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Structure and Origin Insight of Recent Coronavirus with Ongoing Therapeutic Approach

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ABSTRACT

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is highly transmissible zoonotic virus that was initially reported in late 2019 in Wuhan, China. Presently, it has infected approximately 1.5 million person and responsible for more than 0.1 million death across the world. It is likely evolved from bats and pangolin and genetically different from SARS-CoV-1, responsible for SARS disease. In present review, we have discussed the recent finding of its origin from bats and pangolin, its phylogenetic comparison from other coronavirus and ongoing therapeutic approaches against the fight of SARS-CoV-2.

Keywords: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), Non-structural protein (NSPs), human angiotensin converting enzyme-2 (hACE2), TMPRSS2, receptor binding domain (RBD)

INTRODUCTION

Coronavirus (CoV) belongs to RNA virus that forms large protrusions on its surface and externally appear in crown-like appearance (corona means crown in Latin). First time, CoV was reported in 1960 and commonly responsible for respiratory illness in a broad range of mammals and birds. These viruses are classified into *Coronaviridae* family and *Coronavirinae* subfamily along with four genera including *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*. *Alphacoronavirus* and *Betacorona virus* mainly transmit a disease to mammals and responsible for respiratory illness in human while remaining viruses infect mainly birds and rarely mammals. In human population, HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV and SARS-CoV-2 coronavirus have been reported. HCoV-229E, HCoV-NL63 (*Alphacoronavirus)* HCoV-OC43 and HCoV-HKU1 *(Betacoronavirus)* responsible for mild upper respiratory illness while remaining three CoV are more severe including SARS-CoV responsible for severe acute respiratory syndrome (SARS), MERS-CoV responsible for Middle East Respiratory Syndrome (MERS) and recently reported SARS-CoV-2 associated with coronavirus disease-19 (COVID-19) [1].

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In December 2019, first case of COVID-19 was reported in Wuhan city, China and coronavirus was the causing pathogen in these patients based on initial clinical reports. Initially, it was named as 2019 novel coronavirus (2019-nCoV) but subsequently renamed as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) on 11 February 2020. SARS-CoV-2 associated disease was also named as COVID-19. The World Health Organization (WHO) officially announced SARS-CoV-2 outbreaks a Public Health Emergency of International Concern on 30 January 2020 and "pandemic" on 11 March 2020 as confirm cases

were rapidly increasing in China as well as in rest of the world with high transmission rate. At the time of outbreak, no human-to-human transmission was hypothesized but later on, occurrence of human-to-human transmission was accepted. It is not only transmitted through air droplets inhalation coming out from infected person but also through stool and contaminated water also.

During virus outbreak in China, infection was increasing exponentially therefore complete lockdown was imposed in Wuhan on 23 January and further extended to whole Hubei province. Subsequently, virus infection has been controlled significantly now in China and lockdown was uplifted from Wuhan on 8th April 2020 but infections have increased in other parts of the world. Approximately, 27 million corona cases have been reported at the time of submission with 0.9 million deaths across the world. After China, USA, Italy, Iran, Spain, France, and UK were the most affected countries initially, but presently, USA, Brazil, India, and Russia are the most affected countries with SARS-CoV-2 outbreaks. Among these, USA, Brazil, and India are the most adversely affected countries where more than 15 million people have been infected with SARS-CoV-2 virus [2, 3].

Clinical outcomes of COVID-19 are specifically respiratory symptoms specifically sore throat, breathlessness, cough, fatigue, and fever. Mild disease symptoms are usually reported in most of the infected cases but it also leads to the progression of pneumonia, multi-organ dysfunction and acute respiratory distress syndrome (ARDS). Apart from symptomatic patients, it also can be spread through asymptomatic person who is carrying virus but not showing disease symptoms. The average fatality rate in COVID-19 is estimated 2 to 3% depending on various factors. Therefore, rapid expansion of COVID-19 cases are putting extreme burden on health infrastructure and making its control and prevention extremely serious [4]. In this review, mechanistic insight of SARS-CoV-2 life cycle, evolution and phylogenetic relationship with other ancestor and ongoing therapeutic approaches will be discussed.

SARS-CoV-2 STRUCTURE AND GENETIC MATERIAL

SARS-CoV-2 consist of single strand of positive RNA as a genetic material that exemplifies 52 and 79% overall nucleotide sequence homology with other family members, MERS-CoV and SARS-CoV respectively. CoV consist of exceptionally large non-segmented RNA molecule (up to 32 kb) as a genetic material among the RNA viruses with similar genetic composition (Epidemiological and genomic characters of MERS-CoV, SARS-CoV and SARS-CoV-2 are listed in Table 1). Among the CoV, HCoV-NL63 is the first evolved member of CoV family and remaining members are repeatedly evolved during the last 1000 years. CoV synthesizes four type of structural proteins (S, M, N and E-protein) and variable number of nonstructural proteins (NSPs). Structural proteins not only construct viral particles but also perform various vital functions including host receptor interaction, virus assembly and release from host cell and interfered with host immune system. These structural proteins illustrate variable sequence homology among the MERS-CoV, SARS-CoV and SARS-CoV-2 (Figure 1).

SARS-CoV and SARS-CoV-2 both use human angiotensin converting enzyme-2 (hACE2) while MERS-CoV utilizes human dipeptidyl peptidase (DPP4) receptor for entry into host cell. Sequence homology is more among the structural proteins of SARS-CoV and SARS-CoV-2 as compared to MERS-CoV. Among these structural proteins, S-protein is least conserved among three pathogenic coronaviruses as it illustrates 76% (SARS-CoV) and 29.4% (MERS-CoV) sequence homology with S-protein of SARS-CoV-2. Apart from S-protein, remaining structural proteins share high level of similarity (>90%) with respective structural proteins of SARS-CoV-2 (Figure 1) [5].

The hACE2 receptor are present in outer cell membrane of lower respiratory tract, heart, intestine, kidney etc. and exhibit conversion of angiotensin-II to angiotensin 1-7. Angiotensin-II exhibits vasoconstriction through binding with angiotensin AT1 receptor while angiotensin 1-7 exhibits vasodilation through AT2 receptor. Therefore, equilibrium between angiotensin-II and angiotensin 1-7 is decisive and regulates blood pressure. Additionally, hACE2 receptor also acts as a potent negative

regulator that prevents hyperactivity of rennin- angiotensin system (RAS). It likely may be involved in development of lung inflammation. It has a direct involvement, especially in cardiovascular, diabetes and hypertension; therefore, it likely may be one reason for high fatality rate in patients having these diseases during ongoing COVID-19 epidemic [6, 7].

Table 1. Comparative Analysis of Epidemiological and Genomic Characters among MERS-CoV, SARS-CoV and SARS-CoV-2.

Epidemiology Characteristics	MERS-CoV	SARS-CoV	SARS-CoV-2
Natural reservoir	Bat	Bat	Bat
Intermediary host	Dromedary camel	Palm civet	Pangolin (?)
Transmission pattern	Human to Human	Human to Human	Human to Human
Origin place	Arabian Peninsula	Guangdong, China	Wuhan, China
Host receptor	Human dipeptidyl peptidase (DPP4)	Human angiotensin converting enzyme-2 (hACE2)	Human angiotensin converting enzyme-2 (hACE2)
Mortality	Approx. 35%	Approx. 10%	Approx. 2–3%
Disease	Middle East Respiratory syndrome (MERS)	Severe Acute Respiratory Syndrome (SARS)	Coronavirus Disease-19 (COVID-19)
Genomic Characterization			
Genome size	30,119 nts	29,727 nts	29,903 nts
No. of Open reading frames (ORFs)	11	11	12 (putative)
No. of Structural proteins	4	4	4
No. of amino acid in spike proteins	1353 aa	1255 aa	1273 aa
Receptor binding domain (RBD)	367–588	318–510	329–521
No. of non-structural proteins (NSPs)	16	5	15

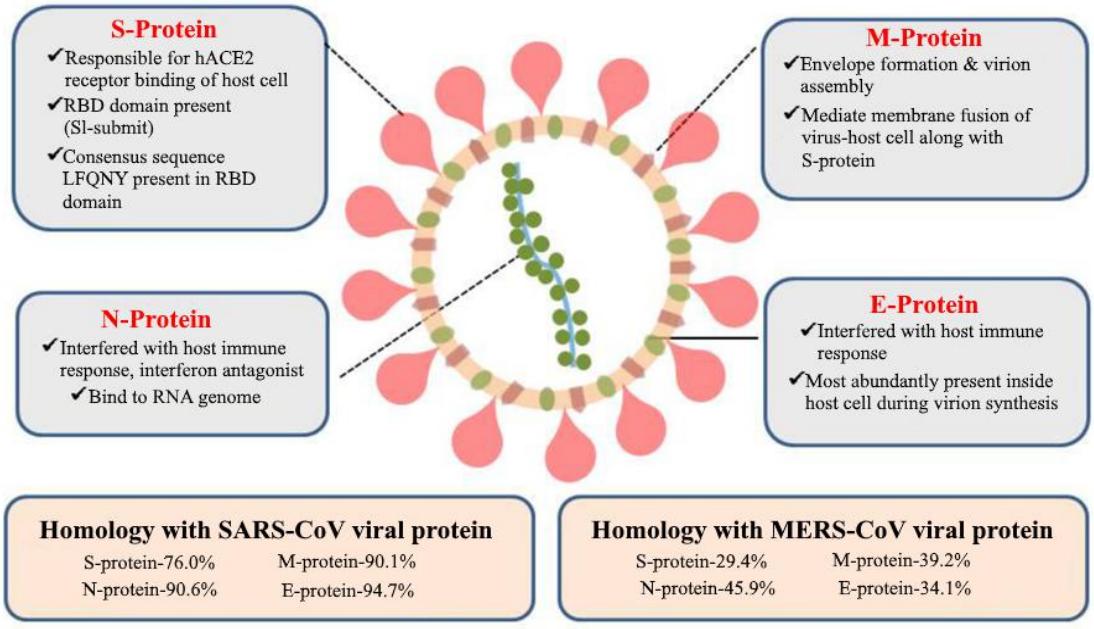

Figure 1. Functional Illustration of Structural Proteins in SARS-CoV-2 Virus and Its Comparative Sequence Homology with Other Pathogenic CoV.

During SARS-CoV-2 pathogenesis, it exhibits entry into host cell through endosome or without endosome formation. Cathepsin and transmembrane serine protease-2 (TMPRSS2) play crucial role during virus entry of SARS-CoV and MERS-CoV but in case of SARS-CoV-2, their role is under intensive research. Recent findings highlighted the involvement of TMPRSS2 in SARS-CoV-2 infection where enhanced SARS-CoV-2 replication was reported in TMPRSS2 expressing cell line. Mechanistic route of its involvement is still not much studied and further research may provide a promising therapeutic approach against present outbreaks [8]. Subsequently, positive RNA strand is released into the host cytoplasm and subsequently forms two polyproteins, pp1a and pp1ab. These polyproteins are encoded from two large over-lapping open reading frames ORF1a and ORF1b. Papain-like protease (PLpro) and chymotrypsin-like protease (3CLpro) are involved in polyprotein processing and form various nonstructural proteins (NSPs) including helicase (Hel) and RNA-dependent RNA polymerase (RdRp). PLpro also exhibits deubiquitinase property along with major protease activity therefore it is also involved in host immune suppression through deubiquitination of NF-κB and interferon factor-3 [9]. Newly synthesized NSPs form replication-transcription complex (RTC) that further produce two types of RNA, positive genomic RNA and overlapping subgenomic negative RNA. Structural and accessory proteins are synthesized from subgenomic RNA and form virion after assembly with genomic RNA, envelope proteins (E) and membrane protein (M). Virion is released outside the cell through exocytosis and similar type of cycle is repeated into another host cell [10].

During virus entry, S-proteins play a vital role in host receptor binding with hACE2 receptor. S-protein consists of three different parts: an outside large ectodomain, single pass transmembrane part and small intracellular part. Ectodomain consists of two subunits S1 and S2 where S1 subunit illustrates receptor binding property through receptor binding domain (RBD) and S2 subunit illustrates membrane fusion property through two domains, heptad repeats 1 (HR1) and heptad repeats 2 (HR2). External part of S-protein appears like trimeric clover-shaped that consists of three S1 heads and three S2 stalks. During infection, RBD binds to hACE2 receptor of host cell and S2 mediates the fusion between virus and host cell membrane. Structural and biochemical studies have revealed that S1 subunit offers more optimized and affirmative binding with hACE2 receptor during SARS-CoV-2 infection in host cell as compared to SARS-CoV. These binding kinetics attract research focus towards S-protein where RBD is the most explored segment of S-protein.

In RBD, there are five critical amino acids that are hypothesized to be involved in receptor binding and determining different hosts including human, cats, ferrets, civet, Chinese horseshoe bat etc. for SARS-CoV-2. Consensus amino acid sequence in RBD is responsible for receptor binding and consists of Y442, L472, N479, D480 and Y491 amino acid residue in RBD of SARS-CoV. In SARS-CoV-2, RBD consensus sequence consists of L455, F486, Q493, N501 and Y505 amino acid residue at defined positions. It is postulated that substitution of L472 (SARS-CoV) to F486 (SARS-CoV-2) may be one of the evolutionary reasons for strong Vander Waals interaction with M82 amino acid residue of hACE2 receptor [11, 12].

In SARS-CoV-2, polybasic furin cleavage site (RRAR) is located at the S1/S2 subunit junction in S-protein present. During host infection, viral genome synthesizes inactive S-protein through host protein machinery that is further cleaved by cellular proteases furin from polybasic furin cleavage site for its activation. Proteolytic cleavage finally activates S-protein through exposure of critical amino acid residues to hACE2 host receptor and exhibits receptor binding and membrane fusion during virus entry. These cleavage sites are present not only in MERS-CoV and HCOV-HKU1 but also in some other RNA virus like avian influenza virus (H5N1), HIV, Ebola virus etc. Furin cleavage site is not present in SARS-CoV as S-protein is uncleaved during virus exit from host cell. Here, TMPRSS2 are present on host cell surface and exhibit proteolytic cleavage of S-protein during SARS-CoV virus infection. Nunberg *et al* (2006) artificially inserted furin cleavage site at R667 position in S1/S2 junction (S-protein) of SARS-CoV to elucidate the role of proteolytic cleavage in viral life cycle. S-

protein was processed accordingly and insertion enhanced cell-cell fusion but viral transmission rate was unaffected. It was concluded that compatible receptor binding and efficient proteolytic cleavage both are crucial barriers in CoV zoonotic infection and should be conquered for zoonotic infectivity in new host [13, 14].

Recently, Menachery *et al*. isolated novel MERS-like coronavirus from Ugandan bat, namely PDF-2180 CoV (MERS-Uganda) [15]. S-protein from MERS-Uganda usually binds with DPP4 receptor of respiratory track. In normal condition, S-protein from MERS-Uganda was able to bind with new host cell but unable to perform virus entry into new host cell. But these outcomes were not similar when trypsin was added exogenously along with MERS-Uganda for virus entry into new host cell. In later experiment, successful virus entry was reported in new host cell even though compatible receptor binding was not there. Such findings illustrated that proteolytic cleavage is more decisive barrier for zoonotic transmission and proposed a new direction to evaluate the future zoonotic transmission [15]. Additionally, furin site is flanked by O-linked glycosylation residues present at S673, T678 and S686 in S-protein of SARS-CoV-2. Biological significance of O-linked glycosylation is still not much explored but hypothesizes to shield critical residues of S-protein. These critical amino acid residues may trigger host immune response therefore these are shielded through O-linked glycosylation. In SARS-CoV-2, there are many other structural proteins that play crucial role in various vital functions during virus life cycle. Membrane (M) protein is another structural protein that mediates envelope formation and virus assembly.

It consists of three membranes spanning transmembrane region, external N-terminus with single N-glycosylation site and long internal C-terminal region. It mediates membrane fusion after S-protein binding with respective hACE2 receptor and plays critical role in virus infectivity. It is considered as central organizer of virus assembly that determines envelope shape through interacting with other structural proteins. M-protein of SARS-CoV-2 illustrates 90.1 and 39.2% homology with SARS-CoV and MERS-CoV respectively.

Envelope (E) protein is the smallest but most mysterious structural protein that interacts with M-protein during envelope formation. It is an integral membrane protein that also consists of three segments; hydrophilic N-terminal, single pass transmembrane domain and large hydrophilic C-terminal. It is abundantly present inside the host cell during virus infection but only small fraction is utilized for virion assembly. Fourth structural protein is Nucleocapsid (N) protein that binds to RNA genome and forms Nucleocapsid with the help of M-protein. The E-protein of SARS-CoV-2 illustrates 94.7 and 34.1% homology with SARS-CoV and MERS-CoV protein respectively while N-protein of these virus are 90.6 and 45.9% homologous with SARS-CoV-2 protein. In SARS-CoV-2 genome, structural and accessory genes cover small part of viral genome where structural proteins play structural role while accessory proteins are involved in interference process with host immune system [15].

EVOLUTION PATHWAY OF SARS-CoV-2

On 10th January 2020, first SARS-CoV-2 genome sequence data was obtained that exhibited close sequence homology with SARS-like viruses (Coronaviridae family). It also exemplifies 52 and 79% nucleotide sequence homology with other coronavirus, MERS-CoV and SARS-CoV respectively. SARS-CoV-2 genomic structure exhibits close similarity with other *betacoronavirus* and consist of gene order 5'-UTR-ORF1a-ORF1ab-spike(S)-envelope(E)-membrane(M)-nucleocapsid(N)-UTR-3'. Positive strand genomic RNA consists of two important eukaryotic RNA features, 5'-methylated cap and 3'-polyadenylated tails.

Comparative genomic analysis of SARS-CoV-2 has revealed its 96% overall sequence homology with bat SARS-CoV-like coronavirus, RaTG13. It was isolated from *Rhinolophusaffinis* (horseshoe bat) in Yunnan province, China in 2013 [17]. There were some reports about additional close

DNA Sequence homology with SARS-CoV-2	96%	91.02%	-
Host entry receptor	Angiotensin-converting enzyme-2(ACE2)	Angiotensin-converting enzyme-2(ACE2)	Angiotensin-converting enzyme-2(ACE2)
Receptor Binding Domain (RBD) sequence in S-protein	L-L-Y-D-H	L-F-Q-N-Y	L-F-Q-N-Y
Furin recognition motif in Sl/S2 cleavage site/Sequence	Absent/-SRSV	Absent/-SRSV	Present/-PRRARSV

Figure 2. Diagrammatic Illustration of SARS-CoV-2 Evolution Pathway and Comparative Analysis of Amino Acid Sequence of RBD and Furin Cleavage Site.

relative of SARS-CoV-2 in *Manis javanica*, pangolin-CoV (*Malayan pangolin*) with 91.02% overall sequence homology. These dead pangolins with pulmonary fibrosis and foamy liquid in lungs were illegally imported into Guangdong province on October, 2019. Interestingly, pangolin sample timing and SARS-CoV-2 outbreaks are very close and possibly may be linked with this outbreak due to close sequence proximity. There are some other SARS-CoV-like viruses also have been reported in bat with varying sequence proximity.

Now, it is important to determine the evolutionary pattern of SARS-CoV-2 among these viruses. There are many possible ways for interspecies transmission in case of SARS-CoV-2 such as direct transmission either from bats or pangolin or both contribute along with some other mediator species through natural selection. Here we analyze all possible ways to understand its origin. First, direct transmission possibility either from bat or pangolin is discussed here. SARS-CoV-2 exhibits efficient binding with host hACE2 receptor through RBD domain of S-protein but RBD sequence of RaTG13 and SARS-CoV-2 are significantly diverse (Figure 2).

RaTG13 consists of LLYDH amino acid sequence while SARS-CoV-2 consists of LFQNY sequence in RBD domain. Out of five, only one amino acid is conserved in RaTG13 that is likely not able to bind efficiently with host receptor. So, it minimizes the direct transmission possibility from bat to human with such weak binding. Another direct transmission is possible from pangolin as it exhibits more close sequence identity between RBD domain of SARS-CoV-2 and pangolin-CoV. RBD domain of both viruses consists of LFQNY amino acid sequence as a consensus sequence that is responsible for binding with host receptor. It primarily illustrates that pangolin-CoV S-protein is optimized for efficient binding with hACE2 like receptor. Sequence proximity in RBD between these two primarily illustrates the possibility of direct transmission from pangolin but another barrier is furin cleavage site. Furin cleavage site at S1/S2 junction is another difference among these as it is absent in RaTG13 and pangolin-CoV but present in SARS-CoV-2. Therefore, there is very least possibility of direct interspecies transmission without any intermediate species either from bats or pangolin [16, 17].

Another possible way of interspecies transmission is through recombination and natural selection. It might be possible that intermediate organism was infected with these two different viruses RaTG13 and pangolin-CoV simultaneously. Recombination occurred between these two divergent viruses in intermediate species and SARS-CoV-2 progenitor was evolved that exhibited optimized receptor binding. Various processes like insertion, deletion and mutation also occur near S1-S2 junction during

evolution either in intermediate species or in human. If it occurred in intermediate species, it is likely possible that an intermediate animal source was present at Huanan market in Wuhan as major initial COVID-19 cases were having visit history of this market. Interspecies transmission occurred from Wuhan market and it spread quickly among human population through various modes of transmission. If it occurs in humans then it is possible that SARS-CoV-2 chimeric progenitor was introduced in humans long before ongoing outbreak. Initially, virulence and antigenicity were very low therefore it was undetected during human-to-human transmission in early phase. It gradually acquired present genomic features including furin cleavage site through natural evolutionary process and became more infective with optimized binding with hACE2 receptor. Out of so many possible ways, researchers are not able to confirm SARS-CoV-2 evolution route based on limited available information. Still, there are so many links which are still unanswered like, which organism served as intermediate animals, what were the factors responsible for recombination, how furin cleavage site is evolved either in intermediate animal or in human etc. Presently, there is no report of any such animal virus that is significantly analogous with chimeric SARS-CoV-2 progenitor but it likely may be reported in other animal species in future studies. Additionally, it also should be studied that any such kind of hidden transmission of SARS-CoV-like coronavirus occurred long before in human population [12, 18, 19].

During SARS-CoV-2 origin, positive natural selection and recombination are primarily responsible for enormous genomic diversity and specifically in S-gene as it mediates use of diverse host receptor for virus infection. Genomic location of positive selection in S-protein may be different in various S-genes of different CoVs. It has been reported that it occurs in the heptad repeats in MERS-CoV while in the RBD region in SARS-CoV and SARS-CoV-2. Additionally, ORF1a is also targeted for positive selection in MERS-CoV, especially nsp3 protein that illustrates function of viral protease and also suppresses the interferon response in host cell.

Present understanding of CoV genome highlights that it is dynamic in terms of gene numbers and recombination. Adaptive mutation chances are also high due to large genome size and any positive selection in S-protein can make it successful to utilize different host receptors. All these features collectively highlight the high probability of interspecies transmission of CoV in near future. Therefore, research should be intensified to understand the mechanistic insight of interspecies transmission and its determinants to prevent any kind of virus outbreaks of CoV in future. Additionally, there are various natural reservoirs where numerous viruses exist since very long time. Human activities like urbanization, agricultural practices, wild life habitat fragmentation and loss and illegal hunting etc. are also responsible for periodic zoonotic outbreak. Therefore, we should follow two-way approaches to control such zoonotic outbreaks. First, mechanistic research should be intensified to understand the mechanism of interspecies transmission, as well, natural barrier should be maintained between natural reservoirs and human.

THERAPEUTIC APPROACH AGAINST SARS-CoV-2

As COVID-19 persist its spread across the world, researchers are intensively exploring the therapeutic potential of various molecules. As of mid-April 2020, it has been reported in 1.5 million persons with total death of 0.1 million person. Numerous therapeutic approaches including interferon therapy, silencing RNA, monoclonal antibody, vaccines, and small molecule etc. are either reported *in vitro* study or under ongoing clinical trial. Unfortunately, there is complete absence of FDA approved drug or vaccine for COVID-19 treatment presently but certain existing antiviral and antimalarial drugs have been recommended in few countries during ongoing epidemics. Similar to SARS and MERS, SARS-CoV-2 genome synthesizes numerous structural and nonstructural proteins that play crucial role during virus life cycle. Out of these, five proteins including PLpro, 3CLpro, RdRp, Hel and S-protein are identified as potential therapeutic targets for COVID-19 treatment. Among these viruses, catalytic sites are conserved and exhibit high sequence homology, therefore large proportions of existing antiviral drugs are being considered for COVID-19 treatment.

In RNA virus, mutation and recombination rate are comparatively higher than DNA virus and responsible for high plasticity in virulence and antigenicity. Mutation rate in coronavirus family is not constant in all members but these viruses unusually consist of high replication fidelity. Therefore, it may be one positive hope in therapeutics against coronavirus because antigenic stability is offered through high replication fidelity. Here, we briefly discuss important drug candidates against COVID-19 treatment to save thousands of lives and ongoing various vaccine clinical statuses for future prevention.

Protease Inhibitor

The 3CLpro protein exhibits dimeric structure and mediates protease activity through Cys-His dyad on its active site. It cleaves initially formed two polyproteins pp1a and pp1ab along with PLpro protein and subsequently releases NSPs. It can be modulated by numerous protease inhibitors like pyrazole analogues, N-phenyl-2-acetamide, C2-symmetric diols, zinc conjugates, benzotriazole, and glutamine peptides with trifluoromethylketone group. The PLpro also cleaves polyproteins pp1a/pp1ab and subsequently forms NSPs (1-3). It consists of LXGG conserved sequence at active site and inhibited by zinc conjugates and benzodioxole. Presently, no inhibitor has been tested clinically against PLpro activity due to less success ratio in previous two CoV outbreaks, SARS, and MERS.

Among these, lopinavir-ritonavir is the most studied broad spectrum protease inhibitor that exhibited *in vitro* inhibition activity in non-randomized trials of SARS. Recently, efficacy of these potent inhibitors was studied with 199 COVID-19 patients through randomized trials. It has not reflected any kind of difference in clinical improvement as compared to control results and even more adverse gastrointestinal issues were associated with lopinavir-ritonavir treatment. Future combinatorial study of lopinavir-ritonavir with other antiviral agents can address the possibility of any enhancement of their efficacy in COVID-19 treatment. Therefore, lopinavir-ritonavir and ribavirin in fixed dose combination is currently under clinical trials as same combination has been reported with reduced fatality rate and clinical enhancement in SARS patients in the 2003 [20].

Darunavir, 3CLpro protein inhibitor has been licensed for HIV treatment by FDA along with ritonavir without any reported resistance. It is under randomized phase III clinical study with low cobicistat dose as cobicistat inhibits cytochrome P450 (CYP3A) and subsequently enhances pharmacokinetics of darunavir. Such combination has been approved for study in China with 800 mg of darunavir and 150 mg of cobicistat dose and estimated primary study completion in August 2020. ASC09F is also a potential 3CLpro inhibitor, initially reported in HIV infection and presently under phase III in combination with oseltamivir for COVID-19 treatment [21].

RNA Dependent RNA Polymerase (RdRp) Enzyme Inhibitor

The RdRp is responsible for the synthesis of genomic and subgenomic RNA during virus life cycle inside the host cell. Guanosine analogue ribavirin is a broad-spectrum antiviral drug with major applicability in hepatitis-C, viral haemorrhagic fevers etc.

Remdesivir, a guanosine analogue has been tested in humans against Ebola, SARS, and MERS virus with promising clinical outcomes. It was studied *in vitro* in Vero E6 cells where it inhibited SARS-CoV-2 (EC_{50}=0.77 μM). In USA, it exhibited positive results in COVID-19 patients where it was administered intravenously. Therefore, two phase III studies were launched to evaluate its efficacy in February in USA, China, and UK with estimated completion in April 2020. Drugs will be administered intravenously with 200 mg on first day, while 100 mg dose for remaining 9 days [22].

Favipiravir, a guanosine nucleoside was developed by Fujifilm Toyama in Japan and presently approved for phase III clinical trials for COVID-19 treatment. It has been approved for influenza treatment in Japan and China with trade name, Avigan. It has potential to inhibit RNA virus

replication through interfering RdRp enzyme activity. Mechanistically, RdRp enzyme is used in its phosphribosylated form as a substrate and subsequently unable to exhibit its activity. Favipiravir entry into host cell converts this into active phosphribosylated form. On 14 February, Clinical Medical Research Centre Shenzhen conducted a clinical trial with 80 patients where two different quantitative doses, 1600 mg on the first day along with two 600 mg dose for remaining time were administered into 70 patients during initial clinical study. During study, it has exhibited efficacy with minimal side effects and after quickly cleared virus as compared to control group that was administered with combinatorial anti-HIV drugs opinavir/ritonavir [23, 24].

Galidesivir (BCX4430, Immucillin-A), BioCryst Pharmaceuticals developed this broad-spectrum adenosine analogue initially for hepatitis-C virus infection as it also interferes the RNA polymerase activity. Presently, it is considered as a potent drug against wide range of fatal virus including Ebola, SARS-CoV, MERS-CoV, Marburg virus and filovirus. Presently, it is under phase I of clinical study in case of yellow fever and Marburg virus but still no clinical study has been initiated specifically for COVID-19 treatments [25].

Virus-Host Cell Fusion Inhibitor

Arbidol (umifenovir), broad-spectrum antiviral indole derivative that inhibits virus entry into host cell as it blocks virus-host cell membrane fusion during infection. Therefore, it has been licensed for influenza and prophylaxis treatment in China and Russia. Since 2004, it is also licensed for SARS treatment with Masterlek trade name without FDA approval. Presently, it has been approved for phase IV study in China into three different combinations. Arbidol is administered with dose of 100 mg for 7–14 days through oral method. Additionally, arbidol is also under clinical study with lopinavir-ritonavir and oseltamivir separately [26].

Oseltamivir was reported as a neuraminidase enzyme inhibitor in influenza virus infection and blocks virus release form host cell surface. In China, it was utilized during recent SARS-CoV-2 outbreaks in different combination with antibiotics and corticosteroids. Presently, it is also approved for phase III study in different combinations with favipiravir and chloroquine [27, 28]. Nafamostat mesylate is licensed for pancreatitis in Japan and recently reported in SARS-CoV-2 access into the host cell. It inhibits virus entry into host cell through TMPRSS2 interference more effectively as compared to camostat mesylate. In Japan, Nafamostat and camostat mesylate are expected to enter into clinical study in April 2020 for efficacy evaluation of these two drugs in COVID-19 treatment [29].

Antimalarial Drugs

Chloroquine and its hydroxy derivative hydroxychloroquine are the most attentive antimalarial drugs against SARS-CoV-2 infection even though their efficacy is still under clinical investigations. These drugs are licensed for malaria treatment through oral mode as it not only restrains malarial infection but enhances time period between initial cure and relapse. Hydroxychloroquine is less toxic and more potent as compared to chloroquine. *In vitro* study of these quinolone drugs has exhibited inhibitory effect against coronavirus.

Mechanistic route is not much explored but it likely inhibits proton movement across the endosome membrane and consequently enhances endosomal pH. *In vitro*, these drugs have exhibited broad-spectrum antiviral activities against some RNA viruses including SARS and SARS-CoV-2. Chloroquine was reported as a potential drug for against SARS-CoV-2 *in vitro* study in Vero E6 cells with EC_{50} value of 1.13 μM. Later on, non-randomized small clinical trial was conducted for hydroxychloroquine efficacy in COVID-19 treatment with promising final clinical outcomes. These clinical findings based on small study have given positive hope to us in the fight against COVID-19. Therefore, hydroxychloroquine is under phase III study (NCT04329923) where 400 mg dose per day is proposed for 5 days in patients with estimated completion in December 2020 [30, 31].

Antibody Based Therapeutic Approaches

SARS-CoV-2 infection into human host not only triggers innate but adaptive immune response into host but impaired and uncontrolled inflammatory response sometime may direct to tissue damage. Therefore, numerous therapeutic approaches are specifically intensifying various immunologic components that participate in inflammatory response. It has been reported that Granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 6 receptor (IL-6R) expressions are significantly elevated during SARS-CoV-2 infection and responsible for inflammation in tissue. These findings highlight the therapeutic importance of IL-6R and GM-CSF and significantly control inflammation so that anti-viral drugs get more time to work. Therefore, numerous monoclonal antibodies specific to these two immune components are under clinical trial (listed in Table 2).

Additionally, convalescent plasma is isolated from the patients after disease recovery and consists of neutralizing antibodies against disease causing agents. It has reduced mortality and hospital stay time in case of SARS patients as compared to study group treated without convalescent plasma. Similar clinical outcomes were reported during influenza A H1N1 virus outbreak during 2009. The WHO has been recommended for Ebola treatment in 2014 and for SARS treatment in 2015. Under numerous clinical studies, convalescent serum is administered into the patients for COVID-19 treatment [32–34].

VACCINE PROGRAMMES AGAINST SARS-CoV-2

SARS-CoV-2 emergence is new in human population and very little is known about its complex life cycle and transmission in host. Intensified research activity was introduced to develop an effective vaccine after publication of SARS-C0V-2 genome. Presently, numerous types of vaccines like RNA, DNA, virus vector based, recombinant protein and live attenuated are under different phases of clinical trial against COVID-19 disease (listed in Table 3).

Recently, Russian vaccine Gam-COVID-Vac is the first registered corona vaccine that is invented by Gameleya Research Institute. It was registered on 11 August 2020 with trade name "sputnik-5' by Russian Health Ministry. It is an adenovirus-based vaccine that consists of two biological components rad26 and rad5. Rad26 and rad5 are recombinant adenovirus that is based on S-protein component of SARS-CoV-2 and trigger host immune system against SARS-CoV-2. Rad26 is injected first and followed by rad5 vaccine with 21 days time period gap.

Table 2. Overview of Different Ongoing Therapeutic Programme for SARS-CoV-2.

Therapeutic Category	Name of Drugs	Mode of Action	Current Clinical Status
Antibody (mAb)	Tocilizumab (Actemr), Sarilumab (Kevzara), Siltuximab (SYLVANT), TZLS-501, Namilumab, Gimsilumab, Mavrilimumab, Lenzilumab, TJ003234, TJM2, Leronlimab (PRO140), Bevacizumab (Avastin), VIR-7831 and VIR-7832, Eculizumab (Soliris), Camrelizumab (Airuka), Meplazumab, Emapalumab (Gamifant) Convalescent plasma	Tocilizumab, Sarilumab, Siltuximab and TZLS-501: Anti-Interleukin 6-receptor (Anti-IL-6R) Gimsilumab, Namilumab, Lenzilumab, Mavrilimumab, TJ003234 and TJM2: Anti granulocyte-macrophage colony stimulating factor (Anti-GM-CSF) Leronlimab: humanized monoclonal IgG4 against to CCR5 receptor (Anti-CCR5) Bevacizumab: Anti vascular endothelial growth factor (Anti-VEGF) Eculizumab: Complement inhibitor	Tocilizumab: NCT04306705, NCT04310228, NCT04317092, NCT04315480, NCT043220615 Sarilumab: NCT04315298, NCT04321993, NCT04324073 Siltuximab: NCT04322188 Gimsilumab and TJ003234 - Phase II Namilumab: Pre-clinical phase Lenzilumab: Phase III Mavrilimumab: NCT04337216 Leronlimab: Phase II Bevacizumab: NCT04305106

		Camrelizumab: specific to programmed cell death protein-1 (PD-1) Meplazumab- Humanized anti-CD-147 antibody VIR-7831 & 7832=Anti S-protein of SARS-CoV-2. Emapalumab= Anti IFN-γ Convalescent plasma= neutralizing antibodies from recovered patient against disease causing agent	Eculizumab: NCT04288713 VIR7831 and 7832: Preclinical phase Camrelizumab: NCT04268537 Meplazumab: Phase II (NCT04275245) Emapalumab: Phase II (NCT04324021) Convalescent plasma: NCT04292340, NCT04332835, NCT04321421, ChiCTR2000030841, ChiCTR2000030929
Antiviral drugs	ASC09, Danoprevir (Ganovo), Darunavir (Prezcobix), Azvudine, Oseltamivir (Tamiflu)	ASC09, Darunavir and Danoprevir: Protease inhibitor Azvudine: Nucleoside reverse transcriptase inhibitor (NRTI) Oseltamivir: Inhibits neuraminidase enzyme activity	ASC09-Pre-clinical phase (NCT04261907) Darunavir: Phase III (NCT04304053) Danoprevir: Phase IV (NCT04291729) Azvudine: ChiCTR2000029853 Oseltamivir: NCT04303299, NCT04255017, NCT04261270
Cytokine inhibitor	Interferon-α2β (IFN- α2β) Novaferon (rIFN-α) Rebif (IFN-β1a) Ruxolitinib (Jakafi)	Induce interferon stimulated genes that participate in inflammation and immunomodulation Ruxolitinib: Janus Kinase 2 (JAK2) inhibitor	IFN-α2β: Phase I (NCT04293887) Novaferon: ChiCTR2000029496 Rebif: Phase III (NCT04315948) Ruxolitinib: Phase II (NCT04334044)
Steroid hormone	Corticosteroids (Methylprednisolone)	Effectively modulate inflammatory response	Different clinical phase (NCT04244591, NCT04263402, NCT04273321, ChiCTR2000029656)
Nuclease inhibitor	Baloxavirmarboxil (Xofluza)	Endonuclease inhibitor and interfere with viral replication	Phase I (ChiCTR2000029544 and ChiCTR2000029548)
Stem Cell Therapies	Mesenchymal stem cell (MSCs), MultiStem, Ryoncil	Stem cell use in repair of various tissue damage during infection	Different clinical phase NCT04273646, NCT04313322, NCT04288102, NCT04315987, NCT04302519
Miscellaneous drugs	Fingolimod (Gilenya), Losartan, Ifenprodil (NP-120)	Fingolimod: Sphingosine-1-phospahte modulator Losartan: Angiotensin II receptor antagonist, approved drug for hypertension Ifenprodil: N-methyl-D-aspartate (NMDA) receptor antagonist	Fingolimod: Phase II (NCT04280588) Losartan: Phase II (NCT04312009, NCT04311177) Ifenprodil: Phase I

Table 3. Overview of Ongoing Vaccine Development Programme for SARS-CoV-2.

Vaccine Type	Vaccine Name	Company	Vaccine Details and Present Status
DNA Vaccine	Fusogenix DNA	Entos Pharmaceuticals	Proteo-lipid vehicle, N/A
	DNA vaccine	Zydus Cadila	Pre-clinical phase
	STI-6991 (I-Cell)	Sorrento Therapeutics Ltd.	Nanoparticle based DNA vaccine, Phase I in mid-2020
	CORVax12	Oncosec Medical Inc.	DNA vaccine, Phase I
	INO-4800	Inovio Pharmaceuticals and Beijing Advaccine Biotechnology	DNA vaccine by Intramuscular, phase I
RNA Vaccine	BNT-162	Pfizer and BioNTech	m-RNA vaccine, Pre-clinical phase
	LUNAR-COV19	Arcturus Therapeutics Ins. and Duke-NUS Medical School	m-RNA vaccine, Pre-clinical phase
	RNA vaccine	CureVac, Germany	Phase-I
	mRNA-1273	Moderna and Vaccine Research Centre	Specific for S-protein, Phase I (NCT04283461)
Recombinant Protein Vaccine	APN01	Apeiron Biologics	Recombinant form of hACE2 receptor, phase-II
	li-key peptide	Generex and EpiVax	Also tested in influenza, HIV and SARS. Pre-clinical phase
	gp-96	Heat Biologics and University of Miami	Induce mucosal immunity, Pre-clinical phase
	IPT-001	Intellistem Technologies Inc.	Peptide based vaccine against S and N-protein, Phase I in Sep 2020
	DPX-COVID-19	IMV Inc and University Laval	Lipid based vaccine, Phase I
	AT-100 (rhSP-D)	Airway Therapeutics	Pre-clinical study
	S-Trimer vaccine	Clover Biopharmaceuticals and GSK	S-protein with adjuvant, pre-clinical study
	COVID-19 XWG-03	GSK and Xiamen Innovax Biotech Ltd.	Based on truncated S-protein, Phase I
Viral Vector Based Vaccine	ChAdOx1 nCoV-19	University of Oxford	Adenovirus vector, phase III study
	AdCOVID	Altimmune and University of Albama	Intranasal mode, pre-clinical study
	Ad5-nCoV	CanSino Biologics	Pre-clinical phase (NCT04313127)
	Gam-COVID-Vac (Sputnik-V)	The Gamaleya Research Institute	Clinical Phase III (Ist registered vaccine)
	Grevax	Greffex	Pre-clinical phase
	Oral vaccine platform	Vaxart Inc.	Pills containing various antigen of COVID-19, Pre-clinical phase
Live Attenuated and Inactivated Vaccine	TNX-1800	Tonix Pharmaceuticals Corp.	Attenuated horsepox virus for percutaneous administration, pre-clinical phase
	Live attenuated vaccine	Codagenix and Serum Institute of India	Pre-clinical phase
	Coroflu	Flugen, Bharat Biotech International Ltd. and University of Wisconsin-Madison	Nasal vaccine and modified form of flu vaccine M2SR, Clinical evaluation phase
Convalescent Serum	TAK-888	Takeda Pharmaceuticals Co.	Polygonal hyper immune antibody from recovered patients, Pre-clinical phase

CONCLUSIONS

SARS-CoV-2 consists of RNA as a genetic material and responsible for ongoing global pandemic Covid-19 with serious respiratory issues and lung infections. It has been hypothesized that it evolved from BatCoV-RatG13 and Pangolin-CoV, close relatives of SARS-CoV-2 but ancestor virus does not consist high rate of infectivity. Presently, researchers are exploring all possible ways to discover an effective vaccine against SARS-CoV-2 under different clinical trial stages. Some vaccines have shown positive hopes against SARS-CoV-2 virus infection and hoping their approval by the end of 2020.

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REFERENCES

1. Cui J, Li F, Shi Z-L. Origin and Evolution of Pathogenic Coronaviruses. Nat Rev Microbiol. 2019; 17(3): 181–192p.
2. Singhal T. A Review of Coronavirus Disease-2019 (COVID-19). Indian J Pediatr. 2020; 87(4): 281–286p.
3. Organization WH. Novel Coronavirus (2019-nCoV): Situation Report. 2020; 3.
4. Organization. WH. Clinical Management of Severe Acute Respiratory Infection (SARI) When COVID-19 Disease is Suspected: Interim Guidance, 13 March 2020. World Health Organization; 2020.
5. Zhang Y-Z, Holmes EC. A Genomic Perspective on the Origin and Emergence of Sars-Cov-2. Cell. 2020; 181(2): 223–227p.
6. Hamming I, et al. Tissue Distribution of ACE2 Protein, the Functional Receptor for SARS Coronavirus. A First Step in Understanding SARS Pathogenesis. J Pathol: A Journal of the Pathological Society of Great Britain and Ireland. 2004; 203(2): 631–637p.
7. Bodiga VL, Bodiga S. Renin Angiotensin System in Cognitive Function and Dementia. Asian Journal of Neuroscience. 2013; 2013: 1–18p.
8. Matsuyama S, et al. Enhanced Isolation of SARS-CoV-2 by TMPRSS2-Expressing Cells. Proc Natl Acad Sci. 2020; 117(13): 7001–7003p.
9. Báez-Santos YM, John SES, Mesecar AD. The SARS-Coronavirus Papain-Like Protease: Structure, Function and Inhibition by Designed Antiviral Compounds. Antiviral Res. 2015; 115: 21–38p.
10. Shereen MA, et al. COVID-19 Infection: Origin, Transmission, and Characteristics of Human Coronaviruses. J Adv Res. 2020; 24: 91–98p.
11. Yan R, et al. Structural Basis for the Recognition of SARS-CoV-2 by Full-Length Human ACE2. Science. 2020; 367(6485): 1444–1448p.
12. Andersen KG, et al. The Proximal Origin of SARS-CoV-2. Nature Medicine. 2020; 1–3p.
13. Coutard B, et al. The Spike Glycoprotein of the New Coronavirus 2019-nCoV Contains a Furin-Like Cleavage Site Absent in CoV of the Same Clade. Antiviral Res. 2020; 176: 104742p.
14. Follis KE, York J, Nunberg JH. Furin Cleavage of the SARS Coronavirus Spike Glycoprotein Enhances Cell–Cell Fusion but does not Affect Virion Entry. Virology. 2006; 350(2): 358–369p.
15. Menachery VD, et al. Trypsin Treatment Unlocks Barrier for Zoonotic Bat Coronavirus Infection. J Virol. 2020; 94(5): e01774–19.
16. Zhang T, Wu Q, Zhang Z. Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak. Curr Biol. 2020; 30(8): 1578p.
17. Zhou P, et al. A Pneumonia Outbreak Associated with a New Coronavirus of Probable Bat Origin. Nature. 2020; 579(7798): 270–273p.
18. Tang X, et al. On the Origin and Continuing Evolution of SARS-CoV-2. Natl Sci Rev. 2020; nwaa036.
19. Li X, et al. Evolutionary History, Potential Intermediate Animal Host, and Cross- Species Analyses of SARS- CoV- 2. J Med Virol. 2020; 92(6): 602–611p.

20. Velavan TP, Meyer CG. The COVID-19 Epidemic. Trop Med Int Health. 2020; 25(3): 278–280p.
21. Sahu KK, Mishra AK, Lal A. Comprehensive Update on Current Outbreak of Novel Coronavirus Infection (2019-nCoV). Ann Transl Med. 2020; 8(6): 393p.
22. Li G, De Clercq E. Therapeutic Options for the 2019 Novel Coronavirus (2019-nCoV). Nature Publishing Group, Nat Rev Drug Discov. 2020; 19(3): 149–150p.
23. Shiraki K, Daikoku T. Favipiravir, an Anti-Influenza Drug against Life-Threatening RNA Virus Infections. Pharmacol Ther. 2020; 209: 107512p.
24. Guo D. Old Weapon for New Enemy: Drug Repurposing for Treatment of Newly Emerging Viral Diseases. Virol Sin. 2020; 35(3): 1–3p.
25. Zumla A, et al. Coronaviruses: Drug Discovery and Therapeutic Options. Nat Rev Drug Discov. 2016; 15(5): 327–347p.
26. Senanayake SL. Drug Repurposing Strategies for COVID-19. Future Science, Future Drug Dicov. 2020; 2(2): 1–3p.
27. Wang D, et al. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA. 2020; 323(11): 1061–1069p.
28. Rosa SGV, Santos WC. Clinical Trials on Drug Repositioning for COVID-19 Treatment. Rev Panam Salud Pública. 2020; 44: e40p.
29. Hoffmann M, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020; 181(2): 271–280p.
30. Owens B. Excitement around Hydroxychloroquine for Treating COVID-19 Causes Challenges for Rheumatology. Lancet Rheumatol. 2020; 2(5): e257p.
31. Gautret P, et al. Hydroxychloroquine and Azithromycin as a Treatment of COVID-19: Results of an Open-Label Non-Randomized Clinical Trial. Int J Antimicrob Agents. 2020; 56(1): 105949p.
32. Chen L, et al. Convalescent Plasma as a Potential Therapy for COVID-19. Lancet Infect Dis. 2020; 20(4): 398–400p.
33. Organization WH. Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (2019-nCoV) Infection is Suspected: Interim Guidance, 28 January 2020. World Health Organization; 2020.
34. Casadevall A, Pirofski L-A. The Convalescent Sera Option for Containing COVID-19. The J Clin Investig. 2020; 130(4): 1545–1548p.

