine coronavirus HKU15* (pCov-HKU15). As seen from the figure, the seven known human coronaviruses are either from genus *Alpha-* or *Betacoronavirus*. Three out of the seven HCoVs are highly pathogenic; these include SARS-CoV, SARS-CoV-2, and MERS-CoV, all belonging to beta-genera

analysis thus revealed that animals can transmit the virus to humans [1]; especially domestic animals can act as intermediate hosts to carry the virus from reservoir animals and pass it to humans. The ability of interspecies jumping has introduced highly pathogenic CoVs in human populations (Fig. 9.3). SARS-CoV was found to have gotten transmitted from bats (reservoir host) to civets (intermediate hosts) and then to humans. MERS-CoV was also found to have originated from bats and got transmitted to humans through camels [14]. Phylogenetic studies of SC2 also revealed bats as the reservoir animal, and Malayan pangolins are being identified as intermediary hosts [1]. These studies further emphasize the potential spillover of new pathogenic coronaviruses into the human populations in the coming future [11]. The alarmingly high diversity of coronaviruses being detected in bats also highlights the importance of using bat coronavirus models for designing strategies against any such future state of viral pandemic.

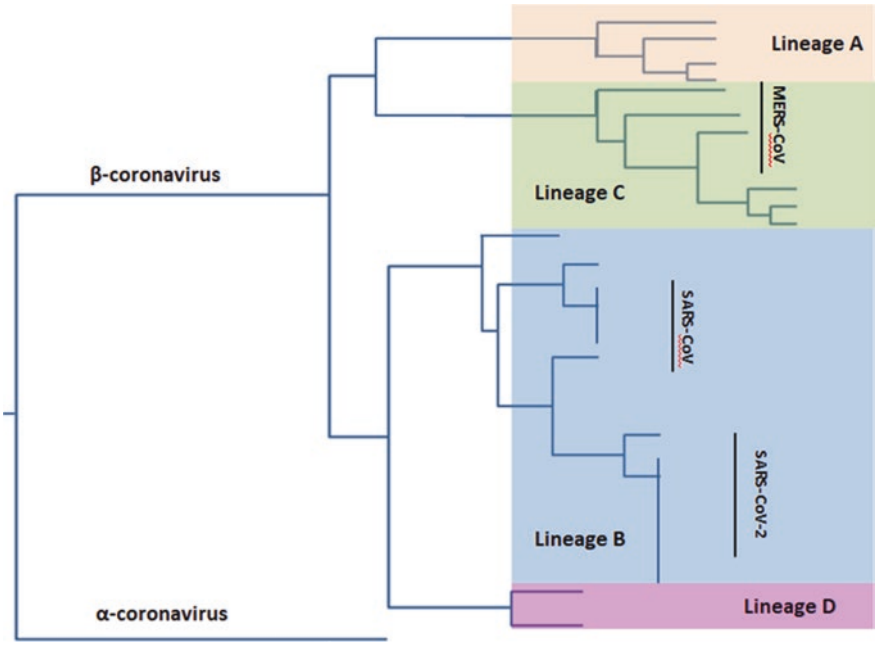

Fig. 9.2 SARS-CoV-2's relatedness with other coronaviruses as evident from protein sequencing studies

Table 9.2 Coronaviruses and their natural hosts

Host animal	Coronavirus
Humans	Human coronavirus 229E (HCoV-229E)
	Human coronavirus OC43 (HCoV-OC43)
	Human coronavirus HKU1 (HCoV-HKU1)
	Human coronavirus NL63 (HCoV-NL63)
	Severe acute respiratory syndrome-related coronavirus (SARS-CoV)
	Middle East respiratory syndrome-related coronavirus (MERS-CoV)
	Severe acute respiratory syndrome 2 coronavirus (SARS-CoV-2)
Cats	Feline infectious peritonitis virus (FIPV)
	Feline coronavirus (FCoV)
Dogs	Canine enteric coronavirus (CCoV)
Chicken	Infectious bronchitis virus (IBV)
Cattle	Bovine coronavirus (BCoV)

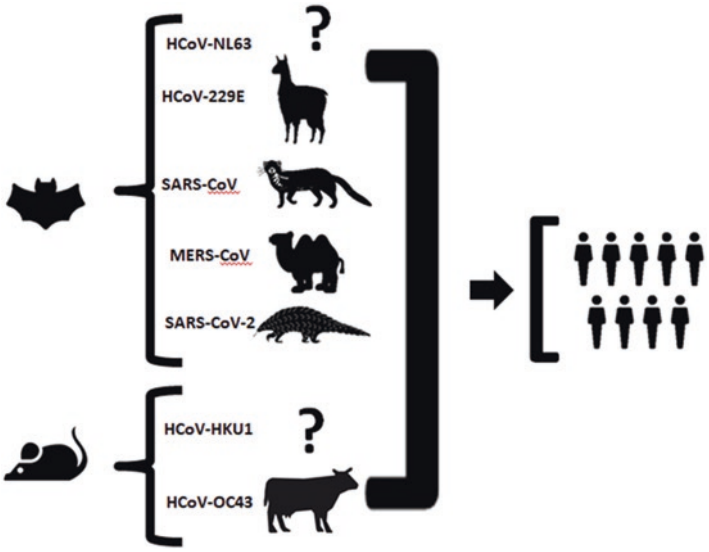

Fig. 9.3 Schematic representation of interspecies transmission route for coronaviruses

Morphology of SARS-CoV-2

COVID-19 has pushed humans in a new struggling battlefield against viral infections. Since December 2019, coronavirology has experienced an expeditious progress revealing morphological and genomic aspects and transmission and replication mechanisms of SC2, the study of which is crucial to decipher its effective therapeutic and preventive strategies.

All coronaviruses share a pronounced morphological and structural resemblance. Corona-virions are pleomorphic to spherical enveloped particles (Fig. 9.4). The diameter of a single corona-virion ranges from 120 nm to 160 nm; the electron microscopy of SC2 revealed a similar range for its diameter, i.e., 60–140 nm [15]. The high similarity shared among SC2 and SARS-CoV's genomic sequence (79%) suggests that the structural characteristics of SC2 would also be quite the same as those of its previously described relative, Table 9.3. The core of coronavirus particle consists of a non-segmented, 3′ polyadenylated, and 5′ capped ssRNA genome of positive polarity [16]. The coronavirus genome is about 27–32 kb which makes it the largest among all known viral RNA genomes [1, 16]. This genome is responsible for the expression of at least four major structural proteins: (N) nucleotide protein, (M) transmembrane protein, (E) envelope protein, and (S) spike proteins [17], all of which are located at the 3′ end of the genome [18]. The RNA genome is found coiled within a helical nucleocapsid which has a diameter of 9–11 nm [16]. The helical nucleocapsid is formed of N-proteins which are known to interact with the C-terminus of the surrounding M-proteins. N-protein is composed of two domains, NTD and CTD, N-terminal domain and C-terminal domain, respectively. Both

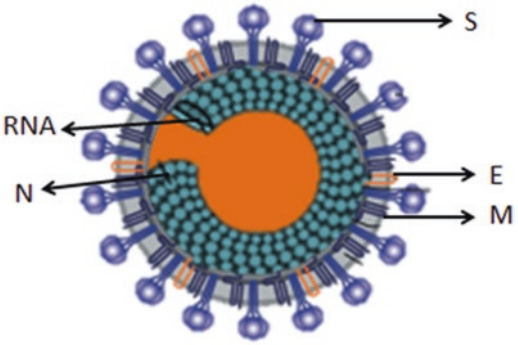

Fig. 9.4 A schematic representation of *Coronavirus* is shown. S spike protein – forms trimmers which are involved in receptor binding, M membrane protein, E envelop protein, N nucleocapsid protein, in all nidoviruses, a single N-protein forms the nucleocapsid which interacts with the genomic RNA as well as the membrane proteins

Table 9.3 Morphological and genomic characteristics of SARS-CoV, MERS-CoV, and SARS-CoV-2

Characteristic	SARS-CoV	MERS-CoV	SARS-CoV-2
Genomic length (nt)	29,727	30,119	29,891
ORFs	11	11	12
Structural proteins	4	4	4
Length of S-protein (aa)	1255	1353	1273
NSPs	5	16	16
Accessory proteins	8	5	6

domains are known to interact with the gRNA but through different mechanisms. The protein itself is found heavily phosphorylated; this phosphorylation is believed to enhance the affinity for viral RNA during viral assembly [17, 18]. The functions of N-proteins [19] have been enlisted in Table 9.4 along with the functions of other common coronavirus proteins [20–25]. The encapsulating membrane is a lipid bilayer derived from the host membrane. All surface proteins including S-, M-, and E-proteins are embedded in this host-derived lipid bilayer [26].

Coronavirus S-proteins are amazing molecules, weighing around 150 kDa [18]. They alone mediate the receptor binding and membrane fusion of viral cells with host cells [20]. S-proteins are heavily glycosylated and [18] exist in two conformations: a pre-fusion and a post-fusion conformation; the understanding of these conformations and factors which trigger its topology has helped identify potential structural targets for therapeutic purposes [57]. Changes in S-glycoproteins (20-nm-long club-shaped protrusions) are largely responsible for the variety in coronavirus tropism.

Table 9.4 Coronavirus proteins and their functions

Protein	Function		[Ref]
Spike (S)	Receptor recognition Membrane fusion		[20]
Envelope (E)	Mediate viral assembly Mediate viral release from host cell Involved in virus' pathogenicity		[22, 23]
Transmembrane (M)	Mediate viral release from host cell		
Nucleocapsid	Viral life cycle	Viral core formation Viral assembly Virus budding/envelop formation mRNA replication	[21]
	Cellular response	Chaperone activity Cell cycle regulation Host translational shutoff Immune system interference Signal transduction	
Accessory proteins	NSP1	Cellular mRNA degradation INF inhibition	[24, 27]
	NSP3	Polypeptide cleavage Blocks host innate response Promotes cytokine expression	[28, 29]
	NSP4	DMV formation	[30, 31]
	NSP5	Polypeptide cleaving IFN inhibition $3CL^{pro}$ M^{Pro}	[32–34]
	NSP6	DVM formation Restricts autophagosome expansion	[35, 36]
	NSP7	Cofactor	[37, 38]
	NSP8	Cofactor Primase	[37–39]
	NSP9	Dimerization and RNA binding	[40, 41]
	NSP10	Scaffold protein	[42–45]
	NSP12	Primer-dependent RdRp	[37, 39, 46]
	NSP13	RNA helicase 5′ triphosphatase	[47–49]
	NSP14	Exoribonuclease N7-MTase	[50–53]
	NSP15	Evades dsRNA sensors Endoribonuclease	[54–56]
	NSP16	2'-O-MTase Negative regulation of innate immunity	[25, 43, 44]

Abbreviations: *$3CL^{pro}$* chymotrypsin-like protease, *DVM* double-membrane vesicle, *dsRNA* double-stranded RNA virus, *IFN* interferon, *mRNA* messenger RNA, *M^{pro}* main protease

Of all the nidoviruses, only coronavirus and arterivirus particles possess conserved (E) envelope proteins [2]. The deletion of E-protein in SARS-CoV results in a dramatic reduction of virus infectivity. Though it is the smallest of all viral proteins (8–12 kDa) [18] which SC2 gRNA expresses, yet it is also the most mysterious one. During cell replication cycle, E-proteins are expressed in a huge amount by the viral genome, but only a small amount of these proteins get incorporated into the new viral envelopes [22]. Studies propose three possible roles which E-proteins serve: (1) it mediates viral assembly, (2) its hydrophobic transmembrane domain is important for viral release from the host cells, and (3) it contributes to virus' pathogenicity [22, 23, 58].

M-proteins play a central role at the junction where viral and host factors meet to produce new virus particles. It is a small protein of up to 25–30 kDa [18]; reverse genetic and VLP (virus-like protein) assembly studies suggest the role of M-protein to exhort viral assembly by interacting with peplomers and viral ribonucleoproteins (vRNP) at the budding site [59]. It exists in the form of a dimer inside the virion and may transit between two different conformations which allow it to bind to the N-proteins or promote membrane curvature during budding stage [18].

Genome Organization of SARS-CoV-2

The study of genomic organization is a definite prerequisite for the functional investigation of any virus' replication mechanism, viral protein expression, host-virus interactions, and the viral pathogenicity.

SC2, like all coronaviruses, carries a large genomic RNA (gRNA) comprised of about 27,000–32,000 nucleotides. In general, a coronavirus gRNA contains seven conserved genes which are common to coronaviruses. Two-thirds of this genome is encompassed by (open reading frames) ORF1a and ORF1b; the rest of the genome harbors ORF3 and genes for all the structural proteins [21]. The conserved order of these genes is illustrated in Fig. 9.5. Genomic sequencing has revealed that SARS-CoV-2 genome holds 96% identity to the genome of bat CoV RaTG13 and 79.5% identity with SARS-CoV [60]. SC2 genome encodes several ORFs. Figure 9.6 shows a schematic presentation of SC2 genomic organization [61]. ORF1a/b are translated to produce nonstructural proteins (NSPs). Polypeptide 1a (pp1a, 440–500 kDa) is encoded by ORF1a, which when expressed is cleaved into 11 further NSPs. ORF1b is translated to produce a larger polypeptide, pp1ab (740–810 kDa). Pp1ab is further cleaved into 15 smaller NSPs. The viral genome

Fig. 9.5 A schematic illustration of coronavirus RNA genome. The genome contains seven genes which are common to all coronaviruses shown in their conserved order. ORF1a and ORF1b make up two-thirds of the entire genome; the rest consists of ORF3 and genes for essential structural proteins; S spike protein, E envelop protein, M transmembrane protein, N nucleocapsid protein

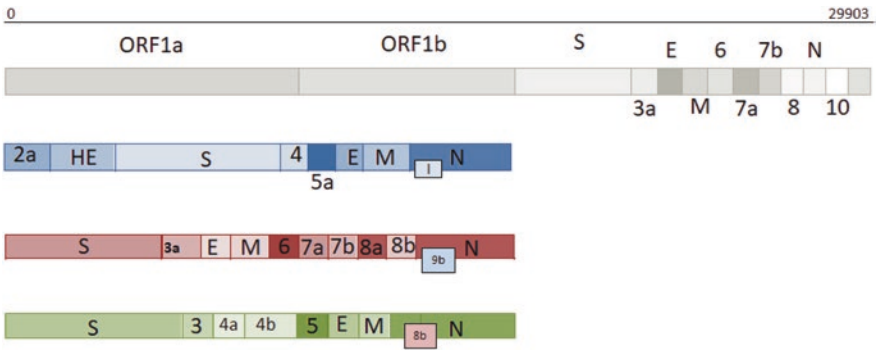

Fig. 9.6 Schematic representation of SARS-CoV-2 genome organization (gray). A 29,903-nt-long mRNA is translated from the entire SC2 gRNA. ORF3a, E, M, ORF6, ORF7a, ORF7b, ORF8, N, and ORF10 are nine major sgRNAs produced by the virus in addition to its gRNA [59, 61]. Illustration of the genomic organization of β-CoVs; MHV, SARS-CoV, and MERS-CoV are shown in blue, maroon, and green, respectively. SC2 SARS-CoV-2, gRNA genomic RNA, sgRNA subgenomic RNA, ORF open reading frame, CoV coronavirus, SARS-CoV severe acute respiratory syndrome-related coronavirus, MERS-CoV Middle East respiratory syndrome-related coronavirus

also serves to encode viral proteases (NSP3/5) which mediate the proteolytic cleavage of pp1a and pp1ab. Nine subgenomic gRNAs (sgRNA) encode for conserved structural proteins (S, E, M, and N) and at least six accessory proteins (3a, 6, 7a, 7b, 8, and 10) according to GenBank: NC_045512.2. CoVs elicit frequent gene recombination which allows them to generate a huge number of variants which may and may also contribute to their better survival and immune evasion activity [62]. Similarly, SC2 gRNA also undergoes frequent events of recombination during its replication cycle, which will be discussed under the next subtopic. The study of its genomic organization opens new directions for investigating SC2's pathogenicity and future pandemic threats.

Replication of SARS-CoV-2

To replicate, the virus cell must first enter into its host target cell. This entry would depend on the host receptor specificity of viral spike glycoproteins (S-glycoP). As for SARS-CoV-2, the S-glycoPs bind to the same ACE-2 receptors on human target cells as SARS-CoV [63]. The S-protein has two subunits: S1 and S2. Subunit 1 consists of an RBD which recognizes ACE-2 receptors specifically on host cell membranes; being the head of S-protein's structure, it is responsible to find and bind to the ACE-2 receptors [64]. Once the binding is done, transmembrane protease serine 2 (TMPRRS2) or cathepsin proteolytically cleaves the S-protein from S2 domains. This cleavage initiates the process of viral-host membrane fusion [18]. This fusion allows the release of SC2 gRNA into host cell's cytosol where pp1a and pp1ab start getting expressed. These large polypeptides are then cleaved into smaller

NSPs by NSP3 and NSP5. Most of these NSPs come together to form the viral replicase-transcriptase complex (vRTC). vRTC is then responsible for encoding the set of sgRNAs which involves the process of ribosomal frame shifting [65]. The viral structure proteins are transported to an ER-Golgi intermediate compartment (ERGIC). Here the gRNA and structure proteins are assembled into new virus particles. After assemblage, the new mature virions are then sent toward the host cell membrane packed in vesicles. As these vesicles reach the budding site, viral M-proteins mediate virus release from the host cell.

Transmission and Pathogenesis of SARS-CoV-2

Viruses also use the known routes of transmission which any other pathogen is known to take. These include:

1. Direct contact transmission – the viral particle gets physically transferred through direct body contact and enters the body through any opening, e.g., eyes, mouth, or a wound.
2. Airborne transmission – this encompasses viral transfer via small droplets or suspended particles which may be inhaled by a host.
3. Ingestion transmission – the virus can be ingested by ingesting contaminated food items.
4. Fomite transmission – the virus gets transmitted via contaminated inanimate objects such as environmental or medical surfaces.
5. Vector-borne transmission – this happens when animals act as carriers/vectors to transfer pathogens; the vectors are mostly arthropods or rodents.
6. Zoonotic transmission – this is spread of diseases from animal populations to human populations and can take any of the five routes of transmission briefed above [66].

Potential for interspecies jumping increases the probability of future zoonotic spillovers (Fig. 9.3); any non-identified intermediate host is one reason that can make it difficult to break the transmission cycle. Figure 9.7 briefly discusses the cross-species transmission mechanisms from host to nonnatural hosts (other humans and animals) and vice versa [1]. Virus transmission from animal to animal relies on the fecal-oral route, whereas the transmission of virus from animal to human may vary and can be depicted through three major stages: (1) viral prevalence and dispersal from animal host; (2) chances of viral exposure, route of entry in host human, and dose of viral particles entering; and (3) genetic, immunological, and physiological state of host human [67]. Transmission from human to human is found to occur due to unprotected and prolonged in-person interaction with the infected individual. Such an exposure builds a constant pathogen pressure on the exposed person, thus leading to the development of infection and possibility of transmission [68]. The major mode of person-to-person transmission is via airborne droplet which can transmit the viral particles in a zone of about 6 ft from the infected individual [69]. The other mode in case of human-to-human transmission is via fomites [70].

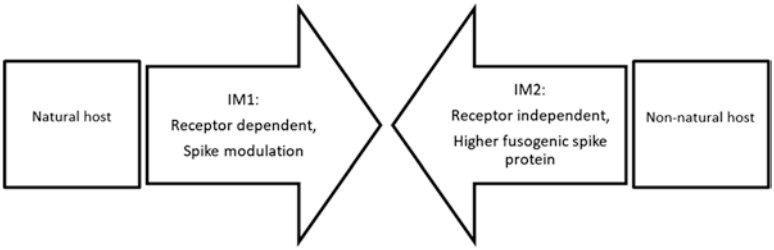

Fig. 9.7 Infection modes (IM1/2) depicting the potential of coronavirus' interspecies transmission. The transmission from natural to nonnatural host is receptor dependent and relies on spike protein modulation (IM1), whereas the transmission in reverse direction is often receptor independent as the spike protein may bypass receptor binding and elicit higher affinity for nonspecific host cell fusion when moving back to a previously known host (IM2)

The airborne droplets can settle on different surfaces and survive for long periods of time. These can then be acquired by healthy individuals through contact with the contaminated surfaces [1].

The pathogenicity of SC2 is thought to be linked with its structural and nonstructural proteins. Although the role of many NSPs haven't been described yet, we do know the crucial role of envelope protein in promoting viral assembly and release [18] and that the NSPs can shut off host immune response. S-proteins, for instance, prominently encourage SC2's pathogenicity by showing high affinity to ACE-2. ACE-2 receptors are widely distributed in the body, but the expression varies between tissues and individuals as well. This also contributes toward the variation in COVID-19's clinical manifestations [63, 71–74] – discussed in "clinical characterization of SARS-CoV-2." The pathogenic mechanism through which SC2 causes pneumonia seems complex. The available data indicates SC2's capability of producing a hyperactive immune response within the host body leading to the development of cytokine storms. That being so, the pathogenic cascade of SC2 implicates several cytokines, such as IL-1β, IL-12, IL-8, TNF-α, IL-6, monocyte chemoattractant protein 1, and macrophage inflammatory protein. IL-6 serves as the prime mover for it can act on numerous types of cells in the body and is mainly involved in the pathogenesis of cytokine release syndrome – which is also the case for COVID-19 – characterized by multiple organ dysfunction and fever. Studies have also proven that the binding of SC2 to TLRs (Toll-like receptors) actuates the release of pro-IL-1β which when cleaved into its active IL-1β form mediates lung inflammation and consequential fibrosis [75].

Clinical Characterization of SARS-CoV-2

The clinical pathology of COVID-19 resembles that of SARS-CoV and MERS-CoV. Once the infection develops in a healthy individual, mild symptoms appear including nonproductive cough, fatigue, low-grade intermittent fever, sore throat, and dyspnea. The disease can then worsen to its severity in 4–5 days from symptom

onset, on average [76–78]. COVID-19 patients may therefore present mild, severe, or critical illness (Table 9.5). Gastrointestinal symptoms such as diarrhea and vomiting also develop but are relatively uncommon. As the disease worsens, the infected individuals develop symptoms of ARDS (acute respiratory distress syndrome) and require mechanical ventilation to survive. Figure 9.8 shows a schematic timeline for onset of symptoms in COVID-19 patients.

Table 9.5 Clinical diagnostic features corresponding to the severity of COVID-19 infection in patients [52]

Severity of illness of COVID-19 patients	Diagnostic clinical conditions
Mild	Mild fever Unproductive cough Sore throat Nasal congestion Malaise Diarrhea Headache Fatigue Sudden loss of taste or smell Vomiting
Severe	Severe dyspnea Hypoxia
Critical	Acute respiratory distress syndrome (ARDS) >50% lung infiltrates Extrapulmonary manifestations Multiple organ failure

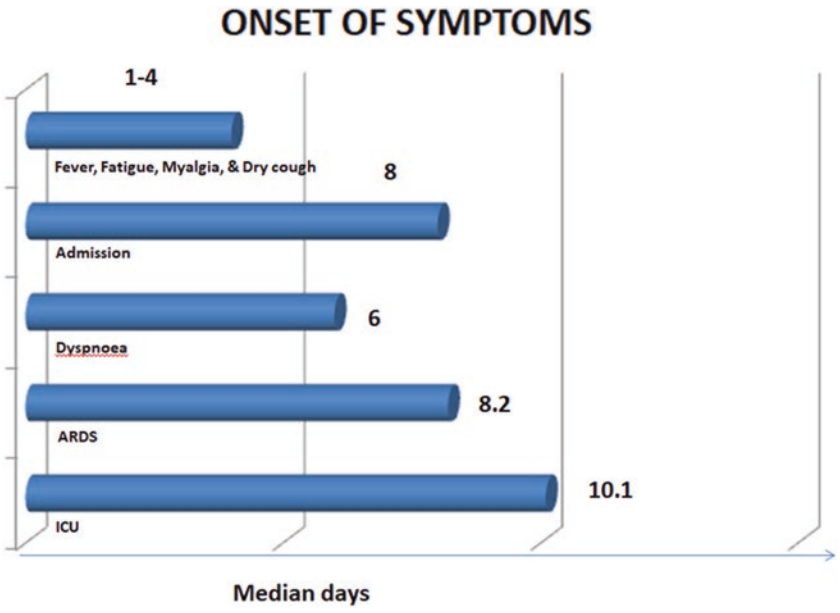

Fig. 9.8 Figure: A timeline of onset of symptoms in COVID-19 patients after development of infection

Underlying clinical complications were found more prevalent in severe COVID-19 cases, the development of which was detected through chest X-rays, T-cell counts (Table 9.4), and tissue biopsy of different organs of the infected person. Rapid progression of pneumonia is often observed in COVID patients' chest X-rays; a reduction in T-cell (CD4 and CD8) counts and hyperactivity of T-cells which triggers a severe cytokine storm [79] are other complications which impede effective treatment. Virus-induced cytopathic effect was observed in intra-alveolar spaces, and moderate microvesicular steatosis and mild lobular and portal activity were observed in liver tissues, while a few inflammatory infiltrates were observed in case of heart tissue. Coronavirus is also suggested to be associated with neurologic manifestations, such as ischemic stroke, axonopathic polyneuropathy, and myopathy [1, 80]. Underlying comorbidities in COVID-19 patients also complicate the course of treatment by increasing the severity of illness and thus the chances of death. The severity of the disease prolongs a patient's stay in ICU and the use of mechanical ventilation, which also adds to the complexity of the disease. Some major underlying comorbidities reported in COVID-19 patients are cerebrovascular disease, hepatitis B, chronic obstructive airway disease, cancer, coronary heart disease, chronic kidney disease, diabetes, hypertension, and immunodeficiency. Table 9.6 enlists the major comorbidities reported in various cohort studies [73, 81, 82].

Table 9.6 Some major underlying comorbidities reported in COVID-19 patients

ARDS
Acute kidney injury
Acute cardiac injury
Cancer
Cerebrovascular disease
Chronic obstructive pulmonary disease
Coagulopathy
Coronary heart disease
Diabetes
ECMO
Hepatitis B
Hypertension
Invasive mechanical ventilation
Pneumonia
Rhabdomyolysis

Clinical and Laboratory Diagnosis of SARS-CoV-2

A basic clinical diagnosis of SC2 depends on the symptoms described in the previous section "Clinical Characterization of SARS-CoV-2," which are also similar for other virus-induced respiratory infections. COVID-19 was initially misdiagnosed as pneumonia before it emerged as a pandemic. Hence, complimentary diagnosis using serology testing, RT-PCR analysis of blood samples, or chest imaging of the patients can help in differential diagnosis of the infection.

Once the symptoms such as intermittent fever, cough, and fatigue are investigated, serological tests and chest imaging should be considered to establish the presence of SC2 infection. COVID-19 provides somewhat similar serological findings as for other viral respiratory diseases; these include alterations in the levels of ALT, D-dimers, lymphocytes, CRP, thrombocytes, etc. – see Tables 9.7 and 9.8 [73, 81, 82]. Chest radiographs show the presence peripheral or rounded opacities with patchy bilateral GGOs (ground-glass opacity) and mixed attenuations. Lesions in the peripheral region or lower lobes are also predominant findings [83]. These structures, however, may not always be due to COVID-19. The radiologist must compare unique features such as reverse halo or atoll signs to confirm the cause of infection. CT scans are more sensitive than radiographs, but confirmed cases can also have normal chest CTs. As these findings might not always be specific for COVID-19, exposure or travel history of the patient can help avoid misdiagnosis [84].

Differential diagnosis is thus strongly recommended for COVID-19 diagnosis, as it might get misdiagnosed as another form of pneumonia due to the high similarity of serological and radiographic findings with those of other virus-induced respiratory infections (caused by adenovirus, influenza, human metapneumovirus, rhinovirus, parainfluenza, and respiratory syncytial virus). A general flowchart shows how a seldom differential clinical diagnosis of COVID-19 can be carried out – Fig. 9.9.

Table 9.7 Laboratory findings in COVID-19 patients [73, 81, 82]

Variable	Variation observed in patients	Approx. values recorded in patient cohort
ALT	Elevated	>40 U/L
D-dimer	Elevated	>1μg/L
CRP	Elevated	–
Creatinine	Elevated	–
LDH	Elevated	>245 U/L
IL-6	Elevated	>7.4 pg/mL
Ferritin	Elevated	>300μg/L
Creatinine kinase	Elevated	>185 U/L
Procalcitonin	Elevated	>= 0.5 ng/mL
Lymphocytes	Lowered	$<0.8 \times 10^9$/L
Thrombocytes	Lowered	$<100 \times 10^9$/L

Table 9.8 List of few drugs with known mechanism of actions (MOA) against SARS-CoV and MERS-CoV [1]

Drug	MOA
Chloroquine	Chloroquine is attributed to a deficit in glycosylation of ACE-2 receptors (SARS) and target type II transmembrane serine proteases (MERS)
IFN-alpha INF-beta-1a NF-gamma IFN-alpha-2b	Inhibits viral replication (SARS, MERS)
Imatinib Dasatinib	Inhibit viral entry (SARS, MERS).
Selumetinib Trametinib	Inhibit viral entry as well as replication of viral particles (SARS, MERS) via ERK/MAPK signaling pathway
Sirolimus	Targets mTOR signaling pathway to reduce viral infectivity (MERS)
Chlorphenoxamine	Inhibits viral entry
Lopinavir	Targets M^{pro}(SARS)
Camostat mesylate	Inhibits TMPRRS-mediated glycoprotein activation (SARS, MERS)
K11777 E-64-D	Inhibits viral attachment to host cells (SARS, MERS)

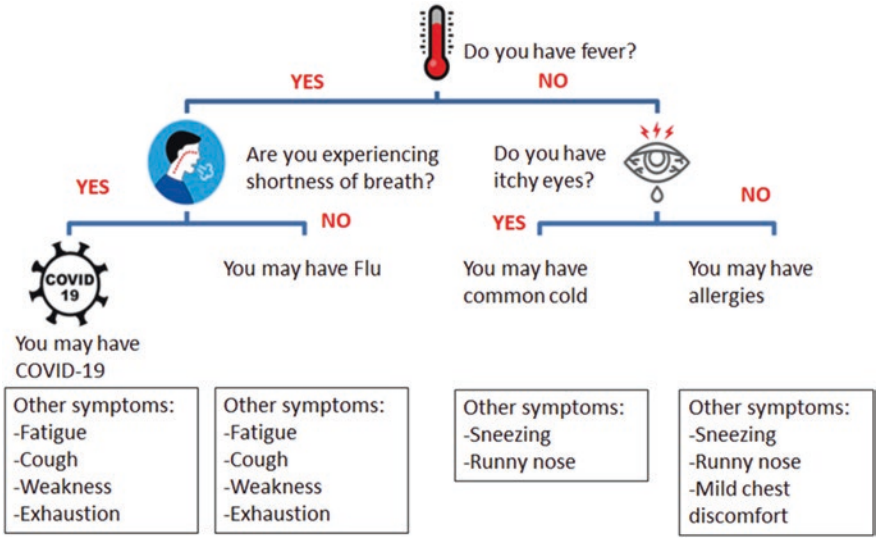

Fig. 9.9 A flowchart to aid in differential clinical diagnosis of COVID-19 and other mimicking conditions

The availability of genome sequence information of SC2 has made it possible for clinicians and biotechnologists to establish an RT-PCR – diagnostic assay. It qualitatively detects the viral nucleic acids in nasopharyngeal or oropharyngeal sputum/swab/aspirates or lavage of the suspected individual. SC2 gRNA is generally detectable during acute phase of infection. Positive results must be correlated with other

diagnostic findings and patient's exposure and travel history to determine infection status. Not every result is precisely definitive to determine the absence or presence of SC2 in samples. RT-PCR can produce false-negative/false-positive results [85]. Again, the decision regarding patient infection status must be based on differential diagnosis to prevent misdiagnosis.

Therapeutic Options for the Treatment of COVID-19

Therapeutic options for COVID-19 are meek; a number of FDA-approved drugs were randomly tested on SC2-infected patients under strict supervision of MEURI (Monitored Emergency Use of Unregistered and Investigational Interventions System) [85]. Other than drugs, therapeutic options include vaccine development, convalescent plasma therapy, corticosteroids, and CRISPR/Cas-mediated therapy. Any specific antiviral treatment for COVID-19 has not yet been approved, and the patients must receive supportive care in order to relieve symptoms [86].

Antivirals or Immunomodulatory Drugs for COVID-19

As SARS-CoV-2 has emerged from a similar pool of viruses as SARS-CoV and MERS-CoV, repurposing of drugs (Table 9.8) with well-established pharmacokinetic profiles against previously known highly pathogenic coronaviruses is an approach that readily got considered. The formerly known potent anti-coronavirus drugs either manifest a virus-targeted strategy or a host-targeted strategy – Fig. 9.10.

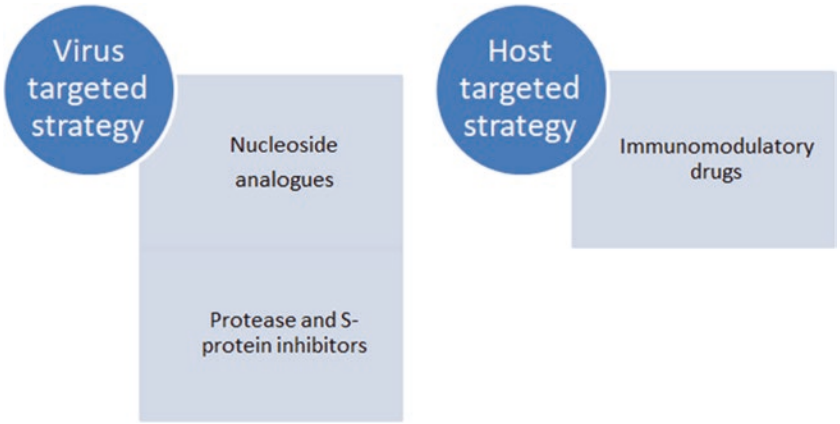

Fig. 9.10 Drug treatment approaches for COVID-19. Two strategies can be considered: (1) Virus-targeted strategy includes any drug which hampers viral development and survival, e.g., nucleoside analogues and protease or S-protein inhibitors. (2) Host-targeted strategy includes treatment with drugs which upregulate host immune response toward viral infection

Nucleoside analogues (NAs) and protease inhibitors (PIs), for instance, demonstrate convincing activity against the progression of SC2 in patients. Fapilavir was the first NA that got approved by NMPA (National Medical Products Administration of China) for the treatment of SC2; ribavirin and favipiravir were also later on approved for the administration in COVID-19 patients [87]. However, the highly frequent ability to mutate provides SC2 with the ability to resist the activity of these NAs. This led to the use of second approach: anti-CoV-NA cocktail therapy which showed enhanced efficacy, especially with coadministration of an additive. The resisting ability of SC2 toward NAs can also be reduced by administering a combination of drugs with different mechanisms of action. Several researchers, however, speculate the use of single broad-spectrum antivirals till a novel anti-SC2 agent is designed/identified [88]. Remdesivir and galidesivir, two experimental NAs, are broad-spectrum antivirals which were also evaluated against SC2 in cell cultures and mouse models [89, 90]. Like typical nucleoside analogues, these drugs inhibit SC2 by causing premature termination of the vRNA chains [87] (Fig. 9.11). Remdesivir is presently in clinical trials' phase for SC2 infection. In animal models, remdesivir was reported to improve pulmonary function and lower viral load (SARS-CoV and MERS-CoV) [90]. Wang et al. studied the effectivity of remdesivir in vitro using human cell line; the study supports the use of intravenous remdesivir as a viable drug treatment for COVID-19 [87].

Protease inhibitors, namely, lopinavir, disulfiram, and ritonavir, possess anti-HCoV activity. Disulfiram is known to inhibit the papain-like protease of SARS-CoV and MERS-CoV in cell-line models [87]. Ritonavir and lopinavir are known to inhibit 3-chymotrypsin-like proteases of SARS-CoV and MERS-CoV. However, the mechanism through which protease inhibitors inhibit proteases still rests in controversy. Clinical trials of lopinavir and ritonavir failed to lower mortality rates in COVID-19 patients and thus have not been considered for furtherance [91]. Other candidate PIs include nafamostat and griffithsin which demonstrate activity against S-proteins, thus hampering viral entry into host cells [92]. A recent study based on activity profiling of SC2 NSP3 (viral papain-like cysteine protease, PL^{pro}) determined crystal structures of two of its proposed potential inhibitors. The study hence

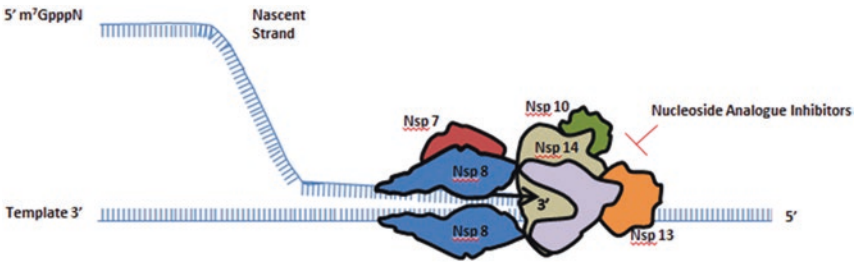

Fig. 9.11 Nucleoside analogues (NAs) are structural mimics of naturally occurring nucleosides. This allows the viral transcriptional machinery to mistakenly incorporate them during RNA translation. NAs either mispair with natural nucleosides to result in lethal mutations or cause premature termination of the viral RNA chain

propounds a framework for the development of protein inhibiting antivirals with promising therapeutic value as an anti-SC2 drug [93].

Chloroquine and hydroxychloroquine (antimalarial drugs) were also repurposed against SC2. 500 mg of chloroquine and 200 mg of hydroxychloroquine every 12 hours were proposed [52]. Gautret et al. [94] reported significant reduction in viral load until complete disappearance associated with the use of hydroxychloroquine. This effect was also documented to have enhanced by the administration of azithromycin with hydroxychloroquine. Like PIs, these drugs were also discontinued due to their inability to lower COVID-19 mortality rate [52]. Howbeit, further studies are recommended to evaluate the use of azithromycin against viral infections despite their established potential to mitigate and modulate immune system in in vivo and in vitro systems.

IFNs are innate components of the human body which naturally respond in defense to viral infections. These have been previously used against hepatitis C virus and in chemotherapy against numerous malignancies [52] (Table 9.8) and thus can be speculated for anti-SC2 activity. However, the data presently available is not enough to decide in favor or against their use for COVID-19 [95].

Vaccine for COVID-19

More than 100 potential vaccines for coronaviruses are being evaluated; these include the following types:

1. Whole virus (live/attenuated)
2. Antibody-based
3. Small subunit-based
4. Vector-based
5. Nucleic acid-based

Live attenuated vaccines help build a long-term immune response toward a specific virus; however, it may sometimes develop complications in the recipient. Antibody-based vaccines are based on (mAbs) monoclonal antibodies. The mAbs are strain specific but only provide a limited protection against the subjected virus. Small subunit-based vaccines are the most safe to use, are simple to produce, and are broad-spectrum as well. Nucleic acid-based vaccines are also referred to as DNA or RNA vaccines. These are also safe to use and provide long-term protection.

Some of the most promising candidates for SC2 as reviewed by the global COVID-19 vaccine R&D landscape were PiCoVacc, INO-4800, Ad5-nCoV, mRNA-1273, LV-SMENP-DC, and aAPC [96, 97] and are currently under clinical trials [52]. S-protein-based vaccines are a type of small-subunit-based vaccines; having demonstrated to be the most effective against both SARS-CoV and MERS-CoV [1], they are now being readily considered as safe, simple, and stable [98] option against SC2 but are presently underway clinical trials. In Wuhan (China), the very first in-human trials for a recombinant adenovirus type 5 (Ad5) vectored SC2

vaccine were carried out between 16th and 27th of March 2020. This vaccine is also an S-protein-based vaccine, and no serious adverse effects were reported within 28 days of its administration. The specific Ab response to SC2 peaked after day 28. This vaccine is currently undergoing phase II trial to confirm its safety and immunogenicity for mass use [99].

Plasma/Serotherapy for COVID-19

The use of convalescent plasma (CP) lowered the mortality rate in SARS- and MERS-infected patients. Likewise, passive immunization with CP in SC2 patients exhibited improved outcomes. CP is speculated to contain therapeutic levels of anti-SC2 neutralizing antibodies (Abs). Studies report the efficacy of convalescent plasma as a treatment without any adverse side/posttreatment effects [100]; however, some known complications otherwise associated with serotherapy include circulatory overload, anaphylaxis, and transfusion-related acute lung injury [101], and so further study is advised. Various treatment protocols mention serotherapy as treatment of last resort; fortunately, no serious unfavorable events have been reported so far. Instead, successful cases of COVID-19 treatment are surfacing [56]. A clinical trial to investigate a cocktail of Abs purified from CP in COVID-19 patients was launched in June 2020 [52]. Anyhow, the maximum benefits or associated complications might be realized through routine administration of these therapies.

Alternative Therapies

Anticoagulant Therapy

Anticoagulation therapy is also being suggested as SC2-infected patients have a higher incidence of venous thromboembolism. The therapy has been associated with reduced ICU deaths in COVID patients. Furthermore, in case of thrombosis or thrombophilia, 1 mg/kg dose of enoxaparin – twice a day for full-intensity anticoagulation – is indicated [102].

Glucocorticoids (GC)

Systemic GCs have been investigated for SARS-CoV back in 2003, but these studies are few and do not provide any conclusive evidence regarding their efficacy [103]. Corticosteroids have been studied in animal models and been extensively

reported as potential therapeutic agents which can help reduce inflammation, lung injury and improve patient survival [104]. These however, were not recommended as a treatment option for critical cases of viral pneumonia or ARDS [52], but as a subset of population infected with COVID-19 may develop cytokine storm syndrome [79, 105] and multiple organ failure, the therapeutic role of glucocorticoids was re-hypothesized for these patients in this current dramatic emergency. A recent study demonstrates dexamethasone to have reduced deaths by 33% among critically ill SC2-infected patients [106]. However, other studies claim that there is no convincing evidence available to prove the efficacy of corticosteroids in decreasing the mortality rate for COVID-19 [107]. The debate on the use of glucocorticosteroids has hence been reignited and needs further experimental evidence to comprehend any benefits it might have for treating COVID-19.

Stem Cell-Based Therapy

A study [108] introduced mesenchymal stem cell transplantation (MSCT) as a promising alternative to antiviral drugs for COVID-19. They report that MSCT can help regulate immune homeostasis and induce specific immune tolerance to help reduce inflammation Based on these findings, the study proposes it as one viable therapeutic approach toward COVID-19 as MSCT were also reported safe and effective for use especially for critically ill COVID-19 patients.

ACE-2-Mediated Therapies

ACE-2 (angiotensin-converting enzyme 2) is a peptidase widely expressed in organs including lungs, kidneys, and the GIT. One major similarity between SC2 and SARS is their affinity for ACE-2 receptors. The entry of SC2 via ACE-2 is followed by the accumulation of angiotensin-II which may mediate acute lung injury. ACE-2 blockers and administration of soluble ACE2 are potential therapeutic approaches for COVID-19 [109]. ARBs (angiotensin receptor blockers) propose another possible treatment option [110]. These therapies are convenient and can be applied.

CRISPR/Cas System

The diversity of HCoVs requires a flexible antiviral technique to pace up with its mutative frequency. CRISPR/Cas9 system has already been successfully used to enhance immunity by reprogramming B- and T-cells as a chemotherapeutic strategy. It can be used as a promising antiviral technique as well, as it can be

used to manipulate immune system against the pathogen or can destroy viral cells directly [111].

Conclusion

SARS-CoV-2 is a highly pathogenic coronavirus which is found to have originated in bats and carried over to humans by Malayan pangolins. COVID-19 emerged as a local outbreak of pneumonia from a seafood market in Wuhan (China) and rapidly transitioned into a global pandemic. SC2 is highly infectious and possesses an aggressive ability of person-to-person transmission, yet the mortality rate in COVID patients is less than that in SARS-CoV and MERS-CoV. COVID patients have been reported with flu-like symptoms in mild to ARDS, cytokine storm, and MOD in critical cases. The most common detection method for SC2 is RT-PCR diagnostic assay, but because RT-PCR is prone to produce false results and other diagnostic findings might share similarity with those of other virus-induced respiratory complications, differential diagnosis must be performed to confirm COVID illness. Various therapeutic options have been explored; however, any potential anti-SC2 agent is currently undergoing clinical trials, and so there is no approved treatment yet. The patients thus rely on supportive treatment to relieve symptoms. Till a treatment is approved, prevention methods including the use of face masks, proper hygiene, and social distancing should be strictly followed, and in case of any symptoms, early detection and treatment must be considered.

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