EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 2
276-289
Year: 2022

NOVEL APPROACHES FOR COVID-19 TREATMENT

[1]*Bharat Lal and [2]Manoj Gadewar

[1]Dept. of Pharmaceutics, K.R. Mangalam University, Haryana, India.
[2]Dept. of Pharmacology, K.R. Mangalam University, Gurgaon, Haryana, India.

***Corresponding Author: Bharat Lal**
Dept. of Pharmaceutics, K.R. Mangalam University, Haryana, India.

Article Received on 22/12/2021 **Article Revised on 12/01/2022** **Article Accepted on 02/02/2022**

ABSTRACT
To date, there is no licensed treatment or approved vaccine to combat the coronavirus disease of 2019 (COVID-19), and the number of new cases and mortality multiplies every day. Therefore, it is essential to develop an effective treatment strategy to control the virus spread and prevent the disease. Here, we reviewed the novel approaches that are used to treat COVID infection. Although it seems that antiviral drugs are effective in improving clinical manifestation, there is no definite treatment protocol. Lymphocytopenia, excessive inflammation, and cytokine storm followed by acute respiratory distress syndrome are still unsolved issues causing the severity of this disease. Therefore, immune response modulation and inflammation management can be considered as an essential step. There is no doubt that more studies are required to clarify immunopathogenesis and immune response; however, new therapeutic approaches including mesenchymal stromal cell and immune cell therapy showed inspiring results.

KEYWORDS: Covid-19, Lymphocytopenia, Immune Response, Immunopathogenesis, and Covid Treatment.

1. INTRODUCTION
1.1 Background of the Study
The Coronavirus belongs to the family of virus that causes viral pneumonia including fever, breathing difficulty, and lung infection (WMHC, 2020).[1] These viruses are common in animals worldwide, but very few cases of them are known to affect humans. The World Health Organization (WHO) used the term 2019 novel coronavirus (2019-nCoV) to refer to the coronavirus that was diagnosed from the lower respiratory tract of patients with pneumonia in Wuhan, China on 29 December, 2019 (Li et al., 2020; CDC, 2019; WHO, 2020).[2-4] It was reported that the human infection of the virus originated from the local Huanan South China Seafood Market in Wuhan, Hubei Province, China (Zhu et al., 2019).[5] Consequently, The Chinese Center for Disease Control and Prevention (China CDC) dispatched a rapid response team to accompany Hubei provincial and Wuhan city health authorities to conduct epidemiological and etiological investigations. The WHO reported that the outbreak of the coronavirus epidemic was associated with the marketplace, but no specific animal association has been identified (WHO, 2020).[6]

Scientists immediately started to research the source of coronavirus. The first genome of 2019 – nCoV was published by the research team led by Prof. Yong-Zhen Zhang, on January 10, 2020 (virological.org). Within one month, this virus spread quickly throughout China and globally during the Chinese New Year when there is high level of human mobility among Chinese. Although it is still early to predict the susceptible population, early patterns have shown a similar trend with Severe Acute Respiratory Syndrome (SARS) and Middle East respiratory syndrome (MERS) coronavirus, showing associations with age, biological sex and other health conditions (Fehr et al., 2017).[7] 2019-nCoV has now been declared as Public Health Emergency of International Concern by WHO (World Health Organization). Given the spread of the new coronavirus and its impacts on human health, the research community has responded rapidly to the new virus and many preliminary research articles have already been published about this epidemic. Till today there is no licensed treatment or approved vaccine to combat the coronavirus disease of 2019 (COVID-19), and the number of new cases and mortality multiplies every day.The scientists and researchers all around the world have showed their interest to study this virus and to find the ways and treatment possibilities for the treatment of this COVID infection. It is essential to develop an effective treatment strategy to control the virus spread and prevent the disease.

In the background of the above, we conducted a review of all the published scientific articles regarding the treatment approaches of new coronavirus in 2020. This review aims to provide a summary and explore the novel approaches for the treatment of the COVID infection (2019-nCoV).

This review can provide meaningful information for all possible treatment options of this infection. The study may also support the future research related to the topic and may support government decision-making on strategies to handle this public health emergency at the community, national and international level.

2. COVID-19

Corona viruses are a large family of enveloped, positive-sense RNA viruses that have the largest RNA genome (rage from 26 to 32 kb) (Zhang et al., 2020; Wang et al., 2020).[8-9] Several coronavirus epidemics such as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) have occurred during the past years (Wang et al., 2020; Shanmugaraj et al., 2020).[9-10] At the end of 2019, a novel coronavirus infection named coronavirus disease of 2019 (COVID-19) was first identified in Wuhan, China (Li et al., 2020; Wang et al., 2020; Cai et al., 2020; Thiel et al., 2008).[2,9,11,12] Due to the fast transmission, it is reported in almost all countries and has become a global crisis. Therefore, COVID-19 pandemic becomes an international threat for human health and economy (Zhang et al., 2020; Heymann et al., 2020).[8,13]

COVID-19 spreads fast among people and the mortality rate is controversial; however, it was less than 2% in some studies. The main manifestations of the disease include fever, dry cough, headache, shortness of breath, pneumonia, acute respiratory distress syndrome (ARDS), septic shock, and even death (Shanmugaraj et al., 2020; Zhu et al., 2020; Frieman et al., 2008).[10,14,15]

The genome sequencing of this virus revealed more than 82% identity to SARS-CoV (Yu et al., 2020).[16] Analysis indicated that the binding affinity of virus S protein to the angiotensin-converting enzyme 2 (ACE2) receptor on human alveolar epithelial cells is higher compared with the SARS-CoV (Yan et al., 2020).[17]

Since SARS-CoV-2 is a new pathogen, little is known about it. Moreover, there is no licensed treatment or approved vaccine and the number of new cases and mortality multiplies daily (Heymann et al., 2020).[13] Therefore, it is vital to develop an effective treatment strategy to control the virus spread and prevent the disease (Zhu et al., 2020; Zhang et al., 2020).[15,18]

2.1 Immunopathogenesis of COVID-19

Although the pathogenesis of this disease has not been fully understood, it seems that the host immune responses play an important role. Aberrant host immune response causes lung tissue damage, reduced lung capacity, and finally respiratory failure (Li et al., 2020). Studies indicated that dendritic cells (DCs) and macrophages are playing crucial role in innate immune responses (Frieman et al., 2008; Liu et al., 2016). These cells produce inflammatory cytokines and chemokines including TNF-α, IL-12, IL-6, IFNγ, and IL-8, and monocyte chemoattractant protein (MCP-1), macrophage colony-stimulating factor (GM-CSF), and granulocyte-colony-stimulating factor (G-CSF) (Thiel et al., 2008; Zhang et al., 2020). These inflammatory responses may lead to systemic inflammation (Thiel et al., 2008; Liu et al., 2016; Wang et al., 2020).

Adoptive immunity plays a major role in viral infections (Janice et al., 2012). Cytotoxic T cells (CD8+ T cells) are the main T cell subsets that destroy infected cells (Ng O-W et al., 2016). Therefore, the number of these cells is one of the major factors for clearance of the viral infection (Liu et al., 2017). Preliminarily, it was indicated that the number of total T cells, CD4+ and CD8+ T cells, reduced significantly in COVID-19 patients. This decrease was more intensive in ICU admitted patients compared with that in non-ICU admitted individuals (Diao et al., 2020). It is also reported that T cell clonal exhaustion occurred during the infection and the expression of certain T cell surface markers like PD1 (programmed cell death protein 1) and TIM-3 (T cell immunoglobulin and mucin domain-containing molecule-3) markedly increased (McLane et al., 2019). The cytokine storm occurred in response to SARS-CoV-2 infection that led to increased expression of NKG2A (natural-killer group 2, member A) on cytotoxic T cells (CTLs) and NK cells. This upregulation suppressed CTL and NK function and cytokine secretion (Diao et al., 2020; Zheng et al., 2020)). It is suggested that inflammatory cytokines, TNF-α and IL-6, mainly originated from apoptotic monocytes (CD14+CD16+) and macrophages and induced T CD4+ and T CD8+ cells. These excessive inflammatory responses might result in respiratory system pathology and dysfunction (Zhou et al., 2020).

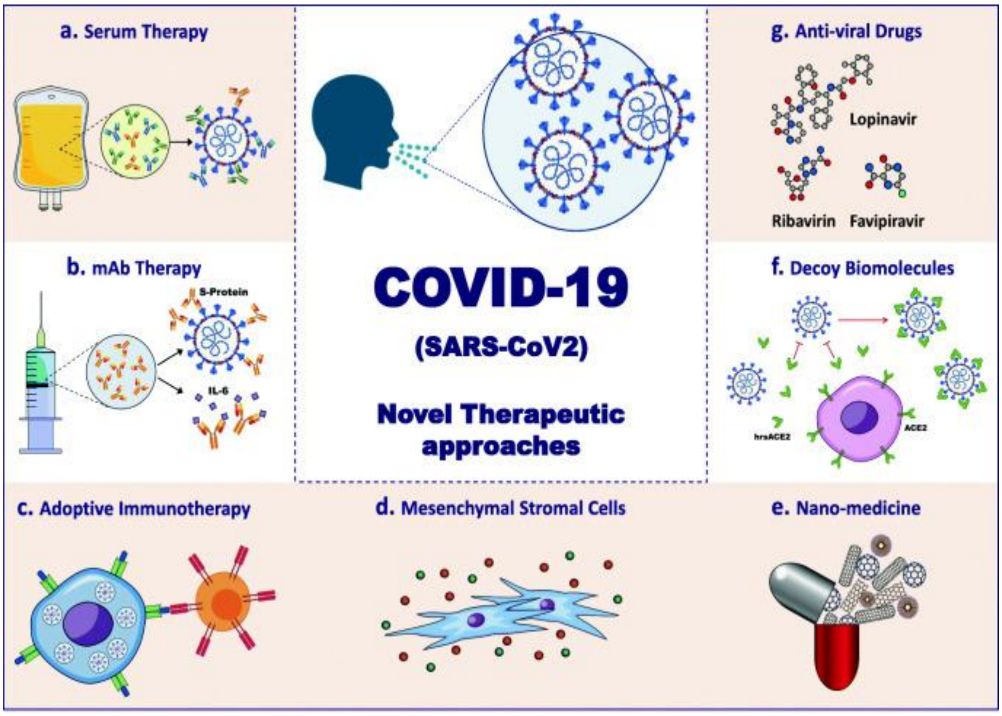

Figure 1: Novel Therapeutic strategies for treatment of clinical complications of COVID-19.

Perhaps it takes many years to achieve a specific and effective therapeutic protocol, efficient vaccine, or suitable medicine for the treatment of COVID-19. There is a wide range of existing and current treatment strategies categorized into antiviral drugs, immunotherapy protocols including convalescent serum and monoclonal antibodies, cell-based therapies, hydroxychloroquine, Chinese medicine, and steroids (just for patients who suffer from ARDS) (Vetter et al., 2020). A schematic figure (Fig.1) summarized the novel therapeutic approaches in treatment of COVID-19 patients. The figure shows the novel therapeutic strategies of clinical complications of this infection: (a) Passive immunotherapy using serum of immunized individuals, (b) Monoclonal antibodies can directly target virus particles. Also, mAbs can be used to eliminate crucial cytokines in progression of inflammation, e.g., IL-6, (c) The effector cells in adoptive immunotherapy can be used to specifically target infected cells and enhance anti-viral immune responses, (d) Mesenchymal stromal cells are key players in immunomodulation of severe immune response. The paracrine effect of these cells can tune down immune reaction, (e) Using nanostructures for drug delivery in different medical applications, (f) Recombinant ACE2 receptor protein in soluble form attaches to viral particles, (g) Antiviral medicines can prohibit viral proliferation.

3. MATERIALS AND METHODS

The ‘Research Methodology’ deals with the description of methods that wasapplied in carrying out the research.

3.1 Literature Search

Literature for this review study was identified by searching the following online databases: medRxiv, ChemRxiv, PMC free article, Google scholar and Pubmed. These online databases contain archives of most biomedical journals. In addition, some white papers published online by World Health Organization (WHO) were also searched and included in the analysis. We searched relevant scientific publications published after the outbreak of COVID-19. The search terms were ‘nCoV’, ‘2019 novel coronavirus’, ‘2019-nCoV’, ‘novel coronavirus’, 'Treatment' and 'Clinical trials for COVID-19'. We included all the relevant scientific publications written in English in the review. Commentary, and news articles were excluded from the analysis. Novel approaches for treatment of COVID infection were searched in the literatures independently also. Duplicate articles were eliminated. Eventually, unique academic publications were included and reviewed in this analysis to assess the aim of the study.

3.2 Data Analysis

The study has included all the relevant unique studies. The research domains, published dates, journallanguage, and authors’ affiliations were analysed respectively. The relevant studies were analysed for all the findings and statements that are mentioned regarding theoutbreak and novel approaches for treatment of this COVID infection. The study has reviewed and analysed the relevant studies to assess the aim of the present study. In this review all the findings and statements regarding the approaches for treatment of corona virus infection are based on published information as listed in the references.

4. APPROACHES FOR TREATMENT OF COVID INFECTION

Moreover, there are a growing number of clinical trials registered for the treatment of COVID-19. Here the study has reviewed novel approaches for the treatment of COVID infection. (See appendix, Table 1)

Passive immunotherapy

Convalescent serum: Antibody injection to the patients and susceptible people provides rapid immunity to treat or prevent the disease. Past experiences from SARS and MERS viral infections indicated that passive immunotherapy could be a potential treatment strategy for the patients (Casadevall et al., 2020; Beigel et al., 2018). It is considered that passive immunotherapy could also be beneficial in SARS-CoV-2 infection (Zhou et al., 2020). Extracting neutralizing antibodies from recovered individuals with high titer of antibodies in sera and transfusion to infected patient could deactivate the virus. However, neutralization activity of these antibodies is not fully understood. It has been showed that neutralizing antibodies are not long lasting and only the recently recovered patients are suitable candidate (Duan et al., 2020). It has also been reported that the neutralizing antibody titers vary among the patients and elderly patients had higher antibody titer compared with young recovered individuals (Tiberghien et al., 2020). It is supposed that convalescent serum administration may induce phagocytosis and antibody-mediated cellular cytotoxicity. One important implication for using convalescent serum is the risk for antibody-dependent enhancement (ADE) (Wang et al., 2014). It is supposed that these neutralizing antibodies may enhance other viral infections (Zand et al., 2020). Another major limitation of this strategy is donor shortage. However, by increasing the number of recovered individuals, this limitation would be solved (Casadevall et al., 2020).

Monoclonal antibodies: It has been shown that monoclonal antibodies (mAbs) could be an effective tool for the treatment of viral infectious diseases. Different techniques have been used to develop mAbs including phage display library, hybridoma, single B cell isolation, and transgenic mice (Jin et a., 2017). Various monoclonal antibodies developed against MERS and SARS infections include m396, 80R, and S3.1 against SARS and LCA60 for the treatment of MERS disease (Jin et a., 2017; Corti et al., 2016). These mAbs limited virus replications and facilitated lung recovery in animal models (Corti et al., 2015; Luke et al., 2016). S protein is also the most immunogenic determinant of coronaviruses (Sui et al., 2004). Several mAbs target receptor-binding domain (RBD) in the virus spike (S) glycoprotein and inhibit the virus to invade the host cell. It is reported that mAbs against SARS-CoV-1 could cross react with SARS-CoV-2 (Chen et al., 2020). It is indicated in the preprint that mAb 1A9 that targets the S protein of SARS-CoV-1 could interact with SARS-CoV-2 (Zheng et al., 2020).

Tocilizumab is a humanized monoclonal antibody against IL-6 receptor cytokine. Tocilizumab targets both membrane and soluble-bound IL-6 receptors. This mAb is used for the treatment of COVID-19 patients (Zumla et al., 2020). It is shown that the IL-6 level is considerably high in severe COVID-19 cases. Treatment of 21 severe COVID-19 cases with tocilizumab indicated that using this monoclonal antibody is an effective treatment and well tolerated in these patients. In the pre-printed study, tocilizumab caused body temperature and CRP returned to the normal levels and improved lung function (Xu et al., 2020). There are also many registered clinical trials on efficiency and safety of tocilizumab for the treatment of COVID-19. (See Appendix, Table 1)

VEGF is one of the main mediators of vascular permeability and progression of ARDS. Bevacizumab is a humanized monoclonal antibody that targets VEGF and employed in a phase II/III clinical trial for the treatment of COVID-19 patients (NCT04275414).

As described earlier, during the SARS-CoV-2 infection, exhaustion of T and NK cells happens. In order to restore these cells, using monoclonal antibodies to block the PD-1/PD-L1 and TIM3 pathways may have beneficial therapeutic effects as well (Chiappelli et al., 2020).

Adoptive immunotherapy

Adoptive transfer of antigen-specific T cells has been developed for the treatment of cancers, autoimmunity, and viral infections including hepatitis B virus (HBV), hepatitis C virus (HCV), and cytomegalovirus (CMV) (Vetter et al., 2020; Wu et al., 2015). In this approach, anti-viral-specific T cell clones are generated, expanded, and purified in vitro (Wu et al., 2015). It is shown that engineered SARS-specific CD8+ T cells had normal activity and function and may be a potential therapeutic tool for SARS infection (Beigel et al., 2018). Recently, it has been indicated that the number of CD8+ T cells decreased dramatically and the ratio of CD4+/CD8+ T cells increased during the SARS-CoV-2 infection. This decrease in the number of CD8+ lymphocytes has been correlated with the disease severity and clinical outcome (Wang et al., 2020). It has also indicated that CD8+ T cells and the CD4+/CD8+ ratio decreased and increased respectively after the treatment. It seems that CD8+ T cells play an important role in COVID-19 and could be a potential biomarker of the disease (Liu et al., 2020). Due to these findings, adoptive transfer of COVID-19-specific CD8+ T cells may be an effective treatment strategy. NK cells are innate immune cells that play a crucial role in host immune response after viral infections (Madera et al., 2016). Pre-printed studies indicated that NK cell population decreased remarkably during the disease (Shi et al., 2020; Wan et al., 2020). It has been indicated that during SARS-CoV-2 infection, increased amount of IL-6 inflammatory cytokine had negative correlation with the number of NK cells (Wang et al., 2020). Thus, it is assumed that adoptive transfer of NK cells may have an effective therapeutic approach. Therefore, recently, an ongoing phase-I clinical trial has been registered in which NK cell therapy in combination with conventional therapies for COVID-19 patients was proposed (NCT04280224). Altogether, it seems that cellmediated immunity plays an important role in host immune response against SARS-CoV-2 (Weiskopf et al., 2020).

Kinase inhibitors

It is suggested that an inhibitor of Janus kinase (JAK) called baricitinib could prevent the entry of SARS-CoV-2 into the host cells and also inhibit the inflammation (Richardson et al., 2020; Stebbing et al., 2020). Cyclin G-associated kinase (GAK) and AP2-associated protein kinase 1 (AAK1) are endocytosis regulators. Baricitinib might inhibit SARS COV-2 entry by disruption of these regulators. Other JAK inhibitors such as fedratinib and ruxolitinib are also candidates for decreasing inflammatory cytokines in COVID-19 individuals (Stebbing et al., 2020). Although JAK inhibitors have wide effects and can inhibit cytokine secretion such as IFN-α, more studies need to confirm their safety and efficiency (Zhang et al., 2020).

Corticosteroids

Corticosteroids are well-known with their immunosuppressive activity, which are essential to stop or delay the progression of the pneumonia and have been proved to be beneficial for the treatment of ARDS (Thompson et al., 2003). Additionally, corticosteroids have an anti-inflammatory effect to diminish systemic inflammation, reduce exudative fluid in the lung tissue, and inhibit further diffused alveolar damage, which can relieve hypoxemia which can protect the lungs effectively and prevent further progression of respiratory insufficiency (Rhen et al., 2005). The use of corticosteroids for the treatment of COVID-19 is controversial due to their negative impact on anti-viral immune responses. However, it has been shown that corticosteroids could improve mortality in severe COVID-19 patients with systemic hyperinflammation (Siddiqi et al., 2020). It is supposed that patient selection, half-life, formulation, and dosage of the corticosteroids are important factors determining the clinical outcome. In this regard, a pre-printed study indicated that in severe COVID-19 patients with ARDS early short-term and low dose of corticosteroid (methylprednisolone) improved clinical manifestation and long lesions (Wang et al., 2020).

Mesenchymal stromal cells

Persistence of inflammatory cytokines in COVID-19 patients leads to lung dysfunction and even death. Using corticosteroids for dampening cytokine storm suppresses immune system and makes delay in virus elimination.

Mesenchymal stromal cells (MSCs) are characterized with their immunomodulatory and anti-inflammatory properties. Because of these characteristics, they have been used for the treatment of various inflammatory and autoimmune disorders including diabetes, graft-versus-host disease (GvHD), and multiple sclerosis (Hashemi et al., 2019). It is proven that MSCs and MSC extracellular vesicle (EV) infusion have beneficial effects in the treatment of virus-induced pneumonia by reducing the lung inflammation (Khatri et al., 2018). EVs are stable, could distribute to the lungs, and have the same immunomodulatory and anti-inflammatory properties of parental MSCs (Bari et al., 2020). MSCs decreased inflammatory cytokines and chemokines in animal model of avian influenza. They could also prevent immune cell infiltration into the lungs and improved alveolar injury. Recently, there are studies evaluating allogenic MSCs and MSC-derived exosomes as potential therapeutic tools for reducing inflammation and improving COVID-19-related ARDS (Metcalfe et al., 2020; Bari et al., 2020). It is indicated that adoptive transfer of allogenic umbilical cord mesenchymal stem cells (UC-MSCs) could inhibit inflammation and attenuate symptoms in patients with advanced COVID-19. Four days after cell therapy, patients are disconnected from the ventilator. UC-MSC therapy also elevated T cell numbers and boosted the immune system. Administration of ACE negative MSCs to seven COVID-19 patients improved clinical symptoms with no side effects just 2 days after injection. The number of inflammatory cytokine secreting cells reduced significantly. Regulatory DC subpopulation (CD14+CD11c+CD11bmid) elevated. The levels of IL-10 anti-inflammatory cytokine increased while TNF-α decreased (Leng et al., 2020). Infusion of MSCs also induced lung tissue regeneration by modulating inflammatory microenvironment in COVID-19 patients (Shetty et al., 2020). There are several ongoing clinical trials using different sources of MSCs for the treatment of COVID-19 (See Appendix, Table 1). Taken together, MSC therapy could inhibit excessive immune system reaction, modulate inflammatory milieu, and prevent virus-mediated cytokine storm. It seems that MSC therapy could be a novel therapeutic approach for the treatment of COVID-19 (Metcalfe et al., 2020).

Decoy biomolecules

As mentioned above, SARS-COV-2 attaches to ACE2 receptor to invade the host cells, particularly alveolar epithelial cells. SARS-CoV-2 spike protein has strong affinity to ACE2 receptor (Cheng et al., 2020). This attachment may enhance viral entry and replication (Ju et al., 2020). It is assumed that targeting this interaction and using soluble form of ACE2 could be a potential therapeutic approach. Studies on COVID-19 indicated that ACE2 injection could competitively neutralize the virus and improve lung injury (Zhang et al., 2020). Recently, a novel therapeutic approach was developed based on soluble ACE2 interaction with the virus. It has been shown that human recombinant soluble ACE2 (hrsACE2) could inhibit SARS-CoV-2 from entering the host cells, decreasing the viral load in a dose-dependent manner. This molecule inhibits viral infection of human blood vessels and kidney organoids. These data indicated that hrsACE2 was effective in early-stage patients (Monteil et al., 2020). Since the inhibitory effects of hrsACE2 were not complete, it is preliminarily considered that the virus may use a second receptor or co-factor such as transmembrane protease serine 2

(TMPRSS2). In this regard, TMPRSS2 inhibitor was approved for clinical application in COVID-19 to inhibit the entry of virus (Hoffmann et al., 2020).

Nanomedicine

LIF (leukemia inhibitory factor) is one of the important cytokines to protect the respiratory system and promote lung homeostasis during viral infections. This cytokine modulates severe adverse events during ARDS (Foronjy et al., 2014). Up to now, there is no study investigating the role of LIF in SARS-CoV-2 infection. However, in respiratory syncytial virus (RSV) model, it has been shown that overexpression of LIF enhanced the recovery of lungs during pneumonia. Neutralization of the LIF induced alveolar damage and chemokine secretion (Poon et al., 2019). According to these data, LIF might also have protective effects in SARS-CoV-2 infection.

LIF nanoparticles (LIF-NPs) indicated clinical benefits in experimental autoimmune encephalomyelitis (EAE) animal models. LIF-NPs possessed immunomodulatory effects and increased self-tolerance in animal models for ARDS (Metcalfe et al., 2015). These inhalable NPs could be a novel strategy for lung tissue repair and cytokine storm inhibition (Metcalfe et al., 2020). Activation and polarization of macrophages play a major role in the initiation and intensity of inflammation, respectively, in ALI/ARDS. Peptide-coated gold nanoparticles could alleviate lung inflammation through inducing M1-to-M2 macrophage phenotype transition and increasing the anti-inflammatory cytokine (IL-10) in the lung of acute lung injury (ALI) mice (Wang et al., 2020).

Antiviral drugs

Remdesivir is claimed to be an option to treat COVID-19 (Al-Tawfiq et al., 2020). It is a nucleoside analog and has broad-spectrum activities against RNA viruses such as MERS; remdesivir can effectively diminish the viral load in lung tissue infected with MERS-CoV and improve lung function in animal model (Sheahan et al., 2020). The in vitro study revealed that, compared with ribavirin or favipiravir, remdesivir in combination with emetine showed the inhibition in viral yield that might achieve 64.9% (Choy et al., 2020). Regarding its clinical application, Grein et al. reported the good improvement among severe COVID-19 cases (68%, n = 53) after treatment with remdesivir. It also showed promising results in the treatment of a patient with COVID-19 in the USA (Holshue et al., 2020). However, its efficacy is doubted because, e.g., in a randomized, double-blind, placebo-controlled, multicenter trial, Wang et al. reported no statistically significant clinical benefits (Wang et al., 2020).

Chloroquine is a drug used to treat malaria (Aguiar et al., 2018). It is taught that chloroquine has a great potential to treat COVID-19 (Cortegiani et al., 2020); chloroquine can prevent pH-dependent steps of the replication of several viruses such as SARS-CoV (Vincent et al., 2005). Additionally, chloroquine has immunomodulatory effects by suppressing the production/release of TNF-α and IL-6. It also might interfere with viral infection and replication, as an autophagy inhibitor. In preprinted paper, Chen et al. showed that hydroxychloroquine use can shorten the time to clinical recovery in COVID-19 patients (Chen et al., 2020). Gautret et al.. (2020) claimed that the treatment of COVID-19 patients with hydroxychloroquine (chloroquine analog) caused the significant viral load reduction/disappearance. However, other researchers did not reveal the same effect. Moreover, high-dose chloroquine diphosphate in combination with azithromycin or oseltamivir resulted in high rates of death and adverse cardiac events (Borba et al., 2020). Clinicians also cautioned that the increased consumption of chloroquine and hydroxychloroquine can lead to their shortage that might create a problem for people suffering systemic lupus erythematosus, other rheumatological disorders, primary Sjögren syndrome, dermatological diseases, and antiphospholipid syndrome (Jakhar et al., 2020).

It has been previously reported that the protease inhibitors such as lopinavir and ritonavir, used to treat infection with human immunodeficiency virus (HIV) (Cvetkovic et al., 2003), could improve the outcome of MERS-CoV- (Arabi et al., 2018) and SARS-CoV (Chu et al., 2004) –infected patients. Initially, lopinavir and ritonavir were hypothesized to inhibit the 3-chymotrypsin-like protease of SARS and MERS, and seemed to be associated with improved outcomes of patients with SARS in a non-randomized open-label trial. In a case report from Korea, it has been shown that the viral loads of a SARS-CoV-2 significantly decreased after lopinavir/ritonavir treatment (Lim et al., 2020). However, it is controversial whether HIV protease inhibitors could effectively inhibit the 3-chymotrypsin-like and papain-like proteases of SARS-CoV-2. HIV protease belongs to the aspartic protease family, whereas the two coronavirus proteases are from the cysteine protease family. Moreover, HIV protease inhibitors were specifically optimized to fit the C2 symmetry in the catalytic site of the HIV protease dimer; however, this C2-symmetric pocket is absent in coronavirus proteases. If HIV protease inhibitors alter host pathways to indirectly interfere with coronavirus infections, their potency remains a concern (Wu et al., 2020).

Favipiravir is a new type of RNA-dependent RNA polymerase inhibitor. Additionally, it is capable of blocking the replication of other RNA viruses (Delang et al., 2018). Favipiravir is converted into an active phosphoribosylated form (favipiravir-RTP) in cells and is recognized as a substrate by viral RNA polymerase, therefore inhibiting RNA polymerase activity (Furuta et al., 2017). Favipiravir may have potential antiviral action on SARS-CoV-2, which is a RNA virus. In a clinical trial on favipiravir for the treatment of COVID-19, the preliminary results indicated that favipiravir had more potent antiviral action than lopinavir/ritonavir (Dong et al., 2020).

BCG vaccine

Bacillus Calmette-Guérin (BCG; weakened strain of Mycobacterium bovis) vaccination could have protective effects against COVID-19 infection. There are several mechanisms that ensure BCG-induced non-specific protection and are actively studied. BCG and viral antigens have similar molecular structure; so, after vaccination, B and T cells can recognize both pathogen types. Moreover, BCG vaccination results in the so-called trained immunity—epigenetic reprogramming of innate immune cell types (Goodridge et al., 2016). Monocytes of vaccinated individuals had higher expression of different surface markers of activation and synthesis of cytokines (IL-1β, IL-6, IFNγ, and TNF) in response to infection than those of non-vaccinated ones; so non-mycobacterium pathogens, e.g., staphylococci, yellow fever virus, and influenza, can be removed faster (Arts et al., 2018). In several preprints, it is claimed that BCG vaccination program could reduce the number of SARS-CoV-2-infected individuals and their mortality (Miller et al., 2020; Dayal et al., 2020). However, the WHO does not recommend BCG vaccination to prevent COVID-19 because there is still no direct evidence that it can protect against SARS-CoV-2 infection, and all related clinical trials are ongoing (WHO, 2020).

Appendix

Table 1: Variety of therapeutic agents used in clinical trials registered to treat COVID-19

Group	Therapeutic agent	Example of clinical trials registered at ClinicalTrials.gov			
		CT number	**Country**	**Recruitment Status**	**Phase**
Serum	Convalescent serum	NCT04327349	Iran	Enrolling by invitation	Phase I
	Convalescent plasma	NCT04372979	France	Not yet recruiting	Phase III
		NCT04343755	USA	Recruiting	Phase II
		NCT04363034	USA	Available	NA
		NCT04333355	Mexico	Recruiting	Phase I
	Inactivated convalescent plasma	NCT04292340	China	Recruiting	Phase I
	Immunoglobulin of cured patients	NCT04264858	China	Not yet recruiting	NA
	Immunoglobulins obtained with DFPP	NCT04346589	Italy	Recruiting	NA
Monoclonal antibodies	Tocilizumab	NCT04322773	Denmark	Recruiting	Phase II
		NCT04317092	Italy	Recruiting	Phase II
		NCT04331795	USA	Recruiting	Phase II
		NCT04377659	USA	Recruiting	Phase II
		NCT04345445	Malaysia	Not yet recruiting	Phase III
	Sarilumab	NCT04315298	USA	Recruiting	Phase II/III
		NCT04324073	France	Recruiting	Phase II/III
	Avdoralimab	NCT04371367	France	Recruiting	Phase II
	Gimsilumab	NCT04351243	USA	Recruiting	Phase II
	Siltuximab	NCT04329650	Spain	Recruiting	Phase II
	Bevacizumab	NCT04275414	China	Recruiting	Phase II/III
	Eculizumab	NCT04288713	USA	Available	Phase I
	Emapalumab	NCT04324021	Sweden	Recruiting	Phase II/III
	Clazakizumab	NCT04381052	USA	Not yet recruiting	Phase III
	Canakinumab	NCT04362813	USA, Spain, UK	Recruiting	Phase III
		NCT04348448	Italy	Not yet recruiting	NA
	Olokizumab	NCT04380519	Russia	Recruiting	Phase II/III
	Otilimab	NCT04376684	UK	Not yet recruiting	Phase III
	Sirukumab	NCT04380961	USA	Recruiting	Phase II
	Emapalumab	NCT04324021	Sweden	Recruiting	Phase II/III
	Lenzilumab	NCT04351152	USA	Recruiting	Phase III
	Leronlimab	NCT04343651	USA	Recruiting	Phase II
	Ravulizumab	NCT04369469	USA	Not yet recruiting	Phase III
	Nivolumab	NCT04343144	France	Not yet recruiting	Phase II
	Meplazumab	NCT04275245	China	Recruiting	Phase I/II

	CD24Fc	NCT04317040	USA	Not yet recruiting	Phase III
	TJ003234	NCT04341116	USA	Recruiting	Phase I/II
	IC14	NCT04346277	Italy	Available	NA
	Anakinra	NCT04362111	UK	Not yet recruiting	Phase III
	Anakinra vs. siltuximab vs. tocilizumab	NCT04330638	Belgium	Recruiting	Phase III
Interferons	IFN-α	NTC04320236	China	Recruiting	Phase III
	Interferon beta-1A	NCT04350671	Iran	Enrolling by invitation	Phase IV
	Recombinant human interferon α1β	NCT04293887	China	Not yet recruiting	Early Phase I
	Recombinant human interferon alpha-1b	NCT04320238	China	Recruiting	Phase III
	Peginterferon lambda-1a	NCT04331899	USA	Recruiting	Phase II
	Pegylated interferon lambda	NCT04343976	USA	Not yet recruiting	Phase II
NK cells	NK cells	NCT04280224	China	Recruiting	Phase I
		NCT04344548	Colombia	Not yet recruiting	Phase I/II
	iPSC-derived NK cells	NCT04324996	USA	Not yet recruiting	Phase I
	IL15-NK cells vs. NKG2D CAR-NK cells vs. ACE2 CAR-NK cells vs. NKG2D-ACE2 CAR-NK cells	NCT04324996	China	Recruiting	Phase I/II
	CYNK-001	NCT04365101	USA	Not yet recruiting	Phase I/II
Kinase inhibitors	Ruxolitinib	NCT04362137	UK	Recruiting	Phase III
		NCT04348071	USA	Not yet recruiting	Phase II/III
		NCT04355793	USA	Available	NA
		NCT04354714	USA	Not yet recruiting	Phase II
		NCT04377620	USA	Recruiting	Phase III
	Baricitinib	NCT04340232	USA	Not yet recruiting	Phase II/III
		NCT04358614	Italy	Completed	Phase II/III
		NCT04346147	Spain	Recruiting	Phase II
	Acalabrutinib	NCT04346199	Spain	Not yet recruiting	Phase II
		NCT04380688	USA	Not yet recruiting	Phase II
	Duvelisib	NCT04372602	USA	Not yet recruiting	Phase II
	Tofacitinib	NCT04332042	Italy	Not yet recruiting	Phase II
	Imatinib	NCT04346147	Spain	Recruiting	Phase II
	Ibrutinib	NCT04375397	USA	Not yet recruiting	Phase II
	Nintedanib	NCT04338802	China	Not yet recruiting	Phase II
Other immunosuppressors	Fingolimod	NCT04280588	China	Recruiting	Phase II
	Sirolimus	NCT04341675	USA	Recruiting	Phase II
	Tacrolimus	NCT04341038	Spain	Recruiting	Phase III
	Lenalidomide	NCT04361643	Spain	Not yet recruiting	Phase IV
	Methotrexate	NCT04352465	Brazil	Not yet recruiting	Phase I/II

Antivirals	Remdesivir	NCT04292899	USA	Recruiting	Phase III
		NCT04280705	USA	Recruiting	Phase III
		NCT04365725	France	Available	NA
	Favipiravir	NCT04336904	Italy	Active, not recruiting	Phase III
		NCT04346628	USA	Not yet recruiting	Phase II
		NCT04349241	Egypt	Not yet recruiting	Phase III
	Umifenovir	NCT04350684	Iran	Enrolling by invitation	Phase IV
	Abidol hydrochloride vs. oseltamivir vs. lopinavir/ritonavir	NCT04255017	China	Recruiting	Phase IV
	Lopinavir/ritonavir	NCT04330690	Canada	Recruiting	Phase II
		NCT04307693	Korea	Recruiting	Phase II
		NCT04346147	Spain	Recruiting	Phase II
		NCT04328285	France	Recruiting	Phase III
	Galidesivir	NCT03891420	Brazil	Recruiting	Phase I
	Danoprevir, ritonavir	NCT04345276	China	Recruiting	Phase IV
	Darunavir/cobicistat	NCT04252274	China	Recruiting	Phase III
	Virazole	NCT04356677	USA	Not yet recruiting	Phase I
	Clevudine	NCT04347915	Korea	Not yet recruiting	Phase II
	Nitazoxanide	NCT04348409	Brazil	Recruiting	NA
		NCT04359680	USA	Not yet recruiting	Phase III
	Hydroxychloroquine	NCT04329611	Canada	Recruiting	Phase III
		NCT04323631	Israel	Not yet recruiting	Early Phase I
		NCT04340544	Germany	Not yet recruiting	Phase III
		NCT04345692	USA	Recruiting	Phase III
		NCT04362332	Netherlands	Recruiting	Phase IV
Antibiotics	Azithromycin	NCT04332107	USA	Not yet recruiting	Phase III
	Doxycycline	NCT04371952	France	Not yet recruiting	Phase III
	Carrimycin	NCT04286503	China	Not yet recruiting	Phase IV
Decoy biomolecules	rhACE2, rhACE2	NCT04287686	China	Withdrawn	NA
		NCT04335136	Austria, Denmark, Germany	Recruiting	Phase II
	rbACE2	NCT04375046	Egypt	Not yet recruiting	Phase I
	PUL-042, PUL-042	NCT04313023	USA	Not yet recruiting	Phase II
		NCT04312997	USA	Not yet recruiting	Phase II
	Rhu-pGSN	NCT04358406	USA	Not yet recruiting	Phase II
	Piclidenoson	NCT04333472	Israel	Not yet recruiting	Phase II
ACE inhibitors and AR	Ramipril	NCT04366050	USA	Not yet recruiting	Phase II

blockers	Valsartan	NCT04335786	Netherlands	Recruiting	Phase IV
	Losartan	NCT04335123	USA	Recruiting	Phase I
	Telmisartan	NCT04355936	Argentina	Recruiting	Phase II
		NCT04360551	USA	Not yet recruiting	Phase II
MSC and other cells	Cardiosphere-derived cells	NCT04338347	USA	Available	NA
	Dental pulp mesenchymal stem cells	NCT04302519	China	Not yet recruiting	Phase I
	Dental pulp stem cells	NCT04336254	China	Recruiting	Phase I/II
	MSC exosomes	NCT04276987	China	Not yet recruiting	Phase I
	MSC	NCT04252118	China	Recruiting	Phase I
		NCT04361942	Spain	Not yet recruiting	Phase II
		NCT04377334	Germany	Not yet recruiting	Phase II
	AD MSC	NCT04362189	USA	Not yet recruiting	Phase II
		NCT04352803	USA	Not yet recruiting	Phase I
		NCT04366323	Spain	Not yet recruiting	Phase I/II
	BM-MSC	NCT04346368	China	Not yet recruiting	Phase I/II
		NCT04345601	USA	Not yet recruiting	Early Phase I
	UC-MSC	NCT04355728	USA	Recruiting	Phase I/II
		NCT04273646	China	Not yet recruiting	NA
		NCT04333368	France	Recruiting	Phase I/II
		NCT04269525	China	Recruiting	Phase II
		NCT04339660	China	Recruiting	Phase I/II
		NCT04366271	Spain	Not yet recruiting	Phase II
	WJ-MSC	NCT04313322	Jordan	Recruiting	Phase I
Corticosteroids	Ciclesonide	NCT04330586	Korea	Not yet recruiting	Phase II
		NCT04381364	Sweden	Not yet recruiting	Phase II
	Budesonide	NCT04355637	Spain	Recruiting	Phase IV
	Dexamethasone	NCT04325061	Spain	Not yet recruiting	Phase IV
		NCT04360876	USA	Not yet recruiting	Phase II
	Prednisone	NCT04344288	France	Recruiting	Phase II
	Prednisone vs. hydrocortisone	NCT04359511	France	Not yet recruiting	Phase III
	Methylprednisolone	NCT04273321	China	Suspended	NA
		NCT04274071	USA	Completed	NA
	Methylprednisolone sodium succinate	NCT04343729	Brazil	Recruiting	Phase II
Sedatives, antidepressants, neuroleptics	Chlorpromazine	NCT04366739	France	Not yet recruiting	Phase III
		NCT04354805	Egypt	Not yet recruiting	Phase I/II
	Thalidomide	NCT04273529	China	Not yet recruiting	Phase II
	Fluvoxamine	NCT04342663	USA	Recruiting	Phase II

	Fluoxetine	NCT04377308	USA	Recruiting	Phase IV
	Dexmedetomidine	NCT04358627	Spain	Not yet recruiting	NA
Others	Azoximer bromide	NCT0438177	Russia	Recruiting	Phase II/III
	Etoposide	NCT04356690	USA	Not yet recruiting	Phase II
	Bicalutamide	NCT04374279	USA	Not yet recruiting	Phase II
	Selinexor	NCT04349098	USA	Recruiting	Phase II
	Melphalan	NCT04380376	Russia	Recruiting	Phase II
	Bromhexine	NCT04355026	Slovenia	Recruiting	Phase IV
	N-acetylcysteine	NCT04374461	USA	Recruiting	Phase IV
	Sargramostim	NCT04326920	Belgium	Recruiting	Phase IV
	Angiotensin peptide (1-7) derived plasma	NCT04375124	Turkey	Recruiting	NA
	Defibrotide	NCT04335201	Italy	Not yet recruiting	Phase II
	Aviptadil	NCT04311697	USA	Not yet recruiting	Phase II
	Dornase alpha	NCT04355364	France	Recruiting	Phase III
	Nafamostat mesilate	NCT04352400	Italy	Not yet recruiting	Phase II/III
	Camostat mesilate	NCT04321096	Denmark	Not yet recruiting	Phase I/II
		NCT04353284	USA	Not yet recruiting	Phase II
	Almitrine	NCT04357457	France	Not yet recruiting	Phase III
	Sildenafil citrate	NCT04304313	China	Recruiting	Phase III
	Progesterone	NCT04365127	USA	Recruiting	Phase I
	Colchicine	NCT04375202	Italy	Recruiting	Phase II
		NCT04355143	USA	Recruiting	Phase II
	Tetrandrine	NCT04308317	China	Enrolling by invitation	Phase IV
	Vazegepant	NCT04346615	USA	Recruiting	Phase II/III
	Dapagliflozin	NCT04350593	USA	Recruiting	Phase III
	Isotretinoin	NCT04361422	Egypt	Not yet recruiting	Phase III
	Deferoxamine	NCT04333550	Iran	Recruiting	Phase I/II
	SnPP protoporphyrin	NCT04371822	Egypt	Not yet recruiting	Phase I
	Ascorbic acid	NCT04363216	USA	Not yet recruiting	Phase II
	BACTEK-R	NCT04363814	Spain	Not yet Recruiting	Phase III
	Traditional Chinese medicine	NCT04323332	China	Not yet recruiting	Phase III
	Huaier granule	NCT04291053	China	Not yet recruiting	Phase II/III
Combined	Favipiravir, hydroxychloroquine	NCT04359615	Iran	Not yet recruiting	Phase IV
		NCT04376814	Iran	Enrolling by invitation	NA
	Favipiravir, tocilizumab	NCT04310228	China	Recruiting	NA
	Hydroxychloroquine, azithromycin	NCT04328272	Pakistan	Not yet Recruiting	Phase III
		NCT04329832	USA	Recruiting	Phase II

		NCT04359316	Iran	Not yet recruiting	Phase IV
	Hydroxychloroquine, nitazoxanide	NCT04361318	Egypt	Not yet recruiting	Phase II/III
	Hydroxychloroquine, azithromycin, tocilizumab	NCT04332094	Spain	Recruiting	Phase II
	Hydroxychloroquine vs. hydroxychloroquine, lopinavir/ritonavir vs. hydroxychloroquine, azithromycin	NCT04359095	Colombia	Not yet recruiting	Phase II/III
	Hydroxychloroquine, famotidine	NCT04370262	USA	Recruiting	Phase III
	Ivermectin, Nitazoxanide	NCT04360356	Egypt	Not yet recruiting	Phase II/III
	Lopinavir/ritonavir, ribavirin, interferon beta-1B	NCT04276688	China	Completed	Phase II
	Met-enkephalin, tridecactide	NCT04374032	Bosnia and Herzegovina	Recruiting	Phase II/III

Note: *ACE* angiotensin-converting enzyme; *AR* angiotensin receptor; *DFPP* double-filtration plasmapheresis; *MSC* mesenchymal stem (stromal) cells; *AD MSC* adipose-derived MSC; *BM-MSC* bone marrow–derived MSC; *UC-MSC* umbilical cord–derived MSC; *WJ-MSC* Wharton jelly–derived MSC; *NK cells* natural killer cells; *rhACE2* recombinant human angiotensin-converting enzyme 2; *rbACE2* recombinant bacterial angiotensin-converting enzyme 2; *Rhu-pGSN* recombinant human plasma gelsolin

5. CONCLUSION

The present review study has successfully reviewed the novel approaches for the treatment of COVID infection. The study signified that it seems that antiviral drugs are effective in improving clinical manifestation and controlling the SARS-CoV-2 infection, until now, however, there is no definite treatment protocol for this novel corona virus infection.

The study concluded that 'Lymphocytopenia' alongside with excessive inflammation and cytokine storm followed by ARDS in these patients are still unsolved problems that cause severity of the disease (Zhang et al., 2020). Therefore, the study has considered that immune response modulation and inflammation management are essential steps. On the basis of aforementioned the present study suggest that more studies needed to be conducted on immunopathogenesis and immune response during the SARS-CoV-2 infection. Although, new therapeutic approaches including mesenchymal stromal cell therapy and immune cell therapy showed promising results. The study also signified that there is still no licensed treatment or approved vaccine to combat the coronavirus. Therefore, more studies are required that are more focusedfordeveloping successful treatment to combat with this COVID infection.

Ethical Considerations

The study adheres to the guidelines described by the ethical committee for the research work. The author declares that there is no any conflict of interest.

Conflicts of Interest Statement

The authors declare no conflict of interest.

REFERENCES

1. WMHC. Wuhan Municipal Health and Health Commission's Briefing on the Current Pneumonia Epidemic Situation in Our City. 2020, http://wjw.wuhan.gov.cn/front/web/ showDetail/2019123108989 (accessed on 30th Sep, 2020).
2. Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., ... & Xing, X.. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *New England Journal of Medicine*. 2020 DOI: 10.1056/NEJMoa2001316.
3. CDC. 2019 Novel Coronavirus, Wuhan,China. https://www.cdc.gov/coronavirus/2019-nCoV/summary.html (accessed on 30th Sep, 2020).
4. WHO. Novel Coronavirus-Japan (ex-China). https://www.who.int/csr/don/17-january-2020-novel-coronavirus-japan-ex-china/en/ (accessed on 30th Sep, 2020).
5. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., ... & Niu, P. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*.
6. WHO. Statement on the second meeting of the International Health Regulations (Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV).2005(https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international -health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-nco v) (accessed on 29th Sep, 2020).
7. Fehr, A. R., Channappanavar, R., & Perlman, S. Middle East respiratory syndrome: emergence of a pathogenic human coronavirus. Annual review of medicine, 2017; *68*: 387-99.
8. Zhang H et al, Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med., 2020; 1–5.
9. Wang, S., Zhang, Y., Liu, S., Peng, H., Mackey, V., & Sun, L. Coronaviruses and the associated potential therapeutics for the viral infections. *J Infect Dis Ther.*, 2020; *8*(417): 2.
10. Shanmugaraj, B., Malla, A., & Phoolcharoen, W. Emergence of novel coronavirus 2019-nCoV: need for rapid vaccine and biologicsdevelopment. *Pathogens*, 2020; *9*(2): 148.
11. Cai, Q., Yang, M., Liu, D., Chen, J., Shu, D., Xia, J., ... & Shen, C. Experimental treatment with favipiravir for COVID-19: an open-labelcontrol study. *Engineering*, 2020.
12. Thiel, V., & Weber, F. Interferon and cytokine responses to SARS-coronavirus infection. *Cytokine & growth factor reviews*, 2008; *19*(2): 121-132.
13. Heymann, D. L., & Shindo, N. COVID-19: what is next for public health?. *The Lancet*, 2020; *395*(10224): 542-545.
14. Frieman, M., Heise, M., & Baric, R. SARS coronavirus and innate immunity. *Virus research*, 2008; *133*(1): 101-112.
15. Zhu, R. F., Gao, R. L., Robert, S. H., Gao, J. P., Yang, S. G., & Zhu, C. (2020). Systematic Review of the Registered Clinical Trials of Coronavirus Diseases 2019 (COVID-19). *medRxiv*.
16. Yu, F., Du, L., Ojcius, D. M., Pan, C., & Jiang, S. Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China. *Microbes and infection*, 2020.
17. Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, 2020; *367*(6485): 1444-1448.
18. Zhang, L., & Liu, Y. Potential interventions for novel coronavirus in China: A systematic review. *Journal of medical virology*, 2020; *92*(5): 479-490.