Pathophysiology and Potential Therapeutic Candidates for COVID-19: A Poorly Understood Arena

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Abstract

Coronavirus disease 2019 (COVID-19), an acute onset pneumonia caused by a novel *betacoronavirus*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in the Wuhan City of China in December 2019 and evolved into a global pandemic. To date, there are no proven drugs or vaccines against this virus. Hence, the situation demands an urgent need to explore all potential therapeutic strategies that can be made available to prevent the disease progression and improve patient outcomes. In absence of clinically proven treatment guidelines, several repurposed drugs and investigational agents are currently being evaluated in clinical trials for their probable benefits in the treatment of COVID-19. These include antivirals (remdesivir, lopinavir/ritonavir, umifenovir, favipiravir), interferon, antimalarials (chloroquine/hydroxychloroquine), antiparasitic drugs (ivermectin, nitazoxanide), biologics (monoclonal antibodies, interleukin receptor antagonist), cellular therapies (mesenchymal stem cells, natural killer cells), convalescent plasma, and cytokine adsorber. Though several observational studies have claimed many of these agents to be effective based on their *in vitro* activities and extrapolated evidence from SARS and MERS-CoV epidemics, the currently available data remains inconclusive because of ill-defined patient selection criteria, small sample size, lack of concurrent controls, and use of intermediary outcomes instead of patient-relevant outcomes. Moreover, there is a need to clearly define the patient populations who warrant therapy and also, the timing of initiation of treatment. Understanding the disease pathology responsible for the clinical manifestations of COVID-19 is imperative to identify the potential targets for drug development. This review explains the pathophysiology of COVID-19 and summarizes the potential treatment candidates which can provide guidance in developing effective therapeutic strategies.

1. Introduction

In December 2019, an outbreak of fatal pneumonia caused by a novel *Betacoronavirus* was reported from the Wuhan City of China's Hubei Province. The virus was initially named by World Health Organization (WHO) as 2019-novel Coronavirus (2019-nCoV). However, due to its similarity with Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), the Coronavirus Study Group later renamed the virus as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the disease was designated as Coronavirus Disease 2019 (COVID-19) [1]. It was declared a global pandemic by the WHO on March 11, 2020 [2]. As of July 19, 2020, COVID-19 has affected more than 14 million people in 216 countries and territories, with 597,583 deaths [3]. USA accounts for the maximum number of cases, followed by Brazil, India, and Russia.

Coronaviruses (CoV) are classified into four distinct genera (alpha, beta, gamma, and delta CoV). Human infections are caused by two genera: α-CoV (HCoV-229E and HCoV-NL63) and β-CoV (HCoV-HKU1, HCoV-OC43, Middle East Respiratory Syndrome Coronavirus [MERS-CoV], SARS-CoV, and SARS-CoV-2). The γ- and δ-CoVs infect birds [4,5,6]. Phylogenetic analysis has revealed that the genome of SARS-CoV-2 bears 96.2% sequence homology with a bat coronavirus RaTG13, and shares 79.5% identity with SARS-CoV [7]. Based on genetic sequence and evolutionary analysis, it has been proposed that both Bat-CoV RaTG13 and SARS-COV-2 might be having a common ancestor, and SARS-CoV-2 might have jumped from bats to humans via some unknown intermediate hosts [8]. Despite overwhelming global efforts, COVID-19 remains a

poorly understood disease with limited success in the field of drug development. Understanding the disease pathogenesis is crucial for choosing effective drug targets. This review explains the pathophysiology of COVID-19, and summarizes the potential treatment candidates, which can provide guidance in developing efficient therapeutic strategies.

2. Virus structure and Pathogenesis

SARS-CoV-2 is a positive sense single-stranded RNA virus belonging to the genus β-CoV (subgenus s*arbecovirus*, subfamily *Orthocoronavirinae*). Two-third of the viral genome is located in the first open reading frame (ORF1a/b), which encodes 16 non-structural proteins (NSP1–NSP16). These NSPs collectively form the replication–transcription complex (RTC) that is involved in transcription and replication. NSP3 and NSP5 encode for Papain-like protease (PLP) and Chymotrypsin-like protease (3CL), respectively, which help in peptide cleaving and host innate immune antagonism. NSP12 and NSP15 encode for RNA-dependent RNA polymerase (RdRp) and RNA helicase, respectively. The structural genes encode four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), and several accessory proteins. The spike consists of a transmembrane trimeric glycoprotein that determines diversity to coronaviruses and host tropism. It has two functional subunits- S1, responsible for binding to the host cell receptor and S2, for the fusion of the viral and host cell membranes. M protein helps in transport of nutrients across the cell membrane, bud release and the formation of viral envelope. N and E proteins help in immune evasion by attenuating host immune responses [9].

2.1. SARS-CoV-2 invasion into host cells

The life cycle of SARS-CoV-2 consists of five steps: attachment, penetration, biosynthesis, maturation, and release. Entry of the virus into the host cells is facilitated by angiotensin converting enzyme 2 (ACE2) receptors which are distributed in various organs such as lungs, heart, kidneys, and gastrointestinal tract. ACE2 mediates entry of the virus into the host cells through interaction with a receptor binding domain (RBD) which consists of a core and a receptor binding motif (RBM). RBM specifically recognises human ACE2 as its receptor. ACE2 mediates human-to-human transmission, and also acts as a receptor for SARS-CoV and respiratory coronavirus NL63 [9]. Following binding of the virus to the host protein, the spike protein undergoes a two-step sequential protease cleavage, one at S1/S2 cleavage site for priming and another at S/2 site for activation. The latter activates the spike for membrane fusion via irreversible, conformational changes. Another receptor which has found to be of importance in viral invasion is cluster of differentiation 147 (CD147), also known as Emmprin or Basigin [10]. A characteristic unique to SARS-CoV-2 is the existence of a novel furin cleavage site ("RPPA" sequence) at S1/S2 which confers the ability to infect organs and tissues where furin is ubiquitously expressed such as brain, lung, liver, gastrointestinal tract, and pancreas [11]. Other proteases that may play a role in virus entry are transmembrane protease serine 2 (TMPRSS2) and cathepsin L. Following entry of the virus into the target cells (penetration), the viral mRNA is released in the cytoplasm which then enters the nucleus where it is translated into two polyproteins- pp1a and pp1ab, together comprising the replication-transcription complex (RTC). RTC causes synthesis of subgenomic RNA which encodes for various structural and accessory proteins (biosynthesis). These proteins are assembled to form new virus particles (maturation) which are subsequently released by budding (release).

2.2. Host immune response to SARS-CoV-2

The entry of virus into the host cells triggers stimulation of innate immune response via antigen presenting cells (APCs) e.g. dendritic cells and macrophages which comprise the first line of defence against viruses. APCs have pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs) present at various locations in host cells such as plasma membrane, endosomal membrane, lysosomes, and cytosol. They recognize various structural components of the virus such as nucleic acids, carbohydrate moieties, glycoproteins, lipoproteins and dsRNA and induce a signalling cascade to produce the immune system effectors. The APCs present the viral antigen to the CD8+ T cells in association with MHC class I causing release of IL-12 as a co-stimulatory molecule, further stimulating Th1 cell activation. In addition, there occurs an upregulation of natural killer (NK) cell activation

and production of pro-inflammatory cytokines via the NF-ϰB signaling pathway, which in turn, leads to further recruitment of neutrophils and monocytes to the site of infection and activation of several other pro-inflammatory cytokines. All these events cause an exacerbation of the inflammatory response, thus initiating cytokine release syndrome (CRS) [12] which is characterised by increased secretion of IL-1β, IL-2, IL-6, IL-7, IL-8, IL-9, IL-10, IL-17, granulocyte-macrophage colony stimulating factor (GM-CSF), TNF-α, IFN-γ and IFN-γ inducible protein 10 (IP10), monocyte chemoattractant protein 1 (MCP-1), mcacrophage inflammatory protein- 1 alpha and -1 beta (MIP-1α and -1β) and chemokines CCL2, CCL3, CCL5, CXCL8, CXCL9, and CXCL10. The cytokine storm induces a hyperinflammatory state causing acute lung injury and various complications like acute respiratory distress syndrome (ARDS), respiratory failure, shock, multiorgan failure and death [13,14]. This complex cascade of inflammatory response triggers platelet activation, endothelial dysfunction, and vascular stasis. Recent studies suggest that COVID-19 induces a hypercoagulable state that may predispose the patients to venous thromboembolic events and worsened outcomes. Furthermore, type I IFN forms complex with its receptor, interferon-alpha/beta receptor (IFNAR) leading to activation of Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling pathway. JAK1 and TYK2 kinases phosphorylate STAT1 and 2 and form a complex with IRF9. This complex migrates to the nucleus and initiates the transcription of IFN-stimulated genes (ISGs), which in turn, lead to suppression of viral replication. During the later phase, CD4+ T cells stimulate humoral immune response via activation of T-dependent B cells leading to production of specific antibodies which play a protective role in controlling the infection [14]. The proposed host immune response to SARS-CoV-2 has been shown in Figure 1.

3. Potential therapeutic candidates: Novel virus, novel targets

Currently, there are no clinically proven antiviral drugs or biologics for the treatment of COVID-19 patients. A protocol issued by National Health Commission of the People's Republic of China states that optimized symptomatic management, together with respiratory support should be the mainstay of treatment [15]. Most existing data on antiviral therapy for COVID-19 are derived from related coronaviruses such as SARS-CoV-1 (2003) and MERS-CoV (2012), and non-coronaviruses such as Ebola virus. How well these data can be extrapolated to SARS-CoV-2 remains unclear. Moreover, a lack of pharmacokinetic/pharmacodynamic or clinical data comparing achievable exposures with treatment outcomes, further questions the clinical relevance of *in vitro* activity of antiviral drugs which may vary widely and therefore, should be compared cautiously. Since the onset of this pandemic, several studies emphasizing the therapeutic benefits of a wide range of antiviral drugs and biologics have been published in medical literature. However, a thorough analysis of these drugs is warranted to ascertain whether the existing evidence supports the currently proposed management strategies. An overview of various repurposed and investigational drugs undergoing clinical trials against COVID-19 has been depicted in Figure 2. There are more than 300 ongoing clinical trials, evaluating the safety and efficacy of these drugs. The major proposed therapeutic candidates that seem promising for the treatment of COVID-19 are summarized in Table 1.

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3.1. Antivirals

3.1.1. Remdesivir

Remdesivir (GS-5734; Gilead Sciences, Inc.) is an analogue of adenosine triphosphate which incorporates into the nascent viral RNA chains and results in pre-mature termination of RNA synthesis. It has broad-spectrum antiviral activity against several RNA viruses including Ebola, Marburg, MERS-CoV, SARS-CoV, respiratory syncytial virus (RSV), Nipah virus, and Hendra virus, and has demonstrated prophylactic and therapeutic efficacy against coronaviruses [16]. Use of remdesivir in SARS-CoV-infected mice resulted in reduced viral loads and improved disease outcomes. Recently, the drug has been shown to possess *in vitro* activity against SARS-CoV-2. Remdesivir seems to possess a favourable safety profile, as evidenced in 500 participants, including healthy volunteers and patients who received remdesivir for Ebola virus disease

[17]. Its prophylactic and therapeutic efficacy was demonstrated in a rhesus macaque model of MERS-CoV infection, in which prophylactic administration of remdesivir 24 hours prior to MERS-CoV inoculation, completely prevented clinical disease, inhibited viral replication, and prevented the development of pulmonary lesions. Therapeutic administration of the drug 12 hours post-inoculation reduced the severity of clinical symptoms, attenuated viral replication, and decreased the pulmonary lesions [18]. Gilead sciences, in a recent case series, considered compassionate-use of remdesivir in 53 COVID-19 patients with severe disease, and reported that 68% of the cases showed clinical improvement after a median follow-up of 18 days, with mortality of 13% and a favourable safety profile [19]. The findings were, however, not compared with a control group that received only standard care. At present, there are six ongoing clinical trials evaluating the safety and efficacy of remdesivir in adult patients diagnosed with COVID-19 (moderate/severe disease): two initiated by Gilead Sciences, one by National Institute of Allergy and Infectious Diseases (NIAID), one by INSERM (France), and two by China-Japan Friendship Hospital. All these clinical trials are currently in Phase III. Formal recommendations regarding the use of remdesivir can be made once these trials come up with some conclusive evidence.

3.1.2. Lopinavir/Ritonavir

Lopinavir/ritonavir (LPV/r; Kaletra) is a combination of protease inhibitors used for the treatment of HIV infection. Ritonavir is also a potent inhibitor of cytochrome P450, a class of enzymes responsible for metabolism of lopinavir, and the co-administration augments the plasma levels of lopinavir, improving its antiviral activity [20]. LPV/r has demonstrated in-vitro antiviral activity against SARS-CoV, and MERS-CoV. Since this combination was not specifically formulated for treatment of coronavirus infections, this alone may not demonstrate a significant advantage over placebo in reducing viral load [21]. A clinical trial involving 199 patients with laboratory-confirmed SARS-CoV-2 infection reported that LPV/r combination did not offer any clinical benefit over the standard management [22]. There are several ongoing clinical trials comparing the efficacy of LPV/r alone and in combination with other drugs like umifenovir, carrimycin, danoprevir/ritonavir, interferon, xiyanping, and traditional Chinese medicines. LPV/r in combination with IFN-β1b reduced MERS-CoV viral load and improved lung pathology in a marmoset model [21]. However, Sheahan *et al.* [23] reported that combining LPV/r with IFN-β did not significantly augment the antiviral activity of the latter against MERS-CoV. In an open label clinical trial involving hospitalized SARS patients, LPV/r in combination with ribavirin was found to decrease the mortality rate and requirement of ventilator support, compared to the control group (median, 6 days versus 11 days; 95% CI, -9 to 0) [22]. Thus, considering the therapeutic benefits in the treatment of SARS and MERS, the safety and efficacy of LPV/r based combination regimen in the treatment of COVID-19 needs to be evaluated.

3.1.3. Umifenovir

Umifenovir (Arbidol, Pharmstandard Ltd.) is a fusion inhibitor that interacts with viral hemagglutinin and prevents the fusion of viral envelope with host cell membrane. The drug is currently licensed for use only in Russia and China for the treatment and prophylaxis of influenza and other respiratory viral infections. Umifenovir has a broad-spectrum antiviral activity due to its dual action as direct-acting antiviral and host-targeting agent. It has been found to be active against several enveloped and non-enveloped RNA and DNA viruses, including Chikungunya virus, Zika virus, foot-and-mouth disease virus, Lassa virus, Ebola virus, HSV, HBV, HCV, chikungunya virus, reovirus, Hantaan virus, and coxsackie virus B5 [24,25]. It also inhibits clathrin-mediated exocytosis and intracellular trafficking by interacting with the cell membrane [26]. Considering its unique mechanism of action, umifenovir alone and in combination with antiretroviral drugs is currently being investigated for treatment and prophylaxis of COVID-19. However, a retrospective study by Lian *et al.*, involving 81 COVID-19 patients showed that umifenovir did not shorten the SARS-CoV-2 negativity time or improve the prognosis in non-ICU patients, compared to the supportive treatment [27]. There are currently four ongoing clinical trials of umifenovir for COVID-19 treatment- one in comparison with the basic treatment [28], and the other three comparing the effects of combination with oseltamivir [29], lopinavir/ritonavir [30], and carrimycin [31].

3.1.4. Favipiravir

Favipiravir (T-705, Toyama Chemical Co. Ltd.), a modified pyrazine analogue, is a potent inhibitor of viral RNA dependent RNA polymerase (RdRp) approved in Japan since 2014, for the treatment of oseltamivir-resistant cases of influenza [32]. Besides influenza A and B, it has been found to be effective against avian influenza. It has also been investigated for the treatment of infections caused by Ebola virus, Lassa virus, and now SARS-CoV-2 [33]. Favipiravir is a prodrug that gets metabolized to an active form favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-RTP), which selectively binds to RdRp and inhibits viral replication. In contrast to the existing antivirals against influenza that primarily block the entry and exit of the virus from cells, favipiravir's novel mechanism of action allows its active form to get incorporated into the nascent RNA strand, thus preventing strand elongation and viral proliferation. The drug has an oral bioavailability of 97.6% and is 54% plasma protein-bound with an elimination half-life of 2-5 hours [37]. The RdRp gene of SARS-CoV-2 is structurally similar to that of SARS-CoV and MERS-CoV, as revealed by genome sequencing [4]. A clinical trial (ChiCTR2000029600) conducted in Shenzhen, China reported that COVID-19 patients who received favipiravir demonstrated significantly shorter viral clearance time and higher improvement in chest imaging, compared to the control group (4 days, 91.4% versus 11 days, 62%) [34]. In another multi-centre randomized trial (ChiCTR2000030254), treatment with favipiravir was found to be beneficial for COVID-19 patients with diabetes and/or hypertension as evidenced by decreased time-to-relief for fever and cough. Also, seven days clinical recovery rate increased from 55.9% to 71.4% [35]. These studies indicate that favipiravir can be a safe and effective treatment option for COVID-19. The drug is currently undergoing Phase III clinical trial, which is expected to be completed by July 2020.

3.2. Interferon

Interferons (IFNs) are a family of inducible cytokines produced by various cell types in response to viral infections. IFNs exert their actions through pattern recognition receptors (PRR) which are largely species specific. Of particular interest are the Type 1 IFNs (viral IFNs), which are secreted by the plasmacytoid dendritic cells and are among the first cytokines produced during a viral infection. IFN-I comprises of several subtypes (α, β, ε, ω and ϰ) [36] which exert their actions after binding with IFNAR. Ligand binding induces phosphorylation of the receptor and activation of signal transducers and several transcriptional factors such as signal transducers and activators of transcription (STAT1 and 2). These form complexes that are translocated to the nucleus, where they activate interferon-stimulated genes (ISG). ISGs include PRRs, interferon regulatory factors (IRFs) and members of the JAK-STAT signalling pathway, which sensitize the cell to pathogens and play a prominent role in inflammation, antiviral innate signalling, immunomodulation, and interfere with several steps of viral replication [37]. Thus, IFN-I plays a vital role in antiviral immunity. Because of their immunomodulatory and antiviral properties, they are often evaluated for the treatment of several emerging viral infections. SARS-CoV-2 bears a close resemblance with other members of the *Coronaviridae* family such as MERS-CoV and SARS-CoV and exhibit similar properties, despite differences in their epidemiology, pathology and several of their structural proteins. Numerous *in vivo* and *in vitro* studies have evaluated the role of IFN-I in the treatment of MERS-CoV and SARS-CoV, either alone or in combination with lopinavir/ritonavir [38], ribavirin [39], remdesivir, corticosteroids, and IFN-γ [40]. Though both IFN-α and-β have demonstrated efficacy *in vitro* and succeeded in certain animal models, they failed to improve the disease in humans. Such difference in therapeutic responses could be attributed to IFN signalling pathway used by the viruses, limited number of study subjects, varied experimental settings or clinical conditions, and IFN-subtype diversity. Studies have shown that IFNβ, particularly the β1 subtype (IFNβ1b or IFNβ1a), is a more potent inhibitor of coronaviruses than IFNα and thus appears to be more relevant in the treatment coronavirus infections [41]. In the lungs, IFNβ1 stimulates the secretion of anti-inflammatory adenosine and promotes maintenance of endothelial barrier function by up-regulating CD73 in pulmonary endothelial cells. This can be a possible explanation to the reduction of vascular leakage in ARDS with IFNβ1a treatment [42]. The timing of IFN-I administration plays a critcal role with positive effects being observed early in the course of infection while delayed administration failed to inhibit viral replication [43]. Based on previous knowledge, it has been hypothesized that SARS-CoV and MERS-CoV are able to disrupt the interferon signalling pathway probably through involvement of ORF6 and ORF3b [44]. However, due to the truncated nature of ORF6 and ORF3b proteins in SARS-CoV-2, they may have lost their

anti-interferon activities. This could be a possible explanation for SARS-CoV-2 displaying substantial *in vitro* sensitivity to IFN-I. Thus, IFN-I is expected to be more promising for the treatment of COVID-19 than for SARS [45]. The assumption is further supported by the fact that IFNα2b sprays minimise the infection rate of SARS-CoV-2 and can be used prophylactically against the virus [46]. All these facts support that IFN-I might be a safe and effective treatment against SARS-CoV-2. The knowledge acquired from studies on MERS-CoV or SARS-CoV indicates that for optimum effects and better safety profile, IFN-I should be administered early in the course of infection. In the later phases, the overwhelming inflammatory response caused by massive release of cytokines might call for anti-interferon drugs to mitigate the pathology. In China, the guidelines for the treatment of COVID-19 recommend administration of 5 million units of IFNα by vapor inhalation twice a day, in combination with ribavirin [47]. Vapor inhalation offers the advantage of specifically targeting the respiratory tract. The efficacy of IFN-I can be further improved if given in combination with lopinavir/ritonavir, ribavirin or remdesivir because of the efficacy of such combinations observed *in vitro* against other coronaviruses [23]. Further research on IFN-based treatment is expected in near future, which should give more accurate information on the efficacy of this therapy and possible outcomes.

3.3. Ivermectin

Ivermectin (Stromectol; Merck & Co., Inc.) is a broad spectrum anthelmintic agent belonging to class of avermectins and is derived from the soil bacterium *Streptomyces avermitilis*. It's selective and high affinity binding with glutamate-gated chloride channels in nerve and muscle cells of nematode, increases the permeability of the cell membrane to chloride ions, resulting in hyperpolarization of cells and paralysis and death of the parasite. It is 93.2% plasma protein-bound and has a half life of 18 hours following oral administration. The drug was originally launched by Merck Laboratories in 1987 for use against onchocerciasis (river blindness) as a part of the Onchocerciasis Control Programme in West Africa. Subsequently, the drug was approved for the treatment of a number of human parasitic infections including strongyloidiasis, ascariasis, trichuriasis, enterobiasis, lymphatic filariasis, and scabies in several countries (Australia, France, Japan, the Netherlands, USA, etc) [48]. Besides its anti-parasitic action, several studies have demonstrated the potent antiviral activity of ivermectin against a broad range of viruses *in vitro* [49]. It has been shown to inhibit the interaction between the HIV-1 integrase protein (IN) and the importin (IMP) α/β1 heterodimer, causing inhibition of HIV-1 replication [50]. Ivermectin has also been reported to limit infections caused by several RNA viruses (dengue viruses 1-4, West Nile Virus, Venezuelan equine encephalitis virus and influenza virus) and DNA virus (pseudorabies virus) [49,50]. Studies have found that host cell division may be affected during SARS-CoV infection, due to a signal-dependent nucleocytoplasmic shutting of the viral nucleocapsid protein, involving IMPα/β1 [51,52]. The antiviral activity of the STAT1 transcription factor is blocked by SARS-CoV accessory protein ORF6, which causes sequestration of IMPα/β1 on the rough endoplasmic reticulum/Golgi membrane [53]. Considering ivermectin's inhibitory action on IMPα/β1-mediated nuclear import, it is presumed to be effective against SARS-CoV-2. Caly *et al.* [49] studied the antiviral activity of ivermectin against SARS-CoV-2 and observed that a single treatment with ivermectin was able to cause 5000-fold reduction of virus titre at 48h in Vero/hSLAM cell culture. Ivermectin has a favourable safety profile in humans with high dose therapy considered as safe as the standard low-dose regimen. However, the therapeutic benefits from multiple drug dosing need to be evaluated in COVID-19 patients. An effective antiviral drug given early in the course of infection can help reduce the viral load and prevent disease progression, while limiting person-person transmission. Ivermectin's unique antiviral action combined with a favourable safety profile allows it for further consideration as a possible treatment option in COVID-19.

3.4. Immunomodulators and biologics

3.4.1. Hydoroxycholoquine and azithromycin

Hydroxychloroquine (HCQ) (Plaquenil; Sanofi-Synthelabo Inc.) is an aminoquinoline like chloroquine and is indicated for the treatment of uncomplicated malaria, prophylaxis of malaria in places without chloroquine resistance, chronic discoid lupus erythematosus, systemic lupus erythematosus, and rheumatoid arthritis. In addition, HCQ has been found to be effective against intracellular bacteria such as *Coxiella burnetii* [54]

and *Tropheryma whipplei* [55]. HCQ has also been shown to possess antiviral properties and is already being used in clinical trials for the treatment of HIV infection. It increases endosomal pH which prevents viral fusion and entry into the host cells, inhibits antigen processing and presentation, blocks dimerization of major histocompatibility complex (MHC) class II, and reduces host inflammatory response by decreasing the release of cytokines like IL-1 and TNF-α. HCQ inhibits terminal glycosylation of ACE2 receptor, the main portal of entry for SARS-CoV and SARS-CoV-2. Non-glycosylated ACE2 interacts less efficiently with the viral spike protein, thus preventing viral entry [56]. Several studies have proposed that repurposing of approved drugs such as chloroquine, HCQ, azithromycin, metformin, losartan, and simvastatin could be useful in the treatment of COVID-19. Clinical trials from China have shown the efficacy of chloroquine in the treatment of COVID-19 patients, as evidenced by subsidence of fever, improvement of radiological findings, and delay in disease progression. Azithromycin (AZ) is a macrolide antibiotic that has demonstrated *in vitro* activity against Zika and Ebola viruses [57]. Several authors have mentioned a synergistic effect of HCQ/AZ combination in the treatment of COVID-19. An open label non-randomized clinical trial from France showed that COVID-19 patients treated with 600 mg HCQ daily had a significant reduction in viral carriage at day 6 post-inclusion, with 70% of the patients having a negative PCR test result, compared to only 12.5% in the untreated control group. Moreover, patients who were treated with a combination of HCQ and AZ (500mg on day 1, followed by 250 mg daily for the next four days) showed complete virological cure at day 6 post-inclusion, compared to 57.1% in the group that received HCQ alone [58]. Another study from France claimed that patients who received a combination of HCQ and AZ had a significant clinical improvement as evidenced by a rapid fall in viral load, with 83% tested negative by quantitative PCR on day 7, and 93% on day 8. Virus cultures of respiratory samples were negative in 97.5% patients on day 5 [59]. However, the apparent beneficial effects of HCQ in the treatment of COVID-19 have been completely negated by a pilot study from China where no significant differences in outcomes were observed between HCQ-treated group and the control group [60]. A large observational study in hospitalized COVID-19 patients in the US also showed that treatment with HCQ was not associated with significant clinical benefits and has no influence on intubation or death [61]. Furthermore, the use of HCQ alone or in combination with AZ is not free from hazards. Both these drugs are associated with an increased risk of QT_c prolongation, torsades de pointes, ventricular tachycardias, and gastrointestinal side effects. It has been observed that patients receiving a five-day course of AZ had an increased risk of sudden cardiac death with a hazard ratio of 2.71 [62]. Considering the cumulative adverse effects of HCQ and AZ on cardiac conduction, it is advised to have baseline and follow-up ECG monitoring, along with careful consideration for other concomitant medications known to prolong the QTc interval, if this combination has to be used. Guidelines published by the Infectious Disease Society of America mentioned that despite a higher proportion of clinical improvement in the HCQ group, the beneficial effect of HCQ on viral clearance or disease progression cannot be judged by the currently available evidence due to certain drawbacks such as small sample sizes, ill-defined patient selection criteria, co-interventions, and methodological limitations [63]. Moreover, none of the studies have addressed patient-relevant outcomes like mortality, rate of disease progression to ARDS and need for mechanical ventilation. Also, the mortality rate among patients receiving HCQ/AZ combination was not compared with an untreated cohort. Though studies have claimed that patients receiving HCQ and AZ experienced less virologic failure (43% pooled virologic failure) as compared to historical controls (100% virologic failure) [59,64], such comparison lacks certainty because of unmeasured confounding and selection bias. Furthermore, these studies have relied mainly on intermediary outcomes such as reduction in development of pneumonia, and less hospital or ICU admission to ascertain therapeutic benefits, which raise question on their precision and feasibility. Therefore, a RCT should be the ideal approach for determining the therapeutic effects of HCQ in COVID-19 patients.

3.4.2. Monoclonal antibodies

3.4.2.1. Tocilizumab

The leading cause of mortality in COVID-19 is respiratory failure from ARDS. A cytokine profile resembling secondary hemophagocytic lymphohistiocytosis (HLH), characterized by a fulminant and fatal hypercytokinemia with multiorgan failure, is associated with COVID-19. There is a massive and uncontrolled release of pro-inflammatory cytokines like IL-2, IL-6, G-CSF, IP10, MCP-1, MIP-1-α and TNF-α [12,65]. A

recent retrospective study involving 150 confirmed COVID-19 cases from Wuhan, China, revealed that elevated levels of serum ferritin and IL-6 were independent predictors of fatality, probably due to virally driven hyperinflammation [66]. Tocilizumab (Actemra, Roche) is a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R) approved for the treatment of seriously ill COVID-19 patients with elevated IL-6, by the National Health Commission of China. Xu *et al.* [67] observed the effects of tocilizumab in 21 COVID-19 patients with severe disease, in addition to routine therapy and reported significant therapeutic benefits as evidenced by subsidence of fever and other symptoms within a few days, and improvement of oxygen saturation in 75% of patients. There were no obvious treatment-related adverse reactions. In another report from China, a case of COVID-19 with pre-existing multiple myeloma was successfully treated with tocilizumab, highlighting its potential therapeutic benefits in the treatment of COVID-19 patients [68]. On March 26, 2020, the drug entered Phase III clinical trial for the treatment of COVID-19 pneumonia.

3.4.2.2. Bevacizumab

The main contributory factors for increased mortality in COVID-19 patients are acute lung injury (ALI) and ARDS, brought about by a cytokine-mediated hyperinflammatory response. Pulmonary edema is the key detrimental feature of ALI/ARDS. COVID-19 is associated with more exaggerated pulmonary mucus exudation than SARS as revealed by autopsy [12]. Pulmonary imaging and histopathological examination also support similar findings. However, specific pharmacotherapy to combat this pathology is lacking. Vascular endothelial growth factor (VEGF) is one of the most potent inducers of increased vascular permeability. Bevacizumab (Avastin; Genentech Ltd.) is a recombinant humanized monoclonal antibody targeted against VEGF and is currently recommended for the treatment of malignancies (colorectal, lung, breast, renal, brain, and ovarian), age-related macular degeneration, and diabetic retinopathy. It acts by reducing the elevated VEGF levels secondary to hypoxia and severe inflammation, occurring as a result of infection [69]. All these are presumed subsidence of pulmonary edema in COVID-19 patients. Qilu Hospital of Shandong University, China is conducting two clinical trials of the bevacizumab, both of which are expected to be over by May, 2020. Thus, bevacizumab holds promise as a potential therapeutic option in the treatment of severe COVID-19 patients.

3.4.2.3. Meplazumab

Studies till date recognize angiotensin converting enzyme 2 (ACE2) as the major entry portal for SARS-CoV-2. However, a novel route of viral invasion through direct interaction between the SARS-CoV-2 spike protein and CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN), expressed on epithelial cells has been recently described by Wang *et al.* [10] Meplazumab (Ketantin, Pacific Meinuoke Biopharmaceutical Co. Ltd.) is a humanized IgG2 monoclonal antibody against CD147 that has demonstrated dose-dependent inhibitory action on SARS-CoV-2 replication and virus-induced cytopathic effect *in vitro* [70]. CD147 binds to cyclophilin A (CyPA), a pro-inflammatory cytokine up-regulated in viral infection, and regulates cytokine secretion and leukocyte chemotaxis. Meplazumab is a monoclonal anti-CD147 antibody that inhibits CyPA-induced T-cell chemotaxis and thus, reduces local inflammation. Bian *et al.* [70] studied the effects of meplazumab in 17 hospitalised patients with COVID-19 at Tangdu hospital, China, and reported that meplazumab treatment significantly improved the clinical outcomes in severely ill patients. Also, the time to virus negativity in the meplazumab group was shortened, compared to the control group. These evidences suggest that meplazumab therapy improves the recovery of patients with SARS-CoV-2 pneumonia and has a favourable safety profile. The drug is currently in Phase II clinical trial, which is expected to be completed by December, 2020.

3.4.2.4. Itolizumab

Itolizumab (Alzumab, Biocon Ltd.) is a humanized anti-CD6 IgG1 monoclonal antibody that was introduced in India in 2013 for the treatment of chronic plaque psoriasis. It binds specifically to domain 1 of CD6 and modulates the activation and proliferation of T-cells by CD6 co-stimulation, without interfering with the interaction between CD6 and activated leukocyte-cell adhesion molecule. It inhibits intracellular phosphoproteins like mitogen-activated protein kinase (MAPK) and STAT3 and interferes with CD6-mediated

intracellular signalling pathways and Th17 development. Itolizumab downregulates the transcription of pro-inflammatory cytokine genes and thus, leads to decreased levels of IFN-γ, IL-6, and TNF-α, causing attenuation of cytokine storm and T-cell infiltration [71]. Considering its unique mechanism of action, the drug has been repurposed for the treatment of CRS which is the leading cause of death in COVID-19. A prospective, multi-centric, randomized phase II study conducted on 30 severely ill COVID-19 patients (20 cases and 10 controls) in India showed significant improvement in blood oxygen levels with reduced levels of proinflammatory cytokines and reduced mortality rate in patients who received itolizumab. A similar trial conducted in Cuba, also indicated positive results with 79.2% of the patients discharged from ICU after two weeks of treatment [72]. Itolizumab has been approved by Drugs Controller General of India for the treatment of CRS in moderate to severe ARDS patients with COVID-19.

3.4.3. Anakinra

Anakinra (Kineret; Amgen Inc.) is a recombinant human IL-1 receptor antagonist that competitively inhibits the binding of IL-1α and IL-1β to the high-affinity IL-1 receptor. It is the first biological agent approved for the treatment of rheumatoid arthritis. It is administered through subcutaneous route and has an absolute bioavailability of 95% [73]. In COVID-19 patients, halting the disease progression from manageable hypoxia to frank respiratory failure and ARDS can have a significant impact on patient management and outcomes. Therefore, a therapy directed at intercepting the cytokine storm may be beneficial in this regard. There is an ongoing prospective, randomized, interventional trial comparing the therapeutic effects of individual and simultaneous blockade of IL-6 and IL-1 versus standard care in COVID-19 patients. The trial will include 342 participants whose clinical status after 15 days of treatment will be assessed to measure the effectiveness of anakinra alone and in combination with tocilizumab and siltuximab, in restoring lung homeostasis [74]. The study is estimated to be completed in December 2020. Considering the role of IL-1 in the pathogenesis of acute lung injury in COVID-19, anakinra seems to be a promising therapeutic option in the management of such patients.

3.5. Cellular therapies

3.5.1. Mesenchymal stem cells

Several studies have recognized the potential benefits of cell-based therapies in a number of disease processes including pulmonary, cardiovascular, hepatic, renal, metabolic, and mulculoskeletal disorders. A guideline published by the Italian College of Anesthesia, Analgesia, Resuscitation and Intensive Care has mentioned that stem cells have the potential to decrease ICU admission and curtail the number of ICU days in COVID-19 [75]. Currently, USFDA recommends autologous bone marrow stem cells as the only candidate for stem cell therapy. Mesenchymal stem cells (MSC) have shown benefit in the treatment of musculoskeletal disorders such as low-back pain and spinal injuries. The other stem cells that can be considered for clinical use include adipose, amniotic, and umbilical cord stem cells. Amongst these, umbilical cord stem cells seem to be the more attractive as unlike bone marrow, umbilical cord (Wharton jelly) has a high concentration of MSC which can be extracted noninvasively [76]. Moreover, they have fast doubling times, more plasticity, greater potency and can be efficiently be expanded in the laboratory to cater the large number of expected coronavirus patients [77]. Despite being allogenic, MSCs can evade the host immune system as they express low levels of MHC I, MHC II and T-cell costimulatory molecules, CD80 and CD86, on their surface. At a cellular level, MSCs demonstrate powerful immunomodulatory activity through secretion of anti-inflammatory molecules by paracrine effect and direct interaction with T and B lymphocytes, dendritic cells, macrophages and NK cells. All these may help in attenuating the cytokine storm [78]. They suppress the hyperactive immune system and promote endogenous repair by improving the cellular microenvironment. Multiple studies have demonstrated the beneficial effects of MSCs in the settings of ALI and ARDS. When given intravenously, MSCs accumulate in the lungs and improve lung function by decreasing inflammation, reducing pulmonary endothelial permeability, facilitating alveolar fluid transport, preventing pulmonary fibrosis, and promoting tissue repair. Several clinical trials have documented the safety and efficacy of MSCs in immune-mediated inflammatory diseases, such as graft versus-host disease (GVHD) and autoimmune disorders [79-81]. MSCs secrete antimicrobial peptides and proteins (AMPs) such as cathelicidin LL-37, human beta-defensin-2 (hBD-

2), hepcidin, and lipocalin-2 (Lcn2), and anti-inflammatory molecules such as indoleamine 2,3-dioxygenase (IDO) and interleukin (IL)-17. AMPs cause disruption of membrane integrity, inhibition of protein and nucleic acid synthesis, and blockade of interaction with intracellular targets [82]. MSCs regulate the host immune response by maintaining a dynamic equilibrium between pro- and anti-inflammatory cytokines. There was a concern that SARS-CoV-2 can infect the stem cells and render them ineffective. However, a study of seven COVID-19 patients (one critically ill, four serious and two mild) in Beijing, revealed that SARS-CoV-2 was not able to infect the injected umbilical cord MSCs. All patients who received single dose of stem cell therapy recovered during the 14 days follow-up period, while two out of three patients (with serious disease) who did not receive stem cell therapy (control group) had unfavourable outcomes (one died and one developed ARDS). There was gradual normalization of oxygen saturation and levels of inflammatory biomarkers like CRP, aspartic aminotransferase, creatine kinase and myoglobin in the treated group with no treatment-related adverse events. Follow-up CT scan of lungs showed significant radiological improvement [83]. Thus, MSCs can be a safe and effective treatment option for patients with COVID-19 pneumonia.

3.5.2. Natural Killer cells

Natural killer (NK) cells (large granular lymphocytes) are innate lymphocyte subsets that constitute the frontline defence system against virus infected and tumor cells. They originate in the bone marrow and represent up to 15% of peripheral blood mononuclear cells. NK cells are phenotypically defined by expression of CD56 and absence of CD3, and do not require prior stimulation to perform their effector functions. NK cells display a diverse range of biological activities that are controlled by several inhibitory and activating receptors. The inhibitory receptors recognize self-MHC class I and prevent NK cell activation. In viral infections, there is upregulation of activating receptors and downregulation of MHC class I expression, which cause activation of NK cells. The major activating receptors include cytotoxicity receptors (NKp46, NKp44), C-type lectin receptors and immunoglobulin-like receptors. Among the inhibitory receptors, the killer-immunoglobulin-like receptors and leukocyte inhibitory receptors have prominent role in defence against viral infections. NK cells lack antigen-specific receptors and kill virus-infected cells through the production of cytokines (TNF-α, GM-CSF, CCL5/RANTES and IFN-γ), perforin-granzyme-mediated cellular destruction, and death receptor-mediated cytolysis [84]. Perforin, a pore forming protein, increases the cell permeability which allows granzymes, a family of serine proteases, to enter into the cell and disrupt cell cycle progression, inflict DNA damage and promote karyolysis [85]. They also cause recruitment and activation of other effector cells, including CD8+ T cells and CD4+ Th 1 cells. Patients with deficient NK cell response are predisposed to recurrent viral infections [86]. Currently, the role of NK cells for immunotherapy in infectious diseases is being explored and results seem to be promising. As hunt for new therapeutic options in the treatment of COVID-19 continue to expand, focus has been on the potential benefits of NK cell-based therapy. On 3rd April 2020, USFDA approved the use of CYNK-001, the only cryo-preserved allogeneic NK cell therapy, derived from placental hematopoietic stem cells, in adults with COVID-19. The agent's manufacturer Celularity, a New Jersey-based therapeutic company, in collaboration with Sorrento Therapeutics is about to launch a Phase I/II clinical trial on CYNK-001, involving 86 COVID-19 patients [87]. The therapy is already being tested in patients with acute myeloid leukemia, multiple myeloma, and various solid tumors. In January 2020, Celularity's CYNK-001 was approved by USFDA for treatment of glioblastoma multiforme. Thus, considering the potent antiviral and immunomodulatory properties of NK cells, their efficacy in the treatment of COVID-19 seems promising and needs to be evaluated in clinical trials.

3.6. Convalescent plasma therapy

Convalescent plasma therapy (CPT) is a passive immunization strategy that has been used for the prevention and treatment of several infectious diseases for more than a century. CPT has been successfully used in the treatment of SARS [88], MERS [89], and influenza A H1N1 [90], with satisfactory efficacy and safety profile. A protocol for the use of convalescent plasma (CP) in the treatment of MERS was established in 2015. CPT is associated with a significant reduction in viral load and pooled mortality as revealed in a large meta-analysis on SARS and severe influenza [91]. In 2014, WHO recommended the use of CP as an empirical treatment

for Ebola virus disease during outbreaks [92]. However, CPT did not offer much survival benefit in Ebola virus disease, as data on neutralizing antibody (NAb) titers were not available for stratified analysis. Since SARS-CoV-2 shares virological and clinical similarities with SARS-CoV and MERS-CoV, and NAbs play a crucial role in virus clearance, CPT might hold promise in the treatment of critically ill COVID-19 patients. Patients with a high titer of NAb, after having recovered from COVID-19 may be a valuable donor for CP. It has been observed that the NAbs titers in COVID-19 patients remain low for the first 10 days following disease-onset and tends to increase thereafter, reaching a peak in 12 to 15 days after the onset [93]. USFDA has laid down eligibility criteria for COVID-19 CP donors which include: i) evidence of confirmed COVID-19 documented by a positive nasopharyngeal PCR at the time of illness or a positive SARS-CoV-2 antibody test after recovery, ii) Complete resolution of symptoms at least 28 days prior to donation, or at least 14 days prior to donation and negative results for COVID-19, either from a nasopharyngeal swab specimen or by a molecular diagnostic test from blood, iii) Male/female donors tested negative for HLA antibodies, and iv) SARS-CoV-2 neutralizing antibody titers [?]1:160 [94]. In a study from China, CPT supplemented with supportive care and antiviral agents, was associated with significant clinical and radiological improvement with a rise in neutralizing antibody titers and a fall in C-reactive protein levels within seven days of initiation of treatment. No treatment-related adverse effects were observed [95]. Similar findings were reported by Shen *et al.* [96]. A systematic review on CPT for the treatment of COVID-19 revealed that CPT is safe, effective, and reduces mortality in critically-ill patients [97]. A clinical trial evaluating the benefits of CP in the treatment of COVID-19 is being conducted by Universidad del Rosario, Colombia (NCT04332380), the results of which are expected to be declared by December 2020.

3.7. CytoSorb therapy

CytoSorb (CytoSorbents Corp.) is an extracorporeal cytokine adsorber that acts by removing the circulating cytokines and redirecting the activated neutrophils to the site of infection. This may help in ameliorating cytokine storm that can otherwise trigger uncontrolled systemic inflammatory response, organ failure, and death. CytoSorb offers significant survival benefits in septic shock as observed in several studies. It has been safely used in over 80,000 cases worldwide, primarily in the treatment of several immune-mediated life-threatening conditions such as septic shock, influenza, ARDS, secondary HLH, liver failure, and pancreatitis. CytoSorb helps in protecting endothelial tight junctions, thus reducing capillary leak syndrome. It also modulates pulmonary metabolism, edema formation, and cell-mediated infiltration and injury to the lungs [98]. On April 10, 2020, the USFDA approved emergency use of CytoSorb for the treatment of adult COVID-19 patients admitted to ICU with features of respiratory failure [99]. SARS-CoV-2 can induce a sepsis-like syndrome and in such cases, since pharmacological approaches fail to give promising results, removal of pro-inflammatory cytokines by hemoadsorption through CytoSorb, should be considered. To date, more than 200 critically ill patients with COVID-19 infection have been treated with CytoSorb across various centers in Italy, China and Germany. Based on positive results in Italy, the Brescia Renal COVID Task Force has formally recommended the use of CytoSorb in severe COVID-19 patients with Stage 3 acute kidney injury, receiving Continuous Renal Replacement Therapy (CRRT). CytoSorb therapy has also been recommended by the National Guidelines for the Care of Adult Patients COVID-19, Panama. In addition, the Handbook of COVID-19 Prevention and Treatment, issued by Zhejiang University School of Medicine, China, is also recommending CytoSorb therapy for the management of cytokine storm in critically ill COVID-19 patients [98]. Currently, an ongoing clinical trial (NCT04324528) is investigating the efficacy of CytoSorb in the treatment of patients with severe COVID-19 disease [100]. It is expected to be completed by November 2020.

4. Conclusion

Formulating appropriate treatment strategies for COVID-19 poses a considerable challenge. During pandemics, in absence of clinically proven treatment guidelines, the tendency is to repurpose drugs based on their antiviral and immunomodulatory activities, as evidenced through observational studies. However, such studies have certain drawbacks like lack of concurrent controls, ill-defined patient selection criteria, small sample size without randomization, and use of intermediary outcomes like viral clearance rather than

patient-relevant outcomes. Though several repurposed drugs have shown promising results, and their potential clinical benefits appear to outweigh the relatively minor risk of adverse events, conclusive evidence is lacking. There is a need to clearly define the patient populations who warrant therapy and the timing of initiation of treatment. Since viral loads are highest early in the course of infection and the disease progression can occur rapidly in stable patients, it is rational to consider rapid initiation of therapy in high-risk populations (old age, hospitalized patients, those with underlying diseases and comorbidities), ideally in the context of a well-controlled, randomized clinical trial. Moreover, the demand for unproven therapies can cause shortages of medications that are otherwise indicated for more prevalent diseases like HIV, malaria, hypertension and diabetes mellitus. The IDSA guidelines for treatment of patients with COVID-19 raise concern upon these aspects. In an attempt to generate and disseminate clinical data on an urgent basis, a phenomenal increase in fast-track publications related to COVID-19 has been observed. However, caution should be exercised because the bulk of the available clinical data are often uncontrolled, not peer reviewed, and subject to publication bias (with an intention to publish outstanding results, there may be a tendency to publish positive outcomes and disregard the negative findings). There are several ongoing clinical trials, some with versatile designs that can reasonably explain the therapeutic benefits offered by these drugs in the management of COVID-19. Given the plethora of uncertainties concerning the reliability of existing data and the safety and efficacy of the proposed treatments, it would be wise to wait for the results of clinical trials than to adopt clinically unproven therapies.

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References

1. Velavan TP, Meyer CG. The COVID-19 epidemic. Trop Med Int Health. 2020; 25: 278–80.

2. The New York Times. Coronavirus Live Updates: W.H.O. Declares Pandemic as Number of Infected Countries Grows. The New York Times. Available at https://www.nytimes.com/2020/03/11/world/coronavirus-news.html#link-682e5b06. 2020. Accessed July 13, 2020.

3. World Health Organization (WHO). Coronavirus disease (COVID-19): situation report, 181. Geneva: WHO; 13 July 2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/ (Accessed July 13, 2020).

4. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Napoli RD. Features, Evaluation and Treatment Coronavirus (COVID-19) [Updated 2020 Apr 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/. Accessed July 13, 2020.

5. de Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ. Host Factors in Coronavirus Replication. Curr Top Microbiol Immunol. 2018; 419: 1–42.

6. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses- drug discovery and therapeutic options. Nat Rev Drug Discov. 2016; 15: 327-47.

7. Zheng J. SARS-CoV-2: an Emerging Coronavirus that Causes a Global Threat. Int J Biol Sci. 2020; 16: 1678–85.

8. Yuen KS, Ye ZW, Fung SY, Chan CP, Jin DY. SARS-CoV-2 and COVID-19: The most important research questions. Cell Biosci. 2020; 10: 40.

9. Astuti I, Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes Metab Syndr. 2020; 14: 407-12.

10. Wang X, Xu W, Hu G, Xia S, Sun Z, Liu Z, et al. SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. Cell Mol Immunol. 2020. doi:10.1038/s41423-020-0424-9

11. Wang Q, Qiu Y, Li JY, Zhou ZJ, Liao CH, Ge XY. A Unique Protease Cleavage Site Predicted in the Spike Protein of the Novel Pneumonia Coronavirus (2019-nCoV) Potentially Related to Viral Transmissibility. Virol Sin. 2020. doi: 10.1007/s12250-020-00212-7.

12. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020; 8: 420–2.

13. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis. 2020. doi:10.1093/cid/ciaa248.

14. Cao X. COVID-19: immunopathology and its implications for therapy. Nat Rev Immunol. 2020. doi:10.1038/s41577-020-0308-3.

15. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Mil Med Res. 2020; 7: 11.

16. Gordon CJ, Tchesnokov EP, Feng JY, Porter DP, Götte M. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. J Biol Chem. 2020; 295: 4773–9.

17. Mulangu S, Dodd LE, Davey RT Jr, Tshiani Mbaya O, Proschan M, Mukadi D, et al. A randomized, controlled trial of Ebola virus disease therapeutics. N Engl J Med. 2019; 381: 2293-303.

18. de Wit E, Feldmann F, Cronin J, Jordan R, Okumura A, Thomas T, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. Proc Natl Acad Sci U S A. 2020; 117: 6771–6.

19. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. N Engl J Med 2020. doi: 10.1056/NEJMoa2007016.

20. Molla A, Mo H, Vasavanonda S, Han L, Lin CT, Hsu A, et al. In vitro antiviral interaction of lopinavir with other protease inhibitors. Antimicrob Agents Chemother. 2002; 46: 2249–53.

21. Yao TT, Qian JD, Zhu WY, Wang Y, Wang GQ. A systematic review of lopinavir therapy for SARS coronavirus and MERS coronavirus-A possible reference for coronavirus disease-19 treatment option. J Med Virol. 2020. doi: 10.1002/jmv.25729.

22. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020. doi: 10.1056/NEJMoa2001282

23. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020; 11: 222.

24. Blaising J, Polyak SJ, Pécheur EI. Arbidol as a broad-spectrum antiviral: An update. Antiviral Res. 2014; 107: 84–94.

25. Kadam RU, Wilson IA. Structural basis of influenza virus fusion inhibition by the antiviral drug Arbidol. Proc Natl Acad Sci U S A. 2017; 114: 206–14.

26. Blaising J, Lévy PL, Polyak SJ, Stanifer M, Boulant S, Pécheur EI. Arbidol inhibits viral entry by interfering with clathrin-dependent trafficking. Antiviral Res. 2013; 100: 215–9.

27. Lian N, Xie H, Lin S, Huang J, Zhao J, Lin Q. Umifenovir treatment is not associated with improved outcomes in patients with coronavirus disease 2019: A retrospective study. Clin Microbiol Infect. 2020. https://doi.org/10.1016/j.cmi.2020.04.026.

28. ClinicalTrials.gov [Internet] Identifier NCT04260594. Bethesda (MD): National Library of Medicine (US); 2020 Mar 12. Clinical Study of Arbidol Hydrochloride Tablets in the Treatment of Pneumonia Caused by Novel Coronavirus. Available from: https://clinicaltrials.gov/ct2/show/NCT04260594. Accessed July 13, 2020.

29. ClinicalTrials.gov [Internet] Identifier NCT04255017. Bethesda (MD): National Library of Medicine (US); 2020 Mar 12. A prospective, randomized controlled clinical study of antiviral therapy in the 2019-nCoV pneumonia. Available from: https://www.clinicaltrials.gov/ct2/show/NCT04255017. Accessed July 13, 2020.

30. ClinicalTrials.gov [Internet] Identifier NCT04252885. Bethesda (MD): National Library of Medicine (US); 2020 Mar 12. The efficacy of lopinavir plus ritonavir and arbidol against novel coronavirus infection (ELACOI) Available from: https://clinicaltrials.gov/ct2/show/study/NCT04252885. Accessed July 13, 2020.

31. ClinicalTrials.gov [Internet] Identifier NCT04286503. Bethesda (MD): National Library of Medicine (US); 2020 Mar 12. The clinical study of carrimycin on treatment patients with Covid-19. Available from: https://clinicaltrials.gov/ct2/show/NCT04286503. Accessed July 13, 2020.

32. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci. 2017; 93: 449–63.

33. Du YX, Chen XP. Favipiravir: pharmacokinetics and concerns about clinical trials for 2019-nCoV infection. Clin Pharmacol Ther. 2020. doi: 10.1002/cpt.1844

34. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. Engineering. 2020. doi: 10.1016/j.eng.2020.03.007.

35. Chen C, Huang J, Cheng Z, Wu J, Chen S, Zhang Y, et al. Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial. medRxiv. 2020. doi: 10.1101/2020.03.17.20037432.

36. Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev. 2001; 14: 778–809.

37. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol. 2014; 32: 513–45.

38. Chan JF, Yao Y, Yeung ML, Deng W, Bao L, Jia L, et al. Treatment With Lopinavir/Ritonavir or Interferon-β1b Improves Outcome of MERS-CoV Infection in a Nonhuman Primate Model of Common Marmoset. J Infect Dis. 2015; 212: 1904–13.

39. Omrani AS, Saad MM, Baig K, Bahloul A, Abdul-Matin M, Alaidaroos AY, et al. Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study. Lancet Infect Dis. 2014; 14: 1090–5.

40. Sainz B Jr, Mossel EC, Peters CJ, Garry RF. Interferon-beta and interferon-gamma synergistically inhibit the replication of severe acute respiratory syndrome-associated coronavirus (SARS-CoV). Virology. 2004; 329: 11–7.

41. Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects. PLoS Med. 2006; 3: 1525–31.

42. Bellingan G, Maksimow M, Howell DC, Stotz M, Beale R, Beatty M, et al. The effect of intravenous interferon-beta-1a (FP-1201) on lung CD73 expression and on acute respiratory distress syndrome mortality: an open-label study. Lancet Respir Med. 2014; 2: 98–107.

43. Channappanavar R, Fehr AR, Zheng J, Wohlford-Lenane C, Abrahante JE, Mack M, et al. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. J Clin Invest. 2019; 130: 3625–39.

44. Kopecky-Bromberg SA, Martínez-Sobrido L, Frieman M, Baric RA, Palese P. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. J Virol. 2007; 81: 548–57.

45. Lokugamage KG, Schindewolf C, Menachery VD. SARS-CoV-2 sensitive to type I interferon pretreatment. BioRxiv. 2020. doi: 10.1101/2020.03.07.982264.

46. Shen KL, Yang YH. Diagnosis and treatment of 2019 novel coronavirus infection in children: a pressing issue. World J Pediatr. 2020. doi: 10.1007/s12519-020-00344-6.

47. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther. 2020; 14: 58–60.

48. Ikeda T. Pharmacological effects of ivermectin, an antiparasitic agent for intestinal strongyloidiasis: Its mode of action and clinical efficacy. Folia Pharmacologica Japonica. 2003; 122: 527–38.

49. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res. 2020. doi: 10.1016/j.antiviral.2020.104787.

50. Wagstaff KM. Ivermectin is a specific inhibitor of importin alpha/beta-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. The Biochemical journal. 2012; 443: 851–6.

51. Timani KA. Nuclear/nucleolar localization properties of C-terminal nucleocapsid protein of SARS coronavirus. Virus Res. 2005; 114: 23–34.

52. Wulan WN. Nucleocytoplasmic transport of nucleocapsid proteins of enveloped RNA viruses. Front Microbiol. 2015; 6: 553.

53. Frieman M. Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. J Virol. 2007; 81: 9812–24.

54. Raoult D, Drancourt M, Vestris G. Bactericidal effect of doxycycline associated with lysosomotropic agents on Coxiella burnetii in P388D1 cells. Antimicrob Agents Chemother. 1990; 34: 1512–4.

55. Boulos A, Rolain JM, Raoult D. Antibiotic susceptibility of Tropheryma whipplei in MRC5 cells. Antimicrob Agents Chemother. 2004; 48: 747–52.

56. Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. Int J Antimicrob Agents. 2020; 55: 105923.

57. Bosseboeuf E, Aubry M, Nhan T, de Pina JJ, Rolain JM, Raoult D. Azithromycin inhibits the replication of Zika virus. J Antivirals Antiretrovirals. 2018; 10: 6–11.

58. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020; 105949. doi:10.1016/j.ijantimicag.2020.105949

59. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study. Travel Med Infect Dis. 2020; 101663. doi:10.1016/j.tmaid.2020.101663.

60. Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). J of Zjeijang University. 2020. doi: 10.3785/j.issn.1008-9292.2020.03.03.

61. Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. N Engl J Med. 2020. doi: 10.1056/NEJMoa2012410.

62. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. N Engl J Med. 2012; 366: 1881-90.

63. Bhimraj A, Morgan RL, Shumaker AH, Lavergne V, Baden L, Cheng VCC, et al. Infectious Diseases Society of America Guidelines on the Treatment and Management of Patients with COVID-19, v 1.0.3. Apr.11, 2020. Available at: www.idsociety.org/COVID19guidelines. Accessed July 13, 2020.

64. Molina JM, Delaugerre C, Le Goff J, Mela-Lima B, Ponscarme D, Goldwirt L, et al. No Evidence of Rapid Antiviral Clearance or Clinical Benefit with the Combination of Hydroxychloroquine and Azithromycin in Patients with Severe COVID-19. Med Mal Infect. 2020. doi: 10.1016/j.medmal.2020.03.006.

65. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020; 395: 1033–4.

66. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020. doi: 10.1007/s00134-020-05991-x.

67. Xu XL, Han MF, Li TT, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. ChinaXiv. 2020; 202003: V1.

68. Zhang X, Song K, Tong F, Fei M, Guo H, Lu Z, et al. First case of COVID-19 in a patient with multiple myeloma successfully treated with tocilizumab. Blood Adv. 2020; 4: 1307–10.

69. Wang Y, Fei D, Vanderlaan M, Song A. Biological activity of bevacizumab, a humanized anti-VEGF antibody in vitro. Angiogenesis. 2004; 7: 335–45.

70. Bian H, Zheng ZH, Wei D, Zhang Z, Kang WZ, Hao CQ, et al. Meplazumab treats COVID-19 pneumonia: an open-labelled, concurrent controlled add-on clinical trial. medRxiv. 2020. doi: 10.1101/2020.03.21.20040691.

71. Menon R, David BG. Itolizumab - a humanized anti-CD6 monoclonal antibody with a better side effects profile for the treatment of psoriasis. Clin Cosmet Investig Dermatol. 2015; 8: 215-22.

72. ctri.nic.in [Internet] Identifier CTRI/2020/05/024959. ICMR-National Institute of Medical Statistics: Clinical Trials Registry- India; 2020 May 01. Efficacy and Safety of Itolizumab in COVID-19 Complications. Available at: http://ctri.nic.in/Clinicaltrials/showallp.php?mid1=42878&EncHid=&userName=itolizumab. Accessed July 13, 2020.

73. Cvetkovic RS, Keating G. Anakinra. BioDrugs. 2002; 16: 303–14.

74. ClinicalTrials.gov [Internet] Identifier NCT04330638. Bethesda (MD): National Library of Medicine (US); 2020 Apr 1. Treatment of COVID-19 Patients with Anti-interleukin Drugs (COV-AID). Available from: https://clinicaltrials.gov/ct2/show/NCT04330638. Accessed July 13, 2020.

75. SIAARTI. Pro Vita Contra Dolorem Semper. Raccomandazioni di etica clinica per l'ammissione a trattamenti intensivi e per la loro sospensione, in condizioni eccezionali di squilibrio tra necessità e risorse disponibili - versione 01 Pubblicato il 06.03.2020.

76. Arutyunyan I, Elchaninov A, Makarov A, Fatkhudinov T. Umbilical Cord as Prospective Source for Mesenchymal Stem Cell-Based Therapy. Stem Cells Int. 2016; 2016: 6901286.

77. Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility. World J Stem Cells. 2014; 6: 195-202.

78. Tipnis S, Viswanathan C, Majumdar AS. Immunosuppressive properties of human umbilical cord-derived mesenchymal stem cells: role of B7-H1 and IDO. Immunol Cell Biol. 2010; 88: 795-806.

79. Behnke J, Kremer S, Shahzad T, Chao CM, Bottcher-Friebertshauser E, Morty RE, et al. MSC-based therapies-new perspectives for the injured lung. J Clin Med. 2020; 3: 9.

80. Li D, Liu Q, Qi L, Dai X, Liu H, Wang Y. Low levels of TGF-β1 enhance human umbilical cord-derived mesenchymal stem cell fibronectin production and extend survival time in a rat model of lipopolysaccharide-induced acute lung injury. Mol Med Rep. 2016; 14: 1681-92.

81. Atluri S, Manchikanti L, Hirsch JA. Expanded Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) as a Therapeutic Strategy in Managing Critically Ill COVID-19 Patients: The Case for Compassionate Use. Pain Physician. 2020; 23: E71-E83.

82. Alcayaga-Miranda F, Cuenca J, Khoury M. Antimicrobial activity of mesenchymal stem cells: Current status and new perspectives of antimicrobial peptide-based therapies. Front Immunol. 2017; 8: 339.

83. Leng Z, Zhu R, Hou W. Transplantation of ACE2 Mesenchymal stem cells improves the outcomes of patients with COVID-19 pneumonia. Aging Dis. 2020; 11: 216-28.

84. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001; 22: 633–40.

85. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. 2008; 9: 503–10.

86. Jost S, Altfeld M. Control of human viral infections by natural killer cells. Annu Rev Immunol. 2013; 31: 163–94.

87. Celularity Announces FDA Clearance of Landmark IND for CYNK-001, an Allogeneic, Off-the-Shelf Cyropreserved NK Cell Therapy [news release]. Warren, NJ. Published: January 22, 2020. Available at: businesswire.com/news/home/20200122005061/en/Celularity-Announces-FDA-Clearance-Landmark-IND-CYNK-001. Accessed July 13, 2020.

88. Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis. 2005; 24: 44–6.

89. Ko JH, Seok H, Cho SY, Ha YE, Baek JY, Kim SH, et al. Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single centre experience. Antivir Ther. 2018; 23: 617–22.

90. Hung IF, To KK, Lee CK, Lee KL, Chan K, Yan WW, et al. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin Infect Dis. 2011; 52: 447–56.

91. Mair-Jenkins J, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. J Infect Dis. 2015; 211: 80–90.

92. WHO. Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks. 2014. http://apps.who.int/iris/rest/bitstreams/604045/retrieve. Accessed July 13, 2020.

93. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. MedRxiv. 2020. 10.1101/2020.03.30.20047365.

94. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Recommendations for Investigational COVID-19 Convalescent Plasma. Available at: https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma. Accessed July 13, 2020.

95. Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci U S A. 2020; 117: 9490-6.

96. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. JAMA. 2020. doi:10.1001/jama.2020.4783.

97. Rajendran K, Narayanasamy K, Rangarajan J, Rathinam J, Natarajan M, Ramachandran A. Convalescent plasma transfusion for the treatment of COVID-19: Systematic review. J Med Virol. 2020; 1-9. doi: 10.1002/jmv.25961.

98. COVID-19 and CytoSorb Therapy. Available at: https://cytosorb-therapy.com/en/covid-19/. Accessed July 13, 2020

99. U.S. Food and Drug Administration. FDA has authorized the emergency use of CytoSorb 300 mL device for Emergency Treatment of COVID-19. Available at: https://www.fda.gov/media/136866/download. Accessed July 13, 2020.

100. ClinicalTrials.gov [Internet] Identifier NCT04324528. Bethesda (MD): National Library of Medicine (US); 2020 Mar. 27. Cytokine Adsorption in Severe COVID-19 Pneumonia Requiring Extracorporeal Membrane Oxygenation (CYCOV). Available at: https://clinicaltrials.gov/ct2/show/NCT04324528. Accessed July 13, 2020.