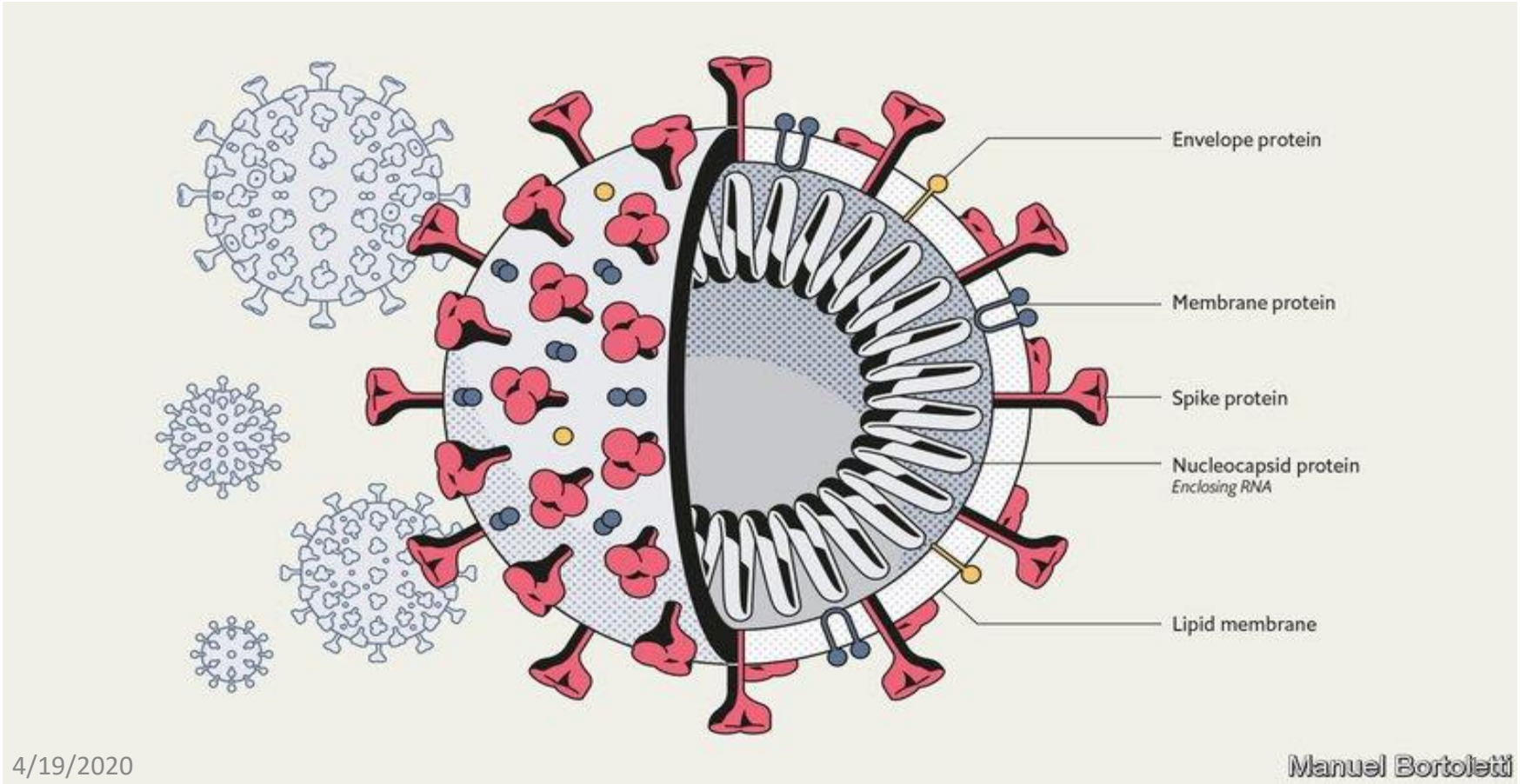
Kermanshah University of Medical Sciences

In the name of God

ACE2-SARS.CoV2 Interplay

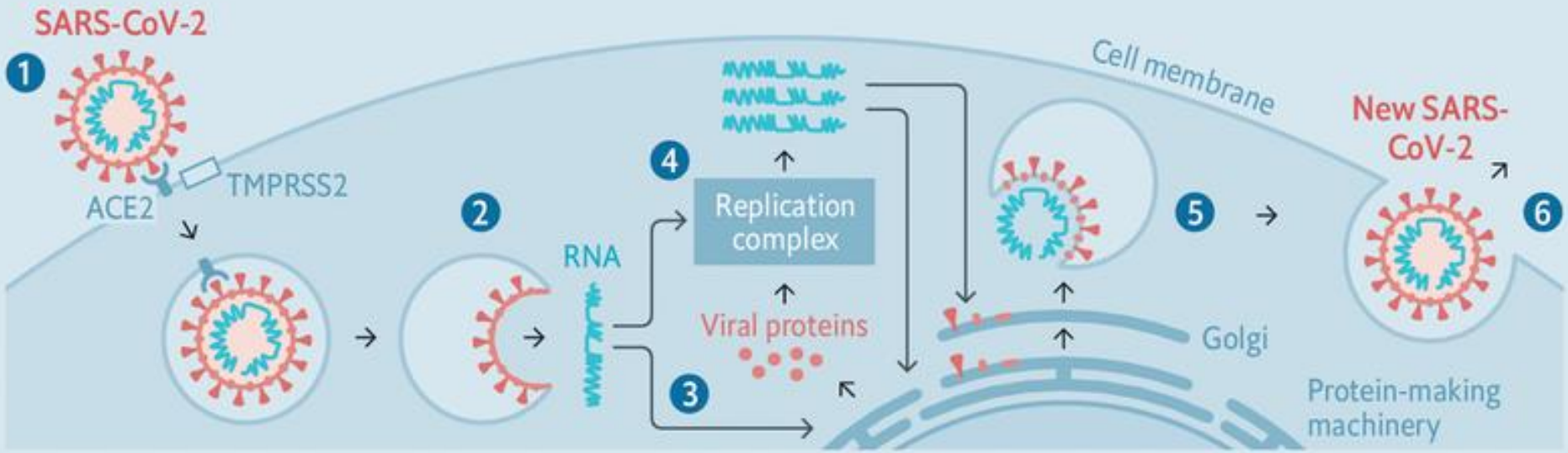
A lesson from SARS-CoV

Reza Khodarahmi, PhD



Hijack

How SARS-CoV-2 replicates itself in the cells of those infected

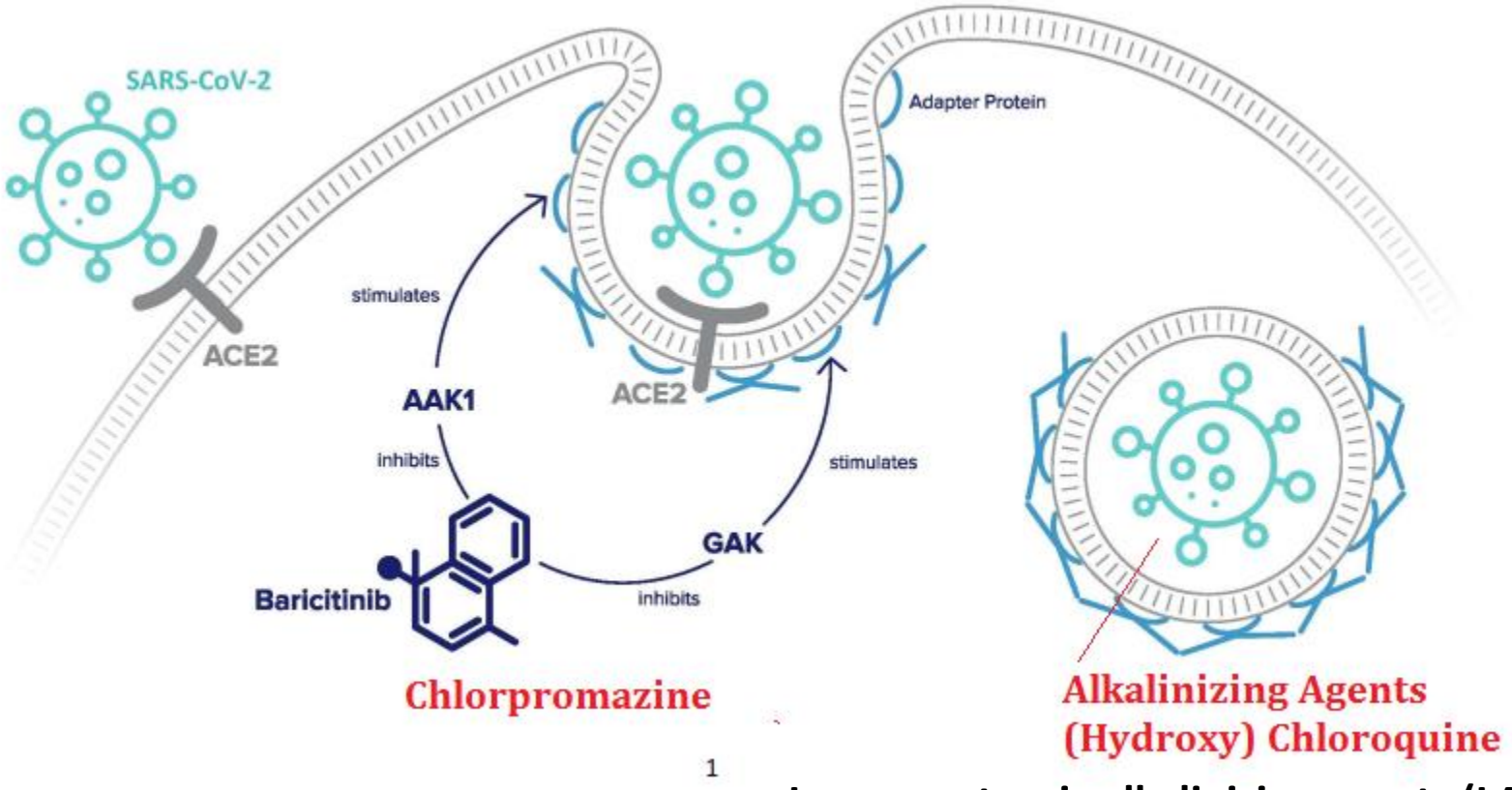


1 Spike protein on the virion binds to ACE2, a cell-surface protein. TMPRSS2, an enzyme, helps the virion enter 2 The virion releases its RNA 3 Some RNA is translated into proteins by the cell's machinery 4 Some of these proteins form a replication complex to make more RNA 5 Proteins and RNA are assembled into a new virion in the Golgi and 6 released

Sources: Song et al., *Viruses*, 2019; Jiang et al., *Emerging Microbes and Infections*, 2012; *The Economist*

The Economist

ACE-2 is a type I transmembrane metallocarboxypeptidase with homology to ACE



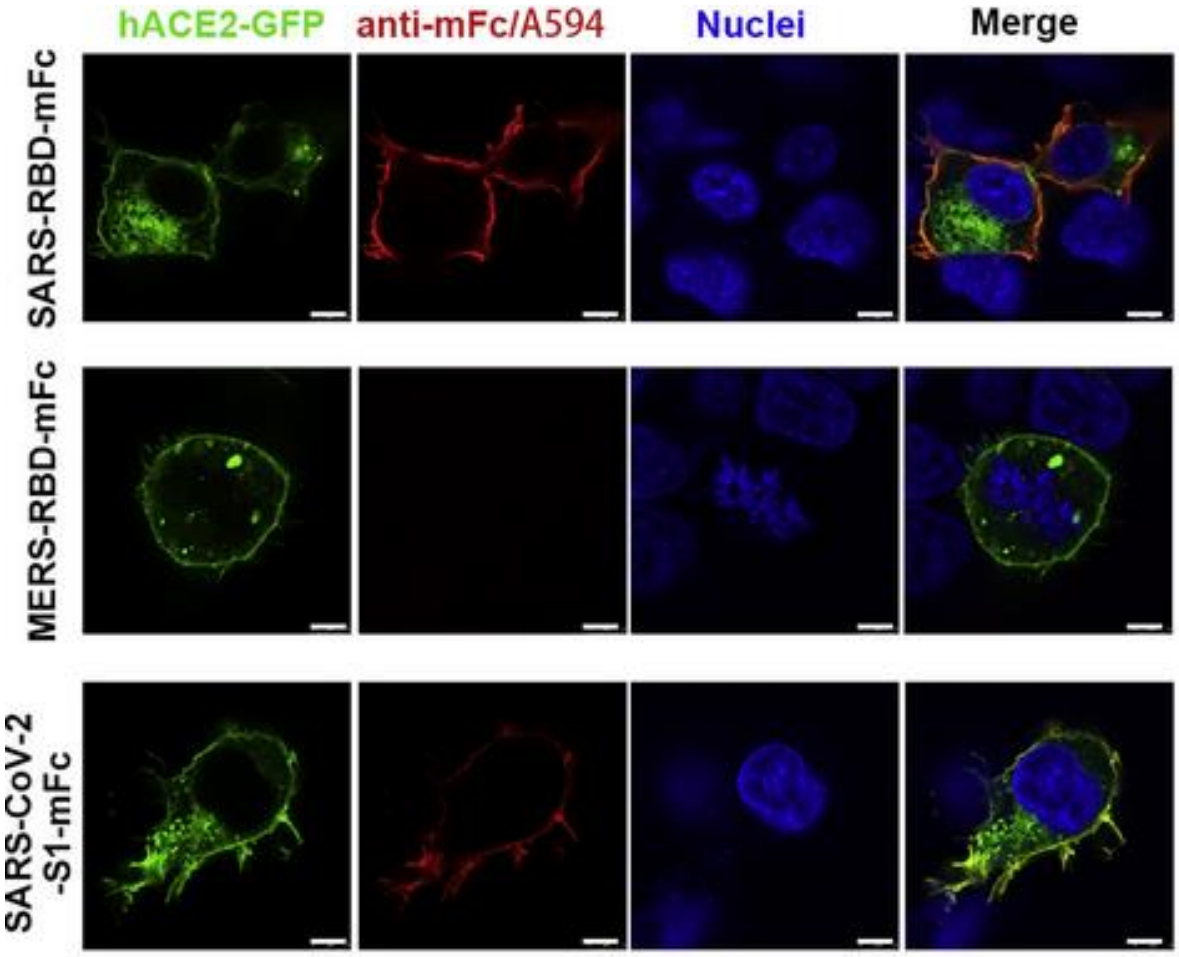
Lysosomotropic alkalinizing agents (LAAs)

https://www.thelancet.com/article/S1473-3099(20)30132-8/fulltext
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4204995/pdf/pone.0110631.pdf

https://www.thelancet.com/cms/10.1016/S1473-3099(20)30132-8/attachment/45cd015a-349f-4ce2-9d41-dc6b75d10f7d/mmc1.pdf

Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2

GFP-tagged hACE2 expressed on the HEK293T cells surface. Using confocal fluorescence microscopy, SARS-CoV-2-S1 and SARS-CoV-2-CTD co-localization with hACE2 was observed on the cell surface



https://www.sciencedirect.com/science/article/pii/S009286742030338X

Available online 9 **April 2020**

- J Shang *et al*, *Nature*, 2020, DOI: 10.1038/s41586-020-2179-y
- J Lan *et al*, *Nature*, 2020, DOI: 10.1038/s41586-020-2180-5

Two parental viruses of SARS-CoV-2

SARS-CoV-2 virus shares about 80% sequencing identity with the original SARS-CoV virus

- The first one is **bat** coronavirus **RaTG13** found in ***Rhinolophus affinis*** from Yunnan Province and it shares **96.2% overall genome sequence identity** with SARS-CoV-2. However, RaTG13 might not be the immediate ancestor of SARS-CoV-2 because it is **not** predicted to use the same ACE2 receptor used by SARS-CoV-2 due to sequence divergence in the receptor-binding domain sharing 89% identity in amino acid sequence with that of SARS-CoV-2.

A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020. https://doi.org/10.1038/s41586-020-2012-7.

Identification of 2019-nCoV related coronaviruses in Malayan pangolins in southern China. BioRxiv. 2020. https://doi.org/10.1101/2020.02.13.945485.

https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-020-00404-4?utm_source=sn&utm_medium=referral&utm_content=RMarketing&utm_campaign=BSLB_4_CA01_GL_BSLB_USG_CA01_GL_LSGR_PubH_Coronavirus_LandingPage

Rhinolophus affinis

Two parental viruses of SARS-CoV-2 have now been identified

- The second one is a group of betacoronaviruses found in the endangered species of small mammals known as **pangolins**, which are often consumed as a source of meat in southern China. They share about **90% overall nucleotide sequence identity with SARS-CoV-2** but carries a receptor-binding domain predicted to interact with **ACE2 and sharing 97.4%** identity in amino acid sequence with that of SARS-CoV-2.

A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020. https://doi.org/10.1038/s41586-020-2012-7.

Identification of 2019-nCoV related coronaviruses in Malayan pangolins in southern China. BioRxiv. 2020. https://doi.org/10.1101/2020.02.13.945485.

https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-020-00404-4?utm_source=sn&utm_medium=referral&utm_content=RMarketing&utm_campaign=BSLB_4_CA01_GL_BSLB_USG_CA01_GL_LSGR_PubH_Coronavirus_LandingPage

Two parental viruses of SARS-CoV-2 have now been identified

- **Many** hypotheses involving **recombination, convergence and adaptation** have been put forward to suggest a probable evolutionary pathway for SARS-CoV-2, **but none is supported by direct evidence**.
- **The jury** is still out as to **what animals** might serve as reservoir and intermediate hosts of SARS-CoV-2. Although Huanan seafood wholesale market was suggested as the original source of SARS-CoV-2 and COVID-19, there is evidence for the involvement of other wild animal markets in Wuhan.

https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-020-00404-4?utm_source=sn&utm_medium=referral&utm_content=RMarketing&utm_campaign=BSLB_4_CA01_GL_BSLB_USG_CA01_GL_LSGR_PubH_Coronavirus_LandingPage

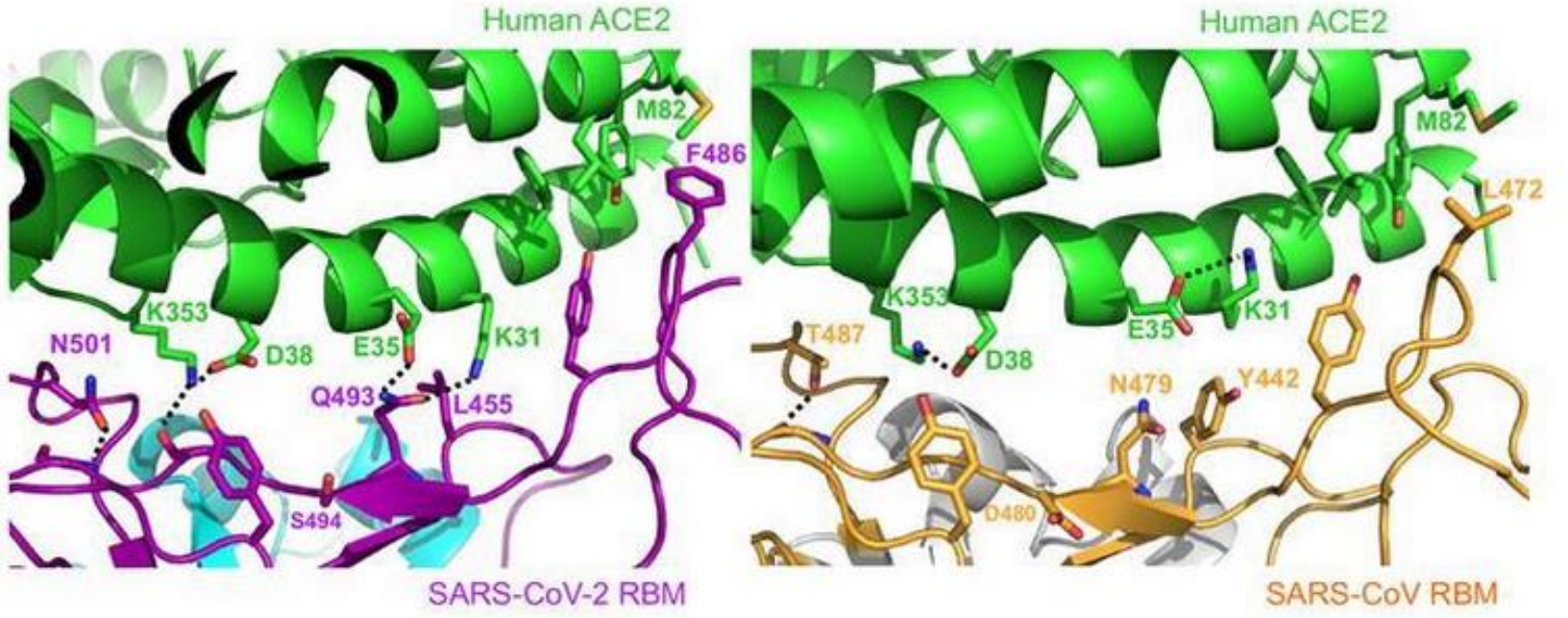
The most closely related virus to Sars-CoV-2

RaTG13

- Structural analysis of its binding domain by Fang's group reveals **it too binds human ACE2**, suggesting it can infect people. The group posit, therefore, that **there does not need to be an intermediate species that passed the virus between bats and people to initiate the pandemic.**

J Shang *et al*, *Nature*, 2020, DOI: 10.1038/s41586-020-2179-y
J Lan *et al*, *Nature*, 2020, DOI: 10.1038/s41586-020-2180-5

Several interactions reveal structural features that **enhance** the binding **affinity** of **SARS-CoV-2 for ACE2, compared to SARS-Cov**



Comparing the interfaces between human ACE2 and (left) the Sars-CoV-2 receptor-binding domain and (right) the Sars-CoV receptor-binding domain reveals structural features that enhance the binding affinity of Sars-CoV-2

J Shang *et al*, *Nature*, 2020, DOI: 10.1038/s41586-020-2179-y
J Lan *et al*, *Nature*, 2020, DOI: 10.1038/s41586-020-2
https://www.chemistryworld.com/news/structural-studies-offer-glimpse-of-how-coronavirus-initiates-human-cell-invasion/4011452.article

Repurposed Drugs

- **While the COVID-19 outbreak continues to spread around the globe, the absence of a clinically proven antiviral therapy or a treatment specifically targeting the critical SARS-CoV-2 receptor ACE2 on a molecular level has meant an empty arsenal for health care providers struggling to treat severe cases of COVID-19**

https://www.sciencedaily.com/releases/2020/04/200402144526.htm

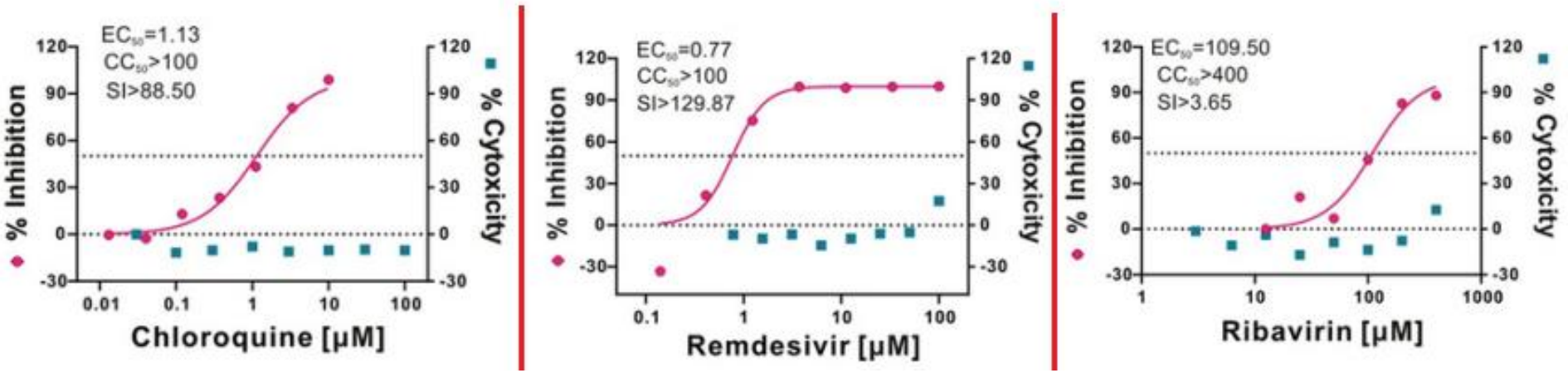
Repurposed Drugs

- **Despite decades of extensive research, there are no specific/effective therapies approved by the U.S. Food and Drug Administration (FDA) for serious coronavirus infections such as SARS, MERS, and now COVID-19**

Chloroquine (CQ) and Hydroxychloroquine (HCQ)

- *In vitro* and limited clinical data suggest potential benefit for chloroquine and hydroxychloroquine. Nevertheless, FDA on 27th March, 2020, issued an emergency authorization for experimental coronovirus treatment using these anti-malarial drugs.

See efficacy/cytotoxicity for CQ



Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, *Cell Research* **volume 30**, pages269–271(2020)

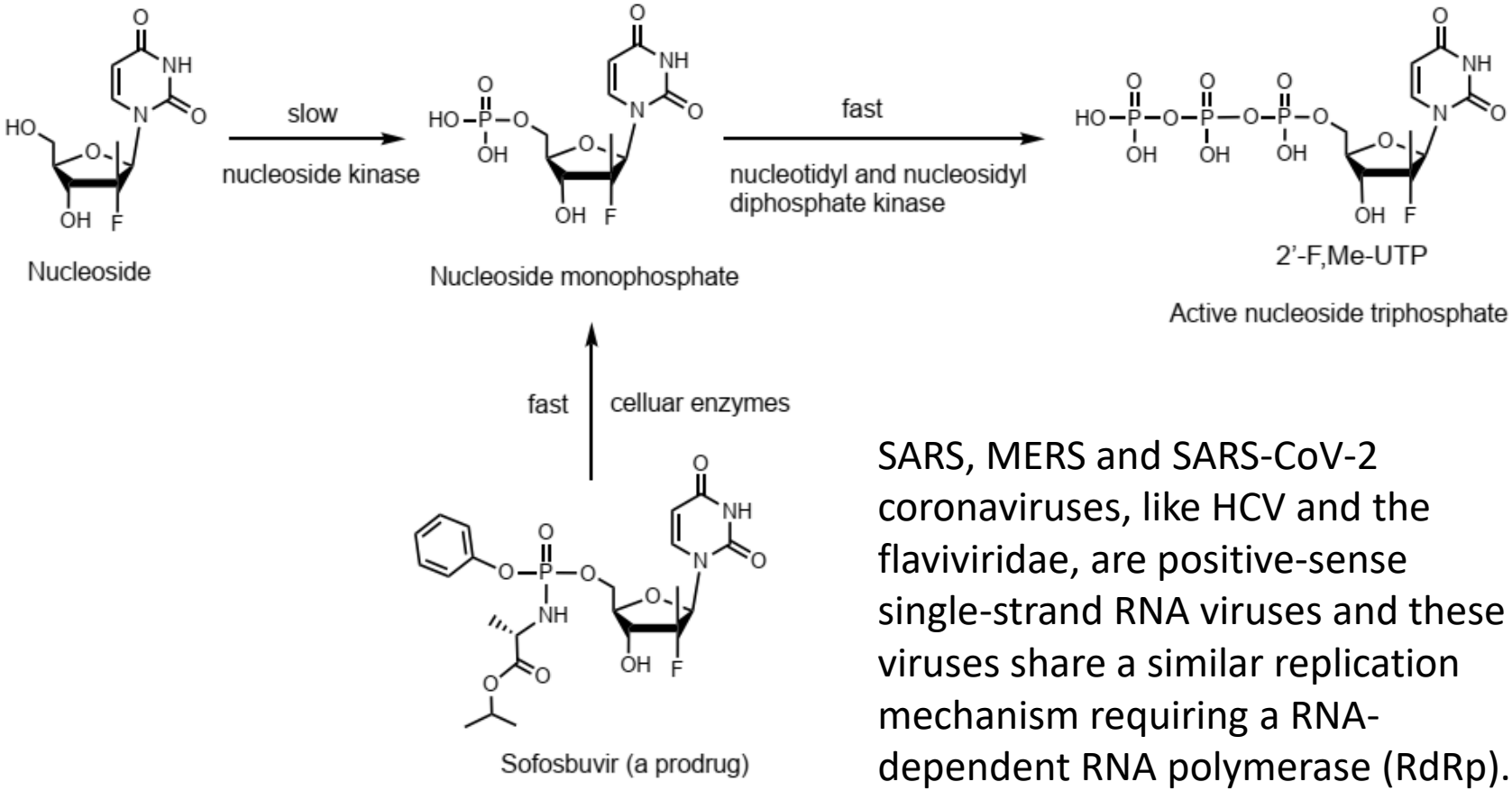
Repurposed Drugs

- On the other hand, although *in vitro* and limited clinical data suggest potential benefit for Kaletra (Lopinavir; Ritonavir), and its actual role in the treatment of COVID-19 is still unclear, some preclinical data suggested potential benefit. However, more recent data has failed to confirm Kaletra efficacy for COVID-19 treatment.
- **Lopinavir** is an antiretroviral protease inhibitor used in combination with other antiretrovirals in the treatment of HIV-1 infection.
- (https://www.elsevier.com/__data/assets/pdf_file/0007/988648/COVID-19-Drug-Therapy_Mar-2020.pdf) (https://www.nejm.org/doi/full/10.1056/NEJMoa2001282)
- While the COVID-19 outbreak continues to spread around the world, the absence of a clinically proven antiviral therapy is a serious challenge for the treatment of severe COVID-19 cases.

https://www.drugbank.ca/drugs/DB01601

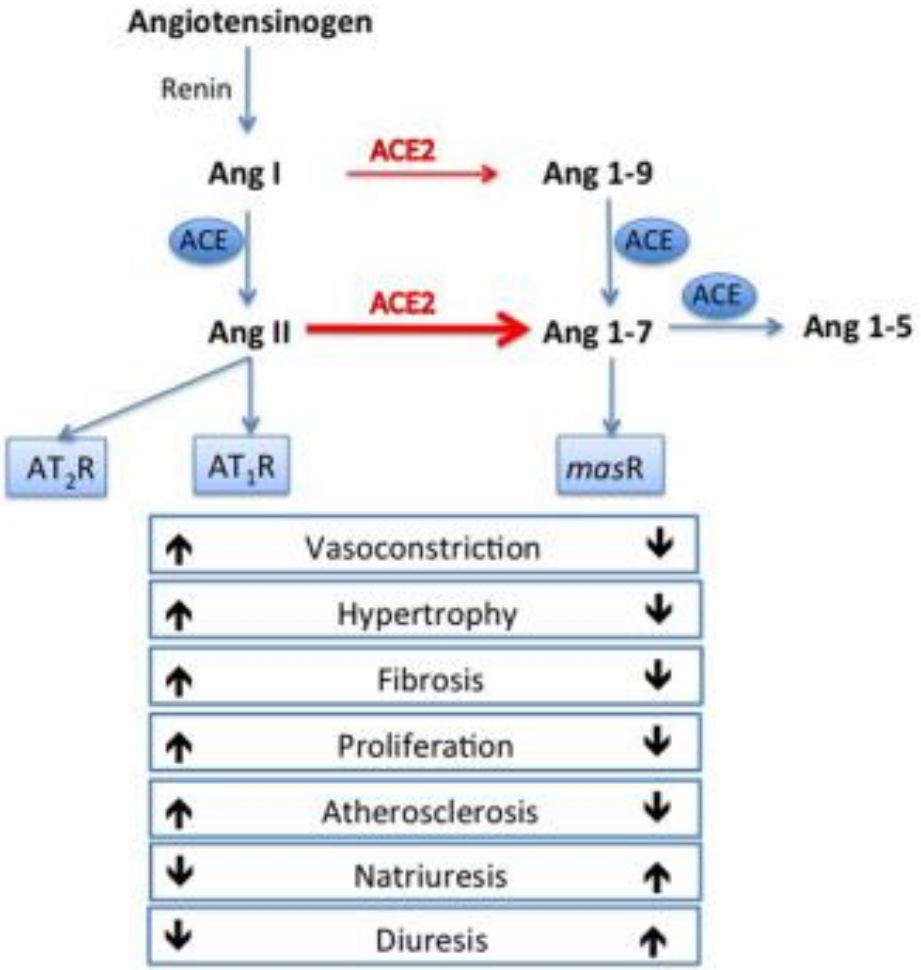
https://www.irct.ir/trial/46790

Babak Sayad*[1], Mahsa Sobhani[2], Reza Khodarahmi[2,3]*
Kermanshah University of Medical Sciences, Kermanshah, Iran



SARS, MERS and SARS-CoV-2 coronaviruses, like HCV and the flaviviridae, are positive-sense single-strand RNA viruses and these viruses share a similar replication mechanism requiring a RNA-dependent RNA polymerase (RdRp).

RAAS



- **The RAAS is one of determinant factors of acute respiratory distress syndrome (ARDS) which is frequently, associated with multiple organ dysfunctions leading to high mortality, is important clinical feature of COVID-19.**

Frontiers in Physiology 5:227
DOI: 10.3389/fphys.2014.00227

Unbalanced RAAS during SARS infection

- ACE is expressed in **entire capillary network of the alveoli in human lung**, resulting in readily conversion of Ang I to Ang II in the lung pulmonary vessels. On the other hand, ACE2 is primarily produced in **Clara cells and type II alveolar epithelial cells**. In COVID-19, due to the virus-mediated ACE2 internalization and epithelial injury as well, the ability of alveolar epithelial cells to produce **ACE2 is severely impaired**.

- Therefore, upon **dominant ACE activities** patients encounter rapid **vasoconstriction/low blood flow in the pulmonary circulation**, followed by **ventilation/perfusion mismatch**.

- Moreover, since **ACE2 is expressed in various tissues including the heart, kidney tubules, the luminal surface of the small intestine, and blood vessels**, SARS-CoV-2 could also infect these tissues, so that clinically **cardiovascular and renal dysfunctions** have been reported for many patients with COVID-19.

Unbalanced RAAS during SARS infection

- Due to the virus-mediated **ACE2 internalization** and **epithelial injury** as well as dominant ACE activities during ARDS, **increased plasma concentration of angiotensin II** is anticipated.
- Patients with COVID-19 have significantly **elevated levels of plasma angiotensin II** compared to that of healthy individual, which were in turn correlated with total viral load and degree of lung injury.
- Moreover, it may be expected that (**at least severe/critically ill**) COVID-19 cases to have some degree of **hypertension.**

Liu, Y., et al. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China Life Sciences *63*, 364-374

Ang II↑ --- High Blood Pressure!

- **Hypertension** being the most common co-morbidity (only 30%), followed by diabetes and coronary heart disease.
- Chronic hypertension was more frequent among **deceased** patients than recovered patients (**48%** vs. 24%)
- Median systolic blood pressure in deceased patients was **137.0 mm** Hg, ~ 10 units greater than that of recovered patients (**125.0 mm Hg**)

- **120 to 139 mmHg is called pre-hypertension; blood pressure 140 to 160 mmHg is called Grade 1 hypertension and above 160 mmHg is called Grade 2 hypertension**

Zhou, F., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet.
Chen, T., et al. (2020). Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ *368*.

Hypertension as underlying disease

- [1]Hypertension prevalence in approximately 17% of the patients, **equal** to the prevalence of hypertension in the entire adult population of China (~23%)
- [2]**Severe** cases accounted for **16.0%** of the study population which was equal to **prevalence of hypertension**, as the most prevalent comorbidity (**16.9%**).
- On **admission**, **10-15%** of patients reported as having hypertension
- **younger patients** (mean age of 44.8±15.2) **had no hypertension comorbidity**

Yang, J., et al. (2020). Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. International Journal of Infectious Diseases.

Guan, et al. (2020). Comorbidity and its impact on 1590 patients with Covid-19 in China: A Nationwide Analysis. European Respiratory Journal.

CVD and susceptibility to SARS-CoV-2 infection

- In patients with SARS-CoV-2 infection, underlying CVD can aggravate the pneumonia and increase the severity of symptoms. Among the people who died from COVID-19 reported by the National Health Commission of China (NHC), **only 11.8%** of patients **without** underlying CVD had substantial heart damage.

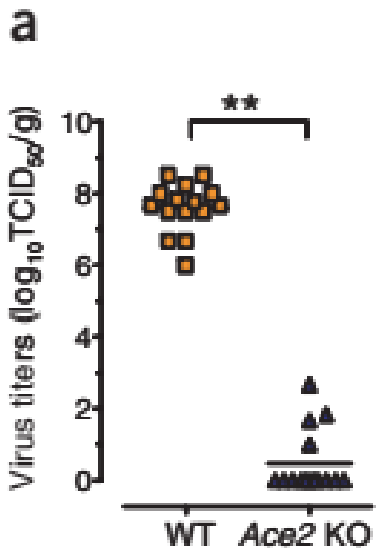
Zheng, Y., Ma, Y., Zhang, J. *et al.* COVID-19 and the cardiovascular system. *Nat Rev Cardiol* **17,** 259–260 (2020). https://doi.org/10.1038/s41569-020-0360-5

- Similarly, according to the Pneumonitis Diagnosis and Treatment Program for New Coronavirus Infection (Trial Version 4, http://www.gov.cn/zhengce/zhengceku/2020-01/28/content_5472673.htm), adult/elderly people with hypertension/coronary heart disease comorbidities are more likely to be infected with SARS-CoV-2 and patients with CVD are more likely to develop severe symptoms and account for a large proportion of deaths from COVID-19.

Susceptibility

- **ACE2 expression** was previously found to correlate with susceptibility to SARS-CoV **entry/infection *in vitro***

In *Ace2* knockout mice, only a very low quantity of infectious SARS-CoV virus could be recovered ($<10^2$ $TCID_{50}$ per gram lung tissue)



Entry ?– viral load?-severity?- survival?

https://www.nature.com/articles/nm1267

[**Biochem Biophys Res Commun**. 2004; 319:1216–1221] [https://www.ahajournals.org/doi/10.1161/JAHA.120.016219].

Susceptibility

- **Lower susceptibility** of **children** to COVID-19 **might** be attributable to the difference in expression of ACE2 between children and adults. However, no evidence exists [Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. **N Engl J Med**. 2020. doi: 10.1056/NEJMoa2001316. Accessed March 22, 2020].

- There is **no difference in activity of ACE2** in bronchoalveolar lavage fluid from neonates, children, adults, and older adults with ARDS [Age-dependent differences in pulmonary host responses in ARDS: a prospective observational cohort study. **Ann Intensive Care**. **2019**; 9:55].

- The **expression of ACE2 was relatively higher** in cells with higher pseudotype **SARS-CoV-2 entry** in agreement with "association of higher initial viral load and worse prognosis in SARS" [Initial viral load and the outcomes of SARS. **CMAJ**. 2004; 171:1349–1352], however, **no direct evidence exists** to indicate a connection between **ACE2 expression** and the susceptibility and **severity** of **SARS-CoV-2 infection**.

Susceptibility

- Preliminary data suggest that a high viral load of the SARS coronavirus is associated with adverse outcomes in ICU, **but** the relation of viral load to survival is unclear, so mortality data (for patients with SARS) should be interpreted in light of **age**, **comorbidity** and **viral load** [https://doi.org/10.1161/JAHA.120.016219, Journal of the American Heart Association. **2020**;9:e016219].

- Other studies suggest that serum ACE2 (Xp22) activity is sex-dependent; with higher levels in **males** compared with females [(2012) Circulating ACE2 activity is increased in patients with type 1 diabetes and vascular complications. J. Hypertens. 30, 375–383]

The report shows that **male gender** and **advanced age** were identified as independent **predictors of enhanced circulating ACE2 activity**

[*Nephrol. Dial. Transplant.* **2015**, *30*,1176–1185].

Let Review!

- Ang II ⬆
- Hypertension!
- Ang II-mediated Inflammation
- Lung Injury
- ARDS

Professor *Ali Mirazimi*

Professor Josef Penninger

- **Seeking a COVID-19 antidote:**
- **the potential of ACE2!**

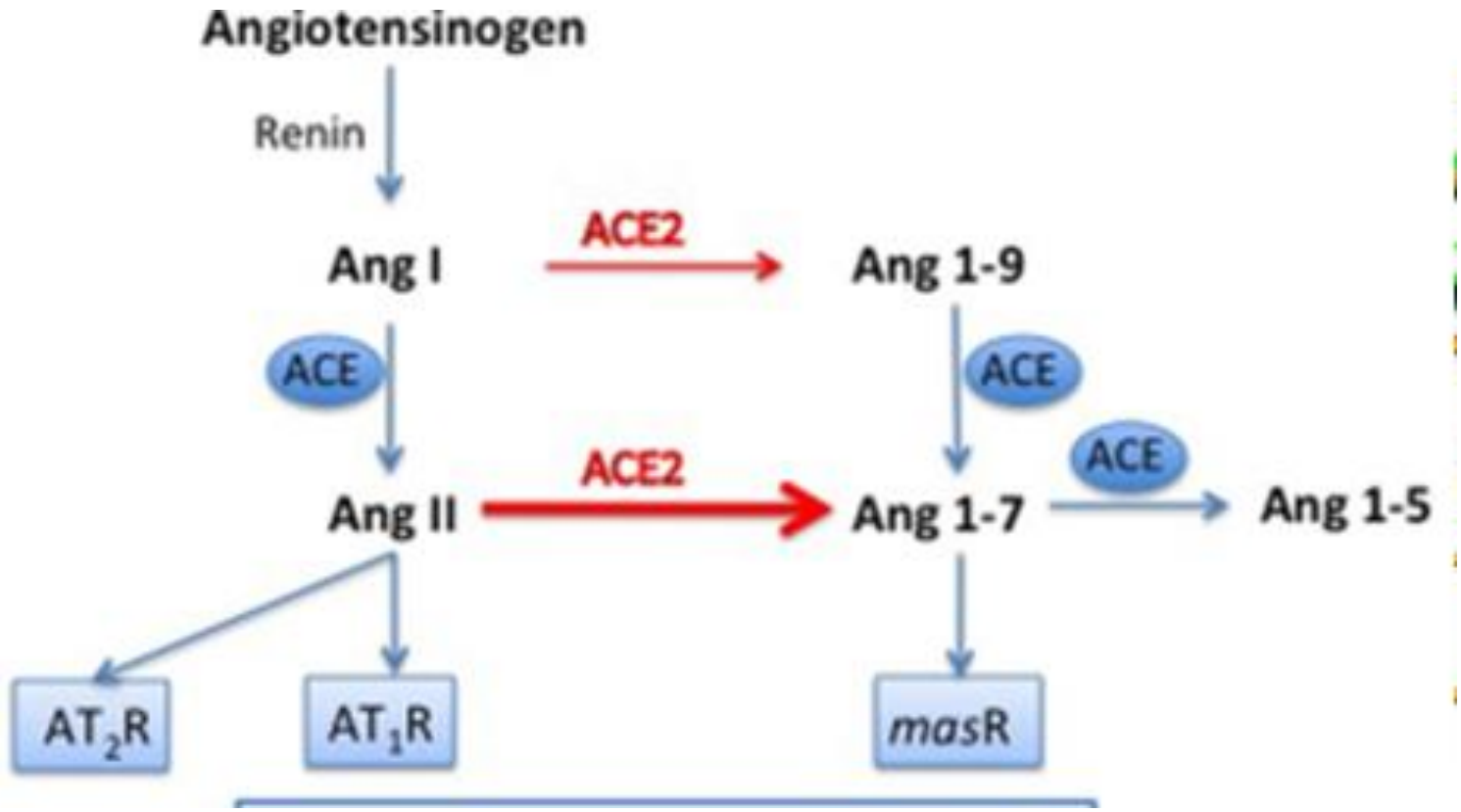
nature Vol 436|7 July 2005|doi:10.1038/nature03712

LETTERS 2005, SARS

Angiotensin-converting enzyme 2 protects from severe acute lung failure

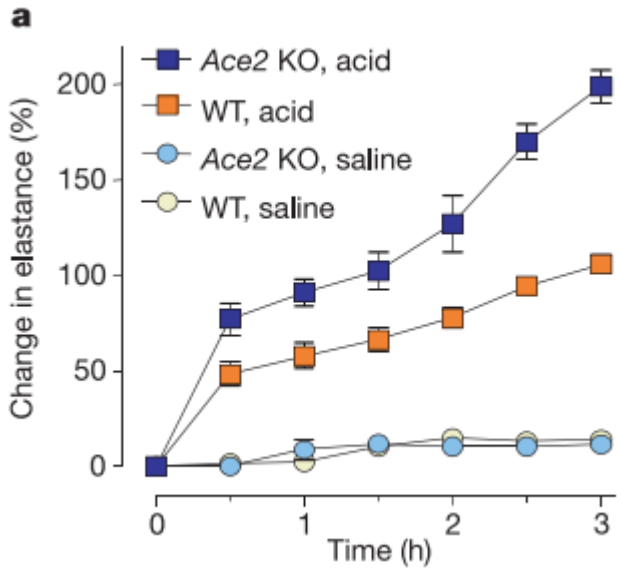
Yumiko Imai[1]*, Keiji Kuba[1]*, Shuan Rao[2], Yi Huan[2], Feng Guo[2], Bin Guan[2], Peng Yang[2], Renu Sarao[1], Teiji Wada[1], Howard Leong-Poi[3], Michael A. Crackower[4], Akiyoshi Fukamizu[5], Chi-Chung Hui[6], Lutz Hein[7], Stefan Uhlig[8], Arthur S. Slutsky[9], Chengyu Jiang[2] & Josef M. Penninger[1]

Angiotensin-converting enzyme 2 protects from severe acute lung failure

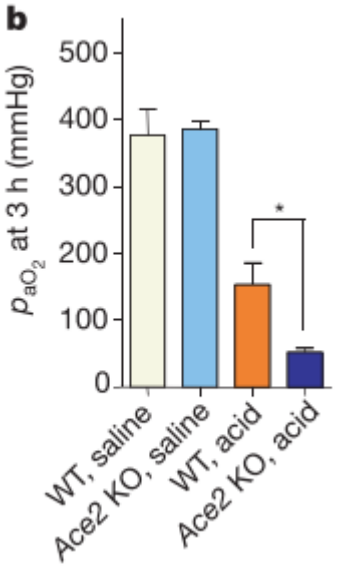


Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

Loss of ACE2 worsens acid aspiration-induced acute lung injury.



Lung elastance after acid or saline treatment in wild type (WT) and *Ace2* knockout (*Ace2* KO) mice



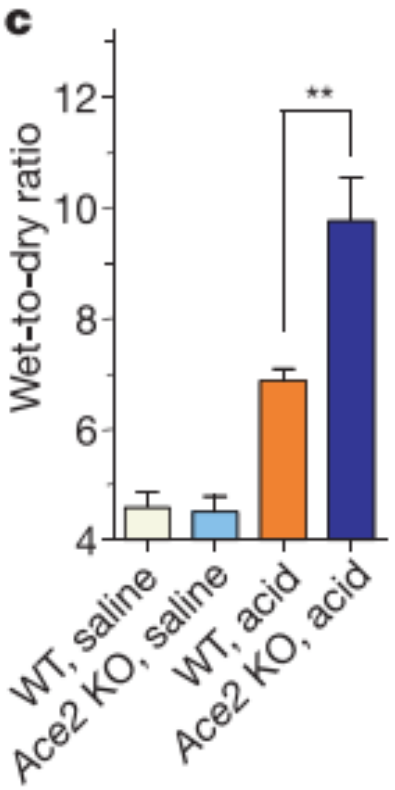
Partial pressure of oxygen in arterial blood (*p*aO2) in acid-induced acute lung injury

Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

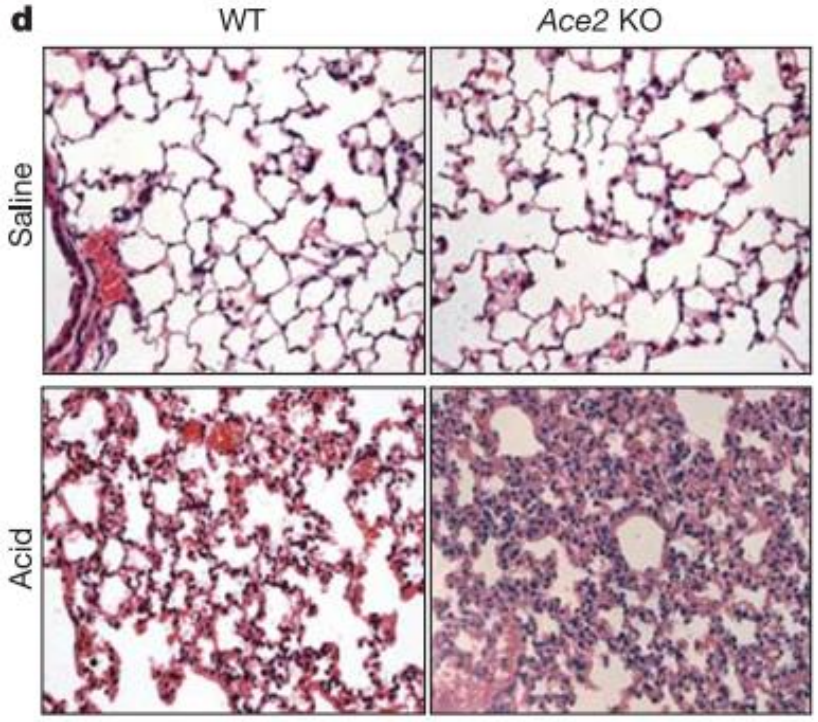
Loss of ACE2 worsens acid aspiration-induced acute lung injury.

c, Wet-to-dry weight ratios of lungs 3 h after acid injury.

d, Lung histopathology. Note the **enhanced hyaline membrane formation**, **inflammatory cell infiltration** and lung oedema in acid-treated *Ace2* knockout mice



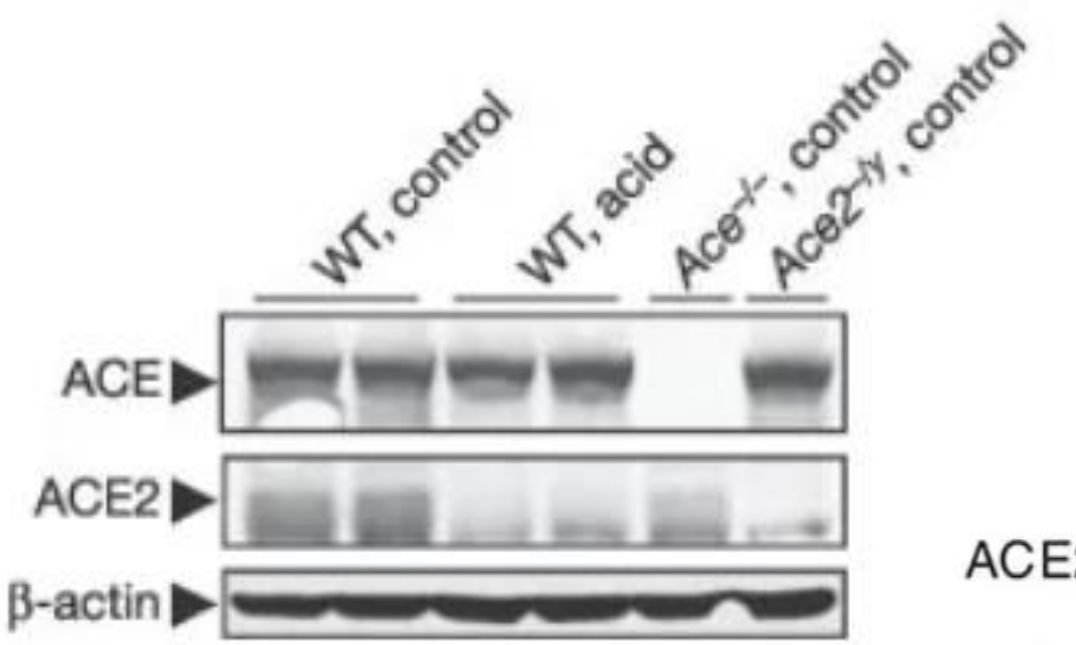
massive lung oedema



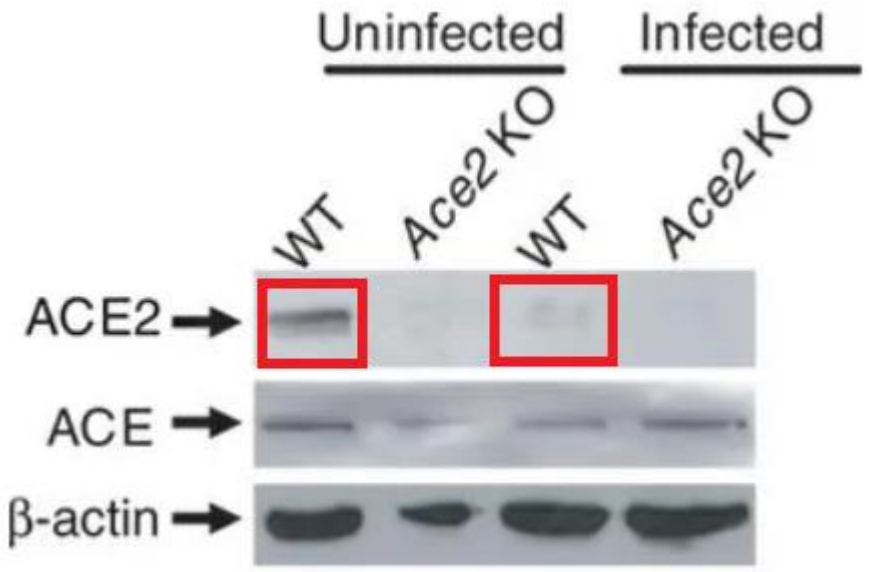
Increased inflammatory cell infiltration and hyaline membrane formations in response to acid aspiration

Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

Loss of ACE2 worsens acid aspiration-induced acute lung injury.



- **ACE2 protein expression is typically downregulated! in wild-type mice following acid challenge/SARS infection**

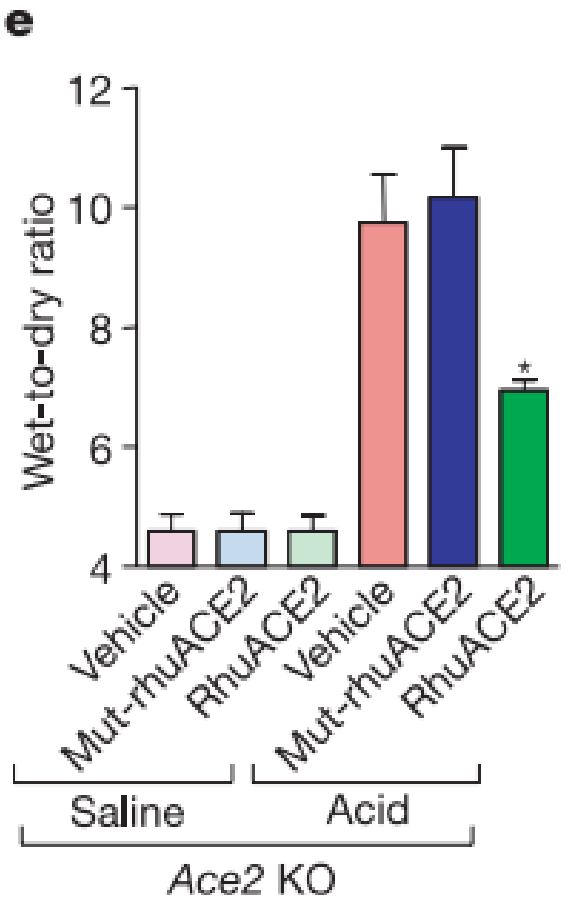


Downregulation of ACE2 expression by SARS-CoV infection

This is a self limitation!

Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116
https://www.nature.com/articles/nm1267

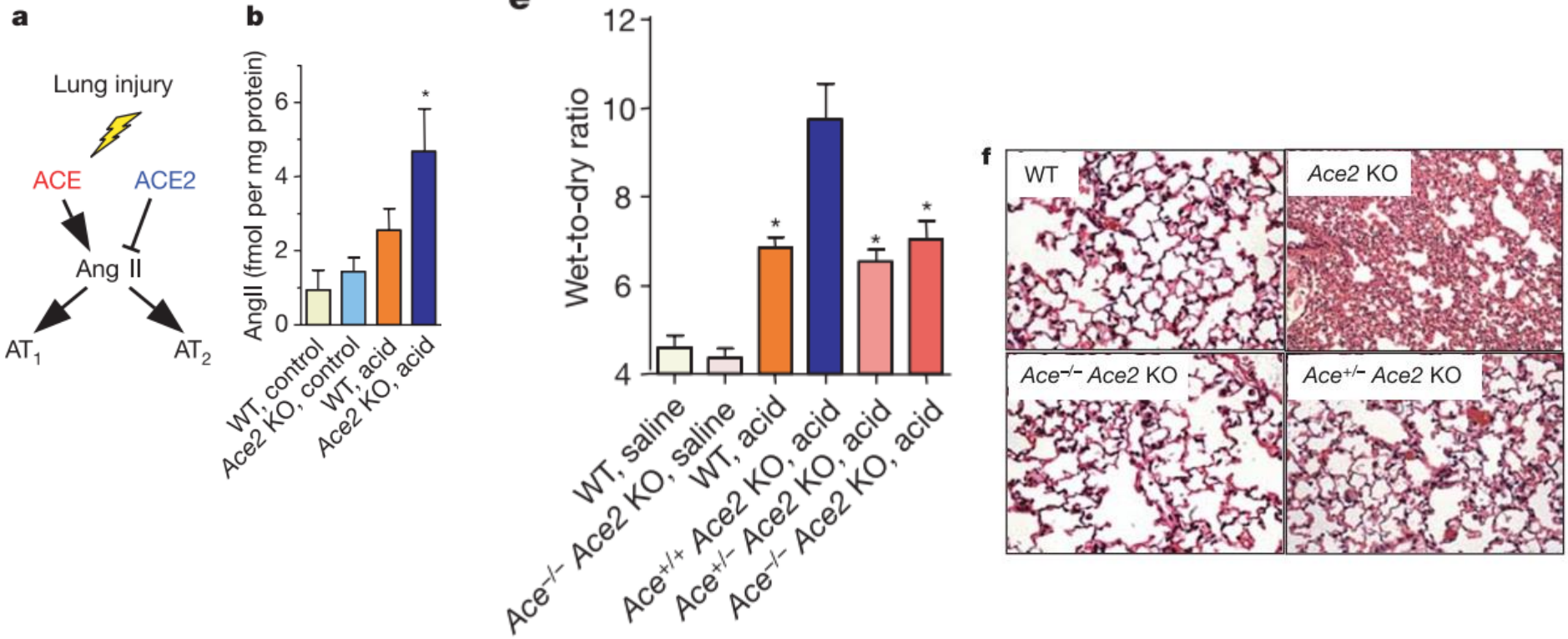
Rescue experiment using recombinant human ACE2 protein (IP)



- **Injection of catalytically active rhuACE2 into acid-treated *Ace2* knockout mice decreased the degree of pulmonary oedema formation**
- **Catalytically inactive ACE2 protein (mut-rhuACE2) did not rescue the severe lung phenotype in *Ace2* knockout mice**

Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

Acute lung injury results in decreased ACE2 expression and increased production of Ang II



Inactivation of *Ace* on an *Ace2* knockout background **rescued** the severe lung failure, oedema formation (Fig. 3e) and histological changes (Fig. 3f) compared with *Ace2* knockout mice

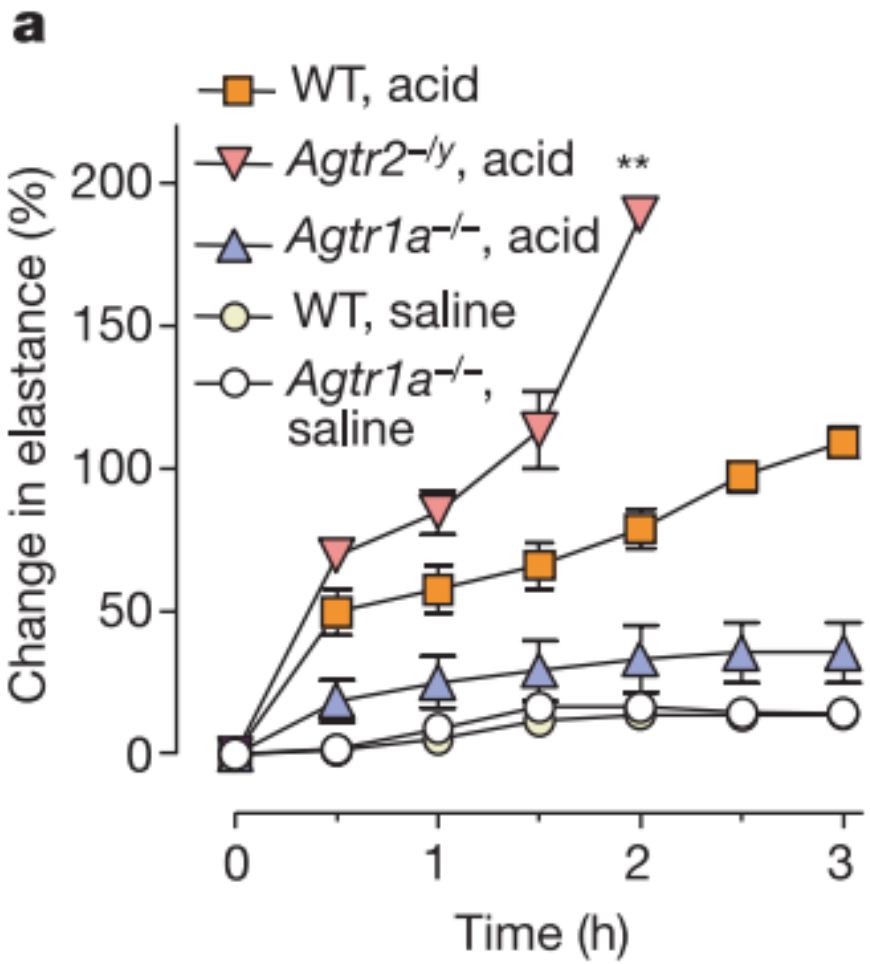
Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

AT_1a and AT_2 receptor expression is found in the **lungs**

AT_1aR: Involved in pathology

AT_2R: Protective

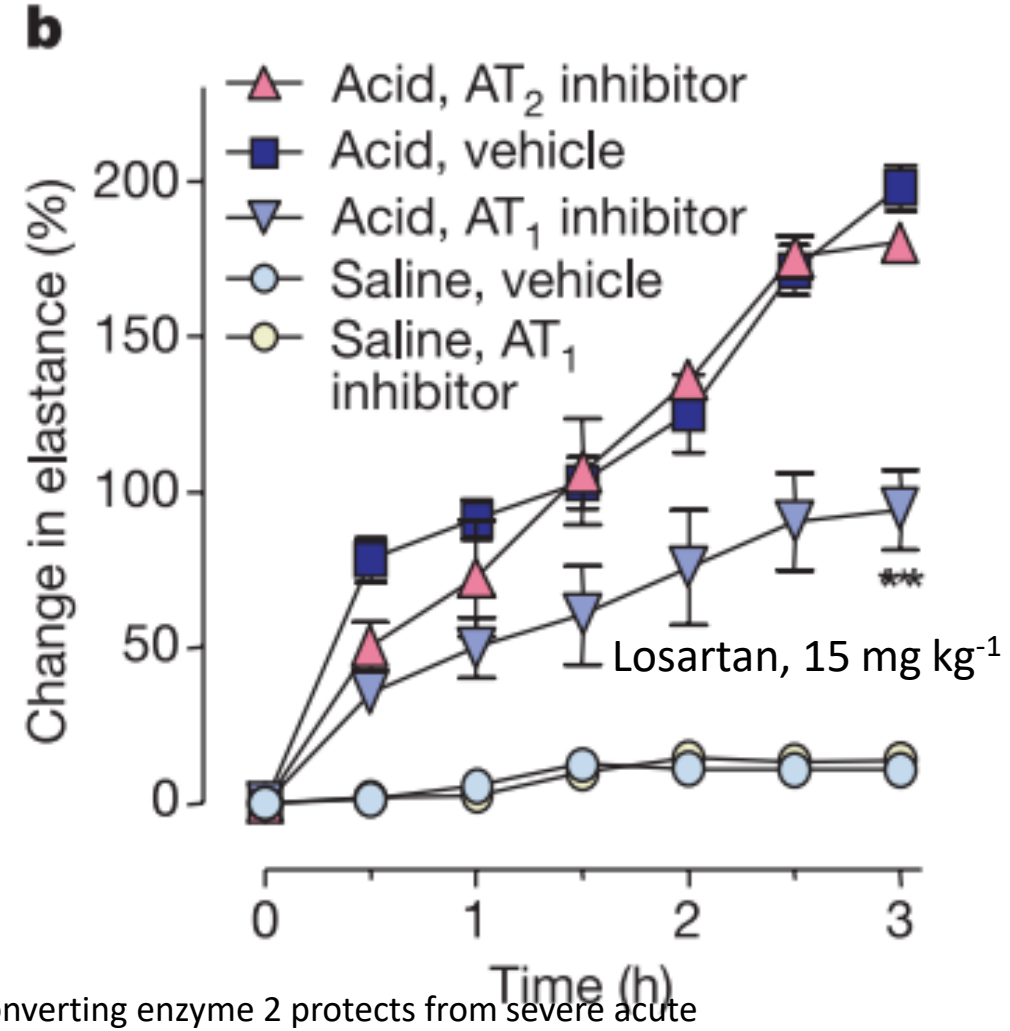
- *Agtr1a*$^{-/-}$ mice markedly improved lung function and reduced oedema formation.
- In contrast, inactivation of the AT_2 receptor (***Agtr2***$^{-/y}$) aggravated acute lung injury



Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

AT_1aR Blocker as RESCUE! (Even for ALI in COVID19?)

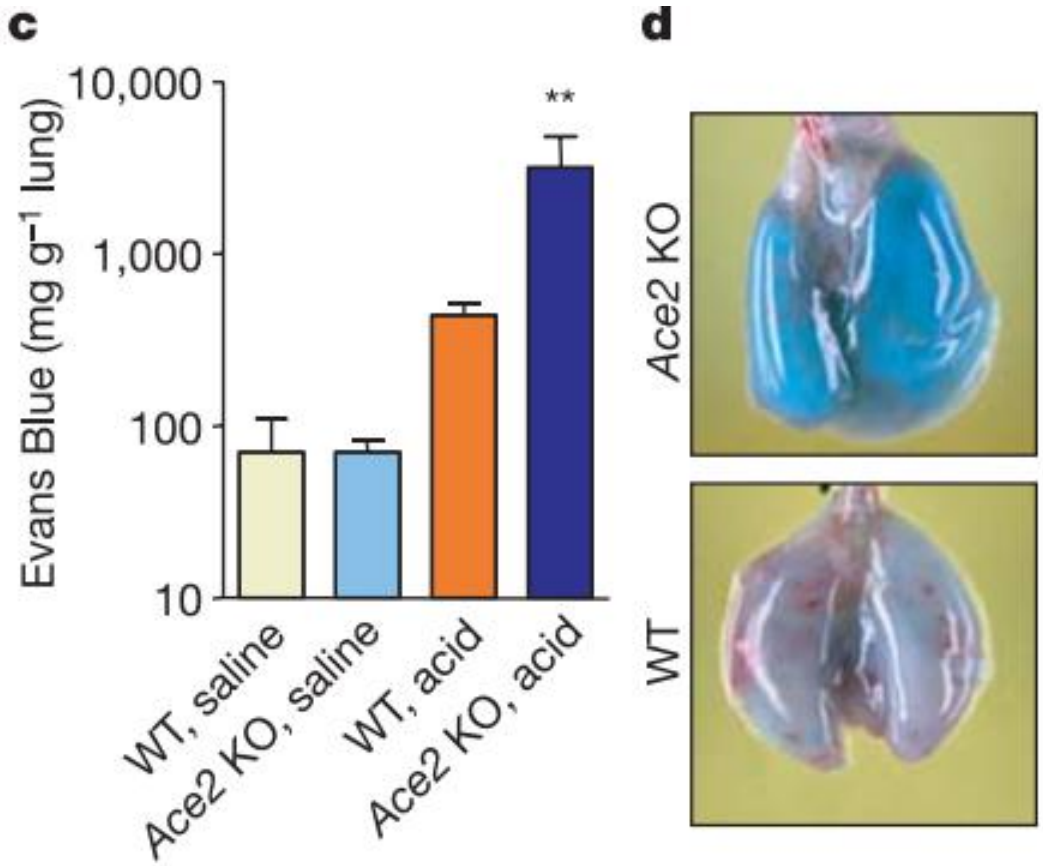
- Pharmacological **inhibition of AT_1 attenuated** the severity of acid-induced lung injury in *Ace2* knockout mice



Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

in increased vascular permeability in ALI

- **Loss of *Ace2* results in increased vascular permeability** using Evans Blue dye injections as an *in vivo* indicator of **albumin leakage**.
- **In *Ace2* knockout mice**, pulmonary **Evans Blue** accumulation **was greatly increased** after acid aspiration



Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

Conclusion of Imai WORK

- **ARDS is characterized by pulmonary oedema due to increased vascular permeability, the accumulation of inflammatory cells and severe hypoxia**
- **Ang II is upregulated by ACE and drives severe lung failure through the AT_1a receptor. On the other hand, ACE2 and the AT_2 receptor protect against lung injury**

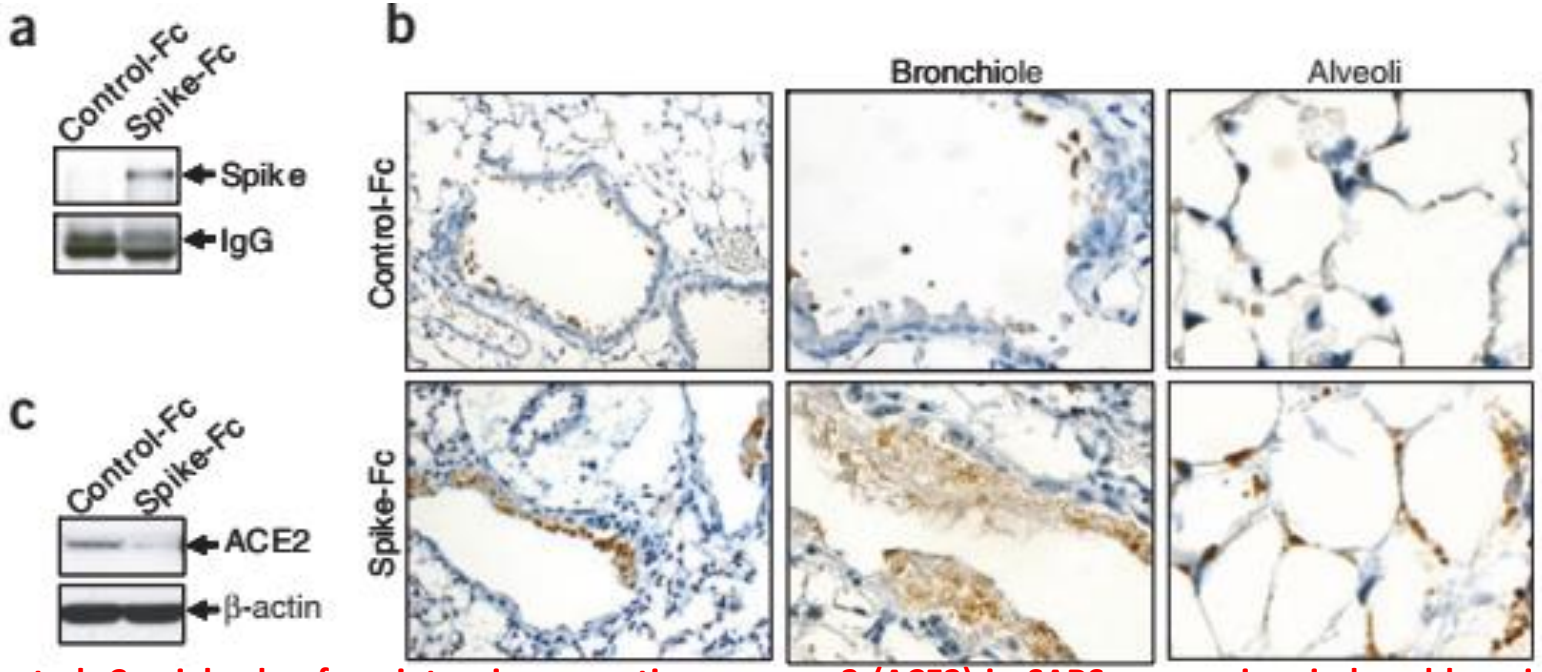
Imai, Y et al, Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **2005**, *436*, 112–116

SARS-CoV-induced ALI

- **SARS-CoV infections and the Spike protein of the SARS-CoV reduce ACE2 expression.**
- **Notably, injection of SARS-CoV Spike into mice worsens acute lung failure *in vivo* that can be attenuated by blocking the renin-angiotensin pathway.**

Kuba, K.; et al. Crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 2005, *11*, 875–879

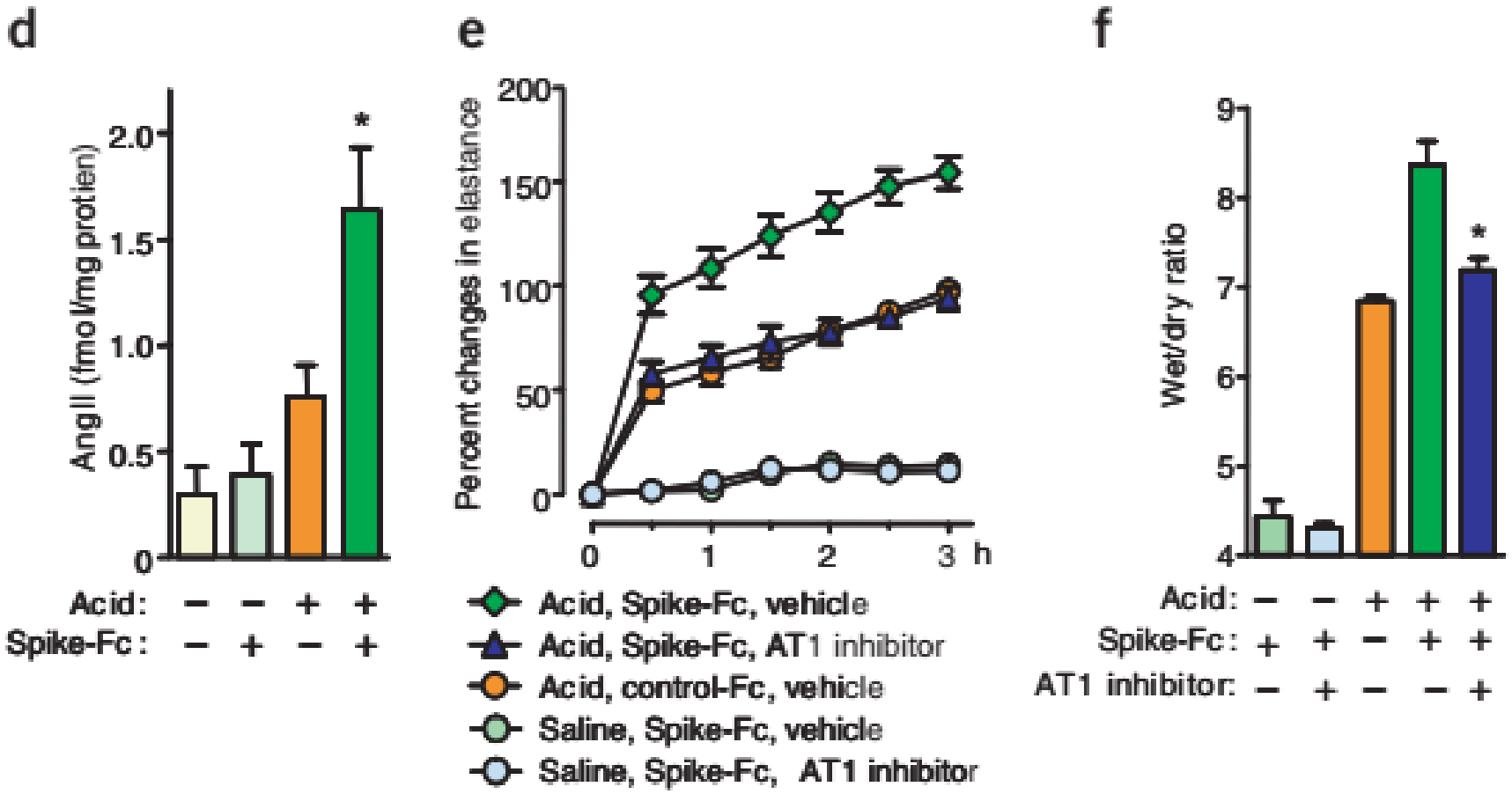
- **Spike-Fc treatment resulted in downregulation of ACE2 protein expression in lungs of acid-treated wild-type mice *in vivo*, consistent with ACE2 protein downregulation in SARS-CoV–infected mice. These results show that the SARS-CoV Spike protein can directly affect the development of severe acute lung failure through ACE2.**



Kuba, K.; et al. Crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 2005, *11*, 875–879.

D **Significant increase in AngII levels in the lung tissue of mice treated with Spike-Fc**

F **nhibition** of the AT1R also **attenuated pulmonary edema**



Kuba, K.; et al. Crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 2005, *11*, 875–879.

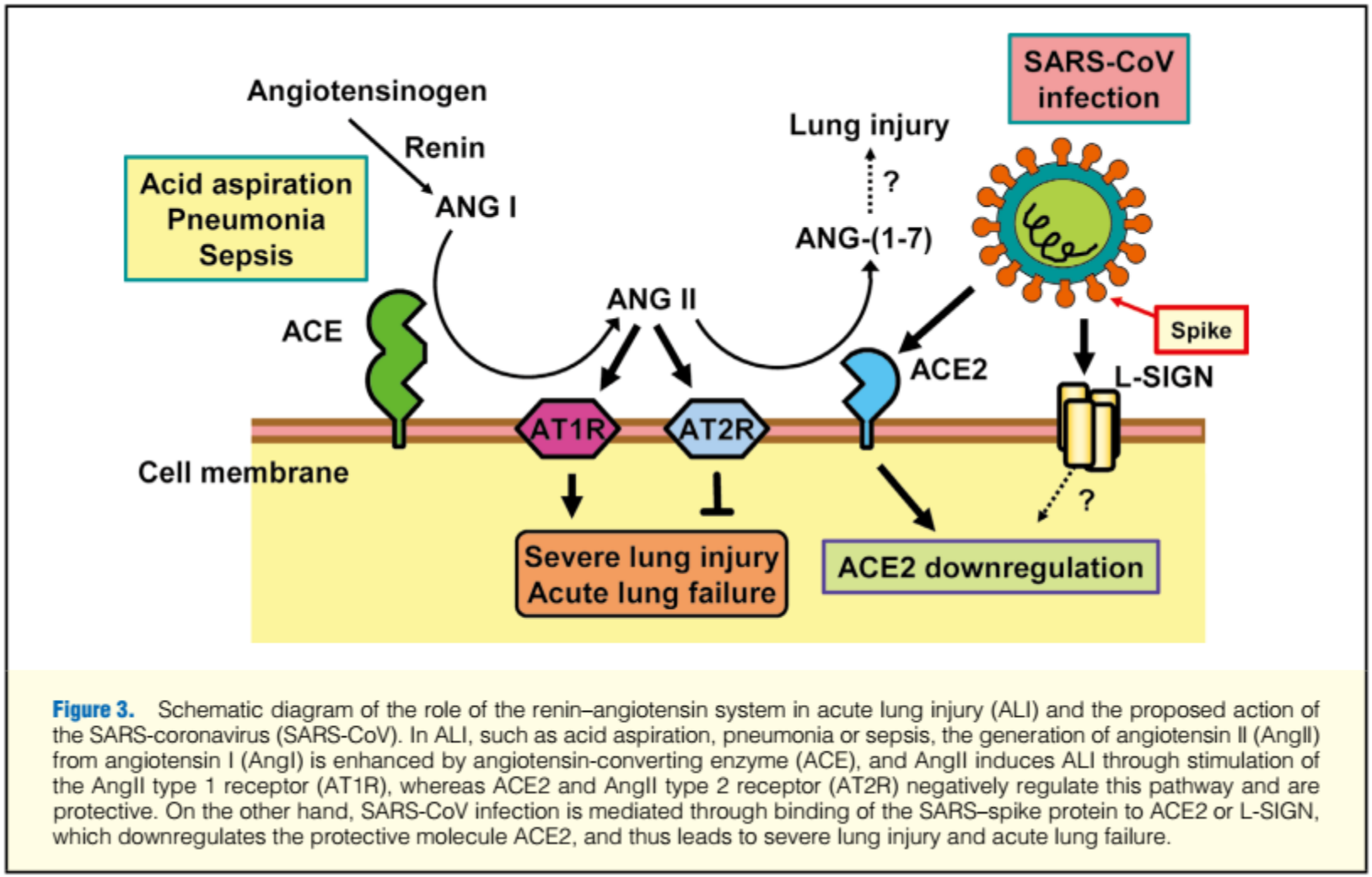


Figure 3. Schematic diagram of the role of the renin–angiotensin system in acute lung injury (ALI) and the proposed action of the SARS-coronavirus (SARS-CoV). In ALI, such as acid aspiration, pneumonia or sepsis, the generation of angiotensin II (AngII) from angiotensin I (AngI) is enhanced by angiotensin-converting enzyme (ACE), and AngII induces ALI through stimulation of the AngII type 1 receptor (AT1R), whereas ACE2 and AngII type 2 receptor (AT2R) negatively regulate this pathway and are protective. On the other hand, SARS-CoV infection is mediated through binding of the SARS–spike protein to ACE2 or L-SIGN, which downregulates the protective molecule ACE2, and thus leads to severe lung injury and acute lung failure.

Circ J 2010; **74:**405–410, doi:10.1253/circj.CJ-10-0045

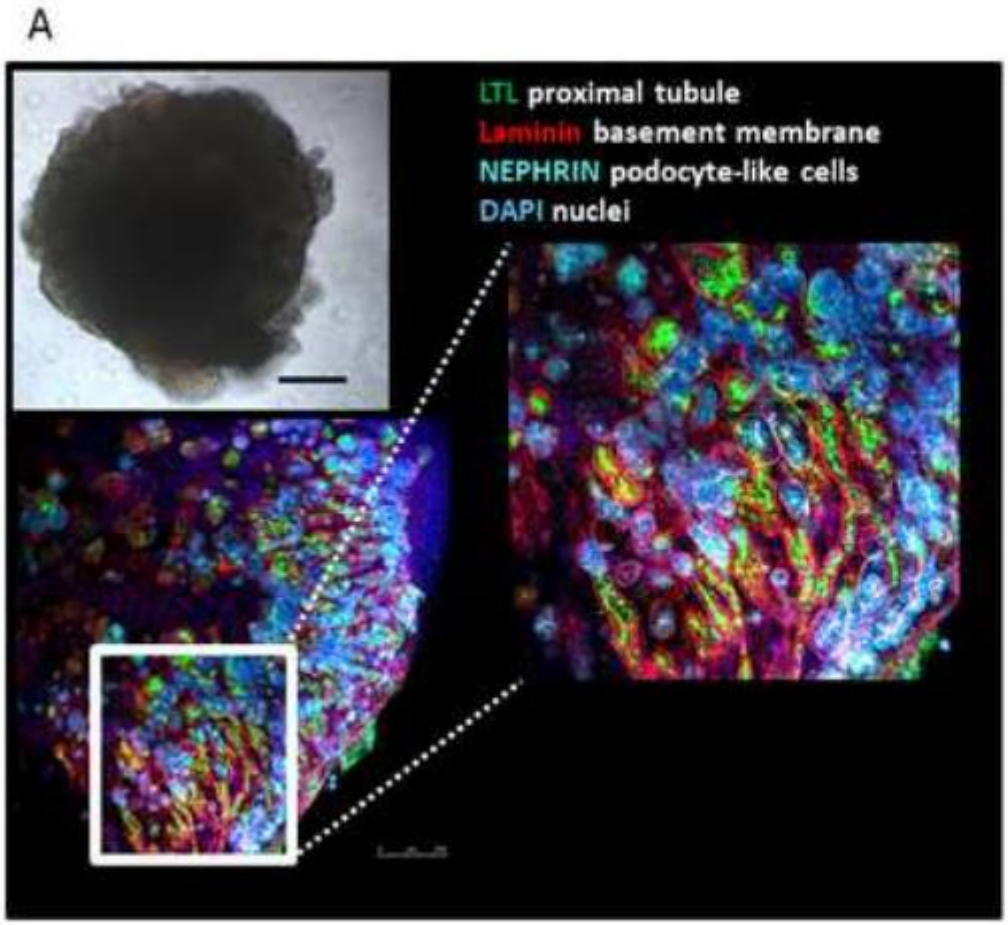
1

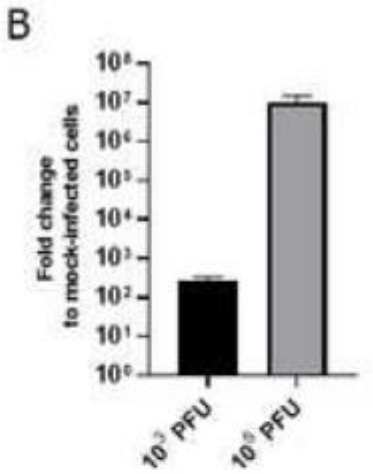
COVID-19: Exogenous ACE2 as Rescue drug

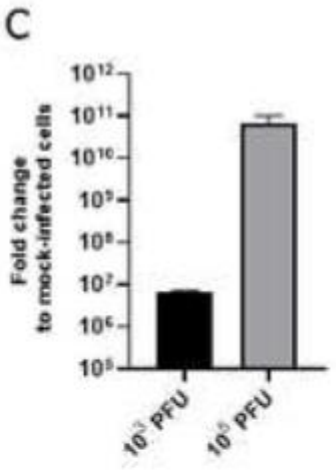
- Clinical grade hrsACE2 reduced SARS-CoV-2 recovery from Vero cells by a factor of 1,000-5,000.

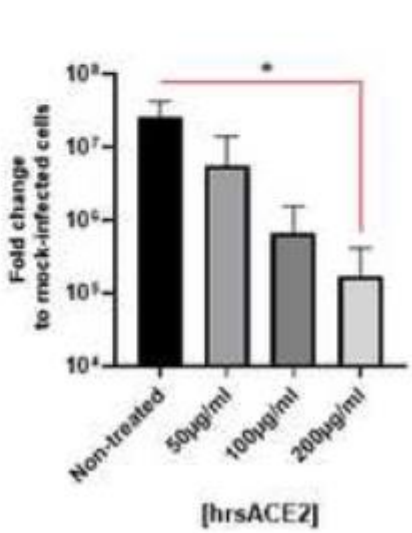
- SARS-CoV-2 can directly infect engineered human blood vessel organoids and human kidney organoids, which can be inhibited by hrsACE2.

DOI: 10.1016/j.cell.2020.04.004, 2020, Cell









LIMITATIONS

This study focused on the early stages of infection; hrsACE2 can block early entry of SARS-CoV-2 infections in host cells.

The RAS system represents a complex network of pathways which are influenced by external processes which are not simulated in this model system.

DOI: 10.1016/j.cell.2020.04.004, 2020, Cell

Active ACE2 as drug candidate

- Researchers already found that injecting exogenous rhACE2 protein could relieve lung injuries in several acute pneumonia experimental models and also show the ability to prevent angiotensin II–induced hypertension, myocardial hypertrophy, diastolic dysfunction, and myocardial fibrosis.

- Soluble ACE2 may act as the bait to neutralize the Spike protein on the surface of the SARS-CoV-2, thus inhibiting the invasion of viruses

Previous **CTs** on hrsACE2

- After **licensing** in February **2010**, several clinical trials from 2014 to 2017 to treat acute lung injury (ALI), acute respiratory distress syndrome (ARDS) and pulmonary arterial hypertension (PAH)
- **Safe and well-tolerated**
- Study **had not been powered** to detect changes in acute physiology or **clinical outcomes**
- **ARDS is characterized by pulmonary oedema due to increased vascular permeability, the accumulation of inflammatory cells and severe hypoxia**

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5588692/pdf/13054_2017_Article_1823.pdf

Upcoming CTs

- **These days, regulatory approvals obtained for the treatment of 200 severely infected COVID-19 patients in Austria, Germany and Denmark, and the first patients are expected to be dosed shortly.**

- **https://www.apeiron-biologics.com/wp-content/uploads/2020/04/20200402_APEIRON_Phase-2-EU-trial_APN01_ENG.pdf**

!

APEIRON BIOLOGICS

APEIRON Biologics Initiates Phase II Clinical Trial of APN01 for Treatment of COVID-19

- Regulatory approvals obtained for the treatment of 200 COVID-19 patients in Austria, Germany and Denmark; Austrian government to provide significant financial support
- APN01 has the potential to block the infection of cells by the novel COVID-19 virus and reduce lung injury
- APN01 was previously proven to be safe and well tolerated in Phase I and Phase II clinical trials
- First patient expected to be dosed shortly

Vienna, Austria, 02 April 2020: APEIRON Biologics AG today announced that it has received regulatory approvals in Austria, Germany and Denmark to initiate a Phase II clinical trial of APN01 to treat COVID-19. APN01 is the recombinant form of the human angiotensin-converting enzyme 2 (rhACE2), and has the potential to block the infection of cells by the novel SARS-CoV-2 virus (COVID-19), and reduce lung injury. The Phase II trial aims to treat 200 severely infected COVID-19 patients, and the first patients are expected to be dosed shortly.

https://www.apeiron-biologics.com/wp-content/uploads/2020/04/20200402_APEIRON_Phase-2-EU-trial_APN01_ENG.pdf

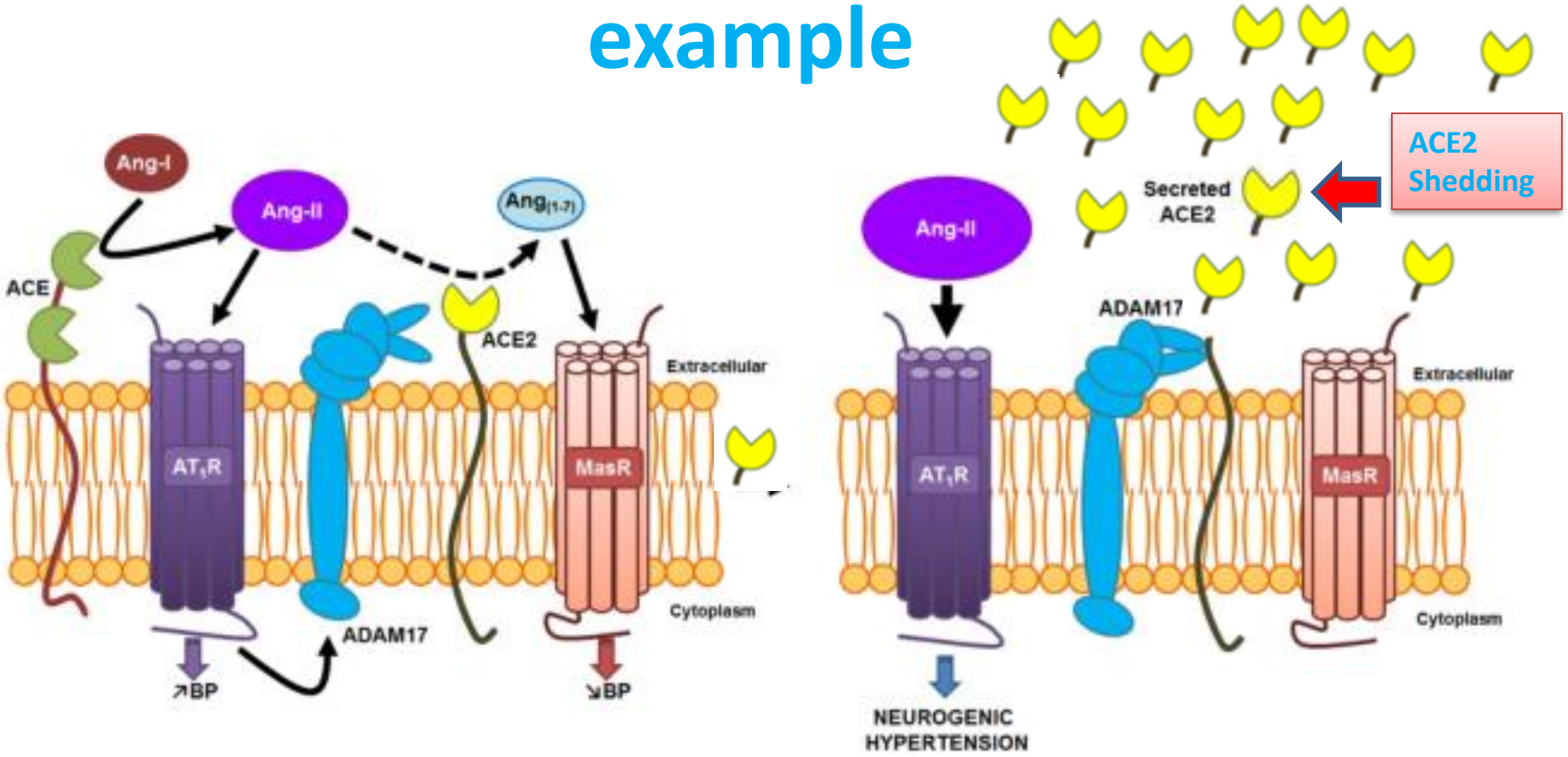
Significant mortality rate in patients with CVD?

- The myocardial dysfunction can be indirectly caused by reduced oxygen supply, severe lung failure, and the cytokine storm after the SARS-CoV-2 infection. However, there is also the possibility that it might be attributable to the (direct cardiocyte infection) decreased activity of ACE2 in the heart, just like SARS [https://www.ahajournals.org/doi/10.1161/JAHA.120.016219].
- However, direct evidence demonstrating that SARS-CoV-2 infects the heart and decreases the ACE2 expression is currently lacking.

2

COVID-19: Endogenous ACE2 as Suspect!

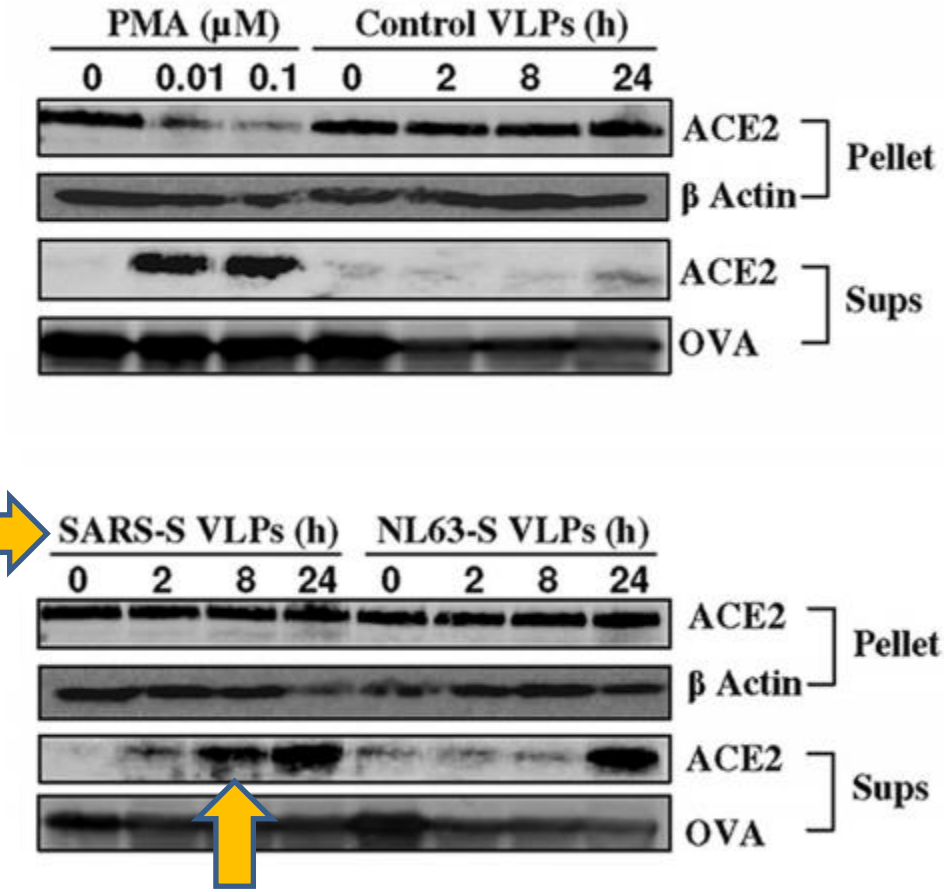
ACE2 Shedding: Definition with an example



RAS overactivity results in **increased** levels of **Ang-II and AT_1R expression**, leading to **increased expression of ADAM17** which in turn **cleaves ACE2**, resulting in **decreased membrane ACE2 levels**, thereby **decreasing Ang-(1-7) formation**, reducing MasR activation and ultimately contributing to the development of neurogenic hypertension

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4655210/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4479408/

VLPs bearing SARS-S triggered ACE2 shedding over time . The levels of cell-associated ACE2 remained constant, suggesting that ACE2 expression is constitutive



For control of comparable precipitation efficiency, **ovalbumin (OVA)** was added to supernatants and also detected by Western blot analysis.

Phorbol myristate acetate (PMA) treatment induced shedding of ACE2 into the cellular supernatants

https://jvi.asm.org/content/84/2/1198, JOURNAL OF VIROLOGY, Jan. 2010, p. 1198–1205 Vol. 84, No. 2 doi:10.1128/JVI.01248-09

When does ACE2 increase?

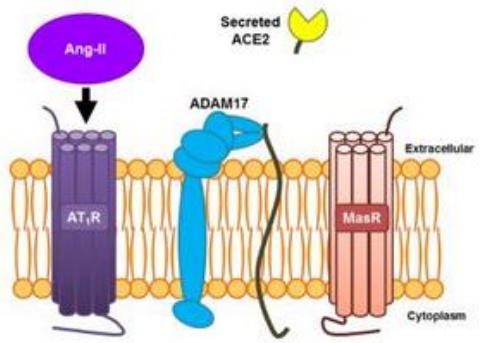
- **Membrane-bound ACE2, expressed in cardiomyocytes, cardiac fibroblasts, and coronary endothelial cells, as regulators for heart function**
- [https://doi.org/10.1161/JAHA.120.016219, Journal of the American Heart Association. 2020;9:e016219][The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7). **Physiol Rev**. 2018; 98:505–553].

- **Idiopathic and ischaemic cardiomyopathy** [Goulter, A. B., Goddard, M. J., Allen, J. C. and Clark, K. L. (2004) ACE2 gene expression is up-regulated in the human failing heart, BMC Med. 2, 19]

- **Higher serum ACE2 activity is correlated with increasing severity of heart failure** [(2008) Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. J. Am. Coll. Cardiol. 52, 750–754].

- **ACE2 activity, either as a cause or an effect of CVD, may be also influenced by intrinsic factors such as genetic variation and single nucleotide polymorphism (SNP)** [https://www.ncbi.nlm.nih.gov/pubmed/23013041]

Extracellular matrix metalloproteinase; ADAM17 (A disintegrin and metalloproteinase 17), also known as TNFα converting enzyme (TACE, as “TACE:ADAM17”)



- Zamami raised an **opposing opinion** and described the rational for **inhibition of ACE2!** pathways as specific targets in patients with critical, advanced and untreatable stages of **SARS-CoV-2 infection**
- **SARS-CoV-2 infection – RAAS Over-activation -- activation of “TACE:ADAM17”)----“ACE2 shedding” and “TNFα shedding/production”**
- **Pro-inflammatory cytokines** (IL-1β, TNFα ⇧), cytokine storm, **trigger** surface ACE2 down-modulation and the free enzyme shedding
- [Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. *Am. J. Physiol.- Lung Cell. Mol. Physiol.* **2009**, *297*, 84–96].

[The rational for administration of ACE2 pathway inhibitors in patients infected by SARS-CoV-2: Devising an administration strategy, doi:10.20944/preprints202003.0338.v2]

“Protective ACE2 shedding feedback”

Bound-ACE2 is yet ACTIVE

[Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2009**, *297*, 84–96] [Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 7809–7814] [Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. *EMBO J.* **2005**, *24*, 1634–1643]

Various researchers found an **intriguing positive correlation** between **ACE2 shedding** (or soluble ACE2 activity) and viral infection**, disease severity/complications**

[Retroviruses Pseudotyped with the Severe Acute Respiratory Syndrome Coronavirus Spike Protein Efficiently Infect Cells Expressing Angiotensin-Converting Enzyme 2. *J. Virol.* 2004, *78*, 10628–10635][TACE antagonists blocking ACE2 shedding caused by the spike protein of SARS-CoV are candidate antiviral compounds. *Antiviral Res.* 2010, *85*, 551–555][Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. *Proc. Natl. Acad. Sci. U. S. A.* 2008, *105*, 7809–7814]

Hypotension, as common clinical features in **SARS-CoV patients**

[Cardiovascular complications of severe acute respiratory syndrome. *Postgrad. Med. J.* **2006**, *82*, 140–144]

Dramatic increase of ACE2 expression in the SARS-CoV-infected bronchial cells (Bioinformatic analyses)

[Integrative Bioinformatics Analysis Provides Insight into the Molecular Mechanisms of 2019-nCoV. *medRxiv Prepr.* **2020**]

Elevated plasma sACE2 activity, Ang (1-7) or sACE2 upregulation have been associated with pathological conditions including GI tract inflammation, inflammatory bowel disease, cirrhosis, lung injury/fibrosis and greater severity of myocardial dysfunction/infarction [Soluble Angiotensin-Converting Enzyme 2 in Human Heart Failure: Relation With Myocardial Function and Clinical Outcomes. *J. Card. Fail.* **2009**, *15*, 565–571] [Role of Circulating Angiotensin Converting Enzyme 2 in Left Ventricular Remodeling following Myocardial Infarction: A Prospective Controlled Study. *PLoS One* **2013**, *8*, 2–9] [Upregulation of circulating components of the alternative renin-angiotensin system in inflammatory bowel disease: A pilot study. *JRAAS - J. Renin-Angiotensin-Aldosterone Syst.* **2015**, *16*, 559–569].

Inhibition of fibrosis/hypertrophy by ACE2 inhibitor in animal model myocardium, confirms the adverse effect of “ACE2 activity [Effects of ACE2 Inhibition in the Post-Myocardial Infarction Heart. **2010**, *16*, 777–785].

- **Upregulated ACE2 (mRNA and protein) expression under hypoxic conditions**
- **ACE2 pathway upregulation induced by hypercapnia/hypoxia (an observed condition in SARS patients**
- **Activation of bax/caspase–dependent apoptotic pathway in lung fibroblasts after Ang-(1-7) treatment**
- **Promoted inflammatory cytokines (including TNF-alfa, and IL-6) upon *in vivo* administration of Ang-(1-7)**
- **Ang-(1-7)-promoted eosinophil apoptosis in lung broncoalveolar lavage (washing) fluid**
- **Up-regulated IL-10/IL-6 cytokines by a nonpeptide angiotensin-(1-7) receptor agonist**
- **Increased circulating ACE2 activity in SARS severe cases with "hypertension or diabetes or dyslipidemia"**
- **All these evidences, together, are reminiscent of a strong positive correlation between ACE2/Ang (1-7) axis activation (circulating sACE2 activity) and SARS-CoV-2 infection and the disease progression.**

References of previous page

- Elevated plasma sACE2 activity, Ang (1-7) or sACE2 upregulation have been associated with pathological conditions including GI tract inflammation, inflammatory bowel disease, cirrhosis, lung injury/fibrosis and greater severity of myocardial dysfunction/infarction [Soluble Angiotensin-Converting Enzyme 2 in Human Heart Failure: Relation With Myocardial Function and Clinical Outcomes. *J. Card. Fail.* **2009**, *15*, 565–571] [Role of Circulating Angiotensin Converting Enzyme 2 in Left Ventricular Remodeling following Myocardial Infarction: A Prospective Controlled Study. *PLoS One* **2013**, *8*, 2–9] [Upregulation of circulating components of the alternative renin-angiotensin system in inflammatory bowel disease: A pilot study. *JRAAS - J. Renin-Angiotensin-Aldosterone Syst.* **2015**, *16*, 559–569]. Moreover, inhibition of fibrosis/hypertrophy by ACE2 inhibitor in animal model myocardium, confirms the mentioned adverse effect of "ACE2 activity [Effects of ACE2 Inhibition in the Post-Myocardial Infarction Heart. **2010**, *16*, 777–785].
- Upregulated ACE2 (mRNA and protein) expression in human hepatocytes [Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. *Gut* **2005**, *54*, 1790–1796] and pulmonary artery smooth muscle cells [Role of HIF-1α in the regulation ACE and ACE2 expression in hypoxic human pulmonary artery smooth muscle cells. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2009**, *297*, 631–640] under hypoxic conditions, possible ACE2 pathway upregulation induced by hypercapnia/hypoxia (an observed condition in SARS patients) [Cyclooxygenase mediates cardioprotection of angiotensin-(1-7) against ischemia/reperfusion-induced injury through the inhibition of oxidative stress. *Mol. Med. Rep.* **2011**, *4*, 1145–1150], ang II-induced lung microvascular permeability and subsequently favored "sACE2 and Ang (1-7)" diffusion in neighbouring lung tissues [The rational for administration of ACE2 pathway inhibitors in patients infected by SARS-CoV-2: Devising an administration strategy, doi:10.20944/preprints202003.0338.v2], activation of bax/caspase–dependent apoptotic pathway in lung fibroblasts after Ang-(1-7) treatment [Angiotensin-converting enzyme 2/angiotensin-(1-7)/mas axis protects against lung fibrosis by inhibiting the MAPK/NF-κB pathway. *Am. J. Respir. Cell Mol. Biol.* **2014**, *50*, 723–736], promoted morphological lung alterations as well as inflammatory cytokines (including TNF-alfa, and IL-6) upon *in vivo* administration of Ang-(1-7) alone in rats [Angiotensin-converting enzyme 2/angiotensin-(1-7)/mas axis protects against lung fibrosis by inhibiting the MAPK/NF-κB pathway. *Am. J. Respir. Cell Mol. Biol.* **2014**, *50*, 723–736], Ang-(1-7)-promoted eosinophil apoptosis in lung broncoalveolar lavage (washing) fluid [Angiotensin-(1-7) promotes resolution of eosinophilic inflammation in an experimental model of asthma] (SARS-CoV-2 infection is associated with Lymphopenia and eosinopenia [The rational for administration of ACE2 pathway inhibitors in patients infected by SARS-CoV-2: Devising an administration strategy, doi:10.20944/preprints202003.0338.v2]), inhibited allergic airway inflammation and reduced asthma symptoms upon ACE2 pathway activation [Angiotensin-(1-7) inhibits allergic inflammation, via the MAS1 receptor, through suppression of ERK1/2- and NF-kB dependent pathways. *Br. J. Pharmacol.* **2012**, *166*, 1964–1976], up-regulated IL-10/IL-6 cytokines by a nonpeptide angiotensin-(1-7) receptor agonist [AVE 0991, a non-peptide mimic of angiotensin-(1-7) effects, attenuates pulmonary remodelling in a model of chronic asthma. *Br. J. Pharmacol.* **2013**, *170*, 835–846] and in the most severe SARS cases [http://www.aginganddisease.org/EN/10.14336/AD.2020.0228], increased circulating ACE2 activity in subjects with "hypertension or diabetes or dyslipidemia" as well as confirmation/identification of "advanced age" as predictor of enhanced ACE2 activity [Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease. *Nephrol. Dial. Transplant.* **2015**, *30*, 1176–1185], increased circulating ACE2 activity in subjects under treatment with angiotensin II receptor blockers (ARBs) but not ACE inhibitors, oral antidiabetic agents and insulin (or smokers, although not significantly), suggesting that ARBs should be avoided to reduce ACE2-mediated viral entry [Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease. *Nephrol. Dial. Transplant.* **2015**, *30*, 1176–1185]; all these evidences, together, are reminiscent of a strong positive correlation between ACE2/Ang (1-7) axis activation (circulating sACE2 activity) and SARS-CoV-2 infection and the disease progression.
- Since these data imply that the SARS coronavirus sustains ACE2 pathway activation and also the clinical picture as a whole (eosinopenia, hypotension and elevated pro-inflammatory cytokine profile, as downstream events stemming from an excessive ACE2 pathway activation/upregulation), is consistent with a ACE2 gain of function (rather than loss of function), inhibition of ACE2/Ang (1–7)/Mas receptor axis has been recently suggested [The rational for administration of ACE2 pathway inhibitors in patients infected by SARS-CoV-2: Devising an administration strategy, doi:10.20944/preprints202003.0338.v2].

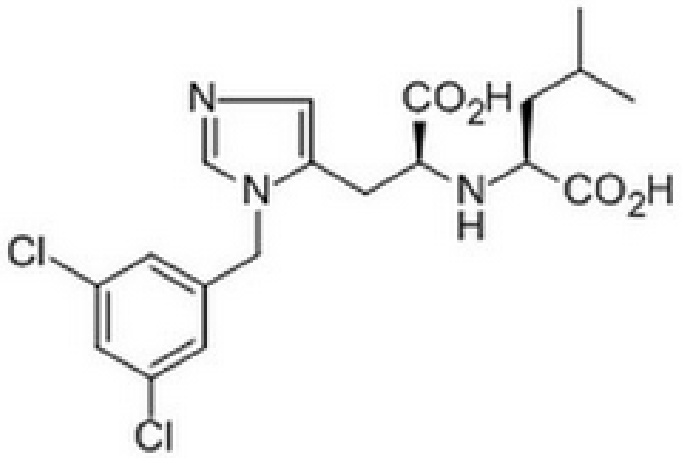
Why ACE2 Pathway Inhibition?

- Since these data imply that the **SARS coronavirus sustains ACE2 pathway activation** and also the **clinical picture as a whole** (eosinopenia, hypotension and elevated pro-inflammatory cytokine profile, as downstream events stemming from an excessive ACE2 pathway activation/upregulation), **is consistent with** a **ACE2 gain of function** (rather than loss of function), **inhibition of ACE2/Ang (1–7)/Mas receptor axis is suggested .**
- [The rational for administration of ACE2 pathway inhibitors in patients infected by SARS-CoV-2: Devising an administration strategy, doi:10.20944/preprints202003.0338.v2].

MLN-4760 is a potent and selective human ACE2 inhibitor (IC_{50}, 0.44 nM), with excellent selectivity (>5000-fold) versus ACE (IC_{50}, >100 μM)

ACE2 Inhibitor, MLN-4760

Pack Size	Price (EUR)
2 mg	217.00 ★

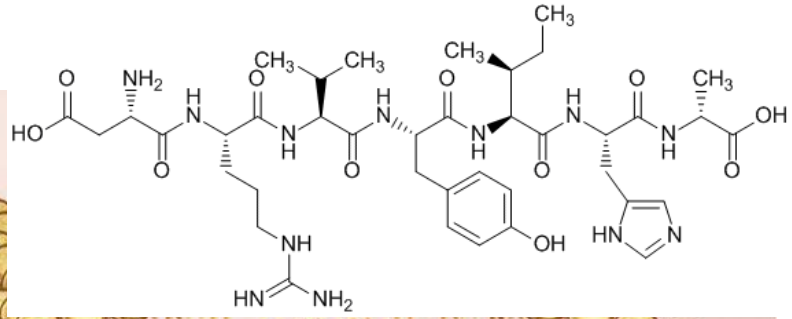


https://www.medchemexpress.com/MLN-4760.html

Joshi S, Balasubramanian N, Vasam G, J.Y. Angiotensin converting enzyme versus angiotensin converting enzyme-2 selectivity of MLN-4760 and DX600 in human and murine bone marrow-derived cells. *Eur J Pharmacol.* **2016**, *774*, 25–33

- Inhibition of Mas receptor activation through **Mas receptor antagonists** [**A-779** and **D-Pro7-Ang-(1-7)**] could be an alternative approach

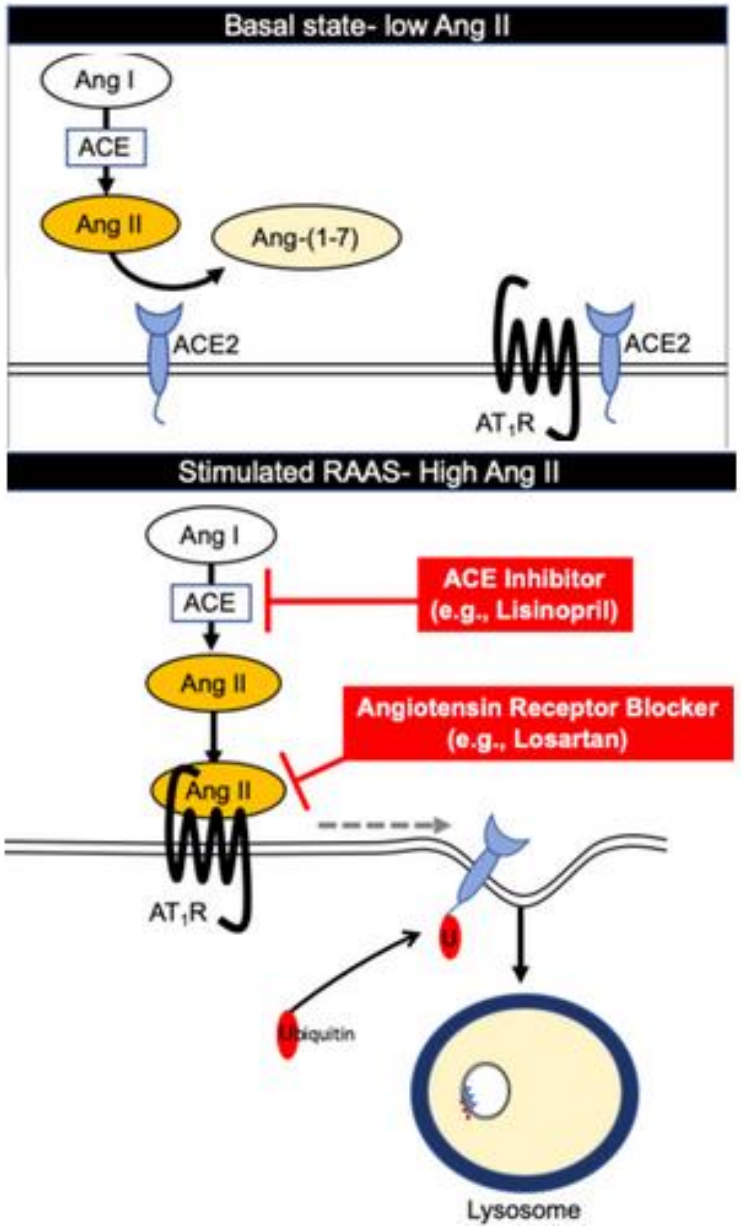
A 779, angiotensin-(1-7) antagonist



http://pharmrev.aspetjournals.org/content/66/4/1080

- Since ACE2 (which is widely expressed in the cardiovascular system) is involved in heart function and the development of hypertension and diabetes mellitus, SARS-CoV-2 might cause chronic damage to cardiovascular system, so attention **should be given to cardiovascular protection during treatment for COVID-19**

- **ACE2 interacts with the type I angiotensin receptor (AT1 receptor, the target of ARBs). Acutely, Ang II decreased ACE2 expression in a tissue-dependent manner, by internalization into lysosomes. This internalization was prevented by AT1 receptor antagonist losartan**



- **Surface ACE2**
- **ACE2 Shedding**
- **Soluble ACE2**

https://www.ahajournals.org/doi/10.1161/HYPERTENSIONAHA.114.03743
http://www.nephjc.com/news/covidace2

- **Increased** circulating ACE2 activity in subjects under treatment with angiotensin II receptor blockers (ARBs. Losartan) but not ACE inhibitors, oral antidiabetic agents and insulin (or smokers, although not significantly), **suggesting that ARBs should be avoided to reduce ACE2-mediated viral entry.**

- [Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease. *Nephrol. Dial. Transplant.* **2015**, *30*, 1176–1185]

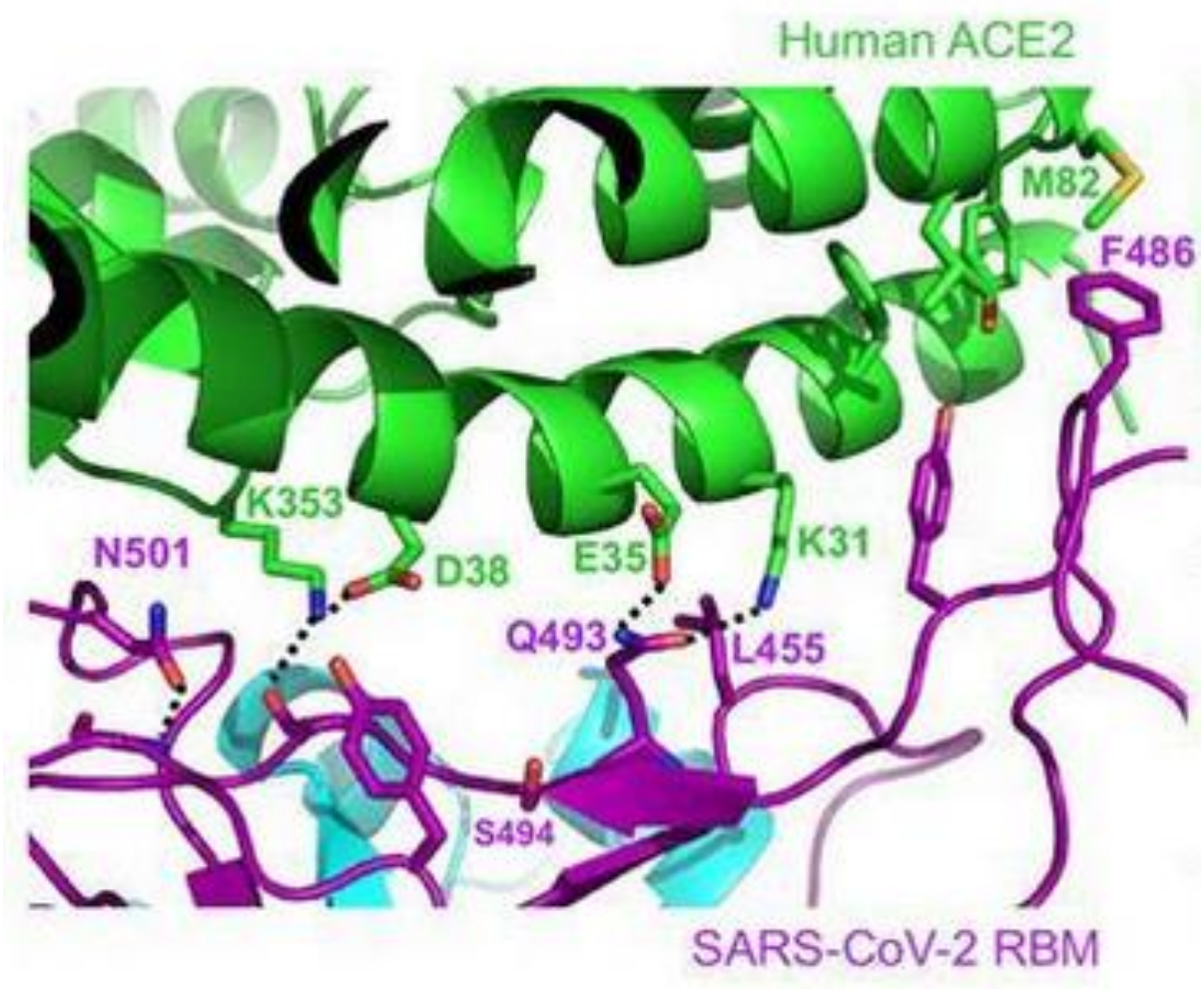
- Considering the highly expression of ACE2, SARS-CoV-2 mainly invades alveolar epithelial cells, resulting in respiratory symptoms. ACE2 is also over-expressed in heart failure, arterial hypertension and diabetes mellitus. The disease symptoms are more severe in patients with CVD, which might be associated with increased secretion of ACE2 in these patients, compared with healthy individuals. Given that ACE2 is a functional (or catalytically active) receptor for SARS-CoV-2, the safety and potential effects of "functional hrsACE2" therapy in patients with COVID-19 should be carefully considered.

Is inactive ACE suitable ?

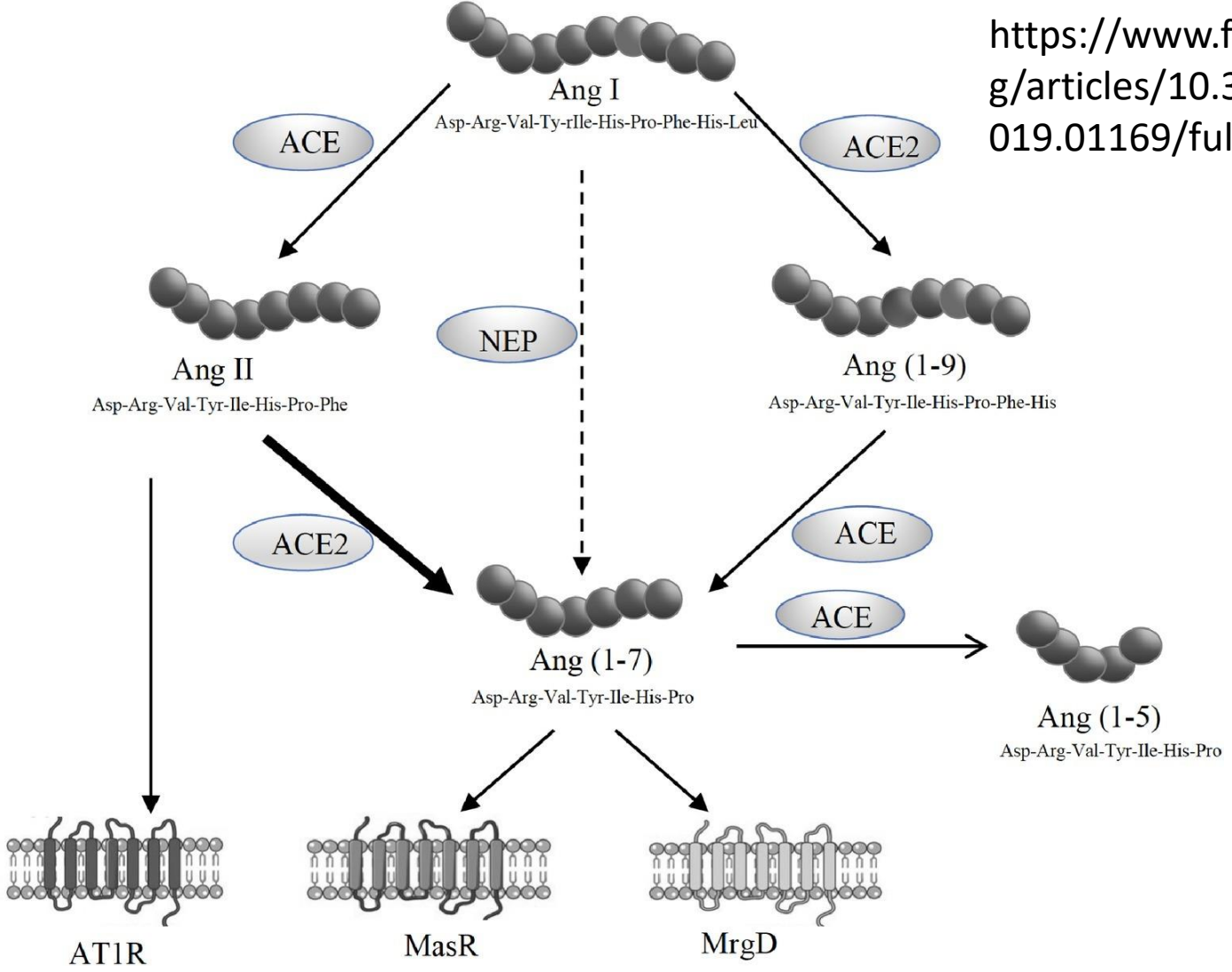
Catalytically inactive sACE2 can potently inhibit SARS-CoV infection

[*J. Virol.* 2004, *78*, 10628–10635]

ACE2 active site: (His^{374}, His^{378}, and Glu^{402})

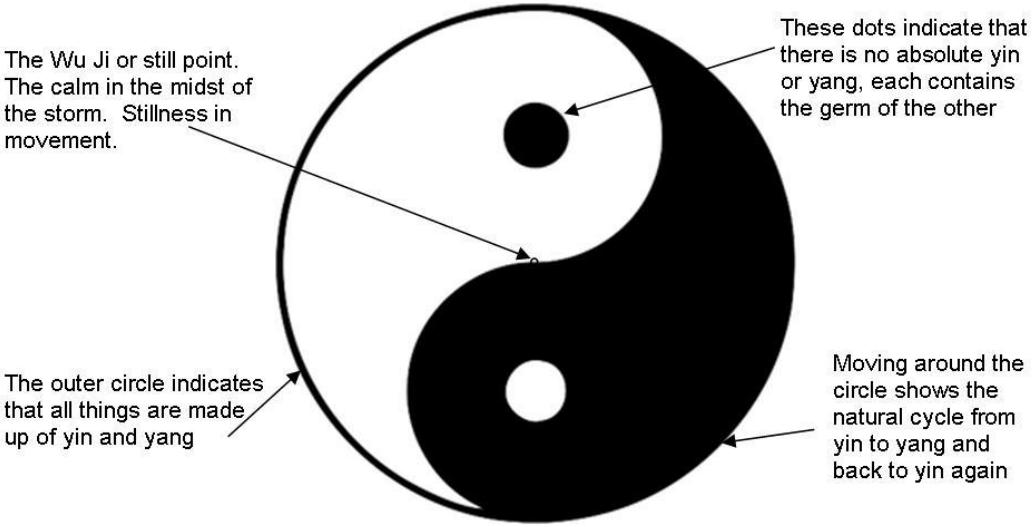


https://www.frontiersin.org/articles/10.3389/fphys.2019.01169/full



Schematic representation of the renin angiotensin system (RAS) showing the pathways responsible for the generation of angiotensin II (Ang II) and angiotensin-(1-7) [Ang-(1-7)]. **Ang II acts *via* its type 1 receptor (AT1R) to exert vasoconstrictive effects**. Ang II is degraded to Ang-(1-7) by angiotensin converting enzyme 2 (ACE2). Ang-(1-7) opposes Ang II effects through its receptors, MasR and MrgD.

Yin-Yang, a Symbol of Balance and Harmony



Yin-Yang balance of ACE/ACE2 pathways

Ref. The rational for administration of ACE2 pathway inhibitors in patients infected by SARS-CoV-2: Devising an administration strategy, doi:10.20944/preprints202003.0338.v2

The classical RAS pathway ACE/AngII/AT1R is balanced by the 'new' arm of the RAS, namely the ACE2/Ang-(1–7)/mas receptor axis

Thank you

- **Any Comment/question, please**

- The classical RAS consists of various axes, including the renin/angiotensin-converting enzyme (ACE)/angiotensin II (Ang II)/Ang II type 1 receptor (AT1R) axis, whose components have been widely identified to play physiological roles in the regulation of cardiovascular and renal function, blood pressure, aldosterone biosynthesis and release, and body salt and fluid balance.
- Angiotensin-converting enzyme 2/angiotensin-(1–7)/mitochondrial assembly receptor [**ACE2/Ang-(1–7)/MasR**] axis, which is one component of the RAS, has recently been identified as a critical component of pulmonary systems, gastric mucosa, and cancer.
- **Counter-regulatory role for Ang-(1-7) by opposing many actions of Ang II on AT1receptors**

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Renin-angiotensin-aldosterone system