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REVIEW

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Microstructure, pathophysiology, and potential therapeutics of COVID-19: A comprehensive review

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Abstract

There have been over seven million cases and almost 413 372 deaths globally due to the novel coronavirus (2019-nCoV) associated disease COVID-19, as of 11 June 2020. Phylogenetic analysis suggests that there is a common source for these infections. The overall sequence similarities between the spike protein of 2019-nCoV and that of SARS-CoV are known to be around 76% to 78% and 73% to 76% for the whole protein and receptor-binding domain (RBD), respectively. Thus, they have the potential to serve as the drug and/or vaccine candidate. However, the individual response against 2019-nCoV differs due to genetic variations in the human population. Understanding the variations in angiotensin-converting enzyme 2 (ACE2) and human leukocyte antigen (HLA) that may affect the severity of 2019-nCoV infection could help in identifying individuals at a higher risk from the COVID-19. A number of potential drugs/vaccines as well as antibody/cytokine-based therapeutics are in various developmental stages of preclinical/clinical trials against SARS-CoV, MERS-CoV, and 2019-nCoV with substantial cross-reactivity, and may be used against COVID-19. For diagnosis, the reverse-transcription polymerase chain reaction is the gold standard test for initial diagnosis of COVID-19. A kit based on serological tests are also recommended for investigating the spread of COVID-19 but this is challenging due to the antibodies cross-reactivity. This review comprehensively summarizes the recent reports available regarding the host-pathogen interaction, morphological and genomic structure of the virus, and the diagnostic techniques as well as the available potential therapeutics against COVID-19.

KEYWORDS

coronavirus, COVID-19, drug, host-pathogen interaction, infection, vaccine

1 | INTRODUCTION

The coronaviruses belong to the family Coronaviradae under the order Nidovirales, and can be further subdivided into four main genera (α, β, γ, and δ). In the large coronavirus (CoV) family, the novel coronavirus (designated as 2019-nCoV), as the seventh family member belonging to β-coronaviruses, had its first outbreak in December 2019 in Wuhan, China. Since, most of the early infected patients of 2019-nCoV were found to be frequent visitors of Huanan South Seafood Market of Wuhan where seafood, bats, chicken, pheasants, and other animals were vended.[1,2] So, it was assumed that associated disease was a zoonotic disease.[3] Out of six former coronaviruses, four species viz. 229E, OC43, NL63, and HKU1 have been found to be mildly pathogenic, while the other two species severe acute respiratory syndrome (SARS)-CoV and middle east respiratory syndrome (MERS)-CoV were highly pathogenic against immune-compromised humans. The SARS-CoV and MERS-CoV spanned over 32 and 27 countries, respectively, with 8422 and 2496 confirmed cases and 916 (10.87%) and 868 (34.77%) deaths over the

period of November 2002 to August 2003 and April 2012 to December 2019, respectively.[4-6] However, the 2019-nCoV spread swiftly across the borders of 216 countries and infected 7 273 958 people, resulting in 413 372 (~6%) casualties till 11 June 2020 (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200611-covid-19-sitrep-143.pdf?sfvrsn=2adbe568_4). As of now, respiratory droplets including those that come in contact with ocular surfaces, have been determined to be a major route of transmission.[7] Epidemiologically, 2019-nCoV is highly infectious to all age groups with elderly patients demonstrating a higher susceptibility, especially considering its incubation period of around 4 to 8 days.[8] The 2019-nCoV causes fatal illnesses including pneumonia, enteric, hepatic, and neurologic diseases. The most common symptoms of 2019-nCoV infection are fever (87.9%), fatigue (69.6%), dry cough (67.7%), and myalgia (34.8%), accompanied with rhinobyon, rhinorrhoea, pharyngalgia, and diarrhea in a few patients.[9] Although, the major clinical manifestations in coronaviruses infection by 2019-nCoV, MERS-CoV, and SARS-CoV are similar, the transmission rate of 2019-nCoV is very high compared to the other two species (SARS-CoV [R0:1.7-1.9] and MERS-CoV [R0 < 1]).[10,11] Due to an increased reproduction rate (R0:2.68), as compared with SARS-CoV and MERS-CoV, 2019-nCoV has posed a substantially greater risk to public health.[12] The details of the majorly affected (no. of deaths > 5000) countries are depicted in Table S1. The most reliable and early diagnosis of 2019-nCoV infection is based on the presence of viral nucleic acid detected by real-time reverse-transcription polymerase chain reaction (RT-PCR) using specimens from the respiratory tract or serum.[13] Despite substantial efforts in research over the past two decades, as well as aggressive therapeutics development in the past 3 months, at present, there is no vaccine or specific drugs available against coronaviruses, particularly the 2019-nCoV.[14] Therefore, to fight the ongoing 2019-nCoV outbreak, it is critical to understand more about the nature of the 2019-nCoV and its family members, especially with regard to clinical and immunological characteristics.[15] In this review, we comprehensively summarize the recent reports available regarding the genomic structure, host-pathogen interaction, mode of transmission, diagnosis, as well as potential therapeutic options (drugs and vaccines) against 2019-nCoV.

2 | HISTORY OF 2019-nCoV INFECTION AND WORLDWIDE CASES

The global outbreak of 2019-nCoV originated in Wuhan, China with a cluster of 44 patients exhibiting pneumonia-like symptoms along with a fever of unknown cause. The viral infection spread rapidly across the borders of several countries to eventually become a pandemic of global concern.[16,17] Soon after China, 2019-nCoV also spread in Taiwan, where it was passed on through a 74-year-old female, whose recent travel history included Wuhan. From here, the virus progressively circulated in 24 other countries. On 12 January 2020, the World Health Organization (WHO) declared this deadly virus as the 2019-novel coronavirus (2019-nCoV) and officially named the associated disease as coronavirus disease 2019 (COVID-19). Furthermore, the International Committee on Taxonomy of Viruses (ICTV) proposed the name of this 2019-nCoV as SARS-CoV-2 on 11 February 2020, and WHO consequently declared COVID-19 to be a public health emergency.[18-21] The case fatality rate (CFR) differs between countries, as given in Table S1. On average, a 3.78% mortality rate has been reported worldwide, as updated on 11 June 2020 at 01:00 AM EDT (https://coronavirus.jhu.edu/data/mortality). However, the testing capabilities (number of COVID-19 tests/day) of different countries exhibit a wide range, and without knowing the number of positive cases, it is still too early to establish an average percentage. Many countries have experienced a more rapid increase in infections than what was observed in China. The countries that have been most extensively affected by COVID-19 other than China include United States, Italy, Spain, France, United Kingdom, India, and Iran (Table S1). WHO has described Europe as the center of the pandemic, wherein Italy has 235 763 cases and 34 114 deaths, Spain has 242 280 cases and 27 136 deaths, France has 151 145 cases and 29 257 deaths, and Germany has 185 416 cases, and 8775 deaths. The United States has declared a national emergency due to rapid expansion of COVID-19 cases that have jumped to 1 968 331 with at least 111 978 recorded deaths, as updated on 11 June 2020 at 2:20 PM CEST (https://covid19.who.int/). The growth of the COVID-19 is shocking with the average mortality rate of 11.44% for the 20 countries currently most affected by COVID-19 worldwide, as updated on 11 June 2020 at 01:00 AM EDT (https://coronavirus.jhu.edu/data/mortality).

3 | GENOMIC STRUCTURE OF 2019-nCoV

From a total 45 726 single-stranded positive-sense RNA genomic sequences of 2019-nCoV submitted as on 12 June 2020 at GISAID (https://www.gisaid.org/), one of the first three 2019-nCoV genomes, namely, Wuhan/IVDC-HB-01/2019 (GISAID accession ID: EPI_ISL_402119) has been shown to consist of 14 open reading frames (ORFs) encoding 27 proteins. The 5′-terminus of the genome contains orf1ab and orf1a genes that encode the replicase polyproteins pp1ab and pp1a (7096 a.a), respectively. They collectively consist of 15 nonstructural proteins (nsps) including nsp1 to nsp10 and nsp12 to nsp16 that are involved in the transcription and replication of the 2019-nCoV genome. The 3′-terminus of the genome includes four structural proteins i.e. the nucleocapsid (N; 419 a.a), the membrane (M; 222 a.a), the envelope (E; 75 a.a) and the spike (S; 1273 a.a) as well as eight accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) with less functional annotation (Figure 1).[19,21-25] The monomer of the S protein consists of two subunits (S1 and S2), which self-assemble naturally into a homo-trimer (S-Trimer), typically similar to the class I membrane fusion protein. Furthermore, the S1 subunit contains two domains namely the N-terminal domain (NTD) and the C-terminal domain (CTD) involving the receptor-binding domain (RBD) while the S2 subunit contains the basic elements required for membrane fusion, including an internal membrane fusion peptide (FP), two 7-peptide repeats (PR), a membrane-proximal external region (MPER), and a transmembrane (TM) domain. The S1 and S2 domains are conserved among the related coronaviruses with 70%

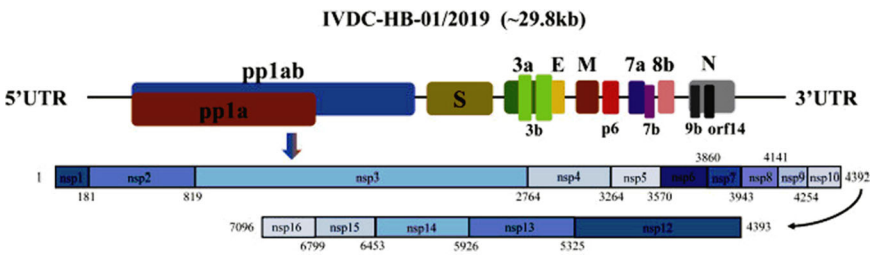

FIGURE 1 Schematic representation of 2019-nCoV genome and encoded major structural and nonstructural proteins for the IVDC-HB-01/2019 (HB01) strain. The longest gene, namely the orf1ab, encodes the pp1ab protein that contains 15 nonstructural proteins (nsps), that is, nsp1 to nsp10 and nsp12 to nsp16. The pp1a protein encoded by the orf1a gene also contains 10 nsps (nsp1 to nsp10). The structural proteins are encoded by the four structural genes viz. spike (S), envelope (E), membrane (M), and nucleocapsid (N). The accessory genes are distributed among the structural genes. (*Source:* Figure 1 of Wu et al. (2020), Cell Host Microbe, https://doi.org/10.1016/j.chom.2020.02.001)

and 99% sequence identity to SARS-CoV, respectively. The RBD of S1 has been reported to come into direct contact with the human receptor angiotensin-converting enzyme 2 (ACE2) expressed by epithelial and lung cells.

Although, the exact origin of the novel coronavirus remains unconfirmed,[1] the phylogenetic analysis of 2019-nCoV based on full genome sequences reveals the closest similarity (~96%) to the bat (*Rhinolophus affinis*) SARS-CoV (RaTG13) reported from Yunnan, China in 2013 (Figure 2). It indicates a transmission of the novel coronavirus from bat to human, exhibiting a sequence identity of approximately 79% and 50% with human SARS-CoV and MERS-CoV, respectively.[16,25-30] However, some of the evidence indicate that the pangolin is the intermediate host of the 2019-nCoV, with 90.55% similarity, which emerged as a result of homologous recombination events in the S genes of pangolin and bat coronavirus.[18,31-33] The S gene of the 2019-nCoV has less than 75% sequence identity to Bat SARS-CoVs (SL-CoVZXC21 and ZC45) as well as human SARS-CoV. Moreover, the genomic sequences of the 2019-nCoV obtained from different available patients exhibited very high similarity (99.9% identity) to each other.[34] This information may further generate fruitful evidence for disease management, if the mutations in such sequences are constantly monitored in infected cases across different geographical/political boundaries.

4 | HOST-PATHOGEN INTERACTION

The S protein of 2019-nCoV has been found to be crucial in determining the host-pathogen interaction through the mediation of receptor-binding and membrane fusion for releasing viral RNA into the cytoplasm for replication. During interactions with humans, the S protein mainly binds to the ACE2 receptor. This receptor is expressed on the cell surface of different organs such as the heart, endothelium, liver, kidney, testis, intestine lung and other tissues, out of which alveolar epithelial type II cells include 83% of ACE2-presenting cells.[35-40] The ACE2 receptor binds with a higher binding affinity to S protein of 2019-nCoV compared with SARS-CoV due to association with some other receptors including TMPRSS2.[32,41-44] The TMPRSS2 is a type II cellular transmembrane serine protease, which is expressed on the surface of epithelial cells and is essential for the activation of S protein, leading to the fusion of the viral membrane into the host cell.[45,46] Besides these, some analytical evidence also suggest that during evolution of 2019-nCoV, certain mutations in the receptor-binding motif (RBM) of RBD favor the binding affinity towards ACE2 and is ultimately responsible for increased transmission rate (Figure 3). The RBM motif includes certain important amino acid residues (Gln493, Asn501) that augments the interaction between S protein and ACE2.[47] Therefore, both the S and TMPRSS2 proteins can be used as drug targets to prevent the invasion of 2019-nCoV in host cells.[48-51]

Apart from S protein activation, other factors like the valosin–containing protein (VCP) also play a role in this infection process. The role of VCP in the release of the virus from the endosome has been shown by mutagenesis.[52] Host factors like interferon-inducible transmembrane protein have also been proposed to play a role in host-pathogen interaction as an antiviral factor in the case of RNA viruses affecting humans including human coronaviruses.[53] The post-infection replication, transcription and translation of viral genome requires formation of many multi-subunit complexes. One such complex was assembled by coronavirus contains nsp14, which acts as exoribonuclease (ExoN) with proofreading ability.[54] Its C-terminal domain causes viral mRNA capping through its N7-guanine methyltransferase (N7-MTase) activity while the N-terminal proofreading ExoN domain plays a role in the prevention of lethal mutagenesis. The ExoN may boost the fidelity of RNA synthesis by correcting nucleotide incorporation errors made by the RNA-dependent RNA polymerase (RdRp). The RdRp activity is encoded in nsp12.[55] The aforementioned role of nsp14 has been made further evident by binding of a cap-precursor guanosine-P3-adenosine-5′,5′-triphosphate to S-adenosyl methionine in the proximity of a highly contracted pocket between two β-sheets to accomplish methyl transfer.[56] The crystal structure of SARS-CoV nsp14 has shed light on the interplay between these two domains, and on nsp14′s interactions with nsp10. The nsp10 is a co-factor that has been shown to strongly enhance ExoN activity, through in vitro assays. Further in vivo and in vitro studies targeting the factors regulating the structure-function relationships of ExoN and its interactions with other (viral and/or host) members of the CoV replication machinery will be key to reveal the enzyme's role in viral RNA

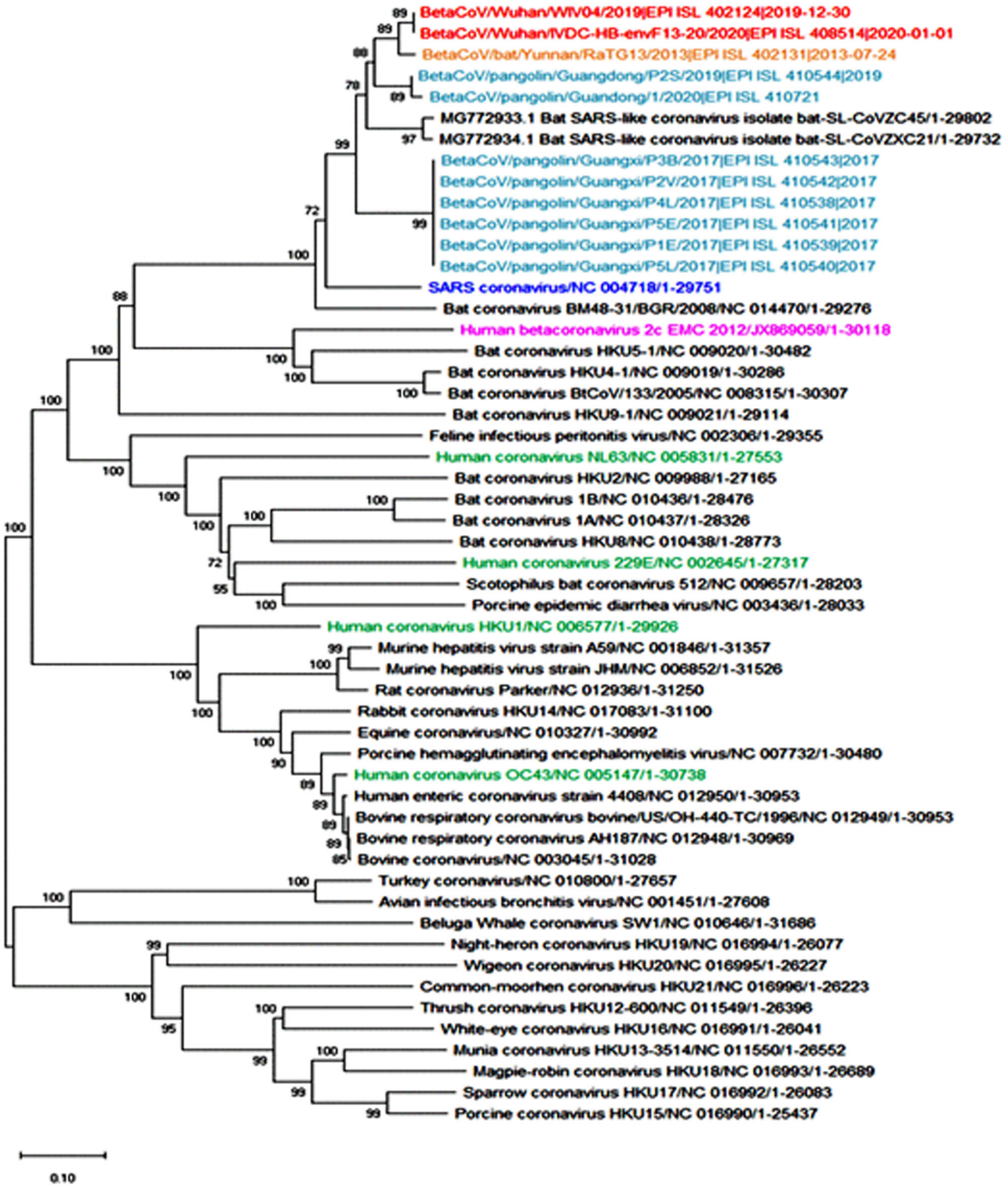

FIGURE 2 The phylogenetic analysis of Wuhan nCOV-19 (based on full genome sequences) with all the representatives of coronavirus family using the MegaX tool. The different color codes represent different families (orange- bat (RaTG13), red: 2019-nCoV, cyan: pangolin-COV, blue-SARS-COV, purple: MERS-COV, green: common cold-COV (*Source:* GISAID, https://www.epicov.org/epi3/frontend#lightbox1353460538)

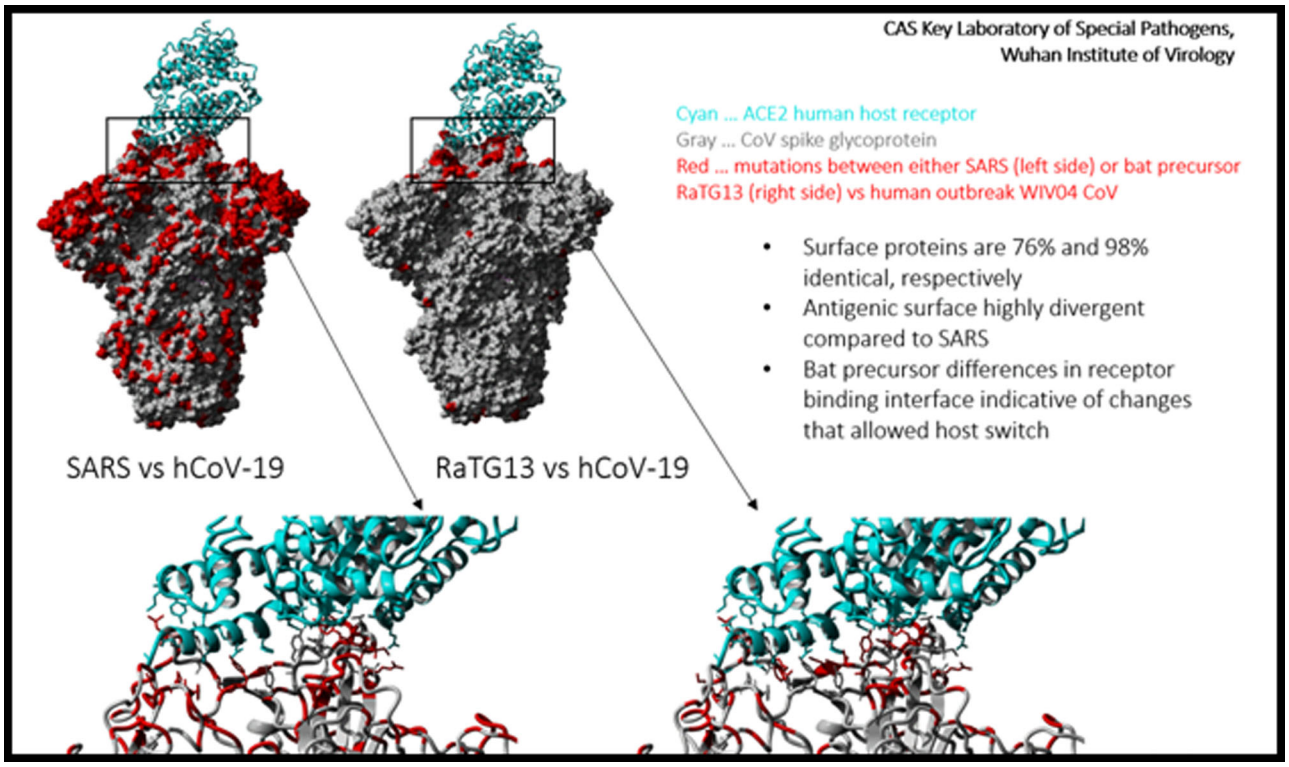

FIGURE 3 The differences between the host receptor (ACE2) binding site of spike protein of human SARS-CoV (SARS), human 2019-nCoV (hCoV-19), and bat SARS-CoV (RaTG13). There are a total of seven different rare variants near the binding interface not known to be linked to severity. V483A in 23 samples (20 USA/WA, 2 USA/UN, 1 USA/CT), V483I in 1 English sample, G476S in 18 samples (13 USA/WA, 2 USA/OR, 1 USA/ID, 1 USA/CT, 1 Belgium), L455I together with F456V in one Brazilian sample, S494P in 1 English sample, and N439K in 1 Scottish sample (*Source:* GISAID, https://www.epicov.org/epi3/frontend#lightbox1353460540)

synthesis and pathogenesis.[57] In addition, the phosphorylation of the N protein (which also act as RNA chaperon and regulates template switching) by glycogen synthase kinase 3[58] and association of N protein by heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) also regulates viral RNA synthesis.[59] All such events are important as far as strategies to combat the viral threat are concerned.

5 | PATHOPHYSIOLOGY OF COVID-19

When 2019-nCoV enters the human body, it interacts with ACE2 receptors and releases its RNA inside the epithelial cells (ECs), where it replicates and is released for further infection to neighboring cells and spread from nasal passage to alveolar area of lung.[60] The gaseous exchange is mediated by alveoli but due to 2019-nCoV infection, there is a vascular integrity defect (increased permeability and leakage), which causes pulmonary edema, activation of disseminated intravascular coagulation (DIC), pulmonary ischemia, hypoxic respiratory failure, and progressive lung damage.[61] Furthermore, it enters the blood from the respiratory tract through infecting ECs and travels throughout the different parts of the body including the brain, gastrointestinal tract, heart, kidney, and liver that may lead to cerebral hemorrhage, neural disorder, ischemic stroke, coma, paralysis, and eventually death.[62] Moreover, the vulnerability and severity of 2019-nCoV infection in individuals is highly impacted by comorbidities including hypertension, diabetes, and lung diseases, and also linked with age and dysregulated innate immune response. This may be due to enhanced expression of ACE2 receptor (an integral membrane protein) on the surface of several organs, including the lung, heart, kidney, intestine as well as ECs of the host.[63] The 2019-nCoV infects ECs through binding with ACE-2 and initiates localized inflammation, endothelial activation, tissue damage, and disordered cytokine release. The severe aggravation of the "cytokine storm" through secretion of vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), and reduced E-cadherin expression on ECs contribute to vascular permeability and leakage, which participate in the pathophysiology of hypotension and pulmonary dysfunction in acute respiratory distress syndrome (ARDS). The majority of the COVID-19 patients die due to ARDS, where pulmonary ECs contribute to the start and broadcast of ARDS by changing vessel barrier integrity, supporting a procoagulative condition, inducing vascular inflammation and reconciling inflammatory cell infiltration.[64] Therefore, understanding the various complications in the vasculature that are attributed to 2019-nCoV is of great significance. The lung ECs are more enhanced in immunomodulatory signatures compared with ECs of other organs including high-level expressions of genes associated with major histocompatibility complex (MHC) class II-mediated antigen processing,

loading, and presentation. This suggests that a subtype of lung ECs is acting as semiprofessional antigen-presenting cells against respiratory pathogens. It has been also hypothesized that ECs play a central role in the pathogenesis of ARDS and multiorgan failure in patients with COVID-19. In severe COVID-19 infection, there is an activation of coagulation pathways with potential development of DIC. As a result of the DIC and clogging/congestion of the small capillaries by inflammatory cells, as well as possible thrombosis in larger vessels, lung tissue ischemia develops, which triggers angiogenesis and potential ECs hyperplasia.[65,66] There are multiple mechanisms proposed for increased vascular permeability and vascular leakage in severe COVID-19 patients elaborately described by Teuwen et al.[61] In brief, (a) the 2019-nCoV can directly affect ECs that exhibit widespread endotheliitis characterized by EC dysfunction, lysis, and death, (b) Furthermore, to enter the host cells, 2019-nCoV binds to the ACE2 receptor, which reduces the activity of ACE2, which indirectly turns on the kallikrein-bradykinin pathway with increased vascular permeability, (c) activated neutrophils, recruited to pulmonary ECs, produce histotoxic mediators including reactive oxygen species, (d) immune cells, inflammatory cytokines, and vasoactive molecules lead to increased ECs contractility and loosening/gap of interendothelial junctions, (e) the cytokines IL-1β and tumor necrosis factor (TNF) activate glucuronidases that degrade the glycocalyx but also upregulate hyaluronic acid synthase 2, leading to increased deposition of hyaluronic acid in the extracellular matrix and promoting fluid retention.[61,67]

Moreover, the high levels of cytokines intensify the destructive progression that leads to additional ECs dysfunction, DIC, inflammation, and vasodilation of the pulmonary capillary bed. Altogether, these disorders ultimately lead to multiorgan failure and death due to alveolar dysfunction and ARDS with hypoxic respiratory failure. Moreover, it has been proposed that denudation of the pulmonary vasculature could lead to activation of the complement system, promoting the accumulation of neutrophils and proinflammatory monocytes that enhance the cytokine storm, which was also observed during influenza virus infection where pulmonary ECs induce an amplification loop, involving interferon-producing cells and virus-infected pulmonary ECs.[68] Normalization of the vascular wall through metabolic interventions could be considered as an added route of intervention and paves the way for future therapeutic opportunities along with anti-inflammatory, anti-cytokine drugs, and ACE inhibitors etc.[67] However, some additional indirect evidence also suggests a link between ECs, pericytes, and COVID-19. Therefore, the consequences of 2019-nCoV on the entire vasculature require more study.[60]

6 | MICROSTRUCTURAL FEATURES OF 2019-nCoV

The microstructural characterization of 2019-nCoV virus has been carried out using both scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The name Corona was given based on the original SEM image which resembles a crown, as shown in Figure 4a,b. In addition, the first microstructure of the 2019-nCoV sample was taken from an infected patient at the Vector Institute in Novosibirsk using JEM-1400 TEM with the negative contrast method (Figure 5).[69] The size of the 2019-nCoV spherical particle was found to be in the range of 60 to 140 nm (Figure 6). In another study, the negative-stained grids and ultrathin sections of the human airway epithelial cell were also observed under TEM by Chinese researchers[70] and these demonstrated some pleomorphism, as shown in Figure 7a. Distinctive spikes of about 9 to 12 nm have been also observed on the external surface of the virus. Additionally, extracellular free viral particles and inclusion bodies filled with virus particles present in membrane-bound vesicles in the cytoplasm were observed as shown in Figure 7b. Indian scientists from the National Institute of Virology, Pune have also obtained the TEM Images of 2019-nCoV from a sample taken directly from the throat swab specimen of a female patient who returned from Wuhan.[71] A total of seven negative-stained viral particles were imaged in the fields scanned as shown in Figure 8, clearly depicting the spherical shape of the virus with a cobbled surface structure having envelope projections of 75 nm in size. The presence of stalk-like projections with round peplomeric structures have been observed, along with patchy

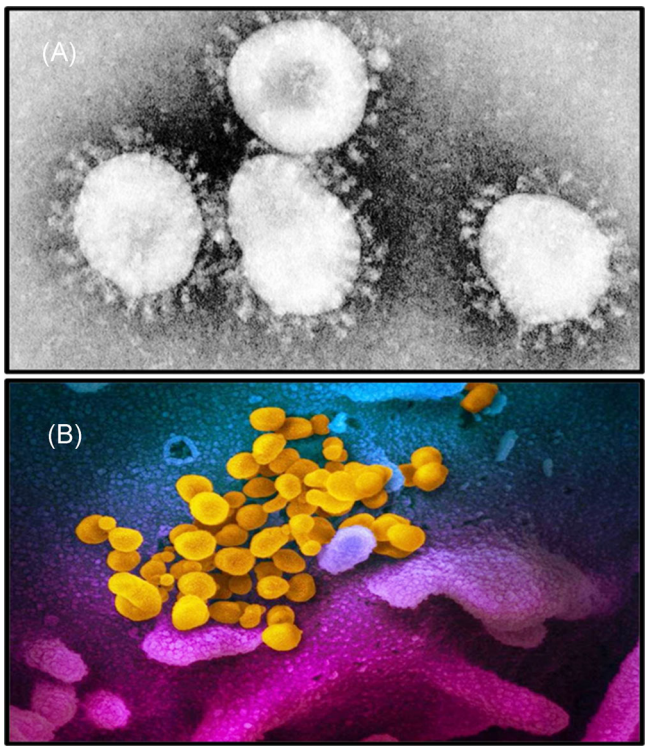

FIGURE 4 A, Image of coronaviruses viewed under a transmission electron microscopy (TEM) (Credit: CDC/Dr. Fred Murphy). B, Scanning electron microscope (SEM) image of SARS-CoV-2 developed by the National Institutes of Health showing yellow virus particles emerging from cells cultured in laboratory conditions (Image Credit: AFP, csm_0320-900_Supp_COVID19_1_In_Article_f7dbbbe8e0)

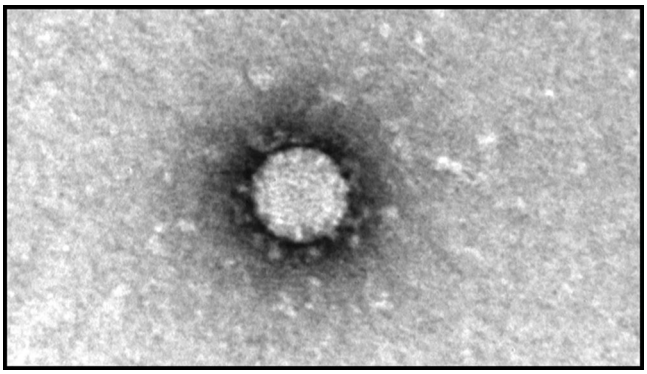

FIGURE 5 The first microstructure of the 2019-nCoV obtained through transmission electron microscopy (TEM) using the negative contrast method (*Source:* Vector Institute in Novosibirsk, TEM [JEM-1400])

stain pooling on the surface and a distinct envelope projection ending in round peplomeric (glycoprotein spike on the viral surface) parts.

7 | IMMUNOLOGICAL RESPONSES AGAINST 2019-nCoV

The immunosenescence, comorbidity, weak immune system, diminished fitness, age related diseases, chronic medical condition, and increased frailty because of aging have been found as the primary reason for the exacerbated rate of infection and mortality.[72] For the development of efficient active and passive immunization against 2019-nCoV, it is necessary to understand the immunopathogenesis of COVID-19.[30] Although, the data available currently on host immune responses against 2019-nCoV is not sufficient, the existing immunopathogenesis data of SARS-CoV could be utilized to hypothesize

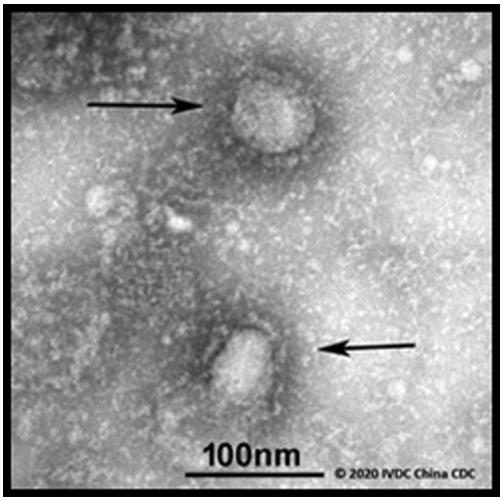

FIGURE 6 Transmission electron microscopy (TEM) image of the 2019-nCoV (*Source*: GISAID, https://www.gisaid.org/, Courtesy: IVDC, the Chinese Centre for Disease Control & Prevention)

an efficient immunization therapy against COVID-19.[73,74] According to available evidence, 2019-nCoV can induce innate, cellular as well as humoral immune responses in humans.[75,76]

There are many reports suggesting the death of COVID-19 patients due to an extreme response of their immune system, that is, abnormal release of circulating cytokines, termed the cytokine release syndrome (CRS). These numerous cytokines released in COVID-19 patients are termed as "cytokine storm" including IL-6, IL-1, IL-2, IL-10, TNF-α, and interferon-γ.[77-79] Huang et al reported the clinical features of 41 hospitalized patients infected with 2019-nCoV, revealing the induction of high levels of proinflammatory cytokines and chemokines such as MCP-1, IL-2, IL-7, TNF-α, G-CSF, MIP-1A, MCP-3, IP-10, which showed a positive correlation with disease severity. In addition, increased level of anti-inflammatory cytokines such as IL-4 and IL-10 were also induced by the human immune system against 2019-nCoV infections.[7,80-84] The over secretion of cytokines could damage the lung, resulting in the death of the COVID-19 patient. Thus, to reduce the lung damage, neutralizing anti- TNF-α, -IL1 and -IL6 antibodies may be utilized to block their biological activity, as they have been previously used in the treatment of other diseases such as cancer, type 2 diabetes, leukemia, etc.[85-87] During a pretreatment study of type I interferon, 2019-nCoV has shown higher sensitivity than SARS-CoV.[88] This variation may be due to several types of modifications in SARS-CoV genome, such as the lack of ORF3b and variations in ORF6 (short truncation). In addition, a high level of follicular helper T cells, antibody-secreting cells, antibodies (immunoglobulin [IgM] and IgG), and activated CD4+ as well as CD8+ T cells were confirmed in the infected patients.[40,89]

With respect to the humoral immune responses, 403 B-cell assays have been reported, which involve different antibodies (IgA, IgG, IgG1, IgG2a, IgG2b, and IgM) including linear and discontinuous B-cell epitopes. A comparative study of known epitopes (432 B-cell epitopes and 164 T-cell epitope) of SARS-CoV with predicted epitopes of 2019-nCoV using contemporary bioinformatics tools has been conducted by Grifoni et al[90] (Table 1). The three-dimensional structure of the S protein of 2019-nCoV in the closed state (PDB ID: 6VXX) and open state (PDB ID: 6VYB) is also available at the RCSB PDB database[37] and the same has been utilized to predict a potential B-cell epitope for designing therapeutics, for example, vaccine and neutralizing antibodies (NAbs).[91,92] These NAbs can be used for passive immunization therapy (eg, convalescent plasma therapy [CPT]), and have shown significant benefits in treatment of severe COVID19 patients.[93,94]

Although, cellular immune responses (CD4+ and CD8+ T cells) against 2019-nCoV have been found activated but due to the innate immune escape mechanism of 2019-nCoV, the T-cell immune response is delayed and it fails to provide significant protection to COVID-19 patients.[95-98] Besides this, a "cytokine storm" also results in functional collapse of T-cell counts in COVID-19 patients. However, during recovery, decrease in the level of IL-6, IL-10, and TNF-α increases the total T cell (CD4+ and CD8+) counts in COVID-19 patients.[99] The immune escape mechanism of 2019-nCoV can be overcome by blocking the overexpression of the NK group 2 member

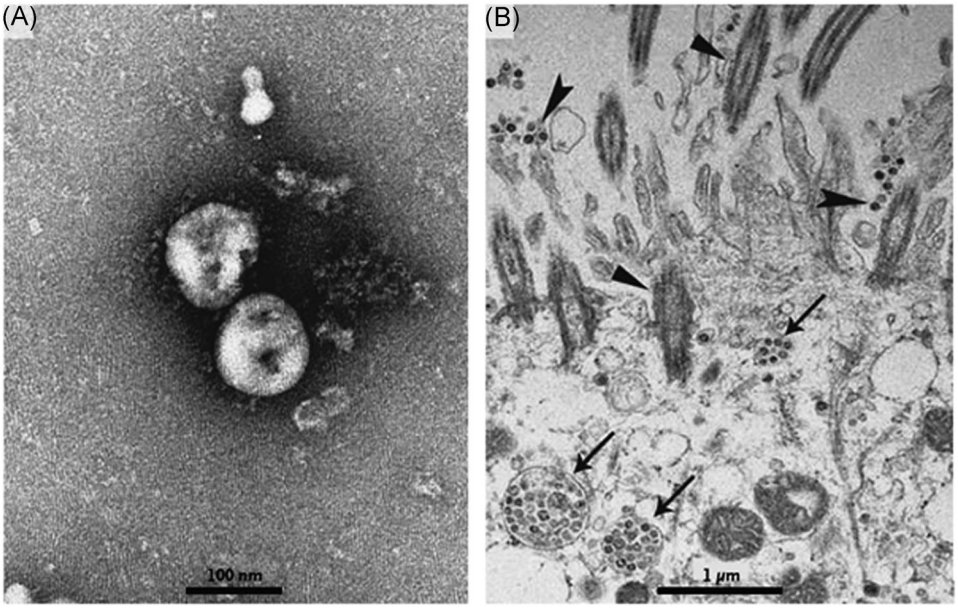

FIGURE 7 Visualization of negative-stained 2019-nCoV via transmission electron microscopy (A) and in the human airway epithelial cell ultrathin sections (B). Arrowheads indicate extracellular virus particles, arrows indicate inclusion bodies formed by virus components, and triangles indicate cilia (*Source:* Figure 3 of Zhu et al, (2020), N Engl J Med, DOI: 10.1056/NEJMoa2001017)

A (NKG2A) receptor using a monoclonal antibody such as Monalizumab.[100,101] For the SARS-related coronaviruses (IEDB ID: 694009), a total of 349 MHC ligand assays have been reported for 123 epitopes including 69 HLA-A, 23 HLA-B, and 31 HLA-DRB1 epitopes. Besides these, in silico prediction of epitopes provides added knowledge towards the development of an efficient vaccine against COVID-19.[90]

8 | DIAGNOSIS METHODS

Diagnosis of pathogenic disorders depends on two aspects (a) the pathogens, their components, and life-cycle stages; and (b) the host response, including the synthesis of micro or macro metabolites. For a virus-like 2019-nCoV, where the genome and nucleocapsids are considerably similar to epidemic predecessors like SARS-CoV or

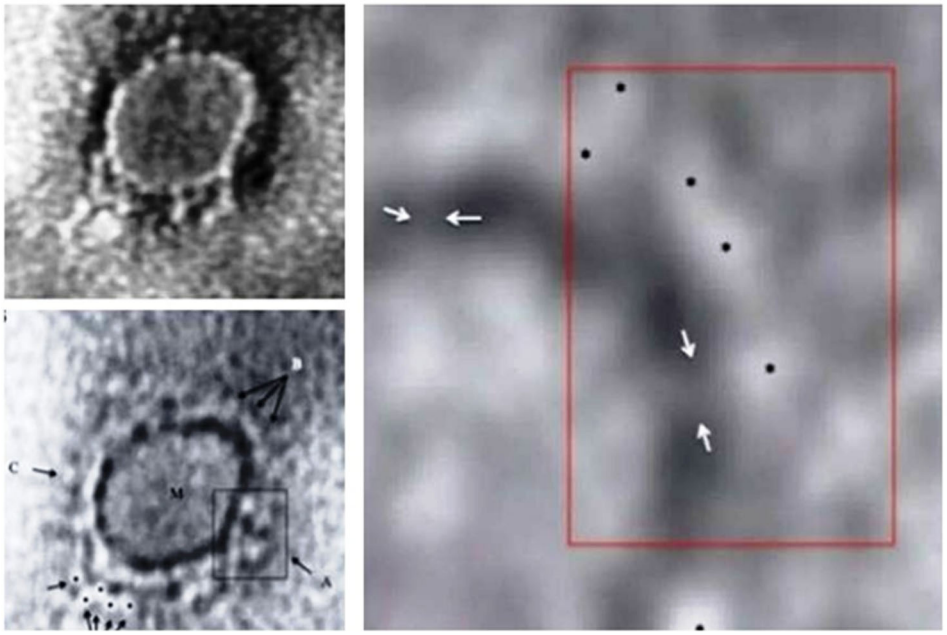

FIGURE 8 Transmission electron microscopy (TEM) imaging of negative-stained 2019-nCoV particle showing morpho-diagnostic features of family Coronaviridae (*Source:* Prasad et al, (2020), Indian J Med Res, DOI: 10.4103/ijmr.IJMR_577_20)

TABLE 1 Details of predicted B- and T-cell epitopes of 2019-nCoV along with similarity to experimentally validated SARS-CoV epitopes

S.No.	Predicted epitope sequence (HLA binding alleles)	Name of source protein	% identity with experimentally validate SARS-CoV epitopes
B cell epitope			
1	FGAGAALQIPFAMQMAYRFNGI	S	100
2	RPQGLPNNTASWFTALTQHGK	N	95
3	NNNAATVLQLPQGTTLPKGF	N	95
4	MADSNGTITVEELKKLLEQWNLVI	M	92
5	NKHIDAYKTFPPTEPKKDKKKKTDEAQPLPQRQKKQPTVTLLPAADM	N	90
6	PLLESELVIGAVILRGHLRI	M	90
7	FSQILPDPSKPSKRSFIE	S	89
8	VCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIAD TTDAVRDPQTLEILDITPCSFGGVSVI	S	80
9	GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS	S	78
10	DAVDCALDPLSETKCTLKSFTVEKGIYQTSN	S	69
T cell epitope			
11	ALNTLVKQL (HLA-A*02:01)	S	100
12	VLNDILSRL (HLA-A*02:01)	S	
13	LITGRLQSL (HLA-A*02:01)	S	
14	RLNEVAKNL (HLA-A*02:01)	S	
15	NLNESLIDL (HLA-A*02:01)	S	
16	FIAGLIAIV (HLA-A*02:01)	S	
17	TLACFVLAAV (HLA-A*02:01)	M	
18	ALNTPKDHI (HLA-A*02:01)	N	
19	LQLPQGTTL (HLA-A*02:01)	N	
20	LALLLLDRL (HLA-A*02:01)	N	
21	LLLDRLNQL (HLA-A*02:01)	N	
22	GMSRIGMEV (HLA-A*02:01)	N	
23	MEVTPSGTWL (HLA-B*40:01)	N	
24	QLIRAAEIRASANLAATK (HLA-DRB1*04:01)	S	
25	KLPDDFTGCV (HLA-A*02:01)	S	≥90
26	TECSNLLLQYGSFCTQL (HLA-DR8)	S	

MERS-CoV viruses, diagnostic development with high species specificity becomes a challenge. As per WHO guidelines and suggestions, the diagnosis of 2019-nCoV may rely upon methods that take into account two or more targets simultaneously. For screening purposes, RT-PCR based methods that target more than one region of viral RNA (one specific for 2019-nCoV and another from related β-coronaviruses) have been recommended for asymptomatic or mildly symptomatic cases. Depending upon expertize and automation, the real-time RT-PCR based diagnostics require 2 to 5 hours. These two requirements make the molecular testing slow and limited as it requires at least BSL-2 and/or BSL-3 facilities for the testing and culture of deadly viruses, respectively. Besides this method, a nucleic acid amplification test (NAAT) is not sufficient and the test for confirmation of beta-coronavirus is also required. As per advice, the NAAT needs to include dual controls (both external and internal). Therefore, a final confirmation needs to be done by nucleic acid sequencing and additional alignment with known strains. The Abbott Company has developed a rapid test kit which produces results within 5 minutes of testing (Abbott ID NowCOVID-19). This molecular test relies on isothermal amplification of nucleic acid and uses an instrument called ID Now to monitor RNA-dependent RNA polymerase (RdRP) of 2019-nCoV.[102] Another German company TibMolBiol has developed a detection system that relies upon RT-PCR and focuses on RdRP as well as E gene assays. It has claimed no cross-reaction with other coronaviruses.[103] In addition, Chu et al[104] have also reported another assay based on RT-PCR of the ORF1b (screening gene) and N gene (confirmatory gene).

Comparatively rapid methods (in 1 hour) SHERLOCK and DETECTR (based on CRISPR technology) have been developed for diagnosis by Sherlock biosciences and Mammoth biosciences, respectively.[105] The former method relies on Cas13, which excises a reporter RNA sequence when activated by 2019-nCoV guide RNA (https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf). The latter one relies on identification of viral E and N specific RNAs by Cas12a using the reporter RNA.[106] Another method called amplicon based metagenomic sequencing[107] (using MiniION based sequencing) has also been proposed, which claims the process can be completed within 8 hours. In a more diverse

approach, a next-generation sequencing (NGS) method for sequencing the whole genome of 2019-nCoV has also been reported, wherein viral RNA has been reverse-transcribed and a sequencing library was created and subjected to sequencing and analysis by Miseq150 PE and CLC workbench.[108]

Alternatively, serological tests are also recommended for investigating the extent and spread of the ongoing outbreak and to verify the efficacy of any tests that will be devised in the future. Although, serological testing for the confirmation of COVID-19 is a challenge due to the antibodies cross-reactivity with other related coronaviruses, they are significant with regard to screening and evaluation of disease status. Recently, an IgM and IgG (produced in response to 2019-nCoV infection) based test has been proposed by Bio Medomics that can be used to assess infectious cases in a mere 10 minutes, after 10 to 30 days and 20 days of antibody production, respectively.[109] Moreover, such tests require only a small volume of blood (10-20 μL). These antibody-based assays not only contribute significantly towards screening the patients and health workers but also indicate their health and immune status.[102] The Diazyme laboratories in the United States have announced the availability of two high throughput serological test kits (50 samples/hr) for the 2019-nCoV, namely the Diazyme DZ-Lite SARS-CoV-2 IgG and the SARS-CoV-2 IgM CLIA test kits, based on a fully automated chemiluminescence analyzer.[110] Many more kits have been designed worldwide to detect COVID-19 infection. A large number of kits that are already in use or in the pipeline have been listed in the 2019-nCoV diagnostic pipeline (https://www.finddx.org/covid-19/pipeline/; accessed on 4 April 2020). To reduce false positives, diverse samples have been recommended for the examination of 2019-nCoV. These include nasopharyngeal and oropharyngeal swab, biopsy tissue of the lung, nasal/nasopharyngeal discharge/aspirates, sputum, serum, whole blood, stool, or urine. All these samples need to be processed quickly at test centers in a strict cold chain or cryopreserved samples may be used if sample transportation to the test center requires an extended duration. International quarantine laws, exchange of biomaterials, and other laws must be followed all over the world, in accordance with the United Nations (UN) conventions. Apart from such molecular examinations, many clinical traits and physiological means of primary investigations have also been published but their ambiguity and qualitative nature seems confusing across different populations and categories of infection. The Indian Council of Medical Research (ICMR) has issued guidelines for clinicians to diagnose COVID-19 suspects and their stages of severity.[111] The clinical traits have been classified into many categories based on stages of disease development. The initial stage is uncomplicated illness (cough, fever, headache, malaise, nasal congestion, sore throat, etc.) followed by mild/severe pneumonia culminating in sepsis and septic attack. Accordingly, the cases are advised with respect to treatment and supporting instrumentation for live-saving and cure.

9 | LEADING VACCINE CANDIDATES

Vaccinations is the most effective and economical way to prevent and control corona infections, but have so far been unsuccessful due to the extensive antigenic sequence diversity of the virus.[112] By now, more than 40 pharmaceutical companies and academic organizations have launched their vaccine development programs against 2019-nCoV, worldwide. Herein, we summarize the latest developments on COVID-19 antigenic candidates, adjuvants, validation, and vaccine technology platforms that are in use in similar research areas. In the past and current decade, much effort has been directed towards the development of vaccines against human coronaviruses. The therapeutic information available on SARS- and MERS-CoV can be exploited to develop an effective vaccine against the emerging COVID-19.[40] Multiple strategies such as virus-like particles, DNA, or viral vectors are currently adopted in the development of coronavirus vaccines, utililizing S glycoprotein as the major inducer of neutralizing antibodies that could block not only viral receptor-binding but also viral genome uncoating.[113,114] A comparative study performed on full-length S protein sequences of 2019-nCoV and SARS-CoV revealed that the highest number of variable residues is located in the S1 subunit, a critical target for developing the CoV vaccine. These results suggest that the specific neutralizing antibodies developed against the mutated region of SARS-CoV might not be effective against the 2019-nCoV.[115] So far, the full-length S protein, as well as its fragments (S1 subunit, RBD domain, NTD, and FP), have been used as antigens in vaccine development against 2019-nCoV. As the RBD of S protein directly interacts with the ACE2 receptor on host cells, specific antibodies developed against the RBD could block this recognition and thus effectively prevent the entry of the virus. Thus, the use of broadly neutralizing antibodies (bnAbs) may represent a good approach to increase humoral protection against COVID-19 by targeting various conserved epitopes of S protein.[44,116] To emphasize, the cross-neutralization ability of specific neutralizing monoclonal antibodies (mAbs) against SARS-CoV RBD greatly depends on the similarity between epitopes of 2019-nCoV RBD.[117,118] As the genomes of coronaviruses are highly variable, it is better to use antibodies targeting different epitopes to avoid immune escape of the virus.[46] Some research groups have also reported the use of NTD of the S1 subunit protein from MERS-CoV as an antigen that induced potent humoral and cellular immunity in mice and was also found to be protective against viral challenge.[119,120] Although the antigenic function of NTD of 2019-nCoV has not been elucidated, it may be similar in function to the MERS-CoV.[49,121] Furthermore, as the FP domain of the S2 subunit is involved in the membrane fusion of the virus, it could also act as a vaccine candidate. Moreover, a high titer of antibodies was detected in mice immunized with the fusion protein of RBD-FP.[122] The possibility of developing a universal coronavirus vaccine was also explored by several research groups, based on the similarity between epitopes of SARS-CoV and MERS-CoV as well as 2019-nCoV.[123,124] In contrast, the most abundant antigenic N protein is highly conserved and performs multiple functions, including the formation of nucleocapsids, signal transduction, virus budding, RNA replication, transcription, etc.[125,126] This protein developed a strong humoral and cellular immune responses in C57BL/6 mice and was effective in viral clearance from the lungs[127-129] but contradictory results were

obtained in hamsters.[130] Similarly, the M protein, a conserved transmembrane glycoprotein that is involved in virus assembly, elicits efficient humoral, and cellular immune response in SARS-CoV patients, thus making it a potential vaccine candidate against 2019-nCoV. It is worth mentioning here that the immunogenicity of E protein is limited.[131] Considering the above-mentioned facts, several researchers have utilized immunoinformatics tools to identify the potential location of B- and T-cell epitopes in proteins S, N, and E of 2019-nCoV and MERS-CoV.[92,132-134] In addition, the attenuated live Avian virus (strain H) of chicken CoV could be considered as another option to develop an oral vaccine against COVID-19 after evaluating its safety in monkeys.[14,25,135-137] Passive immunization with mAbs coupled with drugs (Remdesivir, a cytokine neutralizing compounds) and trans bodies developed against nonstructural intracellular proteins of 2019-nCoV could also be explored as potential therapeutic option against COVID-19.[10,138-140] Trans bodies can traverse across the membrane of the virus-infected cells and stop the replication and transcription of viruses including influenza virus, hepatitis C virus, Ebola virus, and Dengue virus.[138] Along with the aforementioned strategies, some of the important known limitations must be taken into consideration for the successful development of a COVID-19 vaccine. For example, antibody-dependent enhancement of disease is a phenomenon in which a surface exposed antigen-specific antibody facilitates the viral entry into the host cell via the Fc receptor pathway, leading to the enhanced viral infectivity reported in some vaccines against MERS-CoV and SARS-CoV, Dengue, and Zika viruses, but not in 2019-nCoV.[141-144] The aforementioned limitations could be avoided by selection of a nonexposed N protein as a DNA vaccine candidate that can induce antibodies and will not be able to facilitate viral entry, while simultaneously being capable of eliminating the virus from the host through cellular immune response.[129] Another significant hindrance is the higher genetic diversity (hypermutation) of RNA viruses compared to DNA viruses.[145,146] Animal models (mouse and rhesus monkey) related to human ACE2 transgenic of COVID-19 have been well established for vaccine development,[147] including synthetic reconstruction of the SARS-CoV-2 genome.[148]

9.1 | Latest news about vaccine development against COVID-19

There is substantial progress in the design and development of vaccines, involving the characterization of the 2019-nCoV virus, identification of candidate antigens and epitopes, establishment of animal models, characterization of the immune responses, and the formulation of the vaccines themselves. The development of 2019-nCoV vaccines comprises several types, including the inactivated virus, recombinant protein, viral vector-based, messenger RNA (mRNA), and DNA, etc. The RNA and DNA vaccines can be synthesized quickly using synthetic processes, as compared to the other types that require culturing or fermentation, with the exception of the synthetic peptide vaccine. Although there are no approved RNA vaccines to date, some variants have entered clinical trials. The use of next-generation sequencing, reverse vaccinology, immunoinformatics, as well as reverse genetics and human challenge studies, may also reduce the development time of more conventional vaccines during an epidemic.[149,150] The list of major platform types and examples of 2019-nCoV vaccine types being developed is presented in Table 2-5.[76,91,151-154]

Clover Biopharmaceuticals (http://www.cloverbiopharma.com/index.php?m=content&c=index&a=lists&catid=10) has confirmed the mammalian cell culture-based expression of the native-like S-Trimer antigen of the 2019-nCoV and detection of antigen-specific neutralizing antibodies in the sera of fully-recovered COVID-19 patients. The vaccination performance of S-Trimer could be further improved by including the Dynavax based toll-like receptor 9 (TLR9) agonist adjuvant (CpG 1018), and GSK's based adjuvant system with a "molecular clamp" developed by The University of Queensland. A "molecular clamp" is a polypeptide that stabilizes S-Trimer to improve the recognition of the correct antigen by antigen-presenting cells, thereby inducing stronger immune responses (https://www.uq.edu.au/news/article/2020/02/significant-step%E2%80%99-covid-19-vaccine-quest). Inovio Pharmaceuticals (http://ir.inovio.com/news-and-media/news/press-release-details/2020/INOVIO-Initiates-Phase-1-Clinical-Trial-Of-Its-COVID-19-Vaccine-and-Plans-First-Dose-Today/default.aspx) has developed a DNA plasmid vaccine that expresses the 2019-nCoV spike protein and activates T cells against COVID-19. Now, Inovio is planning to conduct phase-I clinical trials with support from the Coalition for Epidemic Preparedness Innovations (CEPI) and Beijing Advaccine Biotechnology.[39] Moderna Inc. has designed an in silico based mRNA vaccine (mRNA-1273) encoding the S protein of 2019-nCoV and planned a phase-I clinical trial (NCT04283461) in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) and CEPI (https://www.modernatx.com/modernas-work-potential-vaccine-against-covid-19).[155] The mRNA-based vaccine is advantageous over DNA vaccines due to non-requirement of host genome integration, the improved immune responses, and production of multimeric antigens.[156]

Researchers from Rocky Mountain Laboratories, Oxford University, University of Queensland, Vaccine Research Center (VRC) of the NIAID, and CEPI have announced their intension to conduct clinical trials of a COVID-19 vaccine candidate in the coming months using a chimpanzee adenovirus-vectored (DNA Medicines) vaccine platform for easier recognition of antigens by the immune system.[135] The vaccine under development utilizes synthetic DNA/mRNA molecules and delivers these into host cells for translation into antigenic proteins of 2019-nCoV, eliciting both humoral and cellular responses (https://www.niaid.nih.gov/news-events/nih-clinical-trial-investigational-vaccine-covid-19-begins). On 16 March 2020, the G7 committed to support the launch of joint research projects for COVID-19 treatments and vaccines. A high-level dialogue is underway to ensure complementarity of efforts and global access to COVID-19 vaccines.[157] On 30th March, Johnson & Johnson (J&J) announced an

TABLE 2 Details of nucleic acid (DNA/RNA) based COVID-19 vaccine candidates as updated on 9 June 2020, prepared by WHO (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines)

S.No.	Vaccine platform	Type of vaccine candidate	Developer institute/organization	Current stage (preclinical/clinical) of evaluation
1.	DNA	DNA plasmid vaccine with electroporation	Inovio Pharmaceuticals	Phase 1 NCT04336410
2.	RNA	LNP-encapsulated mRNA	Moderna/NIAID	Phase 2 NCT04405076 Phase 1 NCT04283461
3.	RNA	3 LNP-mRNAs	BioNTech/Fosun Pharma/Pfizer	Phase 1/2 2020-001038-36 NCT04368728
4.	DNA	DNA Vaccine (GX-19)	Genexine Consortium	Preclinical
5.	DNA	DNA with electroporation	Karolinska Institute/Cobra Biologics (OPENCORONA Project)	Preclinical
6.	DNA	DNA plasmid vaccine	Osaka University/AnGes/Takara Bio	Preclinical
7.	DNA	DNA	Takis/Applied DNA Sciences/Evvivax	Preclinical
8.	DNA	Plasmid DNA, Needle-Free Delivery	Immunomic Therapeutics, Inc./EpiVax, Inc./PharmaJet	Preclinical
9.	DNA	DNA plasmid vaccine	Zydus Cadila	Preclinical
10.	DNA	DNA vaccine	BioNet Asia	Preclinical
11.	DNA	DNA vaccine	University of Waterloo	Preclinical
12.	DNA	DNA vaccine	Entos Pharmaceuticals	Preclinical
13.	DNA	bacTRL-Spike	Symvivo	Preclinical
14.	RNA	LNP-mRNA	Translate Bio/Sanofi Pasteur	Preclinical
15.	RNA	LNP-mRNA	CanSino Biologics/Precision NanoSystems	Preclinical
16.	RNA	LNP-encapsulated mRNA cocktail encoding VLP	Fudan University/Shanghai JiaoTong University/RNACure Biopharma	Preclinical
17.	RNA	LNP-encapsulated mRNA encoding RBD	Fudan University/Shanghai JiaoTong University/RNACure Biopharma	Preclinical
18.	RNA	Replicating Defective SARS-CoV-2 derived RNAs	Centro Nacional Biotecnología (CNB-CSIC), Spain	Preclinical
19.	RNA	LNP-encapsulated mRNA	University of Tokyo/Daiichi-Sankyo	Preclinical
20.	RNA	Liposome-encapsulated mRNA	BIOCAD	Preclinical
21.	RNA	Several mRNA candidates	RNAimmune, Inc.	Preclinical
22.	RNA	mRNA	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Preclinical
23.	RNA	mRNA	China CDC/Tongji University/Stermina	Preclinical
24.	RNA	mRNA	Arcturus/Duke-NUS	Preclinical
25.	RNA	saRNA	Imperial College London	Preclinical
26.	RNA	mRNA	Curevac	Preclinical
27.	RNA	mRNA in an intranasal delivery system	eTheRNA	Preclinical
28.	RNA	mRNA	Greenlight Biosciences	Preclinical
29.	RNA	mRNA	IDIBAPS-Hospital Clinic, Spain	Preclinical

TABLE 3 Details of protein subunit based COVID-19 vaccine candidates as updated on 9 June 2020, prepared by WHO (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines)

S.No.	Vaccine platform	Type of vaccine candidate	Developer institute/organization	Current stage (preclinical/clinical) of evaluation
1.	Protein Subunit	Full-length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	Novavax	Phase 1/2 NCT04368988
2.	Protein Subunit	RBD protein fused with Fc of IgG +Adj.	Chulalongkorn University/GPO, Thailand	Preclinical
3.	Protein Subunit	Capsid-like Particle	AdaptVac (PREVENT-nCoV consortium)	Preclinical
4.	Protein Subunit	Drosophila S2 insect cell expression system VLPs	ExpreS2ion	Preclinical
5.	Protein Subunit	Peptide antigens formulated in LNP	IMV Inc	Preclinical
6.	Protein Subunit	S protein	WRAIR/USAMRIID	Preclinical
7.	Protein Subunit	S protein+Adjuvant	National Institute of Infectious Disease, Japan	Preclinical
8.	Protein Subunit	VLP-reco mbinant protein+Adjuvant	Osaka University/BIKEN/National Institutes of Biomedical Innovation, Japan	Preclinical
9.	Protein Subunit	Native-like Trimeric subunit Spike Protein vaccine	Clover Biopharmaceuticals Inc./GSK/Dynavax	Preclinical
10.	Protein Subunit	microneedle arrays S1 subunit	Univ. of Pittsburgh	Preclinical
11.	Protein Subunit	Peptide	Vaxil Bio	Preclinical
12.	Protein Subunit	Adjuvanted protein subunit (RBD)	Biological E Ltd	Preclinical
13.	Protein Subunit	Peptide	Flow Pharma Inc	Preclinical
14.	Protein Subunit	S protein	AJ Vaccines	Preclinical
15.	Protein Subunit	Ii-Key peptide	Generex/EpiVax	Preclinical
16.	Protein Subunit	S protein	EpiVax/Univ. of Georgia	Preclinical
17.	Protein Subunit	Protein Subunit EPV-CoV-19	EpiVax	Preclinical
18.	Protein Subunit	S protein (baculovirus production)	Sanofi Pasteur/GSK	Preclinical
19.	Protein Subunit	gp-96 backbone	Heat Biologics/Univ. Of Miami	Preclinical
20.	Protein Subunit	Molecular clamp stabilized Spike protein	University of Queensland/GSK/Dynavax	Preclinical
21.	Protein Subunit	Peptide vaccine	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Preclinical
22.	Protein Subunit	Subunit vaccine	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Preclinical
23.	Protein Subunit	S1 or RBD protein	Baylor College of Medicine	Preclinical
24.	Protein Subunit	Subunit protein, plant produced	iBio/CC-Pharming	Preclinical
25.	Protein Subunit	Recombinant protein, nanoparticles (based on S-protein and other epitopes)	Saint-Petersburg scientific research institute of vaccines and serums	Preclinical

(Continues)

TABLE 3 (Continued)

S.No.	Vaccine platform	Type of vaccine candidate	Developer institute/organization	Current stage (preclinical/clinical) of evaluation
26.	Protein Subunit	COVID-19 XWG-03 truncated S (spike) proteins	Innovax/Xiamen Univ./GSK	Preclinical
27.	Protein Subunit	Adjuvanted microsphere peptide	VIDO-InterVac, University of Saskatchewan	Preclinical
28.	Protein Subunit	Synthetic Long Peptide Vaccine candidate for S and M proteins	OncoGen	Preclinical
29.	Protein Subunit	Oral E. coli-based protein expression system of S and N proteins	MIGAL Galilee Research Institute	Preclinical
30.	Protein Subunit	Nanoparticle vaccine	LakePharma, Inc.	Preclinical
31.	Protein Subunit	Recombinant spike protein with Advax adjuvant	Vaxine Pty Ltd/Medytox	Preclinical
32.	Protein Subunit	OMV-based vaccine	Quadram Institute Biosciences	Preclinical
33.	Protein Subunit	OMV-based vaccine	BiOMViS Srl/Univ. of Trento	Preclinical
34.	Protein subunit	structurally modified spherical particles of the tobacco mosaic virus (TMV)	Lomonosov Moscow State University	Preclinical
35.	Protein Subunit	Spike-based	University of Alberta	Preclinical
36.	Protein Subunit	Recombinant S1-Fc fusion protein	AnyGo Technology	Preclinical
37.	Protein Subunit	Recombinant protein	Yisheng Biopharma	Preclinical
38.	Protein Subunit	Recombinant S protein in IC-BEVS	Vabiotech	Preclinical
39.	Protein Subunit	Orally delivered, heat-stable subunit	Applied Biotechnology Institute, Inc.	Preclinical
40.	Protein Subunit	S-2P protein+ CpG 1018	Medigen Vaccine Biologics Corporation/NIAID/Dynavax	Preclinical
41.	Protein Subunit	Peptides derived from Spike protein	Axon Neuroscience SE	Preclinical
42.	Protein Subunit	Adjuvanted recombinant protein (RBD-Dimer)	Anhui Zhifei Longcom Biopharmaceutical/Institute of Microbiology, Chinese Academy of Sciences	Preclinical
43.	Protein Subunit	RBD-based	Neovii/Tel Aviv University	Preclinical
44.	Protein Subunit	RBD-based	Kentucky Bioprocessing, Inc	Preclinical
45.	Protein Subunit	Outer Membrane Vesicle (OMV)-peptide	Intravacc/Epivax	Preclinical
46.	Protein Subunit	Protein Subunit	University of San Martin and CONICET, Argentina	Preclinical
47.	Protein Subunit	Protein Subunit	MOGAM Institute for Biomedical Research, GC Pharma	Preclinical
48.	Protein Subunit	Spike-based (epitope screening)	ImmunoPrecise	Preclinical
49.	Protein Subunit	Outer Membrane Vesicle (OMV)-subunit	Intravacc/Epivax	Preclinical

TABLE 4 Details of inactivated/live attenuated virus and virus-like particles (VLP) based COVID-19 vaccine candidates as updated on 9 June 2020, prepared by WHO (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines)

S.No.	Vaccine platform	Type of vaccine candidate	Developer institute/organization	Current stage (preclinical/clinical) of evaluation
1.	Inactivated	Inactivated	Wuhan Institute of Biological Products/ Sinopharm	Phase 1/2 ChiCTR2000031809
2.	Inactivated	Inactivated	Beijing Institute of Biological Products/ Sinopharm	Phase 1/2 ChiCTR2000032459
3.	Inactivated	Inactivated + alum	Sinovac	Phase 1/2 NCT04383574 NCT04352608
4.	Inactivated	Inactivated	Institute of Medical Biology, Chinese Academy of Medical Sciences	Phase 1 NCT04412538
5.	Inactivated	Inactivated	Beijing Minhai Biotechnology Co., Ltd.	Preclinical
6.	Inactivated	TBD	Osaka University/BIKEN/NIBIOHN	Preclinical
7.	Inactivated	Inactivated + CpG 1018	Sinovac/Dynavax	Preclinical
8.	Inactivated	Inactivated + CpG 1018	Valneva/Dynavax	Preclinical
9.	Inactivated	Inactivated	Research Institute for Biological Safety Problems, Rep of Kazakhstan	Preclinical
10.	Live Attenuated Virus	Codon deoptimized live attenuated vaccines	Codagenix/Serum Institute of India	Preclinical
11.	Live Attenuated Virus	Codon deoptimized live attenuated vaccines	Indian Immunologicals Ltd/Griffith University	Preclinical
12.	Live attenuated virus	Measles Virus (S, N targets)	DZIF—German Center for Infection Research	Preclinical
13.	VLP	VLP + Adjuvant	Mahidol University/The Government Pharmaceutical Organization (GPO)	Preclinical
14.	VLP	Virus-like particles, lentivirus and baculovirus vehicles	Navarrabiomed, Oncoimmunology group	Preclinical
15.	VLP	Virus-like particle, based on RBD displayed on virus-like particles	Saiba GmbH	Preclinical
16.	VLP	Plant-derived VLP	Medicago Inc.	Preclinical
17.	VLP	ADDomerTM multiepitope display	Imophoron Ltd and Bristol University's Max Planck Centre	Preclinical
18.	VLP	Unknown	Doherty Institute	Preclinical
19.	VLP	VLP	OSIVAX	Preclinical
20.	VLP	eVLP	ARTES Biotechnology	Preclinical
21.	VLP	VLPs peptides/whole virus	University of Sao Paulo	Preclinical

investment of $1 billion towards the development of a COVID-19 vaccine, with about half the money coming from the US Biomedical Advanced Research and Development Authority.[139] As of March 2020, two COVID-19 vaccines, Ad5-nCoV and mRNA-1273, developed by the Chinese Institute of Biotechnology of the Academy of Military Medical Sciences and Tianjin Cansino Biotechnology Inc, as well as National Institute of Allergy and Infectious Diseases and Moderna, Inc, respectively, have entered phase-I clinical trials. The Ad5-nCoV is based on the replication-defective adenovirus type 5 as the vector while mRNA-1273 is an mRNA vaccine to express S protein of 2019-nCoV.[155]

10 | POTENTIAL DRUG CANDIDATES

Current clinical practices for patient care and treatment largely rely upon symptomatic treatment and concurrent supportive care.[158] Although some medical centers recommend the use of antibiotics, antimalarials, and antivirals, unselective or inappropriate administration of these drugs should be avoided. Although no antiviral treatments have yet been approved, several approaches have been proposed, such as the administration of lopinavir/ritonavir (400/100 mg every 12 hours), chloroquine (500 mg every 12 hours), or hydroxychloroquine (200 mg every 12 hours). Chloroquine and

TABLE 5 Details of viral vector (replicating and nonreplicating) based COVID-19 vaccine candidates as updated on 9 June 2020, prepared by WHO (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines)

S.No.	Vaccine platform	Type of vaccine candidate	Developer institute/organization	Current stage (preclinical/clinical) of evaluation
1.	Nonreplicating viral vector	ChAdOx1-S	University of Oxford/AstraZeneca	Phase 2b/3 2020-001228-32 Phase 1/2 2020-001072-15
2.	Non-Replicating Viral Vector	Adenovirus Type 5 Vector	CanSino Biological Inc./Beijing Institute of Biotechnology	Phase 2 ChiCTR2000031781 Phase 1 ChiCTR2000030906
3.	Non-Replicating Viral Vector	MVA encoded VLP	GeoVax/BravoVax	Pre-Clinical
4.	Nonreplicatingg viral vector	Ad26	Janssen Pharmaceutical Companies	Preclinical
5.	Nonreplicating viral vector	Replication defective Simian Adenovirus (GRAd) encoding SARS-CoV-2 S	ReiThera/LEUKOCARE/Univercells	Preclinical
6.	Nonreplicating viral vector	MVA-S encoded	DZIF—German Center for Infection Research	Preclinical
7.	Nonreplicating viral vector	MVA-S	IDIBAPS-Hospital Clinic, Spain	Preclinical
8.	Nonreplicating viral vector	adenovirus-based NasoVAX expressing SARS2-CoV spike protein	Altimmune	Preclinical
9.	Nonreplicating viral vector	Ad5 S (GREVAX platform)	Greffex	Preclinical
10.	Nonreplicating viral vector	Oral Ad5 S	Stabilitech Biopharma Ltd	Preclinical
11.	Nonreplicating viral vector	Adenovirus-based + HLA-matche dpeptides	Valo Therapeutics Ltd	Preclinical
12.	Nonreplicating viral vector	Oral vaccine platform	Vaxart	Preclinical
13.	Nonreplicating viral vector	MVA expressing structural proteins	Centro Nacional Biotecnología (CNB-CSIC), Spain	Preclinical
14.	Nonreplicating viral vector	Dendritic cell-based vaccine	University of Manitoba	Preclinical
15.	Nonreplicating viral vector	parainfluenza virus 5 (PIV5)-based vaccine expressing the spike protein	University of Georgia/University of Iowa	Preclinical
16.	Nonreplicating viral vector	Recombinant deactivated rabies virus-containing S1	Bharat Biotech/Thomas Jefferson University	Preclinical
17.	Nonreplicating viral vector	Inactivated Flu-based vaccine + Adjuvant	National Center for Genetic Engineering and Biotechnology (BIOTEC) /GPO, Thailand	Preclinical
18.	Nonreplicating viral vector	Adeno-associated virus vector (AAVCOVID)	Massachusetts Eye and Ear/Massachusetts General Hospital/AveXis	Preclinical
19.	Nonreplicating viral vector	[E1-, E2b-, E3-] hAd5-COVID19-Spike/Nucleocapsid	ImmunityBio, Inc and NantKwest,Inc.	Preclinical
20.	Replicating viral vector	YF17D Vector	KU Leuven	Preclinical

TABLE 5 (Continued)

S.No.	Vaccine platform	Type of vaccine candidate	Developer institute/organization	Current stage (preclinical/clinical) of evaluation
21.	Replicating viral vector	Measles Vector	Zydus Cadila	Preclinical
22.	Replicating viral vector	Measles Vector	Institute Pasteur/Themis/University of Pittsburg Center for Vaccine Research/Merck	Preclinical
23.	Replicating viral vector	Measles Vector	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Preclinical
24.	Replicating viral vector	Horsepox vector expressing S protein	Tonix Pharma/Southern Research	Preclinical
25.	Replicating viral vector	Live viral vectored vaccine based on attenuated influenza virus backbone (intranasal)	BiOCAD and IEM	Preclinical
26.	Replicating viral vector	Recombinant vaccine based on Influenza A virus, for the prevention of COVID-19 (intranasal)	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Preclinical
27.	Replicating viral vector	Attenuated Influenza expressing an antigenic portion of the Spike protein	Fundação Oswaldo Cruz and Instituto Buntantan	Preclinical
28.	Replicating viral vector	Influenza vector expressing RBD	University of Hong Kong	Preclinical
29.	Replicating viral vector	Replication-competent VSV chimeric virus technology (VSVΔG) delivering the SARS-CoV-2 Spike (S) glycoprotein.	IAVI/Merck	Preclinical
30.	Replicating viral vector	VSV-S	University of Western Ontario	Preclinical
31.	Replicating viral vector	VSV vector	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Preclinical
32.	Replicating viral vector	M2-deficient single replication (M2SR) influenza vector	UW–Madison/FluGen/Bharat Biotech	Preclinical
33.	Replicating viral vector	Newcastle disease virus vector (NDV-SARS-CoV-2/Spike)	Intravacc/Wageningen Bioveterinary Research/ Utrecht Univ.	Preclinical
34.	Replicating viral vector	Avian paramyxovirus vector (APMV)	The Lancaster University, UK	Preclinical

hydroxychloroquine have been found to exhibit inhibitory effects on the acidification of endo/lysosomes, owing to their weak basic nature.[159] Endosomes and lysosomes are important organelles required for membrane fusion.[160] Chloroquine also hampers the entry of virus by glycosylating ACE2 and the S protein.[161] Hydroxychloroquine has been found to cause delay in the entry and post-entry stage[162] and therefore, seems to possess a prophylactic role. Accumulation of chloroquine or its analogs leads to the dysfunction of several enzymes of these organelles responsible, particularly those for post-translational modification of viral proteins or proteolytic processing.[163] In a news release, the National Institutes of Health, US Department of Health & Human Services has launched a trial of hydroxychloroquine and azithromycin to assess their impact on COVID-19 patients as therapeutic agent (https://www.nih.gov/news-events/news-releases/nih-begins-clinical-trial-hydroxychloroquine-azithromycin-treat-covid-19). The foundation has been laid by a report where hydroxyxchloroquine supplemented with Azithromycin has been found effective in treatment of COVID-19 patients in a random trial.[164] However, in light of recent publications and uncertainties related to the safety and efficacy of chloroquine/hydroxychloroquine, it is worth being careful while using these drugs in medical prescription until further elevated quality randomized clinical trials are available to elucidate their function in the management of COVID-19 (https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—03-june-2020).[165,166] In addition, the α-interferon (e.g., 5 million units by aerosol inhalation twice per day) is also being used. In Italy, a major investigation led by the Istituto Nazionale Tumori, Fondazione Pascale di Napoli is focused on the use of Tolicizumab. It is a humanized IgG1 mAb, directed against the IL-6 receptor that is commonly used in the treatment of rheumatoid arthritis.[167] Preclinical studies suggested that remdesivir (GS5734), an inhibitor of RNA polymerase with in vitro activity against multiple RNA viruses, including Ebola, could be effective in both prophylaxis and treatment of 2019-nCoV infections.[168] This drug was positively tested in a rhesus macaque model of MERS-CoV infection.[169] Camostat mesylate is another molecule identified as inhibitor of TMPRSS2 activity that eventually prevents entry of 2019-nCoV to the host cell.[170-172] Several in silico studies have also given a ray of hope in the search for potential drugs (Figure 9). In an important study to find RdRP inhibitors, Elfiky[173] has reported the efficacy of sofosbuvir, IDX-184, ribavirin, and remdesivir, based on molecular docking studies. In view of many suggestions from clinical practitioners to proceed with symptom management during COVID-19, the role of anti-inflammatory agents cannot be underestimated. Russell et al[174] have concluded in a review that corticosteroids may be used as anti-inflammatories but not in the acute stages of infection. It has further been reported that intravenous administration of vitamin C may also reduce mortality.[175]

11 | CONVALESCENT PLASMA THERAPY

As the development of efficient and safe drugs and prophylactic vaccines against the 2019-nCoV can take longer (about months or years), fast alternative therapies (e.g., convalescent plasma [CP] therapy) are required. The plasma of recovered COVID-19 patients contains specific antiviral antibodies like IgG and IgM against 2019-nCoV, which can neutralize the viruses in newly infected COVID-19 patient. This CP therapy has already been used in treatment of other respiratory infections including SARS, MERS, and the 2009 H1N1 pandemic with satisfactory efficacy and safety.[176] Although, CP therapy is promising, it has not yet been approved for use by FDA as a safe and effective treatment against COVID-19 and is still regulated as an investigational product (https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma). The principal findings of a systematic review on CP therapy conducted by Rajendran et al[94] on the limited scientific data of COVID-19 patients indicates that it appears safe, clinically effective, and seems to reduce mortality. However, well-designed huge multicenter clinical trials are required to be conducted on urgent basis to establish the efficacy of CP therapy against COVID-19 patients. In the present scenario, implementation of CP therapy might require comprehensive planning before its uses (https://www.isbtweb.org/covid-19resources/covid-19-convalescent-plasma-document-library/). The COVID-19 patient usually develops a primary immune response within 10 to 14 days followed by virus clearance. Therefore, in theory, CP therapy may be more effective in the early stage of COVID-19 infection. However, other treatments might have an effect on the relationship between CP and antibody level, including antiviral drugs, steroids, and intravenous immunoglobulin. Recent guidelines of the FDA's and European Commission's recommend a minimum neutralizing antibody titer of 1:160 (i.e., a 1-in-160 dilution of a given unit of plasma has activity against the virus) and 1:320, respectively.[177]

12 | PUBLIC HEALTH AND SOCIETAL ISSUES

Whenever a healthy individual comes in contact with COVID-19 patients and/or any object contaminated by the infected person through coughing/sneezing, they too have the chance to carry the 2019-nCoV. Such eventualities possibly result in community infection, if not quarantined.[3,31] Thus, for the prevention of COVID-19 infection, some rules have been recommended by different medical agencies and governments, such as maintaining social distancing, prohibiting immigration and social gatherings, appropriate screening of infected persons, home quarantines, compulsory usage of masks, and maintaining appropriate distance when going outside the house, handwashing with soap or use of alcohol-based sanitizers, avoiding touching of eyes, nose, and mouth, and appropriate ventilation in rooms.[178-180] Most of these practices are preventive in nature. Therefore, social awareness for prevention of COVID-19 is extremely important in the present scenario. WHO is regularly outlining the public health and social awareness guidelines which are useful for slowing or stopping the spread of COVID-19 at the

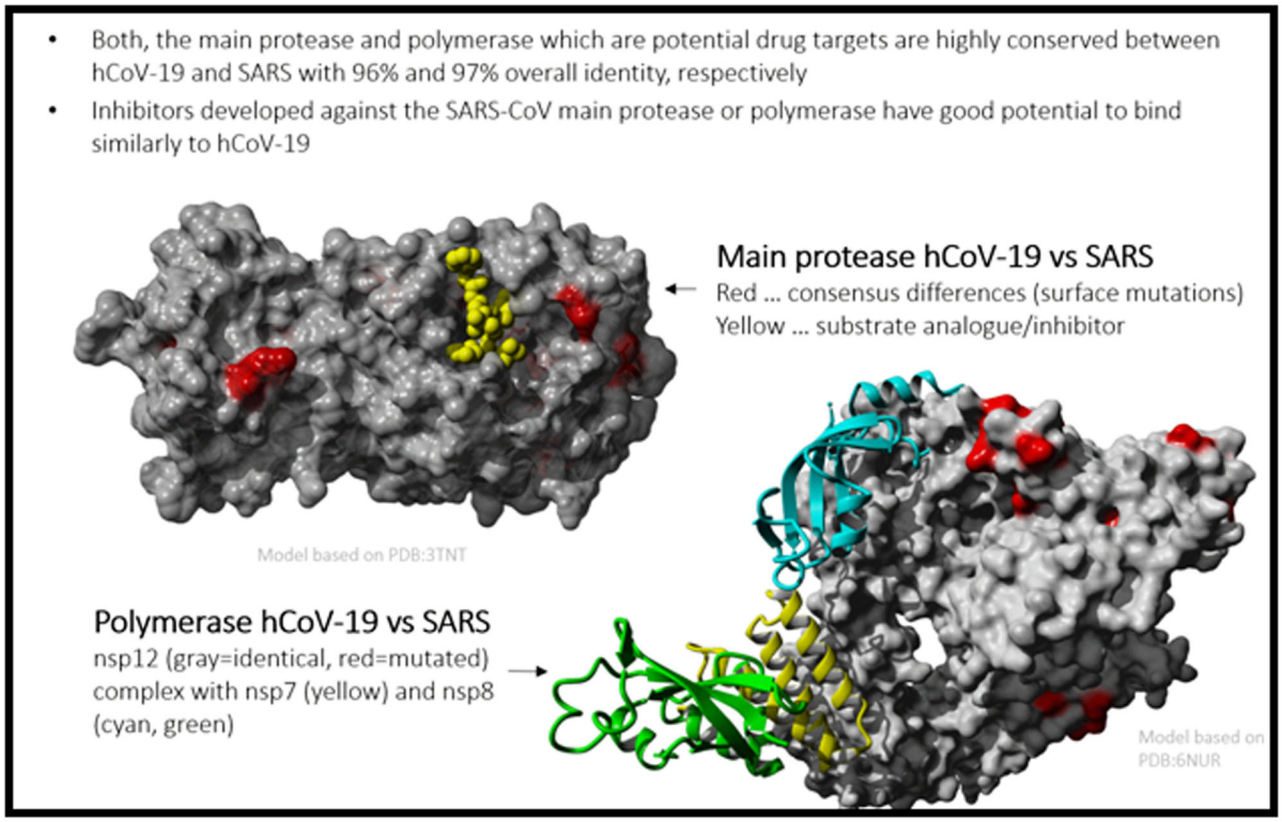

FIGURE 9 Potential drug targets highly conserved between human 2019-nCoV (hCoV-19) and human SARS-COV (SARS) (*Source:* GISAID, https://www.epicov.org/epi3/frontend#lightbox1353460541)

local or community level, based on the data available from different COVID-19-infected nations.[162] The measures to reduce transmission of COVID-19 include individual and environmental measures, detecting and isolating cases, contact tracing and quarantine, social and physical distancing, international travel measures, and vaccines as well as treatments. The treatments are mainly focused on management of life parameters and symptomatic management to avoid worsening of health status. Social and physical distancing measures aim to slow the spread of disease by stopping chains of transmission of COVID-19 while preventing new ones from appearing and have been adopted by most in the suffering zones. Frequent hand washing and appropriate coughing is one of the major health awareness aspects useful in the prevention of COVID-19 spread.[181] Moreover, public health authorities should move against the risk of COVID-19 by promoting spatial distance together with social closeness.[182] Furthermore, some lower- and middle-income countries like African, Asian, and Latin American ones require technical and financial support to successfully respond against COVID-19 by rapidly developing the capacity for testing. Epidemiological reports in China advocate that up to 85% of human-to-human transmission has occurred in family clusters.[183] Based on the data available so far and analysis conducted by Mantovani et al,[184] it has been revealed that COVID-19 infects men and women similarly, but men appear to have a higher risk of death than women due to higher expression of the ACE2 receptor linked with a higher prevalence of smoking. Qazi et al[185] evaluated the influence of information (formal and informal) sources on situational awareness of the public for adopting health-protective behaviors such as social distancing towards COVID-19 using a questionnaire-based survey model. In this study, the information sources, formal ($P = .001$) and informal ($P = .007$) were found to be significantly related to perceived understanding, and furthermore, social distancing is significantly influenced by situational awareness ($P = .000$).

13 | CONCLUSION

- The 2019-nCoV outbreak was declared a Public Health Emergency of International Concern on 30 January 2020 and the disease was named coronavirus disease 2019 (COVID-19) by WHO on 11 February 2020.
- The WHO has formed a group of experts with diverse backgrounds including interdisciplinary scientists, physicians, funders, and manufacturers to accelerate the development of effective diagnostics, vaccines and therapeutics for COVID-19.
- The elucidation of the host-pathogen interaction (morphological and deep structural characterization) performed through electron microscopy techniques (e.g., SEM and TEM) can give

valuable information and may help in developing drugs and vaccines at faster rate.

- Large scale, international, multi-centric, individually randomized controlled clinical trials will facilitate the synchronized evaluation of the benefits as well as risks of each promising drug and vaccine candidate within 1 year.
- Before we get a successful therapy against COVID-19 that may ultimately be helpful in controlling the worldwide pandemic, each person must follow the WHO recommendations and guidelines to prevent the transmission of the novel virus.
- We believe that these coordinated research and development efforts will help to reduce duplication of the work as well as increase the possibility of getting one or more safe and effective therapies for the vulnerable world population.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

SPS: Involved in designing of the study, literature survey, analyzing overall data and finalizing the manuscript. MP: Performed the literature survey and analysed the data related to vaccine candidates. BP: Performed the literature survey and analysed the data related to diagnostics and drug candidates. TPY: Performed the literature survey and analysed the microstructural data of 2019-nCoV.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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