COVID-19: Immune Perspective and Current Treatment Strategies

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Abstract

Coronavirus disease 2019 (COVID-19) is now a well-established and a lethal respiratory pandemic, affecting millions with about 10% fatality rate. Infected patients show mild to severe symptoms that may manifest as mild fever, cough, headache and nausea or they may even remain asymptomatic. World Health Organization has reported over 245,373,039 confirmed cases worldwide with 4,979,421 deaths (October 2021). There are myriads of promising approaches to pharmacologically treat the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the newly reported seventh human coronavirus, which is responsible for this pandemic. Various types of potential drugs; monoclonal antibodies, interferon therapis, peptides, small molecule drugs,
oligonucleotides and vaccines are under consideration and target various structural components of
the virus. A strong host immune system is a key player in combating COVID-19 along with the
effective vaccines that are a game-changing tool. The focus of the review is some worth
mentioning drug candidates; remdesivir, lopinavir, emetine, aloxistatin, viracept, homoharringtonine, ivermectin, favipiravir, tocilizumab, chloroquine and leronlimab against
COVID-19 infections, targeting the membrane nucleocapsid, spike or envelope proteins, either
currently in clinical trials or under consideration. Many drugs directly inhibit the viral infection
while others trigger the immune system to fight against the virus. Furthermore, we also discussed
the current covid vaccines; Pfizer, Moderna, etc. from different technical lines for immunization.
Therefore, here we review how the immune system tries to manage the infection as well as
vaccines and some of the potential therapeutic agents.

**Keywords**

SARS-CoV-2; COVID-19; Innate immune response; Adaptive immune response; Therapeutic
drugs; Vaccine.

**Abbreviations**

Acute respiratory distress syndrome (ARDS), Coronavirus (CoV), Coronavirus disease 2019
(COVID-19), C reactive protein (CRP), Endoplasmic reticulum (ER), Granulocyte-colony
stimulation factor (GCSF), Interferon (IFN), Interferon regulatory factor (IRF), Interleukin (IL),
Immunoglobulin (Ig), Interferon stimulated genes (ISGs), Janus kinase-signal transducer and
activator of transcription (JAK-STAT), Macrophage inflammatory protein alpha (MIP1α),
Monocyte chemotactic protein (MCP), the Middle East Respiratory Syndrome (MERS), NOD-like
receptors (NLR), Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκβ), Natural
killer cells (NK), Pattern association molecular patterns (PAMPs), Pattern recognition receptors (PRR), Personal protective equipment (PPE), Severe acute respiratory syndrome-Coronavirus 2 (SARS-CoV-2), Toll like receptor (TLR), Tyrosine protein kinase (TYK), T helper type 1 cell (Th1), World health organization (WHO).
1. Introduction

Coronaviruses (CoVs), the largest RNA enveloped viruses with crown-like spikes belong to the Coronaviridae family [1]. These are important pathogens to humans and vertebrates. To date, six coronaviruses have been reported to cause respiratory diseases including common cold CoVs (NL63, OC43, HKU1 and 229E) and highly pathogenic CoVs (MERS-CoV and SARS-CoV) [2].

Previously, two major epidemics of human coronavirus (HCoV) were reported including Severe Acute Respiratory Syndrome (SARS) in 2002 with 10% fatality rate [3] and Middle East Respiratory Syndrome (MERS) in 2012 with 39% fatality rate [4]. A novel coronavirus, severe acute respiratory coronavirus 2 (SARS-CoV-2), originated in Wuhan, China [5], found in association with coronavirus disease 2019 (COVID-19). This infection is a respiratory syndrome of the lower respiratory tract, characterized by dry cough, fever, tiredness, sore throat, nausea and less frequently diarrhea [6]. Clinically, pneumonia is the preliminary feature, however, RNAemia and acute respiratory distress syndrome (ARDS) are also observed in critical patients.

COVID-19 outbreak was massive and spread over the globe at an alarming rate. In February 2020, the World Health Organization (WHO) declared it as a public health emergency of international concern [7]. WHO reported 245,373,039 confirmed cases with 4,979,421 deaths till October 2021[8]. The incidence of acquiring disease directly correlates with age and associated comorbidities including obesity, diabetes mellitus, cardiovascular diseases, hypertension, pulmonary disorder, or renal disease [9]. Approximately, the percentage of mortality for younger with this disease is approaching to 7% and 55% for patients older than 60 years [10, 11]. A study comprising 140 COVID-19 patients has reported approximately equal chances (1:1 ratio) of disease in males and females [12]. The following review summarizes the structural features,
immune perspective and potential therapeutic treatment strategies against SARS-CoV-2, along with topical status of vaccination against this ailment.

2. SARS-CoV-2; etiology, genomics and morphology

After entry, the incubation period for SARS-CoV-2 ranges between 5 to 7 days, while 14 days were also reported in some patients [13]. COVID-19 could be categorized into mild, moderate, severe and critical diseases. The majority of the patients (81%) represented mild disease which is categorized by mild fever, sore throat, nasal congestion, muscle ache and malaise without radiographic features. Moderate ones mostly possess tachypnea and shortness of breath while severe cases exhibit sepsis and respiratory distress syndrome (ARDS) which is the leading cause of mortality [14]. Patients with the critical condition have shown respiratory failure, cardiac injury, RNAemia, septic shock, or multiple organ dysfunction [15, 16].

Elevated prothrombin time, creatine kinase, C-reactive protein (CRP), D-dimer and ALT with lymphopenia (CD4 and CD8) [15] are the common clinical findings in COVID-19 sufferers. Some severe patients of corona exhibit high leucocyte and neutrophil-lymphocyte ratio (NLR) with a lower percentage of basophils, eosinophils and monocytes [17]. Furthermore, patients with intensive care unit (ICU) exhibit markedly increased profile of interleukin (IL)-6, IL-2, IL-10, IL-8 (chemokine), interferon gamma-induced protein 10 (IP10), granulocyte colony-stimulating factor (GCSF), monocyte chemotactic protein 1 (MCP1), macrophages inflammatory protein alpha (MIP1A) and tumor necrosis factor (TNF)-α [2, 18]. However, COVID-19 non-survivors have shown elevated ferritin, IL-6, neutrophil count, blood urea, D-dimer and creatinine phosphate [19, 20]. Anti-52 kDa S6SA/Ro antibody, anti-60 kDa SSA/Ro antibody and antinuclear antibodies
were also found prevalent in patients. Ground-glass opacity and bilateral patchy shadowing on chest CT-scan are other important clinical findings [21].

SARS-CoV-2 contains the largest positive-sense RNA genome ranges between 26 to 32kb with a 5’-methyl cap and 3’poly-A tail [22]. Full-length genome sequencing of SARS-CoV-2 revealed 88.99% [23] and 79.5% sequence similarity with SARS-like coronaviruses and SARS-CoV, respectively [24]. The genome of the mature virus is associated with phosphorylated nucleocapsid (N) protein and encased in phospholipid bilayer envelope with embedded spike glycoprotein trimer (S), hemagglutinin-esterase, membrane (M) protein and envelope (E) protein as shown in Figure 1 [1]. Among these, E protein increases the pathogenicity as it is involved in the assembly and release of viruses [25]. Besides these structural proteins, different CoVs also synthesize other structural proteins including HE protein, 3a/b protein and 4a/b protein [26].

**Figure 1. Structure of SARS-CoV-2**

The structure is comprised of positive-sense ssRNA encased in the lipid bilayer, having four structural proteins including S-E-M-N.

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S protein homotrimers make up the spikes of SARS-CoV-2 which bind with an angiotensin-converting enzyme (ACE)-2 receptors for host cell entry [20]. Afterward, conformational changes in S protein promote the fusion of the viral envelope with the host cell membrane. The virus inoculates its RNA genome into the cell where it is translated into polyproteins as shown in Figure 2. Viral proteinases break down the polyproteins into smaller protein products. During discontinuous transcription, genomic mRNAs are produced and translated into viral proteins. Genomic RNA and viral polyproteins are assembled in the endoplasmic reticulum (ER) and Golgi apparatus, from where they are released in the extracellular milieu [6].

It has been observed that infection due to corona primarily begins with particular binding of its spike S protein to the cells’ entry receptors, whose tissue distribution and expression impacts the viral pathogenicity and tropism. Worth mentioning examples include; angiotensin-converting enzyme 2 (ACE2; SARS-CoV, SARS-CoV-2 and HCoV-NL63), aminopeptidase N (APN; HCoV-229E) and dipeptidyl peptidase 4 (DPP4; MERS-CoV).

Moreover, to enhance the therapeutic and prophylactic activities against COVID-19, a deeper understanding of the pathobiology of the virus is of immense importance. As mentioned above Receptor Binding Domain (RBD) of the spike (S) protein is used by the SARS-CoV-2 to bind with ACE2 of the host to enter into the human cell [27]. It is the functional receptor of the outbreak and in fact the structural homology and highly genomic similarity among the S proteins of SARS-CoV-2 and previously known SARS-CoV helped to locate the contact residues that interacting with ACE2. It was similar to SARS-CoV and was also found to be conserved not only in SARS-CoV-2 but also in other members of the species of SARS-CoV [28]. Apart from this, priming of the virus S protein demands the cellular transmembrane serine protease2 (TMPRSS2) that is expressed in the respiratory tract of humans and dominantly assists in the entry of pathogens including SARS-
CoV. Non essentially, the entry can also be assisted through lysosomal/endosomal cysteine proteases cathepsin L and B (CTSL, CTSB). Moreover, in vitro analysis revealed that hindrance of all three proteases inhibits the entry of SARS-CoV while in the case of SARS-CoV-2, suppression of TMPRSS2 was sufficient to prevent its entry into primary lung cells and lung cell lines [28].

More recently, a distinct feature of the S protein of SARS-CoV-2 was identified as the presence of polybasic cleavage site (PRRAR) at the S1-S2 boundary. This acquisition is efficiently cleaved by the prototype proprotein convertase furin that overcomes the species barrier and causes successful infection. This may also enhance the transmissibility along with the expansion of zoonotic potential and cell tropism [28]. After attachment and entry into the host cell, coronavirus now proceeds towards the translation of the replicase gene. For the cleavage of replicase polyproteins, the virus encodes a few proteases and to synthesize RNA, a suitable environment is generated by assembling myriads of non-structural proteins (nsps) (that consist of variable enzyme domains and functions) into the replicase-transcriptase complex (RTC) [29]. Furthermore, it has been observed that there are still various accessory and non-structural proteins present in the coronavirus but their functions and characteristics are not fully known. The discovery of their activity will improve the therapeutic targets to inhibit viral replication [29].
Figure 2. The life cycle of SARS-CoV-2 [6]

The life cycle of SARS-CoV-2 begins when S protein binds to the host cell receptor (ACE2). After that viral envelope fuses with the host cell membrane and the virus releases its genomic RNA into the host cell where it is translated into polyproteins which are then broken down into smaller protein products. During discontinuous transcription, sub-genomic mRNAs are produced by polymerases and then translated into viral proteins. In the Golgi apparatus and ER, genomic RNA and viral proteins are assembled into virions, which are then released out of the host cell.

3. Immunology and novel coronavirus (SARS-CoV-2)

The immune system is the key player in combating coronavirus-related infections [1]. Recent studies have suggested that SARS-CoV-2 regulates both, humoral and cellular division of the immune system; suggesting their role in immune-mediated protection [30].
3.1. Innate immune response against SARS-CoV-2

At present, an assessment of immune response effectiveness against SARS-CoV-2 is not completely known. Interferon (IFN) type I, a component of innate division usually develops as an initial response against viral infections via controlling viral replication [31]. Response against the virus is initiated after a successful recognition of its invasion. In the case of RNA viruses like SARS-CoV-2, pathogen-associated molecular patterns (PAMPS) are recognized by endosomal RNA receptors, toll-like receptors (TLR3 and TLR7) and cytosolic RNA receptors like RIG-I. TLRs are mainly involved in the recognition of lipids, lipoproteins, proteins and nucleic acid of invading parasites at the cell membrane, endosomes and lysosomes [32]. Immediately, after recognition, the oligomerization of receptors regulates the downstream signaling cascade including regulatory transcription factors, most importantly the interferon regulatory factors (IRF3) and nuclear factors (NF)-kB [22]. This results in the expression of proinflammatory cytokines and type I IFN as the first line of defense. TLRs, RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs) are the main pattern recognition receptors (PRRs) which activates the downstream pathways for the IFNs production [33]. In response, type I IFN activates the Janus kinase signal transducer and activator of transcription (JAK-STAT) signaling pathway. Afterward, JAK1 and tyrosine kinase 2 (TYK2) phosphorylate the STAT1 and STAT2, which develops a complex with IRF9 to initiate the transcription of IFN stimulated gene (ISGs) [34]. The successful type I IFN ultimately suppresses the viral infection at an early stage as shown in Figure 3.
Figure 3. Host innate immune response against SARS-CoV-2

Attachment of virus to host cell is recognized by TLR3 & TLR7 and after recognition, oligomerization of receptors regulates the downstream signaling cascades including NF-kb and T signaling pathway. Afterward, JAK1 and TYK2 phosphorylate the STAT1 and STAT2, which IRF3 which results in the expression of type 1 IFN. In turn, type 1 IFN activates the JAK-STAT pathway developing a complex with IRF9 to initiate the transcription of IFN stimulated genes (ISGs) that suppress the viral infection.

The figure is made with BioRender (https://app.biorender.com/)

IFNs, the main immunomodulatory molecules, inhibit viral spread by activating macrophages, natural killer (NK) cells and T and B lymphocytes. Structural and non-structural proteins of CoVs modulated IFN response for inhibiting the signaling pathway [1]. The genomic similarity of SARS-CoV-2 with SARS-CoV and MERS-CoV reveals the possibility of a similar mechanism for modulating the type I IFN response to downregulate the innate immune response [31]. Therefore, their inhibition put an imminent danger to the survival of the host [35]. Various cell and animal
models of SARS-CoV-2 infection exhibit a unique but inappropriate pattern of the inflammatory response which is characterized by the low level of type I and II IFNs. However, IL-6 and chemokines markedly increase. This significant reduction in innate antiviral response along with exuberant inflammatory cytokine production is the defining and deriving feature of COVID-19 [36].

Immunocompromised people, because of underlying morbidities including hypertension, cardiovascular disease and diabetes mellitus, are more susceptible to infection [37]. But a clear mechanism for the immune response against SARS-CoV-2 is yet to discover thoroughly.

3.2. Adaptive immune response against SARS-CoV-2

T helper type 1 cells (Th1) play a critical role in the adaptive immune response against viral infections. Among these, CD4⁺ T cells activate B cells for the immune response while CD8⁺ T cells exhibit cytotoxicity by killing virally infected cells [38, 39]. Production of antibodies and humoral immune response play an important role to prevent the cells from reinfection in the future. In contrast to the humoral immune response, the cellular immune response is another mechanism of adaptive immunity. It can be seen inside the infected cells, which is mediated by the T-lymphocytes [40].

SARS-CoV-2 enters the host cell via the ACE2 cell receptor. Attachment of virus is sensed by the endosomal TLR7 as shown in Fig 4 [41]. Activation of TLR7 leads to the production of α-IFN, TNFα, IL-6 and IL-12 which results in the activation of CD8⁺ cytotoxic T cells and CD4⁺ helper T cells. Then these T cells lead to the production of antibodies and antigen-specific B cells as shown in Figure 4. This type of adaptive immunity plays a critical role in combating viral infection. In the case of immunocompromised patients, the body is unable to produce an adequate adaptive
immune response against the virus due to which severe conditions occur that cause damaging attacks to the body e.g. ARDS, cytokine storm, failure of body organs and even death [42]. With aging, the number of naïve T cells shrinks, which means that the ability of the immune system to react against the pathogen also decreases. In contrast, children have a huge population of naïve T cells ready to fight against viruses [43]. This can be one of the explanations for more severe cases of COVID-19 among elders.

For SARS-CoV, seroconversion was seen on the 4th day after the onset of the disease. Neutralizing antibodies and Immunoglobin G (IgG) were reported in patients as long as after 2 years of infection.
[44]. In the case of MERS-CoV, seroconversion was observed after 2 to 3 weeks of the onset of disease [45]. Infected patients showed high production of Immunoglobulin M (IgM) on the 9th day of infection, while on the 2nd week, a peak of Immunoglobulin G (IgG) is observed [23]. Invitro studies have shown that all sera from the patients of COVID-19 were able to neutralize the novel coronavirus [46].

In the case of SARS-CoV, T cell response was highly investigated. According to a report, the response of CD8+ T cells was highly observed against SARS-CoV, while the dominant response of Th1 cells was observed against MERS-CoV [47]. Furthermore, neutrophils also have a destructive role against many infections, but their function remains unanswered in the case of human coronaviruses [31].

Current studies showed that Th1 cell response is a key factor for successful control of SARS-CoV & MERS-CoV and probably true for SARS-CoV-2. Epitopes of B & T cells were extensively investigated for the structural proteins (S-E-M-N) of previously reported HCoVs [45]. If these epitopes can be identified in the case of SARS-CoV-2, it will be beneficial to create passive immunity by using the serum from recovered patients of COVID-19. It will also contribute to making a more effective vaccine against the HCoVs including SARS-CoV-2 [31]. In this regard, a vaccine platform based on DNA was chosen due to its ability to instigate both the humoral and cellular immune response along with its quick design and manufacturing property. Furthermore, investigations suggest that SARS-CoV-2 DNA vaccine candidates can elude the concerns of VAERD or vaccine-associated enhanced respiratory disease that is caused by Th2-bias vaccine modality, as it urges a balanced Th1/Th2 response [48]
4. **Treatment strategies against SARS-CoV-2**

Contemporary analysis and research are continuously inaugurating various treatment strategies for SARS-CoV-2 [49]. As in the present situation, there is no single antiviral therapy for CoVs instead there are only supportive treatments available. According to physicians and guidelines of WHO, the suspected individual should be quarantined in a room, while the confirmed ones should be isolated and cared for in the hospital or a room with personal protective equipment (PPE) [50]. Paracetamol and guaifenesin can be used as first-line treatment for fever and non-productive cough. Immunocompromised patients with other underlying diseases need to be hospitalized and should be treated with available effective drugs. Only a limited number of cases need to be admitted to ICU as soon as possible, due to the critical condition with severe symptoms e.g. shortness of breath [51]. They require immediate oxygen therapy to maintain the process of breathing.

Many of the drugs were proved effective against SARS-CoV and MERS-CoV. As SARS-CoV-2 is also a member of β-CoVs and has genomic similarity with SARS-CoV and MERS-CoV, so there might be a possibility that the same drugs could be effective against COVID-19. Remdesivir is an antiviral that was effective against SARS-CoV & MERS-CoV because it inhibits the transcription of viral RNA at an early stage [17]. Ribavirin is used against many viral infections including SARS & MERS as it inhibits the synthesis of viral RNA inside the host cell [52]. Griffithsin, a broad-spectrum antiviral agent targets the spike protein to inhibit the attachment of the virus to the host cell [53]. It is highly effective against many viruses including human CoVs. Rapamycin, Imatinib and Dasatinib are the antiviral drugs used to block the entry of viruses by inhibiting the kinase signaling pathways [54].
IFNs are the cytokines of innate immune response and can be expressed by various cell types after viral infection. Upon binding to the specific cell surface receptors, they activate the antiviral pathways to limit the spread of the virus [55]. But all the HCoVs can reduce the response of IFNs, so recombinant IFNs including α & β IFN can be the most effective therapeutic agents against SARS-CoV-2 as they induce the innate immune response against many viruses e.g. SARS-CoV [56, 57].

4.1. Direct therapeutic drugs against the virus

Some of the reported therapeutic drugs directly inhibit the viral infection inside the body. They mainly inhibit the viral replication and protein translation inside the host.

4.1.1. Remdesivir

It is an adenosine analog that was previously used against HCoVs including HCoV-229E, SARS-CoV, MERS-CoV and HCoV-OC43 [58, 59]. It inhibits viral replication at an early stage inside the host cell. The enzyme responsible for the RNA replication from an RNA template is known as RNA-dependent RNA polymerase (RdRp). It is also recognized as nsp-12 and is an indispensable part of RNA viruses as plays a crucial role in their life cycle [60]. In the case of SARS-CoV-1, nsp-7 and nsp-8 ensure the processivity of nsp-12 to perform its RNA-synthesizing activity. This tripartite polymerase complex along with nsp-14 performs the proofreading exonuclease activity. It has been found that there exists a 96% sequence similarity between SARS CoV and SARS CoV-2 concerning RdRp [61]. So, agents targeting RdRp of SARS CoV are also expected to cover that of the SARS CoV-2’s [62]. Deeper analysis has revealed that remdesivir (GS-5734) acts as an RNA-dependent RNA polymerase (RdRp) binding substrate and metabolizes into an adenosine nucleotide analog (GS-441524) that further phosphorylates and converts into a nucleoside
triphosphate (NTP) to be taken as a substrate for SARS-CoV RdRp instead of ATP. Incorporation of these NTPs into viral replication machinery causes incompetent elongation. Hence, the substitution of ATP during polymerization causes chain termination without inhibiting the human mitochondrial RNA polymerases as well as human RNA Pol II [63]. Hence, the mechanism of action of the remdesivir—the nucleoside analog is to prevent the replication of the viral genome by targeting the RdRp enzyme [62]. It was previously tested for the treatment of hemorrhagic fever caused by the Ebola virus, but could not perform well during clinical trials [52, 64]. In December 2019, in-vitro experiments were performed to check the efficacy of Remdesivir against SARS-CoV-2, where the drug reduced the viral infection. In January 2020, it was first used successfully in COVID-19 patients [5]. It is currently in the fourth phase of the clinical trial for the treatment of this pandemic [19]. However, it is found to be effective as no side effects have been reported in patients till now.

4.1.2. Lopinavir

It is used with ritonavir and is known as an HIV protease inhibitor, approved by FDA [65]. Proteases aid in viral replication as they can cleave both structural and functional proteins, enabling the maturation of the virus. Lopinavir, a protease inhibitor can effectively inhibit viral replication to limit the spread of the virus [11]. It was used to treat SARS and MERS and found to be effective. But when used along with ritonavir, it showed many side effects in patients. Lopinavir and ritonavir in combination with or without ribavirin are under clinical trials to treat COVID-19. According to another report, lopinavir without ribavirin was effective against SARS-CoV-2 during in-vitro experiments [65].
4.1.3. Emetine

It is a protein synthesis inhibitor that has antiviral activity against many DNA and RNA viruses [66]. These include ebolavirus, rabies virus, zika virus, bovine herpesvirus 1, influenza virus and buffalo poxvirus [67–71]. It was effective to inhibit the replication of β-CoVs including HCoV-OC43, SARS-CoV, MERS-CoV and HCoV-NL63 [72]. Currently, it is under clinical trials for the treatment of COVID-19 [65]. Its general mechanism of action involves the hindrance of ribosomal protein synthesis. Emetine is found to prevent the polypeptide bond incorporation by inhibiting the transfer reaction of aminoacyl-sRNA [63].

4.1.4. Aloxistatin (E-64D)

This is a cysteine protease inhibitor of cathepsins and calpains and performs regulatory functions in cancer therapy and neurodegeneration. In the case of SARS-CoV-2, aloxistatin inhibits the viral entry by 92.3% as cathepsin L is an obligatory factor for coronavirus cell entry. Structural investigation of the drug revealed its interaction with the main protein (Mpro) of SARS-CoV-2, which is dominant for the synthesis of nonstructural proteins (NSP). Hence, it is considered a promising drug candidate to affect viral proteases [63].

4.1.5. Viracept (nelfinavir mesylate)

It is an anti-retroviral drug that is found to primarily inhibit HIV protease. Mechanistically, viracept targets the SARC-CoV-2 spike S glycoprotein and inhibits the S-o and S-n-mediated cell fusion. This indicates the drug's potential to prevent viral spread and transfer from one cell to another along with its enhanced sensitivity towards neutralizing antibodies [63].
4.1.6. Homoharringtonine

It is an alkaloid derived from the plant *Cephalotoxus fortunei*. It has anti-tumor activity and usually inhibits the viral protein translation. In past years, it has been used against the herpes virus, rhabdovirus, murine hepatitis virus, coronaviruses, echovirus 1 and hepatitis B virus [67, 73]. By mechanism, HHT is found to bind and interact with the A-site of peptidyl transferase of the ribosomes and inhibits the translation of proteins [63]. Omacetaxine, a semi-synthetic homoharringtonine is FDA approved drug for the treatment of chronic myeloid leukemia [74]. In vitro experiments showed that it is also effective to reduce the SARS-CoV-2 infection [65].

4.1.7. Ivermectin

This is an FDA-approved drug that is antiparasitic and possesses the potential to be adopted for neglected topical diseases but is still not approved for the treatment of any viral disease. The proposed mechanism of action of ivermectin suggests the inhibition of a crucial intracellular transport procedure that is utilized by the virus to increase the infection through the suppression of the host’s antiviral response i.e. inhibition of the host importin alpha/beta-1 nuclear transport proteins [75]. Analysis of nuclear transport inhibitory activity of ivermectin suggests it is equally effective as well, in the case of SARS-CoV-2 treatment [76].

4.1.8. Favipiravir

It is another promising drug to treat the infection of coronavirus and is a purine nucleic acid analog. Favipiravir is an antiviral candidate according to various clinical trials and is found to be an RNA-dependent RNA polymerase inhibitor. In cells, the phosphorylated form of Favipiravir is proved to be activated (Favipiravir-RTP), which inhibits the polymerase activity of viral RNA [77]. Mechanistically, the medication is not fully known, but studies reveal that it acts as a substrate of
the viral RNA polymerase that leads to the conversion of an active phosphoribosylated state. The drug is believed to obstruct the genomic RNA synthesis of the virus as a chain terminator [78]. It is found to be an RdRp inhibitor with a prior intervention in the Ebola virus and is now indicated to prevent the SARS-CoV-2 replication with an EC$_{50}$ value to be 61.88 µM [62].

4.1.9. Atazanavir/ritonavir

A deeper analysis of the coronavirus has revealed that Main protease (M$^{\text{pro}}$) or the 3-Chymotrypsin like Protease is a proteolytic enzyme that acts pivotally to cleave the replicase polyprotein 1ab in SARS-CoV-2. Orf1ab of the viral RNA genome is responsible for the translation of M$^{\text{pro}}$ that is also known as non-structural protein 5 (nsp5) and consists of 306 amino acids [79]. Furthermore, it has been recognized that there are 12 nsps (nsp4 and nsp6 to nsp16) involved in viral replication and its assembly, M$^{\text{pro}}$ is found to release these 12 nsps by cleaving the replicase polyprotein at 11 specific sites. Primarily, the enzyme is a dimer in structure with three domains on each monomer. M$^{\text{pro}}$ is a promising drug target due to its functional significance in the SARS-CoV-2 life cycle and the presence of distinct recognition sites which are not possessed by human proteases [62]. In this regard, Fintelman-Rodrigues along with co-workers recognized the potential of atazanavir alone and in combination with ritonavir for COVID-19 treatment [80]. Molecular dynamics and molecular docking revealed that atazanavir possesses the potential to target the active site of M$^{\text{pro}}$. Both the drugs were also analyzed for their potential against SARS-CoV-2 in human epithelial pulmonary cell lines and Vero E6 cells. The drugs were found to inhibit the SARS-CoV-2 in the two cell lines vigorously [62].
4.1.10. EK1C4

SARS-CoV-2 mediated cell-cell fusion is potentially inhibited by the derivatives of EK-1 that were designed by covalently linking with palmitic (EK1P) and cholesterol (EK1C) with their IC_{50} values as 69.2 nM and 48.1 nM, respectively. Based on the success, further peptides were created (EK1C1 to EK1C7) with PEG-based spacers and glycine/serine-based linkers (GSG). Among them, EK1C4 is considered as the most potent lipopeptide, having five optimal residues linker/spacer GSGSG-PGE4 and shown to possess potential activity against live SARS-CoV-2 infection and its pseudovirus infection with IC_{50} value to be 36.5 nM and 15.8 nM, respectively. Hence, EK1C4 can be considered as a promising drug candidate against COVID-19 [62].

4.2. Indirect therapeutic drugs against the virus

These drugs help the immune system fight against the virus but do not directly inhibit viral replication. They are more preferred than direct therapeutic drugs as they do not induce resistance to the virus.

4.2.1. Tocilizumab

Tocilizumab (TCZ) is a recombinant humanized monoclonal antibody, directed against soluble and membrane-bounded interleukin-6 (IL-6) receptors [81]. Monoclonal antibodies provide a quick and better protective effect against these viruses as compared to vaccines [1]. TCZ was used for the treatment of many life-threatening cytokine release syndromes including severe rheumatoid arthritis and giant cell arteritis [82]. It is used to treat the severe patients of COVID-19 as it helps to reduce the level of C reactive proteins [83]. Furthermore, treatment with TCZ showed clinical benefits where patients are at high risk of cytokine storm [84]. It is officially approved for the treatment of COVID-19 in many countries including Italy, Pakistan and China [85, 86]. The aim
of using TCZ is to reduce the risk of invasive ventilation or death in a cohort of severe COVID-19 patients.

4.2.2. Chloroquine

It is an aminoquinoline and a broad-spectrum antiviral drug that is widely used as an autoimmune and anti-malarial drug [87]. It is used for the treatment of COVID-19 as it affects the glycosylation of ACE2 cell receptor which is used by the virus to enter the host cell [88]. It also blocks the viral infection by increasing the endosomal pH required for viral cell fusion. It was highly effective against SARS-CoV and now it is potentially used for the treatment of COVID-19 [52, 89]. Some countries have officially approved the use of chloroquine against SARS-CoV-2 [90, 91].

Chloroquine is found to accumulate itself in Golgi-like organelles and those vesicles which are acidic; for instance the endosomes and lysosomes, in its unprotonated state. These destinations ionized the chloroquine which in turn increases their pH and inactivates various proteolytic enzymes. On the other hand, the virus demands the acidic surroundings to enter, replicate, and cause infection in the endosome-lysosome. Hence, an increase in pH can efficiently prevents viral entry and replication [92]. The promising drug is also found to intervene in the interaction between ACE-2 receptors and viral spike proteins that is vital to allow the entry of the virus into the cell, indirectly. The affinity to SARS-CoV-1 S protein is decreased when chloroquine hinders the Golgi-mediated glycosylation of ACE-2 at N-terminal. Therefore, delicate interaction prevents viral entry into the cell [62].

4.2.3. Leronlimab

This is another suggested humanized monoclonal antibody that is found to bind CC-chemokine receptor-5 (CCR5). Finite evidence is present to suggest leronlimab as an optimistic approach to
treat infection by a coronavirus [93] but still not investigated completely to prove its potential to manage the COVID-19 [78].

4.2.4. Camostat

Apart from ACE-2 receptors, SARS-CoV-2 is also assisted by transmembrane protease serine 2 (TMPRSS2), which is encoded by the TMPRSS2 gene and is a host cellular protease. It is predominantly found in the cell membrane of the epithelial lung cells and assists the spike protein priming that fuses the membranes of the cell and that of the virus, which is also considered as the crucial step for viral entry. Recently, this entry is found to prevent by camostat, an inhibitor of TMPRSS2 [94]. It is a serine protease inhibitor and is previously used to treat pancreatitis and was recently found to inhibit the SARS-CoV-2 via TMPRSS2 inhibition. Currently, under clinical trial, camostat might prove to be an efficient drug to tackle COVID-19 [62].

4.2.5. Nafamostat

It is another serine protease inhibitor and acts as an anticoagulant. It was shown to bind to the TMPRSS2-a transmembrane protease and prevents the SARS-CoV-2 entry into the cell with an EC50 value to be 22.50 µM [89]. Nafamostat can also be considered as a therapeutic drug against COVID-19 [62].

4.2.6. E-64-d

This is a cathepsin L inhibitor and assists the camostat to inhibit the entry of SARS-CoV-2 [95]. Cathepsins are primarily cysteine proteases and are of immense importance during the catabolism of protein in lysosomes and endosomes. Cathepsin L is also fundamental for the entry of SARS-CoV into the host cell. Once inside the endosomes, cathepsin L activates the viral spike protein and hence the release of viral genetic material into the cytoplasm after fusion of endosomal
membranes and the virus [62]. E-64-d is a covalent cathepsin inhibitor and is found to contain an epoxide ring that forms the covalent bond at the catalytic site once undergone nucleophilic attack by Cys-SH [96].
### Table 1. Potential therapeutic drugs against SARS-CoV-2

<table>
<thead>
<tr>
<th>Type of therapeutic drugs</th>
<th>Therapeutic drugs</th>
<th>Bioactivity</th>
<th>Clinical phase</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct therapeutic drugs against the virus</td>
<td>Remdesivir</td>
<td>An adenosine analog that inhibits the viral replication</td>
<td>Under 4\textsuperscript{th} phase of the clinical trial against SARS-CoV-2</td>
<td>[5, 58, 59, 89]</td>
</tr>
<tr>
<td></td>
<td>Lopinavir</td>
<td>Works as a protease inhibitor</td>
<td>Approved for HIV infection Under clinical trial for SARS-CoV-2</td>
<td>[52, 65]</td>
</tr>
<tr>
<td></td>
<td>Emetine</td>
<td>Works as a protein synthesis inhibitor</td>
<td>Approved for severe amoebiasis in China Under clinical trial for SARS-CoV-2</td>
<td>[65]</td>
</tr>
<tr>
<td>Homoharringtonine</td>
<td>Inhibit the replication of SARS-CoV-2</td>
<td>Approved by FDA to treat chronic myeloid leukemia</td>
<td>Under clinical trial for SARS-CoV-2</td>
<td>[65, 73]</td>
</tr>
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</tr>
<tr>
<td><strong>Ivermectin</strong></td>
<td>Inhibition of the host importin alpha/beta-1 nuclear transport proteins</td>
<td>Still not approved for the treatment of any viral disease</td>
<td>[75]</td>
<td></td>
</tr>
<tr>
<td><strong>Favipiravir</strong></td>
<td>It is a purine nucleic acid analog</td>
<td>Another promising option</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td><strong>Aloxistatin</strong></td>
<td>It is a cysteine protease inhibitor of cathepsins and calpains</td>
<td>Promising drug candidate</td>
<td>[63]</td>
<td></td>
</tr>
<tr>
<td><strong>Viracept</strong></td>
<td>Targets the SARC-CoV-2 spike S glycoprotein and</td>
<td>Promising drug candidate</td>
<td>[63]</td>
<td></td>
</tr>
<tr>
<td>Indirect therapeutic drugs against the virus</td>
<td>Action</td>
<td>Promising drug candidate</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------</td>
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<td>--------------------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td><strong>Atazanavir/Ritonavir</strong></td>
<td>Inhibits the S-o and S-n-mediated cell fusion</td>
<td>Faheem et al., 2020;[80]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EKIC4</strong></td>
<td>Possess potential activity against live SARS-CoV-2 infection and its pseudovirus infection</td>
<td>[62]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chloroquine</strong></td>
<td>Interferes the mechanism of Glycosylation in cellular receptors</td>
<td>Approved to used against SARS-CoV-2 in some countries [52]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tocilizumab</strong></td>
<td>A monoclonal antibody used to reduce the level of C-reactive protein in severe patients</td>
<td>Approved for the treatment of COVID-19 in some countries [85, 97]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leronlimab</strong></td>
<td>A humanized monoclonal antibody, bind CC-chemokine receptor-5 (CCR5)</td>
<td>Still not investigated completely to prove its potential to manage the COVID-19</td>
<td>[93, 78]</td>
<td></td>
</tr>
<tr>
<td><strong>Camostat</strong></td>
<td>Inhibit the SARS-CoV-2 via TMPRSS2 inhibition</td>
<td>Promising drug candidate</td>
<td>[94, 62]</td>
<td></td>
</tr>
<tr>
<td><strong>E-64-d</strong></td>
<td>Cathepsin L inhibitor</td>
<td>Promising drug inhibitor</td>
<td>[95, 96]</td>
<td></td>
</tr>
<tr>
<td><strong>Nafamostat</strong></td>
<td>Bind to the TMPRSS2</td>
<td>Promising drug candidate</td>
<td>[62, 89]</td>
<td></td>
</tr>
</tbody>
</table>

4.3 Vaccines against the SARS-CoV-2 virus
The COVID-19 pandemic is continued in its journey to conquer all projections and devastations across the globe even after many months of its cradle. Studies reveal the existence of antibodies against coronavirus as the primary stimulus of protection. This makes the interaction of virus either naturally or by vaccination, a crucial element for the occurrence of population immunity to resist the corona pandemic. However, the mechanism of enduring immunity is still not recognized fully [98].

Vaccines appear to be the most auspicious way to alleviate the new viral strains and to reduce the prolonged incubations and outrageous prevalence of COVID-19. The general strategy to design a vaccine involves the antigen, the adjuvant, the manufacturing system and the functioning strategy to deliver it. The protein structure of the virus and its genome sequence was made accessible in a remarkably short duration, to boost the development of attenuated or inactivated viral vaccines and other treatments along with epitope mapping and bioinformatics predictions [99]. Myriads of efforts are on the way to vaccine development with the majority of the candidate vaccines focusing upon the S-protein of SARS-CoV-2 and based on protein sub-unit, live attenuated virus or inactivated one, nanoparticles, viral vector, RNA and DNA, etc., each possessing its obstacles and precedence. Apart from this, researchers are also considering some adjuvants like CpG 1018 (Dynavax) and AS03 (GSK), etc. to escalate the immunogenicity [100]. COVID-19-associated hospitalization was notably reduced due to corona vaccines and hopes are there to observe a significant turn down in post-corona issues and even deaths. Like a shred of evidence, it was observed that adult recipients of Moderna and Pfizer-BioNTech vaccines showed 94% effectiveness in the case of complete vaccination while partial ones received 64% efficacy with 65+ years of age [101].
United Kingdom was the first country in this regard to execute the vaccination program against COVID-19 on 8th December 2020 and approved the emergency use of Pfizer-BioNTech that is an mRNA vaccine. Later on, it was enlarged to take in the Oxford-AstraZeneca as well which is an adenovirus vector vaccine. Initially, vaccination was given to older people of age $\geq 80$, frontline workers and then extended to other age groups [102]. However, some post-corona vaccine adverse effects were observed that include systematic effects like chills, fever, fatigue and headache, etc. and local ones that involve itching, bruising, redness and swelling, etc. Many patients experience some of these clinical symptoms at the injection site that often vanish after a few days. Quit occasionally, if symptoms do not get better then SIRVA—a shoulder injury related to vaccine administration may occur that is a rare condition due to an improper injection technique [103]. Despite that, the worldwide administration of COVID-19 vaccination at a massive scale has decreased the emerging and inauspicious events. Many options for vaccine development are under consideration to target the proteins and virions of the virus [31, 104] as shown in Table 2.
Table 2. Types of vaccines against SARS-CoV-2

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Bioactivity</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA &amp; RNA Vaccine</td>
<td>Targets the S Protein</td>
<td>• Easy manipulation</td>
<td>• Safety issues</td>
<td>[31, 104]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Immunogenic</td>
<td>• Efficient delivery system</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Rapid production</td>
<td>• Efficient delivery system</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Safety issues</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Efficient delivery system</td>
<td></td>
</tr>
<tr>
<td>Viral vector based vaccine</td>
<td>Targets the S Protein</td>
<td>• Excellent clinical and preclinical results</td>
<td>• Negative effect of vector can disturb viral</td>
<td>[31, 104]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Successful against MERS-CoV</td>
<td>effectiveness</td>
<td></td>
</tr>
<tr>
<td>Inactivated vaccine</td>
<td>Targets the whole virion</td>
<td>• Easy process</td>
<td>• Large amount of infectious</td>
<td>[31, 104]</td>
</tr>
</tbody>
</table>
5. Conclusion

In this review, the progressive literature review has been used to summarize the innate and adaptive immune response and the availability of potential therapeutic agents as well as vaccines against SARS-CoV-2. Type 1 Interferon, the innate immunomodulatory molecules, fight against SARS-CoV-2 by inhibiting viral replication at an early stage. However, adaptive immunity is also

| Live attenuated vaccine | Targets the whole virion | • Excellent in induction of T and B cell response  
• Simple process | • Not suitable  
• Risk of reversion to a viral strain  
• Safety issues | [31, 104] |
|------------------------|--------------------------|-------------------------------------------------|-----------------------------------------------|-------|
| Recombinant protein vaccine | Targets the S Protein | • No infectious virus required  
• Adjuvants may be used | • Limited production | [104] |
observed in patients where the production of antibodies and antigen-specific B cells reduces the viral infection. Furthermore, direct and indirect therapeutic drugs are evaluated against SARS-CoV-2. Remdesivir, Lopinavir, Emetine, and Homoharringtonine are the potentially available direct therapeutic drugs that have been used for the treatment of COVID-19. While Chloroquine and Tocilizumab are the indirect therapeutic drugs used to enhance the immune response against the virus. Indirect therapeutic drugs are comparatively more efficient than direct therapeutic drugs as they do not cause drug resistance in the virus. There are various vaccine candidates under the consideration of many research institutes and pharmaceutical companies around the globe to develop an optimum vaccine against COVID-19. Among them, the worth mentioning is the Moderna and Pfizer-BioNTech with an mRNA platform and having a significant success rate of up to 90%. Despite the rapid progress, some inauspicious effects of the vaccination were observed like headache, pain and fatigue. However, the trial assay sensitivity supported the mRNA vaccine. Yet numerous unknowns continue to prosper this devastating pandemic. Undefined virus mutation is the most significant one and if it occurred in the spike protein, would lead to streamlining analysis of genome sequence to perpetuate sustainable vaccine

Conflict of interest

The authors have no conflict of interest.

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