

A Review on Currently Available Potential Therapeutic Options for COVID-19

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Abstract: A series of unexplained pneumonia cases currently were first reported in December 2019 in Wuhan, China. Official names have been announced for the virus responsible, previously known as “2019 novel coronavirus” and the diseases it causes are, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease (COVID-19), respectively. Despite great efforts worldwide to control SARS-CoV-2, the spread of the virus has reached a pandemic. Infection prevention and control of this virus is the primary concern of public health officials and professionals. Currently, several therapeutic options for COVID-19 are proposed and vaccine development has been initiated for prevention purposes. In this review, we will discuss the most recent evidence about the current potential treatment options including anti-inflammatory drugs, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, nucleoside analogs, protease inhibitors, monoclonal antibodies, and convalescent plasma therapy. Some other agents such as vitamin D and melatonin, which were recommended as potential adjuvant treatments for COVID-19 infection are also presented. Moreover, the potential use of convalescent plasma for treatment of COVID-19 infection was described. Furthermore, in the next part of the current review, various vaccination approaches against COVID-19 including whole virus vaccines, recombinant subunit vaccine, DNA vaccines, and mRNA vaccines are discussed.

Keywords: COVID-19, SARS-CoV-2, treatment, vaccine, coronavirus

Introduction

As of December 2019, the seventh member of the human coronavirus family was recognized. Several pneumonia cases of unknown etiology were first reported in December 2019 in Wuhan, China. Subsequent studies were initiated on identification of a causative agent and control of the epidemic.¹ This virus is named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its related disease is named COVID-19. Currently, the outbreak has become a major clinical threat to the general population and health-care workers (HCWs) all over the world. This virus has rapidly spread across China and several other countries.² The genetic analyses on 103 publicly available SARS-CoV-2 genomes in a more recent study has shown the evolution of these viruses into two major types (L and S). These two types of SARS-CoV-2 are defined by means of two different SNPs.³ The expression and distribution of the receptor apparently determine the pathogenic mechanisms of virus as well as probable therapeutic strategies. Angiotensin-converting enzyme 2 (ACE2) is considered as the major determinant of cell tropism and pathogenesis for SARS-CoV-2.⁴

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In a latest report, the expression of ACE2 was not significantly different between men and women, Asian and white people, or subgroups aged older and younger than 60 years. This study also did not support smoking as a predisposing factor in men for infection with SARS-CoV-2.⁵ The findings of a recent study suggested that elderly patients are more likely to experience disease progression than their younger counterparts.⁶

Impaired immunity can lead to virus dissemination and destruction of the affected tissues, particularly in organs with high ACE2 expression. The damaged cells can induce pulmonary inflammation mainly mediated by pro-inflammatory macrophages and granulocytes.⁷ Pulmonary inflammation is the leading cause of life-threatening respiratory condition at the severe stage.⁸ Therefore, inflammation suppression should be performed at the time of severe lung damage to manage the symptoms. Studies have reported that ALT, LDH, D-dimer and inflammatory markers including hsCRP and ferritin were significantly higher in severe cases than in moderate cases. In addition, serum concentrations of pro-inflammatory cytokines as well as anti-inflammatory cytokines were enhanced in the majority of severe cases and were markedly higher in severe cases than in moderate cases, suggesting cytokine storm might be associated with disease severity.⁹

The most common symptoms of infection with SARS-CoV-2 which have been reported in various studies include fever, followed by dry cough, fatigue, muscle pain, and dyspnea. Some other symptoms such as expectoration, headaches, hemoptysis, nasal obstruction, runny nose, and other upper respiratory rhinorrhea. Gastrointestinal symptoms (vomiting, and diarrhea) have been reported less frequently.^{10,11} The majority of the children infected with SARS-CoV-2 indicate mild symptoms, without fever or pneumonia, and will recover within one to two weeks.¹² The reasons for mild COVID-19 disease in pediatric patients remains unknown. However, several hypotheses exist including: immune differences between children and adults,¹³ and the simultaneous presence of other viruses in the lung mucosa of the young children limiting the SARS-CoV2 growth by direct virus-to-virus competition.¹⁴ Differences in the ACE2 expression could be another possibility for the mild COVID-19 infections in children.¹⁵ Some epidemiological studies have revealed an association between Bacillus Calmette-Guérin (BCG) vaccination and COVID-19 morbidity and mortality. Moreover, the nonspecific beneficial effects of some live

vaccines, such as BCG, measles and oral polio vaccines have previously been reported. Therefore, BCG may have the potential to decrease severity of SARS-CoV-2 infection by induction of innate immune memory.¹⁵

Contemporary diagnostic tests available for SARS-CoV-2 include RT-PCR, real-time RT-PCR, and reverse transcription loop-mediated isothermal amplification (RT-LAMP).^{16,17} Both RT-LAMP and real-time RT-PCR have the same sensitivity.^{18,19}

The first detectable serology marker has been reported, total antibody followed by production of IgM and IgG antibodies, with a median seroconversion time of 11, 12, and 14 days respectively.²⁰ The findings have suggested that antibody testing in addition to RNA tests can provide significant data for diagnosis of SARS-CoV-2 infection.²⁰ Furthermore, technologies based on CRISPR-Cas12 have the potential to provide an in situ diagnostic tool for rapid detection of the SARS-CoV-2 virus. Binding of Cas12a to target DNA can be detected by a reporter molecule providing a fluorescence signal emission. Finally, a visual readout is provided using a paper dipstick.²¹

Chest CT is a significant tool for COVID-19 diagnosis in clinical practice.²² The most frequent finding reported from chest CT is ground glass opacity (GGO)^{23,24} and consolidation, interlobular septal thickening in both lungs has been reported as less frequent. In the primary stages of infection, the total number of the peripheral blood leukocytes is normal or decreased, the lymphocyte count is decreased, and in some cases liver enzymes and myoglobin levels indicate increase. Elevated CRP and erythrocyte sedimentation rate (ESR) are frequent.²⁵ Lymphopenia, leukopenia, elevated level of aspartate aminotransferase are the most common laboratory findings.^{10,11} Primary plasma levels of Interleukin (IL)-1 β , IL-1 α , IL-7, IL-8, IL-9, IL-10, basic fibroblast growth factors (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN γ), IP10, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory proteins (MIP)-1A, MIP1B, platelet-derived growth factor (PDGF), and tumor necrosis factor alpha (TNF- α) has been shown to be higher in patients infected with SARS-CoV-2 than in healthy controls.¹¹

At the time of writing, no vaccine or antiviral drug is available for COVID-19. Infection prevention and control of this virus is the primary concern of public health officials and professionals. Currently, several therapeutic options for COVID-19 are proposed and vaccine

development has also been initiated for prevention purposes. In this review, we will discuss the most recent evidence about the current potential treatment options, vaccine development, and future perspectives of COVID-19 in order to help tackle the SARS-CoV-2 pandemic challenges.

Potential Treatment Options of COVID-19

Medications inhibiting viral dissemination and blocking viral replication may decrease the coronavirus-induced direct cytopathic effects. In addition prevention of inflammatory responses by antibodies or compounds neutralizing cytokines or their cognate receptors may reduce virus-mediated immune pathologies.²⁶ The only treatment currently available for patients with COVID-19 mainly relies on symptomatic treatments as no vaccine has been successfully developed to date. Therefore, effective antiviral therapy and vaccination are under assessment.¹ However, according to the World Health Organization (WHO), empirical antibiotics, antiviral agents, and systemic steroids should be used for treatment of severe cases.²⁷ A recent descriptive study reported that most of the studied patients have received antiviral agents such as oseltamivir (75 mg orally twice daily), ganciclovir (0.25 g intravenously twice daily) and lopinavir/ritonavir tablets (400/100 mg every 12 h orally).²⁸ Empiric antimicrobial and antifungal therapy should also be considered for potential bacterial or fungal coinfection.²⁹ The efficacy or adverse effects of intravenous immunoglobulin and systemic steroids being used in some patients are unclear.³⁰ Invasive ventilation may be used for patients with refractory hypoxemia.¹ Numerous possible drug candidates have been suggested, including chloroquine, DNA synthesis inhibitors (tenofovir disoproxil and lamivudine), lopinavir/ritonavir (Kaletra R®), nucleoside analogues, neuraminidase inhibitors, remdesivir, and umifenovir (Arbidol R®) as well as Chinese traditional medicines (ShuFeng JieDu or Lianhua Qing-wen capsules).^{31,32}

Small Molecules

Identification of the key components of the viral infections is a major step for recognizing medications with high target specificity.³³ Viral entry to the cell and replication within the cell can be considered as a target for designing the antiviral drugs.³³

In addition to several antiviral drugs, chemical small-molecule compounds are also expected to have significant inhibitory properties on various key proteins of viruses including SARS-CoV-2.³⁴ These drugs typically inhibit both viral enzymes such as proteases and components of RNA-dependent RNA polymerase (RdRp).³⁴ Chymotrypsin-like protease (CLPro; 3CLp) and papain-like protease (PLpro) are two main proteases essential for cleavage of viral polypeptides into active components which are required for viral replication and maturation.³⁴ RdRp is an enzyme essential for viral RNA synthesis and catalyzes the replication of RNA.³⁴ The SARS-CoV-2 host cell entry depends on the interaction between ACE2 as the main receptor and viral spike (S) protein.³⁵ Inhibition of this interaction may also represent a potential therapeutic strategy against COVID-19.³⁵ Some studies aim to recognize molecules that inhibit the docking of S protein mediated by receptor-binding domain (RBD) onto cells which express ACE2.^{35,36} A recent study showed that a clinical-grade soluble recombinant human ACE2 (hrsACE2) protein can decrease SARS-CoV-2 recovery from simian Vero E6 cells. In addition, they have demonstrated that infection of kidney organoids and engineered human capillary organoids can be inhibited by hrsACE2. These findings suggest that hrsACE2 can considerably prevent SARS-CoV-2 infection by blocking its entry at the early stage of infection.³⁷

Umifenovir (Arbidol™; CAS number: 131707–25-0) is a small molecule with an indole core and inhibits viral entry into host cells through inhibition of viral membrane fusion.^{38,39} It is approved in Russia and China as an antiviral treatment for oral prophylaxis and treatment of influenza infections.⁴⁰ A recent clinical pilot trial in China reported viral load reduction and decreased mortality rate in COVID-19 patients received arbidol (400 mg; three times; nine days) as compared to the control group.⁴¹ Moreover, there are ongoing clinical trials evaluating the potential efficacy of arbidol alone or in combination with other drugs such as chloroquine and lopinavir/ritonavir against SARS-CoV-2 infection (NCT04255017, NCT04260594, NCT04273763, NCT04286503).

There are several other small molecules that are considered for use in treatment of COVID-19 infection. In further sections of this review, the other small-molecule drugs will be discussed in their respective categories.

Anti-inflammatory Drugs

Immense immune response is common against viral infections to control the pathogen and this can lead to extensive

injury to the host cells. A hyperinflammatory state with extreme release of pro-inflammatory cytokines is the most serious presentation of COVID-19 infection and known as cytokine storm.⁴² The stimulation of pro-inflammatory cytokines by the virus leads to severe organ injury such as acute respiratory distress syndrome (ARDS).^{42,43} Initial studies have reported decreased immune cell populations such as lymphocyte subsets (CD4+, CD8+) and natural killer (NK) cells as well as increased cytokines in the course of severe COVID-19 pneumonia.^{9,11,44,45}

Macrophage activation syndrome as well as elevated levels of ferritin, C-reactive protein (CRP), and cytokines like IL-6, IL-8, IL-1 β , and TNF- α has been shown in the primary stage of COVID-19 infection.⁴⁶ Moreover, a significant escalation in inflammatory monocytes mainly CD14 + IL-1 β + cells at the initial stage of recovery was indicated by single cell sequencing of peripheral blood cells.⁴⁷ The existence of lymphopenia and lower numbers of CD4 and CD8 T cells has been linked to more severe disease.⁴⁸ In addition, both increased inflammatory CCL6 + Th17 cells and decreased circulating regulatory T cells has been reported in severe cases.^{48,49} Lymphopenia, spleen atrophy, and pulmonary macrophage infiltration were observed in later stages of infection and autopsy.⁸ Complement deposition in pulmonary microvasculature due to initiation of alternative complement pathway activation has been reported in COVID-19 infection as well.⁵⁰

Therefore, primary inhibition of inflammatory pathways may be considered as a major therapeutic option,⁵¹ and anti-inflammatory medications can be a supportive treatment for COVID-19. Several anti-inflammatory drugs are currently available including nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, chloroquine/hydroxychloroquine, and immunosuppressants to prevent or diminish the progression of inflammation.⁵²

Moreover, particular anti-inflammatory/immunosuppressive agents such as IL-1 inhibitors (anakinra), IL-6 inhibitors (siltuximab), IL-6 receptor antagonist monoclonal antibodies (tocilizumab, sarilumab), TNF- α inhibitors (adalimumab), and the Janus kinase (JAK) inhibitors (fedratinib, baricitinib)⁵² are being assessed by ongoing clinical trials. However, there is inadequate evidence for their effectiveness and safety against SARS-CoV-2 infection (Figure 1).^{53,54}

However, the dilemma of applying anti-inflammation therapy to COVID-19 patients and using anti-inflammatory/immunosuppressive agents in the presence of an active infection seems to be controversial.⁵⁵ On the

one hand, inflammation control is important to inhibit tissue injury. On the other hand, the anti-inflammation therapy such as corticosteroid may postpone the viral eradication and increase the risk of secondary infection. Although some studies have described the effectiveness of glucocorticoids in the treatment of coronavirus pneumonia (severe acute respiratory syndrome, SARS and Middle East respiratory syndrome, MERS) it remains controversial. Some evidence designated that the advantage of using glucocorticoids is likely outweighed by adverse effects.²⁴ However, several reports have not supported corticosteroid for treatment of COVID-19 lung disease.⁵⁶ According to insufficient evidence, the temporary WHO guideline does not support using systemic corticosteroids for COVID-19 cases. However, there is evidence proposing that cases on chronic anticytokine management for inflammatory diseases mediated by immune responses are not at a greater risk for worse outcomes from COVID-19 infection.⁵⁷

JAK Inhibitors

One of the key parts of immunopathology during infection caused by coronaviruses is cytokine and chemokine responses. Increased levels of serum pro-inflammatory cytokines such as IFN γ , TNF- α , IL-2, IL-4, IL-6, IL-7, and IL-10 were described in severe COVID-19 cases and were associated with the severity of disease.⁵⁸ Numerous cytokines involved in COVID-19 utilize an intracellular signaling pathway mediated by JAKs.⁵⁹ Baricitinib (brand name Olumiant) is a novel small molecule acting as a JAK inhibitor. It is a food and drug administration (FDA)-approved antirheumatoid arthritis medication which has been suggested as a potential candidate for COVID-19 treatment.⁶⁰ However, JAK inhibitors can block the production of a variety of inflammatory cytokines including IFN- α , which is essential against viral infection and may not be a suitable option for COVID-19 treatment. There are some recent registered clinical trials of JAK inhibitor ongoing in China (ChiCTR2000030170; ChiCTR2000029580).

Chloroquine and Hydroxychloroquine

Chloroquine is used either for prevention and treatment of malaria or for the treatment of rheumatoid arthritis and lupus erythematosus. It has wide antiviral effects through increasing endosomal pH essential for virus entry.⁶¹ Chloroquine can lead to inhibition of uncoating as well as modification of posttranslational newly synthesized proteins in several viruses such as HIV. Initial in vivo studies have proposed that the chloroquine alone or in

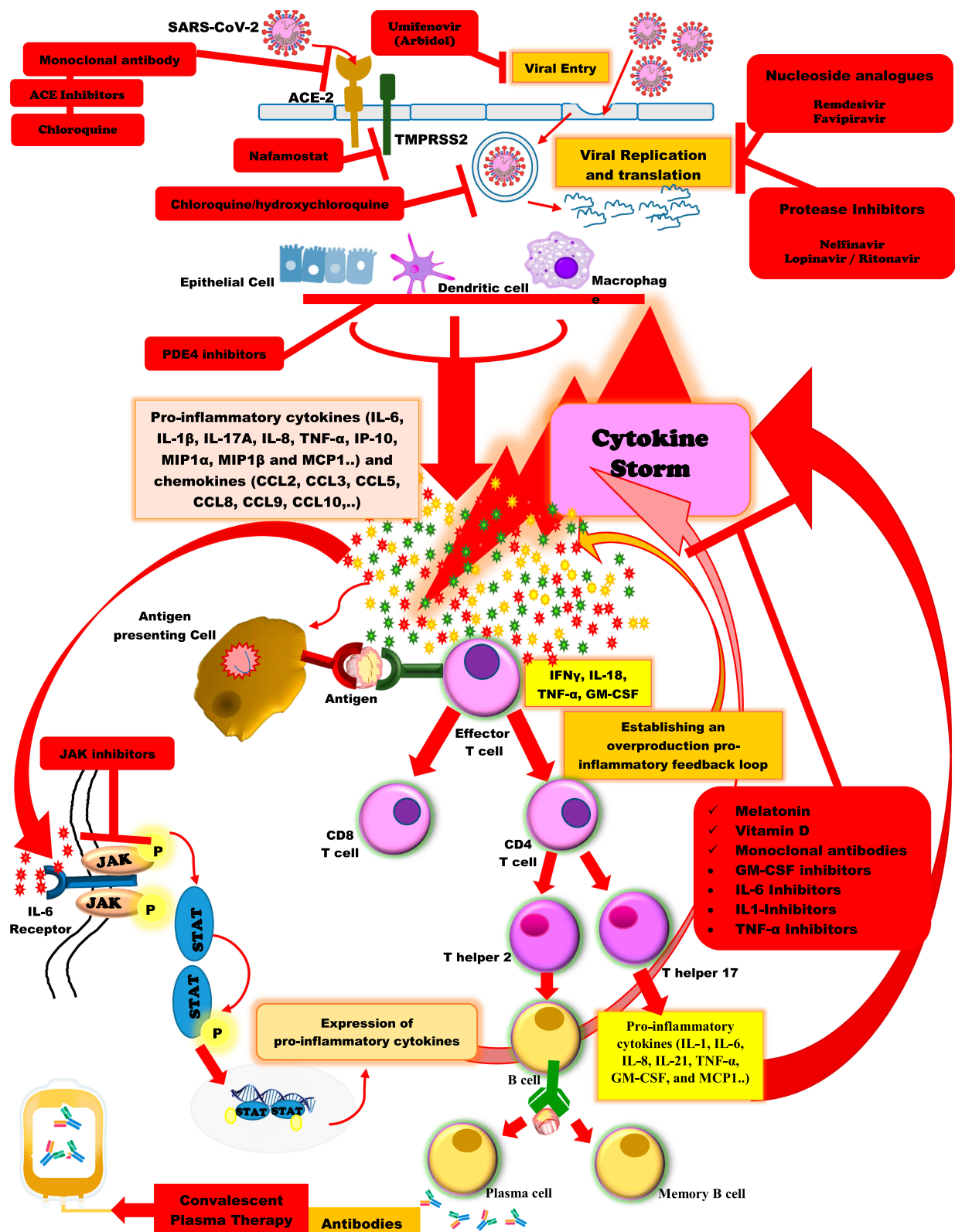


Figure 1 Schematic representation of the immunopathogenesis of SARS-CoV-2 infection and the potential therapeutic options against COVID-19.

combination with antiretroviral agents may play a role in COVID-19 treatment.⁶² It has shown a potent inhibition effect against SARS-CoV through interfering with

ACE2.⁶³ Moreover, its in vitro activity against SARS, MERS, HIV, Ebola, Hendra, and Nipah viruses has been shown.^{63–65} In China, COVID-19 patients were treated

with chloroquine to evaluate its effectiveness and safety. Their results indicated that chloroquine prevents the exacerbation of COVID-19.⁶¹ An *in vitro* study has also indicated high effect of remdesivir and chloroquine combination in COVID-19 control.³² Guidelines (version 6) for COVID-19 treatment recommends oral administration of chloroquine phosphate at a dose of 500 mg (300 mg for chloroquine) for adults, twice/day (no more than 10 days).⁶⁶ Several clinical trials have been registered to evaluate the efficacy and safety of chloroquine in the treatment of COVID-19. A recent clinical trial has reported the short-term efficacy of hydroxychloroquine in the treatment of COVID-19.⁵² There are studies that reported the superiority of chloroquine phosphate to the control treatment in prevention from progression of pneumonia, improving pulmonary imaging results, viral clearance, and shortening the disease duration.⁶¹ The results of an open-label nonrandomized clinical trial showed that hydroxychloroquine treatment significantly decreased the viral load in patients with COVID-19 infection.⁶⁷ However, these studies have some limitations such as small sample size and debatable methodology.

Some potential adverse effects of chloroquine/hydroxychloroquine include retinopathy, cardiac arrhythmias, and muscle weakness.⁶⁸ There are also some reports of ototoxicity following treatment with these drugs, which may lead to hearing loss.⁶⁷ Currently, there is no strong evidence of beneficial potential of chloroquine/hydroxychloroquine for COVID-19 treatment. However, further preclinical and clinical trials are required to prove their effectiveness and safety.

Phosphodiesterase 4 (PDE4) Inhibitors

The PDE4 expression has been established in several inflammatory cells including neutrophils, monocytes, T cells, and eosinophils.⁶⁹ Cyclic adenosine monophosphate (cAMP) as an intracellular regulator has a key role in modulation of cytokine release through the nuclear factor κ B (NF- κ B) and protein kinase A (PKA) pathway. This leads to inhibition of the main pro-inflammatory cytokines including TNF- α , and the stimulation of the anti-inflammatory IL-10.⁷⁰ PDE4 is an enzyme account for the hydrolysis of cAMP and reduces its intracellular levels in inflammatory cells.⁷¹ Thus, inhibition of PDE4 leads to several anti-inflammatory properties in various cells such as neutrophils, macrophages, monocytes, T cells, and B cells.⁷²

The PDE4 by modification of the intracellular cAMP levels can regulate both pro- and anti-inflammatory cytokine

productions.⁷² Higher expression of PDE4 in cases with inflammatory diseases compared to healthy individuals has been shown previously.⁷³ Inhibitors of PDE4 have been suggested as novel anti-inflammatory treatments used in several inflammatory diseases including rheumatoid arthritis, psoriasis, asthma, and chronic obstructive pulmonary disease (COPD).^{72–74} They effectively suppress pro-inflammatory cytokines such as IFN γ , TNF- α , IL-2, IL-12, IL-17 and IL-23 in inflammatory cells and also inhibit the reactive oxygen species (ROS) production.^{72,74,75} IL-17 is a pro-inflammatory cytokine produced by T helper (Th)-17 cells and suggested to have a role in acute pulmonary damage during respiratory viral infections.⁷⁶ Therefore, IL-17 has been suggested as a target for management of pulmonary damage during COVID-19 infection.⁷⁷

In addition, *in vivo* studies on animal models of pulmonary damage injury has shown decreased deposition of pulmonary fibrin and vascular alveolar leakage, as well as improved survival due to the inhibition of PDE4.^{78,79} The use of PDE4 inhibitors leads to not only upstream suppression of several cytokine signaling pathways, but also regulation of the pro-inflammatory/anti-inflammatory equilibrium. Therefore, it has been speculated that PDE4 inhibitors can be considered as potential therapeutic options against COVID-19 infection.⁸⁰

Apremilast (Otezla®) is an oral small molecule belongs to the PDE4 inhibitors. Mugheddu et al. recently reported a 45-year-old obese male with erythrodermic psoriasis who was under treatment with apremilast, has fully recovered from COVID-19 infection.⁸¹ A more recent case report also described a 61-year old male who suffered from moderate psoriasis was receiving apremilast and whose family were diagnosed as being SARS-CoV-2 positive. However, despite his close contact with them, the patient showed no symptoms of COVID-19 infection. The nasopharyngeal swab examination indicated that he was positive for SARS-CoV-2, but the CT results showed no pulmonary changes.⁸² These reports may suggest anti-inflammatory effects of apremilast against COVID-19 infection. Therefore, it has been suggested that the use of PDE4 inhibitors before the cytokine storm and in the primary stage of COVID-19 pneumonia may signify an appropriate treatment option. Appropriate clinical trials are required in order to explore the potential therapeutic effects of PDE4 inhibitors as well as other therapeutic options.⁸⁰

Methotrexate

Methotrexate is an immune system suppressant belonging to antimetabolites. A recent study has provided robust reasoning for high-dose methotrexate usage in treatment of inflammatory syndromes mediated by SARS-CoV-2.⁸³ According to the authors, the major mechanism of action of methotrexate is inhibition of folate-dependent methyl transfer enzymes which interferes with the DNA and RNA synthesis leading to inhibition of cell proliferation particularly in dividing cells.⁸³ Furthermore, methotrexate has several anti-inflammatory effects.⁸⁴ It can affect activated inflammatory T cells which are dividing cells. It also directly binds to high-mobility group box 1 (HMGB1) protein and prevents RAGE–HMGB1 interactions which leads to suppression of cytokine production mediated by macrophages.⁸⁵ Methotrexate in high dose therapy has been indicated to reduce proliferation of monocytes as well as decrease inflammatory cytokine levels.⁸⁶ Special attention should be paid to administration of high-dose methotrexate. It is accessible, cost effective with good CNS penetration.⁵⁵ The use of methotrexate in COVID-19 cases with cytokine storm seems to be an appropriate option. However, this should be evaluated in clinical trials. Management of COVID-19 patients with a combination of recently approved remdesivir with an anti-inflammatory agent will possibly increase treatment efficiency.^{55,87}

Angiotensin-Converting Enzyme Inhibitors (ACEI) or Angiotensin Receptor Blockers (ARBs)

ACE2 as the receptor of SARS-CoV-2 is a significant component of the renin-angiotensin system (RAS). ACEI/ARBs are used primarily for the treatment of individuals with cardiovascular comorbidities.⁸⁸

On the one hand, overregulation of ACE2 can have a potential role in the increased risk of pulmonary infection.⁸⁹ As mentioned above, SARS-CoV-2 uses the receptor ACE2 for entry into the target cells.⁹⁰ It has been reported that both ACEI and ARBs could significantly upregulate mRNA expression of cardiac ACE2.⁹¹ Therefore, it has been hypothesized that the increased ACE2 expression may have an association with increased risk of severe course of COVID-19 cases.⁹²

On the other hand, ACE2 may play a protective role in both cardiovascular and pulmonary diseases.⁸⁹ There is evidence that the ARBs have protective effects against viral spread of SARS-CoV-2.⁹³ It has been observed that

individuals using losartan or telmisartan as antihypertensives agents develop fewer attacks of cold and flu-like diseases.⁹⁴ Both these drugs bind to the AT1 receptors stronger than valsartan.⁹⁴ They can be administered either orally or in the form of a nasal spray. Improved survival has been observed following treatment with losartan in experimentally infected mice models with H5N1 influenza.⁹⁵ Utilization of zinc supplement can also facilitate intracellular killing and phagocytosis.⁹⁶ Therefore, ARBs (telmisartan and losartan) in therapeutic doses along with zinc can be used to control viral replication.⁹³ Since the limited information, rapid clinical trials are required to investigate the use of losartan or telmisartan for inhibition of the viral entry into the host cell.

Collectively, the role of ACEIs and/or ARBs in facilitating SARS-CoV-2 entry and virus replication cannot be ignored. On the contrary, beneficial effect of ACEIs and/or ARBs in clinical outcomes after infection still remains unknown. Therefore, it seems that we are facing a double-edged sword depending on the disease phase. At the early stage of disease, increased expression of ACE2 may enhance infectivity and the use of ACEI/ARBs would be a potential risk factor, but once infection is established, ACE2 downregulation may lead to COVID-19 progression. Accordingly, upregulation of ACE2 in the acute phase of the disease may turn out to be advantageous.⁸⁹

There are several observational studies performed in different countries such as U S,⁹⁷ U,⁹⁸ China,^{99–101} and Italy¹⁰² which were studied on confirmed COVID-19. None of these studies reported association between ACE inhibitors or ARBs and severity of COVID-19 infection. Moreover, there is no study described either benefits or harms of ACE inhibitors or ARBs as treatment for COVID-19 patients.

Nucleoside Analogs

Generally, nucleoside analogs are synthetic nucleosides that mimic their physiological counterparts. They inhibit the cellular nucleotide synthesis pathways and or viral replication through impairment DNA/RNA synthesis. Nucleoside analogs can target the RNA-dependent RNA polymerase that is necessary for viral RNA replication.¹⁰³

Favipiravir (T-705, Avigan, or favilavir) is a guanine analog with activity against various RNA viruses such as influenza, Ebola, yellow fever, and enterovirus.¹⁰⁴ It is a small molecule with a plasma protein binding of about 60%. Therefore, it may appear in breast milk.¹⁰⁵

Favipiravir has shown effective antiviral activity in Vero E6 cells and has been suggested as a possible candidate for treatment of COVID-19.³² Favipiravir in combination with other antiviral agents such as IFN- α or baloxavir marboxil was used against SARS-CoV-2.¹⁰⁶ An experimental treatment with favipiravir indicated its superior therapeutic effects against COVID-19 compared to lopinavir/ritonavir, concerning improvement of disease and viral clearance.¹⁰⁵

Ribavirin is another nucleotide guanine analog (guanosine analog) used for treatment against hepatitis C virus (HCV) and respiratory syncytial virus (RSV) infection as well as against SARS-CoV and MERS-CoV infections.¹⁰⁷ In vitro studies have indicated synergistic effects of ribavirin with both leukocytic IFN- β and IFN- α against SARS-CoV.^{108,109} It may significantly impact the treatment of COVID-19 infection due to its availability and low cost.¹¹⁰ Ribavirin has shown adverse reactions including anemia and altered liver function.^{107,111} Therefore, special dosage consideration should be given.^{108,109}

Remdesivir (GS-5734) (CAS number: 1809249–37-3) is a novel small-molecule adenine nucleotide analog that was originally developed to treat Ebola virus.¹¹² It is considered as a prodrug that is metabolized into its pharmacologically active form recognized as GS-441524.¹¹³ It can block replication of different coronaviruses such as SARS-CoV and MERS-CoV.¹¹² Remdesivir has antiviral activity by inhibition of RdRp which cause interference with the RNA synthesis of virus. It is effective in the primary stage of infection and leads to a reduction of viral RNA levels.¹¹⁴ In vitro and in vivo studies have reported that the antiviral activity of remdesivir and IFN- β combination is superior compared to that of lopinavir/ritonavir-IFN- β against MERS-CoV. Remdesivir has also shown promising results in the treatment of SARS-CoV-2 infection.¹¹⁵ Currently there are several ongoing clinical trials on remdesivir in COVID-19 patients in numerous countries including France (NCT04314817, NCT04315948), USA (NCT04302766), Republic of Korea and Singapore (NCT04280705), and China (NCT04252664, NCT04257656).

Protease Inhibitors (PIs)

PIs are a class of antiviral medications that are mainly used for treatment of HIV and HCV. They selectively bind to viral proteases and block proteolytic cleavage of protein precursors, which leads to inhibition of infectivity and replication. Both lopinavir (ABT-378) and ritonavir (ABT-538) are protease inhibitors, used against

HIV.^{116,117} The lopinavir/ritonavir combination is extensively used in the HIV management. Favorable therapeutic effects have been reported for lopinavir/ritonavir (KaletraTM) with ribavirin against SARS-CoV and MERS-CoV.^{117,118} This combination has been also suggested for COVID-19.¹¹⁹ In a previous study, following confirmed in vitro susceptibility of SARS-CoV to lopinavir/ritonavir, the clinical progress of SARS-CoV positive patients who were treated with a combination of ribavirin and lopinavir/ritonavir were compared with those treated with ribavirin only. The adverse outcome in the group who were treated with lopinavir/ritonavir was significantly lower than that of the other group.¹¹⁷ Furthermore, there is a case report of a patient with MERS-CoV infection who was successfully treated with triple combination of lopinavir/ritonavir, ribavirin and IFN- α and respiratory viral clearance achieved within six days.¹¹⁸

Nelfinavir is also an antiretroviral drug that selectively inhibits the HIV protease.¹²⁰ Its inhibitory effect has been shown for SARS-CoV suggesting its potential therapeutic effect for COVID-19.¹²¹ A previous study suggested that cytopathic effect of SARS-CoV infection could be inhibited by nelfinavir. The use of quantitative RT-PCR indicated that production of virions from Vero cells has been reduced by nelfinavir.¹²⁰

In a recent trial, oral lopinavir/ritonavir (400 mg/100 mg) twice daily for 14 days was prescribed for adults with severe COVID-19 infection. The results were compared to those who received standard treatment. The primary data from this trial indicated no advantage of using lopinavir/ritonavir treatment beyond standard care (ChiCTR2000029308).¹²² Determination of lopinavir/ritonavir effectiveness in COVID-19 patients needs to be further investigated. There are some clinical trials being conducted to assess the efficacy of lopinavir/ritonavir in combination with remdesivir and IFN- β -1a in COVID-19 cases in Hong Kong (NCT04276688), Republic of Korea (NCT04307693), China (NCT04255017, NCT04261907, NCT04286503), and in Europe (NCT04315948).

Type I IFNs

Immune intervention approaches to increase the immunity may help combat against viral infections. IFNs are the most broadly prescribed drugs with strong antiviral activities. IFN is a significant cytokine and represents one of the first lines of host defense against both DNA and RNA viruses.¹²³ IFNs are classified into three groups, type I, II and III IFNs. Type I IFNs include structurally similar

cytokines and comprise 13–14 subtypes of IFN- α together with IFN- β , IFN- ϵ , IFN- κ , IFN- ω , IFN- δ , IFN- ζ , and IFN- τ . In contrast, type II IFNs consist of IFN- γ .¹²⁴

Type I IFNs possess a wide range of biological activities and are the first cytokines produced during viral infections. They induce a wide range of proteins implicated in impairment of viral replication in targeted cells. Experiences from SARS-CoV have demonstrated the better antiviral activity of IFN- β compared to IFN- α .¹²⁵ A previous study evaluated antiviral effect of recombinant IFN- α and IFN- β against two clinical isolates of SARS-CoV (FFM-1 and Hong Kong) which were replicated in Caco2 and Vero cells. Their findings indicated that IFN- β was more effective than IFN- α in Caco2 cells. Moreover, in Vero cells, IFN- β was the most potent inhibitor of SARS-CoV and its selectivity index for SARS-CoV strain FFM-1 was 50 and 25 times higher than that of IFN- α and IFN- γ , respectively.¹²⁶

Nasal drops of IFN can maintain high levels of IFN- α in the nasal mucosa which may prevent the virus passing through the nasal mucosa.¹²³ A recent trial on the protective effect of recombinant human IFN- α (rhIFN- α) nasal drops against COVID-19 in HCWs reported a positive preventive effect of rhIFN- α nasal drops in susceptible healthy individuals against COVID-19 infection.¹²³

Moreover, a recent trial (phase I and II/not yet recruiting) has been registered to evaluate the adverse effects of rintatolimod and IFN- α -2b in management of cancer cases with mild or moderate COVID-19 infection (NCT04379518).

A recent study suggested that treatment with IFN- α 2b considerably decreases viral clearance from the upper respiratory tract as well as decreases circulating levels of the inflammatory markers including IL-6 and CRP in COVID-19 patients.¹²⁷ According to WHO, IFN- α 2b can be considered as a possible antiviral for the management of COVID-19.¹²⁸ Heberon® Alpha R is a human recombinant IFN- α 2b which has indicated antiviral activities.¹²⁹ A multicenter prospective observational study in Cuba enrolled confirmed cases of COVID-19, suggested the therapeutic effects of IFN- α 2b for COVID-19. It also showed that using Heberon® Alpha R may potentially improve the rates of recovery as well as case fatalities.¹³⁰

Melatonin as a Potential Adjuvant Treatment

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone with a variety of functions in health and has been effectively

used to treat sleep disorders, atherosclerosis, respiratory diseases, and viral infections. The efficacy of melatonin has been recognized in experimental animal models and in human studies. Melatonin may have helpful adjuvant utility in treatment of COVID-19 induced pneumonia.¹³¹ Excessive inflammation as a significant feature in COVID-19 patients can depress immune system and trigger cytokine storm. Due to anti-oxidative, anti-inflammatory, and immune enhancing properties, melatonin may have indirect antiviral effects.¹³² A meta-analysis of randomized controlled trials revealed the association between melatonin supplementation and reduced level of TNF- α and IL-6.¹³³ Therefore, while there is no direct evidence of melatonin administration in COVID-19 patients, both anti-inflammatory role of melatonin and its safety profile may indicate its potential beneficial effect in COVID-19 patients.¹³⁴

Monoclonal Antibodies

Neutralizing monoclonal antibodies are an important part of protective immunity against several viral infections.¹³⁵ Some antigenic sites on surface proteins of a virus can be targeted by neutralizing monoclonal antibodies.¹³⁶ Therefore, this strategy can be considered as a potential option for prophylactic and therapeutic purposes. Moreover, effective monoclonal antibodies can be produced by immunization of humanized mice.¹³⁵ Neutralizing antibodies against coronaviruses mainly target S protein that facilitate virus entry into host cells through binding to the human ACE2 protein.¹³⁶ Effectiveness of monoclonal antibodies have been established in animal models for both MERS-CoV and SARS-CoV.¹³⁵ The efficiency of a high-affinity anti-S1 human monoclonal antibody (80R) against SARS-CoV infection through inhibition of syncytia formation between cells expressing the S protein and those expressing the SARS-CoV receptor ACE2 was described previously.¹³⁷

According to the former experience in the treatment of viral infections including SARS, MERS, influenza, and Ebola, mortality rate can be reduced by administration of stimulant plasma.^{138,139} In passive immunization, antibodies specific for a certain epitope of pathogen can lead to a decreased viral proliferation and disease severity.¹⁴⁰ Due to the similarities between the SARS-CoV and SARS-CoV-2, some studies have repurposed the monoclonal antibodies against SARS-CoV for COVID-19 patients.¹³⁹

Currently, RBD on the S protein is the main target of neutralizing monoclonal antibodies against SARS-CoV-2.^{141,142} The findings of recent studies show that B38, H4, as well as 47D11 are novel antibodies that have

indicated promising results in neutralizing the SARS-CoV-2 infection.^{143,144} The 47D11 antibody targets an epitope in the SARS2-S-S1B domain (residues 338–506) and neutralizes SARS-CoV and SARS-CoV-2 through an unknown mechanism.¹⁴³ A recent study has isolated monoclonal antibodies from the B cells of COVID-19 recovered cases as well as from individuals infected with the SARS-CoV in 2003.¹³⁵ A recent study on four human monoclonal antibodies (B38, B5, H2, and H4) isolated from a convalescent COVID-19 patient showed binding between studied antibodies and SARS-CoV-2 RBD. A competition assay to evaluate the ability of each antibody for inhibition of binding between RBD and ACE indicated that B38 and H4 antibodies completely blocked the binding of ACE-2 to RBD.¹⁴⁴ These findings show that monoclonal antibodies have a potential therapeutic efficacy against COVID-19 infection. Several trials are evaluating the efficacy of various monoclonal antibodies for the treatment of hospitalized patients with COVID-19 (Table 1).

GM-CSF Inhibitors

GM-CSF is a cytokine with an important role in regulation of inflammation. In inflammatory conditions, the concentration of circulating GM-CSF is increased. Interaction between ligand and GM-CSF receptor- α can stimulate pro-inflammatory pathways and can lead to elevated production of pro-inflammatory cytokines such as TNF, IL1, IL6, IL-12, and IL23.¹⁴⁵ It has been shown that GM-CSF+T cells are greatly associated with severity of COVID-19 infection.¹⁴⁶ Therefore, clinical trials are initiated, evaluating whether the humanized monoclonal antibodies such as lenzilumab (class IgG1 κ) and mavrilimumab (human isoform IgG4), TJ 003234 (IgG1) that target GM-CSF can alleviate the cytokine release syndrome (CRS) and inhibit respiratory failure and/or death in COVID-19 patients¹⁴⁷ (Table 1).

Lenzilumab is a recombinant monoclonal antibody (class IgG1 κ) with the potential to target GM-CSF.¹⁴⁸ In a recent clinical study lenzilumab was used to treat hospitalized patients with severe COVID-19 pneumonia. Their results indicated clinical improvement in 92% of the patients (11 out of 12). In addition, cytokine analysis revealed a decrease in inflammatory myeloid cells following treatment with lenzilumab. Therefore, according to the results, they suggested that GM-CSF neutralization with lenzilumab was accompanied with improvement of clinical outcomes, oxygen requirement, and cytokine storm. Moreover, it was well tolerated with no adverse effects.¹⁴⁸

In contrast, a case report study has described that despite the use of lenzilumab and hydroxychloroquine for treatment of a male with COVID-19 infection, clinical progression of symptoms continued. His subsequent treatment with tocilizumab showed significant clinical improvement within 48 hours. Consequently, they suggested the superiority of tocilizumab over lenzilumab in the management of CRS.¹⁴⁹ Therefore, clinical trials should be conducted to assess the effect of lenzilumab on treatment and clinical outcome in patients with COVID-19 infection (Table 1).

IL-6 Inhibitors

It has been established that IL-6 has a crucial role in COVID-19 induced cytokine storms.¹⁵⁰ IL-6 is a pro-inflammatory cytokine and is secreted by several cells including monocytes and lymphocytes. Increased serum levels of IL-6 has been correlated with the severe COVID-19 infection and its poor prognosis.¹⁵¹ IL-6 has several biological functions such as modulation of the host immune response and regulation of immune cell proliferation and differentiation and can lead to activation of Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway.^{152,153} Prompt synthesis of IL-6 plays a crucial role in host defense during infection; however, its excessive production can lead to disease pathology.¹⁵⁴

Inhibition of IL-6 is a therapeutic approach in numerous diseases such as rheumatoid arthritis with beneficial effects.^{155–157} Currently, IL-6 inhibitory agents are being evaluated for patients suffering from COVID-19.¹⁵⁸ Therefore, several FDA approved anti-IL-6 agents such as tocilizumab, siltuximab, and sarilumab are currently being repurposed for treatment of COVID-19 infection.¹⁵⁴

Siltuximab (Sylvant) is an FDA approved chimeric monoclonal antibody that binds to IL-6 and is used for the management of neoplastic diseases. Its treatment effects in COVID-19 patients with serious respiratory complications is under evaluation in a clinical trial (NCT04322188).

Tocilizumab is a humanized monoclonal antibody against the IL-6 receptor (IL-6R) which can bind to both membrane-bound and soluble IL-6Rs and block IL-6-mediated signaling.¹⁵⁹ It is an immunosuppressive drug and is used for treatment of rheumatoid arthritis. A retrospective observational study discussed the treatment response of tocilizumab therapy in COVID-19 cases.¹⁶⁰ It has been suggested that tocilizumab is an effective treatment choice in critically ill COVID-19 patients with increased levels of IL-6.^{160,161} However, a recent study reported a potential association between administration of tocilizumab and increased

Table 1 Monoclonal Antibodies Under Trials for Evaluation of Efficacy and Safety as Treatment Options of COVID-19 Infection

Identifier Number	Brief Aim of Trial	Study Phase	Study Sponsor/ Country	Interventions
NCT04425629	To assess the safety, tolerability, and efficacy of anti-spike monoclonal antibodies for the treatment of ambulatory patients with COVID-19	Phase 1 Phase 2	Regeneron Pharmaceuticals/ France	Drug: REGN10933+REGN10987 Drug: placebo
NCT04346277	Using IC14 to block CD14-mediated cellular activation in patients early in the development of ARDS.	-	Implicit Bioscience/ Italy	Biological: IC14 (A recombinant chimeric monoclonal antibody against CD14)
NCT04391309	Assess IC14 in reducing the severity of respiratory disease in hospitalized COVID-19 patients	Phase 2	Implicit Bioscience	Biological: IC14 Other: placebo
NCT04409509	To evaluate CSL312 in COVID 19	Phase 2	CSL Behring/ United States	Biological: garadacimab, factor Xlla antagonist monoclonal antibody Drug: placebo
NCT04351152	To assess whether the use of lenzilumab + current standard of care (SOC) can alleviate the cytokine release syndrome (CRS) and inhibit development to respiratory failure and/or death in high risk COVID-19 patients	Phase 3	Humanigen, Inc./ US	Biological: lenzilumab (anti-human GM-CS monoclonal antibody) Drug: standard of care
NCT04341116	To assess the safety and efficacy of intravenous infusion of TJ003234 in cases with severe COVID-19 under supportive care, and to assess its effect on cytokines levels.	Phase 1 Phase 2	I-Mab Biopharma Co. Ltd/US	Drug: TJ003234 (Anti-GM-CSF monoclonal antibody) Drug: placebo
NCT04432298	To evaluate the efficacy and safety of intravenous pamrevlumab as compared to placebo in hospitalized cases of acute COVID-19.	Phase 2	FibroGen	Drug: pamrevlumab (human recombinant monoclonal antibody against connective tissue growth factor (CTGF) Drug: placebo
NCT04324021	To assess the efficacy and safety of emapalumab or anakinra, vs standard of care (SoC)	Phase 2 Phase 3	Swedish Orphan Biovitrum/Italy	Biological: emapalumab (anti-interferon-gamma (IFN γ) monoclonal antibody) Biological: anakinra (recombinant form of a human receptor antagonist (IL-1ra))
NCT04429529	To assess safety of TY027 as a treatment for COVID-19	Phase 1	Tychan Pte Ltd/ Singapore	Biological: TY027 Other: 0.9% saline
NCT04386239	To assess the efficacy and safety of sarilumab in COVID-19 patients	Early Phase 1	ASST Fatebenefratelli Sacco	Drug: sarilumab (human monoclonal antibody against the interleukin-6 receptor) prefilled syringe
NCT04357808	To assess the efficacy and safety of sarilumab for mild to moderate COVID-19 infection	Phase 2	Maria del Rosario Garcia de Vicuña Pinedo/Spain	Drug: sarilumab Other: standard of care
NCT04324073	To assess the therapeutic effect and tolerance of sarilumab in patients with moderate, severe pneumonia or critical COVID-19.	Phase 2 Phase 3	Assistance Publique - Hôpitaux de Paris/ France	Drug: sarilumab

(Continued)

Table 1 (Continued).

Identifier Number	Brief Aim of Trial	Study Phase	Study Sponsor/ Country	Interventions
NCT04351243	To assess the efficacy and safety of gimsilumab in subjects with acute respiratory distress syndrome secondary to COVID-19.	Phase 2	Kinevant Sciences GmbH/US	Drug: gimsilumab (anti-GM-CSF human monoclonal antibody) Drug: placebo
NCT04343651	To assess the efficacy and safety of leronlimab for mild to moderate COVID-19 infection	Phase 2	CytoDyn, Inc./US	Drug: leronlimab (humanized immunoglobulin (IgG4 monoclonal antibody against CC chemokine receptor 5 (CCR5; CD195) Drug: placebos (700 mg)
NCT04347239	To assess the efficacy and safety of leronlimab for patients with severe COVID-19 infection	Phase 2	CytoDyn, Inc./US	Drug: leronlimab (700 mg) Drug: placebos
NCT04305106	To assess the efficacy and safety of bevacizumab for severe and critical COVID-19 patients.	NA	Qilu Hospital of Shandong University/China	Drug: bevacizumab (humanized anti-VEGF monoclonal antibody)
NCT04413838	To assess the efficiency and security of nivolumab therapy in obese individuals with COVID-19 infection		Hospices Civils de Lyon/France	Drug: nivolumab (fully human IgG4 (S228P) monoclonal antibody) Other: routine standard of care
NCT04435184	To assess the efficacy and safety of crizanlizumab in hospitalized patients with COVID-19 infection.	Phase 2	Johns Hopkins University/US	Drug: crizanlizumab (anti-P-selectin monoclonal antibody) Other: 0.9% saline
NCT04377750	To assess the efficacy and safety of tocilizumab in the treatment of severe COVID-19 cases with suspected pulmonary hyperinflammation	Phase 3	Hadassah Medical Organization/Israel	Drug: tocilizumab (humanized monoclonal antibody against the IL-6 receptor)
NCT04331808	To assess the therapeutic effect and tolerance of tocilizumab in patients with moderate, severe pneumonia or critical pneumonia associated with COVID-19 infection	Phase 2	Assistance Publique - Hôpitaux de Paris/ France	Drug: tocilizumab
NCT04330638	To compare the efficacy of combinations of anti-IL drugs to best standard of care in treatment of patients with severe COVID-19	Phase 3	University Hospital, Ghent/ Belgium	Other: usual care Drug: anakinra Drug: siltuximab Drug: tocilizumab
NCT04341584	To compare the efficacy of anakinra in patients with Covid-19 infection	Phase 2	Assistance Publique - Hôpitaux de Paris/ France	Drug: anakinra
NCT04397497	To assess the efficacy and safety of mavrilimumab in hospitalized patients with severe COVID-19 infection and a hyper-inflammatory status.	Phase 2	Ospedale San Raffaele/Italy	Drug: mavrilimumab (human monoclonal antibody against GM-CSF -R) Drug: placebo
NCT04390464	To assess the potential treatment effect for COVID-19 disease (repurposed drugs)	Phase 4	Cambridge University Hospitals NHS Foundation Trust/ UK	Drug: ravulizumab (humanized monoclonal antibody against C5) Drug: baricitinib Other: standard of care

(Continued)

Table 1 (Continued).

Identifier Number	Brief Aim of Trial	Study Phase	Study Sponsor/ Country	Interventions
NCT04369469	To evaluate the efficacy and safety of intravenous ravulizumab compared to best supportive care in patients with COVID-19 severe pneumonia, acute lung injury, or acute respiratory distress syndrome	Phase 3	Alexion Pharmaceuticals	Biological: ravulizumab Other: best supportive care
NCT04322773	To assess the effectiveness of IL-6 receptor inhibitors in the treatment of patients with severe COVID-19 infection.	Phase 2	Marius Henriksen/ Denmark	Drug: tocilizumab(RoActemra) iv (humanized monoclonal antibody against the IL-6 receptor) Drug: tocilizumab(RoActemra) sc Drug: sarilumab (Kevzara) sc (humanized monoclonal antibody against the IL-6 receptor) Other: standard medical care
NCT04365153	To assess whether early treatment with canakinumab inhibits progressive heart and respiratory failure in patients with COVID-19 infection	Phase 2	The Cleveland Clinic/US	Drug: canakinumab (human monoclonal anti-IL-1 β antibody) injection 600 mg Drug: canakinumab Injection 300 mg Drug: placebos

secondary bacterial infections in critically ill COVID-19 cases.¹⁶² These controversial results highlight the urgent need for evaluation of immunosuppressive agents in randomized controlled trials to recognize their therapeutic role in COVID-19 infection.

Sarilumab (Kevzara) is also a fully human, monoclonal antibody and IL-6 receptor antagonist. It is FDA approved and is under evaluation of efficacy and safety for hospitalized patients with COVID-19 in a recent clinical trial (NCT04315298).

There are some other clinical trials evaluating therapeutic efficacy of anti-IL-6 agents together with other drugs such as hydroxychloroquine, azithromycin, and antivirals such as remdesivir enrolling diverse groups of the COVID-19 patients (Table 1).

IL-1-Inhibitors

IL-1 is one of the key mediators of innate immune system and inflammation.¹⁶³ IL-1 β can stimulate pro-inflammatory signaling in the respiratory tract.¹⁶⁴

Anakinra (Kineret) is a recombinant, nonglycosylated human IL-1 receptor antagonist (IL-1Ra).¹⁶⁵ Anakinra has a very favorable safety profile and is mainly used for management of hyperinflammatory disorders such as Still's disease, as well as in the treatment of cytokine storm syndromes.¹⁶⁶ It also has been used against some severe viral infections including H1N1, EBV, and Ebola.¹⁶⁷ A recent case report described a critical

COVID-19 case who were successfully treated with anakinra.¹⁶⁸ In addition, the use of anakinra in patients hospitalized with severe COVID-19 was evaluated in a prospective cohort study. Their findings indicated that anakinra has resulted in decreased necessity for invasive mechanical ventilation and reduced mortality.¹⁶⁹ However, verification of its efficacy, dosing, and outcome measures will need controlled trials. As of now, there are some ongoing clinical trials using anakinra against COVID-19 infection (Table 1).

TNF- α Inhibitors

TNF- α known as a major pro-inflammatory cytokine produced by stimulated macrophages, NK cells, T cells, and B cells.¹⁷⁰ TNF α is implicated in primary inflammatory events and activates a series of several inflammatory molecules, including other cytokines, and chemokines, such as IL-8, CCL2, CCL3, and CCL4. TNF- α has an important role in the initiation and maintenance of CRS.¹⁷¹ Therefore, TNF- α can also be considered as a potential therapeutic target against COVID-19.

Adalimumab is a recombinant human IgG1 monoclonal antibody against human TNF- α which has been approved for management of rheumatoid arthritis.¹⁵⁰ Currently, a trial has been registered in the Chinese Clinical Trial Registry (ChiCTR2000030089) to assess its efficacy and safety in patients with severe COVID-19 infection.

Convalescent Plasma

Most of the cases that recovered from COVID-19 infection show humoral and cellular immune responses against SARS-CoV-2. Therefore, some research groups have measured SARS-CoV-2 neutralizing antibody titers in the convalescent plasma.¹³⁵ Recently, convalescent plasma has been extensively suggested for COVID-19. However, the effect of convalescent plasma cannot be differentiated from the effects of patient comorbidities, disease severity, or effect of other treatments.¹⁷² It has previously been used for treatment of some diseases including polio, measles, mumps as well as outbreaks of respiratory infections similar to COVID-19 such as H1N1 influenza, SARS, and MERS.^{173–175} According to FDA, antibody-rich plasma from COVID-19 survivors might be effective against the infection.¹⁷⁶

A previous study reported on three HCWs infected with SARS-CoV who were successfully treated with convalescent plasma transfusion. A reduction of viral load was observed and all cases survived.¹⁷³ Another study reported reduced viral load in majority of the patients with Ebola virus disease immediately after initiation of brincidofovir and convalescent plasma therapy.¹⁷⁷ Moreover, potential efficiency of convalescent plasma therapy for clinical improvements of patients infected with MERS-CoV was revealed previously.¹⁷⁴ The promising use of convalescent plasma therapy for influenza A (H5N1) infection was also described previously. The nonresponder patients to oseltamivir treatment alone fully recovered by using a combination of oseltamivir with convalescent plasma therapy. The viral loads decreased within eight hours of transfusion.¹⁷⁵ Patients recovered from COVID-19 with a high neutralizing antibody titer are considered to be the eligible donors. Based on the preliminary results of a trial, convalescent plasma therapy can be a promising option for severe COVID-19 patients. They have reported that one dose of convalescent plasma with a high concentration of neutralizing antibodies can lead to a viral load reduction and tends to improve clinical outcomes.¹⁷⁸ However, randomized clinical studies are required to investigate the optimal dose and treatment time point.¹⁷⁸

In the largest current study, the safety of convalescent plasma was explored in 5000 hospitalized critically ill COVID-19 patients.¹⁷⁹ They have reported less than 1% severe adverse effects and 14.9% mortality rate in seven days, which is consistent with the deadly nature of COVID-19. Therefore, they have suggested that transfusion of convalescent plasma is harmless in hospitalized COVID-19

cases.¹⁷⁹ Another recent study reported the outcomes of convalescent plasma transfusion in patients with severe COVID-19 infection. They have reported its useful therapeutic effects, especially in nonintubated patients.¹⁸⁰ There are some limitations for using convalescent plasma therapy such as the difficulties in collection and possibility of contamination with infectious agents, transfusion-associated circulatory overload (TACO), adverse reactions to transfusion, and variation of neutralizing antibody responses.¹³⁵

Vitamin D

Vitamin D is a fat-soluble vitamin required for regulation of calcium and phosphate levels.¹⁸¹ SARS-CoV-2 uses ACE2 as a functional receptor for entry into target cells.¹⁸² In addition, downmodulation of ACE2 in the pulmonary tissues due to the infection with SARS-CoV-2 leads to severe acute respiratory failure.¹⁸³ Downmodulation of alveolar ACE2 result in decreased angiotensin II level which causes its increased level and increased alveolar permeability and lung injury.¹⁸⁴ Vitamin D3 is able to increase the ACE2 expression in several tissues including lung microvascular endothelial cells.^{185,186}

Elevated number of cell-bound ACE2s may function as a double-edged sword. On the one hand an increased number of ACE2 means an increased number of receptors for SARS-CoV-2 to enter the host cell. On the other hand these excessive ACE2s can competitively bind with the virus and neutralize it. Moreover, excessive ACE2 can protect the lung from injury through negative regulation of the RAS.^{187,188} However, vitamin D3 also plays an important role in innate and adaptive immune responses.¹⁸⁴ It has immunomodulatory effects such as downregulation of pro-inflammatory cytokines.^{189–192} It has an important role in inhibiting the cytokine storm.¹⁹³ Vitamin D deficiency has been correlated with greater susceptibility to viral infections.¹⁹⁴ Therefore, it has been hypothesized that supplementation with vitamin D may reduce the severity of COVID-19 infection.¹⁹⁵ It has been recognized that a number of immune cells express the vitamin D receptor (VDR). Several immune cells including macrophages, monocytes, dendritic cells, T cells, and B cells can convert 25-hydroxyvitamin D into its biologically active form 1,25-dihydroxyvitamin D (1,25-(OH)₂D). Binding of 1,25-(OH)₂D to VDR promotes the expression of numerous genes, including cytokine-related genes.¹⁹⁶

Both pro-inflammatory and anti-inflammatory cytokines are produced by innate immune system in COVID-19 cases.¹¹ The production of Th1 cytokines including IFN- γ and TNF- α can be inhibited by vitamin D.¹⁹⁷

Furthermore, vitamin D supplementation can lead to decreased expression of macrophage mediated pro-inflammatory cytokines and elevated anti-inflammatory cytokines.¹⁹⁸ The protective effect of sufficient vitamin D levels against RSV and influenza infections has been indicated previously.¹⁹⁹ Decrease of serum 25-hydroxy levels with age²⁰⁰ may be attributed to higher case fatality rates (CFRs) in older COVID-19 patients.²⁰¹ In addition, vitamin D deficiency is more frequent in countries with high geographic latitudes.¹⁹⁵ Lack of memory B cells and atypical innate immune response can result in over activation of adaptive immune system in older individuals, which is followed by cytokine storm as seen in COVID-19 cases.⁴⁸ A recent study reported the highest age-specific CFR due to COVID-19 in Spain, France, and Italy where vitamin D deficiency is more frequent than other countries (mean 25OHD concentration <0.25 ng/L).²⁰² Therefore, supplementation with vitamin D may play a role in prevention of infection as well as improve the disease outcomes. Further studies are required to explore the whether 25-hydroxyvitamin D (25(OH)D) levels are associated with prevention and treatment of COVID-19. There are several clinical trials registered in clinicaltrials.gov that are investigating whether vitamin D levels affect outcomes in COVID-19 infection and whether vitamin D deficiency is associated with increased risk of COVID-19 infection (for instance: NCT04394390, NCT04411446, NCT04385940, NCT04334005, NCT04386044, NCT04363840).

Vaccination

Vaccination may offer the best choice for COVID-19 control. Epitopes, mRNA, and S protein-RBD structure-based vaccines have been broadly suggested.^{203,204} Precise recognition of virus surface proteins and the host receptors is essential for understanding of cross-species transmission as well as for the establishment of animal models for vaccine development. Since the S protein is the major inducer of neutralizing antibodies, most of the vaccines being developed for coronaviruses target this protein. Various vaccines have been evaluated based on S protein including full-length and recombinant S protein vaccines, viral vector-based vaccine, DNA-based vaccine, and RBD protein-based vaccines.^{205,206}

There are several challenges in developing an effective vaccine for coronavirus infections including: lack of appropriate animal models that resemble human infection, clinical trials in humans are expensive to perform.²⁰⁷ Moreover, due to the great diversity in antigenic variants,

antibody-mediated protective immunity against coronavirus does not persist for very long. Human ACE2 transgenic mouse and rhesus monkey models have been used for vaccine development,²⁰⁶ and some clinical trials are in progress for SARS-CoV-2 vaccines.^{208,209}

Whole Virus Vaccines

Classic methods for viral vaccinations involve taking a live-attenuated or inactive whole virus. Whole virus vaccines have been shown to be inherently immunogenic and also have ability of toll-like receptors (TLRs) stimulation. A live influenza vaccine that expresses SARS-CoV-2 proteins has been developed by researchers at the University of Hong Kong.²¹⁰ Moreover, Codagenix Inc. has established a “codon deoptimization” technology to attenuate viruses and is currently exploring the SARS-CoV-2 vaccine strategies.²¹¹

Recombinant Subunit Vaccine

Generally, subunit-based vaccines are safer with fewer side effects than the other types of vaccines. They stimulate the immune system without introducing infectious viruses. Studies have reported increased T cell immune responses and production of high titer neutralizing antibodies in vivo due to subunit vaccines.^{212,213} The S protein contains three receptor-binding S1 heads and a trimeric S2 stalk. A recombinant subunit vaccine-based on the trimeric S protein is under preclinical test.²⁰⁴ Recently, The University of Queensland is developing subunit-based vaccines using the “molecular clamp”. A molecular clamp is a polypeptide used to stabilize the shape of proteins in experimental vaccines to improve recognition of the correct antigen, resulting stronger immune responses.^{214,215} The RBD of S protein plays a key role in interaction with the ACE2 as well as in the induction of neutralizing antibody against the virus. Therefore, RBD is being used in development of SARS-CoV-2 subunit vaccines.²¹⁶

DNA Vaccines

DNA vaccines as the third generation vaccines represent a novel approach. They contain DNA encoding particular antigens from a pathogen. The DNA is directly injected into the body and induces adaptive immune responses.²¹⁷ Currently, different methods such as electroporation as a vaccine delivery system and addition of adjuvant to enhance the immune responses have been established to improve the efficiency of vaccines.²¹⁸ Inovio Pharmaceuticals has announced its collaboration with Beijing Advaccine

Biotechnology to start a preclinical trials for COVID-19 DNA Vaccine INO-4800 development.²¹⁹

mRNA Vaccines

During the last two decades, mRNA vaccines have been developed for prophylactic and therapeutic purposes. Preclinical and clinical trials have revealed that mRNA-based vaccines elicit a safe and long-lasting immune response both in animal models and in humans. mRNA-based vaccines consists of mRNAs encoding a disease-specific antigen being translated by the host machinery. These types of vaccines are unable to integrate into the host genome and they can also produce multimeric antigens. Therefore, mRNA vaccines are superior to conventional vaccines in terms of safety and immune responses.^{220,221} A phase 1 clinical trial has been started for mRNA-1273, an mRNA vaccine, encoding a stable form of the SARS-CoV-2 protein of spike protein. In silico design of vaccine immunogens would allow its rapid production and efficacy evaluation.²¹¹

Other Vaccine Approaches

A COVID-19 vaccine is being developed using Hyleukin-7 platform technology (IL-7 fused to hyFc) to enhance the immune responses. Hyleukin-7 is designed to hybridize IgD and IgG4 to generate longer-lasting and more effective Fc fusion proteins. Improved vaccine efficacy in influenza A virus infection model has been reported by Genexine Inc. Fc-fused IL-7 has resulted the accumulation of pulmonary T cells as well as increased plasmacytoid dendritic cells.²²²

Bacillus Calmette–Guérin (BCG) Vaccine

In spite of global strict containment measures to combat the COVID-19 pandemic, increasing number of cases continue, especially in the US and Europe.²²³ There is a great variance in fatality rates across the world.²²⁴ For instance, studies from East Asian countries have reported lower incidence and case fatality rate due to COVID-19 infection.²²⁵

Mycobacterium bovis BCG vaccine is one of the most widely used vaccines worldwide. The main use of this vaccine is for vaccination against disseminated forms of tuberculosis in children. After introduction of BCG, studies revealed the decreased mortality independent of tuberculosis among vaccinated children.²²⁶ Some clinical evidence

supports a role of BCG vaccination in nontargeted protective effects against viral infections. Several studies have demonstrated the effect of BCG vaccination on the reduction of acute lower respiratory tract infections.^{227,228} A study in Guinea-Bissau showed that BCG reduced the incidence of respiratory syncytial virus infection, which may support this hypothesis.²²⁹ Moreover, ecological studies have reported lower mortality due to COVID-19 infection in countries with mandatory BCG vaccination policies than other countries.^{230–232} However, there are several biases that should be considered. For instance, genetic diversity and polymorphism in population structure across regions, dissimilarities in detection and reports of infected cases, and different intervention policies used in different locations.²²³ Due to the controversial reports about the protective role of BCG vaccination against COVID-19 infection, a recent epidemiological study was conducted on a global scale using linear and nonlinear statistical methods. This study suggested a significant association between COVID-19 distribution and mortality rate with BCG vaccination.²²⁴ Furthermore, in another recent study used linear mixed models analysis indicated that mandated BCG vaccination policies significantly affect the mortality rate of COVID-19 infection.²³³ The association between BCG immunization and decreased spread and severity of the COVID-19 pandemic has also been reported in some other studies.^{234–236}

Moreover, effectiveness of BCG as nonspecific immunotherapy against virus-mediated clinical conditions has been previously indicated.²³⁷ Two recent studies have suggested an association between the BCG vaccination and reduced morbidity and mortality due to COVID-19.^{232,234} Moreover, an unpublished study compared COVID-19 fatality rates between countries with high disease burden and those with BCG revaccination policies. They have found significant differences in the COVID-19 fatality rates between the two groups of countries. The finding supports the possible protective role of BCG vaccination against COVID-19.²³⁴ Based on promising research it has been concluded that the BCG vaccination has beneficial off-target effects. However, there are some controversial studies about the effectiveness of BCG vaccination in induction of heterologous immunity. A randomized clinical multicenter trial reported that BCG vaccination has no effect on hospitalization rate of Danish children up to the age of 15 months.²³⁸ Furthermore, it appears that protective effect of BCG vaccine disappears with time. A previous study established no cross protection between BCG and the SARS-CoV.^{239,240}

Table 2 The Clinical Trials to Evaluate Whether Vaccination has any Effect on Prevention and/or Clinical Progression of COVID-19 Infection

Identifier Number	Aim/Study Population	Phase	Sponser/ Country	Intervention/ Treatment
NCT04435379	To assess whether vaccination of elderly with VPM1002 could decrease hospital admissions and/or severe respiratory infectious diseases	Phase 3	Vakzine Projekt Management GmbH/Germany	Biological: VPM1002 Biological: placebo
NCT04387409	To assess whether vaccination of health-care professionals with VPM1002 could decrease the number of days absent from work due to respiratory disease	Phase 3	Vakzine Projekt Management GmbH/Germany	Biological: VPM1002 Biological: placebo
NCT04348370	To assess the efficacy of BCG vaccine for HCWs	Phase 4	Texas A&M University/United States	Biological: BCG vaccine Biological: placebo
NCT04373291	To assess protective effect BCG vaccine in HCWs against COVID-19	Phase 3	Bandim Health Project	Biological: BCG-Denmark Biological: saline
NCT04384549	To assess efficacy of BCG vaccine in the prevention of COVID-19 in HCWs	Phase 3	Assistance Publique - Hôpitaux de Paris/ France	Biological: BCG vaccine Biological: placebo
NCT04414267	To validate whether vaccination of individuals susceptible to COVID-19 with BCG vaccine can reduce their disease susceptibility (using both clinical and immunological criteria)	Phase 4	Hellenic Institute for the Study of Sepsis/Greece	Biological: BCG vaccine Biological: placebo
NCT04369794	To assess the effect of former (the titer of anti-BCG IFN- γ) or recent BCG exposure (boost with intradermal vaccine) on clinical progression of disease	Phase 4	University of Campinas/Brazil	Biological: BCG vaccine Biological: placebo
NCT04327206	To assess the effect BCG vaccination in protection of HCWs following against COVID-19	Phase 3	Murdoch Childrens Research Institute/ Australia	Biological: BCG vaccine Drug: placebo 0.9% NaCl
NCT04347876	To assess whether previous BCG vaccination may modify the prognosis in COVID-19 positive cases	Observational	Assiut University/ Egypt	Diagnostic test: tuberculin test
NCT04417335	To assess the whether BCG vaccine can decrease hospital admission and improve the clinical course of COVID-19 infection in elderly individuals (≥ 60 years old).	Phase 4	Radboud University/ Netherlands	Biological: BCG vaccine Biological: placebo
NCT04350931	To assess whether previous BCG vaccination can be used as immune-prophylaxis among Egyptian HCWs against COVID-19 infection	Phase 3	Ain Shams University/Egypt	Biological: intradermal injection of BCG vaccine Other: placebo
NCT04379336	To assess whether BCG vaccine can decrease morbidity and mortality in HCWs exposed to SARS-CoV-2	Phase 3	TASK Applied Science/South Africa	Biological: BCG Other: placebo
NCT04362124	To assess if BCG vaccination in HCWs can decrease the severity of COVID-19 Infection	Phase 3	Universidad de Antioquia/ Colombia	Biological: BCG vaccine Biological: placebo
NCT04328441	To reduce absenteeism among HCWs with direct patient contacts during the epidemic phase of COVID-19. Secondary objective: to reduce hospital admission, ICU admission or death in HCW with direct patient contacts during the epidemic phase of COVID-19.	Phase 3	UMC Utrecht/ Netherlands	Biological: BCG vaccine Biological: placebo

Abbreviations: HCWs, health-care workers; BCG, Bacillus Calmette–Guérin.

There are some potential confounders that may affect interpretation of correlation between BCG vaccination and COVID-19 related mortality.^{230–232} These include the possible underreporting as well as lower competence for SARS-CoV-2 testing in low income countries.^{230–232} Therefore, randomized controlled trials are required to investigate whether BCG vaccination can induce trained immunity and protect against COVID-19. It is rational to suggest that these should first be initiated in high risk populations including HCWs as well as older individuals. Currently, trials have just begun in some countries such as the Netherlands, Australia, Greece, Britain, Germany, Denmark, and the US to investigate its potential protective effect against SARS-CoV-2 infection.²²³ There are some ongoing trials to explore the possible effects of vaccination in hospital staff.²⁴¹

However, strategies for immunity induction for protection purposes against SARS-CoV-2 infection may not be limited to BCG. For instance, VPM1002 vaccine (Vakzine Projekt Management) which is a novel genetically modified BCG vaccine and is being considered for clinical trials to assess its effectiveness against SARS-CoV-2 infections²²³ (Table 2).

A previous study has reviewed the molecular mechanism of nonspecific effects of BCG vaccine against viral infections.²⁴² The epigenetic reprogramming of monocytes known as trained immunity is recommended as a potential reason for nonspecific effects of BCG. In a previous study, autophagy was recognized as a key component of trained immunity.²⁴³

Conclusion

The knowledge about this novel virus is still limited and the treatment options or vaccination are under assessment. SARS-CoV and MERS-CoV outbreaks have provided comprehensive insights into how to fight the SARS-CoV-2 pandemic. As of now, no vaccine or antiviral drug is available for COVID-19. What we can do currently is take measures to reduce the spread of COVID-19. Based on former experience in management of MERS and SARS infections, the WHO recommends infection control interventions to decrease the risk of transmission of infections. Implementation of control measures should be directed at limiting the possible sources of infection to prevent the risk of disease transmission in the absence of effective treatments. In addition, the asymptomatic spreaders also need to be focused.

Abbreviations

ARDS, acute respiratory distress syndrome; ACE2, angiotensin-converting enzyme 2; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BCG, Bacillus Calmette–Guérin; CFRs, case fatality rates; COVID-19, coronavirus disease COVID-19; CLPro; 3CLp, chymotrypsin-like protease; CRP, C-reactive protein; cAMP, cyclic adenosine monophosphate; CRS, cytokine release syndrome; FGF, fibroblast growth factors; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; GGO, ground glass opacity; HCWs, healthcare workers; HCV, hepatitis C virus; HMGB1, high-mobility group box 1; IFN γ , interferon gamma; JAK, Janus kinase; JAK-STAT, Janus kinase-signal transducer and activator of transcription; MIP, macrophage inflammatory proteins; MERS, Middle East respiratory syndrome; MCP-1, monocyte chemoattractant protein-1; NK, natural killer cells; NSAID, non-steroidal anti-inflammatory drugs; PLpro, papain-like protease; PDE4, phosphodiesterase 4; PDGF, platelet-derived growth factor; PIs, protease Inhibitors; RBD, receptor-binding domain; RAS, renin-angiotensin system; RSV, respiratory syncytial virus; RdRp, RNA-dependent RNA polymerase; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; The Food and Drug Administration; TLRs, toll-like receptors; TACO, transfusion-associated circulatory overload; TNF- α , tumor necrosis factor alpha.

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Disclosure

The author declares no conflicts of interest.

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