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Human recombinant soluble ACE2 in severe COVID-19

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Angiotensin converting enzyme 2 (ACE2) is the crucial severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor and protects multiple tissues, including the lung, from injury as a regulator of the renin–angiotensin system.[1] Therefore, ACE2 has become the focus of COVID-19 research and a plethora of drug development efforts. Among the novel compounds under development is human recombinant soluble ACE2 (hrsACE2 [APN01; Apeiron Biologics, Vienna, Austria]), which has two mechanisms of action that theoretically should be of benefit in COVID-19.[2] The first involves binding the viral spike protein and thereby neutralising SARS-CoV-2,[3] and the second is minimising injury to multiple organs, including the lungs, kidneys, and heart, because of unabated renin–angiotensin system hyperactivation and increased angiotensin II concentrations.[4–6] hrsACE2 has been tested in 89 patients, namely in healthy volunteers in phase 1 studies and in patients with acute respiratory distress syndrome (ARDS) in phase 2 clinical studies, with an acceptable safety profile.[7,8] Moreover, hrsACE2 can reduce SARS-CoV-2 load by a factor of 1000–5000 in in-vitro cell-culture experiments and engineered organoids, directly demonstrating that ACE2 can effectively neutralise SARS-CoV-2.[3] We describe in this Case Report the first course of treatment with hrsACE2 of a patient with severe COVID-19.

A 45-year-old woman was admitted to hospital with a 7-day history of cough, weakness, myalgia, fever, and dyspnoea, and a 4-day history of nausea and diarrhoea. Past medical history revealed type 2 diabetes, controlled non-pharmacologically by exercise and diet, and Grave's disease for which thyroidectomy had been done; the patient was euthyroid on thyroxin. She had a normal body-mass index and reported good exercise tolerance before her present illness. On admission she was febrile (38·1°C), mildly hypoxaemic (PaO_2 56 mm Hg on room air), and was started on oxygen by nasal cannula. A chest x-ray showed bilateral patchy consolidations indicative of viral pneumonia (appendix p 6).

Diagnosis of SARS-CoV-2 infection was made by RT-PCR from a nasopharyngeal swab, and treatment with hydroxychloroquine (400 mg twice daily) and anticoagulation with nadroparin (0·4 mg once daily) were started. The following day, the patient's chest x-ray showed increasing bilateral, multifocal, and peripheral ground glass patterns (appendix p 6**)**. Her fractional concentration of oxygen in inspired air (FiO_2) was 153 mm Hg, and she showed signs of exhaustion while on high flow nasal cannula, with a FiO_2 of 70%. We therefore intubated her and used a lung protective ventilatory strategy. Initial laboratory tests showed mild leukopenia and thrombocytopenia with a profound lymphopenia (0·54 g/L); serum lactate dehydrogenase (636 U/L), ferritin (880 µg/L), C-reactive protein (103 mg/L), and D-dimer (1·3 mg/L) concentrations were all markedly elevated (appendix p 4).

9 days after symptom onset (baseline, day 2 after hospital admission), treatment with hrsACE2 (APN01; 0·4 mg/kg) intravenous infusion for 5 min twice daily was started. This named patient use was initiated after consultations among attending physicians, the patient's family, and external infectious disease and ARDS experts. After administration of the first hrsACE2 dose, the patient became afebrile within hours. Her temperature increased the following day with purulent respiratory secretions, suggesting a bacterial pneumonia, which was treated with cefuroxime. A tracheal aspirate subsequently grew methicillin-sensitive *Staphylococcus aureus* (MSSA). Renin–angiotensin system fingerprinting and angiotensin-based markers, as well as cytokines and injury biomarkers, were measured once daily during the 7-day administration of hrsACE2 and thereafter for a total of 14 days. SARS-CoV-2 viral load and neutralising antibodies were measured once daily in plasma. The presence of viral RNA was also measured in nasopharyngeal swabs and tracheal aspirations (appendix p 2).

Therapy with hrsACE2 was continued as planned for 7 days and was well tolerated without obvious drug-related side-effects. Her clinical course was complicated by slowly resolving pneumonia with repeated detection of MSSA in tracheal suctions and *Enterobacter aerogenes* bacteraemia, and her antibiotics were changed to linezolid and aztreonam. Her clinical condition improved gradually and extubation was done on day 21 after baseline. The patient was then transferred to neurological rehabilitation for treatment of critical illness myopathy, which improved after physiotherapy. On day 57, she was discharged from the hospital.

After the first injection of hrsACE2 we observed a marked reduction of angiotensin II (figure 1A, B; appendix p 4). hrsACE2 administration resulted in a profound increase of the ACE2 products angiotensin 1–7 and angiotensin 1–9 (figure 1B) and their major downstream metabolite angiotensin 1–5 (appendix p 7) throughout the entire observation period. Normalising the sum of these three renin–angiotensin system peptides to renin activity resulted in an angiotensin-based marker that was associated with soluble plasma ACE2 activity (figure 1C), which was confirmed by an angiotensin II-to-angiotensin 1–7 conversion assay (figure 1D); ACE2 activity remained increased up to 7 days following administration of the last dose of hrsACE2. Additionally, the angiotensin

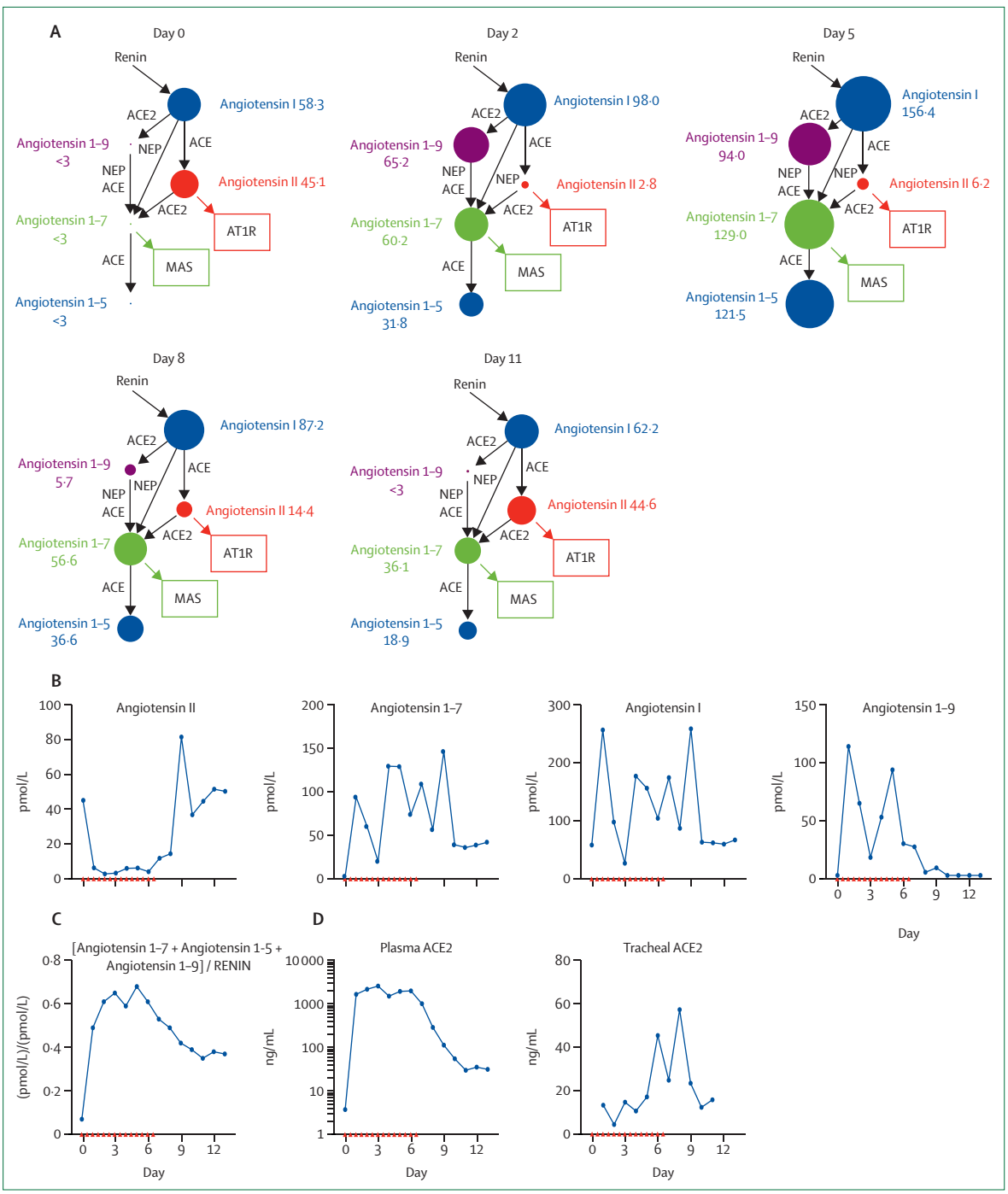

Figure 1: **Human recombinant soluble ACE2 pharmacodynamics and drug concentrations**
(A) Renin–angiotensin system fingerprinting of angiotensin metabolites in relation to human recombinant soluble ACE2 dosing. Circle sizes and angiotensin values correspond to metabolite concentrations (pmol/L). Black arrows mark the catalysing reactions mediated by the indicated enzymes. (B) Plasma concentrations of various ACE2 substrates and catalysed products. (C) ACE2 activity as the sum of ACE2 products and downstream metabolites related to plasma renin activity. (D) ACE2 activity (angiotensin II-to-angiotensin 1–7 catalytic conversion assays) in plasma and tracheal suctions. First dose administered on day 0; red triangles indicate days on which drug was administered. ACE2=angiotensin converting enzyme 2. AT1R=angiotensin II type 1 receptor. MAS=G protein coupled receptor Mas. NEP=neutral endopeptidase.

1–7/angiotensin II and angiotensin 1–5/angiotensin II ratios showed a marked skewing towards ACE2-generated peptides (appendix p 7). The sum of all five angiotensin metabolites as determined by renin–angiotensin system equilibrium analysis showed a strong correlation with renin concentration, as validated in pooled human plasma

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See **Online** for appendix

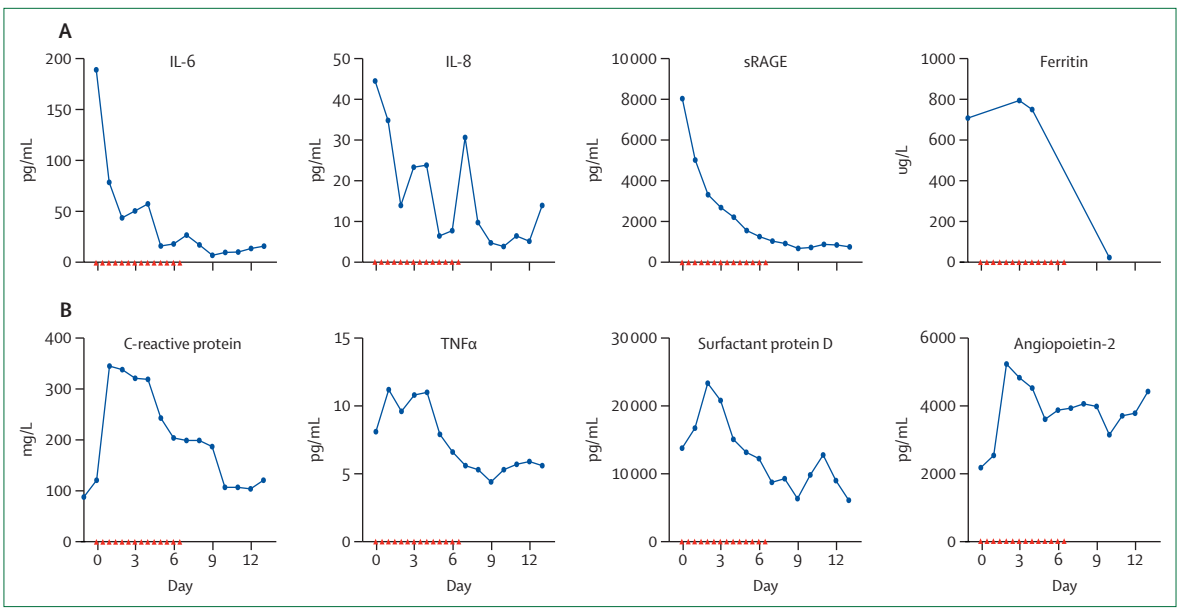

Figure 2: **Inflammatory, endothelial, and alveolar biomarkers**
(A) Plasma concentrations of IL-6, IL-8, sRAGE, and ferritin. (B) Plasma concentrations of C-reactive protein, TNFα, surfactant protein D, and angiopoietin-2. Red triangles indicate days on which drug was administered. IL=interleukin. sRAGE=soluble advanced glycation end product receptor. TNFα=tumour necrosis factor.

spiked with different concentrations of renin and hrsACE2, respectively (appendix p 7). We also observed soluble ACE2 present in tracheal fluid throughout the observation period, showing an increase towards day 6 that peaked 36 h following administration of the final dose of hrsACE2 (figure 1D); whether this reflects shedding of endogenous ACE2 or accumulation of hrsACE2, or both, remains to be elucidated.

Concurrent with reduced angiotensin II concentrations we detected a marked reduction in the inflammatory cytokine interleukin (IL)-6 and chemokine IL-8 (figure 2A; appendix p 4), both of which play an important role in lung injury and cytokine storm.[9] Furthermore, concentrations of soluble advanced glycosylation end product-specific receptor, a marker for lung xa injury associated with higher mortality in ARDS,[10] and the inflammation marker ferritin, were markedly decreased following hrsACE2 administration (figure 2A). Serum concentrations of the inflammatory marker tumour necrosis factor α and the lung damage biomarker serum surfactant protein D showed an initial increase followed by a marked decrease.[11] A similar increase but delayed decrease (possibly because of the bacterial complications) were noted for C-reactive protein and for the endothelial injury marker angiopoietin 2 concentrations (figure 2B; appendix p 4). Therefore, hrsACE2 treatment was associated with a marked decrease in concentrations of critical cytokines implicated in COVID-19 pathology, and hrsACE2 remains catalytically active in the scenario of severe COVID-19.

SARS-CoV-2 was detectable in the plasma 2 days before administration (day −2, 32 000 copies per mL) and on the first day of administration (day 0, 2500 copies per mL; figure 3A). 1 day later, plasma viraemia had decreased to a very low level (270 copies per mL) and thereafter remained undetectable during daily testing, until the end of the observation period when viral RT-PCR plasma testing was stopped. In tracheal aspirates, SARS-CoV-2-specific RT-PCR revealed an increase of the measured viral RNA within 2 days of starting hrsACE2 treatment, followed by rapid clearance until the end of the treatment period (figure 3B; appendix p 4).

hrsACE2 binds to the spike glycoprotein of SARS-CoV-2 at an affinity calculated to be approximately 1·2 nM,[12] an affinity similar to high affinity antibody binding. Because hrsACE2 does not have an Fc region to directly link to the immune system, assessing whether hrsACE2 treatment for 1 week might interfere with the formation of antibodies against the virus was crucial. Therefore, we assessed plasma neutralising anti-SARS-CoV-2 antibodies, which were first detected 7 days after symptom onset and reached a plateau 5 days later (figure 3A)—at a similar timepoint as previously reported.[13] Moreover, hrsACE2 infusions did not interfere with the generation of anti-viral IgA and IgG antibodies in the patient's plasma (appendix p 4). Thus, intravenous treatment with hrsACE2 has no apparent effect on anti-SARS-CoV-2 seroconversion.

ACE2 is the crucial cell surface receptor for SARS-CoV-2.[14] We have previously shown that ACE2 counterbalances the effects of angiotensin II and thereby protects the heart, kidney, and importantly the lung via its enzymatic activity in the renin–angiotensin system.[5,6,15] Furthermore, we showed that ACE2 is the crucial SARS-CoV receptor in vivo,[4] explaining why SARS-CoV and now SARS-CoV-2

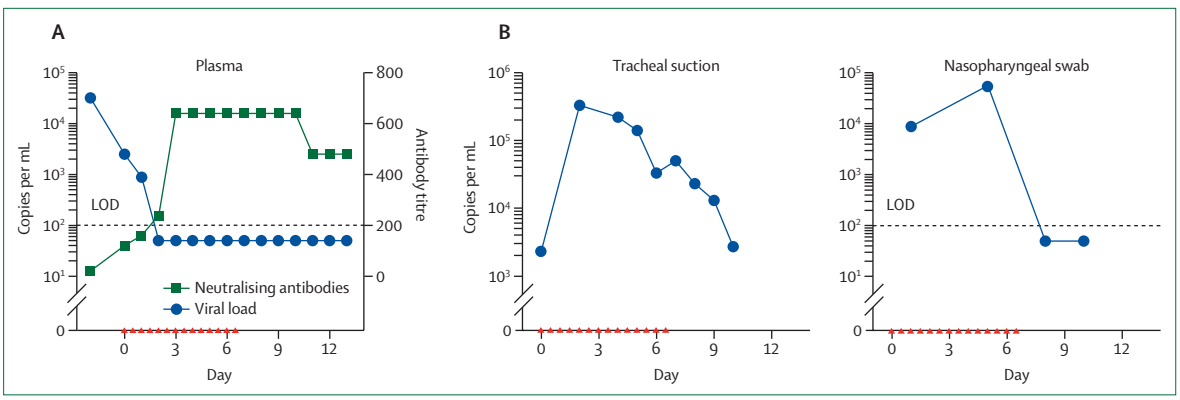

Figure 3: **Viral load and neutralising antibodies**
Viral load (blue circles) and neutralising antibodies (green squares) in plasma (A) and tracheal suctions and nasopharyngeal swabs (B). PCR LOD was 100 copies per mL. Red triangles indicate days on which drug was administered. LOD=limit of detection.

became highly pathogenic viruses, with ACE2 being the receptor for both viruses, and downregulation of ACE2 via virus binding resulting in loss of renin–angiotensin system tissue homoeostasis.[15] These results provide a molecular explanation as to why SARS-CoV and SARS-CoV-2 infections cause severe and often lethal lung failure. Moreover, genetic inactivation of ACE2 causes severe lung injury in influenza A H5N1-challenged and H7N9-challenged mice, while administration of soluble ACE2 protects from lethal avian influenza A H5N1 infections.[16,17]

Angiotensin II plasma concentrations are increased in acute lung injury,[15] predict fatal outcomes in patients infected with H7N9,[18] and, importantly, are also linked to severity of COVID-19.[19] ACE2 converts angiotensin II into angiotensin 1–7, which acts via its G protein coupled receptor Mas receptor to counterbalance induction of the renin–angiotensin system. Angiotensin II can drive cardiac disease, hypertension, fibrotic changes in the lung and liver, diabetic nephropathy, inflammation, blood vessel constriction, and lung injury, all of which are worsened in ACE2 mutant mice and can be alleviated in experimental mouse models using hrsACE2.[20] Angiotensin II infusion in patients has also been associated with increased thrombotic events and induction of the proinflammatory cytokine IL-6.[21,22] Our named patient use in a patient with COVID-19 now shows that hrsACE2 is highly active in cleaving angiotensin II into angiotensin 1–7, as well as cleaving angiotensin I into angiotensin 1–9, starting with the first infusion, and that hrsACE2 remains active throughout the course of the 7-day treatment.

After cessation of the therapy, angiotensin II concentrations returned within 48 h to concentrations before treatment, in line with previous data on the half-life of hrsACE2 in humans.[7] Notably, angiotensin 1–7 concentrations remained elevated for up to 7 days after hrsACE2 therapy was stopped, suggesting long-term beneficial effects. Compared to baseline, markedly increased soluble ACE2 activity was detected in plasma up to 7 days after dosing was stopped, which was paralleled by sustained elevation of the ACE2-dependent renin–angiotensin system markers. Whether low concentrations of hrsACE2 are sufficient to enhance angiotensin 1–7 concentrations for a prolonged time or whether treatment triggered endogenous enzyme activity that can account for these effects needs further analysis. Of note, a second patient with severe COVID-19 symptoms received two doses of named patient use treatment with hrsACE2 for 1 day; however, because of the short term 1-day treatment, we did not include the patient in this Case Report. Importantly, infusion of hrsACE2 in this patient was associated with a rapid and markedly reduced serum angiotensin II concentration, efficiently increasing angiotensin 1–7 concentration and alternative renin–angiotensin system markers for up to 5 days. Thus, hrsACE2 maintains its tissue protective enzymatic activity to reduce angiotensin II concentrations in patients with COVID-19. Notably, our named patient use treatment was started at a late stage of the disease in which inflammatory parameters were elevated and respiratory function had already markedly deteriorated. A phase 2/3 study of hrsACE2 is currently ongoing and includes patients with COVID-19 at earlier stages of the disease (pre-intubation).

Multiple efforts are underway to block SARS-CoV-2 infection, with approaches including blocking viral entry—for example, fusin or transmembrane protease serine 2 inhibitors; soluble ACE2 or antibodies, peptides, or small molecules that mimic ACE2 binding to the viral spike protein; and inhibiting the viral proteases and viral RNA-dependent RNA polymerase (eg, remdesivir). Moreover, vaccines specifically targeting the spike–ACE2 interaction are in development.[23] Therefore, ACE2 is at the core of COVID-19 research and drug development. Providing first data on the effect of blocking the viral spike glycoprotein in patients with COVID-19 is of paramount importance. Here, we have provided data on soluble ACE2 therapy in a patient with SARS-CoV-2 infection.

Acknowledging the limitations of our study, the data show that the virus disappeared rapidly from the serum;

and, albeit at a later time after hrsACE2 (APN01) therapy, from the nasal cavity and lung. Whether this decrease in viral load reflects the effect of hrsACE2 treatment or the natural history of the disease remains speculative. Importantly, hrsACE2 treatment did not interfere with the generation of neutralising antibodies. The same was seen for the patient receiving hrsACE2 for 1 day—rapidly undetectable serum viraemia and generation of anti-viral IgG and IgA antibodies (data not shown). Considering that SARS-CoV-2 can directly infect blood vessels, as determined in our experimental organoid model[3] and post-mortem examinations,[24] intravenous delivery of hrsACE2 could have an important effect on blocking the systemic spread of the virus from the lung to other organs.

Contributors

AZ and JMP designed the study and wrote the manuscript. MP did the renin–angiotensin system analyses, created the figures, and assisted with writing the manuscript. WH, TS, MT, AG, EP, DH, HL, CW, and SN collected data. JHA, KS, and AB did the virological analyses and assisted with writing the manuscript. DH assured quality and regulatory compliance. EP-S, AM, NM, HZ, and ASS contributed to data interpretation and revised the manuscript. All authors discussed the results, commented on the manuscript, and approved the final version of the manuscript.

Declaration of interests

JMP is a founder and shareholder of Apeiron Biologics. ASS is a consultant to Apeiron Biologics. All other authors declare no competing interests.

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References

1 Bourgonje AR, Abdulle AE, Timens W, et al. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol* 2020; **251:** 228–48.

2 Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med* 2020; **46:** 586–90.

3 Monteil V, Kwon H, Prado P, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 2020; **181:** 905–13.

4 Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; **11:** 875–79.

5 Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 2002; **417:** 822–28.

6 Wong DW, Oudit GY, Reich H, et al. Loss of angiotensin-converting enzyme-2 (Ace2) accelerates diabetic kidney injury. *Am J Pathol* 2007; **171:** 438–51.

7 Haschke M, Schuster M, Poglitsch M, et al. Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. *Clin Pharmacokinet* 2013; **52:** 783–92.

8 Khan A, Benthin C, Zeno B, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care* 2017; **21:** 234.

9 Zhang X, Tan Y, Ling Y, et al. Viral and host factors related to the clinical outcome of COVID-19. *Nature* 2020; **583:** 437–40.

10 Jabaudon M, Blondonnet R, Pereira B, et al. Plasma sRAGE is independently associated with increased mortality in ARDS: a meta-analysis of individual patient data. *Intensive Care Med* 2018; **44:** 1388–99.

11 Lopez-Cano C, Lecube A, Garcia-Ramirez M, et al. Serum surfactant protein D as a biomarker for measuring lung involvement in obese patients with type 2 diabetes. *J Clin Endocrinol Metab* 2017; **102:** 4109–16.

12 Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 2020; **181:** 281–92.e6.

13 Okba NMA, Muller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. *Emerg Infect Dis* 2020; **26:** 1478–88.

14 Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579:** 270–73.

15 Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005; **436:** 112–16.

16 Zou Z, Yan Y, Shu Y, et al. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat Commun* 2014; **5:** 3594.

17 Yang P, Gu H, Zhao Z, et al. Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. *Sci Rep* 2014; **4:** 7027.

18 Huang F, Guo J, Zou Z, et al. Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients. *Nat Commun* 2014; **5:** 3595.

19 Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020; **63:** 364–74.

20 Gheblawi M, Wang K, Viveiros A, et al. 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* 2020; **126:** 1456–74.

21 Senchenkova EY, Russell J, Almeida-Paula LD, Harding JW, Granger DN. Angiotensin II-mediated microvascular thrombosis. *Hypertension* 2010; **56:** 1089–95.

22 Han Y, Runge MS, Brasier AR. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. *Circ Res* 1999; **84:** 695–703.

23 Corey BL, Mascola JR, Fauci AS, Collins FS. A strategic approach to COVID-19 vaccine R&D. *Science* 2020; **368:** 948–50.

24 Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020; **383:** 120–28.