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From Adversity to Serendipity

Perspectives of global relevance based on research, experience and successes in combating COVID-19 in Sri Lanka

Volume 01

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Development of novel therapeutics for COVID-19

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ABSTRACT

Angiotensin converting enzyme 2 (ACE2) is the driving force of the protective arm of the renin angiotensin system (RAS). ACE2 plays an unequivocal role in counter-balancing the deleterious effects of the classical RAS comprising ACE. A protective role for ACE2 in antagonising tissue injury and fibrosis has been well characterised in mouse models of cardiac, pulmonary and liver diseases. Despite its highly protective role in disease pathogenesis including in lung epithelial injury, ACE2 has been hijacked by severe acute respiratory syndrome-coronavirus (SARS-CoV) and SARS-CoV-2 to gain entry to alveolar epithelial cells, causing severe respiratory disease in humans. Often SARS-CoV-2 infection causing COVID-19 becomes life-threatening in elderly or people with other medical conditions due to highly virulent and contagious nature of SARS-CoV-2 compared with SARS-CoV. Given an unprecedented number of COVID-19 patients that have been affected globally, there is an urgent need to discover therapeutics targeting the interaction between the receptor binding domain (RBD) of the viral spike protein and the receptor, ACE2. Development and/or identification of already existing drugs used for other medical conditions to treat COVID-19 patients is vital at this stage since long-term efficacy of the vaccines that have been developed for COVID-19 are not yet known. Thus, this paper discusses about the role of ACE2 in recognising and binding of SARS-CoV-2 and provides novel therapeutic interventional strategies to prevent SARS-CoV-2 infection.

Key words: COVID-19, SARS-CoV-2, ACE2, Viraemia, Lung infection, Novel therapeutics, Spike protein

1. INTRODUCTION

Emerging in December in 2019, in Wuhan, China, the novel coronavirus 'severe acute respiratory syndrome-coronavirus-2' (SARS-CoV-2), has rapidly spread across 191 countries with over 108 million positive cases and 2 million and 399,793 deaths by 15th February 2021 (Johns Hopkins CR Centre, 2020). This novel disease was recognized as a pandemic by the World Health Organization

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(WHO), and continues to cause an enormous global health, social and economic impacts. Coronavirus is common and it makes up to 30% of common colds (Mesel-Lemoine et al., 2012). However, this is the third member of the coronavirus family to have caused a pandemic since the turn of this century (Pitlik, 2020). The first coronavirus that crossed this barrier and infected humans in the year 2002 was SARS-CoV which was identified in Guangdong province of Southern China, spreading across 26 countries with more than 8000 infections and 774 deaths (WHO, 2004). The second coronavirus, MERS-CoV (Middle East Respiratory Syndrome Coronavirus), which emerged in 2012 in Saudi Arabia has spread across 27 countries with 2494 infections and 858 deaths (WHO, 2019). It is thought that all these coronaviruses are closely related and came from a common origin in bats (Andersen et al., 2020). However, in contrast with the two other members of the family to have crossed the species barrier and caused a pandemic, SARS-CoV-2, which causes coronavirus disease-2019 (COVID-19), is highly virulent and contagious (Zhu et al., 2020). Despite its devastating global impact on human health and socioeconomic endpoints, a cure for this highly lethal virus has not been established. Therefore, there is a major need to develop novel therapeutics for the prevention and treatment of COVID-19.

2. PATHOGENESIS OF COVID-19

2.1. SARS-CoV-2

SARS-CoV-2 is a single-stranded RNA virus containing ~30 kb genome that consists of up to 14 open reading frames (ORFs) flanked by 5` and 3` untranslated regions (UTRs) (F. Wu et al., 2020). The vital structural proteins of the viral envelope are mainly composed of highly glycosylated spike (S) protein, membrane/matrix protein (M) and envelope protein (E) which are embedded in a lipid bilayer (Wu et al., 2020). Whilst these structural proteins together with nucleocapsid (N) protein are important for virus assembly during viral replication, the subunit proteins of the S protein which exit as a trimeric prefusion state play the leading role in receptor recognition, binding and entry into host cell. Thus, receptor binding domain (RBD) of the subunit 1 (S1) protein binds to the receptor, ACE2, leading to destabilization of the prefusion trimer which results in the cleavage of S1 subunit by host proteases including transmembrane protease serine 2 (TMPRSS2) (Hoffmann et al., 2020). This exposes the S2 subunit containing a fusion peptide sequence which undergoes a conformational change to acquire a stable fusion-able state, leading to host cell membrane fusion and viral entry (Walls et al., 2017; Wrapp et al., 2020).

2.2. SARS-COV-2 cellular receptors

It has been reported that there are multiple receptors involved in recognising SARS-CoV-2. This includes cell membrane proteins angiotensin converting enzyme 2 (ACE2), basigin (CD147) and neurophilin-1. It is possible that basigin may serve as either a co-receptor or it may be of importance in COVID-19 disease pathogenesis through virus dependent functional activation of associated pathways (Faghihi, 2020; Hamming et al., 2004; Helal et al., 2020; Liu et al., 2020). On the other hand, neurophilin-1 which has been shown to bind SARS-CoV-2 in the neuronal system may be important in neuron-specific infection by the virus (Daly et al., 2020). However, in comparison with basigin and neurophilin-1, there is strong evidence to suggest that ACE2 is the leading candidate molecule that directly binds to the SARS-CoV-2 (Li et al., 2003; Turner et al., 2004).

Angiotensin converting enzyme 2 which was discovered in the year 2000 (Donoghue et al., 2000; Tipnis et al., 2000) is a crucial enzyme in the protective arm of the renin angiotensin system (RAS). The RAS is a well characterised essential hormone system with pivotal roles in vascular biology, blood pressure regulation, the nervous system, electrolyte homeostasis, tissue injury, neoplasia and lipid homeostasis (Afsar et al., 2020; Grace et al., 2012; Herath et al., 2007; Putnam et al., 2012). It is now well accepted that the protective RAS has been evolved to counter-regulate the deleterious effects of the classical RAS which consists of the profibrotic and vasoconstrictor peptide angiotensin II working through its G protein-

coupled receptor (GPCR) angiotensin II type 1 receptor (AT1R) (Figure 1). Whilst the effects of the classical RAS are elicited via the activation of the AT1R, ACE2 of the protective RAS elicits its effects by activating distinct GPCRs, Mas receptor and Mas-related G protein-coupled receptor type D (MrgD) (Gunarathne et al., 2019; Paz Ocaranza et al., 2020; Santos et al., 2003; Tetzner et al., 2016).

The effects of the renin angiotensin system (RAS) are determined by the balance between its classical arm and the protective counter-regulatory arm. Classical arm consists angiotensin converting enzyme (ACE), angiotensin II, angiotensin II type 1 receptor (AT1R), which mediate vasoconstriction, cell proliferation and proinflammatory and profibrogenic pathways. The protective counter-regulatory arm consists angiotensin converting enzyme 2 (ACE2), angiotensin (1-7) and the Mas receptor, directly opposing the deleterious effects of the classical RAS.

Over decades of widespread interest in the RAS has resulted in the development and availability of effective pharmacotherapies which target hypertension, cardiovascular and liver disease (Gheblawi et al., 2020; Grace et al., 2012; Mak et al., 2015; Rajapaksha et al., 2019).

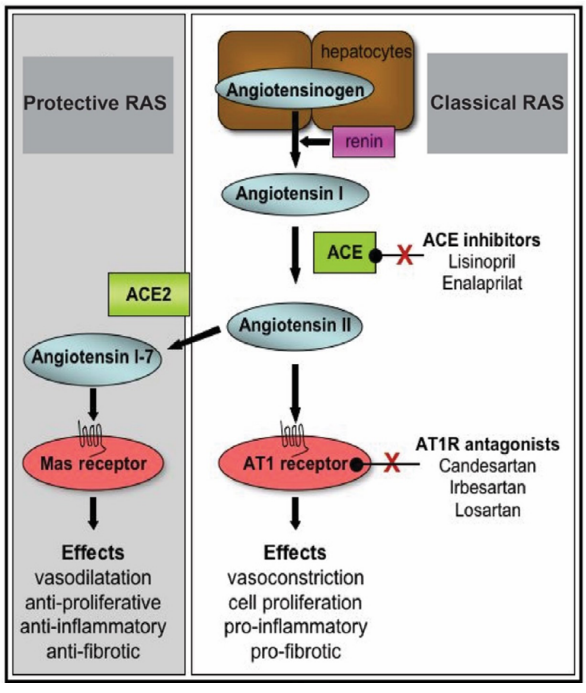

Figure 1. Overview of the renin angiotensin system

Therapeutics that act on the RAS including the angiotensin converting enzyme inhibitors and angiotensin receptor blockers (ARBs) are commonly used in current medial practice. Additional interest in the RAS has been driven by a recognition of the essential roles of this hormone system in tissue injury including pathologies as diverse as the remodelling of cardiac tissue after myocardial infarction, the development of the vasculature in malignancy and progression of tissue fibrosis such as liver fibrosis which can result in cirrhosis (Gheblawi et al., 2020; Warner et al., 2007). With discovery of ACE2, it is now recognised that this enzyme determines the balance between the classical and protective RAS (Santos et al., 2018; Warner et al., 2007). Nevertheless, liver-specific over-expression of ACE2 in mice with liver fibrosis has proved to be highly effective in antagonising liver injury and fibrosis progression and has been suggested to be a potential therapy for liver disease (Mak et al., 2015; Rajapaksha et al., 2019). However, ACE2 has received a great deal of interest as it is the viral entry receptor for the coronaviruses that cause severe acute respiratory syndrome (SARS) (Li et al., 2003; Turner et al., 2004). Thus, despite its highly protective role in disease pathogenesis, ACE2 by serving as a cellular receptor for highly infectious and lethal SARS-CoV-2 contributes to COVID-19, a severe respiratory disease in humans (Hoffmann et al., 2020).

2.3. ACE2 binding of SARS-COV-2

Since viral S protein of both SARS-CoV-2 and SARS-CoV dictates the host cell infection by binding to cell surface receptor, ACE2, which is expressed at relatively high level in lung alveolar epithelial cells (Hamming et al., 2004), the amino acid sequence of the S protein is key to develop drugs and vaccine. Whilst the S subunits from both SARS-CoV-2 and SARS-CoV share a high degree of structural similarity, the binding affinity of the S1 subunit of SARS-CoV-2 to ACE2 is ~6- to 22-fold higher compared to that of SARS-CoV S1 subunit (Lan et al., 2020; Wrapp et al., 2020). The difference between the affinities of the two related viruses may be explained by the observation that although the N-terminal

amino acid residues (SARS-CoV-$2^{331-429}$/ SARS-$CoV^{318-416}$) of the RBD is relatively well conserved, the C-terminal amino acid residues (SARS-CoV-$2^{430-527}$ / SARS-$CoV^{417-513}$) containing the residues of the receptor binding motif (RBM) that interact with ACE2 is more variable (Jaimes et al., 2020). In support of the sequence differences at the C-terminal residues of the RBD of S1 subunit that likely influences the binding affinities, atomic details of binding interface studies provide evidence that natural substitution of key amino acid residues of SARS-CoV-2 RBM led to higher affinity for ACE2 binding with relatively higher Van der Waals bonds than SARS-CoV (Wang et al., 2020). A stronger interaction between ACE2 and the RBM of SARS-CoV-2 as compared with SARS-CoV is further reflected by findings of structure-guided sequence alignment studies which showed that glutamine residue at 493 (Gln493) of the SARS-CoV-2 RBM not only interacts with 3 amino acid residues of ACE2 (Lys31, His34 and Glu35) which are considered as virus binding hotspots (Li, 2008; Wu et al., 2012) but it also forms a hydrogen bond with Glu35 whereas the comparable residue of the SARS-CoV (Asn479) interacts only with His34 of ACE2 (Lan et al., 2020). This concept was further strengthened by studies in which the crystal structure of the RBD of SARS-CoV-2 in complex with ACE2 showed the formation of a hydrogen bond not only between Gln493 of SARS-CoV-2 RBM and Glu35 of ACE2 but also with Lys31 of ACE2 (Shang et al., 2020). A detailed characterization of the structure of the interface between the S1 RBM of SARS-CoV-2

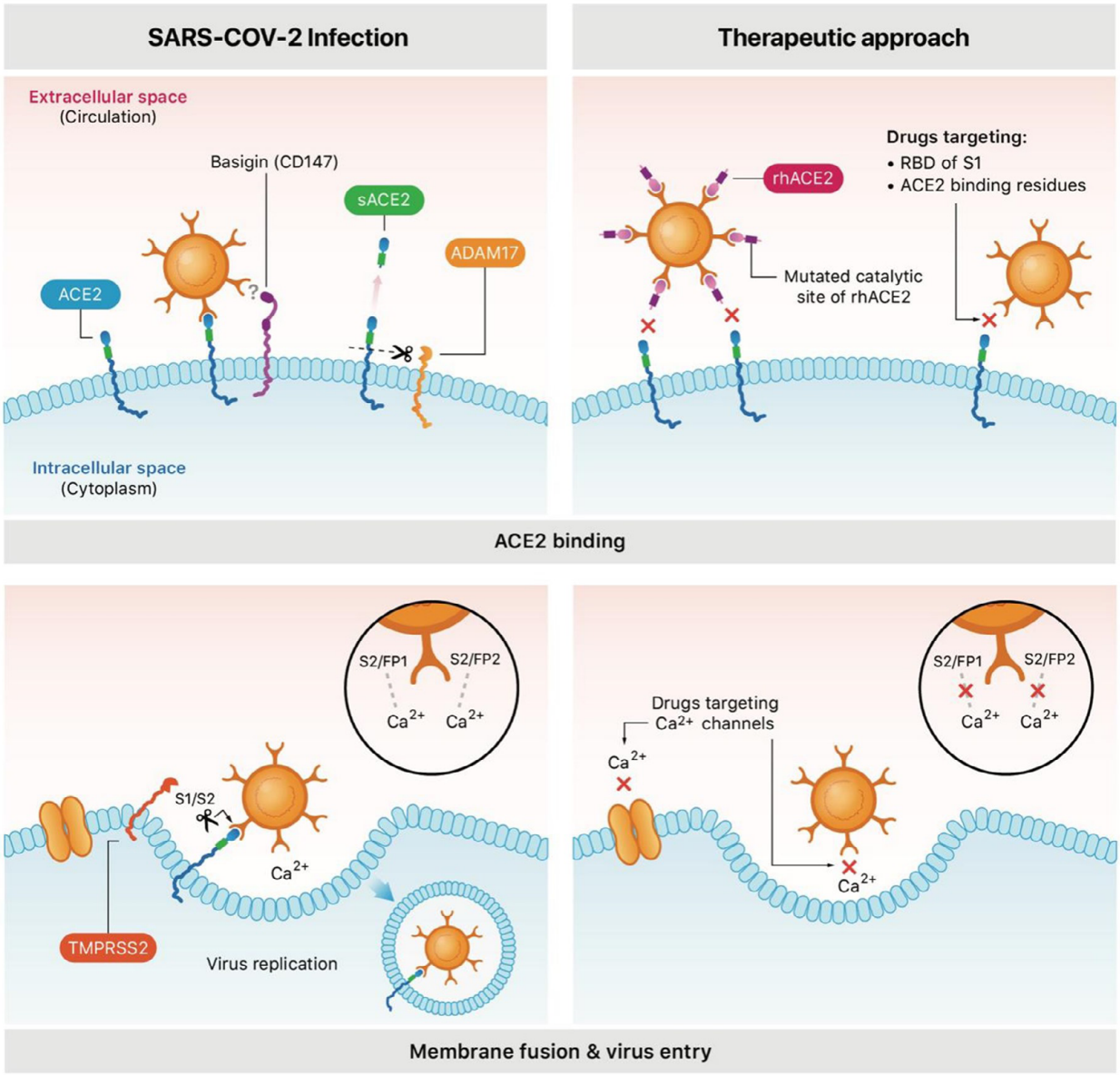

Figure 2. Role of ACE2 in SARS-CoV-2 infection and therapies targeting receptor binding and membrane fusion

and ACE2 residues provides numerous avenues to develop promising therapeutic strategies which may include *insilico*-screening of small molecule drugs targeting the interface between the virus and the receptor.

Left: Spike (S) protein of SARS-CoV-2 (S1 subunit) binds to cellular ACE2 receptor (top), followed by host cell membrane fusion using fusion peptide (FP) of S2 subunit (bottom), leading to endocytosis of the virus and loss of cell surface ACE2. Right: Treatment with recombinant human angiotensin converting enzyme 2 (rhACE2) is expected to mop up SARS-CoV-2 in the circulation by competing with cellular ACE2 for binding to S1 protein (top). Drugs that can be used to target the interface between the ACE2 and the receptor binding domain (RBD) of S1 protein to prevent viral entry to host cell (top). Ion channel inhibitors can be adopted to deplete intracellular and extracellular Ca2+ concentration to prevent the host cell membrane fusion of FP of S2 protein (bottom). Abbreviations: sACE2, cellular ACE2 is shed into the circulation by the activity of metalloproteinase ADAM17; TMPRSS2, transmembrane protease serine 2 cleaves S1 subunit, leaving S2 subunit for host cell membrane fusion.

2.4. Host-cell membrane fusion of SARS-COV-2

Whilst the observed differences between the SARS-CoV and SARS-CoV-2 in binding to ACE2 receptor is relatively well characterized, host cell membrane fusion of S2 subunit shows some striking similarities between the SARS-CoV and SARS-CoV-2 (Millet & Whittaker, 2018; Straus et al., 2020). Proteolytic cleavages at the S1/S2 subunits and S2' site upstream of fusion peptide domains by host proteases such as TMPRSS2 expose two fusion peptide sequences immediately downstream of S2` site, allowing the insertion of the fusion sequences into the host cell membrane (Cannalire et al., 2020; Gierer et al., 2013; Hoffmann et al., 2020; Shirato et al., 2013).

In this membrane fusion process, it has been proposed that Ca^{2+} ions play a dominant role in the ordering of FP domains of most enveloped viruses, thus facilitating the merge of FP domains with host cell membrane (Dube et al., 2014; Lai et al., 2017; Millet & Whittaker, 2018; Nathan et al., 2020). As in host cell membrane fusion of Rubella virus, a strong Ca^{2+} dependency of SARS-CoV FPs has been suggested to be prerequisite events for membrane-ordering effects of the two FP domains with lipid bilayer (Dube et al., 2016; Dube et al., 2014; Lai et al., 2017; Millet & Whittaker, 2018). The requirement for two Ca^{2+} ions to make salt bridges by binding to conserved negatively charged hydrophobic residues in the FPs such as aspartic and glutamic acid residues, has been suggested to promote greater membrane ordering and host cell membrane fusion (Millet & Whittaker, 2018; Nathan et al., 2020). Straus and colleagues in their studies using mutant FP residues and electron spin resonance spectroscopy demonstrated that negatively charged E891 (Glu891) of MERS-CoV FP domain 1 is a critical residue for Ca^{2+} binding, membrane ordering and fusion (Nathan et al., 2020). They further provided evidence using pseudo viral particles decorated with MERS-CoV S subunit protein that the infectivity of Huh-7 cells by pseudo particles was attenuated by depletion of intracellular or extracellular Ca^{2+} concentration whereas intracellular Ca^{2+} depletion completely abrogates SARS-CoV infectivity (Lai et al., 2017). This difference in the ability of membrane fusion and infectivity of the two viruses is supported by isothermal titration calorimetry studies, which in agreement with previous reports (Lai et al., 2017), suggested that unlike MERS-CoV FP which requires one Ca^{2+} ion for membrane ordering, SARS-CoV binds two Ca^{2+} ions, forming a salt bridge with each of the two FP domains (Lai et al., 2017; Nathan et al., 2020). These findings reinforce the importance of targeting Ca^{2+} channels for the design and development of new drugs or repurposing drugs currently in clinical practice to treat patients with COVID-19. Nevertheless, these drugs help prevent the virus from attacking other vital organs in those patients who are in early stages of disease progression.

3. NOVEL THERAPEUTICS FOR COVID-19

3.1. Patients with COVID-19

Among diverse physiological roles of ACE2 in many tissues and vascular beds, the protective role of ACE2 against lung injury is paramount since this multifunctional protein lies in the interface between exterior air and alveolar cells (Imai et al., 2005; Samavati & Uhal, 2020). Intriguingly, despite its protective role in the lung, ACE2 has been hijacked by SARS-CoV and SARS-CoV-2 to gain entry to alveolar epithelia cells, causing severe respiratory disease in humans (Hoffmann et al., 2020; Kuba et al., 2005; Lan et al., 2020; Li et al., 2005; Li et al., 2003). What is remarkable in this process is that not only the virus gain entry into alveolar epithelial cells, but at the same, it destroys the protective machinery driven by ACE2, thus causing dual negative impact in the lungs.

It is likely that the driving force in many gravely ill patients' secondary complications following the appearance of respiratory symptoms is a disastrous overreaction of the immune system known as a 'cytokine storm' where immune cells start to attack healthy tissues (Moore & June, 2020). This phenomenon may lead to blood vessels leak, dropping blood pressure, clot formation, possibly leading to catastrophic multiorgan failure such as stroke, heart and kidney failure (Huang et al., 2020; Klok et al., 2020; Shi et al., 2020; Zou et al., 2020). Whist the lung is the primary battle zone disrupting healthy oxygen transfer with subsequent damage to lung vasculature triggered by cytokine storm, it is highly likely that lung alveolar epithelial cells release a large fraction of the virus into the circulation, leading to viral-mediated direct effects on other major organs such as the kidneys, heart, brain and intestines (Farcas et al., 2005). Indeed, autopsy of dead patients showed viral inclusion bodies and particles in the kidneys, and inflammatory cells and apoptotic bodies in the heart and small intestine, implying that the virus could directly invade other organs (Farkash et al., 2020; Lamers et al., 2020; Puelles et al., 2020; Varga et al., 2020; Zou et al., 2020). This is not surprising given that along with vascular endothelium across the body, these are the organs that highly express ACE2 receptor in both humans and rodents and thus potentially vulnerable to infection (Gembardt et al., 2005; Hamming et al., 2004; Zou et al., 2020). Moreover, the possibility that the virus can directly infect nerve cells, particularly neurons in the medulla oblongata of the brain stem that controls the functioning of the heart and the lungs, and the reported loss of sense of smell and taste in COVID-19 patients, suggests neurological damage and rapid deterioration of patients' condition (Iadecola et al., 2020; Lechien et al., 2020; Liu et al., 2020; Zou et al., 2020).

The strong body of evidence suggests that a significant percentage of COVID-19 patients are transferred to the intensive care unit (ICU) around median day 8 (day 5 to 14) from first admission to the hospital (Huang et al., 2020; Xu et al., 2020) and the median duration from admission to the ICU to death is 7 days (Yang et al., 2020). It could be argued that the latency period between hospitalisation and ICU admission may be enough for the virus to enter and destroy other vital organs including the kidney, heart, liver, gut and neuronal system. Therefore, it appears that secondary invasion of the virus targeting other major organs may be expected to cause catastrophic multiorgan failure (Klok et al., 2020; Shi et al., 2020; Zou et al., 2020).

3.2. COVID-19 in Sri Lanka

On the other hand, a close look at global spread of the SARS-CoV-2 reveals an interesting phenomenon (JHCR Centre, 2020). As of 15th February 2021, India and USA are countries that ranked in the top with ~8% and 8.3% positive cases relative to its population compared to the UK (~6%). Despite its high prevalence in India, death rate of positive cases is low (~1.4%) compared to the USA (1.8%) and the UK (2.9%). What is phenomenal is however that in Sri Lanka positive cases (~0.4%) and death rate (~0.5%) are surprisingly low. This raises an interesting question as to how infection and death rate are reduced in

Sri Lanka? Likely explanations are that in Asian population (1) genetic make-up that triggers an effective surveillance on pathogens including SARS-CoV-2 appears superior; (2) the frequency of occurrence of mutated SARS-CoV-2 variants is low compared to Europe and USA where the variants can escape immune surveillance, resulting in increased number of positive cases and mortality rate, (3) BCG vaccination that triggers a trained immunity may provide a protection against non-specific pathogens such as SARS-CoV-2, and (4) high temperatures and humidity may restrict viral infection.

The genetic background in Asian population may be considered as a possible factor that determines a strong immune surveillance to reduce the lethality of the SARS-CoV-2 infection. Although the infectivity or the spread of the virus in Asian population and in particular in Sri Lanka is considerable, but relatively low compared to the Western world, what matters is the pathogenicity of such infection which determines the severity of illness and mortality in infected patients which is comparatively very low in Sri Lanka. Another possible factor for a low lethality of the virus may be the absence of the occurrence of mutated SARS-CoV-2 variants in Sri Lanka and this includes newer variants found in the UK, Italy and Brazil (Caccuri et al., 2020; Kirby, 2021; Paiva et al., 2020; Tang et al., 2020). Whilst it is yet to be confirmed, it is also possible that BCG vaccination which is performed in most Asian countries including Sri Lanka for tuberculosis may provide some protection against non-specific pathogens such as SARS-CoV-2 (Covian et al., 2020). This phenomenon is known as 'trained immunity' which is defined as an enhanced non-specific immune response to a secondary infection mediated by trained innate immune cells such as monocytes, macrophages and natural killer cells which is characterised as being independent of T and B cell responses (Kleinnijenhuis et al., 2015; Netea et al., 2011).

3.3. Therapeutic strategies for COVID-19

It is imperative that the development of an effective vaccine is key to combat COVID-19. There are many institutions and pharmaceutical companies that are racing to develop and deploy safe and effective vaccines for this highly contagious disease. At present, there are more than 50 COVID-19 vaccine trials around the world. Of which, Pfizer which uses nucleoside modified mRNA and AstraZeneca (Oxford) which uses a recombinant replication defective chimpanzee adenovirus expressing the SARS-CoV2 S surface glycoprotein were the first to make effective vaccines. These vaccines have proved to be effective in clinical trials and several countries have already begun the vaccination. However, it will require a close follow-up in vaccinated individuals for an extended period of time to re-evaluate the long-term effectiveness and safety of the particular vaccine. The crucial question however is that how long a specified vaccine will provide an effective immune protection. This is not surprising as newer variants of the SARS-CoV-2 are being discovered and it is unknown whether these mutated variants can escape the immune surveillance and antibodies produced following vaccination. However, a mutation(s) in the genomic region of the SARS-CoV-2 that contains non-structural genes responsible for virus replication and assembly within host cells such as those occurring in the ORF1ab region may not be expected to confer an advantage for the virus to escape antibodies in vaccinated individuals (Rouchka et al., 2020; Sun, 2020). However, if a mutation(s) occurs in the genomic region that encodes for amino acid residues of the S protein could pose a threat as the current vaccines are being developed to target viral S proteins. This raises a question of whether individuals may require seasonal vaccination against COVID-19 as new variants are found from time to time, a much more similar scenario to that of annual FLU vaccination program. The development of a universal vaccine against COVID-19, which could potentially change the face of a possible seasonal pandemic COVID-19 prevention, is a huge technical challenge. In the backdrop of this scenario as well as future inevitable

coronavirus pandemics due to the recurrent spill over of the virus into humans from their reservoir in bats, it is therefore paramount importance of developing novel therapeutics to treat COVID-19 patients.

Despite many clinical trials undertaken in COVID-19 patients worldwide, an effective treatment for this highly contagious disease is yet to be identified. In addition, there is no accepted *in vitro* model or animal model to screen approved drugs that could be repurposed to treat COVID-19 patients. In our search for novel drugs for COVID-19, it is imperative that potential drug candidates need to be selected by screening large databases of small molecules which will be expected to undergo a rigorous *in vitro* and/or *in vivo* testing for their effectiveness against SARS-CoV-2 infection. The *in vitro* model adopted by Monteil and colleagues used native SARS-CoV-2 on Vero-E6 cells, and organoids derived from human embryonic stem cells (kidney organoid) and induced pluripotent stem cells (capillary organoid) to investigate the effectiveness of recombinant proteins on infectivity of SARS-CoV-2 (Monteil et al., 2020). Whilst this model provides an ideal flatform to investigate the therapeutic potential of drug candidates, the use of native SARS-CoV-2 is apparently not possible in many laboratory settings. Therefore, a simple and robust in vitro-based platform utilising SARS-CoV-2 viral like particles (VLPs) will enable fast, reliable and rapid screening of existing as well as potential new therapeutics in a physical containment 2 (PC2) laboratory setting (Xu et al., 2020). In addition, the recurrent spill over of coronaviruses into humans from their reservoir in bats strongly suggests that future zoonotic transmission events are inevitable and supports the need for ongoing and robust methods of antiviral drug screening and development.

Repurposed drugs or novel drug candidates can be tested to block entry of SARS-CoV-2 to host cells, particularly in those patients who are in the early stage of disease progression such as those patients who only show respiratory illness. It is now clear that therapies targeting the SARS-CoV-2 infection can be implemented at three levels. This includes an early stage of prevention of the virus binding to cellular ACE2 receptor and host cell membrane fusion, and late stage of virus replication within the cell. However, as discussed above, strategies that target the prevention of ACE2 binding and host cell membrane fusion is of paramount importance since these approaches essentially eliminate the viral invasion of vital organs other than the lungs in infected patients. In fact, a combined treatment targeting both receptor binding and membrane fusion is expected to block a higher proportion of the virus from entering target cells. Combined treatments have been successfully adopted for viral diseases; for example, the hepatitis C virus is now being treated using a combined treatment with two antiviral drugs directed at viral replication (Falade-Nwulia et al., 2017).

3.4. Impact of loss of cellular ACE2

Whilst circulating RAS plays a pivotal role in blood pressure regulation and fluid homeostasis in normal physiology (Ferrario et al., 2005; Wu et al., 2018), it is now well recognized that the local tissue RAS plays a predominant role in disease pathogenesis in many organs including liver disease (Crackower et al., 2002; Mak et al., 2015; Rajapaksha et al., 2019; Yang & Xu, 2017). It has been argued that loss of cellular ACE2 due to the endocytosis associated with SARS-CoV-2 infection poses a great risk to patients with COVID-19 as the loss of ACE2 is expected to shift the balance between the two arms of the RAS towards the classical RAS, exacerbating the condition in which Ang II-driven cytokine release can be enhanced (Grace et al., 2012; Mak et al., 2015; Rajapaksha et al., 2019). Moreover, the loss of ACE2 also expected have adverse effects on RAS-independent functions of ACE2 such as the regulation of neutral amino acid transport in the gut epithelial cells (Hashimoto et al., 2012). The possible impact of tissue ACE2 loses in patients with COVID-19 including the impact on those COVID-19 patients who have secondary complications such as type 2 diabetes, heart and kidney diseases, and gut

dysbiosis has been reviewed recently (Gheblawi et al., 2020).

3.5. Recombinant ACE2

The first approach to inhibit the interaction between the host cell membrane ACE2 and the S1-RBM of SARS-CoV-2 can be accomplished by utilising recombinant human soluble ACE2 (rhsACE2). Thus, the approach adopted by Monteil and colleagues using parenterally administered rhsACE2 which competes with SARS-CoV-2 for binding to cellular ACE2 (Figure 2) has a therapeutic potential in patients with COVID-19 (Monteil et al., 2020). Direct translatability of rhsACE2 is highlighted by the finding that this form of ACE2 administered intravenously to healthy volunteers produced no adverse effects and well tolerated (Haschke et al., 2013) Supporting this, work from our laboratory reported that up to 6000-fold over-expression of cellular ACE2 in the liver of healthy mice had no adverse effect (Mak et al., 2015). Although rhsACE2 has been reported to reduce the cellular uptake of the SARS-CoV-2 by up to 5000 folds (Monteil et al., 2020), this has not been tested in *in vivo* model due to a lack of an appropriate *in vivo* model.

Approaches that utilise exogenously administered rhsACE2 may compromise blood pressure since circulating rhsACE2 is expected to breakdown potent vasoconstrictor peptide Ang II, likely causing systemic hypotension and renal failure (Ferreira & Raizada, 2008). The effect on blood pressure can be further impacted by the production of vasodilator peptide, Ang-(1-7), the product of Ang II breakdown (Grace et al., 2012; Mak et al., 2015; Rajapaksha et al., 2019). This potential problem on blood pressure can be circumvented by introducing mutations into the catalytic domain of rhsACE2 (Figure 2) (Guy et al., 2005; Turner et al., 2002). The canonical function of ACE2 is to function as a carboxypeptidase (Guy et al., 2003). On the basis of the ability of ACE2 to cleave biological peptides, a consensus sequence of Pro-X-Pro-hydrophobic has been derived as the target for the ACE2 catalytic activity (Guy et al., 2003). Furthermore, this catalytic activity has been narrowed down to a number of residues located within the catalytic domain of ACE2 (Guy et al., 2005). Particularly, Arg273 has been identified as a critical residue for substrate binding (Guy et al., 2005). Site-directed mutagenesis to replace the arginine with a glutamine residue (R273Q) that represents a positive to neutral change in the side chain while maintaining most of the hydrophobic surface area has demonstrated that the loss of positive charge on the side chain at residue 273 has a profound effect on the substrate binding (Guy et al., 2005). Furthermore, mutations on H345A/L and H505A/L also results in enzyme activity being dramatically reduced. Further investigations have revealed that His345 as the hydrogen bond donor/acceptor during the formation of the tetrahedral peptide intermediate (Guy et al., 2005). Thus, these residues of ACE2 are highly critical for the catalytic activity. Therefore, if the choice is to use rhsACE2 as a therapy in COVID-19 patients, it is important that these critical residues will need to be mutated to make rhsACE2 catalytically inactive to prevent an increased breakdown of circulating Ang II and subsequent effect on blood pressure (Ferreira & Raizada, 2008). Catalytically inactive rhsACE2 could compete with the host ACE2 for binding to the S1-RBM of SARS-CoV-2, thus preventing or reducing the 'effective viral load' in the circulation that is available for host receptor engagement. Because rhsACE2 administration should be performed as an intravenous treatment, this therapy could only be applicable to those patients who are currently admitted to a hospital. Furthermore, since the treatment is a short-term treatment, the risk of developing anti-rhsACE2 antibodies is also minimum.

4. TARGETING ACE2 BINDING INTERFACE

Coronaviruses have been known to use membrane bound ACE2 as a point of entry to the host cell (Bourgonje et al., 2020). The arch shaped α-1 helix on the ACE2 peptidase domain (PD) mainly interacts with the RBM of the S1 protein while α-2 helix and the β3-4 loop of the ACE2 make limited contacts (Huang et al., 2020). The crystal structure of this

interaction (Huang et al., 2020) provides an excellent opportunity to inhibit the viral entry by inhibiting the ACE2-S1 interaction. Binding of the S1 RBM on ACE2 is mainly hinged on the arch shaped α-1 helix of the ACE2 (Huang et al., 2020). Therefore, the surface of the RBM interacts over a significant surface area on the ACE2. This provides two options for the inhibition of SARS-CoV-2 binding to the ACE2; (a) designing small molecule inhibitors that bind to the S1-RBM and inhibit the interaction with the ACE2 (Benítez-Cardoza & Vique-Sánchez, 2020; Hanson et al., 2020), and (b) use the ACE2 residues that interact with the S1-RBM as the target for the drug binding and inhibit the interaction with the SARS-CoV-2 (Figure 3).

The SARS-CoV-2 S1 receptor binding domain (RBD) interaction with the ACE2 is shown as a ribbon diagram using the crystal structure PDB:6m17 (Yan et al., 2020). The right panel shows the residues on ACE2 (Q24(Glu), H34(His), Y41(Tyr), Q42(Glu), M82(Met), K353(Lys), R357(Arg)) interacting with the residues on S1-RBM (K417(Lys), Y453(Tyr), Q474(Glu), F486(Phe), Q498(Glu), T500(Thr), N501(Asp)) (Yan et al., 2020).

Whilst repurposing the approved drugs provides fairly rapid solutions, *insilico* drug screening with molecular dynamics simulations could also be employed to screen and identify novel drug candidates that can be used to inhibit the receptor binding and/or the host cell membrane fusion of not only SARS-CoV-2 (Huang et al., 2020) but also of coronaviruses of future pandemics. Based on the co-crystal structure of the RBD and the ACE2, the S1-RBD forms a pocket around the residues R403(Arg), D405(Asp), Q409(Glu), K417(Lys), I418(Ile), L455(Leu), Y453(Tyr), Y495(Tyr), F497(Phe), N501(Asp) and Y505(Tyr). This pocket is about 17Å long and about 9Å in width and lined with hydrophobic residues. This could be utilised as a drug binding pocket and screen for molecules that bind to this region at a high affinity (Figure 3). Binding a molecule to this pocket could significantly reduce the interactions between the S1-RBD and the ACE2 resulting in lowering the affinity between the virus and the receptor.

Similarly, the same approach can be taken to screen for molecules binding to the ACE2 surface and prevent the RBD interaction with the host receptor. However, the RBD interacting surface of ACE2 does not appear to possess any druggable pockets. Therefore, screening for small molecules that could bind to the ACE2 surface to inhibit the interaction with RBD could be far-fetched. However, if

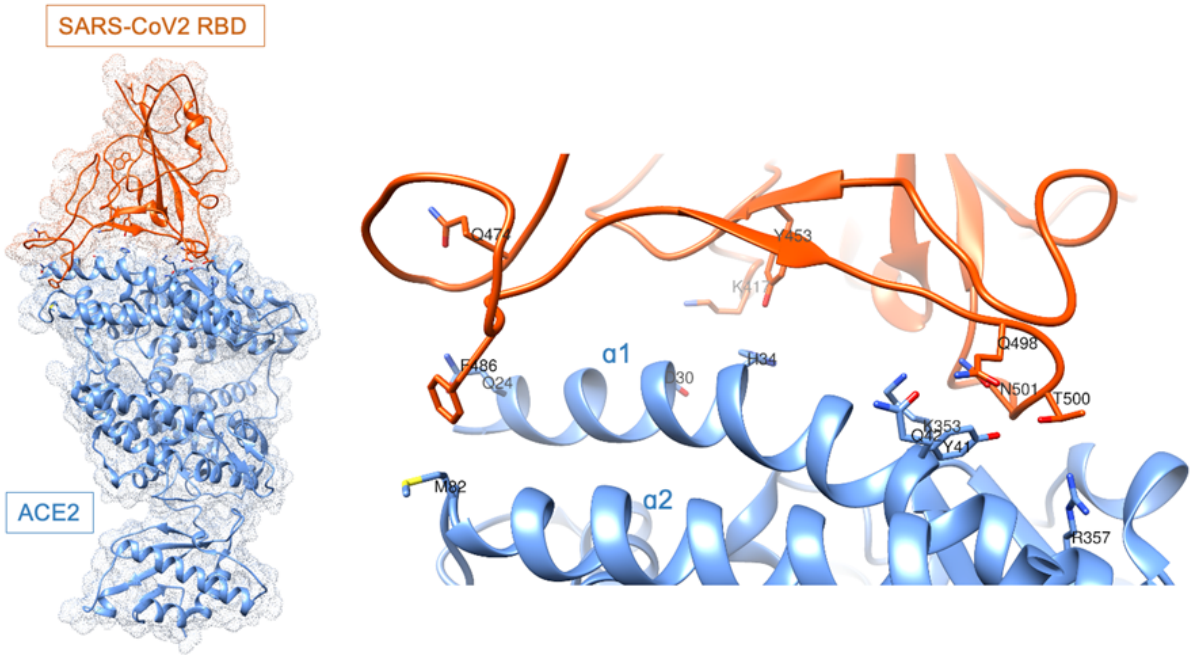

Figure 3. ACE2 interaction with the RBD of the SARS-CoV-2 spike protein

a peptide can be derived from ACE2 RBD interacting surface, this peptide could be used to inhibit the interaction between the ACE2 and S1-RBD. In situations where the protein surfaces do not facilitate small molecule inhibitors, peptide-based inhibitors have become an emerging technique. A recent review has provided a comprehensive review of peptide inhibitors and their application and draw backs (Wójcik & Berlicki, 2016). Given the ACE2 surface characteristics, developing a peptide inhibitor to mimic the RBD binding surface on ACE2 could be equally valuable as developing small molecule inhibitors that bind to the S1-RBD. Furthermore, a similar result could be obtained by using a peptide that binds to the RBD and interfere with the ACE2 binding. One of the disadvantages of using peptide-based inhibitors is their susceptibility to host proteases.

4.1. Targeting host-cell membrane fusion

As described above, Ca^{2+} has been shown to play an important role in the process of fusing SARS-CoV-2 with the host membrane. Viral membrane fusion is the process by which the virus envelop merge with the host cell membrane to deliver the viral genetic materials. SARS-CoV-2 virus membrane fusion occurs after ACE2 binding and once both viral membrane and the host membrane are proximal to each other. During the process of membrane fusion Ca^{2+} ions are believed to be used by the fusion peptides for orienting themselves on the host cell membrane. This provides a unique opportunity for inhibiting the process of virus genetic material delivery to the host cell by inhibiting the peptide orienting process. If a small molecule can be used to compete with the residues that binds Ca^{2+}, it would be possible to inhibit the peptide orientation. However, given the small size of the binding site it would be unwise to take such approach. Therefore, as an alternative if the amount of available Ca^{2+} for the virus can be depleted one should be able to achieve a similar effect.

Calcium transporters located on the cell surface act as regulators of the amount of Ca^{2+} available in the extracellular space. Therefore, if an inhibitor could be used to inhibit Ca^{2+} channels then the amount of Ca^{2+} available can be effectively regulated. Consequently, this would provide a control over the membrane fusion step. Currently there are number of Ca^{2+} channel blockers available that are used to treat patients with hypertension and heart arrhythmia. Thus, it would probably be the right time to revisit those drugs and repurpose them as a treatment for SARS-CoV-2 (Figure 2, Figure 3).

Whilst repurposing the approved drugs such as ion channel inhibitors provides fairly rapid solutions as in the identification of drug candidates to inhibit receptor binding, insilico drug screening can be effectively employed to identify such drugs that have the potential to inhibit host cell membrane fusion of the virus by depleting extracellular Ca^{2+} concentration. A number of open source and proprietary molecular docking software packages are available to be used in the process but Autodock (Morris et al., 2008) and Autodock Vina (Trott & Olson, 2010) has shown to be the most popular with most of the drug screening campaigns. This software is relying on the availability of docking ready compound databases. One of the most commonly used such database is the ZINC database (Irwin & Shoichet, 2005) that contains over 230 million purchasable small molecule compounds in ready-to-dock, 3D formats. Docking algorithms can provide an estimation of the binding affinity and based on the calculated value, top hits can be selected, and further validation could be performed with molecular dynamics simulations. Finally, those promising compounds can be tested in *in vitro* and *in vivo* studies.

5. CONCUSIONS

Since its discovery two decades ago, ACE2 has been the central focus of a large number of studies that investigated the protective role of this protein in many pathological conditions including cardiovascular, renal, lung and liver disease. Intriguingly, two of the coronavirus pandemics of this century, caused by SARS-CoV and SARS-CoV-2, has hijacked ACE2 to

gain entry into alveolar epithelial cells of the lungs, causing a devastating respiratory illness in humans. However, together with findings from early studies conducted since SARS-CoV pandemic in 2003, massive amount of data that generated in response to SARS-CoV-2 in 2020 made it possible to develop vaccines targeting the infection of SARS-CoV-2. Importantly, this knowledge can now be used to develop novel therapeutics that prevent either virus entry or its cellular replication machinery. This effort of developing drugs to target virus entry and/or replication is important for treating COVID-19 patients until such time a universal vaccine that is safe and effective becomes available for this disease and similar coronavirus pandemics in the future. However, it is likely that because of the technical challenges for developing a universal vaccine, the likelihood of ongoing SARS-CoV-2 pandemic with seasonal variants of the virus may require annual vaccination for COVID-19 and thus, there is a need for novel therapeutics to treat COVID-19 patients.

REFERENCES

Afsar, B., Afsar, R. E., Ertuglu, L. A., Kuwabara, M., Ortiz, A., Covic, A., & Kanbay, M. (2020). Renin-angiotensin system and cancer: Epidemiology, cell signaling, genetics and epigenetics. *Clin Transl Oncol*. doi:10.1007/s12094-020-02488-3.

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., & Garry, R. F. (2020). The proximal origin of sars-cov-2. *Nat Med, 26*(4), 450-452. doi:10.1038/s41591-020-0820-9.

Benítez-Cardoza, C. G., & Vique-Sánchez, J. L. (2020). Potential inhibitors of the interaction between ace2 and sars-cov-2 (rbd), to develop a drug. *Life Sci, 256*, 117970. doi:10.1016/j.lfs.2020.117970.

Bourgonje, A. R., Abdulle, A. E., Timens, W., Hillebrands, J. L., Navis, G. J., Gordijn, S. J., . . . van Goor, H. (2020). Angiotensin-converting enzyme 2 (ace2), sars-cov-2 and the pathophysiology of coronavirus disease 2019 (covid-19). *J Pathol, 251*(3), 228-248. doi:10.1002/path.5471.

Caccuri, F., Zani, A., Messali, S., Giovanetti, M., Bugatti, A., Campisi, G., . . . Caruso, A. (2020). A persistently replicating sars-cov-2 variant derived from an asymptomatic individual. *J Transl Med, 18*(1), 362. doi:10.1186/s12967-020-02535-1.

Cannalire, R., Stefanelli, I., Cerchia, C., Beccari, A. R., Pelliccia, S., & Summa, V. (2020). Sars-cov-2 entry inhibitors: Small molecules and peptides targeting virus or host cells. *Int J Mol Sci, 21*(16). doi:10.3390/ijms21165707.

Covian, C., Retamal-Diaz, A., Bueno, S. M., & Kalergis, A. M. (2020). Could bcg vaccination induce protective trained immunity for sars-cov-2? *Front Immunol, 11*, 970. doi:10.3389/fimmu.2020.00970.

Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., . . . Penninger, J. M. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature, 417*(6891), 822-828.

Daly, J. L., Simonetti, B., Klein, K., Chen, K. E., Williamson, M. K., Anton-Plagaro, C., . . . Yamauchi, Y. (2020). Neuropilin-1 is a host factor for sars-cov-2 infection. *Science, 370*(6518), 861-865. doi:10.1126/science.abd3072.

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., . . . Acton, S. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ace2) converts angiotensin i to angiotensin 1-9. *Circ Res, 87*(5), E1-9.

Dube, M., Etienne, L., Fels, M., & Kielian, M. (2016). Calcium-dependent rubella virus fusion occurs in early endosomes. *J Virol, 90*(14), 6303-6313. doi:10.1128/JVI.00634-16.

Dube, M., Rey, F. A., & Kielian, M. (2014). Rubella virus: First calcium-requiring viral fusion protein. *PLoS Pathog, 10*(12), e1004530. doi:10.1371/journal.ppat.1004530.

Faghihi, H. (2020). Cd147 as an alternative binding site for the spike protein on the surface of sars-cov-2. *Eur Rev Med Pharmacol Sci, 24*(23), 11992-11994. doi:10.26355/eurrev_202012_23985.

Falade-Nwulia, O., Suarez-Cuervo, C., Nelson, D. R., Fried, M. W., Segal, J. B., & Sulkowski, M. S. (2017). Oral direct-acting agent therapy for hepatitis c virus infection: A systematic review. *Ann Intern Med, 166*(9), 637-648. doi:10.7326/M16-2575.

Farcas, G. A., Poutanen, S. M., Mazzulli, T., Willey, B. M., Butany, J., Asa, S. L., . . . Kain, K. C. (2005). Fatal severe acute respiratory syndrome is associated with multiorgan involvement by coronavirus. *J Infect Dis, 191*(2), 193-197. doi:10.1086/426870.

Farkash, E. A., Wilson, A. M., & Jentzen, J. M. (2020). Ultrastructural evidence for direct renal infection with sars-cov-2. *J Am Soc Nephrol, 31*(8), 1683-1687. doi:10.1681/ASN.2020040432.

Ferrario, C. M., Trask, A. J., & Jessup, J. A. (2005). Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1-7) in regulation of cardiovascular function. *Am J Physiol Heart Circ Physiol, 289*(6), H2281-2290.

Ferreira, A. J., & Raizada, M. K. (2008). Are we poised to target ace2 for the next generation of antihypertensives? *J Mol Med (Berl), 86*(6), 685-690. doi:10.1007/s00109-008-0339-x.

Gembardt, F., Sterner-Kock, A., Imboden, H., Spalteholz, M., Reibitz, F., Schultheiss, H. P., . . . Walther, T. (2005). Organ-specific distribution of ace2 mrna and correlating peptidase activity in rodents. *Peptides, 26*(7), 1270-1277.

Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J. C., Turner, A. J., . . . Oudit, G. Y. (2020). Angiotensin-converting enzyme 2: Sars-cov-2 receptor and regulator of the renin-angiotensin system: Celebrating the 20th anniversary of the discovery of ace2. *Circ Res, 126*(10), 1456-1474. doi:10.1161/CIRCRESAHA.120.317015.

Gierer, S., Bertram, S., Kaup, F., Wrensch, F., Heurich, A., Kramer-Kuhl, A., . . . Pohlmann, S. (2013). The spike protein of the emerging betacoronavirus emc uses a novel coronavirus receptor for entry, can be activated by tmprss2, and is targeted by neutralizing antibodies. *J Virol, 87*(10), 5502-5511. doi:10.1128/JVI.00128-13.

Grace, J. A., Herath, C. B., Mak, K. Y., Burrell, L. M., & Angus, P. W. (2012). Update on new aspects of the renin-angiotensin system in liver disease: Clinical implications and new therapeutic options. *Clin Sci (Lond), 123*(4), 225-239. doi:10.1042/CS20120030.

Gunarathne, L. S., Angus, P. W., & Herath, C. B. (2019). Blockade of mas receptor or mas-related g-protein coupled receptor type d reduces portal pressure in cirrhotic but not in non-cirrhotic portal hypertensive rats. *Front Physiol, 10*, 1169. doi:10.3389/fphys.2019.01169.

Guy, J. L., Jackson, R. M., Acharya, K. R., Sturrock, E. D., Hooper, N. M., & Turner, A. J. (2003). Angiotensin-converting enzyme-2 (ace2): Comparative modeling of the active site, specificity requirements, and chloride dependence. *Biochemistry, 42*(45), 13185-13192. doi:10.1021/bi035268s.

Guy, J. L., Jackson, R. M., Jensen, H. A., Hooper, N. M., & Turner, A. J. (2005). Identification of critical active-site residues in angiotensin-converting enzyme-2 (ace2) by site-directed mutagenesis. *Febs j, 272*(14), 3512-3520. doi:10.1111/j.1742-4658.2005.04756.x.

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., & van Goor, H. (2004). Tissue distribution of ace2 protein, the functional receptor for sars coronavirus. A first step in understanding sars pathogenesis. *J Pathol, 203*(2), 631-637. doi:10.1002/path.1570.

Hanson, Q. M., Wilson, K. M., Shen, M., Itkin, Z., Eastman, R. T., Shinn, P., & Hall, M. D. (2020). Targeting ace2-rbd interaction as a platform for covid19 therapeutics: Development and drug repurposing screen of an alphalisa proximity assay. *bioRxiv*. doi:10.1101/2020.06.16.154708.

Haschke, M., Schuster, M., Poglitsch, M., Loibner, H., Salzberg, M., Bruggisser, M., . . . Krahenbuhl, S. (2013). Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. *Clin Pharmacokinet, 52*(9), 783-792. doi:10.1007/s40262-013-0072-7.

Hashimoto, T., Perlot, T., Rehman, A., Trichereau, J., Ishiguro, H., Paolino, M., . . . Penninger, J. M. (2012). Ace2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature, 487*(7408), 477-481. doi:10.1038/nature11228.

Helal, M. A., Shouman, S., Abdelwaly, A., Elmehrath, A. O., Essawy, M., Sayed, S. M., . . . El-Badri, N. (2020). Molecular basis of the potential interaction of sars-cov-2 spike protein to cd147 in covid-19 associated-lymphopenia. *J Biomol Struct Dyn*, 1-11. doi:10.1080/07391102.2020.1822208.

Herath, C. B., Warner, F. J., Lubel, J. S., Dean, R. G., Jia, Z., Lew, R. A., . . . Angus, P. W. (2007). Upregulation of hepatic angiotensin-converting enzyme 2 (ace2) and angiotensin-(1-7) levels in experimental biliary fibrosis. *J Hepatol, 47*(3), 387-395. doi:10.1016/j.jhep.2007.03.008.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., . . . Pohlmann, 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 e278. doi:10.1016/j.cell.2020.02.052.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., . . . Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in wuhan, china. *Lancet, 395*(10223), 497-506. doi:10.1016/S0140-6736(20)30183-5.

Huang, Y., Yang, C., Xu, X. F., Xu, W., & Liu, S. W. (2020). Structural and functional properties of sars-cov-2 spike protein: Potential antivirus drug development for covid-19. *Acta Pharmacol Sin, 41*(9), 1141-1149. doi:10.1038/s41401-020-0485-4.

Iadecola, C., Anrather, J., & Kamel, H. (2020). Effects of covid-19 on the nervous system. *Cell*. doi:10.1016/j.cell.2020.08.028.

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., . . . Penninger, J. M. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature, 436*(7047), 112-116. doi:10.1038/nature03712.

Irwin, J. J., & Shoichet, B. K. (2005). Zinc--a free database of commercially available compounds for virtual screening. *J Chem Inf Model, 45*(1), 177-182. doi:10.1021/ci049714+.

Jaimes, J. A., Andre, N. M., Chappie, J. S., Millet, J. K., & Whittaker, G. R. (2020). Phylogenetic analysis and structural modeling of sars-cov-2 spike protein reveals an evolutionary distinct and proteolytically sensitive activation loop. *J Mol Biol, 432*(10), 3309-3325. doi:10.1016/j.jmb.2020.04.009.

Johns Hopkins Coronavirus Resource Centre COVID-19 Dashboard (2020)

Kirby, T. (2021). New variant of sars-cov-2 in uk causes surge of covid-19. *Lancet Respir Med*. doi:10.1016/S2213-2600(21)00005-9.

Kleinnijenhuis, J., van Crevel, R., & Netea, M. G. (2015). Trained immunity: Consequences for the heterologous effects of bcg vaccination. *Trans R Soc Trop Med Hyg, 109*(1), 29-35. doi:10.1093/trstmh/tru168.

Klok, F. A., Kruip, M., van der Meer, N. J. M., Arbous, M. S., Gommers, D., Kant, K. M., . . . Endeman, H. (2020). Confirmation of the high cumulative incidence of thrombotic complications in critically ill icu patients with covid-19: An updated analysis. *Thromb Res, 191*, 148-150. doi:10.1016/j.thromres.2020.04.041.

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., . . . Penninger, J. M. (2005). A crucial role of angiotensin converting enzyme 2 (ace2) in sars coronavirus-induced lung injury. *Nat Med, 11*(8), 875-879. doi:10.1038/nm1267.

Lai, A. L., Millet, J. K., Daniel, S., Freed, J. H., & Whittaker, G. R. (2017). The sars-cov fusion peptide forms an extended bipartite fusion platform that perturbs membrane order in a calcium-dependent manner. *J Mol Biol, 429*(24), 3875-3892. doi:10.1016/j.jmb.2017.10.017.

Lamers, M. M., Beumer, J., van der Vaart, J., Knoops, K., Puschhof, J., Breugem, T. I., . . . Clevers, H. (2020). Sars-cov-2 productively infects human gut enterocytes. *Science, 369*(6499), 50-54. doi:10.1126/science.abc1669.

Lan, J. H., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., . . . Wang, X. (2020). Structure of the sars-cov-2 spike receptor-binding domain bound to the ace2 receptor. *Nature, 581*, 215-220.

Lechien, J. R., Hopkins, C., & Saussez, S. (2020). Sniffing out the evidence; it's now time for public health bodies recognize the link between covid-19 and smell and taste disturbance. *Rhinology, 58*(4), 402-403. doi:10.4193/Rhin20.159.

Li, F. (2008). Structural analysis of major species barriers between humans and palm civets for severe acute respiratory syndrome coronavirus infections. *J Virol, 82*(14), 6984-6991. doi:10.1128/JVI.00442-08.

Li, F., Li, W., Farzan, M., & Harrison, S. C. (2005). Structure of sars coronavirus spike receptor-binding domain complexed with receptor. *Science, 309*(5742), 1864-1868. doi:10.1126/science.1116480.

Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., . . . Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the sars coronavirus. *Nature, 426*(6965), 450-454. doi:10.1038/nature02145.

Liu, C., von Brunn, A., & Zhu, D. (2020). Cyclophilin a and cd147: Novel therapeutic targets for the treatment of covid-19. *Med Drug Discov, 7*, 100056. doi:10.1016/j.medidd.2020.100056.

Liu, F., Long, X., Zhang, B., Zhang, W., Chen, X., & Zhang, Z. (2020). Ace2 expression in pancreas may cause pancreatic damage after sars-cov-2 infection. *Clin Gastroenterol Hepatol, 18*(9), 2128-2130 e2122. doi:10.1016/j.cgh.2020.04.040.

Mak, K. Y., Chin, R., Cunningham, S. C., Habib, M. R., Torresi, J., Sharland, A. F., . . . Herath, C. B. (2015). Ace2 therapy using adeno-associated viral vector inhibits liver fibrosis in mice. *Mol Ther, 23*(9), 1434-1443. doi:10.1038/mt.2015.92.

Mesel-Lemoine, M., Millet, J., Vidalain, P. O., Law, H., Vabret, A., Lorin, V., . . . Tangy, F. (2012). A human coronavirus responsible for the common cold massively kills dendritic cells but not monocytes. *J Virol, 86*(14), 7577-7587. doi:10.1128/JVI.00269-12.

Millet, J. K., & Whittaker, G. R. (2018). Physiological and molecular triggers for sars-cov membrane fusion and entry into host cells. *Virology, 517*, 3-8. doi:10.1016/j.virol.2017.12.015.

Monteil, V., Kwon, H., Prado, P., Hagelkruys, A., Wimmer, R. A., Stahl, M., . . . 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 e907. doi:10.1016/j.cell.2020.04.004.

Moore, J. B., & June, C. H. (2020). Cytokine release syndrome in severe covid-19. *Science,*

368(6490), 473-474. doi:10.1126/science.abb8925.

Morris, G. M., Huey, R., & Olson, A. J. (2008). Using autodock for ligand-receptor docking. *Curr Protoc Bioinformatics, Chapter 8*, Unit 8.14. doi:10.1002/0471250953.bi0814s24.

Nathan, L., Lai, A. L., Millet, J. K., Straus, M. R., Freed, J. H., Whittaker, G. R., & Daniel, S. (2020). Calcium ions directly interact with the ebola virus fusion peptide to promote structure-function changes that enhance infection. *ACS Infect Dis, 6*(2), 250-260. doi:10.1021/acsinfecdis.9b00296.

Netea, M. G., Quintin, J., & van der Meer, J. W. (2011). Trained immunity: A memory for innate host defense. *Cell Host Microbe, 9*(5), 355-361. doi:10.1016/j.chom.2011.04.006.

Paiva, M. H. S., Guedes, D. R. D., Docena, C., Bezerra, M. F., Dezordi, F. Z., Machado, L. C., . . . Wallau, G. L. (2020). Multiple introductions followed by ongoing community spread of sars-cov-2 at one of the largest metropolitan areas of northeast brazil. *Viruses, 12*(12). doi:10.3390/v12121414.

Paz Ocaranza, M., Riquelme, J. A., Garcia, L., Jalil, J. E., Chiong, M., Santos, R. A. S., & Lavandero, S. (2020). Counter-regulatory renin-angiotensin system in cardiovascular disease. *Nat Rev Cardiol, 17*(2), 116-129. doi:10.1038/s41569-019-0244-8.

Pitlik, S. D. (2020). Covid-19 compared to other pandemic diseases. *Rambam Maimonides Med J, 11*(3). doi:10.5041/RMMJ.10418.

Puelles, V. G., Lutgehetmann, M., Lindenmeyer, M. T., Sperhake, J. P., Wong, M. N., Allweiss, L., . . . Huber, T. B. (2020). Multiorgan and renal tropism of sars-cov-2. *N Engl J Med, 383*(6), 590-592. doi:10.1056/NEJMc2011400.

Putnam, K., Shoemaker, R., Yiannikouris, F., & Cassis, L. A. (2012). The renin-angiotensin system: A target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome. *Am J Physiol Heart Circ Physiol, 302*(6), H1219-1230. doi:10.1152/ajpheart.00796.2011.

Rajapaksha, I. G., Gunarathne, L. S., Asadi, K., Cunningham, S. C., Sharland, A., Alexander, I. E., . . . Herath, C. B. (2019). Liver-targeted angiotensin converting enzyme 2 therapy inhibits chronic biliary fibrosis in multiple drug-resistant gene 2-knockout mice. *Hepatol Commun, 3*(12), 1656-1673. doi:10.1002/hep4.1434.

Rouchka, E. C., Chariker, J. H., & Chung, D. (2020). Variant analysis of 1,040 sars-cov-2 genomes. *PLoS One, 15*(11), e0241535. doi:10.1371/journal.pone.0241535.

Samavati, L., & Uhal, B. D. (2020). Ace2, much more than just a receptor for sars-cov-2. *Front Cell Infect Microbiol, 10*, 317. doi:10.3389/fcimb.2020.00317.

Santos, R. A., Simoes e Silva, A. C., Maric, C., Silva, D. M., Machado, R. P., de Buhr, I., . . . Walther, T. (2003). Angiotensin-(1-7) is an endogenous ligand for the g protein-coupled receptor mas. *Proc Natl Acad Sci U S A, 100*(14), 8258-8263.

Santos, R. A. S., Sampaio, W. O., Alzamora, A. C., Motta-Santos, D., Alenina, N., Bader, M., & Campagnole-Santos, M. J. (2018). The ace2/angiotensin-(1-7)/mas axis of the renin-angiotensin system: Focus on angiotensin-(1-7). *Physiol Rev, 98*(1), 505-553. doi:10.1152/physrev.00023.2016.

Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., . . . Li, F. (2020). Structural basis of receptor recognition by sars-cov-2. *Nature, 581*(7807), 221-224. doi:10.1038/s41586-020-2179-y.

Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., . . . Huang, C. (2020). Association of cardiac injury with mortality in hospitalized patients with covid-19 in wuhan, china. *JAMA Cardiol, 5*(7), 802-810. doi:10.1001/jamacardio.2020.0950.

Shirato, K., Kawase, M., & Matsuyama, S. (2013). Middle east respiratory syndrome coronavirus infection mediated by the transmembrane serine protease tmprss2. *J Virol, 87*(23), 12552-12561. doi:10.1128/JVI.01890-13.

Straus, M. R., Tang, T., Lai, A. L., Flegel, A., Bidon, M., Freed, J. H., . . . Whittaker, G. R. (2020). Ca(2+) ions promote fusion of middle east respiratory syndrome coronavirus with host cells and increase infectivity. *J Virol, 94*(13). doi:10.1128/JVI.00426-20.

Sun, W. (2020). The discovery of gene mutations making sars-cov-2 well adapted for humans: Host-genome similarity analysis of 2594 genomes from china, the USA and europe. *bioRxiv*, doi: https://doi.org/10.1101/2020.1109.1103.280727.

Tang, J. W., Tambyah, P. A., & Hui, D. S. (2020). Emergence of a new sars-cov-2 variant in the uk. *J Infect*. doi:10.1016/j.jinf.2020.12.024.

Tetzner, A., Gebolys, K., Meinert, C., Klein, S., Uhlich, A., Trebicka, J., . . . Walther, T. (2016). G-protein-coupled receptor mrgd is a receptor for angiotensin-(1-7) involving adenylyl cyclase, camp, and phosphokinase a. *Hypertension, 68*(1), 185-194. doi:10.1161/HYPERTENSIONAHA.116.07572.

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., & Turner, A. J. (2000). A human

homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem, 275*(43), 33238-33243.

Trott, O., & Olson, A. J. (2010). Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem, 31*(2), 455-461. doi:10.1002/jcc.21334.

Turner, A. J., Hiscox, J. A., & Hooper, N. M. (2004). Ace2: From vasopeptidase to sars virus receptor. *Trends Pharmacol Sci, 25*(6), 291-294. doi:10.1016/j.tips.2004.04.001.

Turner, A. J., Tipnis, S. R., Guy, J. L., Rice, G., & Hooper, N. M. (2002). Aceh/ace2 is a novel mammalian metallocarboxypeptidase and a homologue of angiotensin-converting enzyme insensitive to ace inhibitors. *Can J Physiol Pharmacol, 80*(4), 346-353. doi:10.1139/y02-021.

updates, W. o. Coronavirus disease 2019 - who.Int.

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., . . . Moch, H. (2020). Endothelial cell infection and endotheliitis in covid-19. *Lancet, 395*(10234), 1417-1418. doi:10.1016/S0140-6736(20)30937-5.

Walls, A. C., Tortorici, M. A., Snijder, J., Xiong, X., Bosch, B. J., Rey, F. A., & Veesler, D. (2017). Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion. *Proc Natl Acad Sci U S A, 114*(42), 11157-11162. doi:10.1073/pnas.1708727114.

Wang, K., Gheblawi, M., & Oudit, G. Y. (2020). Angiotensin converting enzyme 2: A double-edged sword. *Circulation*. doi:10.1161/CIRCULATIONAHA.120.047049.

Warner, F. J., Lubel, J. S., McCaughan, G. W., & Angus, P. W. (2007). Liver fibrosis: A balance of aces? *Clin Sci (Lond), 113*(3), 109-118.

Wójcik, P., & Berlicki, Ł. (2016). Peptide-based inhibitors of protein-protein interactions. *Bioorg Med Chem Lett, 26*(3), 707-713. doi:10.1016/j.bmcl.2015.12.084.

World Health Organization (2004). Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003.

World Health Organization. (2019). Middle east respiratory syndrome coronavirus (Mers-Cov).

Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., . . . McLellan, J. S. (2020). Cryo-em structure of the 2019-ncov spike in the prefusion conformation. *Science, 367*(6483), 1260-1263. doi:10.1126/science.abb2507.

Wu, C. H., Mohammadmoradi, S., Chen, J. Z., Sawada, H., Daugherty, A., & Lu, H. S. (2018). Renin-angiotensin system and cardiovascular functions. *Arterioscler Thromb Vasc Biol, 38*(7), e108-e116. doi:10.1161/ATVBAHA.118.311282.

Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., . . . Zhang, Y. Z. (2020). A new coronavirus associated with human respiratory disease in china. *Nature, 579*(7798), 265-269. doi:10.1038/s41586-020-2008-3.

Wu, K., Peng, G., Wilken, M., Geraghty, R. J., & Li, F. (2012). Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *J Biol Chem, 287*(12), 8904-8911. doi:10.1074/jbc.M111.325803.

Xu, R., Shi, M., Li, J., Song, P., & Li, N. (2020). Construction of sars-cov-2 virus-like particles by mammalian expression system. *Front Bioeng Biotechnol, 8*, 862. doi:10.3389/fbioe.2020.00862.

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of sars-cov-2 by full-length human ace2. *Science, 367*(6485), 1444-1448. doi:10.1126/science.abb2762.

Yang, T., & Xu, C. (2017). Physiology and pathophysiology of the intrarenal renin-angiotensin system: An update. *J Am Soc Nephrol, 28*(4), 1040-1049. doi:10.1681/ASN.2016070734.

Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H., . . . Shang, Y. (2020). Clinical course and outcomes of critically ill patients with sars-cov-2 pneumonia in wuhan, china: A single-centered, retrospective, observational study. *Lancet Respir Med, 8*(5), 475-481. doi:10.1016/S2213-2600(20)30079-5.

Zhu, Z., Lian, X., Su, X., Wu, W., Marraro, G. A., & Zeng, Y. (2020). From sars and mers to covid-19: A brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. *Respir Res, 21*(1), 224. doi:10.1186/s12931-020-01479-w.

Zou, X., Chen, K., Zou, J., Han, P., Hao, J., & Han, Z. (2020). Single-cell rna-seq data analysis on the receptor ace2 expression reveals the potential risk of different human organs vulnerable to 2019-ncov infection. *Front Med, 14*(2), 185-192. doi:10.1007/s11684-020-0754-0.