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COVID-19 Pandemic: Insights into Structure, Function, and hACE2 Receptor Recognition by the SARS-CoV-2

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Abstract

SARS-CoV-2 is a newly emerging, highly transmissible, and pathogenic coronavirus in humans, which has caused global public health emergency and economic crisis. To date, millions of infections and thousands of deaths have been reported worldwide, and the numbers continue to rise. Currently, there is no specific drug or vaccine against this deadly virus; therefore, there is a pressing need to understand the mechanism through which this deadly virus enters the host cell. Viral entry into the host cell is a multistep process in which SARS-CoV-2 utilizes the receptor binding domain of the spike glycoprotein (S) to recognize ACE2 receptors on the human cells; this initiates the host cell entry by promoting the viral-host cell membrane fusion through large scale conformational changes in the S protein. Receptor recognition and fusion are critical and essential steps of viral infections and are key determinants of the viral host range and cross-species transmission. In this review, we summarize the current knowledge on the origin and evolution of SARS-CoV-2, roles of key viral factors and discuss the receptor recognition mechanisms of coronaviruses. We provide a comparative analysis of the SARS-CoV and SARSCoV-2 S proteins, receptor-binding specificity, and discuss the differences in their antigenicity based on biophysical and structural characteristics. Finally, we dive into available medications, and the current COVID-19 treatment options, which will be beneficial for the scientific community as well as for the general public.

Key words: COVID-19, SARS-CoV, SARS-like coronavirus, 2019-nCoV, SARS-CoV-2, angiotensin-converting enzyme 2 (ACE2), and neutralizing antibody.

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1. Introduction

Before 2003, only two human coronaviruses, HCoV-229E and HCoV-oC43, causing mild illness were known to mankind[1–3]. However, the emergence of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) changed the view worldwide, because coronaviruses can cause life- threatening infections[4–6]. The ongoing pandemic of a novel strain of coronaviruses, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), is posing an unforeseen public health and economic threats worldwide. As of May 11, 2020, SARS-CoV-2 has infected more than 3.92 million people with 274,488 deaths[7] reported from 215 countries and territories, of which there are 1,245,775 confirmed cases of COVID-19 and 75,364 deaths in the USA alone[8]. Recombination, mutator alleles and mutational robustness are some of the evolutionary mechanisms[9], which makes coronaviruses capable of expending their host ranges, including human beings. Therefore, understanding the virology of the coronaviruses at structural level is of utmost importance, because health threats from these zoonotic viruses are constant, and long term.

Coronaviruses are large, enveloped, positive-stranded RNA viruses, responsible for infecting a wide variety of mammalian and avian species[10]. These viruses contain spike-like projections of glycoproteins on their surface, which appear as crown under the electron microscope; hence, they are referred to as coronaviruses (*coronam* is the Latin term for crown). There are four sub-categories of coronaviruses: alpha, beta, gamma, and delta. Based on phylogenetic evidences SARS-CoV-2 has been classified as a new member of the betacoronavirus family. This novel pathogenic virus is the seventh coronavirus that has been identified to infect humans and the other six are: 229E and NL63 (alphacoronavirus), OC43, HKU1, SARS-CoV, and MERS-CoV (betacoronaviruses). Among these 229E, NL63, OC43, HKU1 are known to infect

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the human population that usually result in mild to moderate respiratory disease[11]. However, infection of SARS-CoV, MERS-CoV[1,6], and SARS-CoV-2 is not only limited to severe respiratory illnesses but can also potentially damage multiple organs, causing death in certain cases (Corman et al., 2019).

The coronavirus genome encodes several structural and nonstructural proteins. The structural proteins are responsible for host infection[12], membrane fusion[13], viral assembly[14], morphogenesis, and release of virus particles[15] among other functions, and the non-structural proteins facilitate the viral replication and transcription[16,17]. The membrane (M), the envelope (E), and the spike protein (S) are part of structural proteins and are associated with the envelope. Among these structural proteins, the trimeric spike proteins protrude from the virus envelope and is a key machinery that facilitate virus entry into the host cell[10,18].

The spike proteins are clove-shaped, type-I transmembrane proteins and has three segments: a large ectodomain, a single-pass transmembrane, and an intracellular tail. The ectodomain of spike proteins consist of S1 subunit containing a receptor binding domain (RBDs) and the membrane fusion subunit (S2). The host cell receptor recognition by the RBDs on spike proteins is the initial step of viral infection, and binding interactions between coronavirus spike and its receptor is one of the most critical factors for host range and cross-species transmission. Human coronaviruses recognize a variety of host receptors, namely HCoV-NL229 recognizes aminopeptidase N (APN)[19] and MERS-CoV binds dipeptidyl peptidase-4 (DPP4)[20], HCoV-OC43 and HCoV-HKU1 bind certain types of O-acetylated sialic acid[21], and HCoV-NL63 and SARS-CoV recognize angiotensin-converting enzyme 2 (ACE2)[22,23]. Recent structures along with functional studies, have suggested that the SARS-CoV-2 spike proteins utilize ACE2 and Transmembrane Serine Protease 2 (TMPRSS2) for host cell entry, which are very similar to the

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mechanisms exploited by SARS-CoV [24]. Readers are advised to check section 5 of this review for more information on the mechanism of coronavirus cell entry mediated by the viral spike-glycoproteins.

Currently, there are over 100 vaccines that are being developed by scientists around the globe to provide immunity against SARS-CoV-2. The basic idea is to expose the human body to an antigen, which should not cause a disease but stimulate the immune response for developing SARS-CoV-2 specific immunity[25]. The spike proteins, common among all coronaviruses, are a major target for eliciting antibodies; therefore, structural and molecular details of spike protein and its interactions with cognate receptor would be vital in developing vaccines and anti-viral drugs against SARS-CoV-2.

In this review, we first talk about the coronavirus classification, then we provide details on SARS-CoV-2 emergence, morphology and key virulence factors, structure, function and antigenicity of spike glycoproteins and its interactions with ACE2 receptor, anti-coronavirus vaccine and drug development, and finally, we talk about environmental factors that might affect the SARS-CoV-2 spread.

2. Emergence of SARS-CoV and SARS-CoV-2

In November 2002, SARS began spreading from the Guangdong province of Southern China, but its reservoir was unknown. In the past, Nipah and Hendra, both zoonotic viruses, originated from bats and this motivated researchers to find if bats are the natural reservoirs of SARS-CoV[26,27]. In 2005, two research groups independently reported that bats (horseshoe bats in particular) are the natural host of genetically diverse coronaviruses, and closely related to those responsible for the SARS outbreak[28,29]. These viruses were termed SARS-like coronaviruses, and they

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displayed considerable genetic similarities to SARS-CoV isolated from human or civets. This suggested that the virus responsible for SARS outbreak was a member of SARS-like coronaviruses group[28]. Of note: since then SARS has reappeared four times: three times due to laboratory accidents (Singapore and Taiwan) and once in Southern China, where the source of infection remains undetermined[30]. In Saudi Arabia MERS-CoV emerged in 2012, when humans were infected through direct or indirect contacts with infected dromedary camels. However, genome analysis suggested that MERS-CoV might have also originated in bats and was transmitted to camels in distant past[31] (Figure 1).

In December 2019, severe pneumonia patients of unknown cause were reported in Wuhan, China and a novel strain was detected from the lower respiratory tract samples of four patients [32]. Viruses were isolated from these clinical samples, and their genomes were sequenced by deep sequencing[33–35]. Phylogenetic analysis of 2019-nCoVs genomes and other coronaviruses were used to establish the evolutionary history and infection sources. Interestingly, this indicated that 2019-nCoV (GenBank: MN908947.3) shares about 96% nucleotide sequence identities to Bat coronavirus RaTG13 (GenBank: MN996532.1), whereas 79.5% and 55% identity to SARS-CoV BJ01 (GenBank: AY278488.2) and MERS-CoV HCoV-EMC (GenBank: MH454272.1), respectively and belongs to the same family of viruses that caused SARS and MERS (Figure 2). Despite high sequence similarities, few most notable and conserved variations arose in 2019-nCoVs genomes that were not previously seen in betacoronaviruses. These notable features, which establish this virus different from SARS-CoV and SARS-like coronaviruses are: (i) multiple mutations in the receptor-binding domains of spike protein that may interact with ACE2 receptor, (ii) polybasic furin-like protease site (RRAR/S) at the boundary of S1/S2 subunits rather than a single arginine observed in SARS-CoV, and (iii) addition of three predicted O-linked glycans

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flanking the protease site[36,37]. Of note: furin-like protease site is a signature of several highly pathogenic avian influenza viruses and pathogenic Newcastle disease virus[38,39]. However, sequencing and evolutionary analysis further suggest that bat is possibly the host of 2019-nCoV origin, and it might have transmitted either directly from bat or through an unknown intermediate host to infect humans[32,40–42]. Originally this virus was called "2019-novel coronavirus" (2019-nCoV), and then the International Committee on Taxonomy of Viruses officially named it "Severe Acute Respiratory Syndrome Coronavirus 2" (SARS-CoV-2) due to its genetic similarity to SARS-CoV on 11 February, 2020[40]. Of note: SARS-CoV and SARS-CoV-2 are two different viruses. SARS-CoV-2 causes the respiratory illness and WHO named it coronavirus disease-2019 (COVID-19). COVID-19 is a contagious and primarily transmitted among people through respiratory droplets and contact routes [43,44]. SARS-CoV-2 is rapidly spreading around the globe and more than four million COVID-19 cases are confirmed worldwide, and WHO has already declared the COVID-19 outbreak a pandemic[45].

3. Classification of coronaviruses

The coronavirus study group of the International Committee on Taxonomy of Viruses has classified coronaviruses under the family *Coronaviridae*, subfamily *Coronavirinae*. Based on genotypic and serological characterization, *Coronavirinae* is divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* [46–49] (Figure 3A). Only six Human Coronavirus species (HCoV) were known until December 2019 that cause human disease. Four of them cause common cold symptoms in immunocompromised individuals, which are HCoV-229E and HCoV-OC43 first identified in mid-1960s [1–3], HCoV-NL63 in 2004 [50,51], and HCoV-HKU1 in 2005 [52]. The other two strains, which cause fatal illness, are namely severe acute respiratory syndrome coronavirus (SARS-CoV) first identified in 2003 [4,6]

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and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 [5]. SARS-CoV-2 has 96% nucleotide sequence similarity to Bat coronavirus RaTG13, a SARS-like coronavirus; therefore, belongs to *Betacoronavirus* genera (Figure 2).

Forsters et al. performed phylogenetic network analysis of 160 complete SARS-CoV-2 genomes sampled from across the world to understand the evolution of this virus in humans and infection sources. They named these closely related genomes in three lineages, namely A, B, and C based on amino acid changes. The lineage A was named for the original bat coronavirus that caused COVID-19, but surprisingly it was not the dominant virus type in Wuhan. The A and C types were found notably in American and Europeans, respectively, while the B types was mostly prevalent in East Asia and needed mutations for spreading outside East Asia. The lineage C differs from its parent lineage B by a mutation at amino acid position 26144 and was prevalent in France, Italy, Sweden, England, California, Brazil, Singapore, Hong Kong, Taiwan and South Korea but absent from mainland Chinese samples. This kind of phylogenetic classification has a potential to accurately trace the infection routes and will prove helpful in designing treatments and vaccines development[53].

4. Morphology, genomic structure, and key viral factors of SARS-CoV-2

Coronaviruses are non-segmented, enveloped viruses with +ve ssRNA ranging between 26 to 32 kb in length. At this length, the coronaviruses genome is the largest among RNA viruses. The electron microscopy of negative-stained SARS-CoV-2 particles suggested spherical shape, diameter ranges from 60-140 nm, with outer surface studded with distinctive 9-12 nm long spikes that gave virions the appearance of a solar corona[32] (Figure 3B). The observed morphology of SARS-CoV-2 is consistent with other members of the Coronaviridae family. SARS-CoV-2 Wuhan-Hu-1 isolate (GenBank: MN908947.3) was among the first complete genome sequenced

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with 29903 bp long RNA. It is 5'-capped, 3'-polyadenylated, and consists of two flanking untranslated regions (UTRs) containing several open reading frames (ORFs) that encode multiple proteins. The genome is arranged in the order of a non-coding 5'-UTR – replicase genes (orf1ab) – structural proteins (S, E, M, and N) – non-coding 3'-UTR[54] (Figure 3C). Notably, it lacks the hemagglutinin-estrase gene, which is a common feature of lineage A betacoronaviruses[34]. The orf1a/b, located at the 5'-end of the genome, is the largest open reading frame and it encodes 16 nonstructural proteins (nsp1-16) in total. Briefly, the orf1a/b has overlapping orfs and produces two polypeptides, pp1a and pp1ab, due to ribosomal frameshifting. The virus genome encodes two cysteine proteases, a papain-like proteases (PL2pro) and a 3C-like protease (3CLpro) from nsp3 and nsp5, respectively. These proteases cleave pp1a and pp1ab polypeptides into 16 nsps. Specifically, PL2pro is responsible for cleaving between nsp1|2, nsp2|3 and nsp3|4 sites and the 3CLpro cleaves at the LQ↓SAG sites to produce nsp4 through nsp16[34,55]. RNA-dependent RNA polymerase (nsp12) and helicase (nsps13) are critical enzymes among these nsps responsible for the transcription and viral RNA replication in complex with nsp7, nsp8. It is a prime target for nucleotide analogue antiviral inhibitors, such as remdesivir, and is under clinical trials for the potential treatment of COVID-19 infections (discussed in the section 7).

The one-third part of the SARS-CoV-2 genome from the 3'-end encode the structural proteins: spike (S), envelop (E), membrane (M), and nucleocapsid (N) proteins. The structural proteins are responsible for virus-host cell receptor binding, virion assembly, morphogenesis, and release of virus particles from the host cell. The envelope (E) protein of SARS-CoV-2 is the smallest of all structural proteins found in the viral membrane and localizes to the ER and Golgi complex in the host cells[56]. The E protein along with M and N are known to facilitate virus-like particle formation[15]. The membrane (M) glycoprotein is a transmembrane protein located in the

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viral membrane and is the most abundant structural protein in a virion, almost ~100 times higher than E protein. The M protein plays a major role in the viral assembly along with E and N proteins[57–59]. The N-protein is responsible for packaging the viral genome RNA (gRNA) into a helical ribonucleocapsid (RNP). SARS-CoV-2 also have six accessory proteins derived from sub-genomic RNA: 3a, 6, 7a, 7b, 8 and 10 (based on the NCBI annotation NC_045512.2), and they are distributed among the structural genes[54,60,61].

Phylogenetic tree-based analysis of the whole genomes and individual genes suggest that SARS-CoV-2 is closer to SARS-like bat coronaviruses than SARS-CoVs. Specifically, the spike gene of SARS-CoV-2 is closer to SARS-like bat coronaviruses, while the 3a and 8b accessory genes are closer to SARS-CoVs[60,62]. In a recent study based on available genomic sequences, it was observed that SARS-CoV-2 (106 sequences) genome has much lower mutation rate and genetic diversity than SARS-CoV (39 sequences), and in particular the spike protein-coding gene is found relatively more conserved than other protein-encoding genes[63].

5. Structure, function, antigenicity and ACE2 recognition by the SARS- CoV-2 Spike Glycoprotein

The spike protein (S protein) is a multifunctional molecular machine that plays key roles in the early steps of viral infection by interacting with host susceptibility factors, including receptors and proteases, and subsequently infecting human cells containing angiotensin-converting enzyme 2 (hACE2) transmembrane proteins[64]. The SARS-CoV-2-S is a transmembrane glycoprotein composed of S1 regions containing the NTD and CTD, S2, a transmembrane region, and a short cytoplasmic domain (Figure 3C, D). Both cryo-EM and crystallographic methods have been used to determine multiple structures of the SARS-CoV-2 spike protein alone, such as ectodomain of S protein (SARS-CoV-2-S), receptor binding domain of S protein (SARS-CoV-2-

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S1-CTD), or in complex with full length ACE2 or soluble ACE2/ B°AT1, in a very short time. These structural studies enable us to understand the molecular basis of SARS-CoV-2 entry into human cells displaying ACE2 receptors[18,65–67]. Several structures of SARS-CoV-2-S were observed in multiple states (the prefusion, closed and partially open conformations and in complex with hACE2 receptor) with the receptor-binding domains (RBDs) either in "up" or "down" conformation (Figure 4A, B). Of note: to engage ACE2 receptor, the RBDs of S1 undergo hinge-like movements that either hide or expose the receptor binding regions and these conformations are referred to as "up" (receptor accessible) or "down" (receptor inaccessible) conformations. SARS-CoV-2-S structures show that protein adopts a clover shaped homotrimeric structure, with three S1 heads that recognize a cognate cell surface receptor and a membrane-anchored trimeric S2 stalk, which contains the fusion machinery and is primarily α-helical[18] (Figure 4C, D). In the prefusion conformation of SARS-CoV-2-S protein, the RBDs rest above the trimeric S2 stalk, exhibiting two protomers in the "down" conformation and one protomer in the "up" conformation, which is a receptor-accessible state required for binding to a ACE2 receptor[18]. Overall the SARS-CoV-2-S ectodomain resembles the closely related SARS-CoV-S structure with a root mean square deviation (RMSD) of 3.8Å over 959 Cα atoms, with a high degree of structural homology when individual domains of SARS-CoV-S and SARS-CoV-2-S were aligned[18].

(5.1) SARS-CoV-2-S-CTD interactions with human ACE2 receptor

Multiple structures of SARS-CoV-2-S-CTD in complex with either full-length hACE2 or soluble hACE2 have shown that the extracellular peptidase domain (PD) of ACE2 recognizes the RBDs of S protein mainly through polar interactions[65,66]. Similar to other betacoronaviruses, SARS-CoV-2-S-CTD structure suggested that it contains two subdomains: a core subdomain with twisted five-stranded antiparallel β sheet (β1, β2, β3, β4 and β7) with a conserved disulfide bond

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between β2-β4, and the other is receptor binding motif (RBM), located between β4 and β7 strand as an extended insertion (Figure 4E). The RBM forms a gently concave surface that accommodate the N-terminal α-helix of the hACE2, and a series of hydrophilic residues were observed along the interface which form a solid network of H-bond and salt bridges interactions (Figure 4F). In brief, strong polar contacts include CTD residues A475, N487, E484, Y453 interacting with S19, Q24, K31, H34 of α1 helix of hACE2, respectively[12]. Residues Q498, T500, N501 on the bulged loop forms a network of H-bonds with Y41, Q42, K353, R357 from ACE2[65]. Thus, overall virus-receptor interactions are dominated by polar contacts mediated by hydrophilic residues[12,65,66] (Figure 4G).

(5.2) Comparison of the SARS-CoV-2-RBD and SARS-CoV-RBD interactions with human ACE2 receptor

Majority of the secondary structure elements between SARS-CoV-S-RBD (PDB ID: 2AJF) and SARS-CoV-2-S-CTD (PDB ID: 6LZG, 6M17) are well superimposed, with an RMSD of 0.475Å over 128 Cα atoms, except the receptor binding loop. Interestingly, these structures revealed that the majority of binding sites of SARS-CoV RBD in hACE2 also overlap with the SARS-CoV-2-S-CTD binding sites suggesting that the SARS-CoV-2-S-CTD: hACE2 complex is strikingly similar to the SARS-CoV-RBD: ACE2 structure with an RMSD of 0.431Å over 669 Cα atoms (Figure 4G, H). However, despite the overall similarity, a number of sequence variations were observed at the binding interface that may account for the change in the affinities for hACE2 receptors. The detailed comparison of the receptor binding interfaces suggested that the SARS-CoV-2-S-CTD: ACE2 complex (PDB ID: 6VW1, 6M17) has larger buried surface areas (1773 $Å^2$ versus 1686 $Å^2$), has additional contacts (21 versus 17), more Van Der Waals interactions (288 versus 213) as wells as H-bonds (16 versus 1) than the SARS-CoV-RBD: hACE2 (PDB ID: 2AJF)

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complex[66]. Strikingly, residues F486 in SARS-CoV-2-S-CTD forms stronger aromatic-aromatic interactions with Y83 of hACE2 than I472 of SARS-Co-V-RBD. Residue E484 in the SARS-CoV-2-S-CTD forms stronger ionic interactions with K31 compared to P470 of SARS-CoV-RBD[66]. A sample collected from the state of Kerala in India on January 27, 2020, showed Arg408→ Ile408 mutation in the SARS-CoV-2-S protein (GenBank ID: MT012098.1), which otherwise is a strictly conserved residue in SARS-CoV, SARS-CoV-2, and bat SARS-like CoVs. The residues R408 is located near to the binding interface of both, the SARS-CoV-2-S-CTD: hACE2 (PDB: 6VW1) and SARS-CoV-RBD: hACE2 (PDB: 2AJF), complexes and appears not to be interacting directly with hACE2 in either case. But R408 forms a H-bond (3.3Å) with the glycan attached to N90 from hACE2; thus, contributes to higher affinities observed for SARS-CoV2-S-CTD: hACE2 complex than the SARS-CoV-RBD: ACE2 complex, where corresponding R395 is located relatively away (6.1Å) from N90 of hACE2. Arg408→ Ile408 mutation that emerged in SARS-CoV-2 strain (GenBank ID: MT012098.1) suggested that a higher hydrophobicity with no H-bond forming potential represents a sample with potentially reduced ACE2 binding affinity[44,46–49]. Consistent with high structural similarities, binding studies from different laboratories have reported that equilibrium dissociation constants (K_D) of hACE2 binding to SARS-CoV-2-S is 15nM[18] or 94.6nM[66] or 4.7nM[12], which are ~10 or 4 or 6-fold higher affinities, respectively, than SARS-CoV-S protein.

Using cryo-EM methodology the structure of full-length hACE2 in complex with SARS-CoV-2-S-CTD and B°AT1 (neutral amino acid transporter) was determined, which revealed that the ACE2: B°AT1complex is assembled as a dimer of heterodimers, where collectrin-like domain of hACE2 drives homodimerization (PDB ID: 6M17)[65]. The SARS-CoV-2-S-CTD is recognized by the extracellular PD of ACE2 as described previously. Further it demonstrates that

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a homodimeric ACE2 can accommodate two S protein trimers, each through a monomer of ACE2[65]. Interestingly, a superimposition of the ternary complex on RBD in the "down" conformation has indicated that PD clashes with the S protein, whereas in the "up" conformation (PDB 6VSB) no clashes are observed. Thus, suggesting that the "up" confirmation of RBD is a receptor-accessible state and is essential for the ACE2-receptor binding. Taken together, the overall interface between SARS-CoV2-S-CTD: ACE2 is very similar to the previously known SARS-CoV-RBD: ACE2 interface, and are dominated by the polar interactions as reported by different investigations[12,65,66]. These evidences further suggest that SARS-CoV-2-S-CTD has increased atomic interactions with hACE2, which results in higher affinities compared to the SARS-CoV-RBD: hACE2 complex, which might be one of the reasons for enhanced human-to-human transmission of SARS-CoV-2.

(5.3) SARS-CoV-2 Exhibits Distinct Epitope Features on the RBD from SARS-CoV

In the past multiple binding and neutralization epitopes have been identified on the spike protein of coronaviruses that makes the S protein an essential target for vaccine design[68–70]. Soon after the emergence of COVID-19 pandemic, some of the initial efforts were focused on screening the SARS-CoV-S specific antibodies to find neutralizing antibody/antibodies for vaccine and drug development against SARS-CoV-2. The hypothesis behind these studies was based on significant sequence as well as structure similarities and, moreover, both viruses bind to the same receptor with overlapping epitopes. Therefore, it was expected that SARS-CoV specific antibody/antibodies alone or in combination can interfere or even inhibit SARS-CoV-2 and hACE2 receptor interactions.

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It has been shown *in vitro* as well in animal models that monoclonal antibodies, such as 80R[71], CR3014[72], S230.15[73] and m396[73] can block binding of the S1 domain and hACE2 receptors by potently neutralizing SARS-CoV. However, CR3022[74] alone did not show neutralization but the mixture of CR3022 and CR3014 both showed neutralization of SARS-CoV in a synergistic fashion by recognizing different epitopes on the RBDs[72]. Of note, some report suggests that CR3022 can also neutralize SARS-CoV alone[75]. Interestingly, researchers from China tested several published SARS-CoV specific monoclonal antibodies and found that CR3022 can bind with the RBDs of SARS-CoV-2 with a K_D of 6.3 nM, whereas other antibodies, such as m396, CR3014 and S230.15 failed to bind to the SARS-CoV-2-S protein[18,76]. However, a low level of binding to SARS-CoV-2-S was observed with a SARS-CoV-S1 specific polyclonal antibody T62 (#40150-T62, Sino Biological Inc., Beijing, China) and it could poorly neutralize SARS-CoV-2-S protein mediated virus entry. Further analysis revealed that the epitope for T62 likely located on the RBDs of SARS-CoV-2-S, but detailed information is lacking[77]. In an exciting study, the Wilson laboratory determined the crystal structure of CR3022 antibody in complex with SARS-CoV-2-RBD (PDB ID: 6W41) and revealed that CR3022 binds a highly conserved epitope that is distantly located from receptor-binding site, which enables cross-reactive binding, but could not neutralize SARS-CoV-2 *in vitro*[75] (Figure 4I). However, whether CR3022 can synergize with other SARS-CoV-2-RBD binding antibodies for neutralization requires further evaluation and study.

The SARS-CoV (GenBank: AY278488.2) and SARS-CoV-2 (GenBank: MN908947.3) spike proteins both share about 76% amino acids sequence identity suggesting that rest 24% amino acids sequences, which are non-conserved might be responsible for antigenic differences between these two proteins. In quest of finding novel antibody binding epitopes on spike proteins, Zheng

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et al. performed antibody epitope analysis, and surface epitope accessibility using bioinformatic tools to identify both weak and strong epitopes, which might be otherwise experimentally ignored[78]. Their analysis identified five shared epitopes along with 40 and 29 unique epitopes on the spike proteins of SARS-CoV and SARS-CoV-2, respectively. Among these unique epitopes, 92.7% were originated from non-conserved regions, which might explain the reason why most of the SARS-CoV specific antibodies discussed in this review did not bind to the spike protein of SARS-CoV-2[78]. Taken together, these results suggest the necessity to develop SARS-CoV-2 specific antibodies and vaccine candidates.

(6) Therapeutic Interventions to COVID-19: FDA approved Small Molecule Inhibitors

Despite the unprecedented rise in COVID-19 cases worldwide, no drug or vaccine has yet been approved to treat the novel human coronavirus SARS-CoV-2. Biologics as a drug option that includes interferon as immune boosters, convalescent plasma therapy, monoclonal antibodies and vaccines are highly promising for COVID-19 treatment[79,80]. Several small molecule inhibitors, such as chloroquine/hydroxychloroquine alone or in combination with azithromycin, remdesivir, and lopinavir-ritonavir are currently being tested, however, the efficacy and safety of these drugs for COVID-19 patients need to be assessed by further clinical trials[81,82]. Amongst the many drugs, which are currently under use for COVID-19 treatments, the most prominent and discussed is chloroquinone and its analogue hydroxychloroquine. Initially, chloroquinone has shown inhibitory effects against SARS-CoV-2 in the *in viro* studies ($EC_{50} = 1.13$ μM in Vero E6 cells)[83,84]. The effect of chloroquine is more pronounced against SARS-CoV-2 when it is used in combination with azithromycin, as evaluated by a non- randomized study[84]. In comparison, Hydroxychloroquine, an analogue of the drug chloroquine, has initially shown to have better

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potency against SARS-CoV-2 (EC_{50} = 6.14 μM, hydroxychloroquine and EC_{50} = 23.90 μM, chloroquine) after 24 hours of growth, and is reported to be less toxic as compared to chloroquinone[85,86]. Chloroquinone and hydroxychloroquine appear to act by blocking the viral entry into cells by inhibiting glycosylation of host receptors, proteolytic processing, and decreasing the acidity in endosomes, and, thus, affecting the endocytotic process[81,87]. These agents are also suggested to have immunomodulatory effects through the attenuation of cytokine production, inhibition of autophagy, and lysosomal activity in host cells[87]. However, in rare cases of severity with pre-existing medical-conditions, such as hypertension, diabetes, and heart conditions are at increased risk of serious-side effects[87]. Therefore, due to these adverse effects, the use of hydroxychloroquine or chloroquine, often in combination with azithromycin (and other QT prolonging medicines) have marred into controversy. The continued investigations are expected to provide more information on the possibility of drastically reducing the effects. Currently, the outcomes of the synergistic action of Vitamins (C &D) and Zinc (Clinical trial NCT04326725) in patients suffering from the coronavirus disease are awaited[82].

Nitazoxanide, another FDA approved drug, which is used for the treatment of diarrhea is also shown to inhibit SARS-CoV-2; however, with a slightly lesser efficacy (EC_{50} = 2.12 μM in Vero E6 cells)[83]. Very recently it has also been reported that an FDA-approved anti-parasitic small molecule drug, Ivermectin, is an inhibitor of SARS-CoV-2, and shows ~5000-fold reduction in SARS-CoV-2 viral RNA at 48 h post-infection (IC_{50} = 2.8 μM in Vero hSLAM cells)[88].

Another important drug that has been allowed for the treatment of COVID-19 patients is Remdesivir, which is a broad-spectrum antiviral drug against RNA viruses. It is an ATP nucleoside analog that acts by inhibiting the viral RNA-dependent RNA polymerase[79,81]. Interestingly, remdesivir was originally used for Ebola treatment but has been demonstrated to be effective

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against SARS-CoV and MERS-CoV in cell cultures and animal models[89]. A recent study has reflected that remdesivir potently blocked SARS-CoV-2 at low concentrations as compared to chloroquine (EC_{50} = 0.77 μM vs. EC_{50} = 1.13 μM in Vero E6 cells)[83]. As of date, several phase III clinical trials have been initiated to evaluate the safety and efficacy of the drug, and a multicenter **A**daptive **C**OVID-19 **T**reatment **T**rial (ACTT) (Clinical Trial NCT04280705) has been launched recently[82]. While the results of the trials are awaited; Japan, on May 7th, 2020, has concurrently approved remdesivir for COVID-19 treatment[90]. A cryo-EM structure of RNA-dependent RNA polymerase is recently determined at 2.9Å resolution, which provides the mechanism of remdesivir binding as well as a blueprint for designing more effective antiviral therapeutics against SARS-CoV-2[16].

Similarly, the anti-HIV drugs Kaletra® (Lopinavir-Ritonavir) has attracted great attention during the initial phase of pandemic[79,81]. However, the *in vitro* data for lopinavir-ritonavir treatments against SARS-CoV-2 are lacking. Lopinavir-Ritonavir are established against HIV protease that belongs to the aspartic protease family, whereas the SARS-CoV-2 coronavirus proteases are from the cysteine protease family, and, thus, concerns have been raised over its applicability to target SARS-CoV-2; therefore, researchers are skeptical of the lopinavir-ritonavir combination[79,81]. The very first trial of lopinavir-ritonavir against COVID-19 has not been encouraging when 199 patients in Wuhan, China, were provided standard care with or without lopinavir-ritonavir, and the outcomes did not differ significantly[91]. However, the ongoing randomized control trials of the lopinavir-ritonavir may shed a light on their applicability in COVID-19 treatment. A very recent randomized trial of the triple drug combination of the drugs interferon beta-1b, plus the antiviral therapy lopinavir-ritonavir and ribavirin (an oral hepatitis C virus drug) is appearing better to reduce the viral load than lopinavir-ritonavir alone[92].

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Although a plethora of studies have been initiated to provide an effective treatment against the COVID-19 infections, the ongoing pandemic still remains a black box, and has become a hotbed for the drug-discovery. In line with the life-saving efforts, researchers are already developing more than 100 treatments and vaccines at both stages, preclinical studies and clinical trials, to stem the COVID-19 pandemic[79,81]. Amongst the several initiatives, the **A**ccelerating **C**OVID-19 **T**herapeutic **I**nterventions and **V**accines (ACTIV); primarily focused on the United States, will inventory drug and vaccine candidates and decide which should get priority. The ACTIV initiative is primarily focused for U.S. funding, however, reported to work with the European Medicines Agency and other COVID-19 research coordination efforts around the world to avoid duplication[80]. Similarly, World Health organization (WHO) has started SOLIDARITY trial, which is an unprecedented and coordinated push involving over 100 countries to find an effective therapeutic, via the large global trial of several drugs[80]. A list of candidate therapeutics has been published by WHO (https://www.who.int/blueprint/priority-diseases/key-action/overview-ncov-therapeutics.pdf?ua=1).

(7) SARS-CoV-2 stability in different environmental conditions

Emerging infectious diseases are a significant distress on public health and global economies. Their emergence is thought to be largely driven by socio-economic, globalization, demographic, environmental and ecological factors[93]. Previous investigations suggest that animal-borne and other infectious diseases like Ebola, SARS, bird flu H5N1 and now SARS-CoV-2 are on the rise and destruction of natural habitat of wild animals and ever increasing human-wild life interactions are few reasons behind these outbreaks[94]. The USA Centers for Disease Control and Prevention (CDC) has estimated that three-quarter of emerging diseases that eventually infect humans originate in nonhuman animals[95].

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Pan Y et al. measured the stability of SARS-CoV-2 at different temperatures. They incubated SARS-CoV-2 in virus transport medium for 14 days at a final concentration of ~6.8 log TCID50/ml and tested for its infectivity and it was found that COVID-19 virus is highly stable at 4°C, while incubation at 70°C for 5 minutes inactivates the virus[96]. They added the droplet of SARS-CoV-2 on different surfaces, up to 7 days at 22°C with a relative humidity of 65%, such as tissue paper, wood, banknote, stainless steel, plastic, and outer layer of surgical mask. They found that no infectious virus could be recovered from printing and tissue papers after 3 hours incubation, smooth surfaces (glass and banknote) on 4th day, stainless steel and plastic on 7th day. However, surprisingly a detectable level of infectious virus could still be present on the outer layer of a surgical mask on the 7th day[96]. Additionally, they have investigated that SARS-CoV-2 is extremely stable in a wide range of pH values at room temperature and requires at least 5 minutes incubation with hand soap to get virucidal effects[96].

Bhattacharjee et al. performed statistical investigation of relationship between spread of SARS-CoV-2 and environmental factors, such as temperature, relative humidity, and highest wind speed for COVID-19 affected cities in China and Italy (Beijing, Chongqing, Shanghai, and Wuhan in China and Bergamo, Cremona, Lodi, and Milano in Italy). Their analysis has indicated that the relationship with maximum relative humidity and highest wind is mostly negligible, while the relationship with maximum temperature is ranging between negligible to moderate for SARS-CoV-2 spread[97]. However, Wang et al. suggested that high temperature, and high humidity can reduce the viability of SARS-CoV-2; thus, their transmission that has been previously observed with the spread of influenza virus[98]. Oliveiros et al. using a linear model on four independent variables (temperature, humidity, precipitation and wind speed) have expected a decrease in the rate of COVID-19 progression with the arrival of spring and summer in the north hemisphere[99].

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So, it would be interesting to see in coming days the direct impact of meteorological parameters on the transmission of SARS-CoV-2 considering the contradicting views of different experts.

(8) Conclusions

The recent global outbreak of COVID-19 has killed almost 275 thousand people and threatened the global economy, causing economic hardships to millions of people. Extensive progress has been made in terms of structure and function of the spike glycoproteins. Specifically, decade-long structural studies on the spike proteins of SARS-coronaviruses have designated six key residues (Y442, L472, N479, D480, T487 and Y491 for SARS-CoV)[64] in the RBDs that are critical for the host cell ACE2 receptor binding as well as for playing important roles in the cross-species transmission. Notably, five out of these six residues differ between the RBDs of SARS-CoV and SARS-CoV-2 S proteins, which has exhibited enhanced binding between the RBDs of SARS-CoV-2 and ACE2 receptors. This might be one of the reasons behind widespread human-to-human transmission of SARS-CoV-2. There are definitely other factors involved in infectivity and pathogenicity of SARS-CoV-2 that are required to be investigated.

The trimeric prefusion structure of the SARS-CoV-2 spike protein was obtained in an asymmetric conformation where one protomer was observed in the “up” and other two in the “down” conformations. A phenomenon known as protein “breathing” was observed in the S1 domain while determining the trimeric prefusion structure, which indicated the technique used by CR3022 to access a cryptic epitope on the trimeric S protein that is otherwise not possible. Interestingly, similar breathing phenomenon was observed to find unique and conserved epitopes in the trimeric interface of influenza hemagglutinin protein recently. The antibodies binding to these cryptic epitopes did not inhibit viral infection *in vitro* but conferred *in vivo*

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protection[100,101]. A similar phenomenon was observed in case of CR3022 monoclonal antibody; therefore, further *in vivo* studies are required as soon as a suitable animal model is established for SARS-CoV-2 studies. When our manuscript is about to complete, two exciting reports became available: (i) an antibody 47D11 is reported that neutralize SARS-CoV-2 as well as SARS-CoV in cell culture through an unknown mechanism, which is different from the virus neutralization process[102], (ii) an inactivated novel coronavirus vaccine (PiCoVacc) is able to induce SARS-CoV-2 specific neutralizing antibodies in mice, rats and non-human primates. Additionally, data demonstrate that PiCoVacc vaccine provides partial to complete protection in macaques against SARS-CoV-2 challenge[103]. Future investigations are required to understand the mechanism of neutralization by these antibodies.

Last but not the least, glycosylation has been an important measure of virus antigenic properties and plays a critical role for the manufacturing of effective vaccines against HIV and influenza. Notably, the SARS-CoV-2 spike protein is densely decorated by host-derived heterogenous N-linked glycans as indicated by the site-specific glycosylation analysis using mass spectrometry. Specifically, each SARS-CoV-2 spike trimer displays 66 N-linked glycosylation sites with an elevation in oligomannose- and hybrid-type glycans compared to typical host-derived glycoproteins[104]. Finally, glycan profiling will be an important addition to measure antigen quality, and should be examined while producing glycoprotein-based vaccine candidates for COVID-19.

Though it is observed that SARS-CoV-2 binds to its receptor on the host cells with higher affinities than SARS-CoV but the fatality rate caused by SARS-CoV-2 (3.4%,) is significantly less than the reported rate of SARS-CoV (9-11%), as reported by the WHO. The reason behind these

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differences remain elusive and future research will shed light on these variations. Recent sequencing data indicate that SARS-CoV-2 is mutating itself at the rate of ~25 mutations per year, if these mutations enables it to spread more efficiently with enhanced pathogenicity, then vaccine development against this virus can be a challenging task. Hopefully, future studies will be able to resolve these questions and come up with medications as well as vaccines against this deadly virus. Even with the vaccine and medications against this virus, future outbreaks of similar viruses and pathogens are likely to continue. Therefore, apart from curbing this outbreak, government policies and efforts should be made to formulate thorough measures to prevent future outbreaks of viruses, bacteria (there is already a significant threat from antibiotic-resistant bacteria), and communicable diseases.

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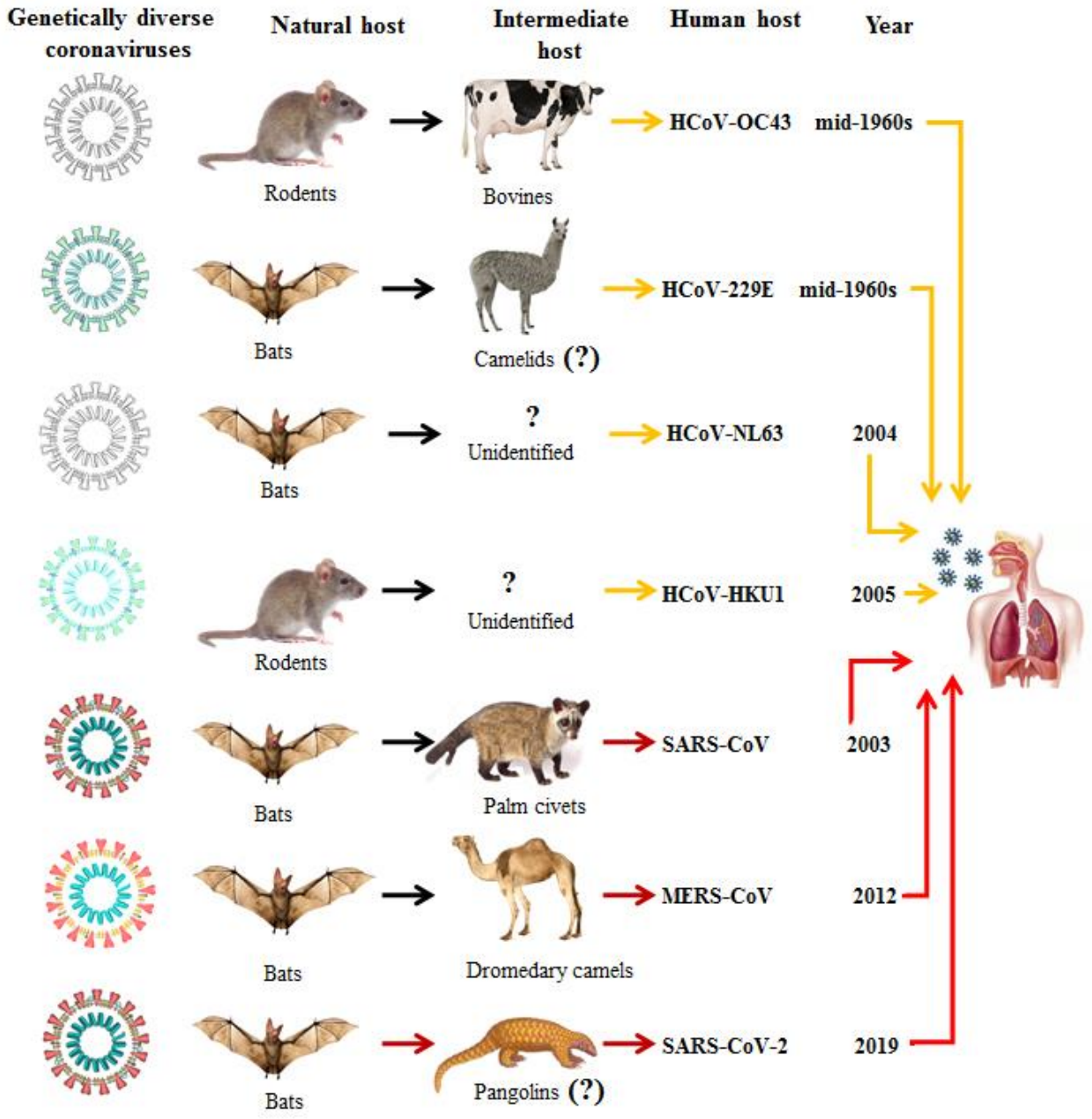

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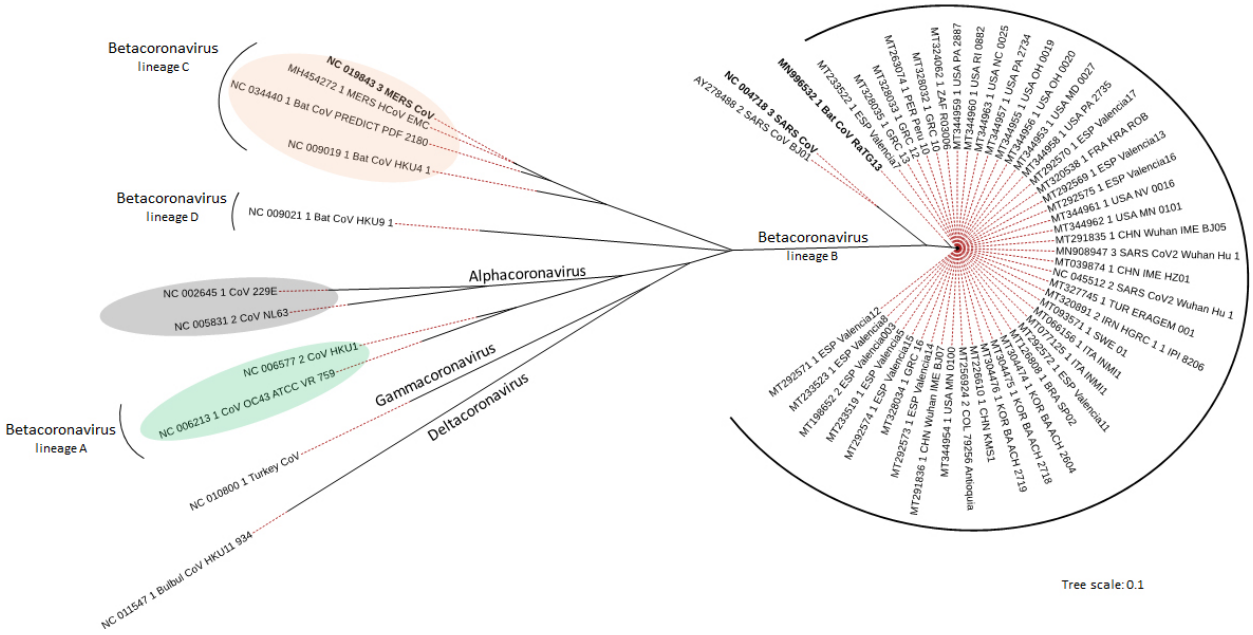

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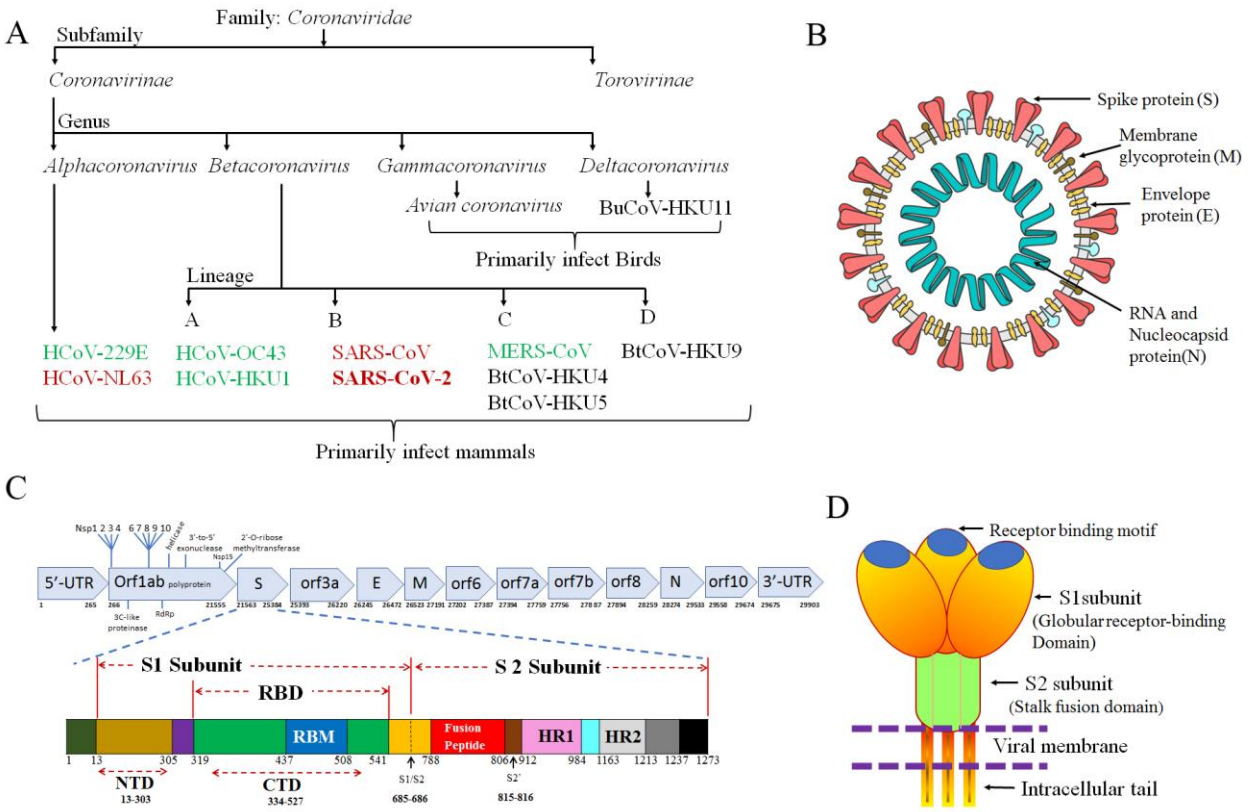

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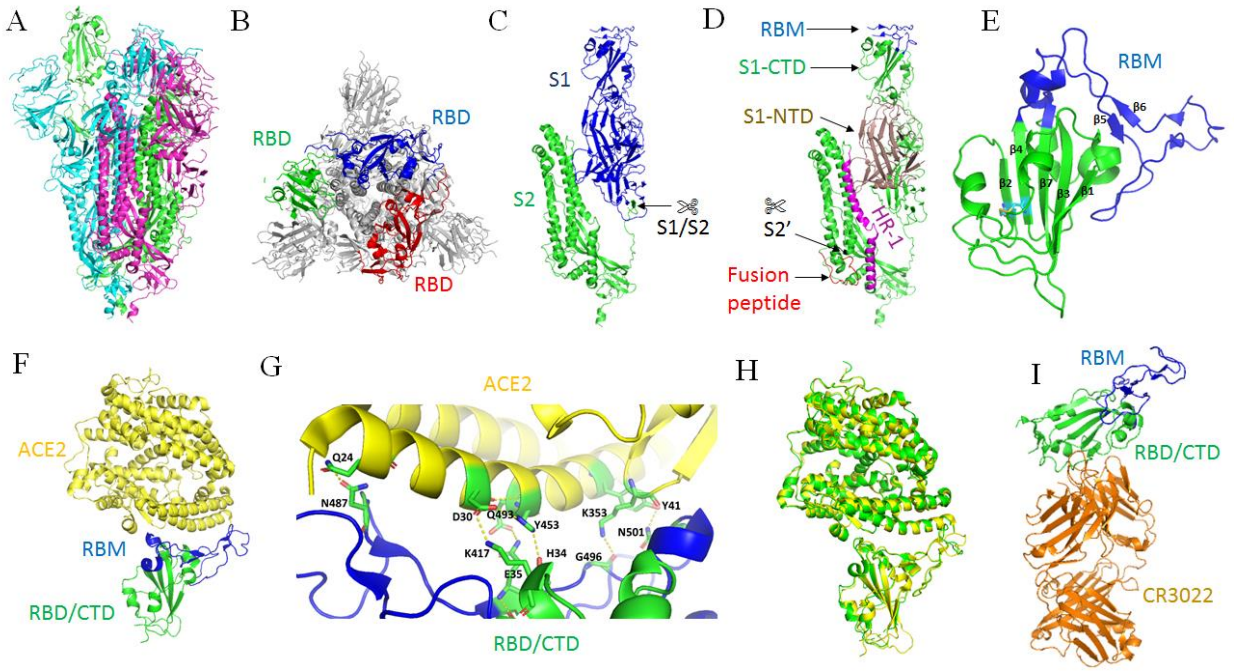

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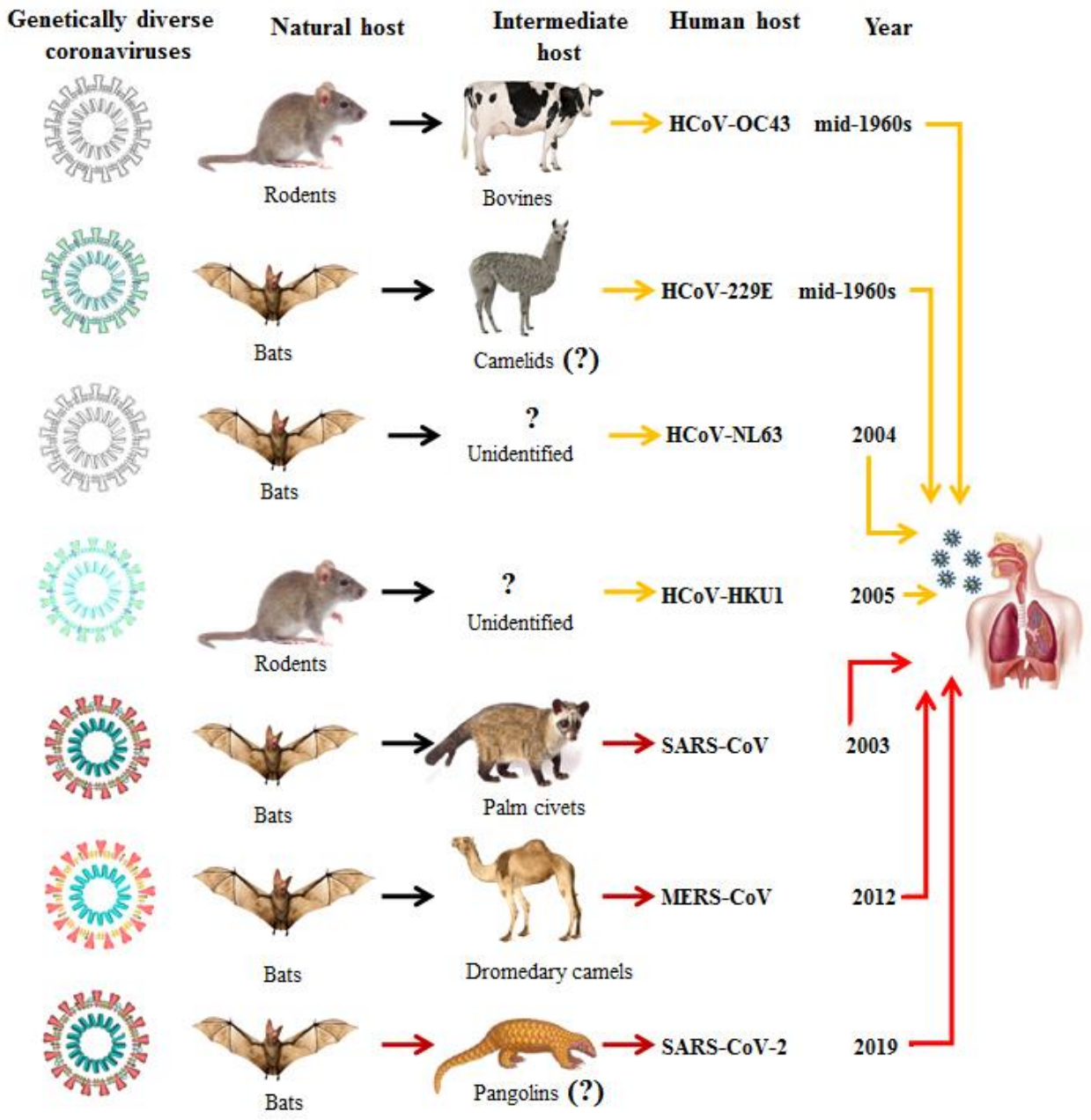

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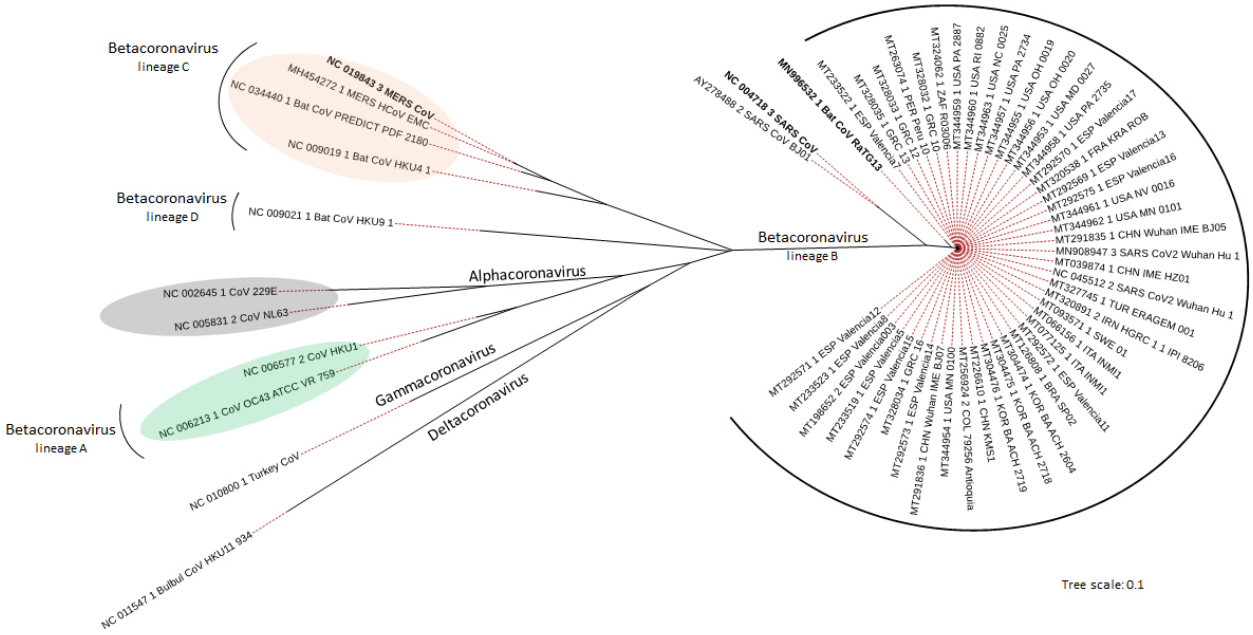

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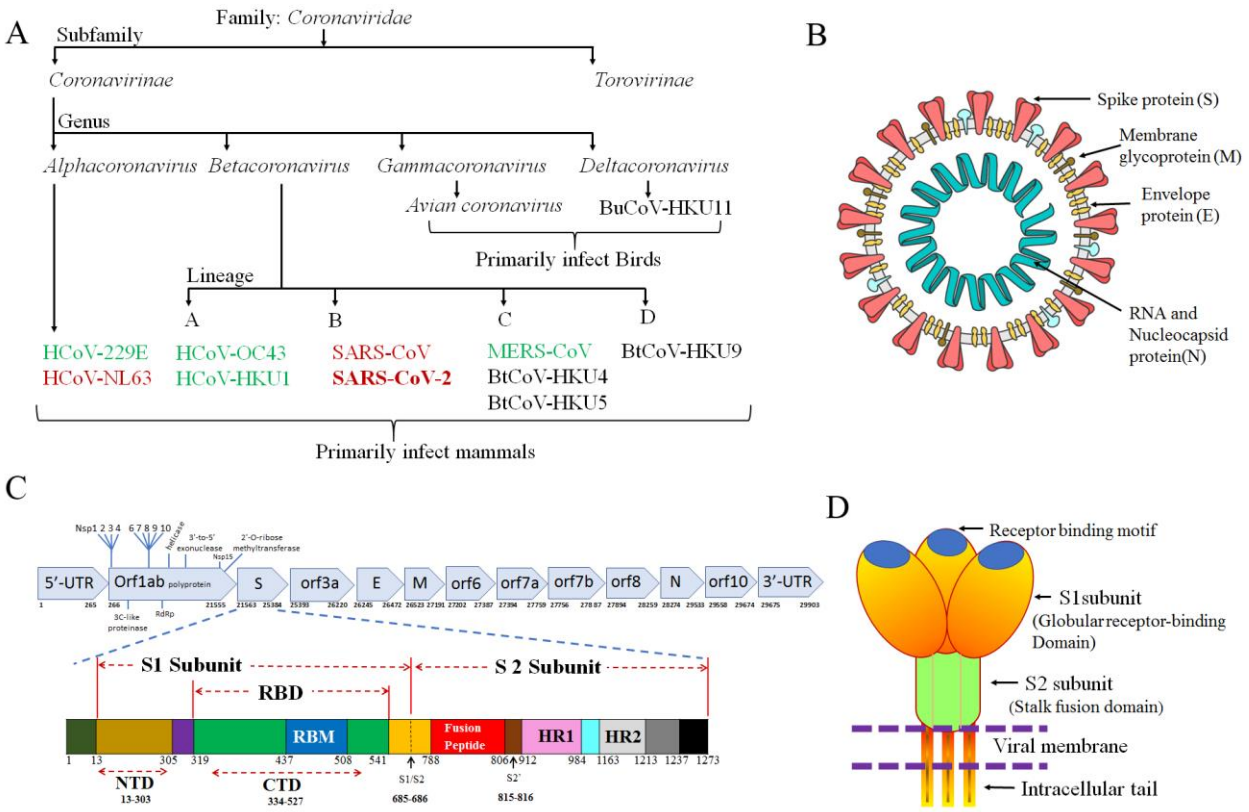

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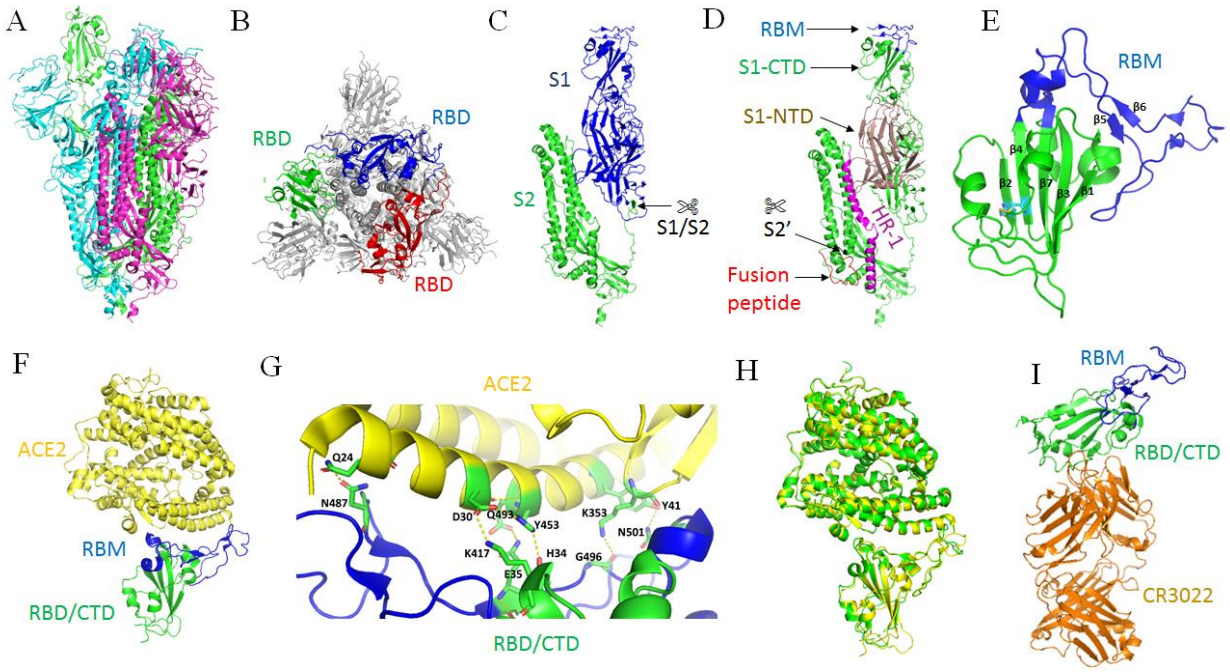

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