Review

The immunology of COVID-19: is immune modulation an option for treatment?

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In December, 2019, an outbreak of COVID-19 emerged in Wuhan, China and quickly spread globally. As of May 7, 2020, there were 3 672 238 confirmed infections and 254 045 deaths attributed to COVID-19. Evidence has shown that there are asymptomatic carriers of COVID-19 who can transmit the disease to others. The virus incubation time shows a wide range (0–24 days) and the virus displays a high infectivity. It is therefore urgent to develop an effective therapy to treat patients with COVID-19 and to control the spread of the causative agent, severe respiratory syndrome coronavirus 2. Repurposing of approved drugs is widely adopted to fight newly emerged diseases such as COVID-19, as these drugs have known pharmacokinetic and safety profiles. As pathological examination has confirmed the involvement of immune hyperactivation and acute respiratory distress syndrome in fatal cases of COVID-19, several disease-modifying anti-rheumatic drugs (DMARDS), such as hydroxychloroquine and tocilizumab, have been proposed as potential therapies for the treatment of COVID-19. In this Review, we discuss the immunological aspects of COVID-19 and the potential implication of DMARDs in treating this disease.

Introduction

In December, 2019, hospitals in Wuhan, China began to report cases of pneumonia of unknown cause. Most of the initially identified patients were geographically linked to a local wet seafood wholesale market, where living or slaughtered wild animals are sold. The virus then rapidly spread to over 200 countries and territories, resulting in 3 672 238 confirmed cases and 254 045 deaths globally according to a report released by WHO on May 7, 2020. Subsequent deep sequencing of lower respiratory tract samples identified a novel coronavirus distinct from the other strains of coronavirus known to infect humans, subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—a highly contagious virus that can be transmitted from person to person.[1] WHO designated the disease caused by SARS-CoV-2 infection as COVID-19. Similar to other diseases caused by coronaviruses, the main transmission route of SARS-CoV-2 is via aerosolised droplets. Other possible transmission routes such as direct contact, oral–faecal route, and mother-to-child transmission have been proposed, but further proof is needed with regard to these.[2] A retrospective study done at the beginning of the pandemic reported an incubation period of SARS-CoV-2 of approximately 5–14 days;[3] however, a more recent report indicates that the incubation period could be as long as 24 days.[4]

There is no effective cure for SARS-CoV-2 infection and the most common treatment for patients with COVID-19 is supportive care. Although multiple anti-viral drugs, including remdesivir and lopinavir plus ritonavir, have been used in clinical practice,[5,6] the safety and efficacy of these are still unclear and are under clinical evaluation. Immune-mediated lung injury and acute respiratory distress syndrome (ARDS) are associated with adverse outcomes in patients with COVID-19.[7] Histological examination of lung biopsy tissue from a patient who died of COVID-19 showed bilateral diffuse alveolar damage and fibroblastic proliferation in airspaces, and laboratory tests indicated a hyperactivated status of circulating CD4

and CD8 lymphocytes.[7,8] Due to the hyperactive nature of the immune system in some patients with severe COVID-19, several disease-modifying anti-rheumatic drugs (DMARDs), such as tocilizumab (interleukin [IL]-6 receptor inhibitor), baricitinib (Janus kinase [JAK] inhibitor), anakinra (IL-1 receptor antagonist), and the antimalarial drug hydroxychloroquine (or chloroquine), have been proposed as potential treatments for COVID-19. In this Review, we discuss the immunological aspects of the SARS-CoV-2 virus infection and the potential implication of DMARDs in the treatment of patients with COVID-19.

Overview of coronavirus

Coronaviruses are a group of highly diverse, enveloped, positive-sense, single-stranded RNA viruses that belong to two subfamilies, Coronavirinae and Torovirinae, in the family of Coronaviridae. These viruses were first discovered in the 1960s and can be further classified into four main genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*, on the basis of their phylogenetic relationships and genomic structures.[9] Among these four genera, alphacoronaviruses and beta-coronaviruses primarily cause respiratory and intestinal infection in mammals, whereas gammacoronaviruses and deltacoronaviruses mainly infect birds. Currently, there are seven strains of coronaviruses that are known to infect humans, including the recently identified SARS-CoV-2, human coronavirus 229E (HCoV-229E), OC43 (HCoV-OC43), NL63 (HCoV-NL63), HKU1 (HCoV-HKU1), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV).[10–12] Domestic or wild animals could have important roles as zoonotic reservoirs that enable virus transmission to humans. On the basis of current sequence databases, the origins of SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-229E, and SARS-CoV-2 are thought to be bats, whereas HCoV-OC43 and HKU1 probably originated from rodents.[13–17] Although most coronavirus infections cause only mild respiratory symptoms,

infection with SARS-CoV, MERS-CoV, and SARS-CoV-2 can be lethal.

SARS-CoV first appeared in southern China and quickly spread around the world between 2002 and 2003. This virus was identified as the causative agent of the global pandemic SARS,[18] which led to substantial morbidity and mortality. A decade after SARS, an outbreak of MERS-CoV emerged in 2012.[19] Most people with MERS had no previous contact with bats, leading to the identification of camels as an intermediate host.[20] Patients with SARS or MERS present with a variety of clinical features, ranging from asymptomatic or mild respiratory illness to fulminant severe ARDS with extra-pulmonary complications.[21,22]

SARS-CoV-2 belongs to the genus of *Betacoronavirus*, and on the basis of evolutionary analysis, is most similar to the SARS-like coronavirus from the Chinese horseshoe bat, with a nucleic acid homology of 84%.[3,10] SARS-CoV-2 also has 78% similarity with SARS-CoV and 50% with MERS-CoV, at the nucleic acid level.[3,10] Only 10 days after the release of the SARS-CoV-2 genome, researchers found a similar coronavirus from fruit bats, BatCoVHKU9–1, based on evolutionary characteristics.[23] Several later reports suggested that snakes, mink, and pangolins could be intermediate hosts, based on codon preference and viral infection patterns.[24–26] At the onset of the COVID-19 pandemic, the main symptoms were fever (98%), cough (76%), and myalgia or fatigue (44%).[27] About half of the patients developed breathing difficulty in one week and the severely ill patients soon developed ARDS, acute cardiac injury, secondary infections, or a combination thereof.[27] The diagnosis of the disease mainly depends on SARS-CoV-2 RNA detection in nasopharyngeal swab by real-time polymerase chain reaction, epidemiological history, clinical manifestations, and lung imaging.

Immune response against SARS-CoV-2

The invasion and pathogenesis of SARS-CoV-2 are associated with the host immune response. The spike glycoprotein (S protein) on the viral envelop binds to its receptor, angiotensin-converting enzyme 2 (ACE2), on the surface of human cells.[15,23] An analysis of the structure of the SARS-CoV-2 S protein and its binding affinity for ACE2 using cryogenic electron microscopy and surface plasmon resonance showed that the structure of SARS-CoV-2 S protein is very similar to that of SARS, although with minor differences.[28] The affinity of SARS-CoV-2 S protein binding to ACE2 is 10 to 20 times higher than that of the SARS S protein, suggesting that SARS-CoV-2 might transmit more readily from person to person.[28]

Innate immunity is the first line of defence against virus invasion. Viral infection of mammals activates intracellular pattern recognition receptors that sense pathogen-associated molecular patterns, such as double-stranded RNA or uncapped mRNA. The recognition of pathogen-associated molecular patterns results in subsequent cytolytic immune responses, mainly through the type I interferons (IFN) and natural killer cells. Adaptive immunity also plays an important part in viral clearance via activated cytotoxic T cells that destroy virus-infected cells and antibody-producing B cells that target virus-specific antigens. Patients with COVID-19, especially those with severe pneumonia, are reported to have substantially lower lymphocyte counts and higher plasma concentrations of a number of inflammatory cytokines such as IL-6 and tumor necrosis factor (TNF).[27,29,30] Another study[29] reported that CD4+ T cells, CD8+ T cells, and natural killer cells were reduced in severely ill patients compared with those with mild disease symptoms. Moreover, a substantial reduction of CD4+ T cell and CD8+ T cell counts in the peripheral blood was also observed in a patient who died.[7] Notably, the proinflammatory subsets of T cells, including IL-17-producing CCR4+ CCR6+ CD4+ (T-helper 17 or Th17) cells and perforin and granulysin-expressing cytotoxic T cells were increased, which could be partly responsible for the severe immune injury in the lungs of this patient.[7]

The anti-viral immune response is crucial to eliminate the invading virus, but a robust and persistent anti-viral immune response might also cause massive production of inflammatory cytokines and damage to host tissues.[31] The overproduction of cytokines caused by aberrant immune activation is known as a cytokine storm. In fact, in the late stages of coronavirus disease, including SARS, MERS, and COVID-19, cytokine storms are a major cause of disease progression and eventual death.[7,32,33] Huang and colleagues[27] found increased plasma concentrations of both Th1 (eg, IL-1β and IFNγ) and Th2 (eg, IL-10) cytokines. Notably, patients admitted to the intensive care unit (ICU) had higher plasma concentrations of IL-2, IL-7, IL-10, granulocyte-colony stimulating factor, IFNγ-induced protein-10 (IP-10), macrophage chemoattractant protein-1, macrophage inflammatory protein 1α, and TNF compared to those not admitted to the ICU. Two other studies[29,34] also showed that plasma IL-6 concentrations were above the normal range in patients with severe symptoms of COVID-19 compared with healthy individuals and those with milder symptoms. Mehta and colleagues[35] suggest that secondary haemophagocytic lymphohistiocytosis (sHLH) could be associated with severe COVID-19 cases. HLH is a disease entity characterised by an uncontrolled cytokine storm and expansion of tissue macrophages or histiocytes that exhibit haemophagocytic activity.[36] HLH can result from genetic defects in cytolytic pathways (familial or primary HLH) or other diseases such as infection, malignancy, and rheumatic disease (sHLH).[37] In 1952, Farquhar and Claireaux first described cytokine storm in patients with HLH.[38] The characteristics of HLH, including hypercytokinaemia, unremitting fever, cytopenias, hyper-ferritinaemia, and multi-organ damage, are commonly seen in seriously ill patients with COVID-19.[27,35] It is suggested that alveolar macrophages expressing ACE2 are the primary target cells for SARS-CoV-2 infection. These activated macrophages may play an important part in HLH-like cytokine storm during COVID-19.[39] Thus, early identification and appropriate treatment of this

	Mechanism of action
csDMARDs	
Chloroquine or hydroxychloroquine	Interference with ACE2 to block virus invasion; increase of endosomal pH required for virus fusion; mild immune suppression
Glucocorticoids	Suppression of immune and inflammatory responses
Leflunomide	Inhibition of virus replication
Thalidomide	Reduction of inflammatory cell infiltration; reduction of cytokine storm; reduction of lung damage and pulmonary interstitial fibrosis
bDMARDs	
Tocilizumab	Blockade of IL-6 receptor and its downstream signalling pathways
Anakinra	Blockade of IL-1 receptor and its downstream signalling pathways
tsDMARDs	
Baricitinib	JAK inhibitor; blockade of viral invasion through the inhibition of AAK1; immune suppression
Ruxolitinib	JAK inhibitor; immune suppression
Cell therapy	
Stem cells	Suppression of inflammation; proviral silencing
Plasma therapy	
Convalescent plasma	Promotion of virus elimination via virus-specific antibodies

AAK1=AP2-associated protein kinase 1. ACE2=angiotensin-converting enzyme 2. bDMARDs=biologic disease-modifying anti-rheumatic drugs. csDMARDs=conventional synthetic disease-modifying anti-rheumatic drugs. IL=interleukin. JAK=Janus kinase. tsDMARDs=targeted synthetic disease-modifying anti-rheumatic drugs.

Table: Repurposing of immune-modulating therapies for COVID-19

hyperinflammatory status is important for reducing the mortality of patients with COVID-19.[35]

Potential immunotherapy in COVID-19

Evidence has shown that asymptomatic COVID-19 carriers can transmit the disease to others and that the virus has a wider range of incubation time than initially thought (0–24 days).[4] In addition, the virus displays a high infectivity. If the virus continues to mutate to lower its pathogenicity, there is a high possibility that it might coexist with humans. Therefore, there is an urgent need to develop therapies to treat SARS-CoV-2. Repurposing of approved drugs is commonly employed to fight against newly emerged diseases, such as COVID-19, as these drugs have known pharmacokinetic and safety profiles. Due to the importance of immune imbalance in the pathogenesis of SARS-CoV-2 infection, several immune-modulating drugs that regulate different aspects of inflammation (table) are being tested for their efficacy in the treatment of severe COVID-19. Hyperinflammation is an important determinant of disease outcome in COVID-19, and immunosuppression might be beneficial to reduce the mortality in patients with severe symptoms.[7,35] Therefore, early identification of such patients is crucial. It has been proposed that laboratory tests of ferritin, lymphocyte or leukocyte counts, platelet counts, erythrocyte counts, and sedimentation rate could be used to screen patients at high risk of hyperinflammation. Application of the HScore, used for the evaluation of patients with sHLH, was recommended by Mehta and colleagues[35] to identify patients with COVID-19 at high risk of hyperinflammation. The HScore combines both laboratory and clinical parameters, including serum aspartate aminotransferase, triglycerides, fibrinogen, ferritin, cytopaenias, body temperature, organomegaly, haemophagocytosis on bone marrow aspirate, and signs of immunosuppression.[35] In addition, evaluation of cytokine profiles and immune cell subsets has important implications for selecting appropriate immunosuppressants (eg, tocilizumab could be considered in patients with high concentrations of serum IL-6). Given the fact that anti-viral immunity is required to recover from COVID-19, the pros and cons of using an immunosuppressant on these patients should be carefully considered. The severity of the hyperinflammation and viral load or replication status needs to be taken into consideration. One way to avoid the suppression of anti-viral immunity is to choose selective instead of broad immunosuppressive drugs. The timing of treatment is also crucial to reduce the side-effects of immunosuppression; unfortunately there is not yet any definitive evidence with regard to the appropriate timing of administration of these agents. Further studies are required to determine the appropriate timing and routes of drug administration.

Biological immuno-modulating drugs

IL-6 is a key inflammatory cytokine that has a critical part in inflammatory cytokine storm and is elevated in patients with COVID-19.[29,34] Tocilizumab, a recombinant humanised monoclonal antibody against the IL-6 receptor, is widely used in treatment for autoimmune diseases, such as rheumatoid arthritis.[40] In patients with COVID-19, IL-6-producing CD14+ CD16+ inflammatory monocytes were significantly increased, and numbers of these cells were further increased in patients with COVID-19 admitted to the ICU.[41] The authors of this study proposed that hyperactivated Th1 cells producing granulocyte-macrophage colony stimulating factor (GM-CSF) and IFNγ in the lung promote IL-6-producing monocytes through release of GM-CSF, suggesting that both IL-6 and GM-CSF might be potential therapeutic targets in patients with COVID-19.[41] Tocilizumab is a first-line drug for the treatment of cytokine release syndrome (a rapid and massive release of cytokines into the blood from immune cells, usually caused by immunotherapy), especially in patients with comorbidities. In terms of mechanism, tocilizumab binds to both the membrane and soluble forms of IL-6 receptor, thereby suppressing the JAK-signal transducer and activator of transcription (STAT) signalling pathway and production of downstream inflammatory molecules.[42,43] There are many ongoing trials assessing the efficacy of tocilizumab in COVID-19 (appendix p 1). However, animal studies have shown that IL-6 is required for the clearance of viruses and control of pulmonary inflammation.[44] Therefore, clinicians should pay close attention to the possibility that blocking IL-6 could interfere with viral clearance or exacerbate lung inflammation. A recent observational study[45] from China reported that tocilizumab treatment in severe COVID-19 cases resulted in improvement in COVID-19 symptoms,

See **Online** for appendix

peripheral oxygen saturation, and lymphopenia within a few days. A substantial remission of lung lesion opacity in chest CT scan was observed in 95% of patients (19 of 20) after 5 days of treatment, and all patients were discharged after an average of 15.1 days of hospital stay.[45]

Blockade of the IL-1 pathway is used for the treatment of some hyperinflammation conditions. The IL-1 receptor antagonist anakinra is approved for rheumatoid arthritis, Still's disease, and cryopyrin-associated periodic syndrome. A phase 3 randomised controlled trial (RCT) for severe sepsis reported that treatment with anakinra was associated with a significantly lower 28-day mortality in patients who were septic with hyperinflammation, without increased adverse events.[46] A retrospective analysis[47] of 44 patients with sHLH who were treated with anakinra indicated that treatment with anakinra resulted in a 57% decrease of ferritin concentrations, and early initiation of anakinra was associated with reduced mortality. Since IL-1 was reported to be increased in some patients with COVID-19,[27] blockade of IL-1 seems a reasonable approach for the treatment of hyperinflammation in these patients.[35] Several trials of anakinra are currently underway, including a phase 2/3 clinical trial evaluating the efficacy and safety of anakinra and emapalumab (IFNγ inhibitor) in reducing hyperinflammation and respiratory distress in patients with COVID-19 (NCT04324021; appendix p 1).

Targeted synthetic immunosuppressants

Baricitinib is a small molecule compound that selectively inhibits the kinase activity of JAK1 and JAK2. Baricitinib can be used in combination with one or more TNF inhibitors and is approved for the treatment of rheumatoid arthritis[48] and psoriatic arthritis.[49] Through searching the BenevolentAI database, Richardson and colleagues[50] predicted that baricitinib might effectively reduce the ability of SARS-CoV-2 virus to infect lung cells.[51] As noted, SARS-CoV-2 binds to the ACE2 receptor on host cells and enters lung cells through receptor-mediated endocytosis. ACE2 is widely expressed in several tissues, including renal, vascular, heart, and lung. High concentrations of ACE2 expression on pulmonary AT2 alveolar epithelial cells makes these cells particularly susceptible to SARS-CoV-2 infection.[52] AP2-associated protein kinase 1 (AAK1) regulates endocytosis via phosphorylation of the clathrin adaptor protein AP2. Richardson and colleagues identified six high-affinity AAK1 inhibitors from 47 clinical candidates in the BenevolentAI database. Baricitinib was then further selected based on its relatively mild side-effects and the feasibility to achieve effective concentrations in the blood. In addition, baricitinib can also bind to cyclin G-related kinases, which also regulate receptor-mediated endocytosis. The immunosuppressive function of baricitinib might also be of benefit to the hyperactive immune status in severe cases of COVID-19 where immune-mediated lung injury and ARDS might occur.

Ruxolitinib, another oral JAK1 and JAK2 inhibitor approved specifically for the treatment of myelofibrosis, has been used for the treatment of sHLH. Ruxolitinib was shown to rapidly improve respiratory, liver, and haemodynamic function in an 11-year-old boy with refractory HLH,[53] and to substantially improve serum ferritin, lactate dehydrogenase, fibrinogen, and liver function in a 38-year-old female patient with refractory Epstein-Barr virus-related sHLH.[54] An open-label clinical trial[55] showed that ruxolitinib was well tolerated and manageable for treating sHLH, with symptoms and cytopenias improved in all (n=5) patients within the first week of ruxolitinib treatment. Concentrations of ferritin, soluble IL-2 receptor, and STAT1 phosphorylation were also reduced after the administration of ruxolitinib.[55] Animal studies showed that inhibition of JAK1 and JAK2 using ruxolitinib improved weight loss, organomegaly, anaemia, thrombocytopenia, hypercytokinaemia, and tissue inflammation in animal models of both primary HLH and sHLH by reducing STAT1-dependent CD8+ T-cell expansion.[56]

Considering the similar hyperinflammatory nature of sHLH and severe COVID-19, JAK1 and JAK2 inhibitors such as baricitinib and ruxolitinib could be potential treatments for the hyperinflammation seen in COVID-19.[50] Several registered RCTs are evaluating the efficacy of ruxolitinib and baricitinib in the treatment of COVID-19 (appendix p 2).

Chloroquine and hydroxychloroquine

Chloroquine and hydroxychloroquine, initially used as antimalarial drugs, have been widely used in several infectious (HIV, Q fever, and fungal infections), rheumatological (systemic lupus erythematosus, antiphospholipid antibody syndrome, rheumatoid arthritis, and Sjogren's syndrome), and other immunological diseases.[57] The mechanism of action of hydroxychloroquine is diverse and includes anti-inflammatory action, immune regulation, anti-infection, anti-tumour, metabolic regulation, and anti-thrombosis. Chloroquine has been shown to have anti-coronavirus effects in vitro. Based on this, and the immunoregulatory actions of these drugs, chloroquine and hydroxychloroquine were proposed in the treatment of COVID-19. These drugs increase the endosomal pH required for SARS-CoV-2 endocytosis and cell fusion (figure). Chloroquine also interferes with the glycosylation of ACE2, which is required for virus attachment to host cells.[58] Chloroquine was first reported in 2020 to be a potent inhibitor of COVID-19 using an in vitro SARS-CoV-2-infected Vero-E6 cell culture model.[59]

Hydroxychloroquine is a derivative of chloroquine that has similar pharmacokinetics and mechanism of action as chloroquine, but substantially fewer side-effects.[60] Compared with other immunosuppressant drugs such as methotrexate, the use of hydroxychloroquine and chloroquine is associated with a reduced risk of infection, even with chronic use.[61,62] Therefore, hydroxychloroquine is more commonly used in patients with rheumatic diseases
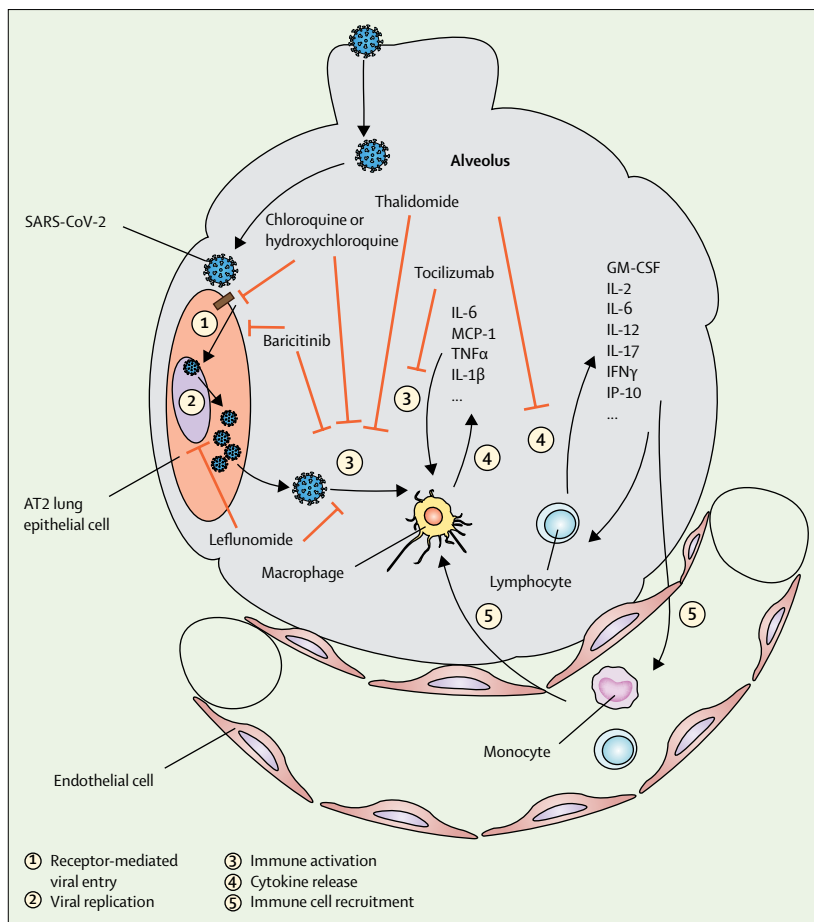
Figure: Potential mechanisms of action for DMARDs in COVID-19
DMARDs could target different stages of the virus infection and immune activation. AT2=alveolar type 2. DMARDs=disease modifying anti-rheumatic drugs. GM-CSF=granulocyte-macrophage colony stimulating factor. IFNγ=interferon gamma. IL=interleukin. IP-10=interferon gamma-induced protein 10. MCP-1=monocyte chemoattractant protein-1. SARS-CoV-2=severe respiratory syndrome coronavirus 2. TNFα=tumor necrosis factor alpha.

and other conditions. Hydroxychloroquine has been used to treat HIV-1 in humans as early as the 1990s. In a randomised, double-blinded, placebo-controlled clinical trial of 40 asymptomatic patients with HIV-1, 800 mg/d hydroxychloroquine treatment for 8 weeks reduced the plasma concentration of HIV-1 RNA, preserved CD4+ T-cell counts and proliferative responses, and lowered serum IL-6 concentrations, compared with the placebo group.[63] Although it takes 1–3 months for hydroxychloroquine and chloroquine to fully take effect in patients with rheumatic disease, the drugs' anti-viral effect is relatively rapid. Hydroxychloroquine treatment as short as 3 days was shown to accelerate virus clearance in patients with COVID-19, and azithromycin reinforced the anti-viral effect.[64] There are a number of ongoing clinical trials testing the efficacy of hydroxychloroquine and chloroquine in COVID-19 (appendix pp 2–5). Although a recent randomised trial has shown that chloroquine and hydroxychloroquine might improve pneumonia symptoms,

laboratory tests, and decrease the progression to severe or critical conditions,[65] other studies reported either no benefits[66–68] or hazardous effects after chloroquine or hydroxychloroquine treatment.[69] Notably, treatment in patients with COVID-19 might cause cardiotoxicity, especially when used at a high dose.[70,71] Therefore, results from ongoing trials are required to assess the efficacy and safety of hydroxychloroquine in COVID-19.

Glucocorticoids
Glucocorticoids and their synthetic analogues have been widely used in rheumatic disease to control autoimmune response.[72] Due to their rapid immunosuppressive effect, glucocorticoids are frequently used in hyperinflammatory syndromes, such as ARDS. In patients with ARDS, glucocorticoid treatment improves oxygen saturation, inflammatory markers, ICU length of stay, and ventilator-free days, although its effect on mortality was not consistent between trials.[73–75] In coronavirus disease, inflammation-induced lung injury and ARDS are associated with adverse outcomes.[26,76] Histological investigations showed severe lung inflammation and diffuse alveolar damage in patients with coronavirus disease.[7,77] Therefore, corticosteroids are commonly used in severe cases of coronavirus disease including SARS, MERS, and COVID-19 to control immune-mediated damage of lung tissue.[27,30,78,79] However, clinical evidence has not supported a beneficial effect of glucocorticoids in coronavirus illness. In a retrospective study[79] of 309 critically ill patients with MERS, after statistical adjustment for time-varying confounders, corticosteroid therapy was not significantly associated with improved 90-day mortality, and resulted in delayed clearance of the MERS coronavirus RNA. In a systematic review of SARS treatments that included 29 studies with cortiocosteroid use, 25 studies were inconclusive with regards to the effect of corticosteroid in SARS and four reported corticosteroids as causing possible harm. Therefore, high-quality RCTs are needed to provide conclusive evidence.

Leflunomide
Leflunomide is a low-molecular weight, synthetic, oral anti-rheumatic drug. The mechanism of its action includes inhibition of pyrimidine synthesis, inhibition of protein tyrosine stimulation, inhibition of nuclear factor kappa beta, and anti-tumour effects.[80–83] Leflunomide has been widely used for the treatment of rheumatoid arthritis,[84] and due to its immunosuppressive function, the drug is also used in organ transplantation.[85] Another important function of leflunomide is that it inhibits virus replication. In vitro studies have shown that the active metabolite of leflunomide (A77 1726) protects umbilical cord epithelial cells and fibroblasts from infection with human cytomegalovirus.[80,86] Electron microscopy revealed that the morphology of virions in the cytoplasm was abnormal and the assembly of virus particles could not be completed in cytomegalovirus-infected cells treated with A77 1726, indicating that leflunomide interferes with the assembly of

virus capsid.[80] In a study[87] of 17 patients with cytomegalovirus disease, 88% (15) of the patients clinically responded to leflunomide therapy (3 doses of 100 mg/day, followed by 20 mg/day—a dose that maintains blood concentrations of 25–50 ng/mL), with viral clearance from blood and healing of involved organs. Therefore, leflunomide is effective for treatment of cytomegalovirus disease.[88]

The anti-viral efficacy of leflunomide has also been observed in transplant recipients infected with BK virus[89] and human papillomavirus (HPV).[90] In patients with polyomavirus type BK nephropathy, those that had blood A77 1726 concentrations higher than 40 µg/mL had either cleared the virus or showed progressive reductions of viral load in blood and urine.[89] In another study,[90] leflunomide successfully cleared verrucae vulgaris and molluscum lesions in four renal transplant patients with cutaneous warts, usually caused by HPV. However, it is worth noting that leflunomide has been associated with interstitial lung disease.[91,92] Leflunomide-associated interstitial lung disease usually occurs within the first 20 weeks of leflunomide initiation. There was a high mortality in patients with pre-existing interstitial lung disease, diffuse alveolar damage, or ground glass shadowing on high resolution CT.[91] The exact mechanism remains unclear, but the presence of pre-existing interstitial lung disease, cigarette smoking, low bodyweight, and the use of methotrexate are risk factors for leflunomide-induced interstitial lung disease.[92] It remains unclear if leflunomide would improve or exacerbate the pulmonary lesions in patients with COVID-19. There is currently one clinical trial aiming to evaluate the efficacy and safety of oral leflunomide tablets against pneumonia caused by SARS-CoV-2 (appendix p 2).

Thalidomide

Another DMARD, thalidomide, which has both anti-inflammatory and anti-proliferative activity, has also been used in viral infections. Animal studies have shown that thalidomide inhibits lung injury in mouse models of H1N1 influenza virus infection, with improved survival, reduced inflammatory cell infiltration, reduced concentrations of cytokines (IL-6 and TNF) and chemokines (RANTES and IP-10), and reduced nuclear factor kappa beta activity.[93] It was concluded that thalidomide could be an alternative treatment when new influenza viruses emerge, especially before new vaccines are developed. A UK study[94] indicated that thalidomide has immunomodulatory and immune remodelling effects by inhibiting TNF, another critical cytokine in COVID-19-associated lung injury. In addition, some studies have shown that thalidomide can treat pulmonary interstitial fibrosis and combat cytokine storm.[95,96] These studies indicate a potential therapeutic value of thalidomide in viral infection. In a case report,[97] a 45-year-old female patient with severe COVID-19 and elevated concentrations of circulating cytokines, including IL-6, IL-10, and IFNγ, on admission was treated with oral thalidomide (100 mg once a day) and low-dose methylprednisolone (40 mg intravenously, every 12 h for

3 days; and then 40 mg intravenously once a day for 5 days) due to the severity of clinical manifestations and lack of response to other treatments. The patient's clinical condition, including oxygen index, fever, nausea, and vomiting resolved within 1 week after thalidomide treatment. Concentrations of IL-6, IL-10, and IFNγ all returned to normal range after 6 days of treatment, SARS-CoV-2 tests in swab specimens were negative after 1 week of treatment, and lung lesions disappeared 12 days after treatment.[97] Although this is a single case, it could provide some useful insight for further clinical investigation. There are two clinical trials evaluating the therapeutic potential of thalidomide in patients with moderate or severe COVID-19 (appendix p 2).

Other immune-modulating therapies

There are also many other immune-modulating strategies under clinical investigation for the treatment of COVID-19, such as stem-cell therapy and convalescent plasma treatment. Mesenchymal stem cells (MSCs) are of increased importance in inflammatory disease due to their anti-inflammatory properties. Animal experiments showed that MSC treatment was able to reduce influenza A H5N1-induced acute lung injury in vivo.[98] Stem cells are able to suppress the activities of viruses via *Chaf1a*-mediated and *Sumo2*-mediated epigenetic regulation (termed proviral silencing).[99] Several phase 1 and 2 clinical trials have confirmed the safety of MSC therapy in patients with ARDS, and have shown beneficial effects.[100,101] However, several issues have limited MSC use in clinic, such as the lack of clarity with regard to optimal dose and route of MSC delivery, difficulties in large-scale production and cryopreservation, and the potential for substantial variability. There are several ongoing clinical trials testing the efficacy of MSC in COVID-19 (appendix pp 5–6).

Convalescent plasma from patients who have recovered from SARS-CoV-2 infection has also been proposed as a potential treatment for COVID-19. Convalescent plasma has been used in many severe infections such as SARS, MERS, and Ebola, as one of the few therapeutic strategies in the absence of vaccines or other specific treatments.[102] The efficacy of such therapy, especially in COVID-19, is being evaluated in ongoing trials (appendix p 6).

Conclusions and outlook

SARS-CoV-2 has spread rapidly since it first emerged in December, 2019, and COVID-19 is characterised as a pandemic by WHO. As a new emerging virus, there is no approved effective drug or vaccine. As of April 16, 2020, several existing drugs are being repurposed for the treatment of patients with COVID-19, with dozens of ongoing clinical trials assessing their potential efficacy. DMARDs, due to their immune-modulating nature, could be a potential treatment option for severe COVID-19. However, there are several issues that need to be taken into consideration. First, the issue of hyperinflammation versus viral replication. Although effective anti-viral immunity is

required for the clearance of pathogens, hyperactivation of immune response causes tissue damage and organ failure. Similarly, there are two sides of immunomodulation therapy in COVID-19, and clinicians should determine in which circumstance to use such medications. Second, there are questions about the timing for immunomodulation therapy. As noted, immunosuppressants could affect anti-viral immune response and the timing should be carefully considered. Although early intervention is considered as a key factor for the success of immunomodulation therapy in infection-associated hyperinflammation,[103] direct evidence from RCTs are required to determine the appropriate timing for patients with COVID-19. Finally, the pharmacokinetics of oral medications in crucially ill patients merit consideration, as physiological alterations in these patients can substantially affect the pharmacokinetics. Some drugs will need to be given parenterally due to gastrointestinal failure (eg, chloroquine has been used parenterally to treat severely ill patients with malaria, although it is absorbed reliably in these patients).[104] Compared with the oral route, parenteral administration of chloroquine results in rapid absorption and transient high plasma concentrations, which is associated with increased risk of acute toxicity.[105] In addition, impaired clearance of the drugs might be problematic in patients with hepatic dysfunction and renal failure. Therefore, smaller and more frequent doses, continuous intravenous infusion, or choosing a less toxic drug (eg, using hydroxychloroquine instead of chloroquine) should be considered in patients who are severely ill.[104,105] The many ongoing trials will hopefully provide a better understanding of the potential effects of immunomodulation therapy on COVID-19-associated hyperinflammation.

Contributors
JZ and JT wrote the manuscript with input from CY and LD. All authors revised the manuscript and approved the final report.

Declaration of interests
We declare no competing interests.

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