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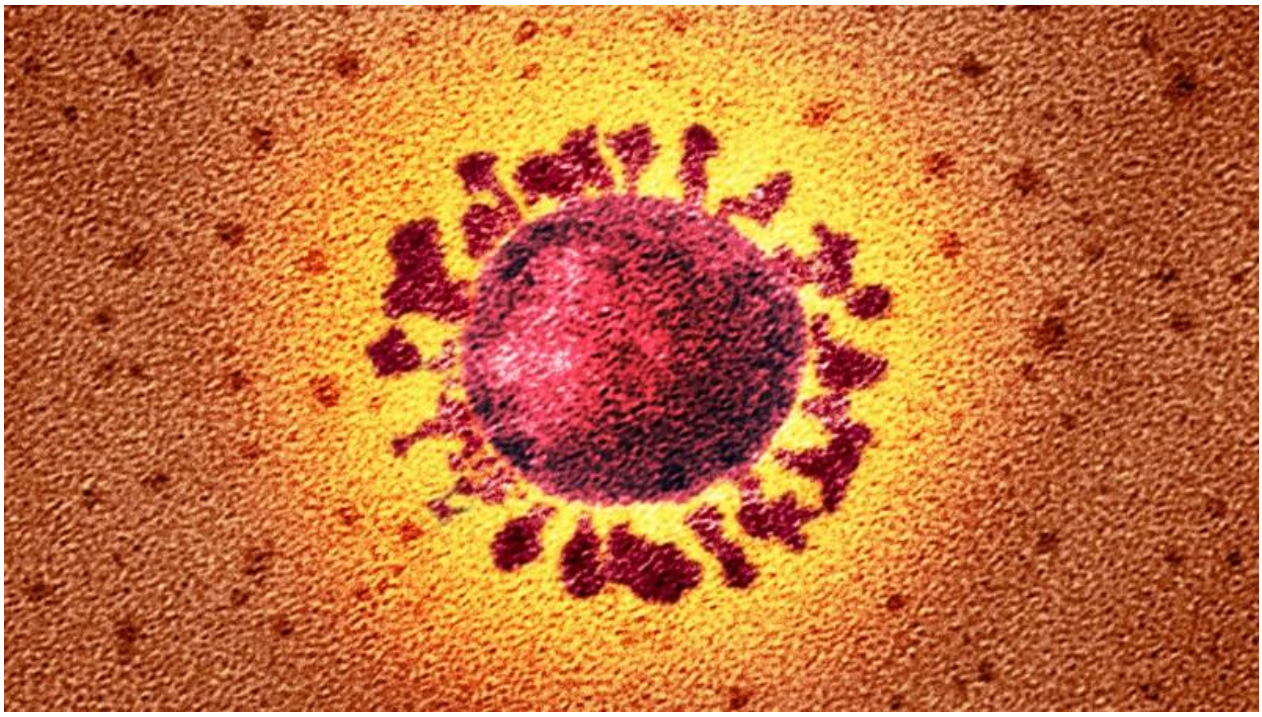
ACE2 and COVID-19

Targeting angiotensin-converting enzyme 2 as a possible treatment for COVID-19



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Summary

In December 2019, a new coronavirus arose in Wuhan, China, which quickly spread across the globe. The virus is called SARS-CoV-2, and can cause a disease named COVID-19. As of October 29, the worldwide number of confirmed cases of COVID-19 is over 44 million, with over 1,1 million confirmed deaths. COVID-19 symptoms can range from fever, cough and muscle pain to acute respiratory distress syndrome, organ failure and shock.

SARS-CoV-2 enters the human host cell via ACE2. ACE2 is a membrane bound enzyme, which can counterbalance the activity of ACE. ACE2 hydrolyses angiotensin II into angiotensin (1-7), which binds to the MAS receptor, leading to vasodilation. Furthermore, this has anti-inflammatory and anti-fibrotic effects. It appears that higher expression levels of ACE2 are correlated with higher diseases susceptibility. SARS-CoV-2 downregulates the expression of ACE2, leading to the imbalance between ACE and ACE2, which causes inflammation, oxidative stress, hypertension and severe tissue damage. Soluble ACE2, produced by the cleavage by ADAM17, still cleaves angiotensin II and can inhibit viral infection partly.

ACE2 is an interesting potential target for treating COVID-19. It is complicated because of the dual role of ACE2. The use of stem cells, stimulation of ACE2 shedding, ACE2 stimulation and ACE2 inhibition are all considered strategies. The most promising appears to be the use of recombinant soluble ACE2, which can inhibit SARS-CoV-2 cellular entry while still protecting against the detrimental effects of the virus.

Introduction

In December 2019, several cases of pneumonia with an unknown cause were reported in Wuhan, China. It was soon found out that these cases were caused by a newly discovered virus, named SARS-CoV-2, with the lower respiratory tract as primary target. The disease caused by the virus was named COVID-19, and only two months later, it had spread over the whole world. In March 2020, the World Health Organization (WHO) stated that this outbreak was a global pandemic (1).

As of October 29, the worldwide number of confirmed cases of COVID-19 is over 44 million, with over 1,1 million confirmed deaths (1). Although these increasing numbers are worrying on their own, there are many more impacts that COVID-19 has had on the world. First of all, it has led to a worldwide reduction in hospital visits for other health problems like heart attacks, strokes and cancer (2). Furthermore, the pandemic has a negative impact on mental health around the world, causing stress, anxiety, depressive symptoms, insomnia and fear (3). Besides health problems, the COVID-19 pandemic also caused the largest global recession in history. Signs of this recession are the stock market crash and rapid increases in unemployment (4). To summarize, the pandemic has a large effect on many aspects of the world.

Right from the beginning of this pandemic, scientists all over the world are researching this virus. They have been researching the virus itself, how it is transmitted and its pathophysiology. As of today, there are no proven vaccines or other antiviral treatments that can cure COVID-19. However, this is a highly researched topic, and many possibilities for treatment have been proposed.

In this essay, I will be discussing one of the treatment possibilities for COVID-19, which is targeting angiotensin-converting enzyme 2 (ACE2). The new coronavirus and COVID-19 will be discussed in more detail, as well as ACE2 and its role in COVID-19. Finally, I will discuss the possibilities of using ACE2 as a target in the treatment of COVID-19.

COVID-19

Pandemic

As briefly described in the introduction, coronavirus disease 2019 (COVID-19) rapidly spread around the globe. On December 31, 2019, the first cases of pneumonia with an unknown cause in Wuhan, China, were reported to the World Health Organization (WHO), by the Chinese authorities. On January 7, 2020, a new coronavirus was identified as the cause of this new outbreak. Only two days later, the first death linked to COVID-19 was reported in China. On January 30, the WHO declared the outbreak a public health emergency of international concern. By then, the virus had already spread to several countries around the world, including the U.S., Australia and Germany. By March 8, over 100 countries had reported cases of COVID-19, and 3 days later the WHO declared the global COVID-19 outbreak a pandemic. Many countries respond with travel restrictions and close their borders. The number of infected people continues to grow exponentially. As of October 29, the worldwide number of confirmed cases of COVID-19 is over 44 million, with over 1,1 million confirmed deaths (1).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus strain that causes COVID-19. It is not clear how SARS-CoV-2 originated, but it is highly suspected it comes from an animal source. The reason for this is the fact that the first COVID-19 patients had almost all visited the Seafood Wholesale Market in Wuhan, where different types of wild and domesticated animals were sold illegally (5). The specific source of SARS-CoV-2 is not known yet. The virus that is most similar to SARS-CoV-2 (approximately 97%), RaTG13, is a bat virus (6). Sequence analyses show that it is probable that the reservoir host was a bat, and an intermediate host was the source of the outbreak (7). A suspected intermediate host is the Malayan pangolin, as viruses similar to SARS-CoV-2 have been identified in these animals (8).

Although the original source of the virus is probably zoonotic, the large pandemic is caused by human-to-human transmission. It is transmitted mainly from the respiratory tract via droplets and aerosols, that spread when an infected person coughs, sneezes or even talks (9). An alternative route of transmission that has been proposed is faecal-oral spread, as coronaviruses can also infect the gastrointestinal tract (10).It is thought that especially asymptomatic infections are a key contributor in COVID-19 spread (11). The reproductive ratio (R0) is the rate of transmission of diseases, which is generally thought to be between 2,0 and 2,8 for COVID-19 (7). This means a COVID-19 patient spreads the virus to 2,0 to 2,8 other people, who will spread it to more people. This means the number of infected people grows at an exponential rate, quickly causing the large pandemic.

More information on SARS-CoV-2 will be discussed in the next chapter.

Virus

SARS-CoV-2 is a positive-sense single-stranded RNA virus (+ssRNA), with an RNA sequence of 29.903 bases in length (12). It belongs to the large family of coronaviruses, which are divided in 4 genera. These are α-, β-, γ-, and δ-coronaviruses. Both α- and β-coronaviruses are known to infect mammals, and SARS-CoV-2 belongs to the β-coronaviruses (13).

The virus particles range from 50 to 200nm in diameter (14). SARS-CoV-2 consists of 4 structural proteins, which are spike (S), membrane (M), envelope (E) and nucleoprotein (N). The structure can be seen in Figure 1. The S, E and M proteins create the viral envelope, and the N protein holds the RNA genome. Coronaviruses enter cells with help of their S proteins. The surface unit of the S protein, S1, can bind to a cellular receptor, allowing the virus to attach to the target cell. Furthermore, before entry, the S protein must be primed by cellular proteases, which allows the fusion of viral and cellular membranes (15). In addition to these structural proteins, there are also non-structural proteins (NSPs) encoded in the RNA genome of the virus. These NSPs have various functions, like transcription regulation, immunomodulation, gene transactivation and countering the antiviral response (16).

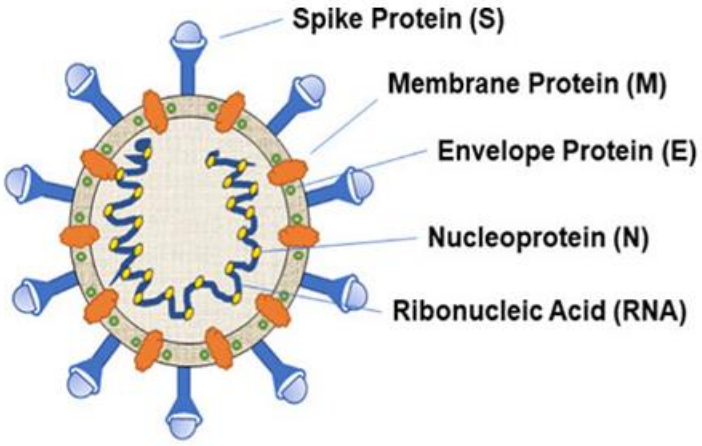

Figure 1. SARS-CoV-2 structure. Adapted from Naqvi et al, 2020 (13).

Once SARS-CoV-2 has entered the cell, its RNA will act as mRNA, which is translated by the host cell ribosomes. This leads to the production of replicative enzymes, which generate new RNA genomes and mRNA for the synthesis of new viral particles (17). How the virus can induce COVID-19 symptoms will be discussed in the next chapter.

Disease

The SARS-CoV-2 virus previously discussed, causes coronavirus disease 2019 (COVID-19). The main symptoms are fever, cough, fatigue, muscle pain, breathing difficulties, and loss of smell and taste (18). Less common are gastrointestinal symptoms, like loss of appetite, diarrhea and nausea (19). About 40% to 45% of the infected people are asymptomatic, and most of the other infected people only experience mild symptoms (20). However, some people can develop acute respiratory distress syndrome (ARDS). This is a form of fluid accumulation in the lungs, impairing the lungs capacity to exchange oxygen and carbon dioxide. This leads to low oxygen levels in the blood. ARDS has a death rate between 35% and 50% (21). Severe COVID-19 can lead to acute cardiac, kidney and liver failure, digestive tract and nervous system injury, as well as shock (22).

The average time between the exposure to the virus and the onset of symptoms is 5 days, it may range from two to fourteen days (23). Usually, loss of smell and taste appear early on in the illness, followed by a fever, and then a cough and muscle pain (24). In patients younger than 50 years, the mortality risk is less than 1%, and it increases exponentially after this age. The highest mortality rate is in people aged 80 years or older, at almost 30% (25).There is a slight predominance in men testing positive for COVID-19 (58,1%) compared with women (41,9%) (26). Patients with obesity have a higher risk of ICU admission and death, compared to patients with a normal weight (27).

The virus enters the body through the respiratory tract, and in the lungs it infects alveolar epithelial type 2 (AT2) cells. The AT2 cells secrete pulmonary surfactant, a group of phospholipids that can decrease the tension in the alveoli. The surfactant prevents the alveoli from collapsing. The infection of AT2 cells leads to their death by apoptosis and pyroptosis. The decrease in AT2 cells will ultimately lead to alveolar collapse and impaired gaseous exchange (28). The infection of the AT2 cells also leads to macrophage activation. Activated macrophages produce several proinflammatory cytokines, such as IL-1, IL-6 and TNFα. This causes vasodilation and increased permeability of the capillaries. Plasma and inflammatory cells will leak into the interstitial spaces of the alveoli, and it will accumulate, leading to the compression of the alveoli. This also contributes to the impaired gaseous exchange (28).

The infiltration of inflammatory cells in the lung tissue results in a cytokine storm, an excessive release of pro-inflammatory cytokines. The cytokine storm is responsible for the multi-organ failure in COVID-19, and is associated with COVID-19 severity (29). The released proinflammatory cytokines do not only affect the lungs locally, but also have systemic effects. In the hypothalamus, it will cause an increase in body temperature, resulting in a fever (30).

ACE2

General information

Angiotensin-converting enzyme 2 (ACE2) is an enzyme, which is attached to different cells in the lungs, arteries, heart, kidney and intestines. It is present mainly on AT2 cells, enterocytes in the small intestine, endothelial cells of the blood vessels and arterial smooth muscle cells in most organs (31). In humans, its gene is located on the X chromosome at position Xp22 (32)

The enzyme is a dimeric metallopeptidase containing zinc. ACE2 consists of 805 amino acids and has a molecular mass of 120 kDa (33). It is a type I bitopic transmembrane protein. Bitopic means it crosses the cell membrane only once. Type I bitopic proteins have their N-terminus on the extracellular side of the membrane, with their signal peptide removed (31). ACE2 has two domains. One is the N-terminus enzymatically active domain, which is exposed on the extracellular side of the membrane. The other is the C-terminus, which is on the intracellular side (33).

Its extracellular domain can be cleaved from the transmembrane domain in a process called shedding. Shedding is mediated by members of the ADAM family (a disintegrin and metalloproteinase), and this is also the case for ACE2. For ACE2, ADAM17 is responsible for the shedding of ACE2. After cleavage, soluble ACE2 (sACE2) is released into the blood and will eventually be excreted in the urine (34).

Function

ACE2 was first discovered in 2000 as a homologue of angiotensin-converting enzyme (ACE); both are part of the renin-angiotensin system (RAS) (33). This system is a cascade of enzymatic reactions, which results in the production of angiotensin II. The RAS is a system which regulates blood pressure, fluid and electrolyte balance and systemic vascular resistance (35).

In the kidney, juxtaglomerular (JG) cells secrete the inactive molecule prorenin, and activation of JG cells leads to the cleavage of prorenin to renin. Renin can cleave angiotensinogen from the liver into angiotensin I, which is biologically inactive. Angiotensin I is cleaved to angiotensin II by ACE. Angiotensin II can cause an increase in blood pressure, total body sodium and total body water, by binding to angiotensin II receptor type I (AT1R) (35). Binding to AT1R can also activate several molecular signalling pathways related to tissue injury, inflammation and fibrosis (36).

The main function of ACE2 is to counterbalance the activity of ACE. ACE2 can cleave the phenylalanine amino acid from the C-terminus of angiotensin II, and hydrolyses it into angiotensin (1-7) (37). ACE2 can also cleave angiotensin I, the inactive precursor of angiotensin

II, into angiotensin (1-9), which in turn can be converted to angiotensin (1-7) by ACE (33). Angiotensin (1-7) is a vasodilator, causing opposite effects of those of angiotensin II. It binds to the Mas receptor, which has anti-inflammatory and anti-fibrogenic effects (36).

A schematic overview of this process is displayed in Figure 2.

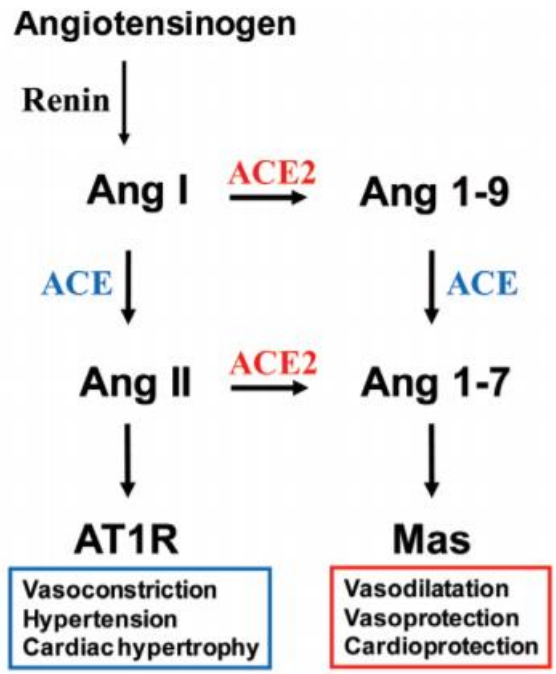

Figure 2. ACE2 function (38).

Furthermore, ACE2 can also cleave several other peptides, including bradykinin, apelin, neurotensin, kinetensin, dynorphin A, casomorphin, and ghrelin (33, 37). ACE2 is also essential for the expression of amino acid transporters (38). To conclude, ACE2 is a multi-functional enzyme with an important role in regulating blood pressure and a protective effect on the whole body.

ACE2 and COVID-19

As mentioned before, coronaviruses enter cells with help of their S proteins. To enter the cell, coronaviruses need S1 (surface unit of S protein) to bind to a cellular receptor, and the S protein must be cleaved by cellular proteases (15).

It is known that the first identified severe acute respiratory syndrome coronavirus (SARS-CoV-1), which caused an outbreak in 2002-2004, used ACE2 as the entry receptor (39). The cellular proteases it uses for S protein priming are the transmembrane serine protease TMPRSS2 (40) and the endosomal cysteine proteases cathepsin B and L (CatB/L) (41). The same was tested for SARS-CoV-2. The receptor binding motif in the S protein that makes contact with ACE2 in SARS-CoV-1, was conserved in SARS-CoV-2, indicating that the new coronavirus uses ACE2 as an entry receptor (15).

Like SARS-CoV-1, SARS-CoV-2 also depends on cellular proteases for S protein priming (15).Cleavage of the S protein happens at two sites; S1/2 and S2'. This cleaving leads to the formation of the subunits S1 and S2, which remain non-covalently bound. The exact of these cleavage sites are at the junction of the amino acids Arg685/Ser686 and Arg815/Ser816 on the S protein (42). The S1 subunit contains the receptor binding domain, the S2 subunit is membrane bound and aids in the membrane fusion (Figure 3). It is thought that the cleavage at the S1/S2 occurs first, and subsequently, the S2' site is exposed and cleaved (43).

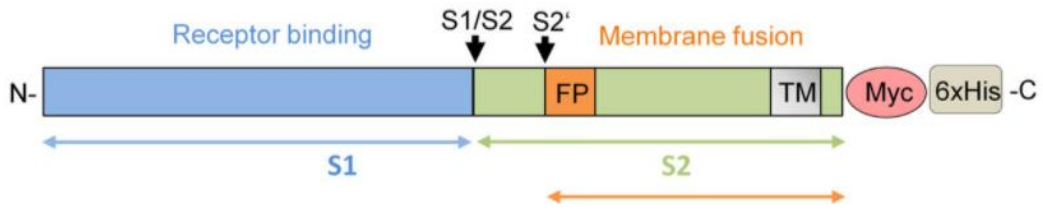

Figure 3. Schematic representation of SARS-CoV-2 spike protein, with S1 and S2 subunits. Black arrows indicate cleavage site. FP = fusion peptide, TM = transmembrane domain (43).

The first event, cleavage of S1/S2, is done by the proprotein convertase furin. This happens when the virus binds to ACE2. By this first reaction, the S protein is pre-activated. Furin pre-activation enhances pseudovirus entry into different human cell lines expressing ACE2 (44). The pre-activation by furin makes SARS-CoV-2 less dependent on other host cell proteases. This is of particular interest in cells with low expression of TMPRSS2 and cathepsins (44).A furin inhibitor could inhibit SARS-CoV-2 S protein processing (45).

After the virus has bound to ACE2 and the spike protein is pre-activated by furin, the S2' site is cleaved by TMPRSS2. Thereby, the S protein is fully activated. SARS-CoV-2 depends on TMPRSS2 for protein priming and cell entry, and entry can be inhibited by a TMPRSS2 inhibitor (15). TMPRSS2 co-expresses, co-localizes and interacts with ACE2 (46). An overview of the entry process can be seen in Figure 4.

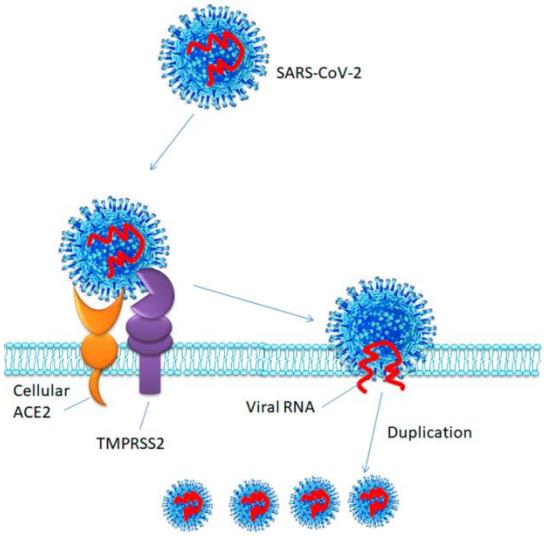

Figure 4. SARS-CoV-2 cell entry, using ACE2 and TMPRSS2. Adapted from Xiao et al, 2020 (47).

As mentioned, SARS-CoV-1 can also use CatB/L for priming the S protein (41). This is also the case for SARS-CoV-2 (15). This appears to be of particular importance in cardiomyocytes, since they do not express TMPRSS2, but do express CatB/L. Indeed, using a cathepsin inhibitor reduced the viral S protein expression (48).

Importantly, ACE2 expression is not limited to the lung, but is also present in the heart, brain, kidney, intestine, esophagus, stomach, nose, testis, pancreas, breast, prostate and thyroid (49). This could mean that SARS-CoV-2 could also directly infect these other organs, directly causing damage and organ failure. SARS-CoV-2 RNA has already been found in cardiac tissue, parts of the gastrointestinal tract, cerebrospinal fluid and urine, and N proteins from the virus have been found in kidney tissue, urine, liver tissue and parts of the gastrointestinal tract (49). These findings suggest that indeed, SARS-CoV-2 can directly infect other tissues where ACE2 is expressed.

Higher ACE2 concentrations in plasma have been found in men, compared to women (50). This might explain the higher incidence of COVID-19 in men, mentioned earlier. It was also mentioned earlier that obese patients have a higher risk of ICU admission and death, which might be explained by the fact that adipose tissue has a higher ACE2 gene expression than human lung tissue (51). Furthermore, patients with certain cancers and chronic diseases have higher ACE2 expression compared to healthy individuals, which might lead to their higher susceptibility to multi-organ injury after infection (52). All these findings suggest that higher ACE2 expression is correlated with higher COVID-19 susceptibility and disease severity. It is important to note that these risk factors such as obesity, cancer and chronic diseases could also be independent of pathogenesis. At this time there is no evidence to prove that elevated ACE2 levels directly cause an increased risk of infection or a worse prognosis for COVID-19 patients.

However, the findings on higher ACE2 expression correlated with higher disease severity are contradicted by another mechanism. SARS-CoV-2 not only uses ACE2 for its entry to the host cell, it also downregulates ACE2 expression and competes with angiotensin II for binding (53, 54). As a result, the balance between the effects of ACE and ACE2 is lost. This means that angiotensin I and II are no longer broken down, so there is more activation of the AT1 receptor. This dysregulation can lead to more inflammation, oxidative stress, hypertension and severe tissue damage (55).This process can be seen in Figure 5.

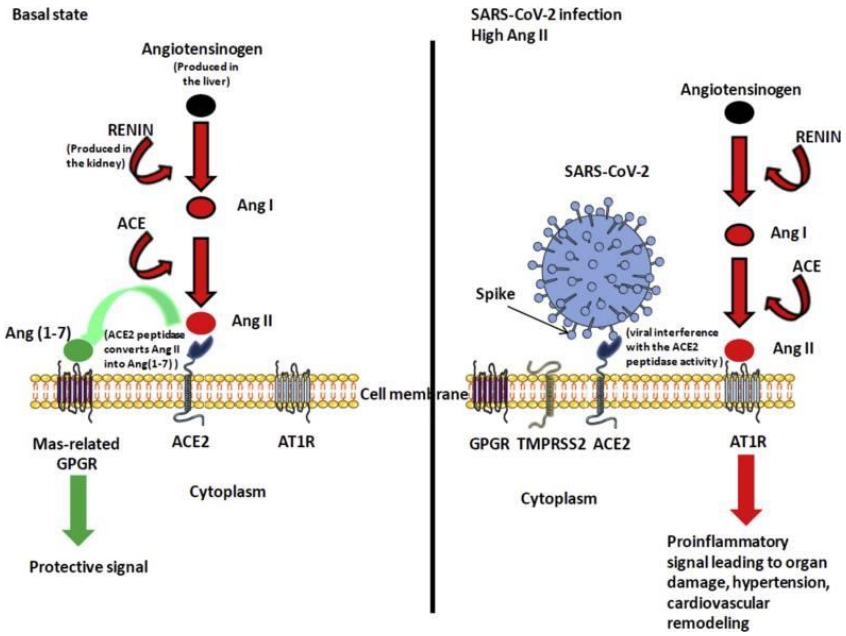

Figure 5. In the basal state, ACE2 converts angiotensin II to angiotensin (1-7), which will send a protective signal through the MAS receptor. When SARS-CoV-2 binds, this interferes with ACE2, leading to angiotensin II accumulation, and proinflammatory signals (54).

Correspondingly, it has indeed been found that angiotensin levels are significantly increased in COVID-19 patients (56). Furthermore, earlier findings show that ACE2 can protect mice from severe acute lung injury. ACE knockout mice show less severe lung injury, while ACE2 knockout mice have worse symptoms compared to wild-type mice (57). These findings suggest that ACE2 has a protective effect.

The interaction between ACE2 and SARS-CoV-2 increases the activity of ADAM17, which can lead to shedding ACE2. Shedding of ACE2 by ADAM17 at first does not appear to be beneficial for COVID-19 patients. This is because ACE2 can protect against severe lung injury (57). The role of ADAM17 and ACE2 shedding is not clear yet in the infection of SARS-CoV2. Inhibiting shedding by mutations in the ACE2 cytoplasmic tail or ADAM17 silencing blocked viral infection (58). This suggests that ADAM17 might actually facilitate viral entry. However, soluble ACE2 (sACE2) that has been shed by ADAM17 still contains the virus binding site, so it can still bind to the virus. Since there is no intracellular environment attached to sACE2, the virus cannot duplicate. By binding to SARS-CoV-2, sACE2 keeps the virus from binding to membrane-bound ACE2, which has a small inhibitory effect on viral infection efficiency (59). Furthermore, sACE2 released by ADAM17 still has enzymatic activity, so it can still cleave angiotensin II to angiotensin (1-7), increasing the protective effects of the Mas receptor (59). This also suggests a protective role for sACE2 in acute lung injury. So, there is evidence suggesting both negative and positive effects of ADAM17 in COVID-19.

To summarize, SARS-CoV-2 uses ACE2 as an entry receptor, with the help of endogenous cellular proteases. Higher levels of ACE2 are associated with higher susceptibility of COVID-19. When SARS-CoV-2 binds to ACE2, it downregulates ACE2 expression. This might be the underlying mechanism for the severe COVID-19 symptoms like organ damage, as ACE2 has a protective and anti-inflammatory effect.

Treatment

Based on the information discussed above, ACE2 is an interesting potential therapeutic target, as it is the point of viral entry and it has a protective effect. Several therapeutic strategies targeting ACE2 have been proposed. In this chapter, these strategies will be discussed.

ACE2 inhibitors

ACE2 is the entry point for SARS-CoV-2 and various investigations have found a positive relationship between ACE2 expression and susceptibility towards COVID-19. This insight led to the idea to inhibit ACE2 expression, and thereby inhibit SARS-CoV-2 from entering the host cell. However, there are no known ACE2 inhibitors, and ACE2 is insensitive to conventional ACE inhibitors (60).

But even if there were any ACE2 inhibitors, it would not be wise to use them to prevent SARS-CoV-2 infection. By doing this, all protective effects of ACE2 would be lost, leading to hypertension, organ damage, cardiovascular remodelling and inflammation (54). This effect is shown in ACE2 knockout mice, which show lung injury and cardiac dysfunction (57, 61).

ACE2 activators

Since ACE2 has many protective effects that can protect from the damaging effects of COVID-19, it might be wise to actually use ACE2 activators as a therapy. Direct activators could have two positive effects. First of all, they can prevent SARS-CoV-2 from binding to ACE2, because the activator is already bound. Secondly, it could also promote the protective effects of ACE2 on different organs. As is the case for ACE2 inhibitors, there are also no pharmacologic ACE2 activators for humans available to date. However, there are several activators that are used in experimental settings and veterinary use, like diminazene acuturate (DIZE), xanthenone (XNT), and resorcinolnaphthalein (RES) (62).

DIZE is an anti-parasitic drug, which is usually used in cattle to treat trypanosomiasis. There are drugs available containing DIZE, like Veriben or Berenil, that are used to treat trypanosomiasis in humans. In rats, chronic administration of DIZE leads to the increase in enzymatic activity of ACE2, and this prevents pulmonary hypertension and lung injury. Furthermore, cardiac remodeling is attenuated after DIZE treatment (Figure 6) (63). This suggests that DIZE could also protect against tissue damage in COVID-19 patients. However, there are very limited data of the use and side effects of DIZE in humans, so much more research is needed (64).

XNT is a chemical compound that can also enhance ACE2 activity. Like DIZE, it reduces hypertension, and can even reverse fibrosis in multiple organs in hypertensive rats (Figure 7) (65). Like with DIZE, very little is known about the use of XNT in humans, so this also requires more research.

RES is another chemical compound, also able to enhance the enzymatic activity of ACE2 (65). RES has been shown to significantly reduce inflammatory cell infiltration and damage in the lungs of mice (Figure 8) (66). Moreover, it attenuates pulmonary vascular remodeling, prevents hypertension and decreases pro-inflammatory cytokines in rats (67, 68). Once again, RES has never been tested in humans before, so this needs a lot of further research.

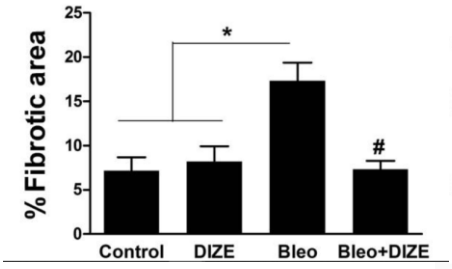

Figure 6. DIZE significantly lowers bleomycin induced cardiac fibrosis (63)

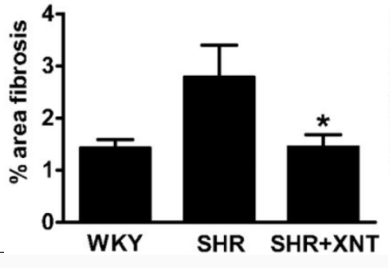

Figure 7. XNT significantly lowers cardiac fibrosis in spontaneously hypertensive rats (65)

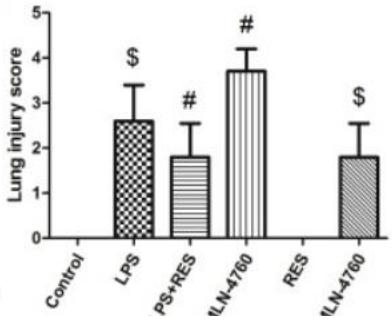

Figure 8. RES significantly lowers lung injury after LPS exposure (66)

A widely used drug in humans, ibuprofen, has also been investigated as a potential therapy for COVID-19. Ibuprofen has been shown to increase ACE2 levels in rats with diabetes, and in those rats it can decrease heart damage (69). However, ibuprofen can also inhibit cytokine production and may weaken the immune system, which can be dangerous in case of a SARS-CoV-2 infection (70). Furthermore, as ibuprofen can mask COVID-19 symptoms like fever, it might delay the diagnosis, causing a further spread of the disease. Ibuprofen has been shown to not prevent the development of ARDS, an important cause of death in COVID-19 (71). At the time, there is no evidence that ibuprofen is either harmful or helpful in COVID-19 patients.

Angiotensin receptor 1 blockers (ARBs) and ACE inhibitors are also widely used in humans, primarily in high blood pressure and heart failure. In a rat study, increased levels or ACE2 mRNA has been found in the heart after ARB treatment (72). The same was also found for the administration of ACE inhibitors, they caused an increase in cardiac ACE2 mRNA and ACE2 activity (73). However, this has not been confirmed in human studies (74). There is no evidence that ARBs and ACE inhibitors can upregulate ACE2 expression in human lungs, and there is no evidence that these drugs have any influence on the susceptibility to COVID-19 or the disease outcome (75).

To summarize, there are some compounds and drugs known to elevate ACE2 expression and thereby have protective effects on the lungs and other organs. However, there is not enough known about their use in humans and about their capacity to actually have protective effects in COVID-19.

Recombinant ACE2

As mentioned in the previous chapter, sACE2 can bind to SARS-CoV-2, preventing it from binding to membrane bound ACE2 and thus preventing viral entry. Moreover, sACE2 can still convert angiotensin II to angiotensin (1-7), thus having protective effects. This makes it an interesting subject for possible treatment. Currently, recombinant human soluble ACE2 (rhACE2) is considered as a therapeutic approach.

Indeed, it has been found that rhACE2 can inhibit SARS-CoV-2 infection in cells and human kidney and capillary organoids, reducing its viral load by a factor of 1000-5000 (76). Furthermore, it has already been shown that administration of rhACE2 can decrease acute lung injury in acid-treated mice (57).

Treatment with rhACE2 appears to be safe, as phase I and II clinical studies with a recombinant version of the catalytic domain of human ACE2 (GSK2586881) have been completed, and they show that it was safe and effective in the treatment of ARDS. The rhACE2 did not significantly change any hemodynamic factors and no adverse reactions were reported (77).

It appears that rhACE2 does not only reduce lung injury, but also blocks SARS-CoV-2 entry in host cells (Figure 9). It seems very interesting as a potential COVID-19 treatment, considering its dual effect. More research is desired to study how effective it is to battle COVID-19 in humans.

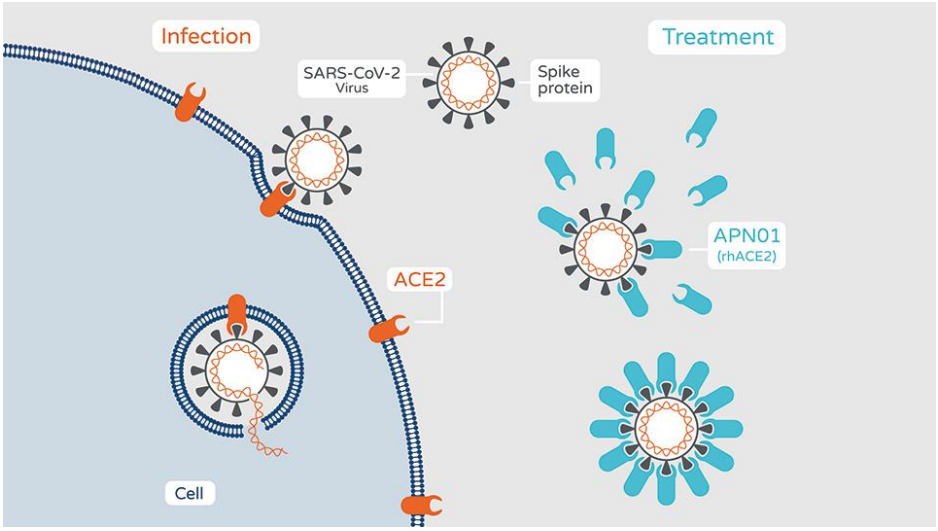

Figure 9. rhACE2 coating SARS-CoV-2, preventing entry into the cell (78).

Shedding

As discussed above, higher levels of soluble ACE2 can prevent SARS-CoV-2 cell entry and reduce organ damage. This indicates that higher levels of shed ACE2 could also inhibit the infectivity of SARS-CoV-2, a hypothesis already stated by Lambert et al, who found that ADAM17 was responsible for ACE2 shedding (34).

On the contrary, it was also found that the S protein of SARS-CoV-1 could induce ADAM17 to shed ACE2, and this also leads to TNF-α production. TNF-α facilitates tissue damage through inflammation. The same study also found that inhibiting shedding reduced viral load and ADAM17 silencing blocked viral infection (58). This suggests that inhibiting ADAM17 and thereby decreasing ACE2 shedding might protect against SARS-CoV-2 infection. However, this study stated that ADAM17 facilitates viral entry for SARS-CoV-1, while for SARS-CoV-2, TMPRSS2 facilitates viral entry (15).

Taken together, ACE2 shedding by ADAM17 increases sACE2, which can reduce SARS-CoV-2 infection and symptoms. However, ADAM17 can also shed other proteins, including IL-6 and TNF-α, which has a negative effect on the pathogenesis of COVID-19. More research is needed to determine the role of ADAM17 and ACE2 shedding in SARS-CoV-2 infection.

Protease inhibitors

There are several host proteases that can prime the S protein of SARS-CoV-2 to allow viral entry to the host cell. By inhibiting the cleaving activity of these proteases and thereby inhibit priming, viral entry can be stopped.

The most important of these proteases is TMPRSS2, which SARS-CoV-2 depends on for cellular entry. It has been shown that a clinically approved inhibitor of TMPRSS2, camostat mesylate, can partially block viral entry (15). Many more TMPRSS2 inhibitors are clinically approved, like bromohexine, aprotinin and nafamostat. Aprotinin has already been shown to inhibit viral entry of SARS-CoV-2 in a dose dependent matter (Figure 10) (43). So, these inhibitors may reduce transmission of COVID-19.

CatB/L can also cleave SARS-CoV-2 S protein. While the TMPRSS2 inhibitor camostat mesylate could only partially inhibit viral entry, adding a cathepsin inhibitor (E64d) led to full inhibition (15). The same inhibitor can decrease the entry of pseudovirions by 92,5% (Figure 11) (79). E64d can decrease the amount of SARS-CoV-2 RNA in cell culture supernatants, with minimal cytotoxicity (80).Multiple cathepsin L inhibitors can also inhibit entry of pseudovirions (79).

As furin pre-activates SARS-CoV-2 by cleaving its S protein, this may be another interesting therapeutic target. S protein processing was inhibited by a furin inhibitor, decanoyl-RVKR-CMK (45).Furthermore, another systemic furin inhibitor, MI-1851 strongly inhibited SARS-CoV-2 replication in human airway cells (Figure 12) (43). Although this seems promising, it must be taken into account that furin is required for normal development (81).Therefore, inhibiting furin might lead to unwanted toxic effects.

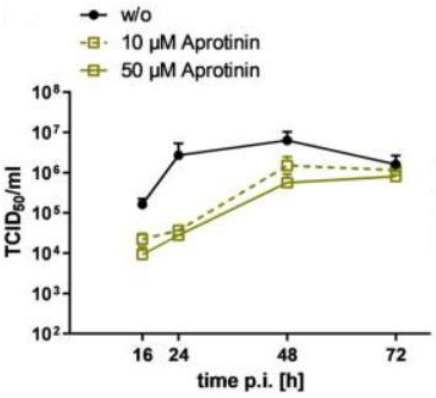

Figure 10. Aprotinin inhibits SARS-CoV-2 entry in a dose dependent matter (43)

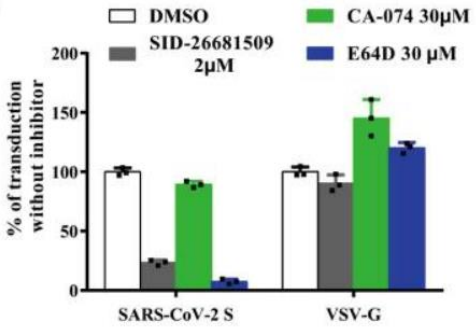

Figure 11. Cathepsin inhibitors E64d and SID-26681509 inhibit SARS-CoV-2 entry (79)

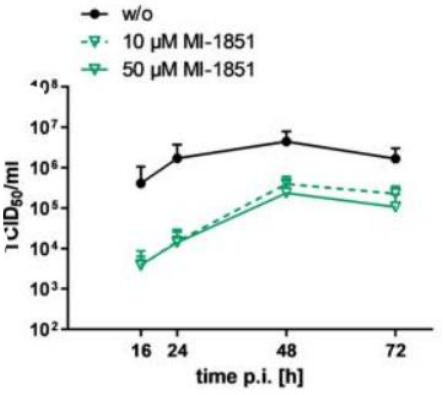

Figure 12. MI-1851 inhibits SARS-CoV-2 entry (43)

Stem cells

As SARS-CoV-2 reduces ACE2 expression, and ACE2 has protective effects, a target for therapy might be to increase ACE2 expression, using gene therapy. Mesenchymal stem cells (MSCs) from the bone marrow can serve as a vehicle for gene therapy. In mice, MSCs alone already show to attenuate lung injury and reduce mortality (82). MSCs overexpressing ACE2 were able to recover ACE2 levels in the lung after injury, decrease angiotensin II levels and increase angiotensin (1-7) levels. Most importantly, MSCs overexpressing ACE2 were able to decrease lung injury and the inflammatory response, and worked better than normal MSCs (83).

A small study with MSCs has been conducted on seven COVID-19 patients. These stem cells did not express ACE2. Injection of MSCs to these patients led to a decrease in pro-inflammatory cytokines and an increase of anti-inflammatory cytokines. Furthermore, it reversed the lymphopenia (low level of lymphocytes in the blood) and CRP levels (84). This information, combined with the information from animal studies discussed above, indicates that MSCs overexpressing ACE2 might be an interesting therapeutic strategy for COVID-19 in humans.

Discussion

In this essay, various options of treatments for COVID-19, targeting ACE2 were discussed. COVID-19 is caused by SARS-CoV-2, a novel coronavirus that is currently causing a pandemic. The virus enters the host cell via ACE2, an enzyme from the RAS. ACE2 converts angiotensin II to angiotensin (1-7), which has protective effects against lung injury, other organ damage, hypertension and inflammation. As ACE2 is the viral entry point and can have protective effects on COVID-19 symptoms, it is an interesting target for treatment.

It can be concluded that targeting ACE2 is a complicated matter, given the dual effect of ACE2. It has detrimental effects by serving as an entry point for SARS-CoV-2, but it also provides protection against tissue damage and inflammation.

When it was found that ACE2 was the cellular entry point for SARS-CoV-2, some studies concluded that downregulating or blocking ACE2 would be protective against COVID-19. This was supported by many studies that found that higher expression levels of ACE2 are present in patients with other diseases, and in men versus women, was correlated with higher disease susceptibility and severity. However, blocking ACE2 will most likely have a counterproductive effect, as the protective properties of will be lost, only worsening the symptoms of COVID-19.

Therefore, it might be a more sensible idea to actually stimulate and enhance ACE2 expression. There are several compounds known to have this effect. All show protection against fibrosis, lung injury, heart damage, hypertension or inflammation, to some extent. However, none of these compounds have any proven effects in humans yet, and little is known about their toxicity. Besides that fact that too little is known about these compounds, it seems only logical that upregulating membrane bound ACE2 will also increase viral entry, making it more infectious.

Alternatively, soluble ACE2 could be a target. sACE2 can still perform its protective effects by converting angiotensin II to angiotensin (1-7), but as it is not membrane bound, it does not facilitate viral entry. On the contrary, recombinant sACE2 can partly inhibit viral entry and thereby reduce the viral load. Treatment with rhACE2 also decreases lung injury.

Inhibiting the priming of SARS-CoV-2 S protein is another interesting therapeutic strategy. It is relatively safe and effective to treat viral infections. For TMPRSS2, there are already many clinically approved inhibitors, and some already show an inhibiting effect on viral entry in vitro. Inhibiting cathepsins and furin also appears to be effective against SARS-CoV-2, but furin might be the least favorable due to its role in development.

Taken together, it appears that treatment with rhACE2 is most favorable at the moment, as it can inhibit viral entry but still help attenuate COVID-19 symptoms. As phase I and II clinical trials with rhACE2 have already been successfully completed, it looks like the best and safest option. Along with rhACE2, inhibiting one or more cellular proteases can help in decreasing SARS-CoV-2 infection and replication.

It is important to put the treatment of COVID-19 in a bigger perspective, and to not only focus on targeting ACE2. Even though it seems like a very promising target, there are many other factors involved in the infection and pathogenesis. Most importantly, the main goal is still to find an antiviral treatment and to develop a vaccine, to directly target the virus. Using ACE2 as a second target, for example by using rhACE2 and protease inhibitors, can be a very effective measure to further prevent infections and to prevent complications caused by the infections.

To conclude, ACE2 can be an important target in treating COVID-19, and especially the use of rhACE2 is very promising. It would probably be most effective when combined with antiviral treatment. A lot of work still remains to be done to find the best possible way to prevent and treat the infection, so this enormous pandemic can be put to a stop.

References

1) *WHO Coronavirus Disease (COVID-19).* (2020). World Health Organization. https://covid19.who.int/

2) Jeffery, M. M., D'Onofrio, G., Paek, H., Platts-Mills, T. F., Soares, W. E., Hoppe, J. A., Genes, N., Nath, B., & Melnick, E. R. (2020). Trends in Emergency Department Visits and Hospital Admissions in Health Care Systems in 5 States in the First Months of the COVID-19 Pandemic in the US. *JAMA Internal Medicine.* Published online.

3) Torales, J., O'Higgins, M., Castaldelli-Maia, J. M., & Ventriglio, A. (2020). The outbreak of COVID-19 coronavirus and its impact on global mental health. *International Journal of Social Psychiatry*, *66*(4), 317–320.

4) Mou, J. (2020). Research on the Impact of COVID19 on Global Economy. *IOP Conference Series: Earth and Environmental Science*, 546, 032043.

5) Ahmad, T., Khan, M., Haroon, Musa, T. H., Nasir, S., Hui, J., Bonilla-Aldana, D. K., & Rodriguez-Morales, A. J. (2020). COVID-19: Zoonotic aspects. *Travel medicine and infectious disease*, 101607. Advance online publication.

6) Malaiyan, J., Arumugam, S., Mohan, K., & Gomathi Radhakrishnan, G. (2020). An update on the origin of SARS-CoV-2: Despite closest identity, bat (RaTG13) and pangolin derived coronaviruses varied in the critical binding site and O-linked glycan residues. *Journal of medical virology*, 10.1002/jmv.26261. Advance online publication.

7) Mackenzie, J. S., & Smith, D. W. (2020). COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don't. *Microbiology Australia*, MA20013. Advance online publication.

8) Lam, T. T., Jia, N., Zhang, Y. W., Shum, M. H., Jiang, J. F., Zhu, H. C., Tong, Y. G., Shi, Y. X., Ni, X. B., Liao, Y. S., Li, W. J., Jiang, B. G., Wei, W., Yuan, T. T., Zheng, K., Cui, X. M., Li, J., Pei, G. Q., Qiang, X., Cheung, W. Y., … Cao, W. C. (2020). Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. *Nature*, *583*(7815), 282–285.

9) Vella, F., Senia, P., Ceccarelli, M., Vitale, E., Maltezou, H., Taibi, R., Lleshi, A., Venanzi Rullo, E., Pellicanò, G. F., Rapisarda, V., Nunnari, G., & Ledda, C. (2020). Transmission mode associated with coronavirus disease 2019: a review. *European review for medical and pharmacological sciences*, *24*(14), 7889–7904.

10) Zhou, J., Li, C., Zhao, G., Chu, H., Wang, D., Yan, H. H., Poon, V. K., Wen, L., Wong, B. H., Zhao, X., Chiu, M. C., Yang, D., Wang, Y., Au-Yeung, R., Chan, I. H., Sun, S., Chan, J. F., To, K. K., Memish, Z. A., Corman, V. M., … Yuen, K. Y. (2017). Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus. *Science advances*, *3*(11), eaao4966.

11) Nikolai, L. A., Meyer, C. G., Kremsner, P. G., & Velavan, T. P. (2020). Asymptomatic SARS Coronavirus 2 infection: Invisible yet invincible. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, S1201-9712(20)30706-2. Advance online publication.

12) Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., Chen, J., … Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet (London, England), 395*(10224), 565–574.

13) Naqvi, A., Fatima, K., Mohammad, T., Fatima, U., Singh, I. K., Singh, A., Atif, S. M., Hariprasad, G., Hasan, G. M., & Hassan, M. I. (2020). Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochimica et biophysica acta. Molecular basis of disease*, *1866*(10), 165878.

14) Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Zhong, W., & Hao, P. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Science China Life Sciences, 63*(3), 457–460.

15) Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, *181*(2), 271–280.e8.

16) J Alsaadi, E. A., & Jones, I. M. (2019). Membrane binding proteins of coronaviruses. *Future virology, 14*(4), 275–286.

17) Romano, M., Ruggiero, A., Squeglia, F., Maga, G., & Berisio, R. (2020). A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. *Cells, 9*(5), 1267.

18) Grant, M. C., Geoghegan, L., Arbyn, M., Mohammed, Z., McGuinness, L., Clarke, E. L., & Wade, R. G. (2020). The prevalence of symptoms in 24,410 adults infected by the novel coronavirus (SARS-CoV-2; COVID-19): A systematic review and meta-analysis of 148 studies from 9 countries. *PLOS ONE*, *15*(6), e0234765.

19) Schmulson, M., Dávalos, M. F., & Berumen, J. (2020). Beware: Gastrointestinal symptoms can be a manifestation of COVID-19. Alerta: los síntomas gastrointestinales podrían ser una manifestación de la COVID-19. *Revista de gastroenterologia de Mexico*, *85*(3), 282–287.

20) Oran, D. P., & Topol, E. J. (2020). Prevalence of Asymptomatic SARS-CoV-2 Infection : A Narrative Review. *Annals of internal medicine*, *173*(5), 362–367.

21) Fan, E., Brodie, D., & Slutsky, A. S. (2018). Acute Respiratory Distress Syndrome. *JAMA*, *319*(7), 698.

22) Zaim, S., Chong, J. H., Sankaranarayanan, V., & Harky, A. (2020). COVID-19 and Multiorgan Response. *Current problems in cardiology*, *45*(8), 100618.

23) Lauer, S. A., Grantz, K. H., Bi, Q., Jones, F. K., Zheng, Q., Meredith, H. R., Azman, A. S., Reich, N. G., & Lessler, J. (2020). The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Annals of Internal Medicine, 172*(9), 577–582.

24) Larsen, J.R., Martin, M.R., Martin, J.D., Kuhn, P. & Hicks, J.B. (2020). Modeling the Onset of Symptoms of COVID-19. *Front. Public Health, 8*, 473.

25) Bonanad, C., García-Blas, S., Tarazona-Santabalbina, F., Sanchis, J., Bertomeu-González, V., Fácila, L., Ariza, A., Núñez, J., & Cordero, A. (2020). The Effect of Age on Mortality in Patients With COVID-19: A Meta-Analysis With 611,583 Subjects. *Journal of the American Medical Directors Association*, *21*(7), 915–918.

26) Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J., Liu, L., Shan, H., Lei, C., Hui, D. S. C., Du, B., Li, L., Zeng, G., Yuen, K.-Y., Chen, R., Tang, C., Wang, T., Chen, P., Xiang, J., … Zhong, N. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. New England Journal of Medicine, 382(18), 1708–1720.

27) Hajifathalian, K., Kumar, S., Newberry, C., Shah, S., Fortune, B., Krisko, T., Ortiz-Pujols, S., Zhou, X. K., Dannenberg, A. J., Kumar, R., & Sharaiha, R. Z. (2020). Obesity is Associated with Worse Outcomes in COVID-19: Analysis of Early Data from New York City. *Obesity (Silver Spring, Md.)*, *28*(9), 1606–1612.

28) Morris, G., Bortolasci, C. C., Puri, B. K., Olive, L., Marx, W., O'Neil, A., Athan, E., Carvalho, A. F., Maes, M., Walder, K., & Berk, M. (2020). The pathophysiology of SARS-CoV-2: A suggested model and therapeutic approach. *Life sciences*, *258*, 118166. Advance online publication.

29) Hu, B., Huang, S., & Yin, L. (2020). The cytokine storm and COVID-19. *Journal of medical virology*, 10.1002/jmv.26232. Advance online publication

30) Vallamkondu, J., John, A., Wani, W. Y., Ramadevi, S. P., Jella, K. K., Reddy, P. H., & Kandimalla, R. (2020). SARS-CoV-2 pathophysiology and assessment of coronaviruses in CNS diseases with a focus on therapeutic targets. *Biochimica et biophysica acta. Molecular basis of disease*, *1866*(10), 165889.

31) Gross, L., Sacerdoti, M., Piiper, A., Zeuzem, S., Leroux, A. E., & Biondi, R. M. (2020). ACE2, the Receptor that Enables Infection by SARS-CoV-2: Biochemistry, Structure, Allostery and Evaluation of the Potential Development of ACE2 Modulators. *ChemMedChem*, 10.1002/cmdc.202000368. Advance online publication.

32) *Gene: ACE2 (ENSG00000130234)*. Ensembl release 89, May 2017.

33) Eriksson, U., Danilczyk, U., & Penninger, J. M. (2002). Just the Beginning: Novel Functions for Angiotensin-Converting Enzymes. *Current Biology*, *12*(21), R745–R752.

34) Lambert, D. W., Yarski, M., Warner, F. J., Thornhill, P., Parkin, E. T., Smith, A. I., Hooper, N. M., & Turner, A. J. (2005). Tumor Necrosis Factor-α Convertase (ADAM17) Mediates Regulated Ectodomain Shedding of the Severe-acute Respiratory Syndrome-Coronavirus (SARS-CoV) Receptor, Angiotensin-converting Enzyme-2 (ACE2). *Journal of Biological Chemistry*, *280*(34), 30113–30119.

35) Fountain, J.H., Lappin, S.L. Physiology, Renin Angiotensin System. [Updated 2020 Jul 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-.

36) Rodrigues Prestes, T. R., Rocha, N. P., Miranda, A. S., Teixeira, A. L., & Simoes-E-Silva, A. C. (2017). The Anti-Inflammatory Potential of ACE2/Angiotensin-(1-7)/Mas Receptor Axis: Evidence from Basic and Clinical Research. *Current drug targets*, *18*(11), 1301–1313.

37) Turner, A. J. (2015). ACE2 Cell Biology, Regulation, and Physiological Functions. *The Protective Arm of the Renin Angiotensin System (RAS)*, 185–189.

38) Kuba, K., Imai, Y., & Penninger, J. M. (2013). Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. *Circulation journal : official journal of the Japanese Circulation Society*, *77*(2), 301–308.

39) Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., Somasundaran, M., Sullivan, J. L., Luzuriaga, K., Greenough, T. C., Choe, H., & Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*, *426*(6965), 450–454.

40) Glowacka, I., Bertram, S., Müller, M. A., Allen, P., Soilleux, E., Pfefferle, S., Steffen, I., Tsegaye, T. S., He, Y., Gnirss, K., Niemeyer, D., Schneider, H., Drosten, C., & Pöhlmann, S. (2011). Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *Journal of virology*, *85*(9), 4122–4134.

41) Simmons, G., Gosalia, D. N., Rennekamp, A. J., Reeves, J. D., Diamond, S. L., & Bates, P. (2005). Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(33), 11876–11881.

42) Hussain, M., Jabeen, N., Amanullah, A., Baig, A. A., Aziz, B., Shabbir, S., & Raza, F. (2020). Structural Basis of SARS-CoV-2 Spike Protein Priming by TMPRSS2. *BioRixv*, Advance online publication.

43) Bestle, D., Heindl, M. R., Limburg, H., Van Lam van, T., Pilgram, O., Moulton, H., Stein, D. A., Hardes, K., Eickmann, M., Dolnik, O., Rohde, C., Klenk, H.-D., Garten, W., Steinmetzer, T., & Böttcher-Friebertshäuser, E. (2020). TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. *Life Science Alliance*, *3*(9), e202000786.

44) Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., & Li, F. (2020). Cell entry mechanisms of SARS-CoV-2. *Proceedings of the National Academy of Sciences*, *117*(21), 11727–11734.

45) Hoffmann, M., Kleine-Weber, H., & Pöhlmann, S. (2020). A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. *Molecular Cell*, *78*(4), 779-784.e5.

46) Shulla, A., Heald-Sargent, T., Subramanya, G., Zhao, J., Perlman, S., & Gallagher, T. (2010). A Transmembrane Serine Protease Is Linked to the Severe Acute Respiratory Syndrome Coronavirus Receptor and Activates Virus Entry. *Journal of Virology*, *85*(2), 873–882.

47) Xiao, L., Sakagami, H., & Miwa, N. (2020). ACE2: The key Molecule for Understanding the Pathophysiology of Severe and Critical Conditions of COVID-19: Demon or Angel? *Viruses*, *12*(5), 491.

48) Bojkova, D., Wagner, J., Shumliakivska, M., Aslan, G. S., Saleem, U., Hansen, A., Luxán, G., Günther, S., Pham, M. D., Krishnan, J., Harter, P. N., Ermel, U. H., Frangakis, A. S., Milting, H., Zeiher, A. M., Klingel, K., Cinatl, J., Dendorfer, A., Eschenhagen, T., Tschöpe, C., … Dimmeler, S. (2020). SARS-CoV-2 infects and induces cytotoxic effects in human cardiomyocytes. *Cardiovascular research*, cvaa267. Advance online publication.

49) Dong, M., Zhang, J., Ma, X., Tan, J., Chen, L., Liu, S., Xin, Y., & Zhuang, L. (2020). ACE2, TMPRSS2 distribution and extrapulmonary organ injury in patients with COVID-19. *Biomedicine & Pharmacotherapy*, *131*, 110678.

50) Sama, I. E., Ravera, A., Santema, B. T., van Goor, H., ter Maaten, J. M., Cleland, J. G. F., Rienstra, M., Friedrich, A. W., Samani, N. J., Ng, L. L., Dickstein, K., Lang, C. C., Filippatos, G., Anker, S. D., Ponikowski, P., Metra, M., van Veldhuisen, D. J., & Voors, A. A. (2020). Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin–angiotensin–aldosterone inhibitors. *European Heart Journal, 41*(19), 1810–1817.

51) Al-Benna S. (2020). Association of high level gene expression of ACE2 in adipose tissue with mortality of COVID-19 infection in obese patients. *Obesity medicine*, *19*, 100283.

52) Li, Y., Xu, Q., Ma, L., Wu, D., Gao, J., Chen, G., & Li, H. (2020). Systematic profiling of ACE2 expression in diverse physiological and pathological conditions for COVID-19/SARS-CoV-2. *Journal of cellular and molecular medicine*, *24*(16), 9478–9482. Advance online publication.

53) Glowacka, I., Bertram, S., Herzog, P., Pfefferle, S., Steffen, I., Muench, M. O., Simmons, G., Hofmann, H., Kuri, T., Weber, F., Eichler, J., Drosten, C., & Pöhlmann, S. (2009). Differential Downregulation of ACE2 by the Spike Proteins of Severe Acute Respiratory Syndrome Coronavirus and Human Coronavirus NL63. *Journal of Virology*, *84*(2), 1198–1205.

54) Devaux, C. A., Rolain, J. M., & Raoult, D. (2020). ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi*, *53*(3), 425–435.

55) Banu, N., Panikar, S. S., Leal, L. R., & Leal, A. R. (2020). Protective role of ACE2 and its downregulation in SARS-CoV-2 infection leading to Macrophage Activation Syndrome: Therapeutic implications. *Life Sciences*, *256*, 117905.

56) Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., Wang, Z., Li, J., Li, J., Feng, C., Zhang, Z., Wang, L., Peng, L., Chen, L., Qin, Y., Zhao, D., Tan, S., Yin, L., Xu, J., … Liu, L. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Science China Life Sciences*, *63*(3), 364–374.

57) Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., Yang, P., Sarao, R., Wada, T., Leong-Poi, H., Crackower, M. A., Fukamizu, A., Hui, C.-C., Hein, L., Uhlig, S., Slutsky, A. S., Jiang, C., & Penninger, J. M. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*, *436*(7047), 112–116.

58) Haga, S., Yamamoto, N., Nakai-Murakami, C., Osawa, Y., Tokunaga, K., Sata, T., Yamamoto, N., Sasazuki, T., & Ishizaka, Y. (2008). Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(22), 7809–7814.

59) Jia, H.P., Look, D.C., Tan, P., et al. (2009). Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. *Am J Physiol Lung Cell Mol Physiol*, *297*(1), L84-L96.

60) Alexandre, J., Cracowski, J.-L., Richard, V., & Bouhanick, B. (2020). Drugs acting on renin angiotensin system and use in ill patients with COVID-19. *Therapies*, *75*(4), 319–325.

61) Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., Oliveira-dos-Santos, A. J., da Costa, J., Zhang, L., Pei, Y., Scholey, J., Ferrario, C. M., Manoukian, A. S., Chappell, M. C., Backx, P. H., Yagil, Y., & Penninger, J. M. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*, *417*(6891), 822–828.

62) Rodríguez-Puertas, R. (2020). ACE2 activators for the treatment of COVID 19 patients. *Journal of Medical Virology*, *92*, 1701–1702.

63) Shenoy, V., Gjymishka, A., Jarajapu, Y. P., Qi, Y., Afzal, A., Rigatto, K., Ferreira, A. J., Fraga-Silva, R. A., Kearns, P., Douglas, J. Y., Agarwal, D., Mubarak, K. K., Bradford, C., Kennedy, W. R., Jun, J. Y., Rathinasabapathy, A., Bruce, E., Gupta, D., Cardounel, A. J., … Raizada, M. K. (2013). Diminazene Attenuates Pulmonary Hypertension and Improves Angiogenic Progenitor Cell Functions in Experimental Models. *American Journal of Respiratory and Critical Care Medicine*, *187*(6), 648–657.

64) Velkoska, E., Patel, S. K., & Burrell, L. M. (2016). Angiotensin converting enzyme 2 and diminazene: role in cardiovascular and blood pressure regulation. *Current opinion in nephrology and hypertension*, *25*(5), 384–395.

65) Hernández Prada, J. A., Ferreira, A. J., Katovich, M. J., Shenoy, V., Qi, Y., Santos, R. A. S., Castellano, R. K., Lampkins, A. J., Gubala, V., Ostrov, D. A., & Raizada, M. K. (2008). Structure-Based Identification of Small-Molecule Angiotensin-Converting Enzyme 2 Activators as Novel Antihypertensive Agents. *Hypertension*, *51*(5), 1312–1317.xant

66) Zhang, X., Zheng, J., Yan, Y., Ruan, Z., Su, Y., Wang, J., Huang, H., Zhang, Y., Wang, W., Gao, J., Chi, Y., Lu, X., & Liu, Z. (2019). Angiotensin-converting enzyme 2 regulates autophagy in acute lung injury through AMPK/mTOR signaling. *Archives of Biochemistry and Biophysics*, *672*, 108061.

67) Yan, D., Li, G., Zhang, Y., & Liu, Y. (2019). Angiotensin-converting enzyme 2 activation suppresses pulmonary vascular remodeling by inducing apoptosis through the Hippo signaling pathway in rats with pulmonary arterial hypertension. *Clinical and experimental hypertension (New York, N.Y. : 1993)*, *41*(6), 589–598.

68) Li, G., Xu, Y. L., Ling, F., Liu, A. J., Wang, D., Wang, Q., & Liu, Y. L. (2012). Angiotensin-converting enzyme 2 activation protects against pulmonary arterial hypertension through improving early endothelial function and mediating cytokines levels. *Chinese medical journal*, *125*(8), 1381–1388.

69) Qiao, W., Wang, C., Chen, B., Zhang, F., Liu, Y., Lu, Q., Guo, H., Yan, C., Sun, H., Hu, G., & Yin, X. (2015). Ibuprofen attenuates cardiac fibrosis in streptozotocin-induced diabetic rats. *Cardiology*, *131*(2), 97–106.

70) Bancos, S., Bernard, M. P., Topham, D. J., & Phipps, R. P. (2009). Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells. *Cellular immunology*, *258*(1), 18–28.

71) Bernard, G.R., Wheeler, A.P., Russell, J.A., et al. (1997). The effects of ibuprofen on the physiology and survival of patients with sepsis. The Ibuprofen in Sepsis Study Group. *N Engl J Med, 336*(13), 912-918.

72) Ishiyama, Y., Gallagher, P. E., Averill, D. B., Tallant, E. A., Brosnihan, K. B., & Ferrario, C. M. (2004). Upregulation of Angiotensin-Converting Enzyme 2 After Myocardial Infarction by Blockade of Angiotensin II Receptors. *Hypertension*, *43*(5), 970–976.

73) Ocaranza, M. P., Godoy, I., Jalil, J. E., Varas, M., Collantes, P., Pinto, M., Roman, M., Ramirez, C., Copaja, M., Diaz-Araya, G., Castro, P., & Lavandero, S. (2006). Enalapril Attenuates Downregulation of Angiotensin-Converting Enzyme 2 in the Late Phase of Ventricular Dysfunction in Myocardial Infarcted Rat. *Hypertension*, *48*(4), 572–578.

74) Walters, T. E., Kalman, J. M., Patel, S. K., Mearns, M., Velkoska, E., & Burrell, L. M. (2016). Angiotensin converting enzyme 2 activity and human atrial fibrillation: increased plasma angiotensin converting enzyme 2 activity is associated with atrial fibrillation and more advanced left atrial structural remodelling. Europace, euw246.

75) Kai, H., & Kai, M. (2020). Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors-lessons from available evidence and insights into COVID-19. *Hypertension Research : Official Journal of the Japanese Society of Hypertension*, *43*(7), 648-654.

76) Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R. A., Stahl, M., Leopoldi, A., Garreta, E., Hurtado del Pozo, C., Prosper, F., Romero, J. P., Wirnsberger, G., Zhang, H., Slutsky, A. S., Conder, R., Montserrat, N., Mirazimi, A., & Penninger, J. M. (2020). Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. *Cell*, *181*(4), 905-913.e7.

77) Khan, A., Benthin, C., Zeno, B., Albertson, T. E., Boyd, J., Christie, J. D., Hall, R., Poirier, G., Ronco, J. J., Tidswell, M., Hardes, K., Powley, W. M., Wright, T. J., Siederer, S. K., Fairman, D. A., Lipson, D. A., Bayliffe, A. I., & Lazaar, A. L. (2017). A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Critical Care*, *21*(1)

78) European Biotechnology. (2020, 16 July). *Targeting coronaviruses*. BIOCOM AG. https://european-biotechnology.com/covid-19/covid-19-detail/targeting-coronaviruses.html

79) Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., Xiang, Z., Mu, Z., Chen, X., Chen, J., Hu, K., Jin, Q., Wang, J., & Qian, Z. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nature Communications*, *11*(1), 1620.

80) Shema Mugisha, C., Vuong, H. R., Puray-Chavez, M., Bailey, A. L., Fox, J. M., Chen, R. E., Wessel, A. W., Scott, J. M., Harastani, H. H., Boon, A., Shin, H., & Kutluay, S. B. (2020). A Simplified Quantitative Real-Time PCR Assay for Monitoring SARS-CoV-2 Growth in Cell Culture. *mSphere*, *5*(5), e00658-20.

81) Roebroek, A.J.M., Umans, L., Pauli, I.G.L, Robertson, E.J., Van Leuven, F., Van De Ven, W.J.M, Constam, D.B. (1998). Failure of ventral closure and axial rotation in embryos lacking the proprotein convertase Furin. *Development, 125*(24), 4863-4876.

82) Gupta, N., Su, X., Popov, B., Lee, J.W., Serikov, V., Matthay, M.A. (2007). Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J. Immunol., 179*(3), 1855-1863.

83) He, H., Liu, L., Chen, Q., Liu, A., Cai, S., Yang, Y., Lu, X., & Qiu, H. (2015). Mesenchymal Stem Cells Overexpressing Angiotensin-Converting Enzyme 2 Rescue Lipopolysaccharide-Induced Lung Injury. *Cell transplantation*, *24*(9), 1699–1715.

84) Leng, Z., Zhu, R., Hou, W., Feng, Y., Yang, Y., Han, Q., Shan, G., Meng, F., Du, D., Wang, S., & Fan, J. (2020). Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging and Disease, 11*(2), 216–228.