Current Knowledge on the Pathogenesis of and Therapeutic Options against SARS-CoV-2: An Extensive Review of the Available Evidence

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Authors’ contributions

This work was carried out in collaboration among all authors. Author OAA designed the study. All authors participated equally in the literature search and the drafting of the initial manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: By May 16, 2020, the SARS-CoV-2 virus had spread to 188 countries, infecting over 4.6 million people and causing 310,520 fatalities. A major factor responsible for the voracious spread of the virus is the lack of specific therapeutics for treatment. Current efforts have focused on repurposing existing agents with proven antiviral properties for the treatment of SARS-CoV-2. In this review, we discuss the pathogenesis of the virus; the current standard of care and state of knowledge on the antiviral effect of some of the therapeutic options, including Chloroquine/Hydroxychloroquine, Remdesivir, Lopinavir/Ritonavir combination and Convalescent Plasma; and examine the efforts so far towards the development of a vaccine candidate against SARS-CoV-2.

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Main Body: The current standard of care which includes supportive treatment and oxygen therapy are crucial in the treatment of SARS-CoV-2 infection. Convalescent plasma has a strong immunotherapeutic potential for the treatment of both MERS-CoV and SARS-CoV infections, which many clinical studies have shown to be applicable for treating SARS-CoV-2. Remdesivir (GS-5734), an experimental Ebola virus drug effectively inhibited SARS-CoV-2 in-vitro and in-vivo, and has recently been given emergency use authorization by the United States Food and Drug Administration (FDA) following early signs of success in human clinical trials. The therapeutic potential of the Lopinavir/Ritonavir combination has been extensively explored, and though promising; it had no significant effect on viral clearance and has been associated with severe adverse reactions. Chloroquine & Hydroxychloroquine have been shown to effectively inhibit the infection in-vitro and in animal models, and had a significant viral clearance. Most vaccine development efforts remain in Phase I stage of development.

Conclusion: The current state of knowledge about the therapeutic options against SARS-CoV-2 shows great promise, however, more structured clinical studies are needed to provided much needed evidence to support the establishment of proper guidelines of therapy to curb the pandemic.

Keywords: SARS-CoV-2; COVID-19; 2019-nCoV; coronaviruses; clinical trial; therapy.

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<tr>
<td>SARS-COV</td>
<td>Severe Acute Respiratory Syndrome Coronavirus</td>
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<td>SARS-CoV-2</td>
<td>Severe Acute Respiratory Syndrome Coronavirus 2</td>
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<td>MERS-COV</td>
<td>Middle East Respiratory Syndrome Coronavirus</td>
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<td>β-CoV</td>
<td>beta Coronavirus</td>
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<td>ORF</td>
<td>Open Reading Frame</td>
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<td>ARDS</td>
<td>Acute Respiratory Distress Syndrome</td>
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<td>ECMO</td>
<td>Extracorporeal Membrane Oxygenation</td>
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<td>ADE</td>
<td>Antibody Dependent Enhancement</td>
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<td>ACE-2</td>
<td>Angiotensin Converting Enzyme 2</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<td>CFR</td>
<td>Case-Fatality Ratio</td>
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<td>Nabs</td>
<td>Neutralizing Antibodies</td>
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<td>RDV</td>
<td>Remdesivir</td>
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<td>EVD</td>
<td>Ebola Virus Disease</td>
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<td>LPV</td>
<td>Lopinavir</td>
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<td>LPV/R</td>
<td>Lopinavir/Ritonavir Combination</td>
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<tr>
<td>MOI</td>
<td>Multiplicity of Infection</td>
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<tr>
<td>SAO₂</td>
<td>Arterial Oxygen Saturation</td>
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<tr>
<td>PAO₂/FIO₂</td>
<td>Ratio of Partial Pressure of Oxygen to Percentage of Inspired Oxygen</td>
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<tr>
<td>CQ</td>
<td>Chloroquine</td>
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<td>HCQ</td>
<td>Hydroxychloroquine</td>
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<td>SLE</td>
<td>Systemic Lupus Erythematosus</td>
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1. INTRODUCTION

In December 2019, a cluster of novel pneumonia cases were reported in people who had come in contact with the Huanan Seafood Wholesale Market in Wuhan, Hubei Province, China [1]. At this point, the cause of this pneumonia was said to be unknown, however, on January 7, 2020, it was confirmed to be associated with a novel strain of human coronavirus called: Severe Acute Respiratory Syndrome – Coronavirus – 2 (SARS-CoV-2) or 2019-novel coronavirus (2019-nCoV) [2]. By January 30, 2020, the outbreak was declared a Public Health Emergency of International Concern (PHEIC) and on February 11, 2020, the respiratory illness caused by the virus was named the new coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) [3]. By March 11, 2020, the WHO elevated the disease to the level of a pandemic [4], and according to data from the Johns Hopkins University Centre for Systems
Science and Engineering (CSSE) Global COVID-19 map, as at May 16, 2020, the virus had spread to 188 countries and territories, infecting over 4.6 million people and causing 310,520 fatalities [5].

A major factor responsible for the voracious spread of the virus thus far is the lack of approved therapeutic agents for the treatment of SARS-CoV-2 as well as the other two coronaviruses causing disease in humans: the SARS-CoV and the Middle East Respiratory Syndrome – Coronavirus (MERS-CoV). Apart from general supportive care, the hallmark of which involves respiratory support in form of oxygen supply for mild cases, and extracorporeal membrane oxygenation for severely ill patients, the search is still on for specific antiviral agents [6]. The process of developing new therapeutic agents is often very tedious, being required to scale several stages of laboratory, clinical and regulatory hurdles, over a period of many months to years. In a recent study, Wong et al. [7] established a success rate of 14% for experimental drug candidates in clinical trials. The urgency of the situation presented by the SARS-CoV-2 outbreak makes this impractical to curtailing the fast-spreading pandemic, thus encouraging researchers to look into the possibility of repurposing existing antiviral drugs or those already in the latter stages of development for treatment of other infections such as: hepatitis B virus (HBV), hepatitis C virus (HCV), HIV and influenza [8], taking into account similar steps conducted for the two other known human coronaviruses: SARS-CoV and MERS-CoV.

![Fig. 1. Literature search methodology chart](image-url)
In this review, we discuss the pathogenesis of the 2019-nCoV; highlight the current standard of care and evaluate the current state of knowledge on the antiviral effect of five of the most promising therapeutic options against SARS-CoV-2 including Chloroquine & Hydroxychloroquine, Remdesivir, Lopinavir & Ritonavir, Interferons and Convalescent Plasma. Finally, we examine the efforts taken so far towards the development of a vaccine candidate for COVID-19. We searched for relevant papers on PubMed Central, Google Scholar and HINARI using the keywords: "COVID-19", "2019-nCoV", "SARS-CoV-2", "novel coronavirus disease", "SARS-CoV", "Drug", "Treatment", "Management", "Therapeutic", "Therapy", "Vaccine" and "Clinical trial". Our search generated 101 results on PubMed Central, 940 results on Google Scholar and 322 results on HINARI, making a total of 1,363 results. After screening the topics and abstracts of the results from the database, we identified 202 articles relevant to our search. 14 duplicates were then excluded giving us a total of 188 articles assessed for eligibility, 12 articles obtained from a manual search were included, making a total of 200 articles considered for the review (Fig. 1). In order to obtain the latest information on our subject of interest, we included review articles, systematic reviews, meta-analysis and clinical trials (including those retrieved from pre-print servers) of potential drugs and vaccine used for the treatment of COVID-19 in this review. Through our work, we aim to answer the question of where we are in the search for the treatment for the rapidly evolving pandemic by exploring in vitro and in vivo experiments as well as clinical trials that have been conducted and published till date.

2. PHYLGENY, VIRION, PHYSIOCHEMICAL PROPERTIES AND PATHOGENESIS OF SARS-COV-2: WHAT DO WE KNOW?

2.1 Phylogeny and Virion Characteristics

Unbiased sequencing and evolutionary tree analysis of the first isolate of SARS-CoV-2 obtained from the bronchoalveolar lavage fluid of the earliest patients of the outbreak in Wuhan, revealed that the virus was a member of the β-CoVs [9]. Generally speaking, the beta-coronaviruses are a class of enveloped, positive-sense, single-stranded RNA viruses known to cause respiratory, hepatic, digestive and neurologic diseases in humans and other animals [10,11]. The family is organized into four sub-families: α-, β-, γ-, and δ-CoVs, however, only members of the α- and β-CoVs sub-families have been known to cause diseases in humans [10,11]. As earlier stated, SARS-CoV-2 is a member of the β-CoVs, so is SARS-CoV and MERS-CoV [10]. Whole-genome phylogenetic analysis within the sub-family reveals that SARS-CoV-2 has a 79.6% sequence similarity with SARS-CoV, and a 50% similarity with MERS-CoV [9,12,13]. With a genome length of 29,900 basepairs, the SARS-CoV-2 virion (Fig. 2) has a nucleocapsid composed of the viral RNA and capsid, all embedded within a phospholipid bilayer, covered with two different types of spike proteins [14]. The first spike glycoprotein trimmer is shared by all sub-families of coronaviruses, while the second protein known as hemagglutinin-esterase is only found in a few members of the family [14]. The genome of SARS-CoV-2 has a 5’ end with a terminal sequence of 265 nucleotides, and a 3’ end with a terminal sequence of 229 nucleotides, a feature common to all β-CoVs, with a gene order 5’-replicase ORF1ab-Spike (S, 3822nt)-envelope (E, 228nt)-membrane (M, 669nt)-Nucleocapsid (N, 1260nt)-3’ [15]. Fig. 3 depicts the relative locations of each gene within the genome of SARS-CoV-2. Other predicted non-coding Open Reading Frames (ORFs) include ORF3ab, ORF6, ORF7ab, ORF8, ORF9ab and ORF10 [15].

2.2 Physiochemical Properties

Most of what we know about the physiochemical properties of SARS-CoV-2, were derived from our previous knowledge from SARS-CoV and MERS-CoV. SARS-CoV-2 is denatured by most disinfectants including: diethyl ether, 75% ethanol, chloroform, chlorine and peracetic acid, and is inactivated by ultraviolet light, or by heating at 56°C for 30 minutes [16]. Van Doremalen et al. [17], reported that SARS-CoV-2 demonstrated a higher degree of stability on plastic and stainless steel surfaces than on copper and cardboard surfaces, with viable viral copies being detectable on plastic and stainless steel up to 72 hours after application of the virus to these surfaces.
Transmission of SARS-CoV-2 occurs majorly through respiratory droplets, person-to-person contact and potentially through feco-oral routes [16]. The virus mainly infects the mucosal epithelial cells of the upper respiratory tract, including the nasal cavity and the pharynx, and less frequently in the lower respiratory tract and the gastrointestinal mucosa, as reported by Xiao et al. [18]. Several reports of the non-respiratory manifestations of SARS-CoV-2 have been published. Cheng et al. [19] reported the association between kidney impairment and death in COVID-19 patients; Guan et al. [20] reported acute liver injury in patients infected by SARS-CoV-2; while in their retrospective analysis of 138 hospitalized COVID-19 patients, Wang et al. [21] described cardiac and enteric complications in their study population; Fan et al. [22], also reported evidence that SARS-CoV-2 could cause testicular dysfunction. All these evidences point to the fact that COVID-19 may very well cause multi-system organ dysfunction. 

Human angiotensin converting enzyme 2 (ACE2) is a receptor broadly expressed in the cells of the nasal mucosa, trachea, bronchus, lungs, esophagus, heart, kidney, stomach, ileum, bladder and frankly anywhere else SARS-CoV-2 has been found to infect [23], and is used by the virus to infect the target cells [24]. Asides from its role in the conversion of Angiotensin I to Angiotensin II, the ACE2 receptor is used as a binding site for the spike (S) protein of the coronaviruses [25]. The S protein exists in a stable pre-fusion state with two sub-units namely: S1 and S2. The process of binding to the ACE2
receptor begins with the S1 subunit destabilizing the stable pre-fusion state of the spike protein which prior to now exists as a pre-fusion S1-S2-ACE2 trimer, resulting in the shedding of the S1 subunit and the transition of the S2 subunit into a stable post-fusion state [15]. A report by Wrapp et al. [26] provided evidence to suggest that the S protein of SARS-CoV-2, binds to the ACE2 receptor with an affinity 10-20 times that of its closest phylogenetic relative SARS-CoV, alluding to the higher virulence of the 2019-nCoV.

The most severe cases of human SARS-CoV-2, SARS-CoV and MERS-CoV infections often result in Acute Respiratory Distress Syndrome (ARDS), a life-threatening clinical condition characterised by a severe diminution of gas exchange in the lungs [27]. Such individuals often require various forms of respiratory support, ranging in severity from oxygen supply to mechanical ventilation and extracorporeal membrane oxygenation (ECMO). Several authors have reported pathological findings consistent with ARDS in patients infected by SARS-CoV2, SARS-CoV and MERS-CoV [28,29]. In a 2013 paper, Meyer and Christie [30] described the various inflammatory cytokines released during ARDS, mediating the inflammatory response, including: interleukin-10 (IL-10), vascular endothelial growth factor (VEGF) and tumour necrosis factor (TNF), while Thompson et al. [27], in their review paper also highlighted that elevated levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) were often closely associated with adverse outcome of ARDS. Ultimately, ARDS remains the leading complication and cause of death following SARS-CoV-2 infections, a finding often attributed also to the overwhelming inflammatory response that accompanies severe infection with the virus. This overwhelming inflammatory response results in the release of excessive amounts of inflammatory cytokines, a situation known as cytokine storm [15]. Several factors have been proposed to be responsible for this effect, including: rapid viral replication resulting in massive cell death, loss of pulmonary ACE2 function due to the high-jacking of the receptor by the virus and antibody-dependent enhancement (ADE) [31]. At the onset of infection, rapid viral replication results in massive shedding and death of mucosal epithelial cells as well as endothelial lining of associated blood vessels, causing vascular leakage and stimulating the production of excessive inflammatory cytokines and chemokines [32].

3. PATTERN OF PRESENTATION

A retrospective study by Huang et al. [33] published in the early days of the SARS-CoV-2 outbreak in China describes the commonest symptoms of the infection in a cohort of 41 hospitalised patients to include: fever (98%), cough (76%) and myalgia or fatigue (44%). They also reported other symptoms such as: sputum production (28%), headache (8%), haemoptysis (5%) and diarrhoea (3%). Dyspnea occurred in 55% of their patient cohort and 63% had reduced lymphocyte count, all patients developed pneumonia with atypical findings on chest computed tomography (CT) scans [33]. Building on this, Wang et al. [21] conducted another retrospective study of a larger cohort of 138 hospitalised SARS-CoV-2 infected patients in Wuhan, and their results agreed significantly with those obtained by Huang’s group. In their paper, they reported the commonest symptoms in their patient cohort to be: fever (98.6%), fatigue (69.6%) and dry cough (59.4%). Milder symptoms included: myalgia (34.8%), diarrhoea (10.1%), nausea (10.1%), and headache (9%). Dyspnea occurred in 31% of the patients, 70.3% had lymphopenia and all patients showed bilateral patchy opacities or ground glass opacities of atypical pneumonia on chest CT [21]. Based on severity, 80% of individuals with SARS-CoV-2 infection will have mild to moderate (pneumonia - and non-pneumonia-associated) illness while 13.8% will have severe disease characterised by dyspnea (with respiratory rate >30 cycles/minute), PaO2 <93% and PaO2/FiO2 ratio <300, while 6.1% would be critical, having severe complications such as septic shock, respiratory failure and multiple organ failure/dysfunction [34]. Asymptomatic infections have also been reported. In the Diamond Princess cruise ship outbreak in Japan, 51% of individuals with laboratory confirmed SARS-CoV-2 infection were asymptomatic at the time of testing [35]. In Japan, as of April 6, 2020, 9.3% of laboratory confirmed cases were without symptoms as well [36], while an European Centre for Disease Control (ECDC) report placed the proportion of asymptomatic cases in Italy at 44% [37] as at March 12, 2020. Based on data from China however, the Joint WHO-China International Mission indicates that about 75% of asymptomatic cases eventually progress into clinical disease [34]. Case fatality differs significantly across geographical regions. Available data on case-fatality ratio (CFR) for China, Italy and South Korea was 2.3%, 2.8% and 0.5% respectively across all age groups [37].
4. CURRENT STANDARD OF CARE

According to the rapid diagnosis and treatment guideline for SARS-CoV-2 infection, developed by Jin et al. [41], the hallmark of treatment of SARS-CoV-2 infection is mainly supportive. Symptomatic patients may require bed rest with frequent monitoring of vitals with fluid resuscitation to correct electrolyte and acid-base imbalances. Some ancillary investigations that are often required include C - reactive protein (often elevated), organ function tests such as: liver enzymes, bilirubin, cardiac enzymes, electrolyte/urea/creatinine, arterial blood gas and chest imaging. Patients may also require oxygen therapy, which may be administered via: nasal catheter, oxygen mask, high-flow nasal oxygen therapy (HFNO), non-invasive ventilation (NIV) or invasive mechanical ventilation [41]. Oxygen therapy is often administered to patients with severe pneumonia, in respiratory distress, with signs of hypoxia, hypoxemia and/or shock. Extracorporeal Membrane Oxygenation (ECMO) is considered in patients whose hypoxemia is not corrected by the afore-mentioned methods.

Another hallmark of treatment involves antibiotic therapy, due to the possibility of superimposed bacterial infection (bacterial pneumonia). Generally a combination of broad spectrum antibiotics therapy is avoided due to the potential of antibiotic resistance. Routine microbial culture and sensitivity should be performed to identify the appropriate antibiotics to be used when secondary bacterial infections occur. In the absence of such laboratory facilities, empirical antibiotics treatment may be commenced using agents effective against community-acquired pneumonia, such as: amoxicillin, azithromycin and fluoroquinolones [41]. Corticosteroid therapy may be indicated in cases of severe ARDS; however their use is controversial, and approached with a great deal of caution. Studies on SARS management strategies have provided ample evidence to suggest that a combination of non-invasive continuous positive airway pressure (CPAP) and Methylprednisolone is effective for improving clinical symptoms of SARS patients, reduce disease progression and encourage absorption of lung lesions; however, it has not been able to shorten hospital stay [41]. It has also been associated with certain severe adverse effects [42] and a recent systematic review by Russell et al. [43] established that clinical evidence did not support the use of corticosteroids treatment for SARS-CoV-2 induced lung injury. In addition, the interim guidance from the WHO on the treatment of SARS due to COVID-19 advises against the use of corticosteroids unless another indication can be established [44].

Other treatment procedures include: symptomatic treatment of fever (temperature >38.5°C) using ibuprofen till a target temperature of 38°C is reached; nutritional support; use of H₂ receptor antagonists and proton pump inhibitors for treatment of stress ulcers and gastrointestinal bleeding in high risk patients (risk factors include: mechanical ventilation >48 hours, liver dysfunction, renal replacement therapy and coagulopathies) and the use of low-molecular weight heparin for prophylaxis against venous embolism and other coagulopathies [41].

5. SPECIFIC THERAPEUTIC ALTERNATIVES

Currently there exists no specific antiviral agent or vaccine for the treatment of SARS-CoV-2 infection or infection by any known human coronavirus specie. As described the previous section, current clinical management guidelines remain heavily reliant on supportive therapy for patients; however, several therapeutic candidates have emerged and currently undergoing several stages of testing and clinical trials to establish their efficacy. In this section, we examine the available evidence on some of the most promising options, highlighting where they have been used and the corresponding outcomes of their application.

5.1 Convalescent Plasma and Therapeutic Neutralising Antibodies (Nabs)

Convalescent plasma treatment is a long-standing basic principle of immunotherapy that has been used in the past for treatment of several viral infections. It involves the use of neutralising antibody containing-plasma or sera obtained from individuals who have been infected by a particular viral pathogen but now in the convalescent phase, for the treatment of patients with active infection or for prophylaxis in high-risk individuals or those who have been exposed to the same viral pathogen. In normal individuals, viral infection stimulates a humoral immune response, which leads to the production
of antibodies responsible for viral neutralisation. These antibodies, known as neutralising antibodies (NAbs) are contained in the plasma, and their concentrations increase gradually as infection progresses, being highest in the convalescent phase [45] and diminishing following recovery from the infection [46]. The NAbs have two functional segments through which they elicit their protective function. The F(\(\text{ab}'\))\(_2\) fragment which is responsible for antigen recognition, by binding to portions of the antigen known as epitopes, and the crystallisable fragment Fc, which mediates immune system activation [47], and they carry out their protective function via three major steps. First, they bind through their F(\(\text{ab}'\))\(_2\) fragment to viral particles, in a way that prevents the particles from binding to their target cells, this is followed by a potent activation of the complement system or the opsonisation pathway through the Fc fragments [48]. Theoretically speaking, such a mechanism would have a potent effect in clearing viral infections and there are several historical precedents involving the use of convalescent plasma, containing NAbs in the treatment of viral infections, to prove this theory true.

In the 20th century, convalescent plasma was used in the treatment of outbreaks of measles [49,50], influenza [51] and mumps [52]. In 2006 meta-analysis by Luke et al. [53] on the use of convalescent plasma in treating patients during the 1918 Spanish flu pandemic, they proved that there was indeed a lower case-fatality rate among patients who received convalescent plasma treatment as compared with those who didn’t. Of recent, convalescent plasma has been used in the 2009-2010 H1N1 influenza virus pandemic and the 2013 Ebola virus epidemic in West Africa. Hung et al. [54], reported that patients who received convalescent plasma treatment during the 2009 H1N1 influenza virus pandemic had a significantly lower mortality rate (20% against 54.8%) as against those who did not receive the treatment. Similarly, a study conducted in Sierra Leone revealed that Ebola virus patients who were given convalescent whole blood had a higher survival rate and lower case-fatality ratio than those who received only routine treatment [55]. More evidence can be found on the successful use of convalescent plasma in the treatment of the H5N1 influenza virus outbreak [56,57] and H7N9 avian flu outbreak [58], as well as the 2003 SARS-CoV [59,60] and 2012 MERS-CoV [61] outbreaks, adding further credence to the point that convalescent plasma is indeed an effective treatment alternative for viral infections.

It is believed that NAbs to SARS-CoV-2 would act via a mechanism similar to those of SARS-CoV, given their phylogetic similarities, and the fact that they make use of the same ACE-2 receptor for cell entry. This mechanism involves binding to the S2 subunit of the virion S protein, with which it normally binds to the receptor binding domain (RBD) of the ACE-2 receptor for cell entry [62]. By so doing, the NAbs are able to prevent the entry of the viral particles into the host cell. While several NAbs targeting the S1 subunit of SARS-CoV have been identified, such as: 80R [63], CR3014 [64], CR3022 [65], m396 [66], 201 [67,68] and B1 [69], a few antibodies with affinity for the S2 subunit have also been identified [70], suggesting an alternative mechanism for antibody-mediated viral neutralisation. Eventually viral clearance is achieved through opsonisation or complement activation [48]. There are several possible sources of antibodies against SARS-CoV-2, including: human convalescent plasma from individuals recovering from COVID-19 as well as monoclonal antibodies (mAbs) generated in animal models or through recombinant techniques. NAbs against SARS-CoV are potential alternatives as well, according to a recent letter by Tian et al. [71], which showed that CR3022 [65] demonstrated potent binding with the 2019-nCoV spike protein. However, the most readily available option is convalescent plasma obtained from recovering or recently recovered COVID-19 patients and this has been used in the few clinical trials conducted so far.

In a case series of 5 critically ill patients with confirmed SARS-CoV-2 infection and ARDS, administration of convalescent plasma resulted in marked clinical improvement of all 5 patients [72]. All 5 patients were receiving mechanical ventilation prior to the treatment and following the transfusion, body temperature of 4 out of 5 patients normalised within 3 days, the sequential organ failure assessment (SOFA) score decreased, PaO\(_2\)/FiO\(_2\) also increased within 12 days (172-275 before and 284-366 following treatment), viral loads were negative in all patients within 12 days and ARDS resolved in 4 patients, while 3 were weaned off mechanical ventilation within 2 weeks [72]. In a larger clinical trial involving 19 patients, ten patients received 200 mL of convalescent plasma containing neutralising antibody with titres above 1:640 in addition to supportive care and antiviral therapy.
While the other 9 patients did not receive the convalescent plasma [73]. Three days following convalescent plasma administration, the clinical symptoms and laboratory parameters of the test patients improved markedly compared with the control. There was an increase in oxyhemoglobin saturation, lymphocyte count (0.65 x 10⁹/L against 0.76 x 10⁹/L) and decreased C-reactive protein (55.98 mg/L against 18.13 mg/L), radiology showed an absorption of lung lesions within 7 days and viral load was undetectable in 7 patients. Two patients were weaned off mechanical ventilation to high-flow nasal cannula, while one patient previously on high-flow nasal cannula was discontinued from oxygen therapy [73]. These two clinical studies show that convalescent plasma therapy so far may useful in the treatment of SARS-CoV-2 infection, however they do not provide evidence on its effectiveness as a stand-alone therapy as both studies have the patients also receiving other forms of treatment such as antiviral drugs and Methylprednisolone.

There are some important considerations of note concerning effective convalescent plasma therapy. First, being an example of passive antibody therapy, convalescent plasma is more effective when used for prophylaxis or shortly after the onset of symptoms, than much later [74]. This important consideration reflects in the results obtained by the trial conducted by Duan et al. [73], in which patients treated before 14 days post onset of illness (dpoi) showed better clinical and laboratory improvement compared with those treated after 14 days. Second, cocktails of NAbs showed greater efficacy for viral neutralisation than single NAbs in Ebola and SARS viruses cases [70,75]. This was explained to be probably due to the synergistic effect of different antibodies that bind to different sites of the viral particles, thus decreasing the probability that the viral particles can escape with decreased sensitivity to neutralisation [76]. In addition, Jawhara explains that for maximal efficacy, convalescent plasma obtained from patients living in the same city as those who would receive the transfusion, should be used [77]. This is because antibodies obtained from a different location may prove ineffective as other factors such as lifestyle, diet and the environment often play a significant role in the development of neutralising antibodies. Also the possibility of different viral strains across geographical regions cannot be ruled out.

Convalescent plasma therapy is not without its risks, one of which is the risk of transfusion reactions and the transmission of other infectious diseases contained in the transfused plasma. This however can easily be circumvented by standard screening protocols guiding the transfusion of blood and blood products. Another risk is that of transfusion related acute lung injury (TRALI) often occurring in critically ill individuals [78,79]. Another risk is the phenomenon of antibody-dependent enhancement (ADE), in which antibodies to one coronavirus could enhance infection to another viral strain, as described by Wan et al. [80]. However, as the proposed use of convalescent plasma relies on the use of sera with high antibody titres for the same virus – SARS-CoV-2, ADE may be unlikely and available evidence lends credence to this, as studies conducted thus far have recorded no adverse reactions [72,73].

5.2 Remdesivir (GS-5734)

Remdesivir (RDV) is a trial drug originally developed by Gilead Sciences Inc. to be used for treating the Ebola Virus Disease (EVD) [81]. It has since been shown to have broad spectrum antiviral activity against a number of RNA viruses both in vitro and in animal models. In a Nature report published by Sheahan et al. [82], they demonstrated that a combination of RDV and Interferon-ß had a superior antiviral activity over the Lopinavir, Ritonavir and interferon-ß combination therapy, against MERS-CoV strains in vitro. In addition, they showed that both prophylactic and therapeutic application of RDV was able to improve pulmonary function and decrease viral loads in MERS-CoV mice models [82]. Its efficacy against SARS-CoV has also been demonstrated both in vitro, and in mouse models [83] and in 2018, Agostini et al. [84] demonstrated that the susceptibility of both human coronaviral strains to GS-5734 was mediated by viral polymerase and the proofreading exonucleases.

Remdesivir (GS-5734) is a monophosphoramidate pro-drug of the Remdesivir triphosphate (RDV-TP), a C-adenosine nucleoside analogue that acts as an inhibitor of RNA-dependent RNA polymerase, an enzyme critical to the RNA viral replication process [84,85]. Remdesivir-TP competes with adenosine triphosphate (ATP) for incorporation into the growing nucleotide chain. The selectivity value for RDV-TP suggests that its incorporation into the nucleotide chain occur through a more
effective process than for ATP and thus, following its incorporation, it triggers an abrupt termination of RNA synthesis at a position 3 nucleotides away [85]. It has been suggested that the addition of these 3 nucleotides possibly protects the RDV-TP from being cleaved by the viral 3'-5' exonuclease activity [85].

In a recent letter, Wang et al. [86] revealed that Remdesivir was an effective inhibitor of SARS-CoV-2 in vitro, using vero E6 cells, at a multiplicity of infection (MOI) of 0.05, a half-maximal effective concentration (EC50) of 0.77 μM, a half-cytotoxic concentration (CC50) greater than 100 μM and selective index (SI) greater than 129.87. They also showed that RDV had the ability to inhibit SARS-CoV-2 infection in human liver cancer Huh-7 cells, which have been shown to possess the ACE-2 receptor used by SARS-CoV-2 for cell entry [12]. In a report of the first case of SARS-CoV-2 infection treated in the USA, RDV was used on compassionate basis following the continued clinical deterioration of the index patient. The administration was accompanied by an almost immediate clinical improvement of the patient, with the discontinuation of oxygen therapy and a rise in SaO2 to 94–96% in room air and the disappearance of bilateral lower-lobe rales seen on chest radiograph, with no adverse effects [87]. On the human clinical trial front, Remdesivir has produces mixed results. The first human clinical trial testing the efficacy of the drug for use against SARS-CoV-2, published by the WHO showed that there was no difference between patients who received Remdesivir and those who received placebo, in terms of recovery time and mortality [88]. Further, the trial results also showed that Remdesivir administration was stopped in 11.6% of patients in the trial due to the drug’s adverse effects. These results contradict 2 other trial results, one by Gilead and the other by the National Institute of Health (NIH). In the Gilead phase 3 trial, they noted that 50% of participants had a time to clinical improvement of 11 days following Remdesivir administration [89], while in the NIH trial known as the Adaptive COVID-19 Treatment Trial (ACTT), preliminary results indicated that patients who received Remdesivir, had a 31% faster recovery time, compared with those who received placebo [90]. It was in light of this encouraging results that Remdesivir received emergency use authorisation from the Food and Drug Administration (FDA) for use in treating SARS-CoV-2 infections [91]. Interestingly, the findings of another randomized, double-blind, placebo-controlled trial in China, published in The Lancet on the same day the preliminary findings of the NIH trial were announced, significantly contradicted the NIH results. In their report, Wang et al. [92], noted that Remdesivir use was not associated with a difference in time to clinical improvement, compared with placebo. They also noted that Remdesivir was stopped in 12% of patients in the Remdesivir group, as against 5% in the placebo group [92].

In spite of the anecdotal evidence suggesting that GS-5734 may indeed be safe and effective against SARS-CoV-2, there is a need for more randomised, controlled clinical trials to settle some of the ambiguities and contradictions of those conducted till date. A search on ClinicalTrials.gov, with the keywords "COVID-19" and "Remdesivir" yielded ten results, of which nine were active trials relevant to the search parameters [93].

5.3 Lopinavir/Ritonavir

Lopinavir (LPV) is an aspartate protease inhibitor developed to be used as a treatment drug for the human immunodeficiency virus (HIV) type I. It has also been reported by several studies to exhibit efficacy in the treatment of human coronaviruses. Theoretically speaking however, this is questionable because while Lopinavir is an aspartate-like protease inhibitor, SARS-CoV and MERS-CoV possess cysteine-like proteases while the SARS-CoV-2 possesses both 3-chymotrypsin-like and papain-like proteases [94]. In addition, the HIV protease inhibitors such as Lopinavir are designed to fit into the C2 symmetry of the catalytic site of the HIV protease dimmer, a configuration that is missing in the proteases of the human coronaviruses, presenting a significant challenge to binding and efficacy [94]. In spite of these theoretical setbacks however, several reports have shown LPV to have some efficacy against SARS-CoV [95–97] and MERS-CoV [98] both in vitro and in vivo. In these studies, LPV is often used together with another agent called Ritonavir, in a Lopinavir/Ritonavir (LPV/r) combination. Ritonavir helps to increase the plasma half-life and bioavailability of LPV, by inhibiting the cytochrome P4503A (CYP3A) mediated metabolism of LPV [99].

In a unique case series reported by Wang et al. [100] involving the treatment of a cohort of four SARS-CoV-2 infected patients using a combination of LPV/r (400 mg Lopinavir/100 mg
Ritonavir), arbidol and Shufeng Jiedu Capsule (SFJDC), a traditional Chinese medicine, in addition to supportive care, three patients showed significant improvement in their clinical symptoms and laboratory parameters, two of whom were confirmed SARS-CoV-2 negative and had been discharged by the end of the study period, and the last patient showing significant improvement as well. This study provides evidence that LPV/r may be beneficial in the treatment of symptomatic SARS-CoV-2 infections. However, due to the combination of other antiviral drugs and treatment modalities, it is difficult to assess how much of the improvement was in fact due to LPV/r.

In the earlier days of the SARS-CoV-2 outbreak, Cao et al. [99] launched an urgent open-label randomised, controlled clinical trial to test the safety and efficacy of LPV/r combination for the treatment of hospitalised COVID-19 patients in Wuhan. Their cohort of 199 patients involved 99 test subjects and 100 control subjects. All patients were recruited on the basis of having a $\text{SaO}_2 < 94\%$ in ambient air and a ratio of $\text{PaO}_2/\text{FiO}_2 < 300\text{mmHg}$. The test subjects received the LPV/r combination (400mg LPV and 100mg Ritonavir) in addition to standard care for 14 days, while the control subjects received standard care alone [99]. Unfortunately, the results of the trial were disappointing as concerning its primary end point, which was the time to clinical improvement of the patients, with both groups requiring a median of 16 days [99], however, the results for the secondary end points were quite interesting.

First, there was a slightly lower (but statistically insignificant) number of deaths among the LPV/r group ($14/99$ vs. $25/100$), but this could be explained by the fact that the standard care group were at baseline in a slightly worse condition clinically than the LPV/r group [101]. It has also been said that the open-label nature of the trial could have left the trial susceptible to interpretation bias from the clinicians. Also, it is quite difficult to separate the effect of the LPV/r administered from that of other supportive treatments such as glucocorticoids and interferon [101]. In addition, the LPV/r combination had no significant effect on viral clearance rate, a contradiction to its mechanism of action which involves the inhibition of a protease crucial to the process of viral replication. This could be a reflection of the reduced potency of the drug as a result of its conformational mismatch being an aspartate-like protease inhibitor of HIV and not an inhibitor of the 3-chymotrypsin-like and papain-like proteases of SARS-CoV-2 [94].

There have been several other observational studies on the use of the LPV/r combination in the treatment of COVID-19 patients. In a report of the 3rd case of COVID-19 confirmed in South Korea, who received the LPV/r combination, the drug was able to reduce the viral load significantly [102]. In another case report published by Han et al. [103], involving the use of LPV/r combination together with Methylprednisolone, recombinant human interferon alfa-2b and supportive therapy, for the treatment of a case of COVID-19 in China, they reported marked clinical improvement of the patient and discontinuation of ventilator support on the 8th day of treatment. In a retrospective study comparing the efficacy of an Arbidol + LPV/r combination against LPV/r alone, in the treatment of SARS-CoV-2 infection, the authors noted that while the virus was undetectable in 75% (12 of 16) of patients who received the Arbidol + LPV/r combination, only 35% (6 of 17) of patients who received LPV/r alone demonstrated similar results [104], suggesting a possible synergistic effect of both medications.

Adverse reactions to the LPV/r combination have also been frequently reported in literature. In the trial conducted by Cao and colleagues [99], 14% of the patients receiving the LPV/r combination were unable to complete the full 14-day course of treatment due to gastrointestinal adverse reactions such as: anorexia, nausea, diarrhoea and abdominal discomfort, as well as two cases of acute gastritis. Two patients also had skin eruptions, which were self-limiting. In a case series of the first 18 COVID-19 patients to be diagnosed in Singapore, of the five patients who were given the LPV/r combination, four developed similar gastrointestinal adverse events, and only one patient was able to complete the full 14-day treatment course [105]. These adverse events offer an additional area of consideration for the use of the LPV/r combination for treating SARS-CoV-2 infections.

### 5.4 Chloroquine/Hydroxychloroquine

Chloroquine (CQ), a drug on the WHO list of essential medicines is world renowned for the prophylaxis and treatment of Malaria. It has also been used in the management of extra-abdominal amebiasis [106]. Hydroxychloroquine (HCQ) sulphate on the other hand is a derivative of CQ which was synthesized in 1946 by
introducing a hydroxyl group into CQ, and was proven to be 40% less toxic than its parent CQ in animal models [107], and is being used not just as an antimalaria medication but also in the treatment of SLE and Rheumatoid Arthritis [108]. First discovered as “resochin” in 1934, Chloroquine has since fallen out of favour in the treatment of malaria in many countries, due to a combination of reasons ranging from the widespread development of resistance, to concerns about its toxicity in humans [109], however, the drug has in recent times been a subject of renewed attention, following reports of its potential antiviral activity [110,111]. These reports made CQ/HCQ an immediate option for consideration against SARS-CoV-2 in the early days of the pandemic, and its efficacy has been demonstrated in several published literature.

Both CQ and HCQ are weak bases capable of increasing the pH of certain acidic intracellular organelles including endosomes (EE) and endolysosomes (EL) which are crucial for membrane fusion, a step in the process of viral cell entry [112]. In addition, it has been reported that CQ was able to inhibit SARS-CoV entry into its target cell, by interfering with the glycosylation of the ACE-2 receptors employed by the virus for entry [113]. This shows that CQ and its derivative HCQ are able to block the entry and post-entry steps of viral infection. In a recent letter, Wang et al. [86] demonstrated that CQ was able to inhibit SARS-CoV-2 infection in vitro, using Vero E6 cells, with an EC\textsubscript{50} value of 6.90 μM, which has been achieved clinically in the plasma of rheumatoid arthritis patients receiving 500 mg of the drug [114]. In another correspondence on the efficacy of HCQ in inhibiting SARS-CoV-2 infection in vitro, Liu et al. [115] showed that HCQ had a lower 50% cytotoxic concentration (CC\textsubscript{50}) compared with that of CQ (249.50 μM vs. 273.20 μM), suggesting it is a less toxic alternative to CQ. Also they showed that apart from their effect on lysosomal fusion and ACE-2 glycosylation, both drugs also have anti-inflammatory properties [108] that helps decrease the production of cytokines, alleviating the cytokine storm often associated with SARS-CoV-2 infection and responsible for many of its adverse events such as ARDS.

In a recent trial on the safety and efficacy of HCQ treatment of COVID-19 patients, conducted in Shanghai, the authors reported no significant difference between the test (400 mg HCQ + supportive therapy) and control groups (with supportive therapy only), at any of the end points investigated [116]. In another open-label, non-randomized clinical trial on the efficacy of HCQ and azithromycin conducted in France, following the administration of 600 mg of HCQ daily, together with supportive therapy and azithromycin, at day 6 post-inclusion, 70% of the HCQ treated patients had complete viral clearance as demonstrated by RT-PCR of the nasopharyngeal swab samples, as compared with 12.5% in the control group who received only supportive therapy [117].

A search on ClinicalTrials.gov with the keywords “COVID-19”, “Chloroquine” and “Hydroxychloroquine” revealed 11 active trials investigating the use of these agents for treating SARS-CoV-2 infections [118]. By extending the search to include the Chinese clinical trials registry and the WHO international clinical trial registry platforms, the number of active trials increases to about 40, with completion dates ranging from as early as April 2020 to as late as April 2021. It is believed that the results of these trials will provide sufficient data to establish the efficacy of CQ/HCQ in treating SARS-CoV-2 infections.

### 5.5 Vaccine

Vaccines have been responsible for the eradication of small pox [119] and the impending eradication of polio [120] globally. Such rich pedigree makes them an indispensable force in the fight between humans and infectious diseases, being able to protect against infection, especially in those regarded as most vulnerable to the adverse events of such contagions. They are also often times regarded as one of the two cheapest methods of preventing infections, the other being good sanitation and hygiene. Relating to the SARS-CoV-2 infection, the discovery of a vaccine is to be a game changing turning point, and a push towards elimination and possible eradication of the deadly virus, by preventing new infections and creating herd immunity to control disease transmission [121].

The advantages of a vaccine go beyond an improvement of health outcomes, as described by Lu [122], but also in the stabilization of the global economy, bringing it back on track as soon as possible. Several groups have already taken up the challenge of developing an effective vaccine against SARS-CoV-2, and more entities, both private and public institutions are entering the scene, in a similar manner as during the SARS-CoV outbreak [123]. For example,
Johnson & Johnson [124] will be using an experimental adenovirus vector platform while Sanofi [125] will be using a known platform which was used in developing the approved Flublok recombinant influenza virus vaccine [126]. However, the vaccine production process is one that is known to be time-consuming, requiring several stages of clinical trials before each vaccine can make its way to clinical use. In a recent paper, Zhang and Liu [127] highlighted the fact that SARS-CoV-2 seldom affects children and even when it does, is less severe than in adults. They noted that this could be a result of the several recent required vaccinations they received and thus recommended that the RNA virus vaccines such as those for: polio, measles, influenza, rabies and Japanese encephalitis virus may be administered to health workers and other high risk individuals as a form of prophylaxis, pending the discovery of the SARS-CoV-2 vaccine [127].

Recently, the Coalition for Epidemic Preparedness Innovation (CEPI) pledged funding for three vaccine development programmes, being run by The University of Queensland, Inovio and Moderna Inc., with a 16 week deadline (June 2020) for clinical testing, using a range of delivery platforms [128]. Due to the phylogenetic similarities SARS-CoV-2 has with both SARS-CoV (79.6%) and MERS-CoV (50%), it has been suggested, that vaccines against either or both of these pathogens may offer some degree of cross-protection against SARS-CoV-2 [121]. Following the last MERS-CoV outbreak, a number of vaccines candidates have been in development and have been reviewed by Young et al. in [129]. However, as at March 2020, only candidate has had the result of its phase I clinical trial published by Modjarrad et al. [130]. The SARS vaccine trials conducted by the US National Institute of Allergy and Infectious Diseases (NIAID) reported positive results in Phase I trials but only one of the vaccines will be proceeding to Phase II so far [121].

6. CONCLUSION

In conclusion, we have discussed extensively, the pathogenesis, highlighted the current standards of care and explored the current situation as regards the therapeutic options against the SARS-CoV-2. We have also examined the efforts thus far in the process of vaccine development. We have demonstrated that while the current standard of care remains the bedrock of SARS-CoV-2 therapy, there is an urgent need to identify and commission the several potential therapeutic agents under investigation, following the results of ongoing clinical investigations. This would be essential in the fight against the raging pandemic and would have a ripple effect on the push towards a quick recovery of the global economy post-pandemic. Finally, the discovery of a vaccine would be crucial towards the elimination or the possible eradication of the disease. Further understanding of the pathogenesis of SARS-CoV-2 would be instrumental in this regard.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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