Exploring the pathogenesis and potential therapeutic candidates for COVID-19: A quest into the unknown

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Abstract

COVID-19 is an acute respiratory disease caused by a novel coronavirus SARS-CoV-2 that emerged in the Wuhan City of China in December 2019 and evolved into a global pandemic, resulting in unprecedented morbidity and mortality. 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. With growing knowledge regarding the pathogenesis of COVID-19, several repurposed drugs and investigational agents are currently being evaluated in clinical trials for their probable benefits in the treatment of this condition. Though several observational studies have claimed some of these drugs to be effective based on *in vitro* or extrapolated evidence, 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. This review explains the pathophysiology of COVID-19 and summarizes the potential treatment options which can provide guidance in developing effective therapeutic strategies.

Introduction

In December 2019, several patients in the Wuhan City of Hubei Province, China were diagnosed with a rapidly progressive pneumonia caused by a novel *betacoronavirus* [1]. 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) by WHO [2]. The disease was declared a global pandemic by WHO on March 11, 2020 [3]. As of May 05, 2020, COVID-19 has affected more than 3.5 million people in 215 countries and territories, with 243,540 deaths. USA accounts for the maximum number of cases, followed by Spain, Italy, and France.

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), responsible for mild respiratory infections, and β-CoV (HCoV-HKU1, HCoV-OC43, SARS-CoV, and Middle East Respiratory Syndrome Coronavirus [MERS-CoV]), causing severe and potentially fatal respiratory infections. The γ- and δ-CoV infect birds [4,5,6]. The SARS-CoV-2 is an enveloped, non-segmented, positive-sense RNA virus, belonging to the genus β-CoV (subgenus sarbecovirus, subfamily Orthocoronavirinae). Phylogenetic analysis has revealed that the SARS-CoV-2 genome bears 96.2% sequence homology with a Bat-CoV 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]. This review explains the pathogenesis of COVID-19, and summarizes the current evidence regarding potential treatment options which can provide guidance in developing effective therapeutic strategies.

Virus structure and Pathogenesis

The genome of SARS-CoV-2 encodes for four essential structural proteins i.e. S (spike glycoprotein), E (small envelope protein), M (matrix protein) and N (nucleocapsid protein). S protein mediates entry of the virus into the host cells through interaction with a receptor binding subunit (RBD) that has a core and a receptor binding motif (RBM). RBM specifically recognises human angiotensin converting enzyme 2 (hACE2) as its receptor. ACE2 mediates human-to-human transmission, and also acts as a receptor for SARS-CoV and respiratory coronavirus NL63 [9]. It is expressed in type 2 pneumocytes of alveoli, lung parenchyma as well as and enterocytes of small intestine. 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]. The S protein of SARS-CoV-2 contains a novel furin cleavage site which confers the ability to infect organs and tissues where furin is ubiquitously expressed such as brain, lung, liver, gastrointestinal tract, and pancreas, leading to systemic infection [11]. 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 response [9].

Following entry of the virus particle by membrane fusion, viral RNA is released into the cytoplasm which then translates into two polyproteins i.e. pp1a and pp1ab, together forming the replication transcription complex (RTC). RTC causes synthesis of subgenomic RNA which encodes for various structural and accessory proteins. Viral replication initiates hyperinflammatory conditions resulting in activation and recruitment of neutrophils, monocytes and macrophages. Also, there is activation of T helper cells type 1 (Th1) and type 17 (Th17), which exacerbate the inflammatory response by causing release of pro-inflammatory cytokines, thus initiating cytokine release syndrome (CRS) [12]. This is characterised by increased serum levels 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), and mcacrophage inflammatory protein- 1 alpha and -1 beta (MIP-1α and -1β). This 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. Innate immune response is initiated following viral recognition by pathogen associated molecular patterns (PAMPS), as a result of which IFN-I is secreted which helps in controlling viral replication and also induces adaptive immune response (Th1 cells play a major role). During the later phase, activation of humoral immune response leads to production of antibodies which plays a protective role in controlling the infection [14].

Potential therapeutic options: 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 effect, further questions the clinical relevance of *in vitro* activity of antiviral drugs (defined as half-maximal effective concentration [EC50] values). Also, *in vitro* data may vary widely across studies due to potential variability in testing methodologies 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 findings is warranted to ascertain whether the existing evidence supports the currently proposed management strategies. At present, there are more than 300 ongoing clinical trials, evaluating the safety and efficacy of these drugs. The major proposed therapeutic candidates that hold promise for the treatment of COVID-19 are summarized in Table 1.

1. Antivirals

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.

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.

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].

1.4. Favipiravir

To date, no antiviral drugs have been approved for the treatment of COVID-2019. In addition to the agents that hold promise such as remdesivir, lopinavir/ritonavir, ribavirin, interferon-α and hydroxychloroquine sulfate, some clinical trials are right now focusing on RNA dependent RNA polymerase (RdRp) inhibitors. Favipiravir (T-705, Toyama Chemical Co. Ltd.), a modified pyrazine analogue, is a potent inhibitor of viral RdRp. It has been approved for use in Japan since 2014, for the treatment of 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 [32]. 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, 91.43% 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.

2. 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) [36]. Besides its anti-parasitic action, several studies have demonstrated the potent antiviral activity of ivermectin against a broad range of viruses *in vitro* [37]. 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 [38]. 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) [37,38]. 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 [39,40]. 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 [41]. Considering ivermectin's inhibitory action on IMPα/β1-mediated nuclear import, it is presumed to be effective against SARS-CoV-2. Caly *et al.* [37] 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. Interferon

a family inducible cytokines produced types in response to infections. IFNs exert their actions through recognition (PRR) which are largely species specific. Type 1 IFNs (viral IFNs), secreted by the plasmacytoid dendritic cells and are the infection. IFN-I comprises subtypes (α, β, ϰ) [42], which exert their actions after binding with a heteromeric surface receptor, IFNAR. Ligand binding phosphorylation of receptor and activation of signal transducers and several signal transducers and activators of transcription (STAT1 2). These form complexes that are translocated the genes interferon regulatory factors (IRFs) and members of the JAK-STAT signalling pathway, sensitize pathogens play a prominent role in inflammation, antiviral innate signalling, immunomodulation, and several steps of [43]. Thus, IFN-I plays vital their immunomodulatory and antiviral often evaluated for several emerging viral infections. resemblance other family MERS-CoV despite in structural have role SARS-CoV, in [44], [45], corticosteroids, [46]. and animal disease in attributed the study or and IFNβ, β1 of coronaviruses more in the treatment [47]. stimulates the anti-inflammatory promotes function by up-regulating CD73 in pulmonary endothelial cells. can possible ARDS treatment [48]. The administration critcal positive the delayed viral [49]. previous been to the ORF6 and [50]. due to truncated nature of proteins in SARS-CoV-2, they could *in* IFN-I. Thus, is expected to promising the treatment of than [51]. assumption is IFNα2b minimise infection used virus [52]. All these facts support that IFN-I against knowledge SARS-CoV for optimum effects and better safety profile, in the course of infection. later overwhelming inflammatory call anti-interferon the treatment 5 inhalation combination [53]. the of specifically IFN-I can lopinavir/ritonavir, combinations other Clinical evaluating of and and of with treatment the DisCoVeRy launched by of trials, with is alone, remdesivir (NCT04315948). IFN-based near more efficacy this possible outcomes.

4. Immunomodulators and biologics

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 [56,57]. Several studies have proposed that repurposing of approved drugs such as chloroquine, HCQ and 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 [58]. 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 group that received HCQ alone [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]. 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 [61]. However 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. To date, there are two RCTs evaluating the therapeutic efficacy of HCQ and three trials evaluating the efficacy of HCQ/AZ combination therapy on the clinical outcomes of COVID-19 patients. 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 pitfalls such as small sample sizes, ill-defined selection criteria, co-interventions, and methodological limitations [63]. Moreover, the studies failed to address patient-relevant outcomes like mortality, rate of disease progression to ARDS and need for mechanical ventilation. Studies evaluating HCQ/AZ combination therapy did not compare failure of virologic clearance between the treatment group and historical controls. Also, the mortality rate among patients receiving HCQ/AZ combination was not compared with an untreated cohort. Studies have claimed that patients receiving combination therapy with HCQ and AZ experienced less virologic failure (43% pooled virologic failure) as compared to historical controls (100% virologic failure) [61,64]. However, such comparison lacks certainty because of high-risk selection bias. Furthermore, these trials have relied mainly on intermediary outcomes such as reduction in development of pneumonia, and hospital or ICU admission to ascertain therapeutic benefits, which raise question on their precision and feasibility.

4.2. Monoclonal antibodies

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.

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.

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.

5. Cellular therapies

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 [71]. 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 [72]. 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 [73]. 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 [74]. 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 [75-77]. 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 [78]. 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 [79]. Thus, MSCs can be a safe and effective treatment option for patients with COVID-19 pneumonia.

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 [80]. 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 [81]. 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 [82]. 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 [83]. 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.

6. Convalescent plasma

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 [84], MERS [85], and influenza A H1N1 [86], 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 [87]. In 2014, WHO recommended the use of CP as an empirical treatment for Ebola virus disease during outbreaks [88]. 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 [89]. 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 [90]. 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 [91]. Similar findings were reported by Shen *et al.* [92]. A systematic review on CPT for the treatment of COVID-19 revealed that CPT is safe, effective, and reduces mortality in critically-ill patients [93]. 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.

7. 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 [94]. 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 [95]. SARS-CoV-2 can induce a sepsis-like syndrome and in such cases, since pharmacological approaches fail to give promising results, removal of proinflammatory 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

[94]. Currently, an ongoing clinical trial (NCT04324528) is investigating the efficacy of CytoSorb in the treatment of patients with severe COVID-19 disease [96]. It is expected to be completed by November 2020.

Conclusions

Formulating appropriate treatment strategies for patients with 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 in *in vitro* and 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 seem to give promising results, and their potential clinical benefits appear to outweigh the relatively minor risk of adverse events from short-term therapy, the evidence remains inconclusive and is subject to change frequently. 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. However, the therapeutic benefit of such a strategy needs to be weighed against the potential adverse events (that remain ill-defined). Also, 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. Though several clinical trials and institutional protocols recommend 5–7 days of therapy for uncomplicated disease, the duration of therapy in COVID-19 should be carefully monitored and individualized to the patient. In an attempt to generate and disseminate clinical data related to COVID-19 on an urgent basis, a phenomenal increase in fast-track publications has been observed recently. 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 insignificant variables). 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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Author contributions

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