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FULL LENGTH ARTICLE

Pandemic COVID-19: Current status and challenges of antiviral therapies

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Abstract The pandemic COVID-19, caused by a new coronavirus SARS-CoV-2 infection, has infected over 12 million individuals and caused more than 55,200 death worldwide. Currently, there is no specific drug to treating this disease. Here we summarized the mechanisms of antiviral therapies and the clinic findings from different countries. Antiviral chemotherapies have been conducted by in multiple cohorts in different counties. Although FDA has fast approved remdesivir for treating COVID-19, it only speeds up recovery from COVID-19 with mildly reduced mortality. The chloroquine was suggested a potential drug against SARS-CoV-2 infection due to its *in vitro* antiviral effects, it is imperative high-quality data from worldwide clinical trials are necessitated for an approved therapy. In terms of hydroxychloroquine (HCQ) therapy, although WHO has stopped all the clinic trials due to its strong side-effects in COVID patients, large scale clinical trials with a long-term outcome follow-up may warrant HCQ and azithromycin combination in combating the virus. Convalescent plasma (CP) therapy suggested its safety use in SARS-CoV-2 infection; but both CP immunotherapy and NK cellular therapy must be manufactured and utilized according to scrupulous ethical and controlled conditions to guarantee a possible role of these products of human origin. Further research should be conducted to define the exact mechanism of SARS-CoV-2 pathogenesis, suitable animal models or *ex vivo* human lung tissues aid in studying replication, transmission and spread of the novel viruses, thereby facilitating highly effective therapies.

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Introduction

After outbreak of severe acute respiratory syndrome (SARS) in 2003, Middle East respiratory syndrome (MERS) in 2012, another Coronavirus Infectious Disease 2019 (COVID-19) has become a global catastrophe in the twenty first century. According to World Health Organization (WHO), COVID-19 is referred to the cluster of viral pneumonia occurring in Wuhan, Hubei Province, since December 2019. It is found that the causative agent of this pandemic was caused by a novel coronavirus SARS-CoV-2.[1] This novel infectious disease is encountered by over 210 countries and territories around the world,[2] leading to a newly emerging global crisis.

Up till now, SARS-CoV-2 has spread internationally with over 12 million patients diagnosed and over 55,200 deaths worldwide (worldometers.info, 2020) by July 9, 2020. In the Western Pacific Region, China is the country hit most severely with a total confirmed case of 83,396.[8] Whereas the USA, UK, Spain and Italy have become the most unfortunate countries exposed to this overwhelming disease in the region of the Americas and the European region respectively. The US has taken over 3.1 million confirmed cases with over 130,000 deaths,[9] while Italy had reported the percentage of patients in intensive care who are actively infected was consistently between 9% and 11% from March 1 up until Match 11.[10] At a glance, the spread of COVID-19 and its overwhelming virulence have turned into an unstoppable crisis which has already reached the required epidemiological standard for it to be declared a pandemic, with infected cases exceeding 100,000 in 100 countries in just three months and only 12 days to reach the next 100,000.[11]

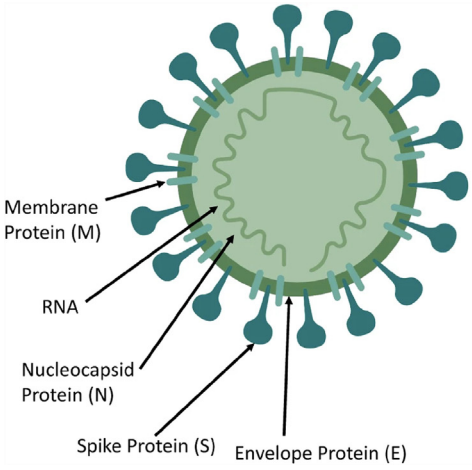
It is of paramount importance to implement a series of medical interventions to overcome the challenges brought by COVID-19 and govern this grievous plague efficiently. While awaiting the development of vaccination, prompt and precise medical treatment plays a crucial role in this demanding situation. However, there are no specific therapies approved officially by the U.S. Food and Drug Administration (FDA) to treat COVID-19.[12] Consequently, identifying effective therapies to combat the disease is a pressing need. Either antiviral treatment or cellular therapy may lead to the complete or partial success of a cure for the patients, depending on its level of effectiveness. Thus, the effectiveness of treatment is critically responsible for the overall improvement of this pandemic disease and deserves to be studied extensively to enhance the clinical intervention.

Based on the mortality rate of COVID-19 is about 6% globally, learning more about the effectiveness and differences between drug therapies help to contribute to a more successful treatment. This literature review will summarise and discuss different types of therapies, including compassionate uses of antiviral drugs (which are now undergoing their clinical trials owing to a lack of clinical experience) and on in-vitro activity against this novel virus. Besides, we will also explore cellular and immunotherapy to highlight their therapeutic potentials. This review would give an insight of feasible development of potent medications and provide a framework for possible intervention strategies in a foreseeable future.

SARS-CoV-2

Structure of SARS-CoV-2

SARS-CoV-2 belongs to Beta-coronavirus, the same genus as contagious Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) in coronavirus family. Their genome is composed of a positive-sense single-stranded RNA (+ssRNA). SARS-CoV-2 comprises of four structural proteins which aid in assembling and infection – the transmembrane spike glycoprotein (S), membrane protein (M), envelop protein (E) and nucleocapsid protein (N) (Fig. 1).[13] Among these proteins, viral attachment and entry into host cells are mediated by the S protein. It is surface-exposed and can be cleaved by host proteases into S1 and S2



Structural Protein	Function of Protein
Nucleocapsid Protein (N)	• Bound to RNA genome to make up nucleocapsid
Spike Protein (S)	• Critical for binding of host cell receptors to facilitate entry of host cell
Envelope Protein (E)	• Interacts with M to form viral envelope
Membrane Protein (M)	• Central organiser of CoV assembly • Determines shape of viral envelope

- It has been noted that some CoVs do not need to have the full ensemble of structural proteins to make virions, highlighting that certain proteins may be dispensable or compensated by the function of non-structural proteins.

Figure 1 Basic structure of coronaviruses.[3]

subunits.[14] The cleavage allows initiation of the membrane fusion via a large scale irreversible conformational changes.[15,16] The S1 subunit consists of the receptor-binding domain(s) (RBD) that can undergo hinge-like conformational movements, leading to a transient hidden or exposure of receptor binding determinants and thus mediates the engagement of a host receptor.[17] Therefore, it has been suggested that the critical RBD(s) is responsible for the determination of cell tropism, host range and zoonotic transmission of coronaviruses.[18] Subsequently, the S1 subunit stabilizes the profusion conformation of the membrane-bound S2 subunit which contains the fusion machinery.[19,20] Hence, the intricate processes of host receptor binding and proteolytic cleavage of the S protein are essential for the viral entry into susceptible targets. The comprehension of structure and function of the S protein help to formulate a timely strategy for a specific drug such as monoclonal antibody drugs, as well as the development of vaccines in a foreseeable future.

The life cycle of SARS-CoV-2

Upon entering the host cell with the aid of S protein, SARS-CoV-2 releases its RNA genome into the cytoplasm, it translates into structural proteins and two polyproteins, pp1a and pp1ab, they are then split into small products via viral proteinase cleavage.[21] Afterwards, the viral genome begins to replicate, the positive-strand genome undergoes discontinuous transcription to synthesize the subgeneric negative-strand templates, templates for mRNA synthesis are thus generated, while the full-length negative-strand template is served as a genomic RNA template. Followed by the formation of viral nucleocapsids via a combination of genomic RNA and N protein in the cytoplasm, as well as the insertion of the newly formed envelope glycoproteins into the membrane of the endoplasmic reticulum (ER) or Golgi, the viral particles are then budded into the lumen of ER-Golgi intermediate compartment (ERGIC). It is then followed by the release of virions through exocytosis. Finally, the vesicles containing virions fuse with the plasma membrane to infect other host cells (Fig. 2).[22] It must be pointed out that the above process is a replication basis of SARS-CoV, the exact pathophysiological mechanisms underlying the emergence of SARS-CoV-2 still awaits exploration.

SARS-CoV-2 mutations impact the viral pathogenicity

Noteworthily, the pathogenicity of SARS-CoV-2 are found to be influenced by patient-derived mutations. It is identified that 19 of the 33 mutations are novel in the collection of 11 viral isolates, in which 6 mutations are localized in spike protein.[23] This indicates that the polymorphism of SARS-

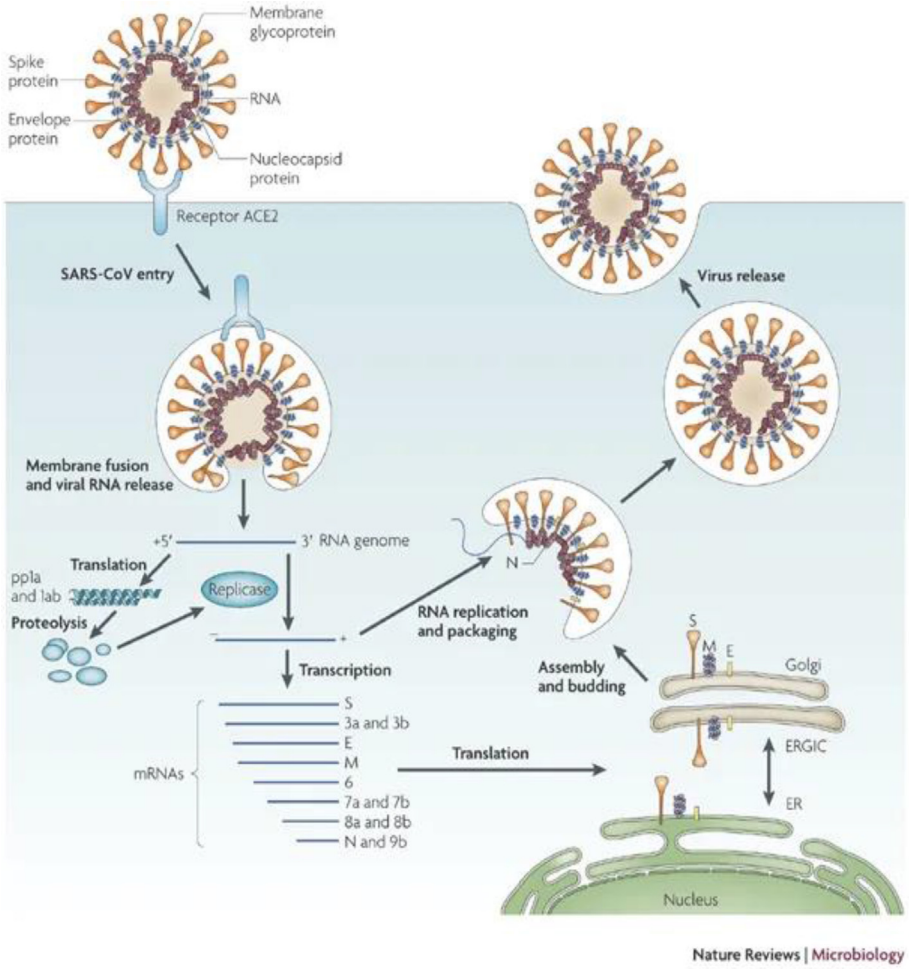


Figure 2 The life-cycle of SARS-CoV in host cells.[4]

CoV-2 is much more than expected and the site of mutation may highly affect the viral pathogenesis. Strikingly, two viral isolates at 24 h post-infection (p.i.) have reported a nearly 270-fold difference in viral load. In addition, an unexpected tri-nucleotide mutation was observed in one viral isolate, which resulted in viral positivity for a sustainable period of 45 days.[24] Further studies should be directed towards the mutational impact of tri-nucleotide on the viral pathogenicity. Although this non-peer-reviewed journal cannot be served as a clinical guideline, it has provided a significant insight for therapies development by taking the impact of the accumulating mutations into account to prevent potential pitfalls.

The similarity between SARS-CoV and MERS-CoV

SARS-CoV-2 shares more than 85% of its identity with SARS-CoV.[25] What is more, a greater similarity of (>90%) sequence identity is observed in the RNA-dependent RNA polymerase (RdRp) between them. This poses a suggestion of RdRp inhibitors being the candidate drug for COVID-19.[26] Revealingly, the same receptor of SARS-CoV, angiotensin-converting enzyme 2 (ACE2) is also used by SARS-CoV-2,[27] which is highly expressed in a small subset of cells in the lung called type 2 alveolar cells.[28] Interestingly, SARS-CoV-2 conserves 8 binding-residues of 14 these amino acids in SARS-CoV, which are responsible for direct interaction with ACE2.[29] To note, there is a nearly 10- to 20-fold higher affinity in the SARS-CoV-2 S protein binding to ACE2 than that of SARS-CoV, implying an increased ease in spreading between humans.[17] This indicates that SARS-CoV-2 is highly similar to the receptor-binding domain in S protein of SARS-CoV.

In contrast, SARS-CoV-2 is less related to MERS-CoV; only 50% of its genetic sequence identity is shared with SARS-CoV-2.[30] Moreover, the primary receptor of MERS-CoV present in the lower respiratory tract, dipeptidyl peptidase 4 (DPP4), is not exploited by SARS-CoV-2.[31] Nonetheless, MERS-CoV shares highly similar pathological characteristics with COVID-19, including viral cytopathic-like changes of giant cells multinucleation along with atypical enlarged pneumocytes in the intra-alveolar spaces,[32] suggesting to some extent the epidemiological characteristics of SARS-CoV-2 is similar to that of MERS-CoV. General principles of the same Coronaviridae family may govern its biological activities.

Besides, these three CoVs all show a similar innate immune response. A highly pro-inflammatory condition was observed in patients with SARS-CoV-2 infection who needed intensive care unit (ICU). They showed increased plasma levels of many innate cytokines.[33] Both SARS and MERS also have this noticeable growth, signifying a potential similar cytokine storm-mediated disease severity.[34,35] Altogether, identifying the similarity of pathological and immunological characteristics with SARS-CoV and MERS-CoV could facilitate our knowledge of pathophysiological mechanisms underlying the emergence of SARS-CoV-2. The genetics similarity with SARS-CoV also aids in the interpretation of the resulting inflammatory response that may trigger the onset of severe pneumonia.[29] By comprehending these similarities with the presidential SARS-CoV and MERS-CoV, it is believed that it increases the likelihood for the physicians to design efficient therapeutic measures for the COVID-19 patients and reduce fatality.

Cytokine storm

While the pathogenesis of SARS-CoV-2 is still elusive and there is uncertainty with respect to its mechanism of viral transmission, the similar mechanisms of other pathogenic CoV provide useful information to enhance our recognition of COVID-19. Since SARS-CoV-2 requires the same receptor as SARS-CoV (ACE2) for viral entry, aerosol and fomites transmission of SARS-CoV-2 is plausible.[36] Upon entry into alveolar epithelial cells via exploitation of ACE2, the virus replicates rapidly by infecting pneumocytes and macrophages.[37] This triggers the antigen presentation in CoV infection, leading to a strong cellular and humoral immune response. Ultimately, the cytokine storm and pulmonary tissue damage are induced (Fig. 3). The cytokine storm is an overwhelming systemic inflammatory response resulting from the overproduction of pro-inflammatory cytokines by the immune effector cells in SARS-CoV infection. The cytokine storm is one of the crucial causes of acute respiratory distress syndrome (ARDS), multiple organ failure (MOF) and death from severe SARS-CoV infection.[38] In fact, of the 99 cases of COVID-19, critically ill patients had shown cytokine storm syndromes and 17 patients suffered from ARDS, in which 11 patients deteriorate in a short period of time, and died of MOF.[39] It is also reported that 6 of 41 SARS-CoV-2 infected patients died from ARDS and speculated the death of COVID-19 is mainly attributed to ARDS.[33] An important note is that there is a decrease in the numbers of total T cells in patients with COVID-19, with the remaining CD4+ T and CD8+ T cells functionally exhausted. This indicates that the SARS-CoV-2 infection can attenuate human immune function.[40] Once the weakened immune response or ARDS exists with concomitant secondary infection, it may result in respiratory failure.

Besides, as mentioned above, this deadly cytokine storm-mediated condition is also observed in SARS and MERS.[34,35] An increased level of IL-6, IFN-α, and CCL5, CXCL8, CXCL10 in serum was observed in patients with severe MERS-CoV infection in contrast to those with the mild-moderate disease.[41] Also, the dead has a distinctively higher level of IL-18, IP-10, MG, and MCP1 than in those of the survivors of severe SARS-CoV infected patients.[42] Likewise, the immune response of some patients against the SARS-CoV-2 causes an elevation of IL-6.[33] The data have strongly emphasized that these three CoVs share the common immunopathological events which provoke a vigorous attack by the immune system to the host body, giving rise to ARDS and MOF (Fig. 3).

Clinical features and transmissibility of COVID-19

The main clinical manifestations of COVID-19 include fatigue, fever, nonproductive cough, myalgia, dyspnea, normal or declined leukocyte counts, and radiographic evidence of pneumonia.[33] These features are also observed in SARS-CoV and MERS-CoV infection,[43,44] indicating a resemblance in clinical features between these three CoVs.[45] However, gastrointestinal symptoms were uncommon in COVID-19, whereas diarrhoea was observed in about 20–25% of patients with SARS-CoV or MERS-CoV infection.[46] It is possible that such difference in viral tropism may attribute to the potentially improved transmissibility of SARS-CoV-2 relative to SARS-CoV.[17] Compared with SARS-CoV, SARS-CoV-2 might reinforce the infection of ACE2

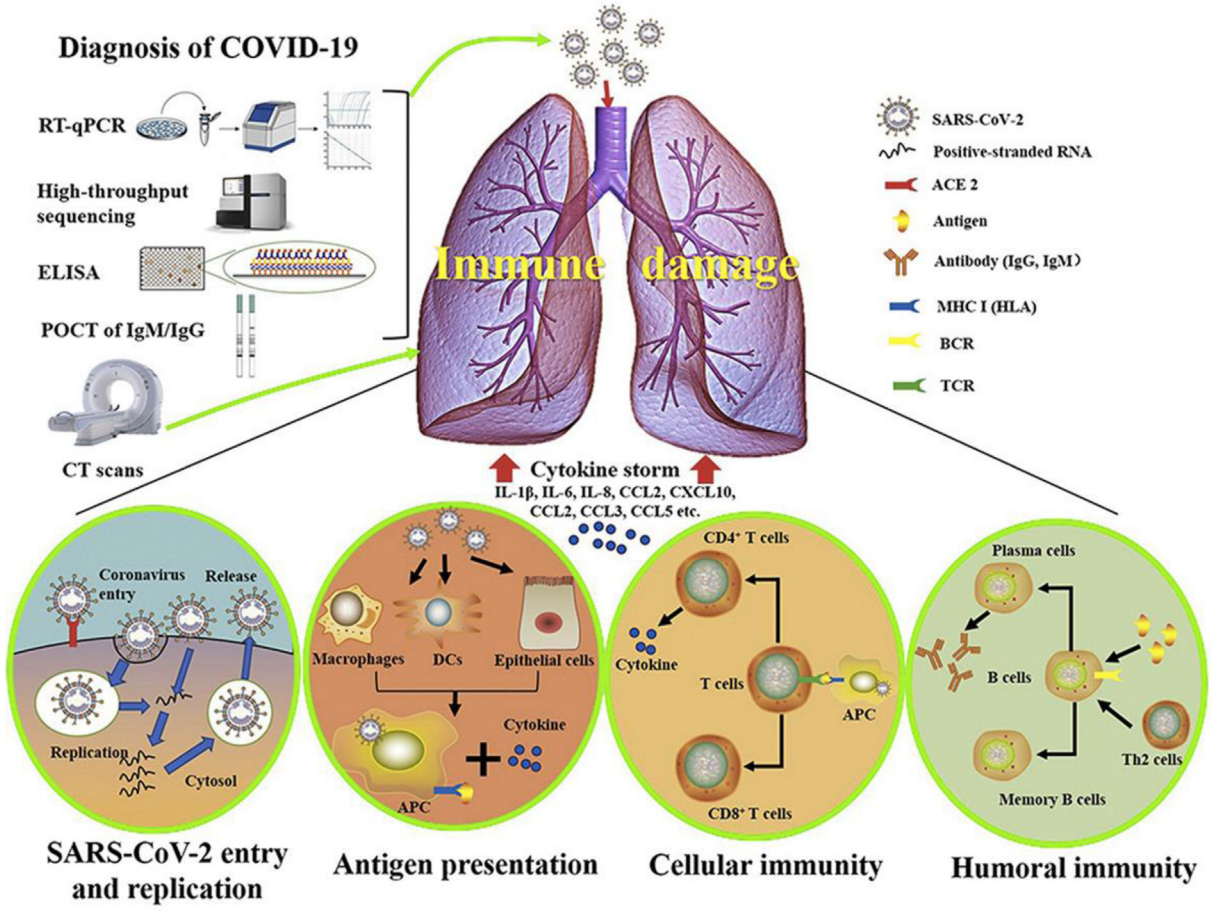


Figure 3 Stimulation of cytokine storm in CoV infection.[5]

cells in the upper respiratory tract via superior cellular attachment-promoting factors.[31] Future investigations are needed to determine if SARS-CoV-2 is capable of determining the main site of infection.

Furthermore, a model of *ex-vivo* human lung tissue revealed that SARS-CoV-2 generated 3.2 folds higher amounts of infectious viral particles than that of SARS-CoV within 48h p.i. Surprisingly, despite the more efficient replication, SARS-CoV-2 did not trigger significant levels of interferons in infected human lung tissues.[47] The preliminary data have explained the high person-to-person transmissibility of this novel virus. Moreover, its low degree of innate immune activation accounts for the false-negative in patients who have mild or even asymptomatic infections, which promotes the COVID-19's spread by "stealth transmission". In short, SARS-CoV-2 acts as a ninja which is not easily detected while maintaining its potency. If the mechanism that renders these viral characteristics of high transmissibility and symptomatic infection can be elucidated in the future researches, more precise treatment strategies and infection controls can be developed for COVID-19 patients, especially for those who immunocompromised.

Although advancement in drug improvement occurs frequently, de novo drug development is costly and timely. In this moment of global emergency, a drug to cure the ailment is needed as soon as possible. Inventing a new drug from scratch would cost too much time, which in this case, cost too many lives. Instead, repurposing existing drugs for a new disease is always the preferred first step since animal and safety trials are not required which could shorten the time to roll out the drug and reduce its cost. A possible way for drug exploration with considerable interest is to test if the currently existing drugs are potent for related viral infections, along with a full comprehension regarding their safety profile, side effects and drug interactions, thereby facilitating the drug approval for COVID-19 treatment. In light of the genetic similarity with SARS-CoV and pathological features with MERS-CoV infection, those genomic regions and phenotypic interests help facilitate therapeutic modulation. Thus drugs used in treating patients with SARS and MERS may also exert their antiviral efficiency on SARS-CoV-2. Hence, it is possible to reposition the existing drugs to treat the novel virus infection.

Antiviral chemotherapy

Remdesivir

Remdesivir was suggested to treat SARS-CoV-2 due to its effective inhibitory effect on SARS-CoV-2 *in vitro*. Remdesivir is a RdRp inhibitor of Ebola virus and expected a broad-spectrum antiviral effects.[48] It is a prodrug of remdesivir triphosphate (RDV-TP) and is an adenosine nucleotide analogue to disrupt viral replication. Once it is metabolized

into its active form, it obscures the RdRp by competing with adenosine triphosphate (ATP) for incorporation into the nascent RNA strand.[48] As a result, RDV-TP terminates the RNA synthesis prematurely and the growth of the RNA strand is ceased after the incorporation of additional nucleotides. Since the RNA synthesis is not halted instantly, remdesivir escapes from proofreading process of CoVs done by viral exoribonuclease, an enzyme that renders resistance to drugs by detecting and excising nucleotide analogue inhibitors.[49] Thus, by utilising the delayed cessation of nascent viral RNA strand of CoV and surpassing the viral proofreading activity, remdesivir has sufficient antiviral activity that makes it possible to become a candidate novel drug for treating COVID-19.[50,51]. In fact, both prophylactic and therapeutic remdesivir treatment have provided adequate protection against SARS-CoV and MERS-CoV infection in a mouse model.[52,53]

In preclinical trials, remdesivir has demonstrated effective antiviral potency against CoVs. It has shown excellent potency towards viral infection blockage at a low micromolar concentration. In addition, the *in vitro* efficacy in the inhibition of virus replication was proven by its high selectivity index (SI). Whereas favipiravir, ribavirin and penciclovir were precluded from this study as they required higher concentrations to whittle down the viral infection *in vitro*, reflecting a relatively inferior *in vivo* antiviral effect against SARS-CoV-2.[54] Furthermore, in non-human primates (NHP) models, remdesivir all showed satisfactory antiviral activity with full protection against Ebola virus infection.[55] Although the excellent antiviral efficacy of remdesivir has been demonstrated in different studies, its resistance mutations have not been identified. Wild-type murine hepatitis virus (MHV) in co-infected cell cultures without exposure to remdesivir have outpaced the strains with induced mutations that were resistant to remdesivir.[49] The mechanism of naturally developed resistance mutation still remains unknown. Because of this, we cannot rule out the possibility of remdesivir resistance, since the same study had shown that remdesivir was more sensitive towards the mutated MHV that lacked proofreading capability.[49] This implies there is a likelihood for either a mutation that enhances proofreading or strengthens replication fidelity that ends up in the emergence of drug resistance.[56] Further studies should be conducted to understand such resistance mutations for improving the effectiveness of remdesivir.

Nonetheless, remdesivir has been widely applied to over a hundred patients infected with SARS-CoV-2 in the China, US, Europe, and Japan through Expanded Access or Compassionate Use programs.[57,58] Presently, evaluation of remdesivir efficacy in patients have been conducted by randomized, controlled, and double-blind clinical trials. More recently, remdesivir showed clinical improvement in 36 of 53 (68%) patients with COVID-19 in a preliminary report.[59] The mortality was 13% over a median follow-up of 18 days, compared to the 28-day mortality of another randomised, controlled trial of lopinavir-ritonavir in patients, which was 22%.[60] Although this trial is conducted in a small size of the cohort and there is a lack of viral load data to confirm the antiviral effects of the drug, the mortality and clinical improvement shown in this cohort are optimistic. In May, report from big size of cohort revealed improved therapeutic effects with speed up recovery from COVID-19 although it only mildly reduced mortality. Therefore, FDA has fast approved this drug for treating COVID-19.

Chloroquine

In vitro data have shown its influential antiviral activity against a clinical isolate of SARS-CoV-2. Similar to remdesivir, chloroquine has a high selection index (SI) and inhibits SARS-CoV-2 at a low micromolar concentration ($EC50 = 1.13\ \mu M$; $CC50 > 100\ \mu M$, $SI > 88.50$), suggesting a decent chance of it becoming a potential treatment candidate.[54]

As an antimalarial drug, chloroquine has been used for over 70 years. It is chosen from the tens of thousands of existing drugs because of the reported antiviral effects in patients with SARS-CoV.[61] Chloroquine had become less relevant as the SARS outbreak died and there were no new cases of CoVs until now. Recognising that the current SARS-CoV-2 is similar to SARS-CoV genetically, chloroquine was considered again as a therapeutic measure for the novel pandemic.

Principally, several antiviral functions are involved in the use of chloroquine. A well-known function is that chloroquine can act as an inhibitor to the endosomal acidification to block CoV replication. A previous study declared as the viral entry is mediated by fusion competent SARS-CoV S protein in a pH-dependent manner, they require endosomal acidification for entry.[62] The endosomal acid proteases are used to cleave the S protein, whereby activating fusion.[63] Chloroquine being a lysosomotropic agent to reduce the transduction of SARS-CoV via an elevation of lysosomal pH, optimal cleavage is prevented and thus negatively affects the S protein. This in turn restricts the fusion of autophagosomes with lysosomes and the degradation of the lysosomal protein[64,54]. These end up inhibiting the viral endocytotic entry and consequently suppressing a series of fusion events between the virus and endosomes. Apart from pH alternation, pretreatment effect of chloroquine is attributable to an interference with the glycosylation of cellular receptors of SARS-CoV. The impairment of terminal glycosylation of ACE2 was mediated by a potent anti-SARS-CoV concentration of chloroquine, purposing that the pretreatment of chloroquine is capable in generating the surface expression of the under-glycosylated ACE2, which may reduce binding affinities for ACE2.[61] Past research had undoubtedly reflected the prophylactic value of chloroquine and give an insight into its potential role for COVID-19 prevention.

Other than the inhibition of pre-entry step and early stage of the virus replication, a recent publication has suggested an antiviral effect of chloroquine on post-translational modification of the viral protein. By altering the normal proteolytic processing of M protein, couple with inhibiting budding with an accumulation of M proteins in the Golgi network, SARS-CoV-2 infectivity can be possibly limited since its M proteins are accumulated in the Golgi complex and acts as a CoV budding determinant, thereby impeding the replication cycle.[65] Besides, chloroquine is also involved in immunomodulation of cell-signalling and cytokine release. The essential signaling for viral replication, mitogen-activated protein kinase (MAPK), was

inhibited by chloroquine in a model of HCoV-229E,[66] presuming that molecular crosstalk of SARS-CoV-2 with target cell can be disturbed by chloroquine induced anti-phosphorylation of the kinase. Besides, chloroquine suppresses the cytokine storm by limiting CD154 expression in T cells.[67] Furthermore, the excessive synthesis of TNFα, IL6, and IFNγ may induce ARDS via a cascade of events. Chloroquine aids in reducing the production of pro-inflammatory cytokines to prevent ARDS (Fig. 4).[6] Therefore, the immune-modulating activities of this drug pose a synergistic reinforcement for its antiviral potency *in vivo*, encouraging the clinical application for COVID-19 treatment.

Taken together, the multiple mechanisms of chloroquine can potentially achieve a promising therapeutic effect against SARS-CoV-2 infection. The reduction of SARS-CoV infectivity and cell spread was attained in the presence of 1—10 μM chloroquine, which is clinically achievable in the plasma of malaria-infected patients.[68] The EC90 value of this drug against the SARS-CoV-2 in Vero E6 cells was 6.90 μM, which is the plasma concentration achievable for rheumatoid arthritis treatment.[54]

These data support that chloroquine can interfere with SARS-CoV-2 in a feasible and rather effective dosage. Due to the numerous pros as mentioned above, preliminary trials of chloroquine have been conducted in many countries. 70% of COVID-19 patients were considered cured in a small-scale clinical trial in Marseille of France.[69] The promising result had led to several new large-scale trials for

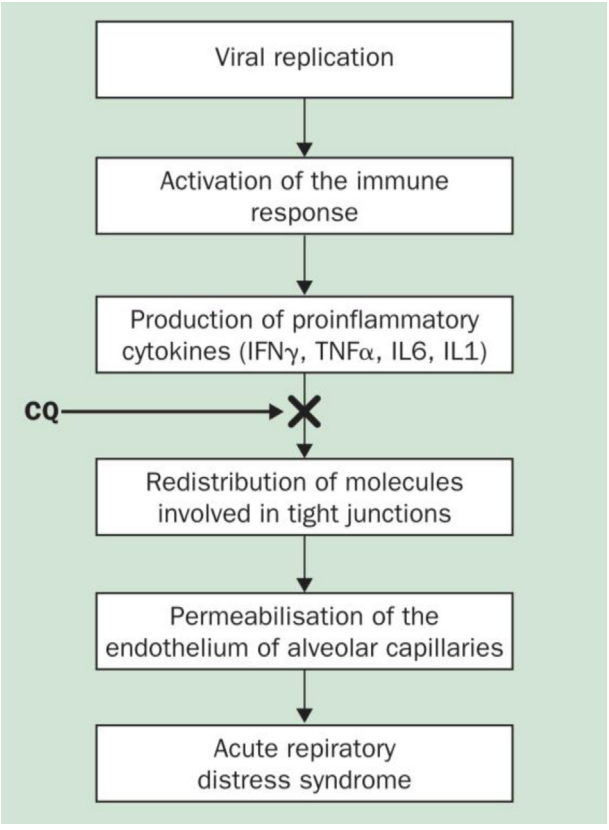


Figure 4 Postulated model for the immunomodulating activity of chloroquine on ARDS.[6]

reaching conclusions on chloroquine effectiveness, including a European programme of large-scale clinical trials which was launched on March 12,[70] as well as a main trial that is open-label, randomised and controlled trial involving 240 patients called The Vietnam Chloroquine Treatment on COVID-19 (VICO) conducted on the first of April.[71,72] Yet, currently, there is no published scientific journal reporting the precise data from the clinical trials, which poses a difficulty in interpreting results. Likewise, although the inhibitory effect of chloroquine was observed in the pneumonia patients with COVID-19, no specific data were available for result interpretation, even though a significant effect in terms of clinical outcome and viral clearance has been shown when compared to controls groups.[73] In light of the foregoing, it is imperative high-quality data from worldwide clinical trials are necessitated for chloroquine to become an approved therapy. Given the fact that chloroquine exerts remarkable inhibitory antiviral effects on the susceptible cells, it is highly recommended to be considered as the treatment candidate for both prophylactic and therapeutic use.

Hydroxychloroquine

Hydroxychloroquine (HCQ) was also suggested as candidate drug.[74] It is a derivative of CQ by introducing a hydroxyl group into CQ. Hence it is not surprising that it shares almost the same pharmacological properties as CQ.[75] The effectiveness of both drugs on systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) has been reported.[76] In fact, a time-of-addition experiment has already demonstrated that HCQ was able to stop the entry step and post-entry stages of SARS-CoV-2.[5] Even in the SARS outbreak, HCQ was already found to be inhibitory against SARS-CoV *in vitro*.[77]

The reason for choosing HCQ as a candidate is not to replace the existing CQ, but to provide another possibility for COVID-19 therapies. Compared to CQ, the addition of the hydroxyl group renders HCQ 2—4 times less toxic while conserving its efficacy. The toxicity was relatively less 40% than CQ in animals.[78] More importantly, HCQ may be more effective than CQ. The EC50 values (6.14 and 0.72 μM at 24 and 48 h) for HCQ is always lower than that for CQ (23.90 and 5.47 μM at 24 and 48 h), signifying that HCQ has a relatively superior anti-SARS-CoV-2 ability.[24] However, after 48 h of infection at four different multiplicities of infection (MOIs), a lower EC50 for CQ (2.71, 3.81, 7.14, and 7.36 μM) relative to that of HCQ (4.51, 4.06, 17.31, and 12.96 μM) was observed. Coupled with the SI indexes of CQ (100.81, 71.71, 38.26, and 37.12) which are higher than those of HCQ (55.32, 61.45, 14.41, 19.25) suggests that the antiviral efficacy of HCQ is less influential compared to CQ's.[79] Besides, other factors including the concentration of drugs, culture medium or procedures may also contribute to the inconsistent results.

In spite of the contrary proposition given out by different papers, the antiviral effect of HCQ can be reinforced by azithromycin. An open-label and non-randomised clinical trial reported that all patients treated by HCQ in conjunction with azithromycin were virologically cured at day 6 post-inclusion compared to 57.1% in patients treated with HCQ only, and 12.5% in the control group.[80] A clear synergistic effect of HCQ in conjunction with azithromycin

was demonstrated in these preliminary data. Azithromycin is a macrolide antibiotic used for treating a number of bacterial infections; it was reported to have the antiviral potency in cystic fibrosis airway epithelial cells.[81] Moreover, inhibitory effects of azithromycin were found to be applicable on Zika and Ebola virus.[82,83] Hence, it is possible that the action of azithromycin can contribute purely to the improved viral clearance when HCQ coupled with azithromycin. This even gives rise to another approach of testing azithromycin as a first-line treatment for COVID-19 by postulating its lone drug effect may be sufficient to eliminate SARS-CoV-2 at the initial stage of the disease. This possibly requires confirmation by clinical trials. All in all, more studies are necessary to explore this combination therapy as it has a great potential to be a promising antiviral therapy for COVID-19 and prevent bacterial infections, particularly in treating severe patients who may expose to opportunistic infections. To note, the study of Gautret et al is limited by a small sample size, thus large scale clinical trials with a long-term outcome follow-up should be initiated to warrant HCQ and azithromycin combination in combating the virus.

However, by May 26, WHO has announced to stop all treatments regarding HCQ due to the increased risk of death and de-novo ventricular arrhythmia.[84] The lower toxicity of HCQ still poses a risk of poisoning if prolonged and overdose used.[85] It particularly provokes QT prolongation that may result in dangerous arrhythmias.[86]

Ivermectin

The aforementioned drugs are well known among the candidates in case of COVID-19; they are the first to be investigated owing to their preexisting data from the previous CoVs outbreak. Noteworthy, ivermectin, an antiparasitic drug that is less concerned during the early outbreak of COVID-19, was postulated to have potential for repurposing by showing an overwhelming antiviral activity towards SARS-CoV-2 *in vitro*.

Ivermectin is a common anti-parasitic medication for treating human nematode infections, such as onchocerciasis[87] and lice.[88] Recently, it displayed a valuable clinical role for the treatment of viral infection. Its antiviral activity *in vitro* against RNA viruses has been demonstrated in HIV-1,[89] Dengue fever virus (DENV),[90] and influenza virus.[91] The broad-spectrum antiviral activity of ivermectin is attributed to its inhibition of importin α/β (IMPα/β)-mediated nuclear import. It binds to nuclear localization signal (NLS)-binding pocket of IMPα, thus avoiding the recognition from NLS-containing cargoes, resulting in a blockage of the viral cargoes entering the nucleus.[89,92] This mode of action is noticed in both HIV-1 and DENV, either integrase or nonstructural protein 5 (NS5) is inhibited by ivermectin to bind with IMPα/β.[89] Since IMPα/β may be responsible for the signal-dependent nucleocytoplasmic shuttling of N protein during SARS-CoV- infections;[93] altogether, it have been hypothesised that ivermectin displays a valuable clinical role in treating COVID-19.[94]

It has been shown that 5 μM of ivermectin reduced 99.8% viral RNA in SARS-CoV-2 RNA within 24h p.i. The antiviral effect is enhanced to a ~5000-fold reduction of viral RNA within 48h p.i., reflecting a time-dependent decline of viral RNA. It was suggested a single treatment to achieve SARS-CoV-2 inhibition within 24—48 h.[94] However, its effectiveness towards COVID-19 is doubted by another study because of impossibility of 5 μM dosing regimens of this drug.[95] It is found that the 5 μM (4375 ng/ml) *in vitro* inhibitory concentration (I.C.) was 17 times higher against the largest C max value (247.8 ng/ml). Furthermore, $>$50 times more than the levels achievable after 700 μg/kg (clinically relevant C max $=$ 96.2 ng/ml) was observed in the same concentration. Based on the recognition of the known dosing regimens, the study of the pharmacokinetics of ivermectin emphasised unlikely to complete reduction of viral RNA at 5 μM, at least in the range of clinically relevant dosage.[95] Moreover, Momekov denied the feasibility of a single treatment with a reason for continuous exposure of SARS-CoV-2 clinical isolate to an unreachable concentration even with an overdose of ivermectin.[95] This drug is only approved for human use by prescription for the parasitic treatment, and for preventing heartworm disease in animals.[96] Since the drug called Heartgard containing ivermectin is available in veterinary clinics,[96] Momekov have raised a concern about that a trend of self-medication for COVID-19 may be evoked.[95] Taken together, ivermectin repurposing appears to be implausible in treating COVID-19. Nonetheless, the attempt in exploring the therapeutic value of the anti-parasitic drug in SARS-CoV-2 should be appreciated. Presumably, more investigation on the dosing regimen can be conducted to assure the true inhibitory concentration of ivermectin in COVID-19, thereby determining the antiviral ability of ivermectin.

Despite the effectiveness of ivermectin on COVID-19 remains controversial, in conjunction with HCQ, it may hypothetically exert a consequential and synergistic effect on SARS-CoV. Given that HCQ and ivermectin inhibit the viral entry and reduce the viral RNA respectively, the antiviral activity can be reinforced.[97] More importantly, a clinical trial on a long-acting formulation of ivermectin for COVID-19 treatment is going to be launched by a French venture.[98]

After a detailed review of synthetic antiviral drugs, a guide concerning indications and application of promising antiviral therapy for COVID-19 is presented. Remember, to treat COVID-19 thoroughly, improving the quantity of life, shortening the duration of illness and minimising infectivity are necessary for effective antiviral therapy. Unlike other antimicrobial agents, antiviral drugs exert a robust inhibitory effect on viral replication instead of deactivating or destroying the virus. This way the viral load can be prevented from elevating to a point where pathogenesis could be induced. However, therapy involving combinations of drugs should be carefully considered, particularly if the antiviral drugs may interact with other therapeutic drugs, the potential risk should be taken into account. Once there are intolerable side effects, the use of related drugs must be ceased. Side effects raised by these drugs such as QT prolongation should also be monitored and cautioned. Further investigations of the efficacy of antiviral drugs in wide clinical applications should be undertaken to combat this deadly pandemic as soon as possible.

Convalescent plasma therapy

The therapeutic value is highlighted in immunotherapy as like as antiviral therapy. While awaiting the approved specific antiviral agents for COVID-19 via clinical trials, convalescent plasma (CP) therapy is also approached as a potential candidate. It is a classic adaptive immunotherapy which provides the plasma from recovered patients with a high neutralising antibody titer to a susceptible person. Upon circulation of the blood, the antibodies reach the target tissue and confer antiviral protection. Several historical precedents have shown the efficacy of CP therapy on different contagious infections. A significant decline in the relative risk of mortality was observed in CP received patients with severe influenza A (H1N1) infection compared with those in the control group (20% vs 54.8%, $P = 0.01$). Serum-treated individuals had also shown alleviated viral load and cytokine response.[99] The effectiveness of CP was also noticed in H5N1 and H7N9 outbreaks with all patients surviving.[100,101] A meta-analysis demonstrated a similar result of reduced mortality with no adverse events or complications in patients suffered from SARS-CoV infection and severe influenza after receiving various doses of CP.[102] For the 2013 West African Ebola epidemic, survival was significantly prolonged in CP-treated patients in contrast with those who undertaken standard treatment.[103] Because of this, a recommendation of the use of CP as an empirical treatment during outbreaks of Ebola virus was announced by WHO.[104] Even though the efficacy of CP therapy varied with the virus and the study, these significant historical precedents confer a valuable experience, reaching a consensus that this intervention was useful and feasible.

Since SARS-CoV-2 shares similar virological and clinical features with SARS-CoV and MERS-CoV, CP therapy may be another choice for COVID-19 treatment other than antiviral agents. In 2015, a protocol for the use of CP in the treatment of MERS-CoV was established.[105] Its satisfactory efficacy and safety were testified in a small sample study. Notably, the satisfying efficacy could be achieved by exceeding 1:80 of the neutralising antibody titer,[106] suggesting that the neutralising antibody titer plays the major factor associated with CP therapy. Whereas the largest study of patients with SARS in Hong Kong reported a higher day-22 discharge rate among those who received CP before day 14 of illness (58.3% vs 15.6%; $P < 0.001$),[107] which highlights the treatment time point is another major factor associated with the efficacy of CP therapy. Besides, the level of neutralizing antibody against SARS-CoV declined continuously 4 months after the disease stage, an undetectable level of 16.1% neutralizing antibodies of patients is attained at 36 months after disease status.[108] Of 99 samples of convalescent sera from patients with SARS, 87 had neutralising antibody with a geometric mean titer of 1:61.[109] For the MERS-CoV infection, patients showed a rapid decrease in antibodies titer within 3 months.[110] Also, only 2 of 3 patients with MERS in South Korea had neutralizing antibody in their serum.[106] Taken together, the data suggested the antibody will diminish with time or some who recover from the disease may not produce a high-titer response. Both short-lasting humoral immune response and difficulty in finding eligible donors pose a challenge in using CP therapy.

Regardless of the challenge encountered by CP therapy, several studies have proved its effectiveness to COVID-19. It is reported a decreased viral load in 5 severely ill patients with COVID-19 during the period of CP treatment, cycle threshold (Ct) value became negative in all patients within 12 days. The clinical conditions were improved as indicated by Sequential Organ Failure Assessment (SOFA) score, ranging from 2 to 10 before plasma transfusion and declined to a range of 1–4 at 12 days following transfusion.[111] Another experiment to study the feasibility of CP therapy in COVID-19 demonstrated a satisfactory negative result of SARS-CoV-2 RNA in all investigated patients associated with increased oxygen saturation (before 93.00%; after 96.00%) and lymphocyte counts (before 0.65 $\times$ 109/L; after 0.76 $\times$ 109/L), along with improved liver function and C-reactive protein. No severe adverse reactions were observed throughout the study.[112] More importantly, the case-fatality rates (CFR) of SARS-CoV-2 infected patients were 0% relative to CFRs in SARS which was 12.5%.[107] Both studies have reflected the therapeutic value of CP in alleviating the viral load and ameliorating the immune system, thereby minimize symptoms and mortality. The efficacy of these manifestations is attributed to the neutralizing effect of antibodies that may suppress viraemia.[113] The viral clearance and restriction of entry into susceptible cells can be accomplished by the virus-specific antibody via viral neutralization, antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis.[114] Also, an *in vivo* trial of HIV-1 has shown an accelerated infected cell clearance by antibodies.[115] In addition, high virus-specific IgG and IgM titers were detected in 5 critically ill patients with COVID-19, and the restriction of viral infection was elevated distinctly in all of them.[111] The above trials have highlighted the importance of viral clearance accomplished by neutralizing antibody in CP. Typically the viraemia peaks in the first week and be eradicated by days 10–14 after infection[107] suggested CP therapy could be administered at an early stage of the disease to maximize its efficacy. To note, the synergetic or antagonistic effect of CP therapy was found in some concurrent treatments such as steroids and polyclonal intravenous immunoglobulin[116], thus the interaction between CP therapy and other treatment must be considered.

As transfusion is involved in this therapy, it is necessary to consider contraindications to serum constituents such as serum sickness. Transfusion-transmitted infection (TTI) is one of the main concerns, such as a recognized risk of hepatitis E within UK blood donor population;[117] consequently, the risks should be investigated during the larger clinical trials of COVID-19. Besides, the risk of transfusion-related acute lung injury (TRALI), a severe complication of blood transfusion, is critically concerned. TRALI has been reported in an Ebola virus disease woman who was given CP therapy.[118] Since it can be induced by anti-human leukocyte antigen (HLA) antibodies,[119] in case of COVID-19, it is recommended to implement an anti-HLA antibody screening.[120] Although it is a rare adverse reaction, it should be alerted for those in the critical condition associated with significant pulmonary injury.[121] What is more, the antibody-dependent enhancement of disease (ADE) is

worth noting. It involves the suppression of innate immune systems that occurs at sub neutralizing concentration, this may lead to enhanced intracellular growth of the virus.[122] This was observed in SARS-CoV infection *in vitro*.[123] Nonetheless, ADE may be unlikely to happen in COVID-19 as the proposed use of CP would rely on preparations with high titers of neutralizing antibody and timely transfusion.[112] Furthermore, given the advanced blood banking techniques, the risks of the above contraindications and inadvertently TTI can be minimized via screening the suspected pathogens and matching the blood type of donors and recipients.

Overall, given the experience and current preliminary data on the use of CP therapy suggest its safety use in SARS-CoV-2 infection (worldometers.info, 2020). This treatment would be a boon for critically ill patients to improve their survival rate. It is also beneficial if administered at an early stage of the disease, more randomized clinical studies should be conducted for a risk-benefit assessment in individual variables. Moreover, SARS-CoV-2 specific hyperimmune serum such as Takeda's TAK-888[124] and Kamada's anti-COVID-19 IgG[125] are now developing, these have undoubtedly provided additional options for COVID-19 rescue.

Natural killer cell therapy

Natural killer (NK) cells are innate cytotoxic lymphocytes essential for host-rejection of both tumours and virus-infected cells. Therefore, NK cell therapy is a common therapeutic approach for solid tumours and haematological malignancies, whereas recently NK cells are evolutionarily designed to detect and eliminate virus-infected cells, which can be developed into a practical treatment for COVID-19. It is presumed that NK cell lyses antibody-coated virus-infected cells via ADCC, a key effector function.[126]

Normally stimulation and effector function of NK cell relies on the integration of signals derived from activating and inhibitory receptors. The surface of normal cells has expressed major histocompatibility complex (MHC) class I molecules to act as ligands for inhibitory receptors and conduce to the self-tolerance of NK cells. Whereas to evade T-cell recognition, viruses reduce expression of the MHC I molecules and result in a low inhibitory signal of NK cells. Plus, DNA damage response (DDR) activated by the virus increases cellular stress. Altogether these shift the balance of the signal towards NK cell activation, followed by stimulation of NK cells to remove virus-infected cells directly via ADCC or indirectly through secretion of pro-inflammatory cytokines at the times before replicating virion assembly and spreading of virus.[127]

Apart from the positive signaling, this defense mechanism also requires a cell to cell contact to initiate. Therefore, it is necessary to form a lytic synapse between the target cells and NK cells. The actin cytoskeleton is reorganized followed by polarization of microtubule organizing centre and secretory lysosome toward the lytic synapse. After docking to the plasma membrane of NK cells at the synapse, the secretory lysosome, eventually, is fused with the target cell plasma membrane to liberate its cytotoxic granules.[7] Perforin, a membrane pore-forming molecule which can permeabilise the cells.[128] This allows the entry of a serine protease, granzyme, into the target cells and activate caspase molecules to induce apoptosis of target cells (Fig. 5).[129] As a result, the cell cycle progression is disrupted by damaging the DNA, thereby dissolving the nucleus. Notably for ADCC, since NK cells express both FcγRIIC and FcγRIIIA,[130] they bind to the Fc portion of an

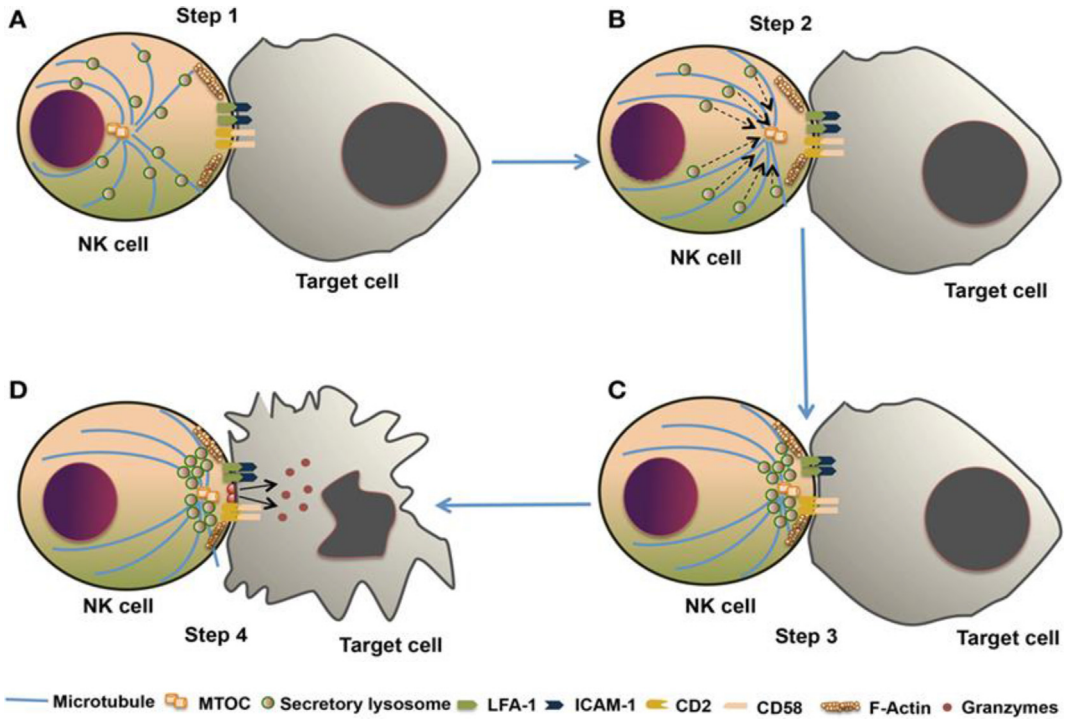


Figure 5 The cytotoxic response of NK cells.[7]

immunoglobulin. Both mediate ADCC via phosphorylation of immune tyrosine-based activating motifs (ITAM), which contained in the cytoplasmic tail of FcγRIIC and that of FcεRI-γ chains or CD3-ζ chains associated with FcγRIIIA. These ITAMs are phosphorylated upon binding of Fcγ receptors, followed by activating signals transduction within NK cells.[131] Once Fc receptors of NK cells recognize antibodies bound with the antigen of the virus-infected cells, degranulation of NK cells are stimulated into the immunological synapse, and the subsequent effector responses are the same with cellular cytotoxicity. The important role of ADCC is represented in several viral infections, including dengue virus infections,[132] cytomegalovirus infections,[133] and HIV-infected patients *in vitro*.[134] Given the studies from the past, NK cells may be also specific to SARS-CoV-2 infected cells in this way. For this purpose, investigations of SARS-CoV-2 using an animal model would be welcome, these studies could lay the groundwork for a suggestion that NK cell therapy be considered for a compelling strategy for COVID-19 pandemic.

Another irreplaceable effector response of activated NK cells would be the production of interferon-γ (IFN-γ). It directly exerts on host cells to provide resistance to the virus and prevent infection spread to other cells.[135] It also recruits and activates nearby immune cells such as cytotoxic T lymphocytes and CD4 T helper type 1 cells to promote MHC surface expression and adaptive immune response;[136] Smyth et al[137] thereby creating the second wave of durable antiviral response.[137] Noticeably, previous experimental infections have induced interleukin 12 (IL-12), can stimulate NK cells for IFN-γ production. It is observed during murine cytomegalovirus (MCMV)[138] and influenza virus;[139] however, it is not demonstrated in lymphocytic choriomeningitis virus (LCMV).[138] The above infections have shown the NK cell response is dependent upon the endogenous IL-12, conclusively suggesting that the IFN-γ production by NK cells is an outcome of IL-12 during viral infection. It would be reasonable to consider NK cell therapy for treating COVID-19, particularly for those who are unrelieved by antiviral medicine. On these grounds, to clarify the immune-mediated component of NK cells of COVID-19, efforts to develop an animal model would be appreciated. Be alert, cytokine storm induced by the exaggerated production of IFN-γ may be involved in the immunopathological damage in patients with COVID-19. Therefore, NK cell therapy may not be suitable for those patients who are suffered from cytokine storm syndrome, especially the critical illness individuals as aforementioned in this review.[33] The therapeutic value of this therapy, alternatively, may be reflected in immunocompromised patients with COVID-19.[140] In view of this, a thorough risk-benefit assessment must be committed to investigating the potential immune impact, particularly dose—response relationship should be taken into account during the clinical trial.

Unlike the antiviral drugs, NK cell therapy is fully dependent on harnessing the human immune system to fight the virus. Since NK cells can be differentiated from induced pluripotent stem cells (iPSCs) and gained from umbilical cord blood, they are a promising source of allogeneic NK cells and are potential to be true off-the-shelf cellular therapies, whereby providing NK cells with a patient-specific basis. Allogeneic induced NK therapy does not require a treatment involving patient-specific cells that spend weeks to synthesize, and it can be applied repeatedly, with the potential to improve clinical benefit based on optimisation of dose and schedule. In addition, NK cells are capable in recognising various infected cells without the reliance of the presentation of a single antigen, it is superior to T-cell based therapy as it omits some of the resistance mechanisms.[141] Most importantly, a clinical and manufacturing collaboration is designed to extend the therapeutic use of CYNK-001 to COVID-19.[142] If this allogeneic, off-the-shelf, placental-derived NK therapy shows a satisfactory result, it is feasibly to become a potential novel treatment for COVID-19.

Lastly, for both CP immunotherapy and NK cellular therapy, CP/NK must be manufactured and be utilized according to scrupulous ethical and controlled conditions to guarantee a possible role of these products of human origin.

Current challenges of clinical trials and future prospect of therapeutic interventions

The novel COVID-19 situation has escalated into an unprecedented worldwide crisis in the last few months. The scientists and physicians continue to actively monitor the developments, particularly paying attention to the health and rescue of sufferers and the community at large. With the concerted efforts and commitment to researches, many therapeutic regimens have been explored and advocated in the absence of definitive management protocols. Some therapies may be developed from desperation; fortunately, some of these show initial promise and potential efficacy. Nevertheless, published results of rigorous clinical trials are awaiting interpretation and evaluation of clinical impacts. Moreover, the research response may be insufficient to resolve problems relevant to optimal clinical practice. These can be attributed to the long lead times which are indispensable for design, implement, approval attainment and patient recruitment into randomised trials.[143] Noted that registration of all clinical trials should be done in publicly available domains before they are considered for publication.[144] Currently, over 300 interventional studies are focusing on the treatments of patients with COVID-19 are analysed,[145] these ongoing clinical trials have reflected an urgent unmet that need to determine the optimal treatment including specific antiviral therapy, modulating rationale of the immune system, as well as the capability of supporting failed organ systems. However, it is a pity that some of the studies have not been further analysed since they lack scientific rationale, insufficient provision of information regarding active ingredients and the applicability to mainstream medical practice is finite.[145] These indeed pose an obstacle towards a successful intervention for COVID-19, if no improvement for these limitations is done, the postponed development of efficient therapy, in particular, concerning the area of investigations on unmet medical need and life-threatening diseases, will lead to a delay in patients' access to treatment in hospitals. Hence it is essential to overcome the challenges to achieve satisfactory and undisrupted access to treatment in hospitals.

Although the limitations emerge among the clinical investigations, they provide valuable insights into a future perspective of treatment strategies. With much of the interest in remdesivir has been raised following the intravenous treatment of the first COVID-19 infected patient, and subsequent recovery, in the USA,[146] such inspiring result brings about the current 10 registered trials taking place globally to investigate efficacy for COVID-19.[145] While the efficacy of this drug is shown *in vivo* and *in vitro*.[59,54] Among other treatments in that top-tier category are chloroquine and hydroxychloroquine. There are 35 ongoing trials are now evaluating the use of these antimalarial drugs, both beneficial clinical and virological impacts have reported in CQ[73] and HCQ treatments.[80] Even though only 4 reports are using a robust double-blind randomised controlled protocol to evaluate the efficacy of CQ and HCQ,[145] the implication of their potency drive the implementation of therapeutic interventions. It is not difficult to imagine these antimalarial treatments are listed into approved therapies for COVID-19 in a foreseeable future. In addition, the affordable price of these drugs enables high cost-effectiveness, this alleviates the burdens of the medical cost of COVID-19.[73,67] While CP therapy has 12 registered trials at the present, with only 2 double-blind randomised clinical trials are being conducted.[145] The upcoming preliminary data may not be sufficient to warrant CP therapy as an official therapy but to provide supportive evidence in proving its competence and feasibility. It is estimated that there is still a distance for plasma-based therapy to reach the standard authorised treatment for COVID-19. Whereas there are 5 trials involving NK cell for investigations,[145] compare to the above treatment strategies, it is relatively far away to be developed as an intervention strategy in COVID-19. Lastly, ivermectin is still under investigation with a pending clinical trial, potential role in COVID-19 must be fully confirmed before deployment of global use (Table 1). Further clinical trials are expected to be conducted to obtain high-quality data for assessing potentials therapies objectively, so as to assure its effectiveness is satisfactory for COVID-19 qualified therapy. It is believed that there are more international collaboration and the globalisation of clinical trials with larger sample size which have a reliable scientific rationale and statistical rigour for providing conclusive results.

By and large, many clinical trials are focusing on antiviral treatments with certain amounts of them targeting in antimalarial drugs, this stands for some point of view that repositioning and repurposing the existing drugs are the main trends in the future therapeutic strategies. The repurposed drugs raise considerable interest in being the optimal therapeutic option in managing CoV infections, owing to their advantage of easy availability, known pharmacokinetic properties, sides effects and also well-established regimens.[147] It is forecasted that developmental antiviral treatments would be the fastest approved therapeutic intervention for COVID-19, efforts should also be directed toward evaluating immunotherapies and cellular therapies that are effective against viruses similar to SARS-CoV-2.

Conclusion

The recent COVID-19 outbreak has been deemed a global misfortune, especially the COVID-19 cases on the Diamond Princess Cruise Ship, which have raised ethical consideration concerning the psychological fragility and quality of life of the isolated passengers and crew members.[148] Apart from feeling their desperation and fear for tomorrow etched in the worried lines of their face, it is realized that the spread of COVID-19 cannot be fully restricted by quarantine alone. Such pathogenic COVID-19 is, therefore, demanding efficient therapeutic interventions to precisely treat the sufferers and prevent the new spread.

Although there are no specific therapies approved by the FDA for COVID-19, many potential therapies are now under development given the similarities and epidemiological characteristics of SARS-CoV. It has been confirmed that SARS-CoV-2 is genetically similar to SARS-CoV and share the same receptor ACE2. Cytokine storm is also observed in all SARS-CoV-2, SARS-CoV and MERS-CoV infections, therefore several antiviral treatments for SARS-CoV and MERS-COV infection have been considered for the COVID-19 rescue. Among the various treatment methods, remdesivir is one of the most promising antiviral therapeutics by acting an inhibitor of RdRp to terminate viral RNA synthesis. While CQ and HCQ exert multiple mechanisms to inhibit the viral entry, viral receptor glycosylation, post-translational modification of the viral protein, couple with immunomodulating activities, reflecting they have robust therapeutic values of COVID-19 treatments with pharmaceutical drugs, in which a synergistic effect was demonstrated in the combination of HCQ and azithromycin. Ivermectin is an anti-parasitic drug which shows a potential antiviral ability against SARS-CoV-2 *in vitro*, its true inhibitory effect is yet to be determined. Besides, the neutralizing effect of antibodies is shown in CP therapy to suppress viral load and improve the immune system, which heightens its value in immunotherapy for COVID-19. Likewise, superior effector functions in NK cell therapy to enhance immunity also features its possible role in cellular therapy for COVID-19 pneumonia. While urgent, the impact of patient-derived mutation should be taken into account to prevent unexpected risk. Nevertheless, the present review is limited by the non-stopping changing of the COVID-19, ever-updating statistics and the continued unravelling of new study findings. Although researches are in progress to evaluate the clinical impacts of each therapy, the documented clinical data on different treatment approaches for SARS-CoV-2 are under demand.

Undoubtedly, the novel COVID-19 pandemic is one of the biggest humanity challenges. The near and distant future is accumulated by all those irreplaceable days. Lessons from the 2003 SARS outbreak have already warned us not to exclude the possibility of new outbreaks of SARS.[6] There is no time for us to sacrifice the lives of today; instead, it is now the time for us to demand a concerted endeavor for COVID-19 rescue. Further research should be conducted to define the exact mechanism of SARS-CoV-2 pathogenesis, suitable animal models or *ex vivo* human lung tissues aid in studying replication, transmission and spread of the novel viruses, thereby facilitating the development of the

Table 1 A summarised table of therapeutic approaches in this review.

	Remdesivir	Chloroquine	Hydroxy-chloroquine	Ivermectin	CP therapy	NK cell therapy
Classification	Nucleotide analogue	Antimalarial drug	Antimalarial drug	Antiparasitic drug	Immunotherapy	Cellular therapy
Rationale for use	Antiviral activity against SARS-CoV-2 *in vitro*; Treat the first infection case in the USA	Potential antiviral activity against SARS-CoV-2 *in vitro* coupled with immunomodulating activities	Less toxic than CQ; in conjunction with azithromycin to strengthen antiviral activity	Show a robust inhibitory effect on SARS-CoV-2 *in vitro*, yet its dosage regimen remains controversial	Historical precedents including SARS and MERS showed proven efficacy; improved clinical outcomes in COVID-19 patients	Harness immune system to combat with the virus; can be true off-the-shelf cellular therapy
Mechanism of action	Inhibitor of RdRp, competes with ATP by inserting into viral RNA chains to induce premature termination; its potency is also warranted by escaping viral enzyme proofreading	Viral entry is inhibited by elevated lysosomal pH; Virion assembly and release is inhibited by post-translational modification	Similar to that CQ owing to its similar chemical structures	Inhibit IMPα/β-mediate d nuclear import by binding to NLS-binging pocket of IMPα	Virus specific antibodies from the donated plasma exert a neutralizing effect on the infected host cells	NK cells remove virus-infected cells directly via ADCC or indirectly through secretion of pro-inflammatory cytokines
Status of clinical trials	Currently 8 registered trials	Currently 48 registered tials		One clinical trial pending	Currently 12 registered trials	Currently 2 registered trials
Clinical trial phases and registration numbers (As registered on WHO)	NCT04409262: Phase 3 NCT04410354: Phase 2 NCT04292730: Phase 3 NCT04292899: Phase 3 NCT04401579: Phase 3 EUCTR2020-001366-11-RO: Phase 3 EUCTR2020-001052-18-DK: Phase 3 NCT04315948: Phase 3	NCT04345692: Phase 3 NCT04345653: Phase 2 NCT04381936: Phase 2 NCT04344951: Phase 2 NCT04339816: Phase 3 NCT04421664: Phase 3 NCT04345861: Phase 2/Phase 3 NCT04341727: Phase 3 NCT04332991: Phase 3 NCT04329832: Phase 2 EUCTR2020-001270-29-GB: Phase 3 EUCTR2020-001270-29-CZ: Phase 3 EUCTR2020-001270-29-DK: Phase 2/Phase 3 RBR-95yjmq: Phase 3 NCT04344379: Phase 3 RBR-9d8z6m: Phase 3 NCT04410562: Phase 3 NCT04405921: Phase 3 NCT04428268: Phase 2 ISRCTN99916292: Phase 3		NCT04422561: Phase 2/ Phase 3 NCT04343092: Phase 1 ISRCTN40302986: Phase 3 IRCT20200422047168N2: Phase 2/Phase 3 NCT04407130: Phase 2 NCT04391127: Phase 3	NCT04425915: Phase 3 NCT04407208: Phase 1 NCT04346446: Phase 2	NCT04324996: Phase 1 & 2 NCT04280224: Phase 1

EUCTR2020-001257-51-DK: Phase 3
NCT04372017: Phase 3
NCT04408456: Phase 3
NCT04363827: Phase 2
NCT04322123: Phase 3
NCT04411433: Phase 3
NCT04403100: Phase 3
NCT04330690: Phase 2 LBCTR2020043495: Phase 3
EUCTR2020-001366-11-RO: Phase 3
PACTR202004893013257: Phase 3
IRCT20200428047228N2:
Phase 3
IRCT20200523047550N1: Phase 3
IRCT20200502047268N1: Phase 3
SLCTR/2020/011: Phase 4
IRCT20200325046859N2: Phase 2-3
NCT04358068: Phase 2
EUCTR2020-001482-37-DE: Phase 3
NCT04420247: Phase 3
NCT04334967: Phase 4
EUCTR2020-001363-85-DK: Phase 3
PER-011-20: Phase 3
PACTR202005622389003: Phase 3
NCT04316377: Phase 4
NCT04391127: Phase 3
NCT04347512: Phase 3
NCT04429867: Phase 4
NCT04414241: Phase 3
NCT04333914: Phase 2
NCT04333654: Phase 1
PACTR202004801273802: Phase 3

therapeutic intervention. Also, clinical trials should be focused on the determinants of therapies safety and efficacy.

Conflicts of interest

All authors declare no conflicts of interests.

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