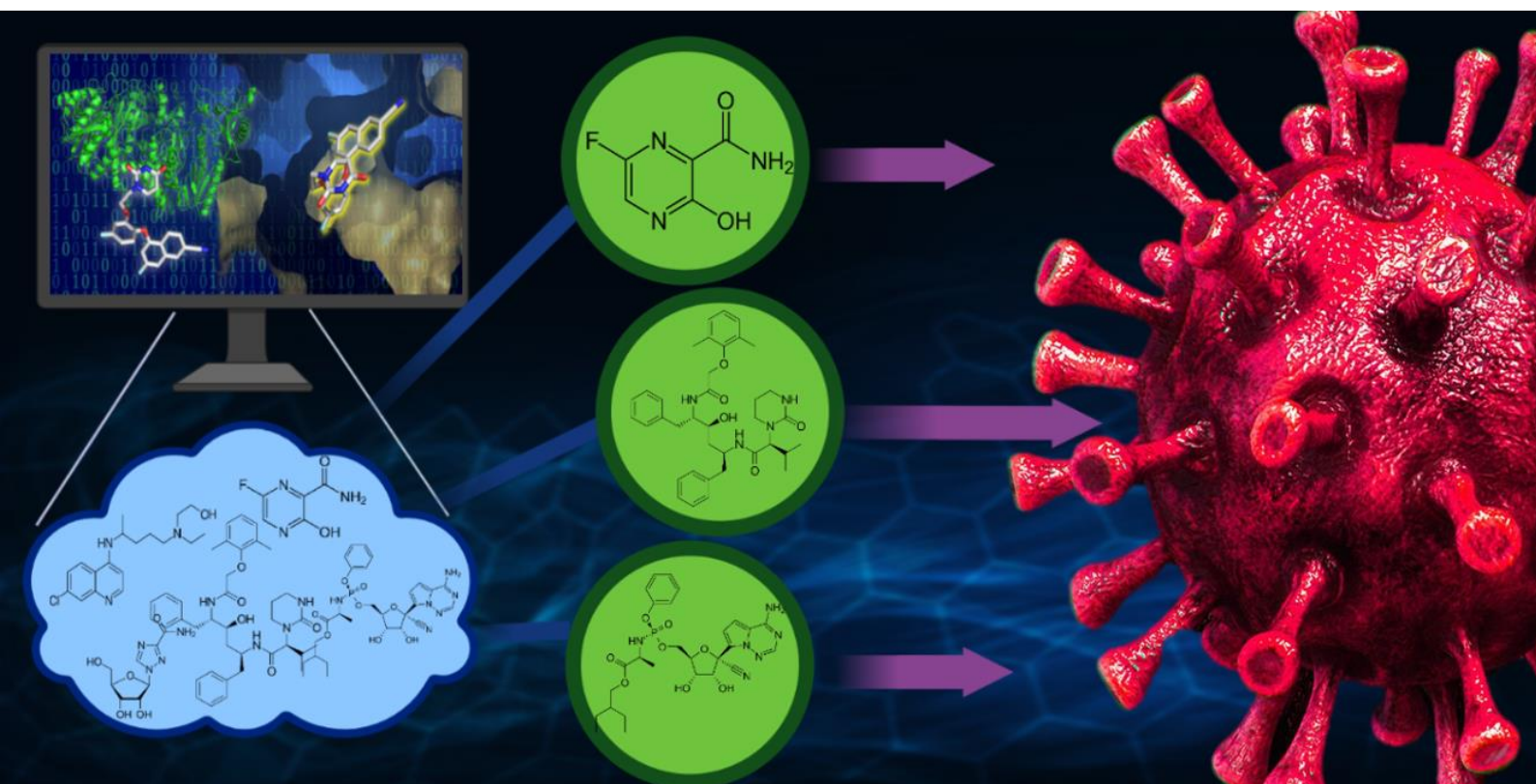


# Potential therapeutics and vaccine candidates for coronavirus disease-2019

An insight into the characteristics of zoonotic coronaviruses and the current development of therapeutics and vaccines for coronavirus disease-2019



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## Abstract

The novel coronavirus, a single-stranded positive-sense RNA, emerged from Wuhan has caused a global pandemic, causing surge of COVID-19 patients in hospital systems worldwide. Previous zoonotic coronaviruses outbreaks provided us knowledge on the immunopathology and the function of viral components, paving the way for the COVID-19 therapeutics and vaccine development. This article provides insight into pharmaceutical interventions, identifying potential COVID-19 therapeutics and vaccines.

As of May 23<sup>rd</sup>, 2020, there were 223 therapeutics and 141 vaccines in clinical trials. Some of the COVID-19 therapeutics and vaccines showed promising preliminary results and other negative results. However, it is difficult to determine whether they have an essential role in COVID-19 treatment in the future since most of the trials have their shortcomings. Remdesivir is the only drug approved for COVID-19. Lesson learned from this pandemic is that new clinical trial design needs to be designed to speed up therapeutic development for future pandemic

**Keywords:** COVID-19, Drug development, vaccine, therapeutic, clinical trial.

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## List of Abbreviation

ACE2 : Angiotensing-converting enzyme type 2 .....	6
AKT : Protein kinase B.....	21
ARDS : Acute Respiratory Distress Syndrome .....	13
ATP : Adenosine Triphosphate.....	19
CAS : Chemical Abstracts Services.....	23
CC5R : Chemokine receptor type 5 .....	22
CI : Confidence Interval.....	27
COVID-19 : Corona Virus Disease 2019 .....	4
ECMO : Extracorporeal Membrane Oxygenation .....	15
ERGIC : Endoplasmic reticulum Golgi intermediate compartment .....	10
FDA : Food and Drug Administration .....	18
G-CSF : Granulocyte Stimulating Factor.....	14
HFNC : High Flow Nasal Cannula .....	15
HR : Heptad Repeat .....	18
IBM : International Bussiness Machine.....	23
ICU : Intensive Care Unit.....	11
IFN : Interferon .....	7
IgG : Immunoglobulin G .....	15
Il : Interleukin .....	13
IP : Chemokine .....	13
Jak : Janus Kinase .....	21
MAPK : Mitogen activated protein kinase .....	21
MCP : Monocyte chemoattractant protein.....	13
MERS : Middle Eastern Respiratory Syndrome .....	5
MERS-CoV : Middle Eastern Respiratory Syndrome Coronavirus .....	5
MHC : Major Histocompatibility Complex .....	14
MIP : Macrophage Inflammatory Protein.....	14
NIAID : National Institute of Allergy and Infectious Disease.....	26
NLRP : NOD-like receptor protein.....	20
NPV : Negative Pressure Ventilation.....	16
nsp : nonstructural protein .....	9
OR : odds ratio.....	27
PD-1 : programmed cell death protein 1 .....	22
PDG : Prostaglandin .....	14
PI3K : Phosphoinositide 3-kinase .....	21
PICALM : Phosphatidylinositol binding clathrin assembly protein .....	9
PPV : Positive Pressure Ventilation.....	16
RdRp : RNA dependent RNA polymerase .....	19
ROS : Radical Oxygen Species.....	13
RTC: Replication Transcription Complex .....	19
SARS : Severe Acute Respiratory Syndrome .....	5
SARS-CoV : Severe Acute Respiratory Syndrome Coronavirus .....	5
SIMPLE : Shockless IMPlant Evaluation.....	26
Stat : signal transducer and activator of transcription.....	21
TMPRSS : Transmembrane protease serine .....	18
TNF : Tumor Necrosis Factor.....	13

WHO : World Health Organization ..... 4

## Introduction:

On March 11th, the WHO (World Health Organization) declared the COVID-19 outbreak a global pandemic.<sup>1</sup> The pandemic that is caused by the novel coronavirus has affected many peoples' lives in so many ways. Since people do not have a natural immunity to this novel coronavirus, many people's health is, therefore, at risk, especially immunocompromised people and people with comorbidity. Thus, many countries are implementing social distancing measures to protect vulnerable people and minimize the spread of this virus, thereby "flattening the curve." However, social distancing measures harm the global economy and people's mental health, so it is not a permanent solution to eradicate COVID-19.

The outbreak is caused by a novel coronavirus and was first detected in Wuhan.<sup>2</sup> In December 2019, hospitals in Wuhan were receiving several patients with an unknown pneumonia-like illness, which led doctors in Wuhan to believe that an infectious disease might cause this illness.<sup>3</sup> Shortly after detecting this unknown pneumonia-like illness, they took CT scans and fluid samples from several patients. After analyzing the CT scan results from the patients and the laboratory results, they suggested that this unknown infectious disease has a noticeable similarity to the virus that caused the SARS outbreak back in 2003. They also indicated that most of the cases of unknown pneumonia-like illnesses are linked to a wet market named Huanan seafood wholesale market in Wuhan.<sup>4</sup> Some critics claimed that the novel coronavirus might have originated from the Wuhan P4 lab.<sup>5</sup> However, there is no real evidence that suggests that the novel coronavirus came from the lab in Wuhan. It is more likely that the virus is originated from wild animals and spread to humans.

On December 29th, a doctor in Wuhan named Li Wenliang shared an RNA test results with his colleagues from other hospitals via WeChat, warning them about a SARS-like coronavirus circulating in Wuhan.<sup>6</sup> However, the messages from Dr. Li gained significant attention on Chinese social media. For this reason, Chinese Officials reprimanded Dr. Li and other doctors for spreading rumors and misinformation. However, the World Health Organization (WHO) was notified about this unknown pneumonia-like disease and asked for more clarification from the Chinese authorities.

On December 31st, the Chinese health official reported 44 cases of unknown pneumonia-like illness to the WHO.<sup>7</sup> Subsequently, a Chinese laboratory based in Shanghai determined the genetic sequence of this respiratory pathogen and shared it to the rest of the world.<sup>8</sup> Based on the determined genetic sequence, the respiratory pathogen was identified as a novel coronavirus, enabling scientists and researchers worldwide to develop vaccines and diagnostic screening tests for COVID-19 quickly.

As of May 15th, there are 302,025 deaths and 4,437,442 cases reported worldwide. COVID-19 poses not only a significant problem to the global economy but also global health systems.<sup>9</sup> Since the outbreak has started, hospitals are receiving many more patients than they can handle. Therefore, the health systems around the world are at risk of collapsing since there is not enough capacity, medical equipment, and personnel available to treat those patients. As a result, fewer patients are being treated in hospitals, which can lead to more deaths. To prevent hospitals from being overwhelmed by COVID-19 patients, governments worldwide issued social distancing measures and other non-pharmaceutical public health measures. In response, medical biotech companies and pharmaceutical companies are trying to develop effective treatment strategies, preventative and diagnostic strategies intending to

decrease the number of infected people and deaths. Therefore, it is essential to understand the progress that is currently being made in the development of COVID-19 interventions. It is also important to know how such interventions can contribute to the fight against COVID-19.

This article outlines different pharmaceutical interventions that are currently in development. It also provides insight into the drug and vaccine development and identifies potential therapeutics and vaccine candidates for COVID-19 infections.

## Characteristics of COVID 19

### Origin

The novel coronavirus (COVID-19) that causes the current pandemic belongs to the Beta coronavirus family, a group of coronaviruses mostly found in mammals. To this day, there are four groups of coronaviruses identified, namely alpha, beta, gamma, and delta coronaviruses.<sup>10</sup> Only the alpha and beta coronavirus groups are known to infect humans and other mammals. Gamma and delta coronavirus families are mainly found in birds. However, there are currently seven species of coronaviruses identified that can cause respiratory and gastrointestinal diseases in humans. Four of them (229E, OC43, NL63, and HKU1) are found in humans and are known to cause common cold symptoms in immunocompromised individuals.<sup>11</sup> The other three viruses are zoonotic and are responsible for the outbreaks that started in 2003 (SARS), 2012 (MERS), and 2019 (COVID-19).<sup>12</sup> These zoonotic viruses are known to cause severe acute respiratory syndrome, which can be life-threatening for immunocompromised patients.

Zoonotic viruses are viruses that originated from non-human animals and may be transmitted to humans. Most of the zoonotic viruses require a second or intermediate host, which serves as a vector for viral transmission to humans. Previous studies reported that both SARS-CoV and MERS-CoV are closely related to bat coronaviruses CoV HKU3 and CoV HK4/5, respectively, which indicates that bats are the natural reservoir of these zoonotic coronaviruses.<sup>13 14</sup> Previous studies have demonstrated that the intermediate hosts of SARS-CoV and MERS-CoV are palm civets and dromedary camels, suggesting that intermediate hosts were responsible for the viral transmission to humans for both SARS and MERS.<sup>15 16</sup>

Recently, a study has found that the genomic sequence of COVID-19 is 96% identical to the genomic sequence of RaTG13, a coronavirus previously found in bats, which indicates that bats could be the potential natural reservoir of COVID-19.<sup>17</sup> However, other researchers claimed that the potential intermediate host for COVID-19 is a snake based on Ji *et al.* (2020) study.<sup>18</sup> However, the study Ji *et al.* (2020) included a limited number of snake's CDS (coding regions of a gene) for the bioinformatics analysis, indicating that the results from the study Ji *et al.* are inconclusive.

Therefore, there is no evidence that snakes are the potential intermediate host for COVID-19. On the other hand, several studies, Zhang *et al.* (2020) Lam *et al.* (2020), and Xiao *et al.* (2020) suggested that the pangolin is the potential intermediate host for COVID-19.<sup>19 20 21</sup> According to these studies, the genome sequence of pangolin coronavirus shares 91% similarity to the genome sequence of COVID-19. On top of that, the spike protein of COVID-19, which is a crucial viral component for the host cell entry, is found to be 92% identical to the spike protein of coronavirus found in pangolin.<sup>22</sup> These results imply that pangolin is the intermediate host for COVID-19. Although these studies did provide strong evidence that



pangolin can be the potential intermediate host, more research on the origin of COVID-19 needs to be done because identifying natural or intermediate hosts is crucial when it comes to the development of potential treatments for COVID-19. It is known that most virus hosts can carry the virus without generating immune responses, which indicates that these virus hosts must have exhibited a unique mechanism that suppresses the virus's pathogenicity.

### Structure of coronavirus

It is essential to understand the structure of coronavirus and knowing the functions of different structural components, which can be served as potential drug targets for the development of novel COVID-19 therapeutics.

All coronaviruses have the same structural components. However, the type of accessory proteins expressed, the mechanism of entry and replication vary depending on the coronavirus type.

The coronavirus's genetic material is a positive-sense single-stranded RNA with a size of approximately 26 to 30 kilobases. Also, the RNA contains a 3'-poly-A tail and 5'cap structure.<sup>23</sup> The genetic material is encapsulated in a helical nucleocapsid made of nucleocapsid (N) proteins, a phosphoprotein with two RNA-binding domains, which packages the viral RNA into a helical nucleocapsid by forming a complex with the viral RNA. Nucleocapsid proteins are also involved in viral assembly and viral RNA replication of coronaviruses.<sup>24 25</sup>

Furthermore, the viral helical nucleocapsid complex is encapsulated by a lipid bilayer membrane containing the spike (S) proteins, envelope (E) proteins, and membrane (M) proteins.<sup>26</sup> The spike (S) protein on the surface of the viral envelope is a trimeric glycoprotein consisting of two domains, namely S1 and S2 domain. The spike protein mediates the viral-cell fusion by interacting with the host ACE2 receptors. The membrane protein is a dimeric glycoprotein that spans three times across the viral membrane.<sup>27</sup> A study has shown that M protein can facilitate the viral assembly by converting cellular membrane into a viral membrane and by incorporating different viral proteins into the viral membrane at the budding site of the endoplasmic reticulum Golgi intermediate compartment.<sup>28</sup>

Moreover, the envelope (E) protein is a glycoprotein incorporated into the viral envelope, playing a role in viral assembly and the release of virus-like particles.<sup>29</sup> The viral envelope and the viral helical nucleocapsid complex constitute the viral particle with a diameter of approximately 200 nm.<sup>30</sup> (figure 1).

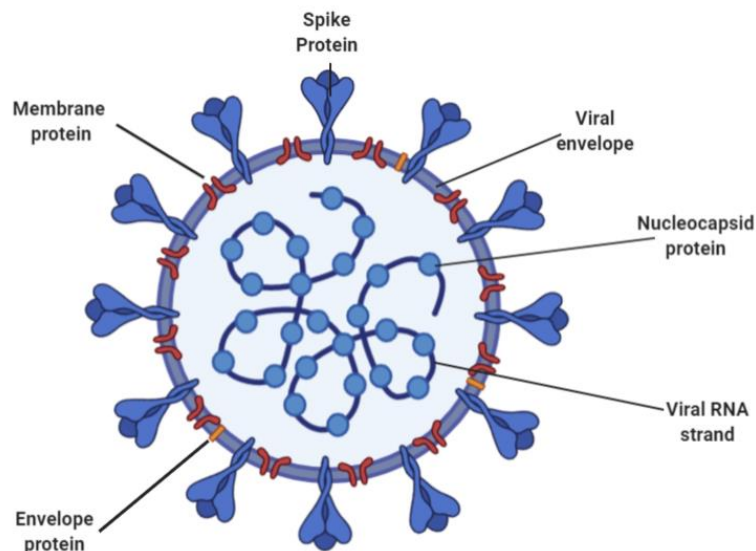


Figure 1. Structure of coronavirus



SARS-CoV, MERS-CoV, and COVID-19 have similar structural viral components. However, the differences between these zoonotic coronaviruses lie in the genome sequences and the types of accessory proteins encoded in the genome. The genomic sequence organization of SARS-CoV, MERS-CoV, and COVID-19 is displayed in figure 2. As shown in figure 2, the RNA of both coronaviruses encodes for the same viral proteins, such as pp1a, pp1b, and four structural proteins such as N, M, S, E. It is also noticeable that all three viral RNAs express different accessory proteins. For instance, SARS-CoV express eight different accessory proteins (3a, 3b, 6, 7a, 7b, 8a, 8b, and 9b).<sup>31 32</sup> Whereas MERS-CoV only express five different accessory proteins (3, 4a, 4b, 5, and 8b).<sup>33</sup> A study by Ren *et al.* (2020) was able to sequence and determine the genome of the novel coronavirus (COVID-19). The determined RNA sequence of COVID-19 shows that the COVID-19 genome encodes the same viral proteins (pp1a/1b, S, M, E, and N) as the other two coronaviruses.<sup>31</sup>

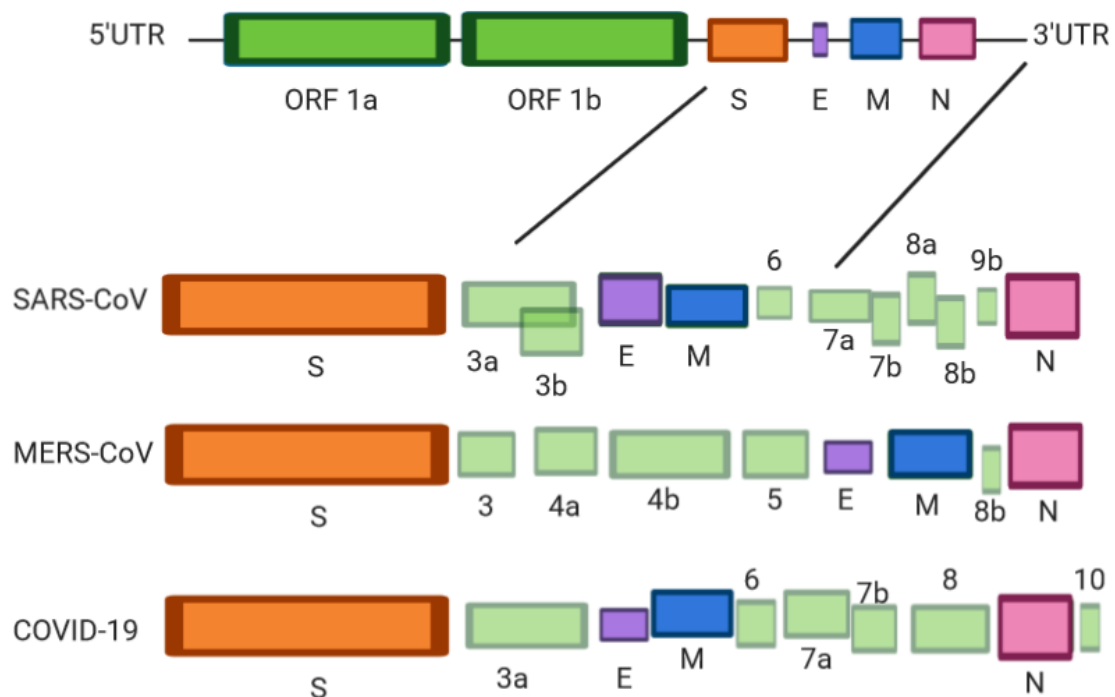


Figure 2. Genomic organization of SARS-CoV, MERS-CoV and COVID-19.

Moreover, Ren *et al.* (2020) suggested that the genome sequence of COVID-19 shares 79.0% and 51.8% similarity to SARS-CoV and MERS-CoV, respectively. The study Ren *et al.* (2020) also showed that the RNA of COVID-19 only express six different accessory proteins, such as 3a, 6, 7a, 7b, 8, and 9b, which differ from the type of accessory proteins expressed in SARS-CoV and MERS-CoV. These findings show that COVID-19 shares some degree of similarity to the other two coronaviruses in terms of structural proteins. However, the infectivity and the severity of COVID-19 might be different compared to SARS-CoV and MERS-CoV since all three viruses express different kinds of accessory proteins.<sup>34</sup> The function of different types of accessory protein is displayed in table 1.<sup>35</sup> As shown in Table 1, most accessory proteins inhibit the production and signaling of type 1 IFN, which prevents the host immune system from eliminating virus infection.

Table 1. The function of different accessory proteins

Accessory proteins	Functions
1a	Polyprotein 1a replicative enzyme components
1b	Polyprotein 1b replicative enzyme components
2	Spike protein
3a	Increases NF-kB expression, stimulate the production of IL-8, induce cell apoptosis and necrosis
3b	Inhibits type 1 INF signaling and production, induces cell apoptosis
4	Envelope protein
5	Membrane protein
6	Inhibits type 1 IFN signaling and production, stimulates viral RNA-replication.
7a	Stimulates NF-kB expression, MAP kinase activity and inhibits host translation
7b	Promotes Golgi localization
8a	Induces host cell apoptosis
8b	Stimulates host DNA replication
9a	Nucleocapsid protein
9b	Induces host cell apoptosis via caspase
10	Unknown

### Mechanism of entry

It is known that most coronaviruses interact with the host cell receptor to get into the host cell. The mechanism of entry differs between species of coronaviruses. Previous study Ren *et al.* (2020) and Shang *et al.* (2020) have shown that SARS-CoV invades respiratory cells by interacting with the host angiotensin-converting type 2 (ACE2) receptors through its spike protein on the surface of the virus.<sup>30 36</sup> The study Ren *et al.* has demonstrated that the spike protein of COVID-19 is 75.5% identical to the spike protein of SARS-CoV, which indicates that COVID-19 uses the same mechanism to enter the host cell.

The spike protein facilitates the host cell-virus attachment by directly binding to the ACE2 receptors on the host cell. As mention earlier, the spike protein consists of two subunits, namely, S1 and S2 subunits. The studies Babcock *et al.* (2004) and Wong *et al.* (2003) have demonstrated that the receptor-binding domain is located in the S1 subunit, which can specifically interact with the host ACE<sub>2</sub> receptors.<sup>37 38</sup> ACE2 receptors are highly expressed in lung epithelial cells and enterocytes in small intestines.<sup>39</sup> It is believed that the receptor-binding domain of the S1 subunit goes through conformational changes. The receptors binding domain of S1 subunit changes from down conformation to up conformation, which allows the spike protein to recognize the ACE2 receptors directly, thereby binding to the host receptors.<sup>40</sup> Once the spike protein is attached to the receptor, the adjacent host protease such as TMPSS2, Tmprss11d, and furin will cleave the spike protein at the S1/S2 or S2 sites, which allows the fusion peptide of S2 subunits to induce fusion between viral and cellular membrane.<sup>41 42 43</sup>

However, some studies Burckard *et al.* (2014) and Wang *et al.* (2008) have demonstrated that coronaviruses such as SARS-CoV and COVID-19 can also enter the host cell through endocytosis mediated by clathrin. Clathrin is a protein responsible for shaping rounded

vesicles during endocytosis by forming a coat around the vesicle.<sup>44</sup> The binding of spike protein to the ACE2 receptor activates the phosphatidylinositol binding clathrin assembly protein (PICALM), an adaptor protein. The activated adaptor proteins (PICALM) will recruit clathrins to the site of the membrane and initiate polymerization to form a coat around the vesicle. The formed vesicle containing the virus is then transported to the endosomes, where pH-dependent membrane fusion takes place. The fusion between viral and endosomal membrane is induced by cathepsin, a pH-dependent protease mainly found in endosomes and lysosomes. It is known that this protease can cleave the spike protein into S1 and S2 subunits, thereby inducing the fusion between the viral membrane and the endosomal membrane.<sup>45 46</sup> The two different pathways of entry mechanism are illustrated in figure 3.

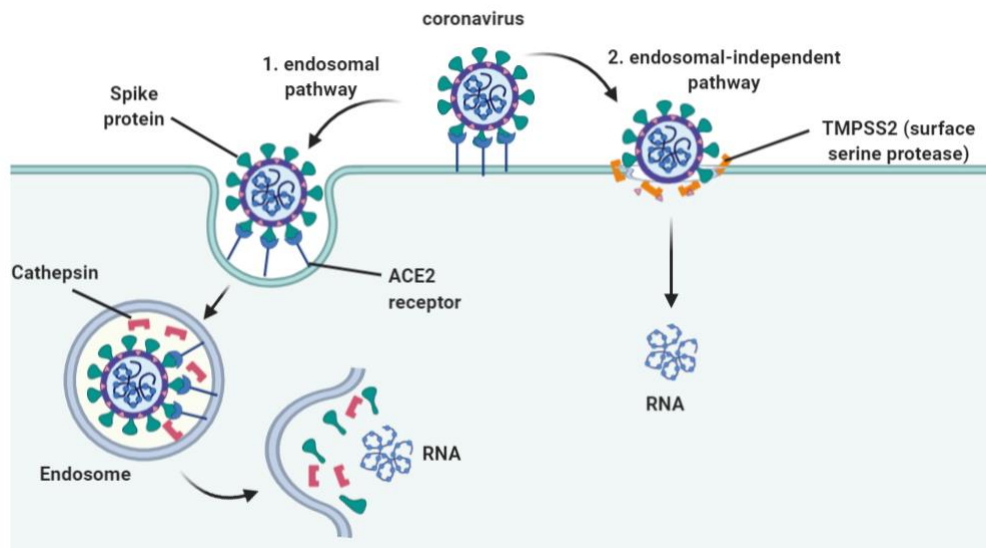


Figure 3. Endosomal and endosomal-independent entry pathways of coronavirus.

### Viral replication:

Once the virus is released into the cytoplasm, the virus will undergo uncoating, a process in which the viral envelope disassembles, releasing the nucleocapsid containing the viral RNA. Subsequently, the viral positive-sense RNA is translated by the host ribosomes, producing polyprotein pp1a and pp1b and other structural proteins such as S, M, E, and N proteins.<sup>47</sup> The host and viral proteases such as papain-like protease (nsp 3) and 3C-like protease (nsp 5) will degrade these polyproteins into 16 different nonstructural proteins (NSP).<sup>44</sup> Each NSP has its own functions. Nsp 7, 8, 9, 10, 12, 13, and 14 are replicative enzymes that jointly form the replication-transcription complex, which plays a role in the synthesis of viral mRNA. The replication-transcription complex is mainly located in a double membrane vesicle, a vesicle formed by nsp 4 and nsp 6. It is believed that this membrane vesicle protects the replication-transcription complex from the host immune response.<sup>48 49</sup>

Moreover, several nonstructural proteins (nsp 1, 2,3,5, and 16) involved in the viral evasion mechanisms (see table 1).<sup>50</sup> These proteins inhibit the IFN signaling in host cells, counteract the innate immune response and prevent the viral RNA from getting recognized by the host immune system.<sup>40</sup>

Table 2. The function of different nonstructural proteins

Nonstructural proteins	Function
<b>Nsp1</b>	Inhibits gene expression by inducing endonuclease cleavage on host RNA, thereby preventing IFN signaling in the host cell
<b>Nsp 2</b>	Disrupts intracellular signaling (cell growth, cell division, and apoptosis) in the host cell by interacting with host proteins such as PHB1 and PHB2
<b>Nsp 3</b>	The papain-like protease cleaves polyprotein into nonstructural proteins and counteracts innate immune systems
<b>Nsp 4</b>	Induce the formation of the double-membrane vesicle.
<b>Nsp 5</b>	3C like- protease; cleaves polyprotein into nonstructural proteins and counteracts IFN signaling in the host cell.
<b>NSp 6</b>	Stimulates double-membrane vesicle formation
<b>Nsp 7</b>	Serves as a cofactor for nsp 8 and nsp 12
<b>Nsp 8</b>	Primase; forms complex with nsp seven and nsp12
<b>Nsp 9</b>	Is an RNA-binding protein that interacts with nsp 8
<b>Nsp 10</b>	Is cofactor; regulates the activity of replicase enzymes
<b>Nsp 11</b>	Unknown
<b>Nsp 12</b>	RNA directed RNA polymerase
<b>Nsp 13</b>	Helicase
<b>Nsp 14</b>	Exhibits exonuclease activity and is responsible for proofreading
<b>Nsp 15</b>	Exhibits endonuclease activity, which prevents the activation of the dsRNA sensor, thereby inhibiting the IFN signaling
<b>Nsp 16</b>	2'-O-MTase; is responsible for RNA capping, which prevents the viral RNA from getting recognized by the host innate immune system.

The formed replication-transcription complex binds to the positive-sense viral RNA and reads the RNA, which produces a negative-sense RNA template. Subsequently, the newly formed N nucleocapsid proteins interact with the negative-sense RNA, forming a helical

nucleocapsid around the negative-sense RNA. The helical nucleocapsid containing the RNA is then transported to the Endoplasmic reticulum Golgi intermediate compartment (ERGIC).<sup>21 22</sup> The M membrane proteins and other structural proteins are assembled at ERGIC. The M membrane protein converts the Golgi membrane into a rounded shape viral membrane, which allows other structural proteins to come together. Subsequently, the membrane proteins interact with other structural proteins and the nucleocapsid to incorporate them into the viral membrane.<sup>25</sup>

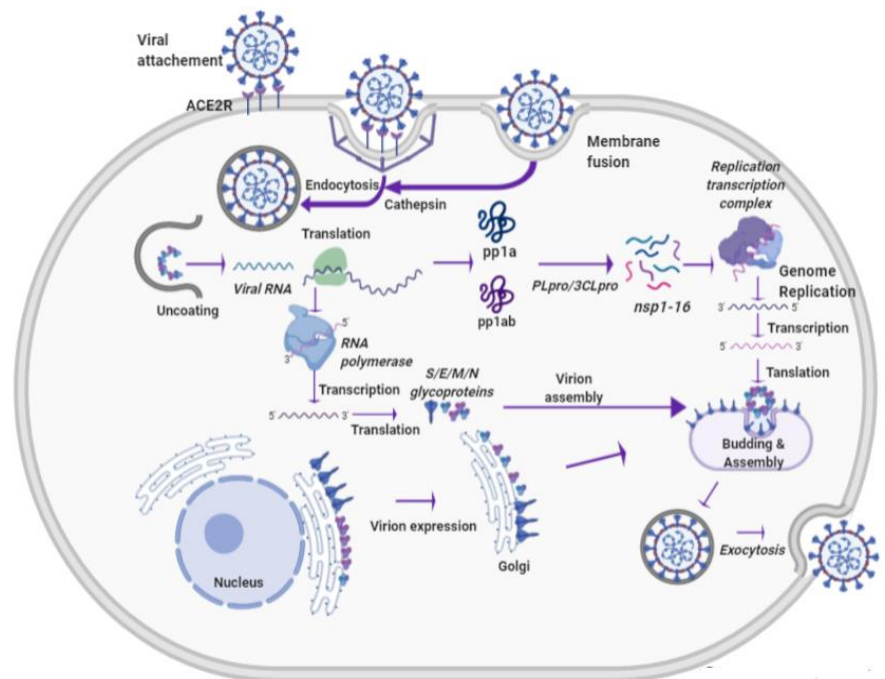


Figure 4. Replication process of coronavirus.

The envelope proteins and the membrane protein are involved in budding, a process by which the virion splits from the Golgi membrane compartment, forming a viral particle.<sup>24 25</sup> This viral particle is then transported to the plasma membrane by a vesicle. Finally, the viral envelope fuses with the plasma membrane, releasing the newly formed virus particle outside the cell. The viral replication process is illustrated in figure 4.

### Clinical characteristics of COVID-19

It is known that coronaviruses such as SARS-CoV, MERS-CoV, and COVID-19 can actively replicate in lung cells, causing respiratory inflammation. Patients infected with this novel coronavirus will start developing symptoms after 2 to 14 days<sup>51</sup>. According to several studies, the average incubation period of SARS-CoV and MERS-CoV is 4.0 days and 4.5-5.2 days, respectively, which means that COVID-19 has a more extended incubation period compared to SARS-CoV and MERS-CoV.<sup>52 53</sup> During this incubation period, patients who are carrying this virus are capable of infecting other people before developing symptoms, and this phenomenon is called pre-symptomatic viral transmission. This finding is supported by several studies (Wei *et al.*, 2020 and Furukawa *et al.*, 2020) suggesting that people infected with COVID-19 can still be contagious before showing any sign of symptoms.<sup>54 55</sup> For this reason, COVID-19 can spread rapidly from person to person, making it challenging to trace and contain this novel coronavirus.

Recent data have shown that COVID-19 has an average  $R_0$  is between 2.2-2.6.<sup>42</sup>  $R_0$  stands for basic reproductive number, and it says something about the transmission rate of the virus.<sup>56</sup> Viruses with an  $R_0$  greater than 1 are capable of spreading in the population, whereas viruses with  $R_0$  lower than 1 cannot spread in the population.<sup>57</sup> In this case, an individual infected with COVID-19 is capable of spreading the virus to 2.2-2.6 other people. The COVID-19's  $R_0$  is higher compared to SARS-CoV and MERS-CoV. According to the data from previous studies, the average  $R_0$  of SARS-CoV and MERS-CoV is 0.67-1.23 and 0.29-0.80, respectively.<sup>58 59</sup> This suggests that COVID-19 spreads faster compared to SARS-CoV and MERS-CoV.

Moreover, a study with 41 COVID-19 patients has determined the clinical characteristics of COVID-19.<sup>60</sup> The primary symptoms of COVID-19 observed in these patients are fever (98% of patients), cough (76% of patients), dyspnea (55% of patients), fatigue (44% of patients), and sputum production (28% of patients). The minor symptoms observed are headache (8% of patients), hemoptysis (5% of patients), and diarrhea (3% of patients). In addition, 32% of the patients were in critical condition and needed ventilation support in the ICU.<sup>48</sup> The observed symptoms of COVID-19 are similar to the symptoms of both SARS and MERS. A study with 47 MERS patients showed that the most common symptoms observed were fever (98%), cough (83%), dyspnea (72%), and fatigue (32%). In this study, 89% of the patients infected with MERS were admitted to the ICU for ventilation support, which indicates that MERS infection is more severe than COVID-19.<sup>61</sup> The mortality rate of MERS is 34.4%, according to WHO, which is higher than the mortality rate of COVID-19 (2%).<sup>62 63</sup> On the other hand, the most common symptoms observed in SARS patients are also fever (100%), cough (56%-72%), dyspnea (40%-42%), and fatigue (45%-61%).<sup>64</sup> According to a study, 20% and 36% of the patients infected with SARS were admitted to ICU for ventilation support.<sup>65</sup> The SARS mortality rate was 9.6%, which is higher than COVID-19 (1.4%), but lower than MERS (34.4%).<sup>50 51 66</sup>



The severity of viral infections and the fatality rate vary between person to person. It is believed that the severity of COVID-19 infection depends on factors such as gender, age, and comorbidity

A recent descriptive, explanatory study from the Chinese health official has found that 80,9% of the patients showed mild symptoms (without pneumonia or mild pneumonia) while 13.8% and 4.7% of the patients are in severe (severe pneumonia and hypoxemia) and critical condition (sepsis, multiple organs failures), respectively.<sup>67</sup> This study showed that the fatality rate for men is 2.8%, whereas the fatality rate for women is 1.7%, indicating that there is a correlation between gender and the severity of the infection. The gender differences in patients are still unknown, and more research needs to be done. However, several studies suggested that men express a higher level of circulating ACE2 compared to women.<sup>68 69</sup> It is known that ACE2 facilitates viral entry to the host cells. This means that a higher level of ACE2 can increase viral entry, which leads to increased viral infection. Hence, Men are more susceptible to develop severe or critical complications, which can lead to hospitalization and death.

Furthermore, this study also showed that the fatality rate for the age between 0 and 39 is 0.2%, and it is noticeable that the fatality rate started to increase at the age of 40 (see figure 5). This finding indicates that the elderly are more likely to be at risk.

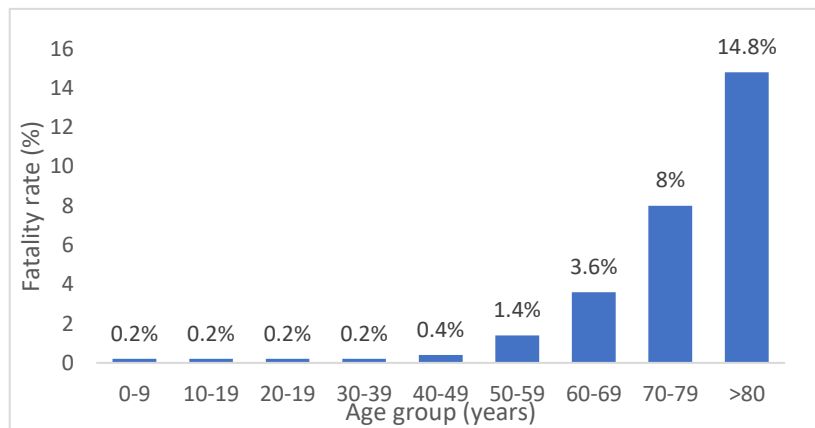


Figure 5. Fatality rate (%) in different age groups.

In addition, this study shows that patients with underlying conditions are more likely to die compared to patients without comorbid conditions. The fatality rate for different comorbid conditions is illustrated in figure 6. As shown in figure 6, the fatality rate for hypertension is the highest, followed by cardiovascular diseases and diabetes. This is because people with cardiovascular diseases or diabetes express a high level of ACE2.<sup>57 70</sup> Therefore, there is an increased viral infection in these patients, leading to more severe complications. For this reason, these

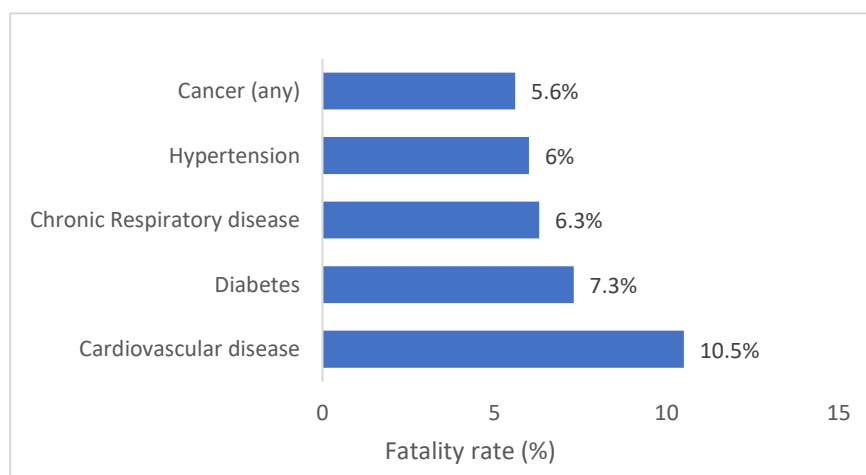


Figure 6. Fatality rate (%) for different comorbid condition.



patients are more likely to die from severe COVID-19 infection compared to patients with comorbidity.

## Immunopathology of COVID-19

### Innate immune response

There are still not enough information and knowledge on how COVID-19 affects our immune systems. However, we can utilize previous data of SARS and MERS to predict the immunopathology of COVID-19 since this novel coronavirus is closely related to SARS-CoV and MERS-CoV.

Infected cells and damaged cells release chemokines and IFN type 1 to recruit local innate immune cells such as dendritic cells, natural killer cells, and macrophages to the site of inflammation. Subsequently, these innate cells are activated by recognition of viral particle or intracellular components released from the damaged cells via pattern recognition receptors such as NOD-like receptors Toll-like receptors and RIG-like receptors.<sup>71 72</sup>

A previous study on SARS immunopathology has suggested that the activated innate immune cells in SARS patients can release high levels of cytokines and chemokines such as IL-1B, IL-6, IL-12, IFN- $\gamma$ , IP10, and MCP1.<sup>73</sup> On the other hand, high levels of IFN- $\gamma$ , TNF- $\alpha$ , IL-17, and IL-15 were observed in patients with MERS.<sup>74</sup> Elevated levels of cytokines can attract a vast number of immune cells, such as neutrophils and macrophages to the lungs. The recruited neutrophils and macrophages are capable of producing cytokines and chemokines to activate and attract other immune cells, creating a positive feedback loop. As a consequence, more and more immune cells will infiltrate the lungs.<sup>75</sup>

The activated macrophages and neutrophils produce a massive number of proteolytic enzymes and ROS, which damages the alveolar epithelium and endothelium, thereby causing vascular leakage and liquid build-ups in lung alveoli.<sup>76</sup> Furthermore, activated local mast cells can release inflammatory mediators such as histamine, leukotriene, and prostaglandin, which increase the blood flow and vascular permeability, thereby causing exudation of complement proteins and plasma leakage to the tissues.<sup>77</sup> The complement proteins also play an essential role when it comes to inflammation. They stimulate the phagocyte activity of macrophages and neutrophils and attract immune cells such as macrophages, T-cells, and neutrophils. Moreover, complements proteins can form a membrane attack complex, which perforates infected cells, thereby causing cell lysis.<sup>78</sup>

The combination of continuous production of cytokines, activation, and infiltration of different immune cells to the lungs can cause significant damage to the lung, causing lung edema and lung fibrosis, resulting in severe pneumonia. These clinical characteristics are, therefore, indicative of acute respiratory syndrome. Acute respiratory distress syndrome (ARDS) is a form of lung injury and is characterized by local alveolar damage and liquid build-ups.<sup>79</sup> Alveolar scarring can occur when the local tissue is continuously damaged by activated neutrophils and macrophages, leading to arterial hypoxemia and multiple organs failure.<sup>80</sup>

Besides, the overproduction of cytokines can lead to cytokines storm syndrome. Cytokine storm syndrome is one of the pathophysiological characteristics of SARS and MERS infection. It is triggered when the host immune system overreacts and activates a large

number of immune cells, which can overproduce cytokines.<sup>60</sup> Cytokine storm syndromes can cause septic shock and multiple organ failures such as lung injury, cardiac arrest, and renal failure, leading to death.

Recent studies Huang *et al.* (2020) and Chen *et al.* (2020) observed high levels of IL-6, MIP1 $\alpha$ , MIP1 $\beta$ , IP-10, G-CSF, MCP1, and increased amount of neutrophil and macrophages were also observed in the lungs of patients with COVID-19.<sup>48 81</sup> These findings are in accordance with previous studies on SARS and MERS immunopathology, which indicates that COVID-19 might exhibit similar immune responses as SARS-CoV and MERS-CoV. The recruited immune cells in most individuals, especially younger people, eliminate the viral infection in the lungs. After the viral infection is cleared, the immune response will diminish, and fewer immune cells will infiltrate the lungs. Consequently, the local alveolar tissues are no longer damaged by the recruited immune cells, which allows the patients to recover. However, some patients, especially older people, can develop dysfunction T-cell response, which causes cytokine storm syndrome. As a result, the immune cells are recruited continuously to the lungs, producing cytokines and chemokines consistently, which induces widespread inflammation in the lung. It is not known how this defective immune response in older people is triggered. It is believed that older people have different lung microenvironment than young people. The study Zhao *et al.* (2011) has demonstrated that aging can change the lung microenvironment, thereby altering the T-cell immune response. This study observed a high level of PDG<sub>2</sub> in the lungs of aged mice infected with SARS-CoV. PDG<sub>2</sub> is both anti-inflammatory and pro-inflammatory mediators produced by different cells, such as mast cells, epithelial cells, and macrophages. The study Zhao *et al.* (2011) suggested that PDG<sub>2</sub> exhibits anti-inflammatory activity, inhibiting the maturation and migration of dendritic cells to the lymph node, thereby interfering with the activation and the priming of T-cells.

Consequently, T-regulatory cells are not recruited to the site of inflammation to control and eliminate viral infection, which causes continuous production of cytokines. Besides, an increased level of PDG<sub>2</sub> in the lung can also induce neutrophils and macrophages infiltration to the lungs, causing cytokine storm and widespread inflammation.<sup>82</sup> Hence, older patients are more susceptible to develop cytokine storm syndrome than younger patients.

### Adaptive immune response

The dendritic cell plays an essential role in the activation of the adaptive immune system to fight off the virus. When the dendritic cell gets activated after engulfing the viral particles, it migrates to the lymph node to prime. It activates the naïve T-cells by presenting the antigen via an antigen-presenting receptor (MHC II). The activated naïve T-cell will differentiate into CD4<sup>+</sup> T-helper cells or CD8<sup>+</sup> cytotoxic T lymphocytes. CD8<sup>+</sup> cytotoxic T lymphocytes and natural killer cells target and eliminate infected cells, whereas CD4<sup>+</sup> T-helper cells are responsible for the activation of macrophages, cytotoxic T-cells, and B cells.<sup>83</sup> Several studies Oh *et al.* (2012) and Shin *et al.* (2018) have demonstrated that the T-helper cells in SARS and MERS patients are able to produce high levels of IL-2, IFN $\gamma$ , and TNF, which indicates that the T-cell response is mediated by T helper 1 cells.<sup>84 85</sup> Since COVID-19 is closely related to SARS-CoV, COVID-19 might induce T-cell response mediated by Th1-cells. The activated T-helper cells can prime and activate B-cells in the bone marrow. Subsequently, the activated B cells will differentiate into B memory cells and plasma cells that produce antibodies specifically against SARS-CoV or COVID-19. One recent report has

demonstrated that patients who were infected with COVID-19 produced IgM in the first nine days after developing symptoms. However, after the second week, the patients started producing IgG, a long-lasting immunoglobulin circulating in the blood after the infection.<sup>86</sup>

Overall, all three coronaviruses trigger both innate and adaptive immune responses. Typically, immune cells in most patients are able to eliminate the viral infections but some patients, especially elderly, have deficit T-cell mediated immune responses, which causes the overproduction of cytokine and excessive influx of immune cells to the lungs, leading to cytokine storm syndrome, which causes acute respiratory distress syndrome and, eventually, hypoxia in vulnerable patients (see figure 7).

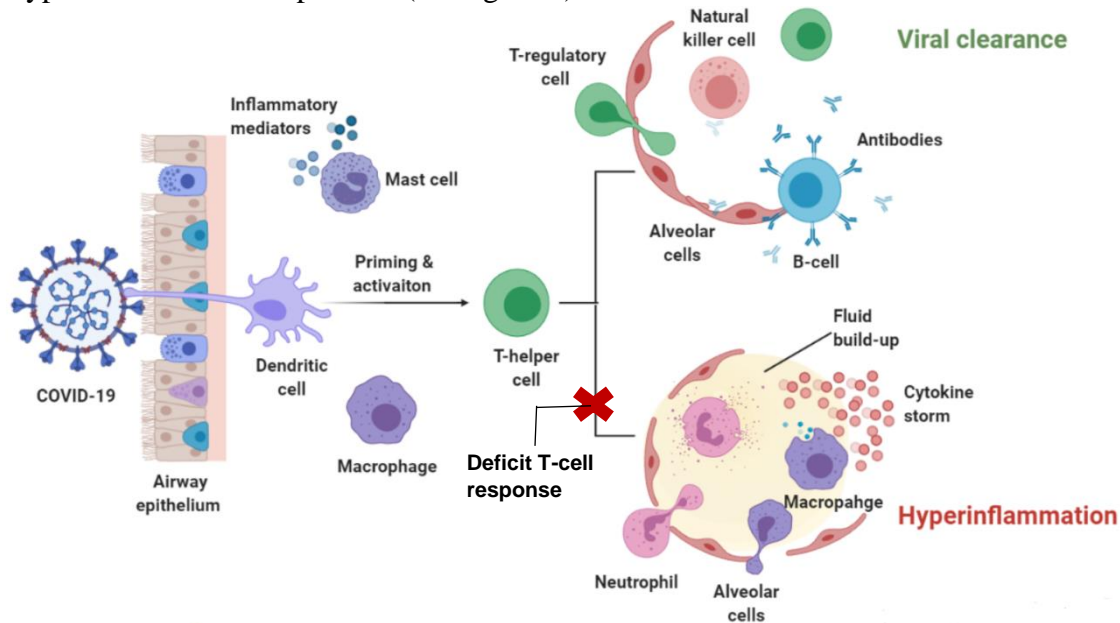


Figure 7. Immunopathology of COVID-19.

The incidence of acute respiratory distress syndrome in COVID-19 patients is 14.8%.<sup>87</sup> Early studies on the clinical course of COVID-19 patients in China have shown that between 23%-32% of the patients who developed hypoxia and ARDS required ICU.<sup>48 68 88</sup> Different respiratory support strategies, including a nasal cannula, non-invasive ventilation, invasive mechanical ventilation, and ECMO (extracorporeal membrane oxygenation), were used to treat patients who had ARDS and hypoxia. Since there are no drugs available to treat critically ill patients, oxygenation therapy is currently the only treatment strategy to treat those patients. It is, therefore, essential to understanding how different types of oxygenation therapies work.

### Oxygenation therapies:

Early studies in China have shown that between 66-76.8% of the patients admitted to the ICU received oxygenation therapy.<sup>48 68 75</sup>

Oxygenation therapy is mainly used as a supportive treatment for patients with hypoxia due to respiratory insufficiency. During this therapy, oxygenated air is delivered to the patient's lungs through a nasal cannula, prongs, or mask with a maximum flow rate of 15 L/ min.<sup>89</sup> According to the guidelines of Critical Care Medicine, oxygen therapy is used in COVID-19 patients if the peripheral oxygen saturation level is lower than 90%.<sup>90</sup>

Other oxygenation therapies used in these studies were HFNC and non-invasive mechanical ventilation. These studies have shown that between 24%-56% of the ICU patients with COVID-19 received HFNC and non-invasive mechanical ventilation.<sup>48 68 75</sup>

HFNC stands for high flow nasal cannula and is one of the oxygenation therapies used for patients with hypoxia and ARDS. During HFNC, increased oxygenated airflow is delivered to the patients' lungs via a nasal cannula. The maximum flow rate used in HFNC is 60 L/min. In addition, humidified and heated gas is utilized in HFNC, which allows the patients to use less energy to heat inhaled air.<sup>76</sup> Non-invasive mechanical ventilation is also an oxygenation therapy, which supports the patient's respiration without using invasive techniques such as intubation. This therapy delivers oxygenated air into the patient's lungs via face mask, thereby enhancing and maintaining the patient's oxygen blood level.<sup>91</sup> The guidelines of Critical Care Medicine suggested that the peripheral oxygen saturation level in patients receiving oxygenation therapy needs to be maintained between 91% and 96%.<sup>77</sup>

Moreover, the guidelines of Critical Care Medicine recommend the use of HFNC over conventional oxygenation therapy and non-invasive mechanical ventilation.<sup>77</sup> According to the data of several randomized controlled trials, HFNC has a lower intubation rate compared to traditional oxygenation therapy and non-invasive mechanical ventilation, indicating that patients who received HFNC have a lower risk of requiring invasive mechanical ventilation to support their respiration.<sup>92 93</sup> These data also suggest that HFNC did not have a significant impact on the mortality rate of the patients who received HFNC. In addition, HFNC is found to be more comfortable according to the patients, and the use of HFNC is more comfortable compared to non-invasive ventilation.<sup>94 95</sup> Other studies have shown that non-invasive ventilation is linked to a high risk of nosocomial infections in healthcare workers due to the exhaled air dispersion produced by the patients.<sup>96 97</sup> Thus, the use of HFNC is more preferred for patients with COVID-19 compared to non-invasive ventilation according to Critical Care Medicine's guidelines.<sup>77</sup>

Invasive mechanical ventilation is used when patients have severe ARDS and are unable to breathe. Invasive mechanical ventilation is an oxygenation therapy used to assist the patient's respiration by delivering oxygenated air into the patient's lungs via an endotracheal tube.<sup>98</sup> There are two types of invasive mechanical ventilation: positive pressure ventilation (PPV) and negative pressure ventilation (NPV).<sup>99</sup>

During PPV, a positive pressure is applied to the patient's lungs using an endotracheal tube, which creates a pressure difference between the atmosphere and the lungs, thereby causing air to flow into the patient's lungs.<sup>100</sup> NPV, on the other hand, assists the patient's respiration by applying negative pressure to the patient's body using a mechanical ventilator, which allows the patient's chest to expand, enabling oxygenated air to flow into the patient's lungs.<sup>86</sup> Currently, there are no studies concerning the use of mechanical ventilation for COVID-19 patients.

Even though the use of mechanical ventilation for COVID-19 patients is still recommended by the guidelines of Critical Care Medicine and WHO, invasive mechanical ventilation can induce lung injury by overstretching the lung alveoli and rupturing the alveolar tissues. As a consequence, inflammatory mediators are released from the damaged alveolar cells, which causes an influx of immune cells and, ultimately, inflammation.<sup>101</sup> A previous study with 34 critically ill SARS patients has demonstrated that 25% of intubated SARS patients showed lung injury associated with mechanical ventilation.<sup>102</sup> In addition, a recent study with 52

critically ill COVID-19 patients has been demonstrated that only 2% of the ventilated patients developed ventilation-induced lung injury.<sup>103</sup>

ECMO (extracorporeal membrane oxygenation) is an oxygenation therapy and is used to supply oxygen to patients with heart and lung failure. Several studies from China have shown that between 3% and 15% of ICU patients infected with COVID-19 received ECMO.<sup>48 68 75</sup>

This type of oxygenation therapy is only used when other mechanical ventilation fails to support and enhance the oxygen blood level in patients with hypoxia. During this oxygenation therapy, blood is temporarily drained from the patient's vascular system.<sup>100</sup> Subsequently, the drained blood circulates outside the patient's and passes through an oxygenator, replenishing red blood cells with oxygens and removing carbon dioxide from the red blood cells. After the blood is oxygenated, the blood will recirculate back to the patient's vascular system.<sup>104</sup>

According to a previous study with 35 MERS patients, ECMO is found to be effective in reducing the mortality of patients.<sup>105</sup> In addition, a meta-analysis suggests that ECMO lowered the mortality in patients with ARDS. However, a recent study with 52 COVID-19 patients showed that 5 out of 6 patients who received ECMO died.<sup>89</sup> The finding of a recent study is not in line with the results of previous studies. The guidelines of Critical Care Medicine recommend the use of ECMO for COVID-19 patients with hypoxia, and only selected patients can receive ECMO. This is because ECMO is not widely available in all hospitals and it requires specialized personnel who have experience with this type of oxygenation therapy.<sup>77</sup>

Overall, the mortality rate of patients who received mechanical ventilation is between 45%-94%, suggesting that supportive care alone is not sufficient to improve COVID-19 patients' outcomes.<sup>77 106 107</sup> Most of the patients who died in the ICU had severe ARDS, multiple organs failure, and septic shock. Since there are no drugs for COVID-19 available, more patients with mild complications will develop severe complications and eventually be admitted to the ICU. As a consequence, a surge of COVID-19 patients will occur in the ICU of most hospitals. Most hospitals around the world have limited ICU capacity, medical equipment, and medical staff to handle many critically ill patients in the ICU. This will be the most concerning issue for most of the low-income countries with underdeveloped healthcare systems. Underdeveloped healthcare systems are at risk of collapsing since there are enough ICU capacity and medical equipment available in the hospitals to treat such a large number of COVID-19 patients.

According to a study, the average ICU bed per 100,000 population in low-income Asian countries and high-income Asian countries is 2.56 and 14.4.<sup>108</sup> Moreover, Italy and the USA, both are high-income countries with 12.5 and 29.4 ICU beds per 100,000 population, respectively, still had a difficult time managing large numbers of critically ill COVID-19 patients in hospitals.<sup>109 110</sup> Therefore, it is important to develop safe and effective treatment and vaccines for this novel coronavirus to prevent and reduce exacerbation of COVID-19 complications in patients. So, in that way, less COVID-19 patients will be admitted to the ICU, thereby reducing the number of patients in the ICU.

## Viral components

Since the SARS and MERS outbreak, viral components of coronavirus have been extensively studied and researched, providing knowledge on coronavirus entry mechanism and replication, which paves the way for the development of COVID-19 therapeutics and vaccines.

### Viral entry

As mentioned earlier, Zhou *et al.* (2020) have demonstrated that COVID-19 uses its spike glycoprotein to enter the host cell by interacting with the angiotensin-converting enzyme 2.<sup>111</sup> The receptor-binding domain is located in the S1 subunits, which directly binds to ACE2.<sup>112</sup>

S2 subunit, on the other hand, contains a fusion peptide responsible for the virus-cell fusion.<sup>113</sup> Previous research has demonstrated that people who were infected with SARS-CoV contained circulating immunoglobulin that can specifically bind to the receptor-binding domain, preventing the virus from attaching to host ACE2.<sup>114 115</sup> This finding enables drug developers to identify and generate a monoclonal antibody that can neutralize the spike proteins of the virus by blocking the receptor binding proteins in the S1 domain.<sup>116</sup> Other monoclonal antibodies targeting the S2 domain were also identified in the study Coughllin *et al.* (2012).<sup>117</sup> In this study, several monoclonal antibodies were identified that could target different epitopes of the spike protein, which suggests that different monoclonal antibodies can potentially be used as a cocktail to increase the inhibition effect on the virus-cell fusion. Synthetic antiviral peptides analogs were also designed to mimic different domain regions of S1 and S2 subunits to prevent viral entry. It is hypothesized that both HR1 and HR2 domains bind to each other to form a fusion core, allowing the viral envelope and the host cell membrane to be at close approximate for the virus-cell fusion.<sup>118 119</sup> Previous studies identified and designed synthetic peptides (CP-1 and HP2P-M2) that are analog to HR2 domains of S2 subunits. These peptides exert anti-SARS and anti-MERS activity by binding to HR1, interrupting the formation of fusion core.<sup>120</sup>

Moreover, spike protein is shown to be a perfect viral antigen for the COVID-19 vaccine. Previous studies have demonstrated that vaccine containing spike proteins elicited a strong immune response, producing high titer of IgG that specifically target the spike protein.<sup>121 122</sup>

Host components such as ACE2, cathepsin, clathrin, and TMPRSS2 are also involved in viral entry, potentially serving as a drug target. A previous study has demonstrated that coronaviruses enter the cell through different pathways, including endosomal pathway and non-endosomal pathway.<sup>123</sup> Both ACE2 and TMPRSS2 are involved in the non-endosomal pathways. During the non-endosomal pathway, the spike protein binds to the ACE2 forming a receptor complex. Subsequently, the bound spike protein is recognized and cleaved by surface serine proteases (TMPRSS2), thereby inducing viral fusion and allowing the viral nucleocapsid to enter the cell.<sup>124</sup> NAAE is a small molecule and was previously identified as a novel ACE2 inhibitor, which inhibits the binding between spike protein and ACE2.<sup>125</sup> However, recent studies suggest that ACE2 inhibitors upregulate the expression of ACE2, causing more viral entry. For this reason, some physicians do not recommend the use of ACE2 inhibitors during COVID-19 infections.<sup>126</sup>

One of the drugs that target TMPRSS2 and are proven to be effective against SARS-CoV and MERS-CoV infections in vivo is camostat mesylate. Camostat mesylate is a small molecule



and is currently marketed as a drug for chronic pancreatitis.<sup>127</sup> Several studies have demonstrated that Camostat mesylate blocks the entry of coronavirus by inhibiting the serine protease activity.<sup>128 129</sup>

Both cathepsin and clathrin are involved in the endosomal pathways. The spike-ACE2 complex activates numerous adaptor proteins, which in turn activate and recruit clathrin to initiate endocytosis by forming vesicles around the viral particle at the cell surface. Subsequently, the vesicle containing the viral particle is transported to the endosome. The virus particle is then released into the endosome, which contains cathepsin, an endosomal protease. Cathepsin recognizes the spike protein and exerts pH-dependent proteolytic activity, which cleaves the spike protein into S1 and S2, inducing the fusion between viral envelope and the endosomal membrane, which leads to viral entry.<sup>130 131</sup>

There are several drugs identified that exert anti-SARS-CoV and MERS-CoV activity by inhibiting clathrin and cathepsin. Chlorpromazine is an FDA-approved drug for schizophrenia and was previously identified as a potential antiviral drug for MERS-CoV in a study. According to this study, chlorpromazine inhibits the viral entry of MERS-CoV by directly targeting the clathrin proteins, which prevents the formation of the rounded vesicle, thereby inhibiting the endocytosis of the virus.<sup>132 133</sup> Moreover, Chloroquine and Oxocarbazate can hinder the cathepsin activity, thus preventing virus entry. According to several studies, chloroquine inhibits viral replication of SARS-CoV in vivo.<sup>134 135</sup> Oxocarbazate was previously found to exert anti-SARS-CoV activity by inhibiting cathepsin L reversibly, preventing viral fusion.<sup>136</sup>

On the other hand, chloroquine accumulates in the endosome and gets protonated due to the acidic environment in the endosome.<sup>137</sup> Consequently, the pH-level in the endosome increases, which impairs the low pH-dependent proteolytic activity of cathepsin. A recent study has shown that chloroquine exerts an inhibitory effect on the COVID-19 infection in vivo.<sup>138</sup>

#### Papain-like protease and 3C-like protease

Papain-like protease and 3C-like protease are both responsible for the formation of the Replication-transcription complex and the production of immune evasion components by cleaving the polyprotein pp1a and pp1b into 16 different nonstructural proteins with each their functions (see table 1).<sup>41</sup> Drugs that target these proteases can inhibit viral replication and remove the virus's ability to evade the host immune system. Since the SARS and MERS outbreak, many compounds and previously approved drugs were screened against these proteases to determine whether these compounds can be the potential inhibitors for papain-like protease or 3C like protease. CE-5, 6-thioguanine, 6-mercaptopurine, TL-3, JMF 1586 were previously identified and proved to exert anti-SARS and anti-MERS activity by inhibiting 3C like protease and papain-like protease in vivo.<sup>139 140 141 142</sup> In addition, lopinavir and ritonavir were both already approved by the FDA for the treatment of HIV/AIDS and were shown to exert anti-3C like protease activity in several studies.<sup>143 144 145 146 147</sup>

Recently, research led by Zihe Rao has determined the crystal structure of 3C like protease using X-ray crystallography. In addition, a covalent inhibitor (N3) targeting 3C like protease

was also identified using computer-aided drug design.<sup>148</sup> The identification of the 3C like protease crystal structure of this novel coronavirus enables other scientists to design novel compounds that target the active site of this protease. A research group (Frank von Delft, Dave Stuart, and Martin Walsch) from Diamond Light Source performed a large crystal-based fragment screening against the 3C like protease at the XChem facility of UK's diamond light source, yielding 78 fragments in total that can bind to the active site. 48 of which inhibit to the active site covalently, and 23 binds non-covalently to the active site.<sup>149 150</sup>

### Transcription-replication complex

The replication-transcription complex (RTC) is an essential component for the viral replication and is formed by the assembly of seven different nonstructural proteins (Nsp 7,8,9,10,12,13 and 14).<sup>151</sup> Drugs targeting the replicative components of RTC can inhibit the synthesis of negative-sensed viral RNA, blocking the expression of structural proteins. Most of the drug and experimental compounds are targeting helicase (nsp 13) or RNA-dependent RNA polymerase (nsp 12). This is because both components are directly involved in the synthesis of viral RNA. Helicase unwinds double-stranded RNA into single-stranded RNA in an ATP-dependent manner, allowing RNA dependent RNA polymerase (RdRp) to directly read the single-stranded RNA, which synthesizes negative sensed RNA.<sup>152</sup> The catalytic site of helicase and RdRp are highly conserved in all groups of coronaviruses, making it easier for the scientists to design novel compounds that specifically target the helicase and RdRp.<sup>153</sup> After the SARS and MERS outbreak, many compounds targeting helicase or RdRp were developed or repurposed. Several helicase inhibitors were identified in the previous studies. There are two types of helicase inhibitors, one that inhibits both ATPase and unwinding activity of helicase, and the other inhibits only the unwinding activity. Idobananin and Vanillinbananin are both baninin derivatives and exert anti-SARS activity by inhibiting the unwinding and ATPase activities of helicase in vivo. However, these compounds are shown to cause cytotoxicity due to the inhibition of ATPase.<sup>154 155</sup> For this reason, the development of baninin derivatives for SARS-CoV is put on hold. On the other hand, SSYA10-001, a triazole derivative, can only inhibit the unwinding activity of helicase, which does not cause cytotoxicity.<sup>156</sup>

Most of the drugs targeting the RNA dependent RNA polymerase are nucleoside analog. Nucleoside analogs share similar structural similarities with the natural nucleotides, which can be incorporated into the growing RNA strand by the RNA dependent RNA polymerase. Consequently, the RNA-dependent RNA polymerase is blocked by the nucleoside analog, terminating the elongation of RNA strands.<sup>157</sup> All nucleoside analogs are activated intracellularly by viral kinases and host kinases.<sup>158</sup>

Nucleoside analogs that were previously proven to exhibit anti-SARS and anti-MERS activity in vivo were ribavirin, galidesivir, acyclovir fleximer, and GS-5733, currently known as remdesivir.<sup>159 160 161 162</sup>

### Envelope glycoproteins, membrane glycoproteins, and nucleocapsid phosphoprotein proteins

E proteins and M proteins are glycoproteins responsible for the formation and the release of virus-like particles. E proteins pinch off the virion from the endoplasmic membrane, thereby facilitating the budding viral process at the ERGIC.<sup>163</sup> Most of the E proteins reside within the ERGIC. However, only a small fraction of the E proteins are incorporated into the viral

envelope and serves as viroporins.<sup>164</sup> Viroporins are membrane protein channels responsible for the transport of different ions such as  $\text{Ca}^{2+}$  and  $\text{Na}^+$  across the membrane, maintaining the viral membrane potential.<sup>165</sup> M proteins are responsible for shaping the viral envelope and incorporating different structural proteins such as S and E proteins into the viral membrane. They also interact with the nucleocapsid proteins, thereby stabilizing the nucleocapsid complex and incorporating it into the viral particle.<sup>166</sup> N proteins interact with the viral RNA, forming a helical coat around the RNA, which serves as a protective coat for the viral RNA. Also, N proteins are involved in viral assembly, viral budding viral replication, and translation.<sup>167</sup>

All three proteins are capable of eliciting an immune response, indicating that these structural proteins can be the perfect base for vaccine development. The study has demonstrated that E proteins can elicit host immune response by activating the inflammasome complex (NLRP3), producing IL-1 $\beta$ .<sup>168</sup> In addition, M and N proteins are also able to elicit an immune response. In the study (Jin *et al.*, 2005), mice were immunized with vaccines containing genes that encode for N, M, and E proteins of SARS-CoV. This study's results suggested that N protein induced the highest immune response, followed by M protein and E protein.<sup>154</sup>

On the other hand, the E protein induced the lowest immune response. These findings indicate that both N protein and M protein are stronger immunogens compared to E protein.<sup>169</sup> The strength of the immune response induced by these structural proteins depends on the number of the epitopes present on the protein. E proteins contain the least amount of epitope compared to the other two proteins since it only contains 76 amino acid residues. On the other hand, M and N proteins contain 220 and 420 amino acid residues, respectively. These proteins contain more epitopes than E proteins and therefore induce higher immune response compared to E protein.<sup>154</sup>

## Host components

The continuous overproduction of cytokines and chemokines large numbers of immune cells recruits and activates large numbers of immune cells at the site of inflammation. Chemokines and cytokines attract and activate immune cells, respectively, by binding to the inflammatory receptors. All immune cells express cytokine and chemokine receptors responsible for the activation of immune cells through different signaling pathways. Activated cytokine receptors promote cell survival, production of cytokines, and cell proliferation. Most of the interleukin receptors such as IL-1R, IL-6R, IL-10R, IL-2R, and IL-7 R activate the adaptor protein JAK through phosphorylation, which in turn propagates downstream signaling via three different pathways; JAK-STAT pathway, PI3K-AKT pathway, and MAPK pathway

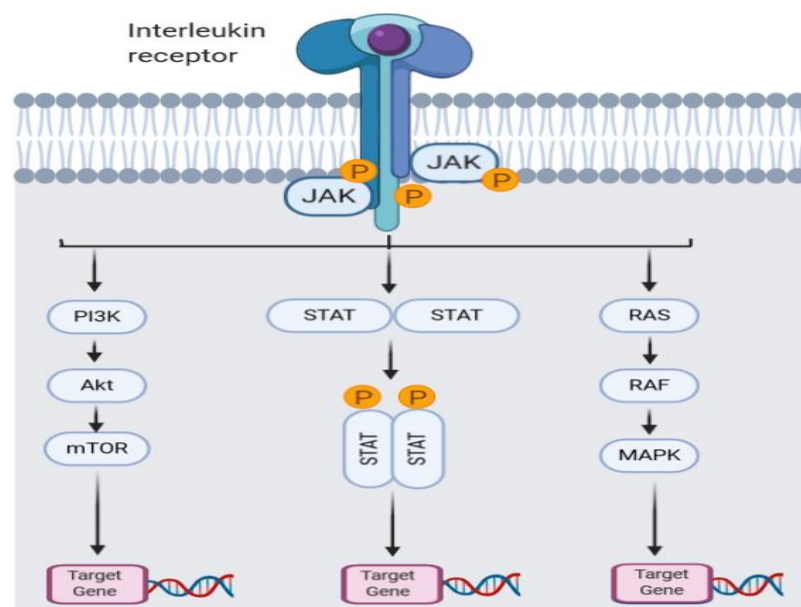


Figure 8. Three different intracellular signaling pathways with their corresponding kinases. All three pathways lead to upregulation of inflammatory genes.

(see figure 8).<sup>170 171</sup> Drugs that target the JAK kinases might block the downstream signaling initiated by interleukin receptors, preventing overproduction of cytokines during COVID-19 infection.

JAK inhibitors are generally used to treat tumors and inflammatory diseases such as rheumatoid arthritis.<sup>172 173 174</sup> Recently, baricitinib, a JAK inhibitor, is identified as a potential drug for COVID-19 through BenevolentAI.<sup>175</sup> Currently, baricitinib and other JAK inhibitors such as tofacitinib, ruxolitinib, and pacritinib are in the clinical trial.<sup>176</sup>

The chemokine receptors and cytokine receptors are continuously activated during cytokine storm syndrome, causing more production of inflammatory mediators. Drugs targeting the inflammatory components, inflammatory receptors can potentially inhibit the production of cytokines, preventing cytokine storm syndrome in COVID-19 patients (see figure 9).

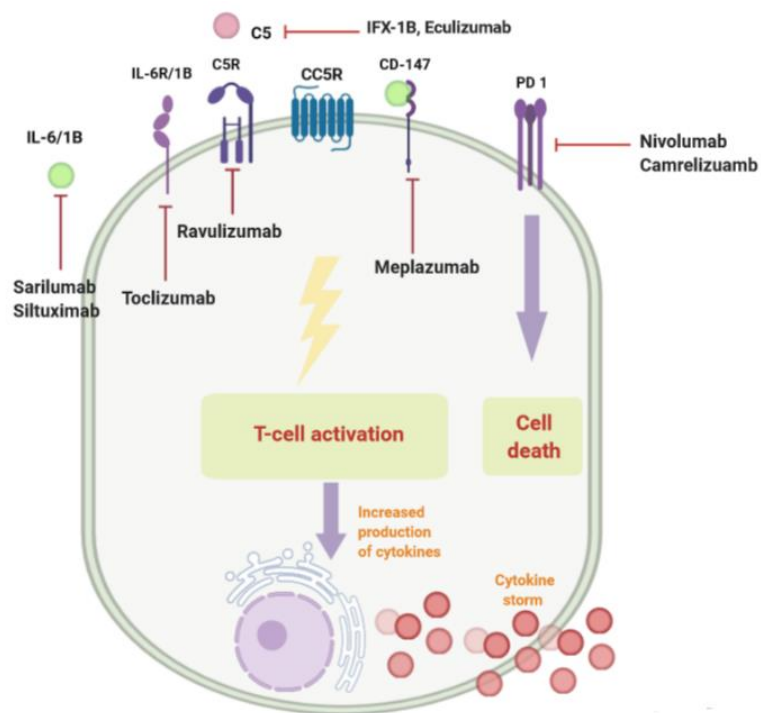


Figure 9. Drugs targeting different inflammatory receptors on T-lymphocytes

Several monoclonal antibodies targeting these immune components are currently in the clinical trial as of May 23rd, 2020 (see table 3).<sup>161</sup>

Table 3. Drugs identified in Milken institute's database that target the inflammatory receptors and cytokines

Drug	Mechanism
Leronlizumab	CC5R antagonist
Siltuximab	Anti-interleukin 6
Tocilizumab	IL-6 receptor antagonist
Sarilumab	IL-6 receptor antagonist
Canakinumab	Anti-Interleukin 1B
Eculizumab	Complement C5 inhibitor
Ravulizumab	Complement C5 inhibitor
IFX-1	Complement C5 inhibitor
Nivolumab	PD-1 inhibitor
Camrelizumab	PD-1 inhibitor
Otilizumab	Anti- colony macrophage stimulating factor
Lenzilumab	Anti- colony macrophage stimulating factor
Gimsilumab	Anti- colony macrophage stimulating factor

## COVID 19 therapeutics in the clinical trial

During the COVID-19 pandemic, many patients and healthcare workers are desperately in need of effective and safe COVID-19 treatments and vaccines.

Drug developers and scientists are trying to accelerate drug and vaccine development using supercomputers and artificial intelligence. Most of the pharmaceutical companies are using cloud-based technologies to speed up this process of collecting and integrating different types of data.<sup>177</sup> Most of the pharmaceutical companies are using cloud-based technologies provided by several companies such as IBM and Google Cloud platforms to speed up this process of collecting and integrating different types of data. Cloud-based artificial intelligence technologies provide researchers with relevant datasets containing data of different chemical substances, drug targets, and pharmacology that are extracted from numerous research papers. In addition, it can rapidly analyze and perform a large number of calculations in order to filter and select the right chemical substances from the databases. These compounds are selected based on their target affinity. The interactions between the drugs chosen and target are examined using 3D virtual screening, such as molecular docking. Once a chemical substance has been identified as a potential drug for a particular target, a high throughput screening method is used to determine the side effects. With this approach, researchers can also identify and screen previously approved drugs (drug repurposing).<sup>178</sup>

CAS (Chemical Abstracts Services) registry is a collection of drugs and chemical compounds that are extracted from numerous scientific papers.<sup>179</sup> The registered drugs and chemical substances are assigned with a unique numerical code, which can be identified in the CAS database. The CAS registry recently identified numerous patented drugs and potential compounds that target SARS-CoV and MERS-CoV viral compounds, which can be used as a potential treatment for COVID-19.<sup>180</sup> The number of patents and potential compounds with their corresponding targets are illustrated in Figure 10. As shown in this figure, most of the drugs and experimental compounds target 3C-like protease and RNA-dependent polymerase due to the fact that the catalytic sites of these enzymes are highly conserved and well-studied compared to the other viral components.

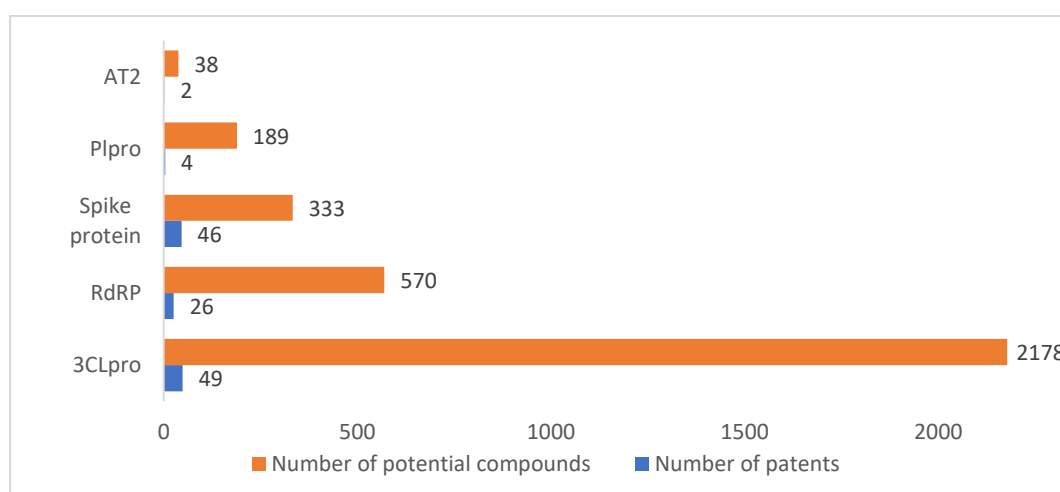


Figure 10. Different viral components of coronavirus with their corresponding number of patents and potential compounds identified in the CAS registry.

In addition, several existing drugs that can be the potential COVID-19 treatment are also identified in the CAS data (see table 4), and most of these existing drugs are already in the clinical trial for COVID-19.<sup>163</sup>

Table 4. Existing drugs identified in the CAS registry that target coronavirus components.

Existing drugs	Target
Baricitinib	JAK kinase
Darunavir	3CLpro/ PLpro
Remdesivir	RdRp
Ribavirin	RdRp
Galidesivir	RdRp
Favipiravir	RdRp
Ritonavir	3CLpro/Plpro
BCX-4430	RdRp
Arbidol	Spike protein, ACE2
Chloroquine	Endosome
Nitazoxanide	N/A
Lopinavir	3CLpro/PLpro

As of May 23<sup>rd</sup>, 2020, there were 223 COVID-19 therapeutics in preclinical and clinical trials identified in the database provided by Milken institute. Moreover, 47 of which were previously approved by the FDA. COVID-19 therapeutics currently in the clinical trial are divided into five categories: antibodies, antivirals, immunomodulators, RNA, and stem cell therapy based on their mode of action.<sup>161</sup> Therapeutics that do not fall into these categories are classified as "other." The chart below illustrates different therapeutic types as a percentage of the total COVID-19 therapeutics that are currently in the clinical trial (figure 11). Looking at the chart, immunomodulators, antibodies, and antivirals account for 18.8% (42), 19.3% (43), and 10.3% (23) of the 223 COVID-19 therapeutics in the clinical trial, respectively. Furthermore, only 2.69% (6) and 6.72% (15) of the 223 COVID-19 therapeutics in the clinical trial are RNA based therapies and cell-based therapies, respectively.

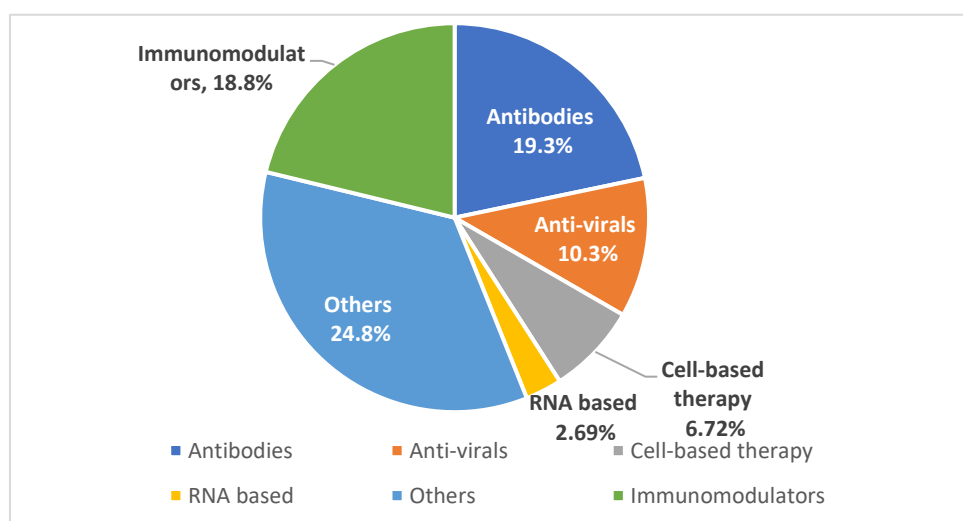


Figure 11. Six drug categories as percentage of 223 COVID-19 therapeutics in clinical trial. Note: the data was published on May 23<sup>rd</sup>, 2020.



As of May 23<sup>rd</sup>, 2020, 14 treatments received the authorization of emergency use, which allows critically ill COVID-19 patients to have access to these experimental treatments (Table 4).<sup>161</sup>

Table 5. 14 drugs that received emergency use authorization. Note: the data was collected on May 23<sup>rd</sup>, 2020

Drug	Type	Sponsor, partners
Hydroxychloroquine	Antimalarial	Sanofi, Novartis, Bayer, Teva ,and Mylan
Chloroquine	Antimalarial	Bayer, Teva, Zydus, Prasco Labs, Sun, Rising
Nitric oxide	Vasodilation	Bellerophon Therapeutics, Vero Biotech
Lopinavir + ritonavir	Antiviral	AbbVie
Atlizumab	IL-6R antagonist	Roche, Chugai, Cipla
Remdesivir	Antiviral	Gilead, NIH, USAMRIID, CDC
Giapreza	Vasodilator	La Jolla Pharmaceutical Company, HealthCare Royalty Partners
Eculizumab	Complement inhibitor	Alexion
Convalescent plasma therapy	Antibodies	Multiple global research
IC14	Anti-CD14	Implicit bioscience
ADMCs	Cell-based therapy	Celltex
Ryoncil	Cell-based therapy	Mesoblast, cardiac surgical trial network
CAP-1002	Cell-based therapy	Caprior Inc.
Ruxolitinib	JAK inhibitor	Novartis, Incyte.

Moreover, as of May 23<sup>rd</sup>, there were 1133 ongoing trials worldwide, and 63 countries are actively developing treatments and vaccines for COVID-19 (figure 12).<sup>181</sup>

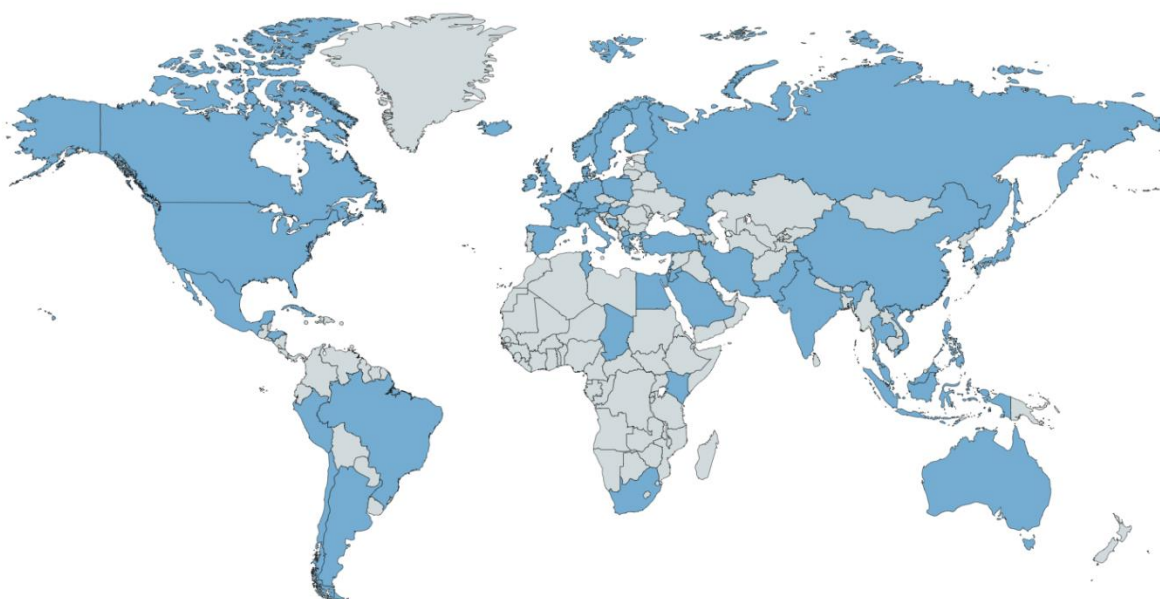


Figure 12. 63 countries actively developing COVID-19 therapeutics and vaccines as of 23<sup>rd</sup>, 2020 are highlighted in blue.

The chart below illustrates the ten countries with the most ongoing trials for COVID 19 treatments and vaccines (figure 13). Looking at the chart, the number of trials in China, the USA and Iran are 343, 215, and 138, respectively. China has the most ongoing trials for COVID-19 since China was the first country affected by this novel coronavirus. Besides, China also previously experienced a similar outbreak back in 2003 with SARS-CoV.

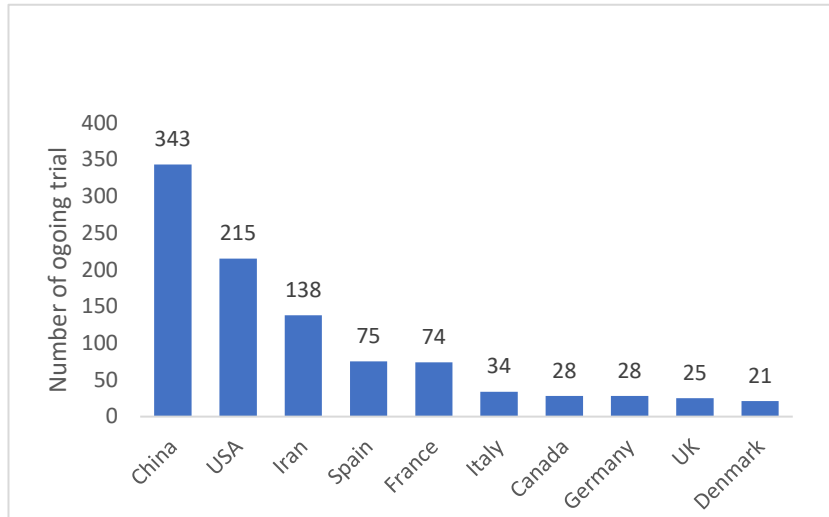


Figure 13. Top ten countries with their corresponding number of ongoing trials. Note: the data was collected on May 23<sup>rd</sup>, 2020

## Clinical trials of COVID-19 therapeutics with results

### Favipiravir

Favipiravir (Avigan) is an antiviral drug and was first developed by Toyama Chemical Co. to treat influenza virus.<sup>182</sup> Favipiravir exhibits antiviral activity by inhibiting the RNA-dependent RNA polymerase. Favipiravir is activated through phosphorylation by cellular kinase, which forms an active metabolite called favipiravir-ribofuranosyl-5'-triphosphate that acts as a pyrimidine nucleoside, which directly inhibits the RdRp.<sup>183</sup> Recently, a study of favipiravir showed promising results in terms of effectiveness in treating COVID-19 patients. The study (Cai *et al.*, 2020) is an open-label non-randomized controlled trial with 80 COVID-19 patients. This study compared favipiravir with lopinavir/ritonavir (control group). This study's results suggested that patients who were treated with favipiravir showed shorter viral clearance time compared to the control group. The median viral clearance time for the favipiravir group and control group was four days and 11 days, respectively. Besides, the improvement rate of favipiravir group (91.45%,  $P=0.004$ ) was found to be higher compared to the control group (62.22%,  $P=0.004$ ). However, a more gastrointestinal complication such as diarrhea was observed in the favipiravir group (2.9%,  $P=0.44$ ) compared to the control group (0%,  $P=0.44$ ).<sup>184</sup> This study has its limits, which can negatively affect the validity of the results. For instance, the trial was not randomized and the sample size was not sufficient to provide a significant evidence supporting the effectivity of favipiravir in the treatment of COVID-19 infection.

### Remdesivir

Remdesivir is an antiviral drug and was initially developed by Gilead Sciences, Inc. to treat hepatitis C infections. Remdesivir exerts antiviral activity by inhibiting the activity of viral RNA dependent RNA polymerase. In addition, remdesivir is activated intracellularly by

kinases, which converts it into an activate metabolite that acts as adenosine analog, which binds to RNA dependent RNA polymerase, terminating the elongation of the RNA strand.<sup>185</sup> Gilead recently announced that the two trials of remdesivir, SIMPLE trial, and NIAID trial, showed positive results. The SIMPLE trial is an open-label randomized multicenter phase 3 trial containing 397 patients, in which 200 patients received the five- day dose regimen of remdesivir, and 197 patients received the ten-days dose regimen. This trial showed that the discharge rate of 5 days dose regimen group and ten days dose regimen group was 60% and 53.2%, respectively, suggesting that both treatment regimens showed similar clinical improvement (OR: 0.75, CI: 0.51-1.12). Moreover, 1.5% and 2.0% of the patients in 5 days dose regimen and ten days dose regimen developed drug-related severe adverse reactions.<sup>186</sup>

The second trial was conducted by the US National Institute of Allergy and Infectious Diseases and is double-blinded randomized controlled trials with 1063 participants, in which 538 patients were assigned to remdesivir and 521 patients to placebo.<sup>184</sup> The preliminary result of this study suggested that patients who were treated with remdesivir had a shorter recovery time (11 days,95% CI: 9-12) compared to the placebo group (15 days, 95% CI:13-19) with an odds ratio of 1.32, 95% CI: 1.12-1.55. In addition, the remdesivir group had a lower mortality rate than the placebo group. The remdesivir group and placebo group's mortality rate was 7.4% and 11.9%, respectively, with an odds ratio of 0.70, 95% CI: 0.47-1.04. Besides, 21.1% of patients in the remdesivir group with adverse reaction was observed, whereas 27% were observed in the placebo group.<sup>187</sup> The results from both studies demonstrated that remdesivir is effective in improving the clinical outcome of COVID-19 patients. Moreover, fewer drug-related severe adverse reactions were observed in patients who were treated with remdesivir. Because of both trials' promising results, the Japanese health official approved the use of remdesivir on COVID-19 patients on May 7<sup>th</sup>, 2020.<sup>188</sup> However, remdesivir is not approved yet in other countries, and as of May 24<sup>th</sup>, there are nine ongoing trials of remdesivir to further assess the safety and efficacy.<sup>189</sup>

#### Tocilizumab

The FDA previously approved tocilizumab for the treatment of rheumatoid arthritis.<sup>190</sup> It is a humanized monoclonal antibody that targets the interleukin receptors, which dampens the host immune responses, thereby reducing the production of cytokines and infiltration of immune cells.<sup>191</sup> A retrospective study with 21 critically ill patients, suggested that patients showed clinical improvement after five days of tocilizumab treatment. 75% of the patients who received tocilizumab required lower oxygen intake. Moreover, a decrease in peripheral Il-6 level was observed in 85% of the patients, and 84.2% showed a significant reduction of C-reactive protein. As for the adverse effect of tocilizumab, no drug-related adverse effects were observed in patients within the five days treatment of tocilizumab. Although this study did show promising results regarding the efficacy and safety of tocilizumab, the sample size of this study is limited, which means that the evidence found in this study is not significant enough to support the conclusion that tocilizumab is indeed safe and effective for COVID-19 patients.<sup>192</sup>

#### Stem cell therapy

Stem -cell therapy utilizes mesenchymal cell-derived from the placenta, umbilical cord, adipose tissue, and bone marrow to regulate host immune responses by modulating the

activity of immune cells, thereby decreasing the production of cytokines and the influx of immune cells. A pilot trial with ten patients showed that mesenchymal cell therapy was effective and safe in treating critically ill COVID-19 patients.<sup>189</sup> Patients infused with mesenchymal cells showed clinical improvement after stem cell therapy. Symptoms such as fever, cough, and dyspnea were cleared after two days of stem cell therapy.

In addition, patients who received stem cell therapy showed a decreased level of pro-inflammatory cytokine (TNF- $\alpha$ ), and an increased level of anti-inflammatory cytokine (IL-10) compared to the placebo group.<sup>193</sup>

In the beginning, all critically ill patients had high levels of peripheral T-lymphocytes and natural killer cells, which cause cytokine storm syndrome. However, after six days of cell therapy, an increase in regulatory lymphocytes, and a decrease in effector lymphocytes were observed in severe critically ill patients. This pilot trial's preliminary result showed positive results regarding the efficacy of mesenchymal cell therapy in treating COVID-19. Recently, Mesoblast, an Australian-based regenerative medicine company, reported that its mesenchymal stem cell therapy, remestemcel-L showed promising results. In this report, 12 critically ill patients in New York City's Mt Sinai hospital were treated with remestemcel-L. According to Mesoblast's report, 81% (10/12) of the patients who received two shots of remestemcel-L in the first five days survived and 75% (9/12) of the intubated patients were able to recover without ventilator support.

On the contrary, only 9% (38/445) of the patients who did not receive remestemcel-L were able to recover without ventilation support. Besides, the survival rate of these patients was 12%, which is lower than the patients who received remestemcel-L.<sup>194</sup> Results from both studies suggested that stem cell therapy did show benefits in treating critically ill COVID-19 patients. However, both studies' sample size is not sufficient to show strong evidence, and extensive trials are therefore needed.

#### Lopinavir /ritonavir

Lopinavir and ritonavir are both inhibitors of HIV-1 protease and were first developed by AbbVie as a treatment for HIV-aids.<sup>195</sup> As mention earlier, a study demonstrated that ritonavir and lopinavir exhibited anti-SARS-CoV activity by inhibiting the 3C like protease in vitro.<sup>196,197</sup> This encourages researchers and pharmaceutical companies to initiate a trial of lopinavir/ritonavir to treat COVID-19 infections. A randomized controlled trial with 199 hospitalized patients, in which 99 patients received lopinavir/ritonavir treatment and 100 received standard-care, suggested that lopinavir/ritonavir did not show benefit in treating COVID-19 infections. No significant difference in clinical improvement observed between the patients who received lopinavir/ritonavir and standard-care treatments with a hazard ratio of 1.31 and 95% confidence interval between 0.95 and 1.80.<sup>193</sup> Besides, no significant difference in mortality rate was observed between these two groups. The mortality rate of the lopinavir/ritonavir group and standard-care group was 19.2% and 25.0%, respectively, with a percentage difference of 5.8%.<sup>193</sup> Gastrointestinal complications such as diarrhea, vomiting, and nausea were more observed in the lopinavir/ritonavir treatment patients compared to the standard-care treatment patients.<sup>198</sup>

The main problem of this study is that it is not blinded, which might negatively affect the validity of the results. Therefore, it is not possible to confirm the role of lopinavir/ritonavir in the treatment of COVID-19 infections based on this study.

## Hydroxychloroquine/chloroquine

Hydroxychloroquine and chloroquine are both prescribed as a treatment for malaria, lupus, and rheumatoid arthritis.<sup>199</sup> As mentioned earlier, several studies have shown that both drugs exert antiviral activity by increasing the endosomal pH level, thereby inhibiting the viral entry through the endosomal-dependent pathway.<sup>200 201</sup> Previous studies have demonstrated that chloroquine showed an inhibitory effect on SARS-CoV in vitro, which encourages researchers to conduct a study on chloroquine and hydroxychloroquine to determine whether these drugs are effective in treating COVID-19 infections.<sup>202 203</sup>

A recent study from France has suggested that the combination treatment hydroxychloroquine/azithromycin showed benefit in treating COVID-19 infection in patients.<sup>204</sup> The study Gautret *et al.* (2020) is an open-label non-randomized controlled trial with 36 patients, in which 20 patients were assigned with hydroxychloroquine/azithromycin and 16 with a placebo. According to this study, a significant decrease in viral load was observed in patients who received the combination treatment hydroxychloroquine/azithromycin compared to the placebo group. The finding of this French study had attracted attention from both the media and politicians, creating hypes around these antimalarial drugs. However, some critics claimed that the preliminary result from this study is not valid since it is not randomized, meaning that biases might occur in this study. Besides, the sample size of this study was also not sufficient enough to provide strong evidence to support the effectivity of hydroxychloroquine/azithromycin in treating COVID-19 infection. Recently, a study showed contradicting results regarding the effectivity of hydroxychloroquine.

The observational study with 1446 patients showed that hydroxychloroquine did significantly associate with the mortality rate of patients with COVID-19 (HR: 1.04, 95% CI: 0.82-1.32).<sup>205</sup> In addition, hydroxychloroquine as monotherapy and the combination therapy hydroxychloroquine/azithromycin were shown to be associated with a high risk of QT prolongation. A cohort study with 143 patients, of which 90 received monotherapy hydroxychloroquine and 53 received combination therapy hydroxychloroquine/azithromycin.<sup>202</sup> According to this study, 19% of the monotherapy patients developed QT-prolongation, whereas QT-prolongation was observed in 21% of the combination therapy patients.<sup>206</sup> The result of Zimetbaum *et al.*, (2020) suggested that the combination therapy (hydroxychloroquine/azithromycin) was associated with a higher risk of QT-prolongation compared to the monotherapy and that both hydroxychloroquine and azithromycin can induce QT-prolongation.

Based on the results of all three studies, it is still difficult to determine whether hydroxychloroquine is safe and effective in treating patients with COVID-19 infection. This is because the first study was non-randomized and had a small sample size. Moreover, the other two studies are observational studies, which are not suitable for determining the safety and effectivity of drugs since confounding and biases are more likely to occur in this type of study, and, therefore, cannot provide strong evidence. More results from large size randomized controlled trials are therefore needed to truly confirm the effectiveness and safety of hydroxychloroquine for the treatment of COVID-19.

## Vaccine candidates in clinical trials

It is known that this novel coronavirus has a high transmission rate with an  $R_0$  of 2.2, which means that the virus can spread rapidly in a population, increasing the number of infected people. On top of that, our body does not exhibit protective immunity against COVID-19 since our immune system is naïve to this novel coronavirus. As a consequence, more waves of infection will occur in the population, causing more infected people and deaths. It is, therefore, important to develop COVID-19 vaccines to prevent the spread of this novel coronavirus. In normal circumstances, it takes years to develop vaccines for infectious diseases. In this pandemic, many biopharmaceutical companies are aiming to fully develop safe and efficient vaccines for this novel coronavirus within 12-18 months.

Recently, researchers have proposed a new approach called human challenge trials that can speed up vaccine development in clinical trials. Typically, vaccines spend a large portion of their time in phase II/III clinical trials, which is 2 to 4 years to confirm the efficacy and safety in humans.<sup>207</sup> In this human challenge trial, the vaccines candidates are first tested on animals and healthy volunteers to determine the ideal dose. In phase II/II, large numbers of young and healthy participants are recruited, which can be divided into a placebo group and intervention group. Both groups are then injected with the effective vaccine dose. Subsequently, these groups are purposely infected with the virus in order to determine the effectiveness and safety of the vaccine.<sup>208</sup>

Multiple vaccine platforms are currently being developed for COVID-19 vaccines. Each vaccine platforms have their characteristics, which are listed in table 6.<sup>209 210</sup> Two vaccine platforms are ideal for the development of COVID-19, namely nucleic acids (DNA and RNA) and recombinant viral vectors. The production of both vaccine platforms can adapt platform manufacturing technologies, which enable large-scale manufacturing, thereby speeding up the production process.<sup>211</sup>



Table 6. Different vaccine platforms with their characteristics, advantages, and disadvantages

Vaccine platforms	Immunity	Advantages	Disadvantages
<b>Live attenuated vaccines</b>	Humoral and Cell-mediated	<ul style="list-style-type: none"> <li>• Small dose</li> <li>• Long-lasting immunity</li> </ul>	<ul style="list-style-type: none"> <li>• Incorporation into host's genome</li> <li>• The attenuated virus can revert to a virulent form</li> </ul>
<b>Inactivated virus</b>	Humoral	<ul style="list-style-type: none"> <li>• The inactivated virus cannot return to its virulent form</li> </ul>	<ul style="list-style-type: none"> <li>• Booster shots are needed due.</li> <li>• Induce weak immune response</li> </ul>
<b>Protein-based, liposaccharide and virus-like particle vaccine</b>	Humoral	<ul style="list-style-type: none"> <li>• Induce strong immune response</li> </ul>	<ul style="list-style-type: none"> <li>• Low immunogenicity</li> <li>• Booster shots are needed</li> </ul>
<b>Viral vector</b>	Humoral and cell-mediated	<ul style="list-style-type: none"> <li>• Induce a strong cell-mediated and humoral response.</li> <li>• Large-scale production is possible.</li> </ul>	<ul style="list-style-type: none"> <li>• The production processes are complex and expensive.</li> <li>• Pre-existing host immunity can neutralize the viral vector.</li> </ul>
<b>Nucleic acid (DNA and RNA)</b>	Humoral and cell-mediated	<ul style="list-style-type: none"> <li>• Production is faster and inexpensive.</li> <li>• Induce long-lasting immune response.</li> </ul>	<ul style="list-style-type: none"> <li>• Induce the production of antibodies that target DNA.</li> <li>• DNA can incorporate into the host's genome.</li> </ul>

As of May 23<sup>rd</sup>, 2020, there were 141 vaccine candidates in the preclinical and clinical trial identified in the database provided by Milken Institute.<sup>161</sup> Vaccine candidates are divided into ten different categories based on their vaccine platform.

The pie chart below illustrates nine different types of vaccines that are under development as a percentage of the total vaccine candidates in the clinical trial (figure 14). The chart illustrates the number of vaccine candidates from each vaccine platform that is currently in the clinical trial. Looking at figure 14, Protein subunits, RNA-based, and non-replicating viral vector account for 31% (44), 13% (19), and 11% (16) of the 141 COVID-19 vaccine candidates in the clinical trial. In addition, figure 15 illustrates the number of vaccine candidates of different vaccine platforms in clinical trials. Both figures show that most of the vaccine candidates in clinical trials are protein subunits based.

This is because most of the subunit vaccines use COVID-19's spike protein and receptor binding domain as a base to elicit immune responses against COVID-19. It is shown that the spike protein and the receptor-binding region can induce a robust immune response, which is why most pharmaceutical companies are developing vaccines based on these viral components.

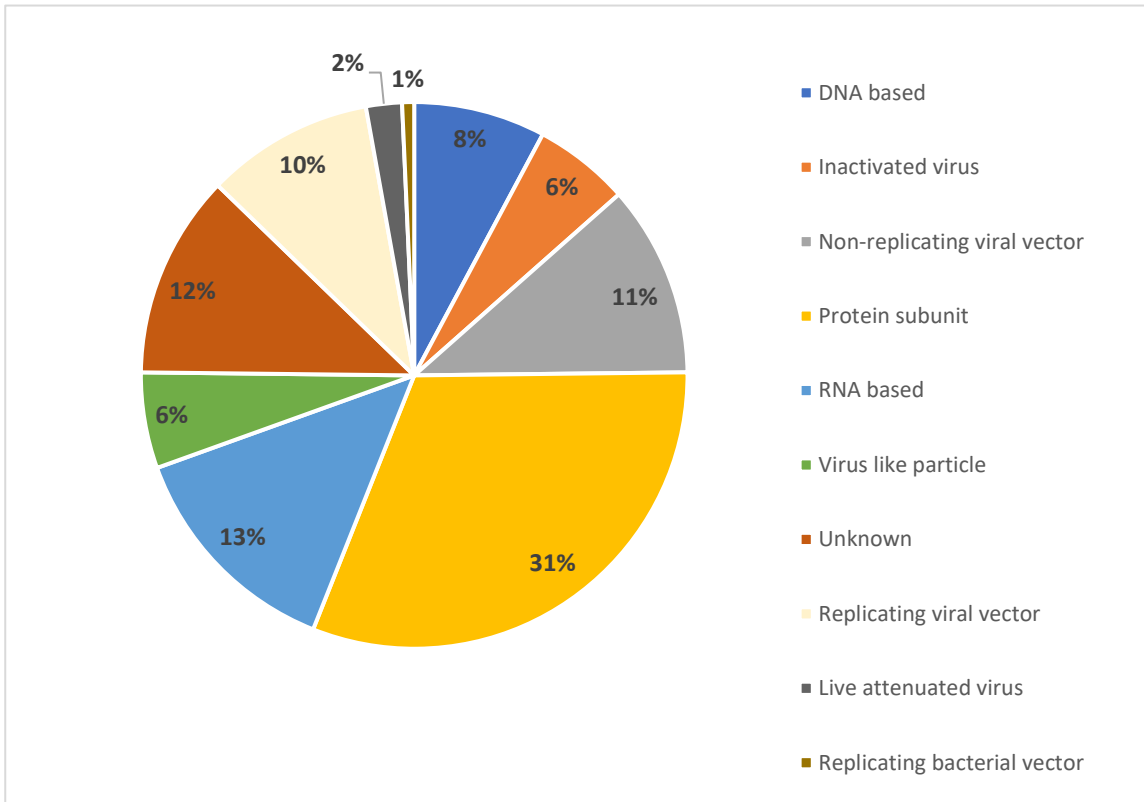


Figure 14. 10 different vaccine platforms as percentage of 141 vaccine candidates in clinical trial. Note: the data was collected on May 23<sup>rd</sup>, 2020

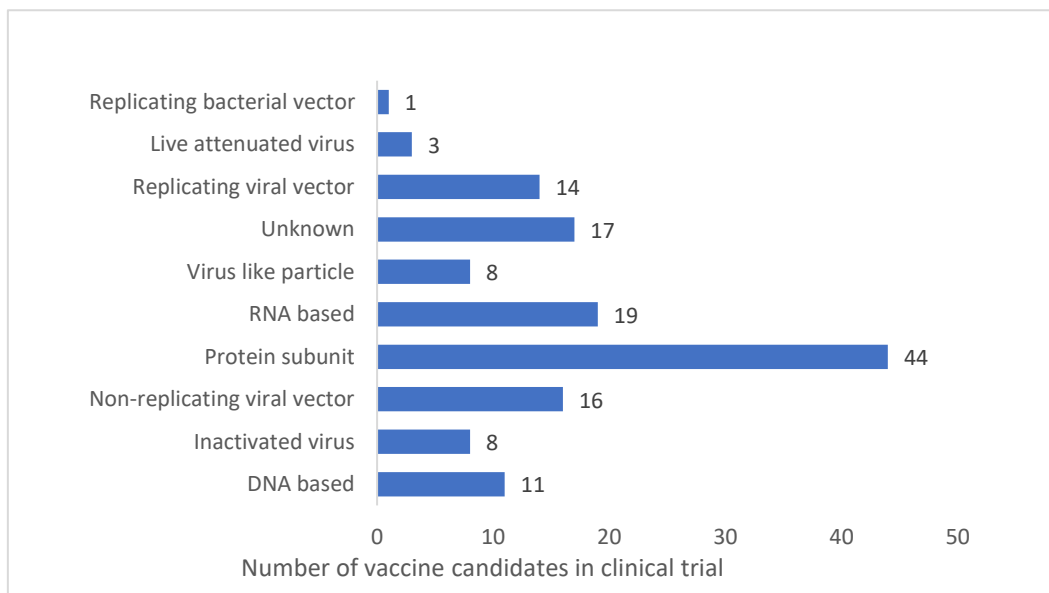


Figure 15. Ten vaccine platforms with their corresponding number of vaccine candidates in clinical trial. Note: the data was collected on May 23<sup>rd</sup>, 2020

As of May 27<sup>th</sup>, 2020, there were 13 most advanced vaccine candidates in the clinical trial (table 7).<sup>161</sup>

Table 7. 13 most advanced vaccines in clinical trials as of May 23<sup>rd</sup>, 2020.

Vaccines candidates	Phase	Type	Sponsors, partners
<b>BCG vaccine</b>	Phase III	Live attenuated	Multiple
<b>PreP-001</b>	Phase II	RNA-based	PreP Biopharm
<b>Ad5-nCoV vaccine</b>	Phase II	Viral vector	CanSino Biologics
<b>NVX-CoV2373</b>	Phase I/II	Protein subunit	Novavax
<b>PiCoVacc</b>	Phase I/II	Inactivated	Sinopharm
<b>LV-SMENP-DC vaccine</b>	Phase I/II	Viral vector	Shenzhen Genoimmune Medical institute
<b>BNT 162 mRNA vaccine</b>	Phase I/II	RNA-based	Pfizer, BioNTech AG
<b>RUTI Vaccine</b>	Phase I	Subunit	Archivel Farma SL.
<b>Pathogen-specific aAPC vaccine</b>	Phase I	Viral vector	Shenzhen Genoimmune Medical institute
<b>mRNA-1273</b>	Phase I	RNA-based	Moderna, NIH, Lonza
<b>INO-4800 DNA vaccine</b>	Phase I	DNA-based	Inovio
<b>ChAdOx1 nCov-19</b>	Phase I	Viral vector	AstraZeneca
<b>bacTLR-Spike</b>	Phase I	DNA-based	Symvivi Corporation

### Clinical trials of COVID-19 vaccines with results

Recently, an open-label non-randomized phase I trial with 195 individuals (Zhu *et al.*, 2020) has demonstrated that Ad5-nCoV, a non-replicating viral vector vaccine developed by CanSino, was well-tolerated and was able to induce high humoral and cell-mediated responses in vaccinated participants effectively. In this trial, a low dose, medium dose, and high dose of Ad5-nCoV vaccine were tested on participants. All vaccine recipients were able to produce high specific T-cell-mediated and humoral antibodies at days 14 and 28, respectively. Also, no serious adverse effects were reported in these participants. However, the most common side effects observed in vaccinated participants were muscle pain (17%), fever (46%), headaches (39%), and fatigue (44%).<sup>212</sup> This study did not include a randomized controlled group. In addition, this study had a short follow-up time and an insufficient sample size, which is why it is not possible to measure the efficacy and safety outcome of this vaccine. The tolerability and the immunogenicity of the vaccine were only determined in this study. Hence, the efficacy and safety of this vaccine need to be further investigated in phase II.

Other vaccine candidates such as INO-4800 and PicCoVacc showed positive results from their preclinical trial.

The preclinical study of INO-4800, a DNA-based vaccine developed by Sanofi, has shown that the elicited specific T-cell mediated and humoral antibodies responses in immunized mice and guinea pig were able to prevent COVID-19's spike protein from binding to host ACE2, thereby blocking viral entry, which provides protective immunity against COVID-19

infection. This finding suggests that the INO-4800 vaccine is immunogenic and is able to induce potent immune responses against COVID-19 in multiple animal models.<sup>213</sup> Currently, INO-4800 is in phase I study, testing 40 participants for its safety and immunogenicity in humans.<sup>214</sup>

According to a preclinical study, PiCoVacc, an inactivated virus vaccine developed by Sinopharm, was able to induce specific T-cell mediated and humoral responses in macaques, mice, and rats that were immunized with three different doses (1.5µg, 3µg, and 6µg). Macaques that were vaccinated two times with different doses (3µg and 6µg) were able to exhibit partial and complete protective immunity against COVID-19. It was also reported that the produced COVID-19 specific antibodies could neutralize ten other coronaviruses, which suggests that this vaccine can induce broad-spectrum humoral responses against coronaviruses. As for the safety of this vaccine, no severe adverse reactions were observed in immunized macaques. This finding suggests that PiCoVacc is immunogenic and tolerable in non-human macaques.<sup>215</sup>

Both INO-4800 and PiCoVacc is shown to be immunogenic and tolerable in animal models. However, the results from animal models cannot be translated to humans due to the immunophysiological differences between non-human animals and humans. Therefore, it is not yet possible to determine whether these vaccine candidates currently have a role in the prevention of COVID-19 infections.

## Discussion

Overall, some of the drug candidates show positive preliminary results, while others showed negative results. It is difficult to determine which drugs are safe and effective for the treatment of COVID-19 patients based on these studies. This is because most of the studies described above included a small number of patients, and some studies were not blinded or randomized. These limitations can affect the validity of the results and the strength of evidence. Also, clinical trials of these drugs are still ongoing to further assess safety and effectiveness. Therefore, we can only know which drugs are safer and more effective in treating COVID-19 infections, once these trials are completed.

Nevertheless, the preliminary results of these studies can give us an early indication of whether these drug candidates can have a role in COVID-19 treatment in the future. So far, remdesivir is the only drug that is approved for the treatment of COVID-19 infections in Japan, which suggests that remdesivir could be the potential COVID-19. Usually, the anti-infectious drugs' approval rate in phase I, II, and III is 16%, 27%, and 71%, respectively, which means that COVID-19 therapeutics currently in phase III trial are more likely to be approved.<sup>216</sup>

As for the experimental vaccine candidates that showed promising results in preclinical and phase I/II trial, it is still too soon to determine whether they can be approved for human use to prevent COVID-19 in the future. Typically, the approval rate for infectious disease vaccines that are undergoing preclinical and clinical trials is 6%, according to the study. Also, the average development duration of vaccines is 10.71 years.<sup>217</sup> The traditional vaccine development usually takes years, mainly because experimental vaccines spend a large portion of their time (2 to 6 years) in phase II and III trial, testing on healthy participants for their

safety and efficacy. Therefore, it is unrealistic to develop safe and effective COVID-19 vaccines in a short amount of time.

For this reason, new approach such as "human challenge trial," is being proposed with the aim to speed up the process in phase II/III trial, which involves rapid vaccine testing on a large number of participants and deliberately injecting the participants with the novel coronavirus after the vaccination. However, such approach is not ethical and dangerous since we have limited knowledge of the immunopathology of COVID-19, which can potentially put participant's lives at risk. Furthermore, there are limited data and insight on the mutation rate of this novel coronavirus, which can affect the performance of approved vaccines.

There are currently no approved vaccines and therapies available worldwide to treat and prevent COVID-19 infection. The majority of critically ill patients are provided with supportive care in the ICU, which potentially overwhelms the hospital system in countries with an underdeveloped health system since the number of cases keeps increasing. As for now, social measures are the only solution, but certainly not a permanent solution, to slow down the spread of this virus. Our technologies and knowledge of infectious diseases have advanced dramatically in the last two decades, but yet it takes, on average, 10 years to fully develop safe and effective therapeutics and vaccines. In the setting of a pandemic, where drugs and vaccines are urgently needed to prevent the number of infected and death from rising, the traditional approach of drug/vaccine development is, therefore, not ideal since it is slow. What we can learn from this pandemic is that more efforts and research should be put into designing a new clinical trial design that can accelerate the process of therapeutic/vaccine development during a pandemic in the future.

## Conclusion

The novel coronavirus (COVID-19) that caused the current global pandemic was found to share sequence similarities with SARS-CoV, MERS-CoV, and RaTG13, suggesting that COVID-19 might come from bats. It was also found that pangolin could be the intermediate host since COVID-19 shares 91% sequence similarities with coronavirus found in pangolins. Studies have demonstrated that COVID-19 invade host cells by interacting with the host ACE2 via its surface spike protein.

Moreover, the novel coronavirus is shown to exhibit similar immunopathology and clinical characteristics to SARS-CoV and MERS-CoV. 80% of infected people develop mild complications. Some people, especially elderly, who develop severe complications caused by cytokine storm syndrome, are admitted to ICU for ventilation support. There are several oxygenation therapies currently used to treat patients with severe ARDS, including nasal cannula, non-invasive ventilation, invasive mechanical ventilation, and ECMO. However, due to the increasing number of patients who require ICU admission, hospital systems around the world are at risk of collapsing. Therefore, therapeutics and vaccines for COVID-19 are urgently needed. Researchers and scientists are using different approaches and methods such as artificial intelligence services and human challenge studies to speed up the drug/ vaccine development for COVID-19.

Since the SARS and MERS outbreak, several drugs have been identified that exhibit anti-CoV activity by targeting the viral components and the host components, which can be the potential therapeutics for COVID-19 infections. As of May 23<sup>rd</sup>, 2020, there were 223

COVID-19 therapeutics and 141 vaccines in preclinical/clinical trials. The majority of therapeutics and vaccines under development were antibodies and protein subunits, respectively. Several therapeutics and vaccine candidates showed promising results in preclinical/clinical trials such as favipiravir, remdesivir, tocilizumab and Ad5-nCoV but only remdesivir was approved in Japan. Most of the studies included small numbers of patients and were not blinded or randomized, which negatively affect the validity of the results, which is why it is hard to determine whether these therapeutics and vaccine candidates will have a role in the treatment of COVID-19 in the future. What we can learn from the current pandemic is that more efforts and research should be put into designing a novel clinical trial design that can be used in the setting of a pandemic, where drugs and vaccines are urgently needed.

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