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COVID-19

Credible Protein Targets and Curative Strategies for COVID-19: a Review

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Abstract

The pandemic of coronavirus infection 2019 (COVID-19) due to the serious respiratory condition created by the coronavirus 2 (SARS-CoV-2) presents a challenge to recognize effective strategies for management and treatment. In general, COVID-19 is an acute disease that can also be fatal, with an ongoing 10.2% case morbidity rate. Extreme illness may bring about death because of enormous alveolar damage and hemorrhage along with progressive respiratory failure. The rapidly expanding information with respect to SARS-CoV-2 research suggests a substantial number of potential drug targets. The most encouraging treatment to date is suggested to be with the help of remdesivir, hydroxychloroquine, and many such repurposed drugs. Remdesivir has a strong in vitro activity for SARS-CoV-2, yet it is not the drug of choice as affirmed by the US Food and Drug Administration and presently is being tried in progressing randomized preliminaries. The COVID-19 pandemic has been the worst worldwide general health emergency of this age and, possibly, since the pandemic influenza outbreak of 1918. The speed and volume of clinical preliminaries propelled to examine potential treatments for COVID-19 feature both the need and capacity to create abundant evidence even in the center of a pandemic. No treatments have been demonstrated as accurate and dependable to date. This review presents a concise precise of the targets and broad treatment strategies for the benefit of researchers.

Keywords COVID-19 · SARS-CoV-2 · Protein targets · Antisense therapy · Repurposed drugs

Abbreviations

COVID-19	Coronavirus infectious disease 2019
SARS-CoV-2	Severe acute respiratory disorder coronavirus 2
US	United States
HIV	Human immunodeficiency virus
2019-nCoV	2019- coronavirus
HCoV-229E	Human coronavirus 229E
HCoV-NL6	Human coronavirus NL6
HCoV-HKU1	Human coronavirus HKU1
MERS-CoV	Middle East respiratory syndrome
HCoV-OC43	Human coronavirus OC43
CoV	Coronavirus
RNA	Ribonucleic acid
M protein	Membrane protein
E protein	Envelope protein
S protein	Spike protein
N protein	Nucleocapsid protein
HE	Hemagglutinin esterases
ACE2	Angiotensin-converting enzyme 2
TM	Trimeric
ED	Ectodomain
MHV-A59	Mouse hepatitis virus strain A59
SM	Small membrane protein
RBD	Receptor-binding domain
TMD	Transmembrane domain
HnRNPA-1	Heterogeneous nuclear ribonucleoprotein A1
M_{Pro}	Membrane proteases
$3CL_{Pro}$	Cysteine-like protease
PL_{Pro}	Papain-like protease(s)
TGEV	Transmissible gastroenteritis virus

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Nsp	Non-structural protein
USPs	Ubiquitin-specific proteases
RMSD	Root-mean-square-deviation
HAUSPs	Monoclonal antibody for studying USP7
BCV	Bovine coronavirus
BSM	Bovine submaxillary mucin
HCV-OC43	Human coronavirus OC43
Neu5-9Ac-2	9-O-acetyl-5-*N*-acetyl sialic acids
HEF-1	Hemagglutinin esterases fusion protein
DNA	Deoxy ribonucleic acid
NTP	Nucleotide triphosphate
RSV	Respirational syncytial virus
vRNP	Viral nucleoproteins
CRM	Cellular export receptor
GTP	Guanosine triphosphate
ESCRT	Endosomal sorting complex required for transport
3D	Three-dimensional
RAS	Renin-angiotensin system
ACEIs	Angiotensin-converting enzyme antagonists
ARBs	Angiotensin II type 1 receptor blockers
CT	Clinical trials
IL	Interleukin
mRNA	Messenger ribonucleic acid
PMO	Phosphorodiamidate morpholino oligomers
WHO	World health organization
ICMR	Indian council of medical research
ARDS	Acute respiratory distress syndrome
RT-PCR	Reverse transcription polymerase chain reaction
SiRNA	Small inhibitory ribonucleic acid

Symbols

kb	Kilobase
nm	Nanometer
kDa	Kilodalton
Å	Angstrom

Introduction

Corona virus at first probably surfaced in Wuhan, a city with a population of 11 million [1]. Coronavirus disease (COVID-19) is triggered via a novel coronavirus that leads to coughing, fever, and trouble in breathing. Symptoms may appear 5–6 days after infection. This virus rapidly spread worldwide, including China, Japan, the US, South Korea, Britain, Italy, and India. The confirmed cases of infection (COVID-19) are multiplying at an exponential rate as well as the death rates [2].

The COVID-19 is deadly and stands answerable for many deaths by a respiratory infection [3].

At present, no confirmed treatment for COVID-19 exists and the treatment for infection from COVID-19 is yet in its early stages, although the step is frantic. Some preliminary studies have investigated potential combinations that include anti-malarial drug chloroquine, drug ivermectin, and anti-HIV vaccines which may provide some relief against COVID-19 infections [4].

Phylogenetic analysis of this virus indicated that it is different (~ 80% nucleotide identity) but related to SARS-CoV-1. Because the world is threatened by the possibility of a SARS-CoV-2 pandemic, the broad-spectrum antiviral effects of chloroquine warranted particular attention for repurposing this drug in the therapy of the disease caused by SARS-CoV-2, named coronavirus disease 2019 (COVID-19) [5].

Scientific Categorization and Structure of Human Coronaviruses

Coronaviruses are placed in two subfamilies; coronavirinae and torovirinae within the family of coronaviridae, which is contained in the order of nidovirales. The coronavirinae subfamily is characterized into four essential genera: alpha-coronavirus, beta-coronavirus, gamma-coronavirus, and delta-coronavirus as per the Worldwide Committee for Logical Classification of Infections [6]. COVID-19 affects mammals and is thought to spread from bats [7]. Coronaviruses include single-stranded positive-sense RNA infection through the biggest genome size, extending around 26–32 kb quantitated [8]. This genome is wrapped inside the helical capsid which is made up of the nucleohelical capsid protein. In these viral envelopes, there are three structural and functional proteins, i.e., the membrane protein (M), the envelope protein (E), and the spike protein (S). The membrane and envelope proteins help in wrapping the genome whereas the spike proteins mediate virus entry into host cells to infect the same. Some coronaviruses additionally encrypt an envelope-related hemagglutinin-esterase protein (HE) [9].

In these structural proteins, the spikes of the surface of the genome give coronaviruses the resemblance of a crown from outside hence named *corona* (Latin word) which means crown. The main functioning protein is spike (S) protein because it determines the range and immune responses of the host cells. The spikes of coronaviruses contain three segments: a large ectodomain, a single-pass transmembrane anchor, and a short intracellular tail. The S protein enters the human alveolar epithelial cells through binding angiotensin-converting enzyme 2 (ACE2) receptors [10–12].

India's first microscopic photos of the SARS-CoV-2 indicate that it is circular like other coronaviruses, around 70–80 nm (a human hair is around 80,000 nm), and has a cobbled surface layer [13].

Protein Targets in the Structure of COVID-19

Spike Protein The S protein is a clove-like type I TM protein. It has three fragments which are the ectodomain (ED) region,

TM zone. Also, intracellular space comprises short tail fragments. The receptor restricting the S1 area (three S1 heads) and the layer combination subunit S2 (trimeric tail) on the C-terminal, together, form the ED [14]. The ectodomain of all CoV spike proteins shares the same structure in two domains: an N-terminal domain called S1 which is responsible for receptor-binding and a fusion-related C-terminal S2 domain [15].

Spikes can be seen as simple, 20 nm elongated spherical surface projections on the virion membrane under an electron microscope [16]. Certain receptors can also mediate the entry of SARS-CoV-2 through T cells, such as CD147, on the surface of T lymphocytes, a novel intrusive path for SARS-CoV-2 [17].

Membrane Protein SARS-CoV-2 reveals a 96% similarity to the bat coronavirus [18]. The mode of entry of the HIV1 is almost similar to that of coronavirus and both are able to fuse to the cell and elicit severe devastating effects [19].

The glycoprotein membrane (M) covers the viral envelope and has three regions, namely the cytoplasmic domain, the transmembrane dominion, and the membrane's hydrophilic end N. M protein is a glycoprotein largely responsible for making up viral particles and tiny membrane protein, used together to create a homogeneous matrix. Proteins like S, N, and E that associate with this protein form heterogeneous complexes. M protein alone cannot cause virion formation; it must be triggered until E protein acts so it can be formed into virions. The association between the virus and the host can be linked to M protein glycosylation [20].

Envelop Protein On studying the primary and secondary structures, it is revealed that E includes a little, hydrophilic amino end composed of 7–12 amino acids, with a wide hydrophobic transmembrane (TMD) space of 25 amino acids, and wraps up with a long hydrophilic carboxylic end containing the remaining portion of the protein [21].

E is disproportionately distributed inside the infected cell throughout the replication process but only a limited amount is integrated into the virion envelope [22]. Recombinant CoVs missing E show significantly decreased viral titers, weakened viral development, or awkward offspring for producing, outlining the centrality of E in infection advancement and development [23].

Studies reveal a significant portion of TMD consists of two non-polar, neutral amino acids, valine, and leucine, which offer the E protein a heavy hydrophobicity [24]. In both CoVs, membrane (M) and spike (S) proteins are the bulk of the protein found in the viral shell, although only a few E protein molecules are available [25].

Nucleocapsid Protein The nucleocapsid protein collaborates through hydrophilic and hydrophobic cooperations with the RNA genome. Because 1500 nucleocapsid molecules are present per viral core and are assumed to cover the genome widely, each molecule will bind with average 11–12 nucleotide residues if equally spread [26]. N protein self-affiliation has been seen in numerous infections and is required to shape the viral capsid that shields the viral genome from extracellular agents [27].

The N protein is a 46-kDa protein consisting of 422 amino acids [28]. Chang et al. (2006) conducted a biophysical experiment which indicated that this protein consisted of two distinct structural domains and a linking zone [29]. The first domain, within the putative RNA binding domain, is located at the N-terminus. The second domain is the C-terminal region capable of self-association [30].

Proteases The primary proteases (M_{Pro} also named $3CL_{Pro}$) are one of the better-described drug targets of coronaviruses. This enzyme is significant for the handling of the polyproteins that are translated from the viral RNA, alongside the papain-like protease(s) [31]. The M_{Pro} acts on the broad polyprotein-1-ab (replicase-1-ab, ~ 790 kDa) at no fewer than 11 cleavage sites; the sequence of identification at most locations is Leu-Gln replication (Ser, Ala, Gly) (marks the cleavage site). Halting this enzyme's function will prevent viral replication. Because no human proteases are reported to have a comparable cleavage range, inhibitors are unlikely to be harmful [32].

Given the substantial challenge faced by SARS-CoV and associated viruses, it is clear that the amount of cases so far does not justify the commercial production of a targeted antiviral medication [33].

3C-like Proteases Coronaviruses produce the largest documented RNA genome, varying in size from 27 to 32 kb for different CoVs and encoding a viral protease-processed replicase polyprotein, papain-like protease (PLP), and 3C-like protease ($3CL_{Pro}$, also documented as the central protease) [34]. The various available X-ray structures of the 3C-like proteases from SARS-CoV, rhinoviruses, HCoV-229E, and transmissible gastroenteritis infection (TGEV) render structure-based medicate plan reasonable [35].

All proteases are deemed promising targets for antiviral therapy, owing to their important function in viral replication [36] (Fig. 1).

Papain-Like Proteases The SAS-CoV PL_{Pro} framework appears a design of thumb-palm-fingers coordinating cellular ubiquitin-specific proteases (USPs) [36]. SARS-CoV PL_{Pro}'s dynamic location comprises of a canonical catalytic Cys-His-Asp group of three, near to that of USP14 [37].

The USPs are cysteine proteases forming strongly differing chains and show similarity primarily in the areas surrounding the catalytic Cys; these are the so-called Cys Box (B19 amino acids) and the His Box (60–90 amino acids) domains [38].

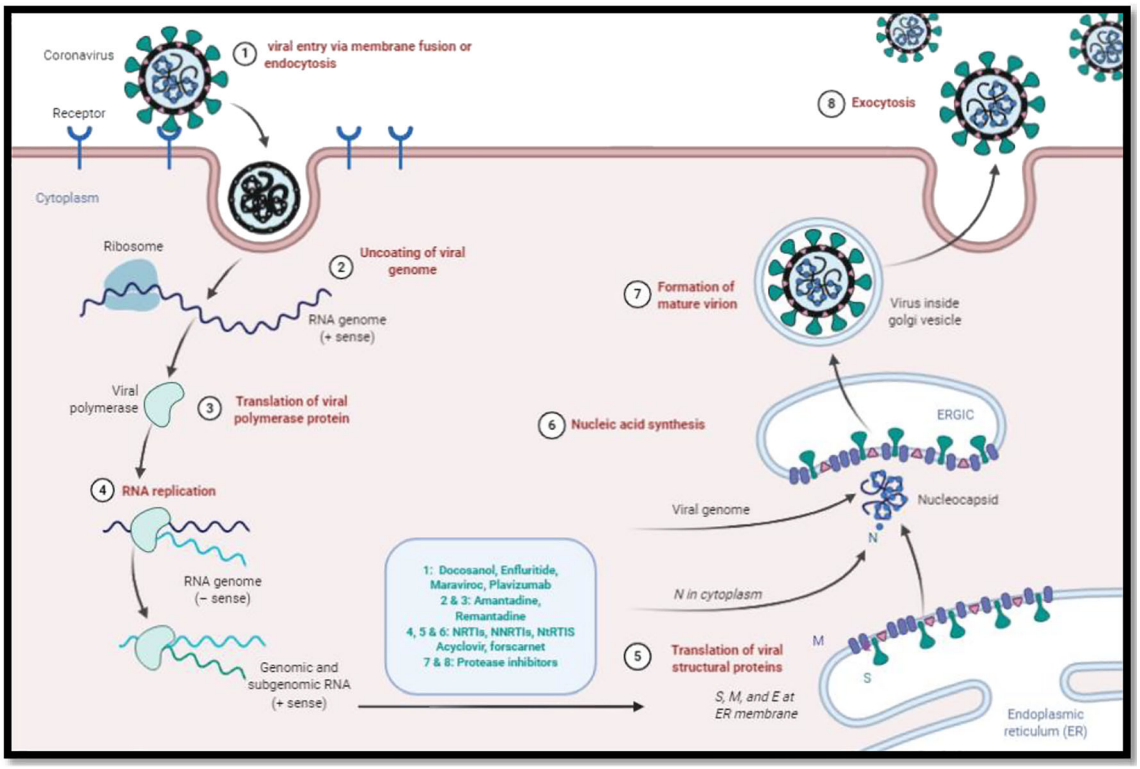

Fig. 1 Viral replication and their inhibitors

Hemagglutinin Esterase Hemagglutinin esterases (HEs) are a class of glycoproteins in the viral envelope that mediate reversible binding towards *O*-acetylated sialic acids by functioning as both lectins and receptor-destroying enzymes [39].

HE proteins of influenza infections, especially flu C, were known to tie to cell receptors containing 9-*O*-acetyl-5-*N*-acetyl sialic acids (Neu5, 9Ac2) as the significant receptor determinant [40].

Reports uncover that the CoV HE monomer comprises of three regions, each with a flu C HEF subunit 1 (HEF1) comparable: a receptor-binding space (R), an acetyl esterase space E, and a membrane-proximal space [41].

NTPase/Helicase Helicases are competent in enzymatically loosening up duplex DNA or RNA structures by disturbing the hydrogen bonds that keep the two strands together [42].

NS3 helicases are thought as a potential target for anti-coronaviridae compounds [43]. NTPase/helicase (Hel) proteins are both known to be significant for the viability of nidoviruses and are thus possible targets for anti-SARS drug production [44].

Drug Targets for COVID-19 Antiviral medicines may be divided into fusion, non-coating, nucleic acid synthesis, absorption, and protease release inhibitors [45, 46].

Fusion/Entry The virus-host cell layer or receptor(s) relationship is the primary stage of the viral neurotic life arrangement, called the fusion/entry space. Advance fusion/entry inhibitors have been given for the administration of human immunodeficiency infection (HIV) and respirational syncytial infection (RSV) as a prophylactic measure. Targeting chemokine receptors and glycoprotein (GP)-receptor interactions, as important entry co-transporters are also among the most desirable candidates for viral entry/fusion inhibition [47].

M_2 is a type III transmembrane protein forming tetramers whose transmembrane domains form a loop serving as a source of proton-selective ions. Opening the pores of the M_2 ions acidifies the viral nucleus [48]. This acidic state in the virion releases the viral nucleoproteins (vRNP) from M_1 so that vRNP will reach the cytoplasm of the host cell-free [49].

Non-coating/Uncoating "Uncoating" diversely defines the removal of the structural proteins from the capsid. Furthermore, both the removal of structural proteins from the capsid and structural rearrangements within it can be called priming activities, since they are a requirement for effective release of the genome [50].

Nucleic Acid Synthesis The other approved antiviral drugs influenced the DDX3 (Asp-Glu-Ala-Asp (DEAD)-box polypeptide 3. DDX3 executes important steps in the metabolism of RNA which includes encoding, translation, and production of membranes stress granules. DDX3 is also involved in the in-built response of the body in case of viral invasions. Interestingly, certain RNA viruses, for example, HCV and

HIV-1, use DDX3 to execute various phases of the replication processes [51].

Maturation and Release After de novo synthesis of viral genome and proteins, viral proteins are assembled into new viruses that are ready for release from the host cell with freshly replicated viral genome. This phase is referred to as maturation. vRNPs tend to be transported inside the nucleus through the nuclear pores through the CRM1-based pathway. Nucleoprotein appears to connect with CRM1 although there is no identification of GTP hydrolysis behavior. This suggests an uncommon export process if the transfer of vRNPs is essential in connecting NP to CRM1 [52]. The endosomal sorting complex necessary for the transport pathway (ESCRT) is a cellular system for the external budding and fission of vesicles away from the cytoplasm [53].

Drug Repurposing Drug repurposing from existing licensed products is a successful product development technique, which could shorten the period and lower costs relative to de novo product investigation [54].

This strategy provides numerous advantages over the creation of a specific indication of a completely new drug. The reused medication has already been indicated to be reasonably secure in preclinical models as well as in human studies, and since the early-stage study has already been completed, the chance of failing is lower and possibly most significantly, as, in subsequent efficacy trials, it is less probable at least from a side effect or toxicity point of view that it fails [55]. Second, the timeline for drug expansion can be shortened, because much of the preclinical testing, safety evaluation, and, in some cases, development of the formulation may have already been completed. Thirdly, less investment is required, although this may differ greatly depending on the repurposed candidate's stage and phase of development [56].

The detection of novel drug-disease interfaces is the main concern of medication repositioning. A variety of methodologies have been established to challenge this problem including quantitative strategies, biological strategies, and many more permutations and combinations of experimental strategies [57]. Most current computational strategies are centered on the quality expression reaction of cell lines after treatment or combining numerous shapes of information around a hypothesis [58].

However, novel product repurposing methods are expensive and time-consuming. Computational methods deliver new, testable theories for repositioning systemic drugs. Orthodox structure-based approaches are therefore restricted where three-dimensional (3D) protein structures are inaccessible, as is sadly the case with most human and viral targets. Furthermore, attacking single virus proteins also poses a high risk of drug resistance through rapidly changing virus genomes [59–61].

Drug Repurposing Challenges Drug repurposing presents the following challenges:

1. Intellectual property and economic considerations
2. Data and compound availability
3. Overcrowding of repurposing space [62].

Drugs Used in the Treatment of COVID-19 Infection An antiviral medication, Tamiflu, is targeted at inhibiting the enzyme that cleaves sialic acid on the exterior of human cells and thereby interferes with the capacity of the virus to enter the host. Newer medications, currently in clinical testing for the diagnosis of COVID-19 infection, such as chloroquine which is a well-known anti-malarial medication, and its variant hydroxychloroquine have shown better efficacy [4] (Table 1).

ACE2 has been reported to be the 2019-nCoV key host cell receptor and plays a critical role in a viral entry inside the cell to induce the infection. To explore the possible route of 2019-nCoV attack on oral cavity mucosa, bulk RNA-sequence profiling has been undertaken [69].

Renin-angiotensin framework (RAS) dysfunction has been recognized in patients enduring from coronavirus disease (COVID-19), but whether RAS antagonists, such as angiotensin-converting protein opponents (ACEIs) and angiotensin II sort 1 receptor blockers (ARBs), are connected with clinical impacts, however, remains vague [70]. The awareness of MERS-CoV and SARS-CoV immunotherapies in recent years may expand prospects for the successful use of the same treatments for modern coronavirus [71] [72].

Antisense Therapy In 1978, Zamecnik and Stephenson discovered the ability of oligodeoxynucleotides to function as antisense agents inhibiting viral repetition in cell culture [73]. The idea behind it is that the base of an antisense nucleic acid sequence pairs with its complementary sense RNA strand and evades its conversion into a protein. By attaching to mRNA, antisense drugs disrupt and interfere with the development of different proteins associated with the diseases [74].

The basic principle is that whenever an oligonucleotide (a short RNA or DNA molecule complementary to an agency-generated mRNA) may be inserted into a cell, it can precisely attach to its target mRNA by the excellent specificity of complementary base pairing the same process that guarantees the gene's adherence to DNA replication and RNA transcription. This attachment creates an RNA dimer in the cytoplasm and prevents the synthesis of proteins [75]. Reports indicate that the role of cyclodextrin derivatives as a transferor for the transmission of phosphodiester oligonucleotides in viral infections could be an important strategy for improving the therapeutic ability of such compounds. Viral infections can occur when the oligonucleotides in the antisense complement viral RNAs [76]. Many antisense medications have reached clinical

Table 1 Repurposed drugs in COVID-19 infections

Drug	Therapeutic use	The original mode of action	Being tested?
Chloroquine	Anti-malarial	Heme-polymerase inhibitor	Under clinical trials
Kaletra (ritonavir and lopinavir)	HIV	Protease inhibitor	Yes
Interferon alfa-2b	Hepatitis C	Immune modulator	Under clinical trials
Remdesivir	Experimental	Nucleotide analog	Approved
Favipiravir	Influenza	RNA polymerase inhibitor	Yes
Actemra [63] (tocilizumab)	Rheumatoid arthritis; COVID-19	Anti-inflammatory	Yes
Kevzara (sarilumab)	Rheumatoid arthritis	Anti-inflammatory	Yes
Rapamycin	Anti-tumor	Immunosuppressant	Yes
Arbidol [64]	Antiviral	Fusion inhibitor	Under CT
Thymosin [63]	Immunotherapy	Polypeptide hormone for the maturation of T cells	Yes
Siltuximab [63]	COVID-19	Against interleukins-6 receptor (IL-6R)	Yes
Sofosbuvir	Hepatitis virus C	Viral RNA synthesis inhibitor	Under CT
IDX-184 [65]	Immunotherapy	Lymphokine-activated killer cells	CT
Ribavirin [66]	Broad-acting antiviral drugs	Nucleoside inhibitor	CT
Thymopentin [67]	Hepatitis B	Immunostimulants	Not approved
Levamisole	Anti-parasitic agent	Polypeptide hormone for the maturation of T cells	Yes
Cinanserin	Serotonin receptor antagonist	Replication inhibitor	Yes
Emodin	Anti-SARS-CoV	S protein interactions	CT
Promazine	Antipsychotic agents	Replication inhibitor	Yes
Nitric oxide	Anti-inflammatory agent	Viral RNA synthesis inhibitor	CT
Mucroporin M1	Broad-spectrum antiviral agents	COVID-19	Yes
Estradiol and phytoestrogen	Immunotherapy	Potent MERS-CoV	Yes
Melatonin [68]	Anti-oxidants	Activation of melanin receptors	CT

trials during the last 30 years for the prevention of a large range of diseases. Fomivirsen is a first-generation antisense cytomegalovirus medication approved for the treatment of cytomegalovirus retinitis, a serious opportunistic infection in HIV patients at the time [77]. Phosphorodiamidate morpholino-oligomers (PMO) and β-cyclodextrins are the main antisense agents that are used in the coronavirus infection [78] (Fig. 2).

Plasma Therapy The wellbeing network over the world is taking aid of plasma or immunizer treatment to fight COVID-19 [79] (Fig. 3). According to the WHO, the experience in the past suggests that the experimental use of convalescent plasma can be a possibly useful treatment for COVID-19. In India, numerous states have looked for and gotten the endorsement of ICMR for plasma treatment. Be that as it may, up until this point,

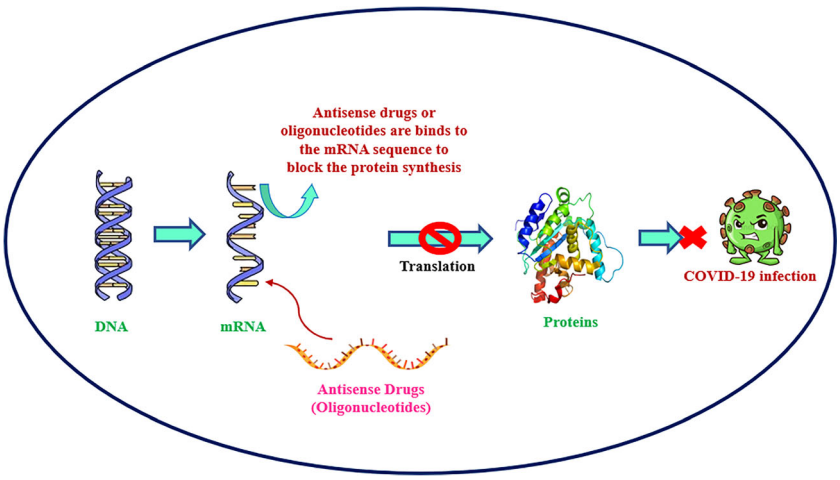

Fig. 2 Antisense therapy mechanism on COVID-19 infection

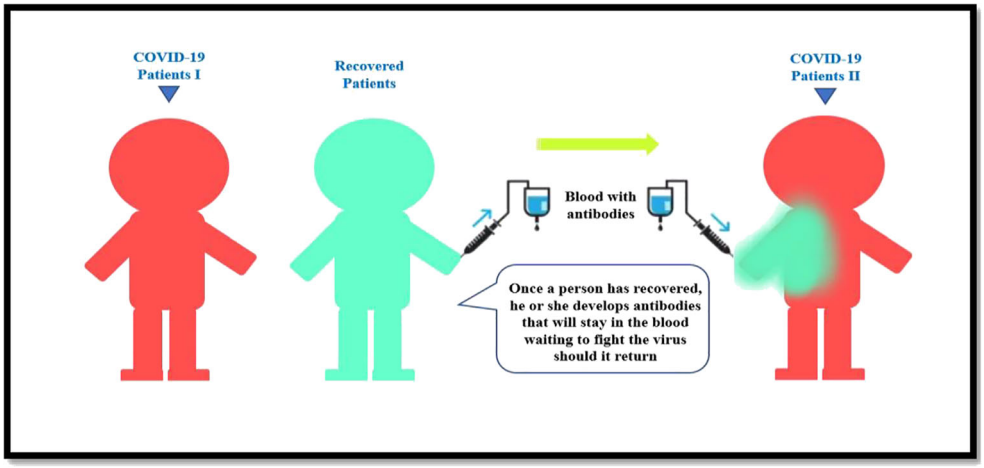

Fig. 3 Plasma therapy for COVID-19 patients

ICMR does not suggest it as a treatment alternative outside clinical trials [80].

Discussion

The basic protein and drug targets for COVID-19, which help in the systemic identification of putative repurposable medications and medication mixes for treatment of 2019-nCoV/SARS-CoV-2, have been detailed above [54]. Two drugs, namely hydroxychloroquine and remdesivir, as well as steroids are the front runners in the quest for treatment for COVID-19 infection [81]. At this moment, our knowledge of the 2019-nCoV infection clinical framework is very restricted. Complications such as extraordinary pneumonia, respiratory distress where fluid accumulates in the lungs, and cardiac arrest are a few symptoms which have been recorded in patients all over the world. A few unspecific signs and indications of gentle illness early within the 2019-nCoV contamination similar to numerous other respiratory disorders, especially amid the winter respiratory infection season and related mild diseases, make it difficult to identify a patient [11]. The cases of asymptomatic patients also exist. Asymptomatic individuals disclose mishaps of sense of smell which is perhaps a sign [82].

If one gets to know that he/she has been in touch with an asymptomatic carrier of the disease, one must quarantine him/herself for 14 days, without any delay. This will ensure that one does not spread the virus to others [83]. Agents targeting host signaling pathways or receptors may induce immunopathology [59].

At the active site of COVID-19, the numerous drug candidates with proven properties such as anti-malarial, anti-hepatitis C, anti-bacterial, anti-fungal, and anti-inflammatory have also been identified to be attached. The medicines retained unbonded associations with metabolites from the active site [84]. We discussed numerous mechanism demonstrating the significance of target sites in developing potential new therapies and developing new vaccines [85]. In the meantime, several antiviral drugs are being launched that have close properties to target 2019-nCoV single-stranded RNA and may serve as potential candidates for further research against 2019-nCoV [86].

Conclusion

The HCVs are smart enough to use the human physiological system to enter, multiply, show pathological symptoms, and then go on to utilize other human hosts to multiply and proliferate. Such rapid and efficient transmission is making it virtually impossible for humans to intervene and disrupt the life cycle of the virus. Even after vast-concentrated research and utilization of different methods to date, no effective treatment for HCoVs is available.

One of the key explanations for these is that for in vitro and in vivo research, most of the established agents have not been adequately evaluated. The main challenge is the race against time in this case, but eventually, this large body of research shall help us in the future to tackle any other types of coronaviruses that may emerge.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical Approval Not applicable.

Informed Consent No clinical trials were involved, hence not applicable.

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We the undersigned declare that the manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.