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COVID-19: A Compendium of SARS-CoV-2 Invasion and Host Defense

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ABSTRACT

Corona Virus Disease (COVID-19) caused by novel SARS-CoV-2 has spread like a wildfire, causing respiratory distress in humans at an alarming rate since its inception at Wuhan, China in December 2019. The mortality rate is 2-3%, causing more complications in elderly people, males with pre-existing medical conditions like diabetes, asthma, cardiac anomalies. Self-isolation is the best strategy to effectively contain the virus and prevent the spread. Like the SARS and MERS outbreaks in previous decades, this transmission has also been from animals to humans. However, due to novel alterations in spike protein, SARS-CoV-2 has a high affinity towards host cell Angiotensin Converting Enzyme (ACE2) receptor, which has allowed for its rapid human to human transmission. Therefore, it is of the utmost importance to gain knowledge about the virus in detail and our own immune system proficiency in combating the infection. In the present review, we focus on the genomic and proteomic features of SARS-CoV-2, the infection mechanism and immune response of the host to the virus along with potential therapeutic interventions. Understanding the crux of infection will aid in devising therapeutic strategies to control the COVID-19 pandemic.

Keywords: COVID-19, SARS-CoV-2 entry, Coronavirus pathogenesis, Host immune response, Immunotherapy.

Introduction

COVID-19 (Corona virus Disease 2019) with its epicenter at Wuhan, China has been a catastrophe since December 2019 spreading to 214 countries and has resulted in about 2,21,823 deaths out of total 31,45,407 cases as of May 1, 2020 [1]. It has been announced as a Pandemic on March 11, 2020 by WHO. The causative agent of it is termed as SARS CoV-2 by the International Committee on Taxonomy of Viruses (ICTV) due to its similarity to the SARS outbreak in 2002-2003. The SARS CoV-2 belongs to the *betacoronavirus* genus of *Coronaviridae* family. The major concern with this novel virus infection is its ability to cross species barrier, being rapidly transmitted from animals to humans and then from humans to humans. The bats were found to be the original source of coronovirus in the past outbreaks, from which it

was transmitted to intermediate hosts like the Himalayan palm civet and dromedary camel for SARS-CoV and MERS-CoV respectively before infecting humans[2]. Likewise, it is speculated that SARS CoV-2 also has an animal origin, owing to its inception at Huanan Seafood Wholesale Market of Wuhan, China. The probable modes of transmission of the virus are by respiratory droplets during cough or sneeze and increased levels of aerosol in packed spaces. COVID-19 is characterized by respiratory distress, ranging from mild symptoms like sore throat, lethargy, congestion, fever to severe dyspnea, pneumonia and possibly multi-organ dysfunction or failure in 5% cases[3]. In addition to the respiratory system, few reports suggest its effect on the gastrointestinal, hepatic and central nervous system [4]. Diagnosis of the disease is currently done by RT-PCR based viral RNA detection, NGS in some cases and CT scan where ground glass opacity in lungs shows positivity for SARS-CoV-2 infection.

In order to devise efficient strategies to prevent and treat the COVID-19 outbreak, there is an unmet need to have a detailed understanding about SARS CoV-2, its mode of entry into the human host and the response of the host to the causative agent.

Features of novel SARS-CoV-2

Genome:

The SARS CoV-2 belongs to the *betacoronavirus* genus of *Coronavirinae* subfamily under *Coronaviridae* family and *Nidovirales* order [2].The genome is 29,903 bp long, which shows 87.99% and 80% sequence identities with bat and human SARS-CoV at 99% and 98% coverage respectively. Phylogenetically, it is nearer to the bat-SL-CoV ZC45 and bat-SL-CoV ZXC21 than SARS-CoV, and MERS-CoV. Like other coronaviruses, the genetic material is positive sense RNA which is single stranded with 5' cap and 3' poly-A tail, serving as the mRNA template for synthesis of polyproteins (pp1a/1ab) involved in replication. These polyproteins aid in formation of replication-transcription complex (RTC), which further facilitate synthesis of sub-genomic RNAs (sgRNAs) [5].There are six ORFs in the genome, the first two (ORF 1a/1b) encode for the polyproteins, encompassing two-third of the genome. The polyproteins in turn are processed by chymotrypsin-like protease (3CLpro) or main protease (Mpro), as well as one or two papain-like proteases to form 16 non-structural proteins (nsps). Four main-structural proteins in the particular order: spike (S)-membrane (M)-envelope (E)-nucleocapsid (N) are encoded by the ORFs at the 3'end along with accessory proteins like HE, 3a/b, 4a/b. These proteins encompass the remaining one-third of the genome [6]– [8]. Among different coronaviruses, the non-structural proteins are more conserved, approx. 58% as compared to the 43% identical structural proteins. This implies the dependence of the coronaviruses on structural proteins to adapt in new hosts [5]

Proteome:

The proteome of SARS CoV-2 consists of 9860 amino acids. The non-structural proteins are mostly involved in viral replication and the structural proteins help in virus particle assembly. The heavily glycosylated, 150 kDa Spike protein, organized in trimers is involved in recognition and fusion with the host cell membrane receptor. This protein gives the virus, the crown shaped appearance which lead to its nomenclature [9], [10]. The highly abundant M protein of 25-30 kDa imparts shape and curvature to the viral membrane. The pathogenicity of the virus is imparted by the less structured E protein of about 8-12 kDa. Finally, the highly phosphorylated N protein is involved in packaging of the genome to form virions by interaction with nsp3 proteins and tethering RTC to the genome [11].

The non-structural proteins on association with each other are mostly involved in processing of polypeptides, mRNA degradation, RNA binding, inhibition of IFN signalling, thereby protecting from the host immune system[5]

Mechanism of SARS-CoV-2 infection

Virus attachment:

The first step for entry into the host cell is interaction between the viral S protein and the host cell receptor. The S protein consists of the receptor binding domain (RBD) at the edge of the S1 subunit, involved in receptor recognition and the stalk called the S2 subunit, which is involved in fusion. It is a type 1 membrane fusion protein. Several structural and in-silico docking studies predict Angiotensin converting Enzyme 2(ACE2) to be the receptor for SARS CoV-2 due to 8 conserved residues in the RBD which determine receptor tropism [12]–[14].

Normally, the Spike proteins binds to the host cell surface as a trimer and the RBD can exist in two states- standing up and lying down. Upon binding, conformational changes in the spike protein mediate its cleavage to occur at two sites, one at the S1-S2 interface to separate the two domains and the other within S2 to expose the fusion peptide. This facilitates entry into host cell cytosol and fusion between the host and viral cell membranes by formation of six-helix bundle. The fusion process occurs in the endosomes at low pH [7]. Coutard et al. showed presence of additional cleavage site upstream of the first one in SARS CoV-2 by protease furin, abundant in lungs which might give it a selective advantage for human transmission [15]. In addition to this, the coronavirus entry can also be mediated by antibody dependent non-exosomal pathways as shown by Wan et. al that Mersmab 1 controls MERS-CoV entry in FC-receptor containing cells by causing conformational changes in the spike protein [14]

Phylogenetic analysis reveals significant difference of 16.1% in the minimal RBD of SARS CoV-2 S protein to that of other SARS coronaviruses implying altered specificity of binding and infectivity. On characterizing the RBD, Tai et al. found that the SARS CoV-2 could bind to ACE2 receptors of human and bat origin. Moreover, the novel coronavirus has additional glycosylation sites and antigenic epitopes. Despite sequence variation, the structural heterogeneity of the spike protein is very low which tells us that blocking agents used in previous SARS outbreaks can be effective against the SARS CoV-2 [16]

Viral replication and transcription:

Following entry into the host cell, the polyproteins, which upon cleavage code for the non-structural proteins start to be synthesized by the genomic ORFs. Then, they facilitate assembly and formation of replication-transcription complex which in turn helps in synthesis of genomic RNAs by transcription. The nsps are RNA-dependent RNA polymerases, helicases, exo-ribonucleases and methyltransferases. They also have a role in inhibiting host cell immune system [17].

Virus assembly and release:

Mature virions are assembled following entry into the secretory compartment of endoplasmic reticulum and Golgi complex with encapsulation been provided by the N protein. Protein-protein interactions are mediated by the highly abundant M protein with the help of sparse, E proteins which help in formation of virion like particles. S proteins, though not necessary for assembly are incorporated in this step. M protein also interacts with N protein for completion of this step. After being transported to the host cell surface, the assembled virions are released via exocytosis. The unassembled S proteins mediate host cell recognition and fusion [17]–[19].

Host immune response to SARS-CoV-2

In order to understand the variable outcome of novel Coronavirus disease (COVID19) caused by SARS-CoV-2 and possibly diagnose and treat it in an early stage, it is imperative that we get a clear idea about how the host immune system responds to the virus entry and subsequent infection. The differences in immune

response elicited during recovery and in case of fatality may shed some light on to how the disease can be controlled or modulated.

SARS-CoV-2 like the previous coronavirus infection causing diseases SARS and MERS, has been shown to induce Innate as well as Cell mediated adaptive immune response in patients [20]. Foreign particles like bacteria or viruses are recognized by immune system based on their antigenic pattern presentation called Pathogen Associated Molecular Patterns (PAMP). A viral infection is first recognized by its dsRNA in a host cell by endosomal RNA receptors TLR3, TLR7 [21] and expression of Type I Interferons (IFNα and β) is induced. They act by inhibiting the viral RNA replication in the cell. Soon, humoral responses with production of IgM and IgG take place against the viral surface proteins like protein S in case of SARS-CoV. In a typical virus infection, the virions get cleared by 7th day and CTL activity is established to eliminate the infected cells by cytotoxic activity. Immune responses in case of severe COVID-19 show dysregulation in cytokine production, lymphocyte functioning and sometimes CTL activity.

Cytokine storm:

Most severe cases of SARS-CoV-2 infection are accompanied by an abnormal surge in proinflammatory cytokine production called the "Cytokine Storm" [21]–[23]. This includes secretion of deadly amounts of IL-2, IL-6, IL-7, IL-8, IFN-γ, TNF-α, TGF-β, CXCL8, CCL3, CXCL10, MIPIA, MIPIB from effector immune cells in response to the infection [22]–[24][25]. Although there are no clear reasons yet known for this storm in COVID-19, speculations from correlating studies suggest it to be a result of antibodies produced against Spike protein of SARS-CoV-2 which induce the macrophages and Lymphocytes. Also, the blood characteristics of severe patients show Neutrophilia [21], [26] which may explain high production of chemokines. These highly inflammatory cytokines along with reduced expression of Type-I IFN favour a Th2 type immune response rather than antiviral Th1 type response leading to severe inflammation, tissue damage and ARDS (Acyte Respiratory Distress Syndrome) in sever COVID19 cases [25], [26]. In a rare extreme case, Leukocytosis followed by Leukoerythroblastic reaction was recently reported [27].

Antibody profile and viral load clearance:

Multiple studies with COVID-19 patient sera have reported that humoral response is efficiently elicited in patients with mild as well as severe conditions [28], [29]. A study with a cohort of 23 patients confirmed production and increase in IgM and IgG levels in serum at day 10 after symptom onseot. 100% seroconversion was seen within 3 weeks [30]. Another timeline analysis of blood sample collected over 7 weeks from 34 COVID19 patients indicated an antibody profile similar to that induced by SARS-CoV infection [31]. Both the reports suggested an initial high IgM response staying until approximately week 3 after the onset of symptoms and then declining. While IgG response was initiated later that IgM and lasted longer, even after the recovery in some cases. An independent recent study with a bigger cohort of 173 patients revealed the antibody profile in relation with changing viral load of the patients [29]. Seroconversion rate among patients for total antibody was higher than IgM followed by lowest for IgG. Also, correlating with other studies, Antibody levels increased after first week where viral RNA was highest indicating high probability for transmission [29], [30]. Antibody titre overtook viral RNA during 2nd week and followed the trend. In recovered cases, even after days 15-39 of symptom onset, IgG was detected indicating efficient body response to virus with undetectable viral load. This notion is also supported by a small study conducted on blood serum collected from infants born to COVID19 positive mothers. The samples showed higher positivity for both IgG and IgM than normal along with high IL-16 [32]. Whether these antibodies would give immunity to those infants against SARS-CoV-2 infection is not clear though. This retention of antibodies in recovered patient sera opens an opportunistic window for utilizing it as neutralizing immunotherapy for severe patients where humoral response is impaired as is evidenced by

Antibody neutralization study carried out with 5 COVID19 patient sera on N protein and Spike protein antigens from SARS-CoV and SARS-CoV-2 respectively [28]. However, clearance of viral load is case dependent and can even persist after recovery and resolution of symptoms indicating active possibility of transmission from such patients [33].

Functional exhaustion of Lymphocytes:

SARS-CoV-2 infection is characterized by Lymphopenia and Neutrophilia [20], [22], [26]. In a study with 68 patients out of which 55 had severe disease and 13 showed mild disease, Lymphocytes in total had a drastically reduced count in severe cases than mild cases which was even lower than healthy patients. In the acute phase of the disease, the first immune effector cells against a viral invasion, CD8+ CTL and NK cells showed significantly lower count than healthy samples. Moreover, their functionality was reduced as evidenced by their cytokine profile [26]. These results suggest the dysfunctional CTL activity in acute phase owing to disease progression to severe stages where incidentally, there is a high infiltration of CD8+ T cells observed [22]. This sudden upregulation of dysfunctional CTLs and hyperinflammation as mentioned above in severe phase of COVID19 leads to extensive lung tissue damage, severe pneumonia and ARDS.
In light of these factors, it follows that SARS-CoV-2 infection induces two types of responses in patients. Both starting with the initial acute phase with low IFN-I, low Lymphocytes and high neutrophils soon followed by Antibody response. After the current antiviral treatment initiation, the second phase can either lead to a recovery with viral load clearance as in most cases or a severe pneumonia and ARDS as in cases with co-morbidities like old age, diabetes mellitus, Lung disorder, Heart disorder and kidney disorder.

Therapeutic interventions

The multiple stages of SARS-CoV-2 infection and pathogenesis are being investigated as potential windows for treatment and vaccine development. Currently, though there are no vaccines or FDA approved drugs to combat COVID-19, various anti-viral agents are in pre-clinical and clinical trials, following the guidelines of National Health Commission (NHC). Blocking agents to prevent the viral entry or its replication and immunomodulators are under use.

Strategies to inhibit viral pathogenesis

Neutralizing antibodies: The first step for viral entry involves interaction between viral spike protein and host cell receptor (ACE2) followed by fusion of viral and host cell membranes. Thus, inhibition of this critical step would be of great benefit. In this context, neutralizing antibody against the viral S protein of SARS CoV, CR3022 was found to be potent against SARS CoV-2 as well [34]. However, distinct differences in the RBD of SARS-CoV-2 S protein, necessitate development of new antibodies specific to SARS-CoV-2 [35].In addition, a large randomized clinical trial to see the effect of recombinant ACE2 is in progress. Heptad repeat inhibitors, HR2P and EK1 along with drugs like baricitinib and antimalarial agents like chloroquine, hydroxychloroquine for fusion and viral endocytosis inhibition are being used [36]– [39].
Antiviral compounds: The next strategy being used is inhibition of enzymes involved in viral replication, transcription. Compounds like Ramdesvir blocking RNA-dependant RNA Polymerase, lopinavir and ritonavir to inhibit proteases are in clinical trials [40]. In addition, in-silico protein docking studies predict use of Zanamivir, previously used to treat influenza, Indinavir and Saquinavir against HIV virus can also inhibit SARS CoV-2 $3CL^{Pro}$ main protease[41]. However, presence of new cleavage sites in SARS-CoV2 might render such protease inhibitors ineffective [15]. Very recently, CRISPR-Cas13d system approach to target the SARS CoV-2 RNA genome is also underway [42].

Immunotherapy

Cases in which COVID-19 presents itself as a life-threatening disease, severe acute respiratory syndrome is the causative factor. Immunosuppression by the viral proteins and delayed type hypersensitivity reaction from the host immune system being the deciding factors on whether the patient will follow a path to recovery or severity of the disorder, it is only fitting that immunotherapy is being evaluated as the most potential and sustainable intervention.

Reinforcing innate immunity: An effective and precisely regulated innate immune response from host is necessary to eliminate viral infection. Following the reports with low Type-I IFN production during early phases of COVID-19, exogenous Interferon α treatments are being evaluated [22], [43], [44]. In a study conducted by Zheng et al on 68 COVID19 patients treated with Antiviral therapy and IFN α along with Chloroquine showed significant change in lymphocyte functionality after treatment which correlated with lower viral titre [26].

Convalescent Plasma therapy: Another way of augmenting antiviral immune response of the host is by providing 'Convalescent Plasma'. It is obtained from a donor who has recovered from SARS-CoV-2 infection and is non-symptomatic for at least 14 days prior to donation. The rationale behind this is providing the virus specific antibodies from human host to a critically threatened patient so as to boost the anti-viral response. [43], [45]. This has been shown to be effective *in vitro* where patient sera of 5 recovered patients were shown to be effectively neutralizing SARS-CoV-2 as well as SARS-CoV antigens [28]. However due to the concerns of purity, sensitivity, possibility of blood borne infections, safety and availability, the treatment has only been approved for trial only in patients with life-threatening severity.

Targeting hyperinflammation: The severity of the disease COVID-19 is mostly attributed to hyperinflammation, high cytotoxic T cell infiltration, vasodilation, lung tissue damage and acute respiratory distress syndrome. Therefore, treating patients with the later stages of the disease may require immunomodulation and suppression rather than augmentation. Although general corticosteroids are not recommended due to concerns of worsening the antiviral immunity, many anti-inflammatory drugs targeting a specific molecule or a pathway are being investigated [22], [43], [44]. Patients with SARS-CoV-2 infection have high IL-6 levels in plasma along with high C-reactive protein. Tocilizumab a monoclonal antibody that binds to both soluble and membrane bound receptor of Il-6 is approved for clinical trials [22]. Xu et al in their study on 20 patients treated with Tocilizumab along with antiviral drugs showed that the cytokine levels, CRP levels positively decreased after treatment with Lymphocyte count returning back to normal in more than 50% patients [46]. Cytokine responses involving Th17 and Th2 type generally lead to JAK-STAT pathway activation with nuclear localization of transcription factors like NFkB causing stimulation of effector T lymphocyte proliferation [47]. JAK inhibitors, Baricitinib in particular having an inhibitory effect against GAK and AAK kinases too, has been proposed as a potential therapeutic agent in COVID-19 [25], [48], [49]. Apart from these, other anti-autoimmunity drugs, anti-rheumatoid drugs, anti-TNFα agents like adalimumab are under evaluation for their efficacy in treating SARS-CoV-2 infection. The currently in use antiviral drugs including Chloroquine and Hydroxy-chloroquine both also show some immunomodulatory activity by inhibiting cytokine signalling [22], [43], [50].

In spite of these studies and potential therapeutic strategies, providing an immune-suppressive therapy to patients with severe life-threatening illness will always be a difficult decision to make.

Vaccines

Since the outbreak of first SARS-CoV, there have been tremendous efforts put into developing vaccines against *betacoronaviruses*. Although the vaccines developed against the modifications of S protein of SARS-CoV and MERS-CoV, their efficacy was limited to animal models[51], [52]. Fading antibody titre in the

recovered patients as well as in vaccine trails poses another difficulty in imparting sustainable immunity against coronaviruses. Strategies for SARS-CoV-2 vaccine development are still based on the knowledge from previous CoV infections and bioinformatic tools to predict a potential epitope target for novel CoV [53]– [55]. In one such study, Lucchese and group compared the viral and human proteome and proposed 107 oligopeptides with potential for vaccine testing against SARS-CoV-2 [56]. Development of prophylactic vaccines using biomolecules like nucleic acids, proteins are underway by various biopharmaceutical companies using viral full-length spike protein or S1 subunit harbouring the RBD as the antigen[57]. A multi-dimensional approach for vaccine development is being taken by various companies and research laboratories including mRNA vaccine, recombinant protein-based vaccine, DNA vaccines, live attenuated vaccines (Serum Institute of India) and inactivated virus vaccines [52], [58]. Although a fully functional vaccine for human use takes years for development, recent reports of newer vaccines being approved for clinical trials like the purified inactivated SARS-CoV-2 virus vaccine candidate (PiCoVacc) show promising future[59].

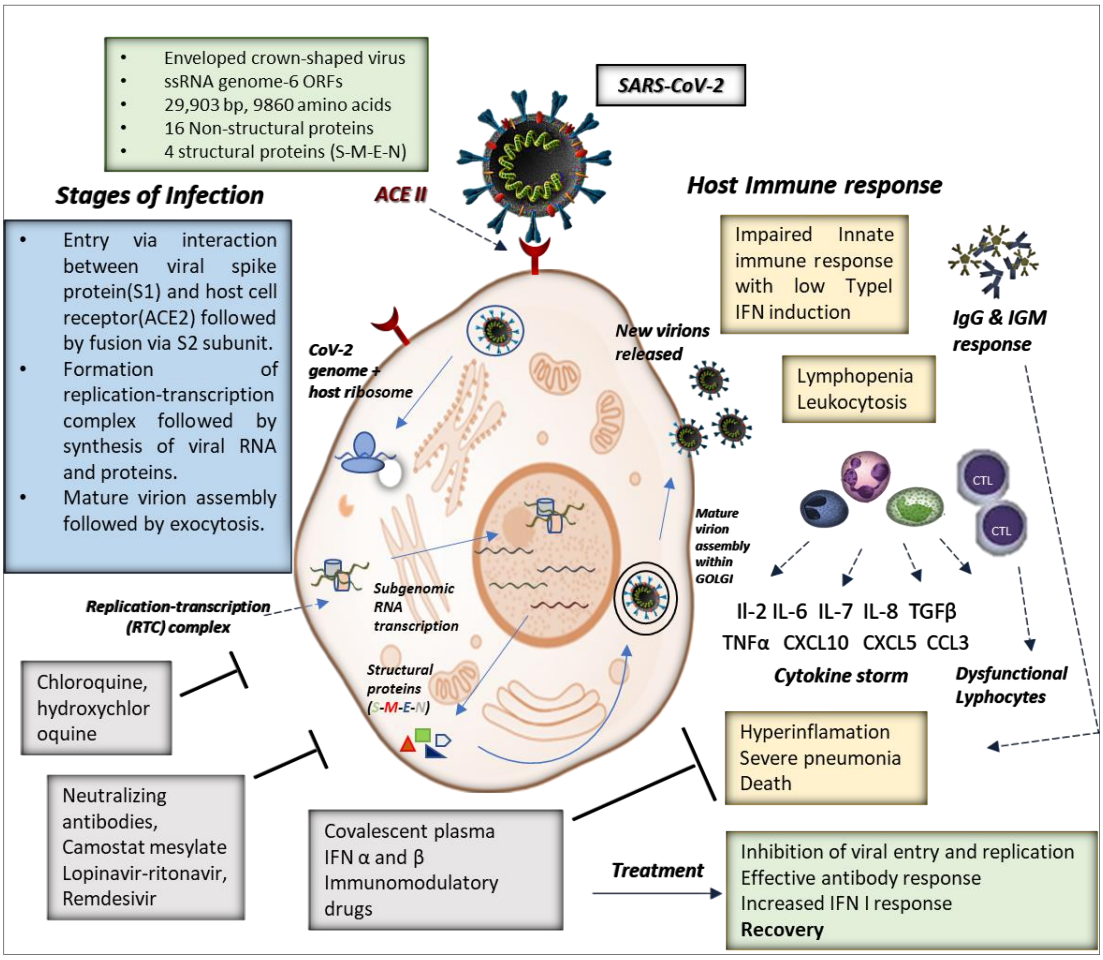

Fig.1 Graphical representation of SARS-CoV-2 fate after entering the host cell

This illustration depicts the stages of SARS-CoV-2 infection, immune response elicited by the body and the potential therapeutic interventions

Conclusion and future perspective

COVID-19 with growing number of cases and deaths each day has emerged to be a world health emergency. Despite similarities to SARS-CoV and MERS-CoV, the novel modifications in the receptor binding domain of S protein, increases the affinity of SARS-CoV-2 to host ACE2 receptor. Hence, even very low viral titre

can cause a havoc [13]. Therefore, primary goal of the health sector worldwide is to increase and implement the right control measures and treat the patients as effectively as possible. However, due to the lack of specific therapy or vaccine against SARS-CoV-2 infection and the prevalence of human to human transmission of the disease, safety of the health workers and management of the disease is still compromised. In context of this, vast amount of resources is being put towards development of potential vaccines and treatment options [60]. Recently, WHO started the randomized clinical trial called 'SOLIDARITY' using a combinatorial approach for application of drugs: Remdesivir, lopinavir and ritonavir; lopinavir and ritonavir + interferon; and chloroquine or hydroxychloroquine which were found to be potent in-vitro [61]. These efforts, current treatments and the potential immunotherapies under clinical trials show a promising future for combating this novel coronavirus. However, testing these vaccines and medications like immunosuppressive agents should be tightly monitored to avoid unwarranted risk of complications and setback during this pandemic [62].

Competing Interests

The authors declare no potential conflicts of interest.

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