

**Humanized mouse models
to study infections such as SARS-Cov-2 and
their treatments**

**Humanizované myší modely
pro studium infekcí jako je SARS-Cov-2 a jejich
léčby**

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SARS-CoV-2

What we know and which question remains

- more than 12 million confirmed cases later, the COVID-19 pandemic has become the worst public-health crisis in a century
- more than 540,000 people have died worldwide
- we have learnt how the virus enters and hijacks cells, how some people fight it off and how it eventually kills others.
- Drugs have been identified that benefit the sickest patients, and many more potential treatments are in the works.
- Nearly 200 potential vaccines have been developed— the first of which could be proved effective by the end of the year.

... for every insight into COVID-19, more questions emerge and others remains

SARS-CoV-2

What are the pathogenic features, transmission routes, and infection mechanisms of SARS-CoV-2?

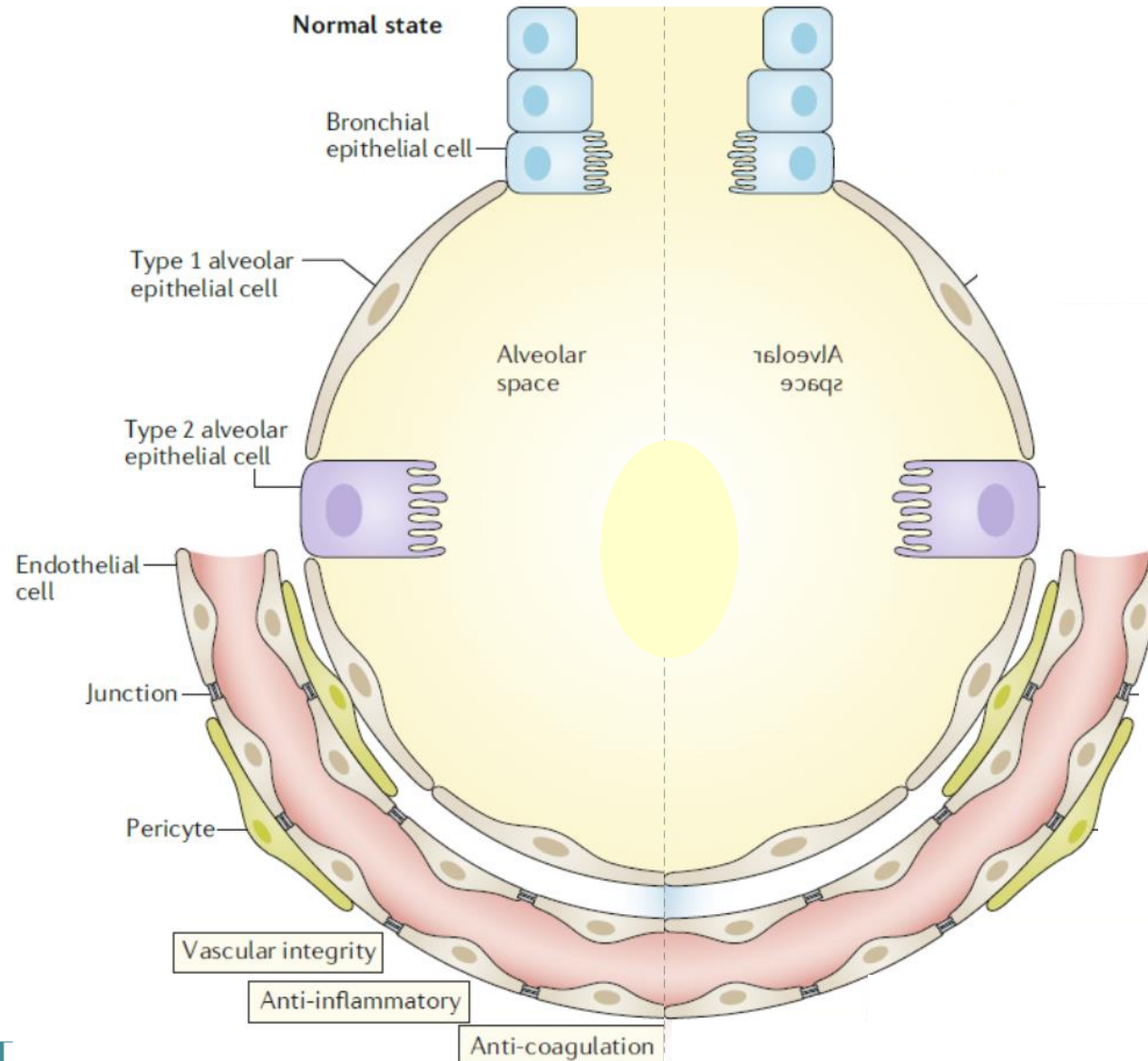
- How did the new coronavirus get into people?
- What drives mortality in people infected by the coronavirus?
- What coronavirus causes in our body?
- Are there any safe and effective drugs to treat COVID-19?
- What are the long-term consequences for those who survive COVID-19?

.... and many other

Proposed vessel–lung tissue interface in normal state and in CoVID-19 disease

On the left side:

the normal interface between the alveolar space and endothelial cells is depicted



the right side:

pathophysiological features of COVID-19 in the lung, including loss of vascular integrity (1), activation of the coagulation pathway (2) and inflammation (3).

Where is ACE2 expressed - in which tissues can we find it?

in human organs and tissues

lung
stomach
small intestine and colon
Skin
lymph nodes
thymus
bone marrow,
spleen
liver
kidney
testes
brain

ACE2 is highly expressed

in the **lungs** and **small intestine**

and

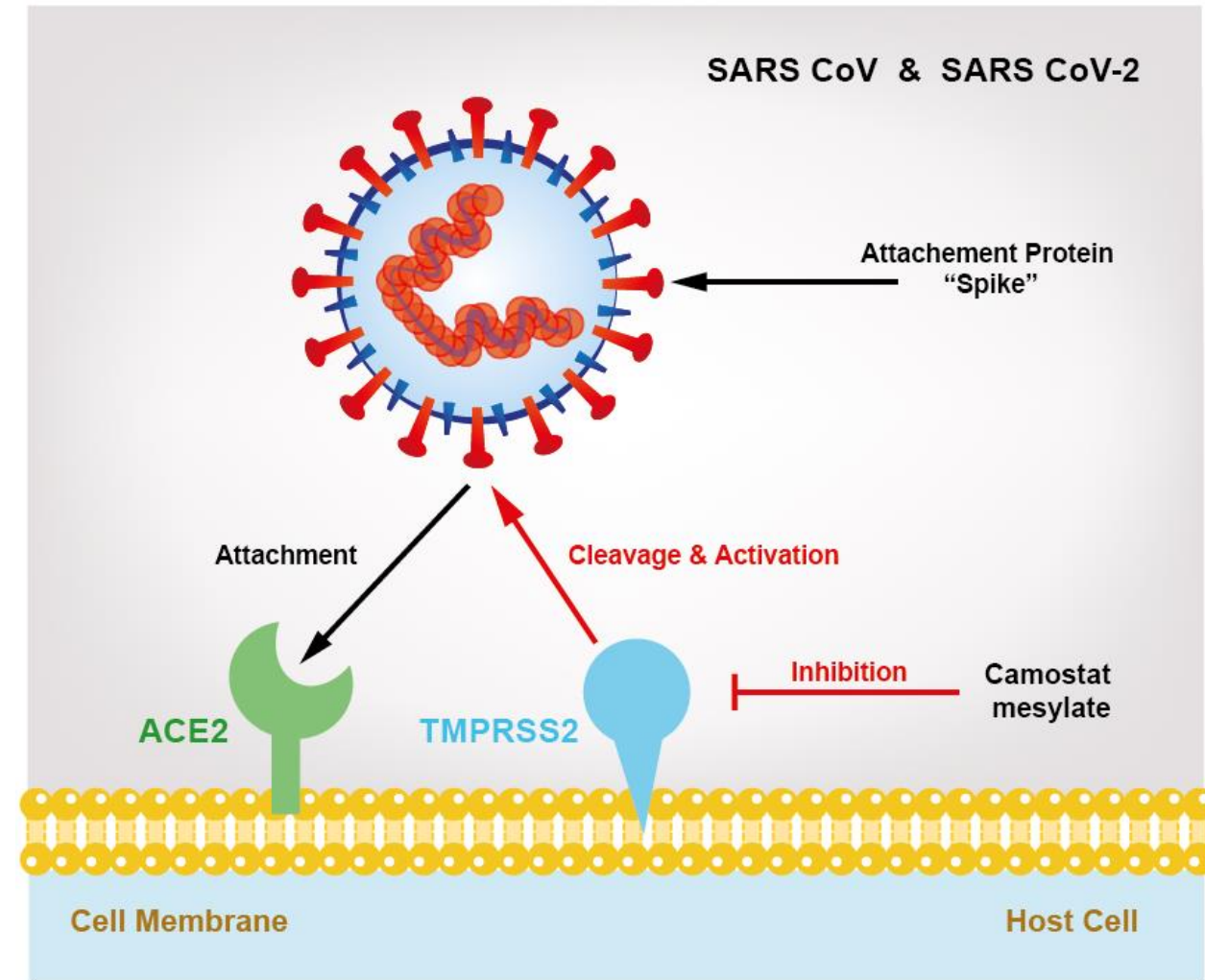
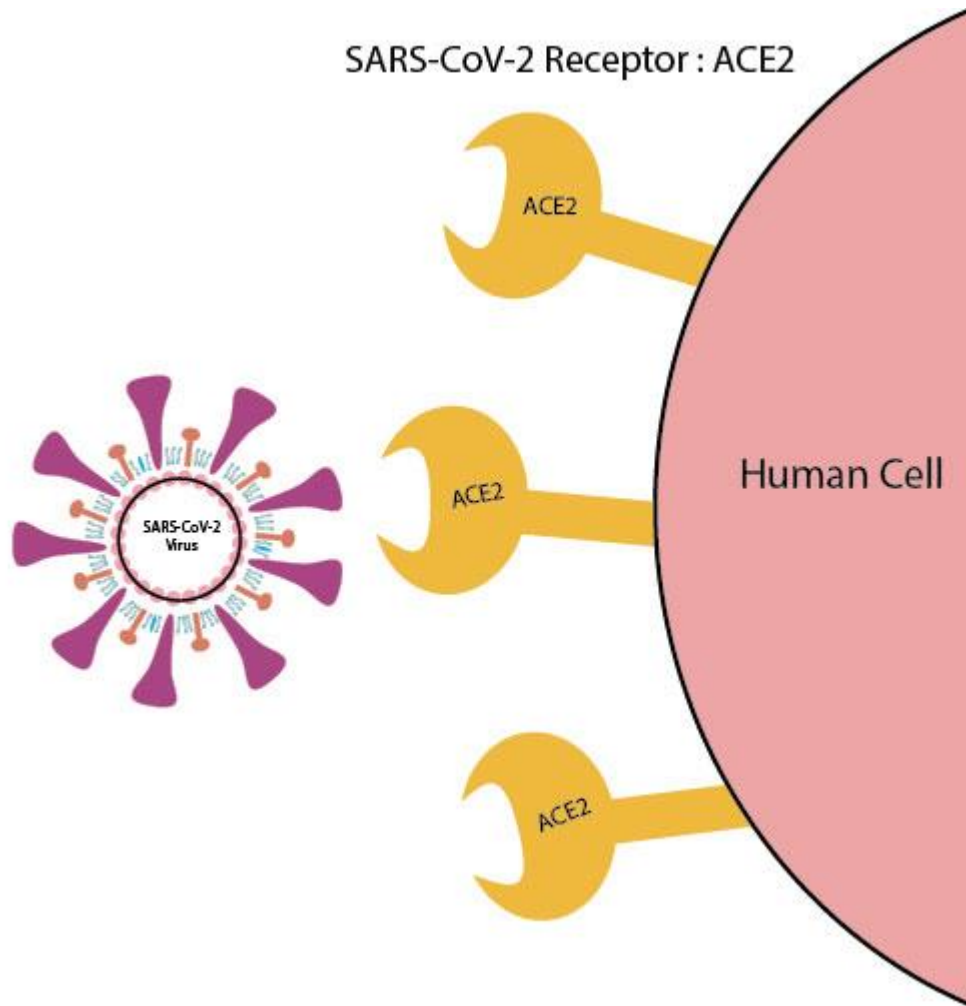
in **endothelial cells and smooth muscle** cells of virtually all organs

Consequence:

SARS-CoV-2 is likely to spread via blood flow

Once in the circulatory system, SARS-CoV-2 not only affects the respiratory system but is also **a potential threat to the digestive system, urogenital system, central nervous system, and circulatory system**

angiotensin- converting enzyme: ACE2 and transmembrane serine protease 2 (TMPRSS2)

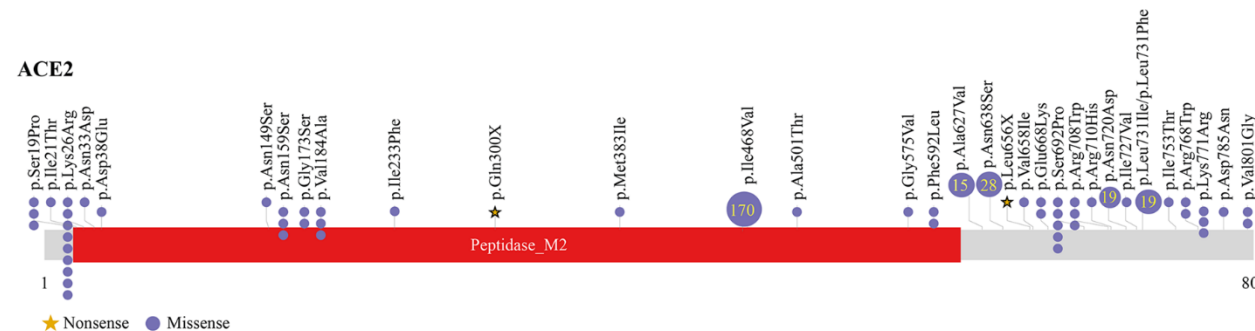


ACE2

- ❑ **Peptidase, zinc-dependent metalloprotease, expressed at the surface** of cell including lung epithelial cells and other tissues, that regulates the renin-angiotensin-aldosterone system
- ❑ converts the octapeptide AngII to the heptapeptide Ang (1e7) by hydrolysis of the C-terminal residue.
 - ACE2 limits the adverse vasoconstrictor and profibrotic effects of AngII.
 - Ang (1e7) was reported to have vasodilatory and antifibrotic actions
 - The hydrolysis of AngII into Ang (1e7) reduces the oxidative stress of AngII on endothelial cerebral arteries.
- ❑ In the pancreas ACE2 play an important glycemia-protective role
- ❑ Low ACE2 expression in the kidney is also associated with progressive renal diseases including diabetic nephropathy
- ❑ The transcriptional regulation of ACE2 is under the control of Sirtuin 1 (SIRT1) & human interferon-stimulated gene (ISG)

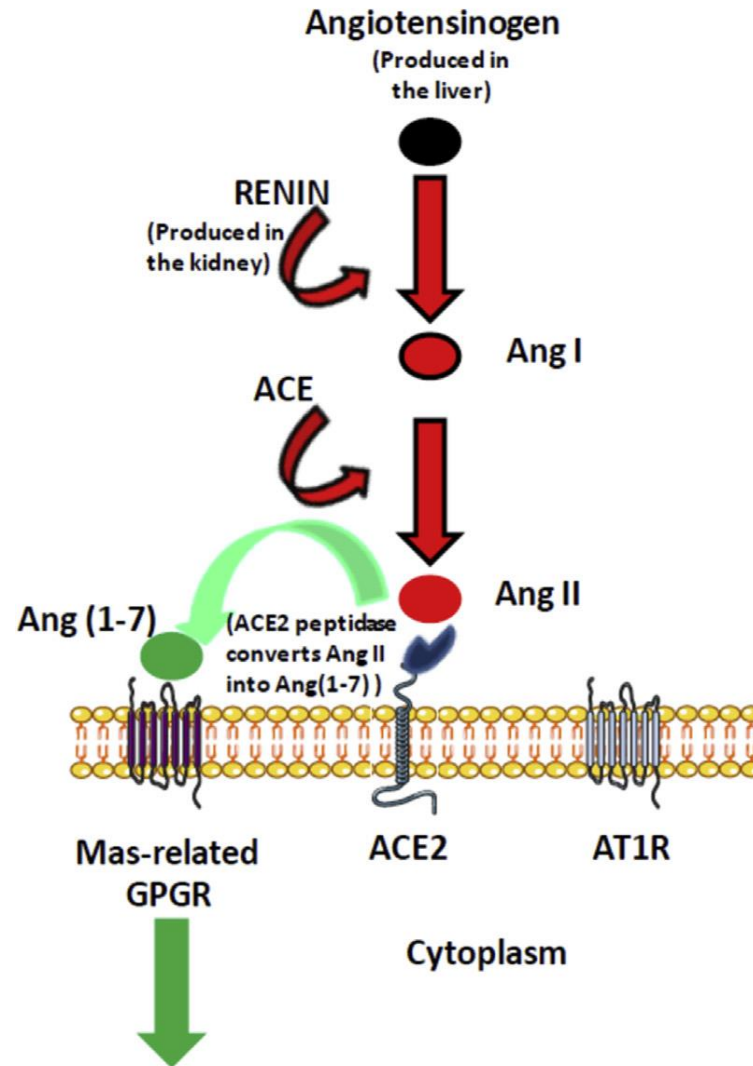
ACE2 receptor polymorphism

- ❑ Humans are not equal with respect to the expression levels of the cellular ACE2
- ❑ The expression level and expression pattern of human ACE2 in different tissues might be critical for the susceptibility, symptoms, and outcome of 2019-nCoV/SARS-CoV-2 infection
- ❑ Males and black ethnicity are at a higher risk for Covid-19 infection and disease progression compared to females and white ethnicity, respectively
- ❑ ACE2 polymorphisms were recently described in human populations:
 - ❑ **1700 variants** in ACE2 gene - ChinaMAP, 1KGP (1000 Genomes Project), other largescale genome databases
 - ❑ **62 variants** located in the coding regions of ACE2
 - ❑ **32 variants** potentially affecting the amino acid sequence of ACE2



ACE2 – biological role

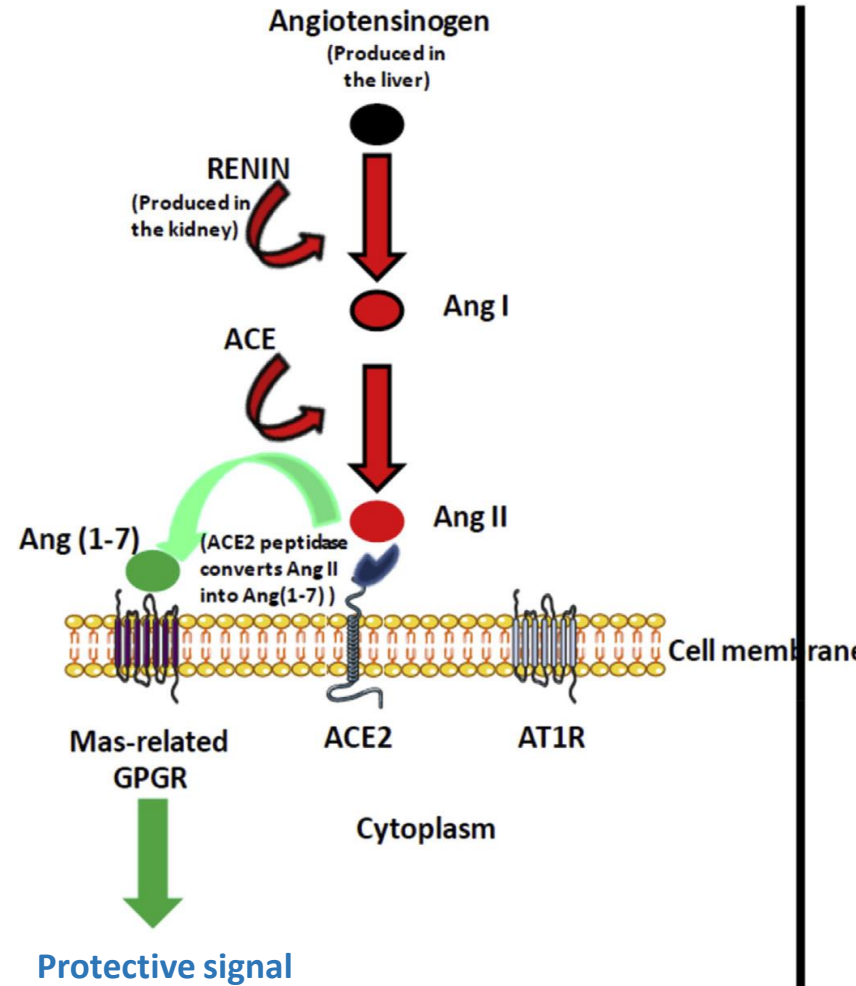
Basal/physiologic state



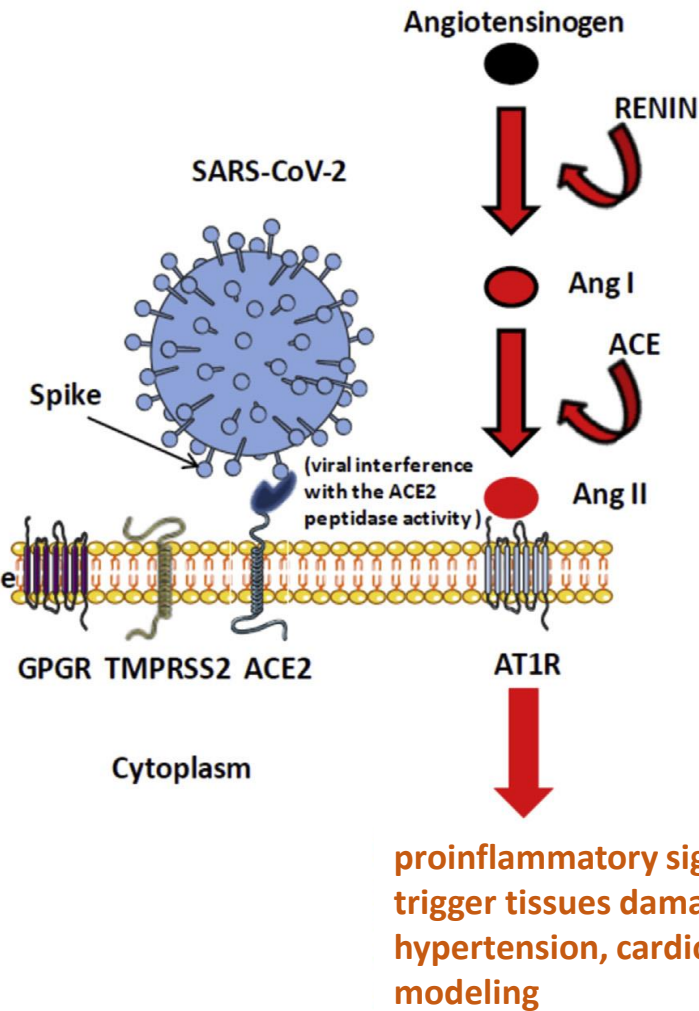
Protective signal

ACE2 – biological role

Basal/physiologic state



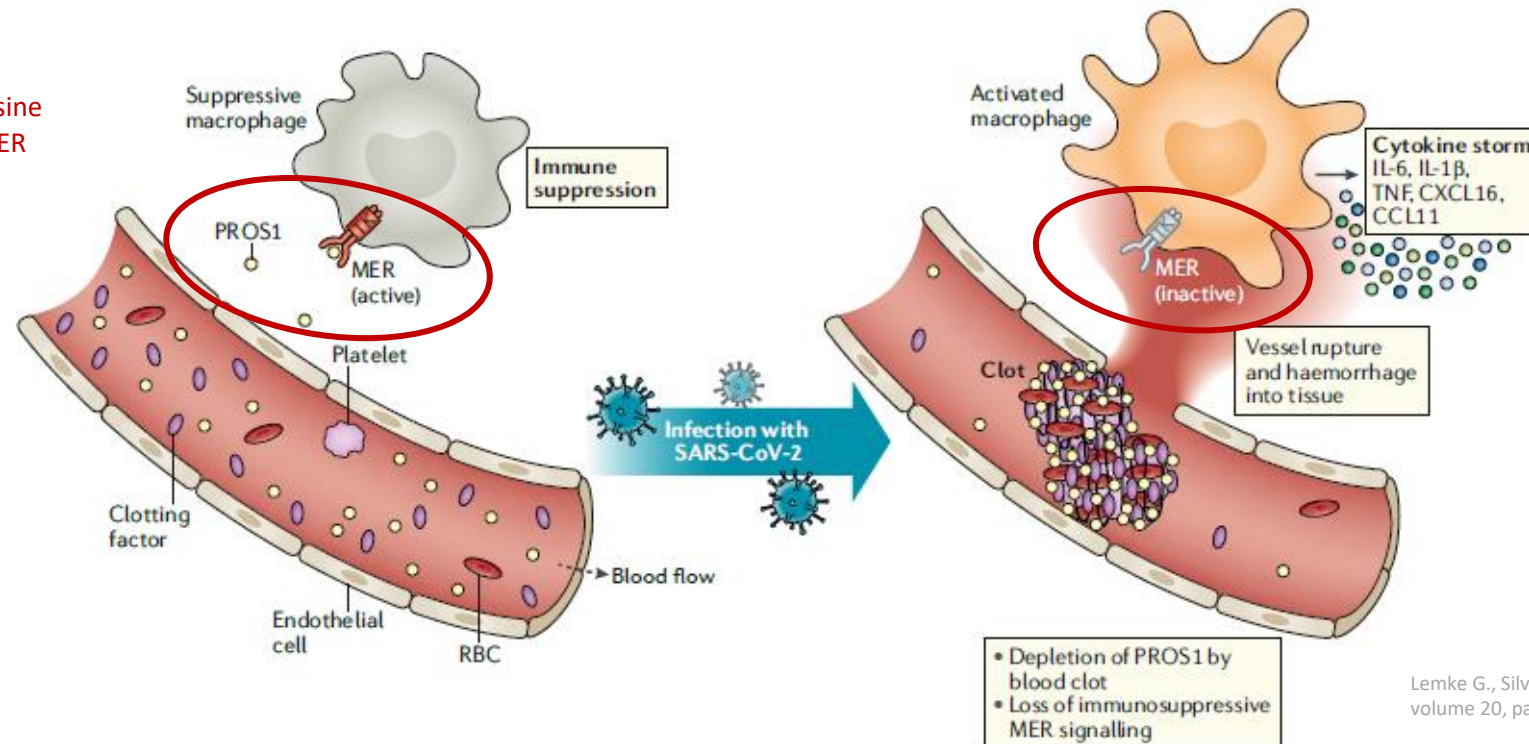
Sars-Cov-2 infection High AngII



blood clotting and Sars-Cov-2

The role of protein S

PROS1 is one of two activating
Ligands TAM family of receptor tyrosine
kinases (RTKs) — TYRO3, AXL and MER



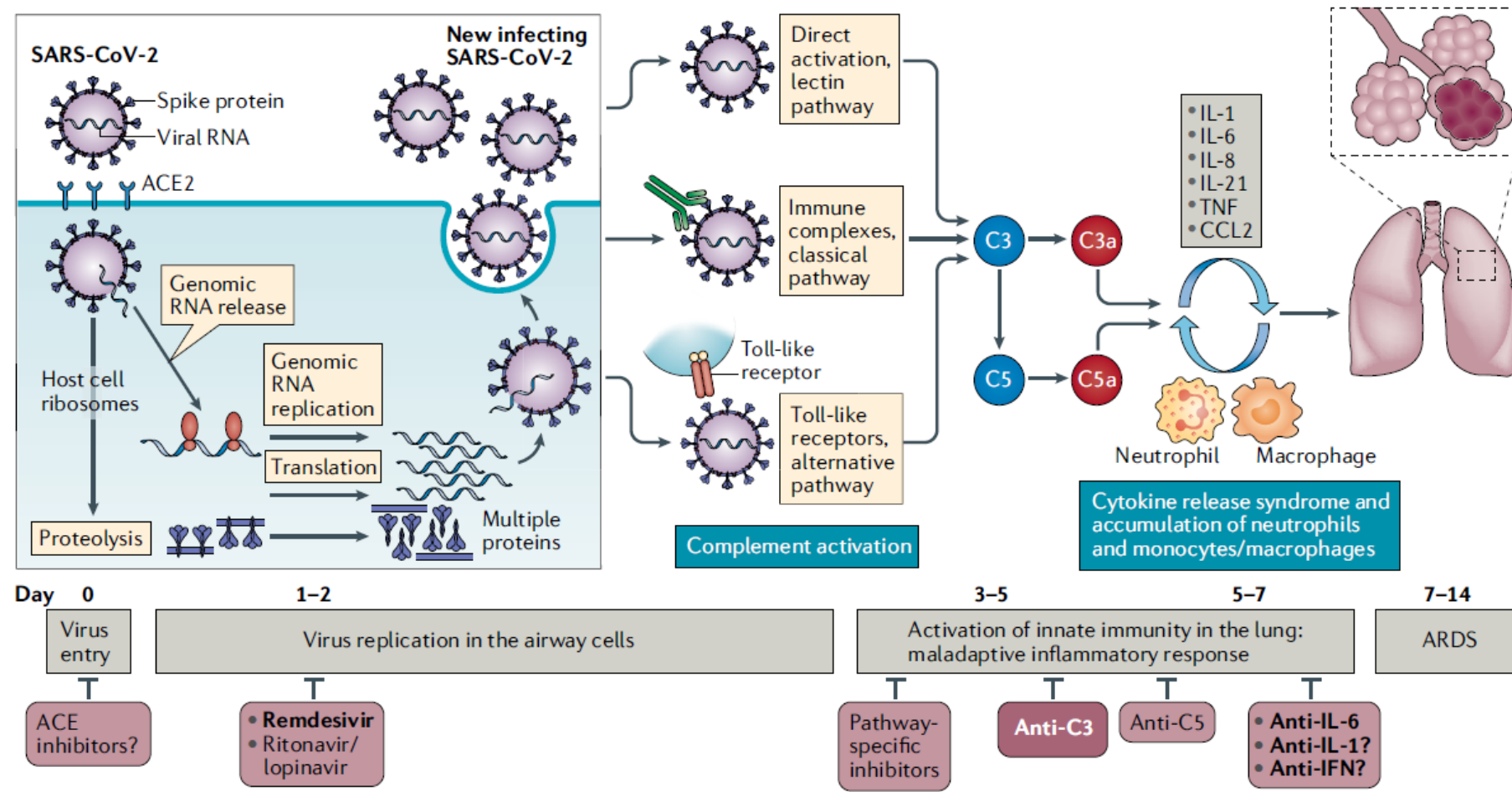
Lemke G., Silverma GJ. Nature Reviews Immunology
volume 20, pages395–396(2020)

Infection by SARS-CoV-2: what is going on in blood vessels

- vessel walls weakening results in their local rupture and hemorrhage
- The growing clot consumes clotting factors, including the **anticoagulant protein S (PROS1)**, which is also a ligand for the immunosuppressive receptor tyrosine kinase (TK) MER, expressed by macrophages and other immune sentinels. PROS1 depletion may silence MER signaling and **activate sentinel cells to express and secrete inflammatory cytokines**.

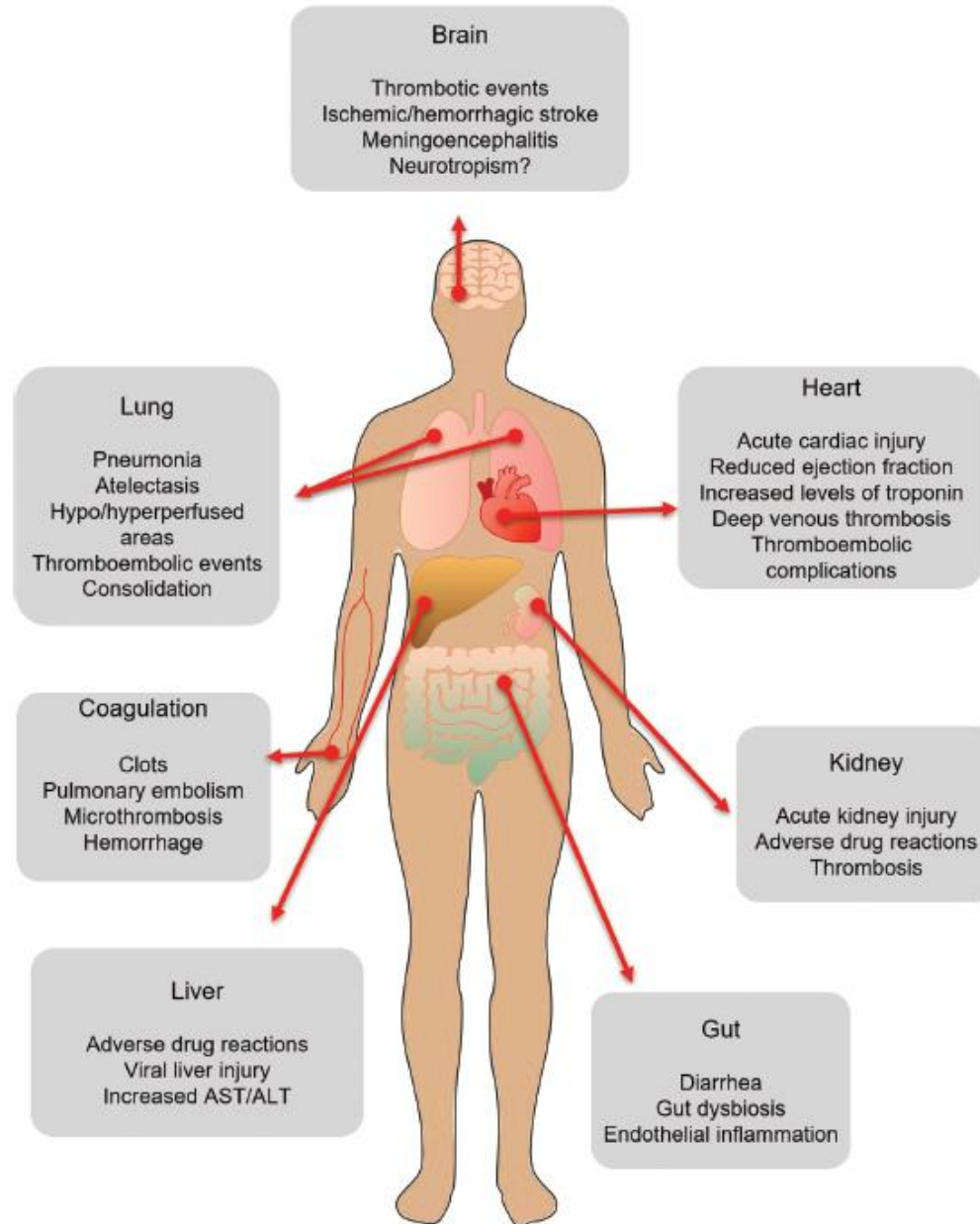
Targeting complement in SARS-CoV-2-associated lung injury.

Complement activation may contribute to the maladaptive inflammatory response seen in some patients with severe COVID-19. Inhibition of C3 or C5 may have therapeutic potential. ARDS, acute respiratory distress syndrome.



Gao, T. et al. Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2- mediated complement over- activation

Multiple organ dysfunction in SARS-CoV-2



Chiara Robba , Denise Battaglini , Paolo Pelosi & Patricia R. M. Rocco
(2020):Multiple organ dysfunction in SARS-CoV-2: MODS-CoV-2, Expert Review of Respiratory Medicine

Animal Models to study biology of SARS-Cov-2 and other infectious diseases

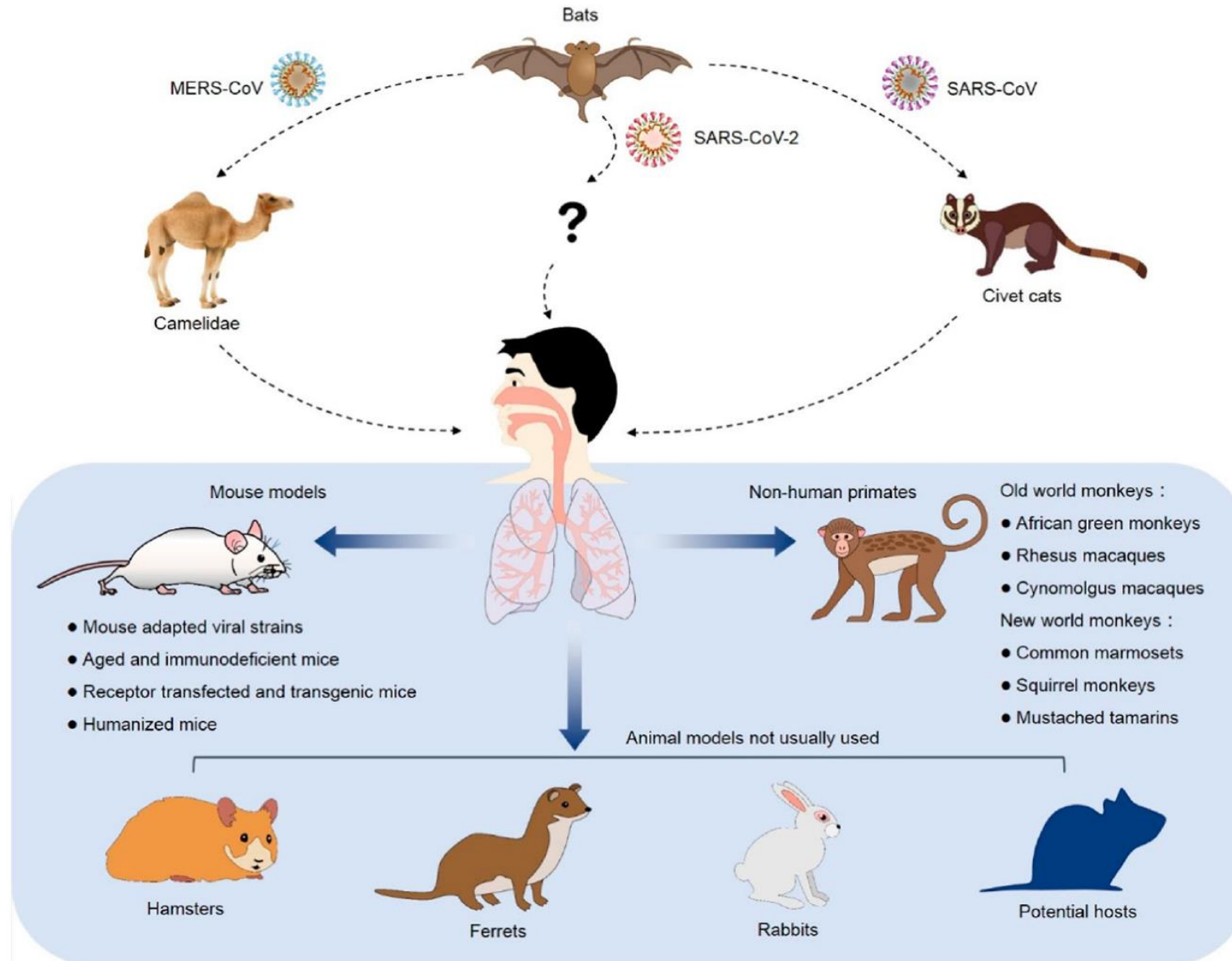
Animal Models to study biology of SARS-Cov-2

- ❑ **Animal models are vital for understanding viral pathogenesis, vaccine development, and drug screening.**
- ❑ **Non-human primates (NHPs)** are instrumental for the preclinical evaluation.
- ❑ However, the application of NHPs is restricted by high costs, availability, and the complexity of husbandry facilities required
- ❑ **Mouse models** are popular because their affordability, availability, and clear genetic backgrounds and have been widely used for studying pathogenesis of human coronaviruses

Models for SARS-Cov-2

- Current clinical treatment at hand is inadequate to suppress viral replication and inflammation, and reverse organ failure.
- Intensive research efforts have focused on increasing our understanding of viral biology of SARS-CoV-2, improving antiviral therapy and vaccination strategies.
- The animal models are important for both the fundamental research and drug discovery of coronavirus.

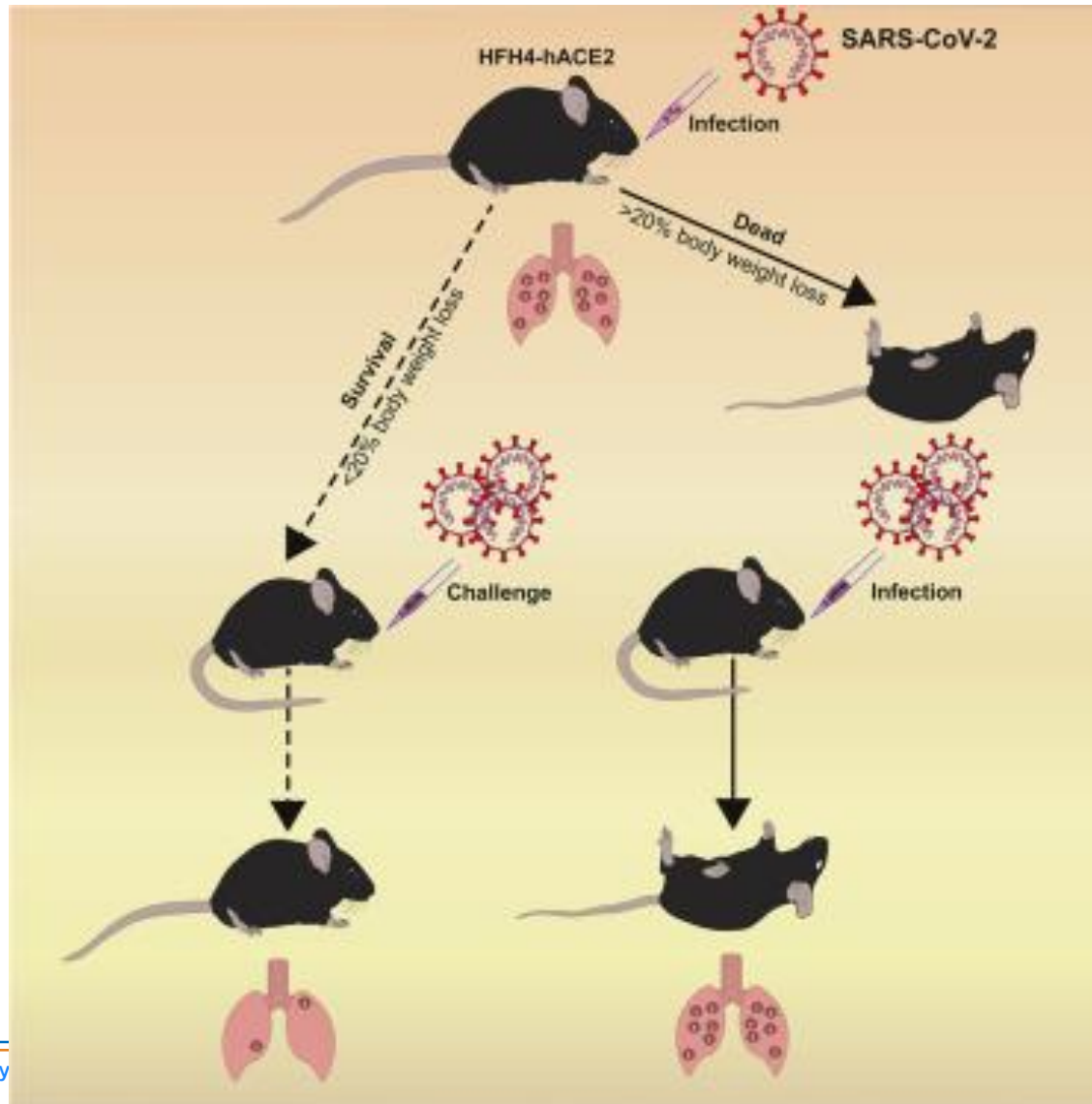
Experimental animals of SARS-CoV, MERS-CoV and SARS-CoV-2



L. YUAN ET AL., Emerging Microbes & Infections2020, VOL. 9

hACE2 transgenic mouse infection model

SARS-CoV-2 hACE2 transgenic mouse (HFH4-hACE2 in C3B6 mice) infection model

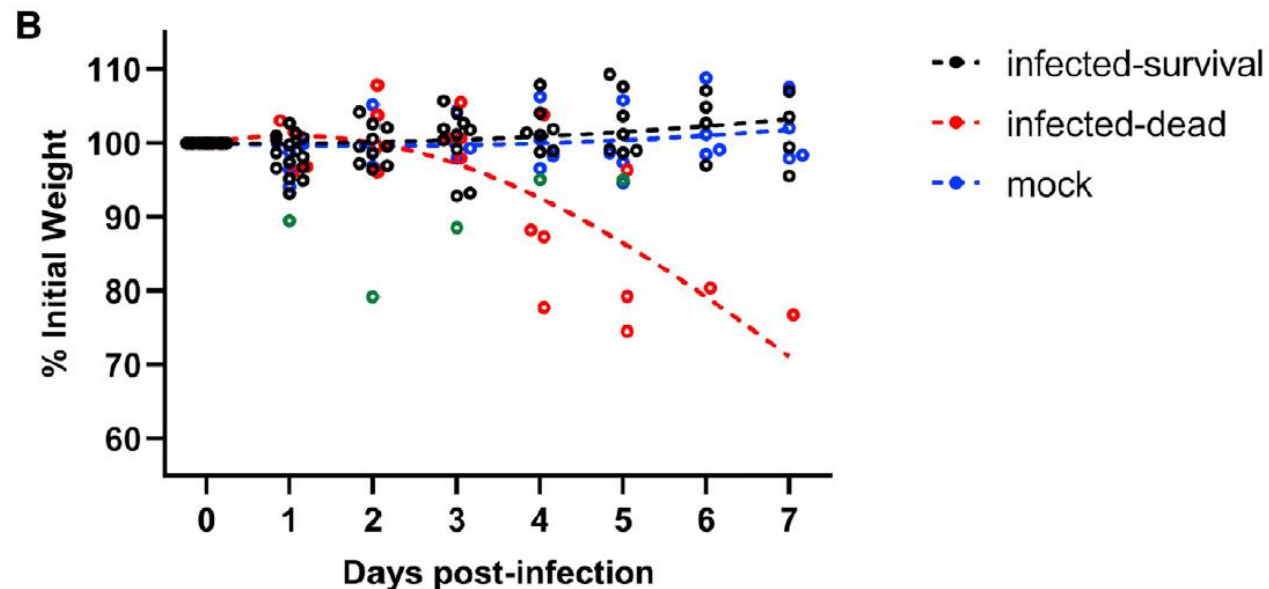
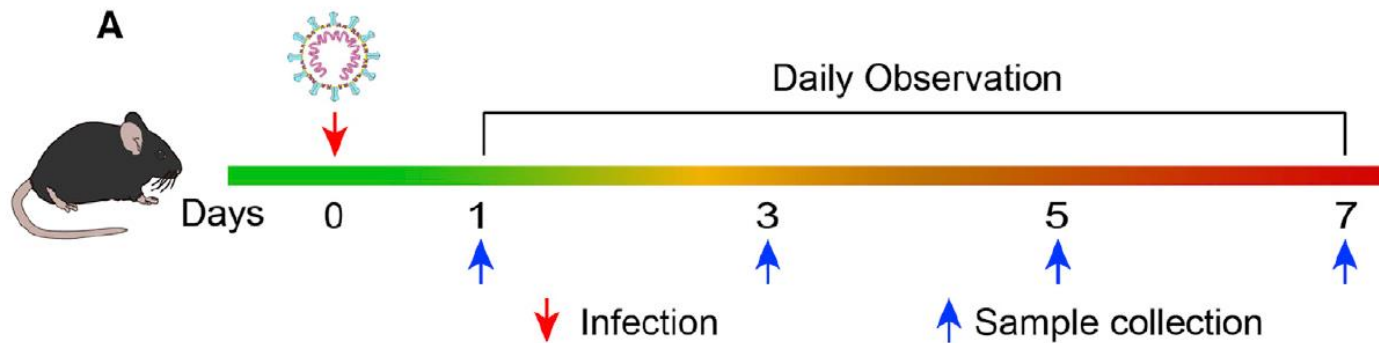


A transgenic mouse model (HFH4-hACE2 in C3B6 mice) expressing human ACE2 has been constructed under the control of a lung ciliated epithelial cell-specific HFH4/FOXJ1 promoter

- hACE2 transgenic mouse infection model recapitulates a number of infection symptoms and pathology in COVID-19 patients
- Pre-exposure to SARS-CoV-2 was able to protect mice from severe pneumonia

Jiang et al., 2020, Cell 182

hACE2 transgenic mouse infection model



Highlights

- SARS-CoV-2 could infect HFH4-hACE2 mice and cause death
- SARS-CoV-2 infection localizes to lungs of mice and causes typical interstitial pneumonia
- Pre-exposure to SARS-CoV-2 protects mice from lethal challenge

Jiang et al., 2020, Cell 182

Models for SARS-Cov-2

- Animal models are critical for us to understand the viral infection and pathogenesis
- animal models are essential for development and preclinical evaluation of a vaccine or an antiviral agent

An ideal animal model: should reflect the clinical signs, viral replication and pathology seen in humans

Non-animal models

Animal models

- expressing **human-like** ACE2 : non-human primates

- Models that do not have **human** ACE2:
 - **mouse as a preferred model:**
 - **Genetically modified -> humanized**
 - **Not-modified/wild-type - using viruses (AAS)**

Use of AAVs for rapid humanized ACE2 mouse model



made by



Use of AAVs for rapid humanized ACE2 mouse model

Advantages

- Rapid approach compared to transgenic mouse generation
- Generation of experimental cohort can be speed up and large number of animals can be prepared
- Multiple viral receptors can be delivered in once
- Can be used for more pathogens

Use of AAVs for rapid humanized ACE2 mouse model

Disadvantages

- Higher variation in level of humanization of the mouse model
- Only organ specific (lung)
- Limited size of gene construct, which can be delivered

Use of AAVs for rapid humanized ACE2 mouse model

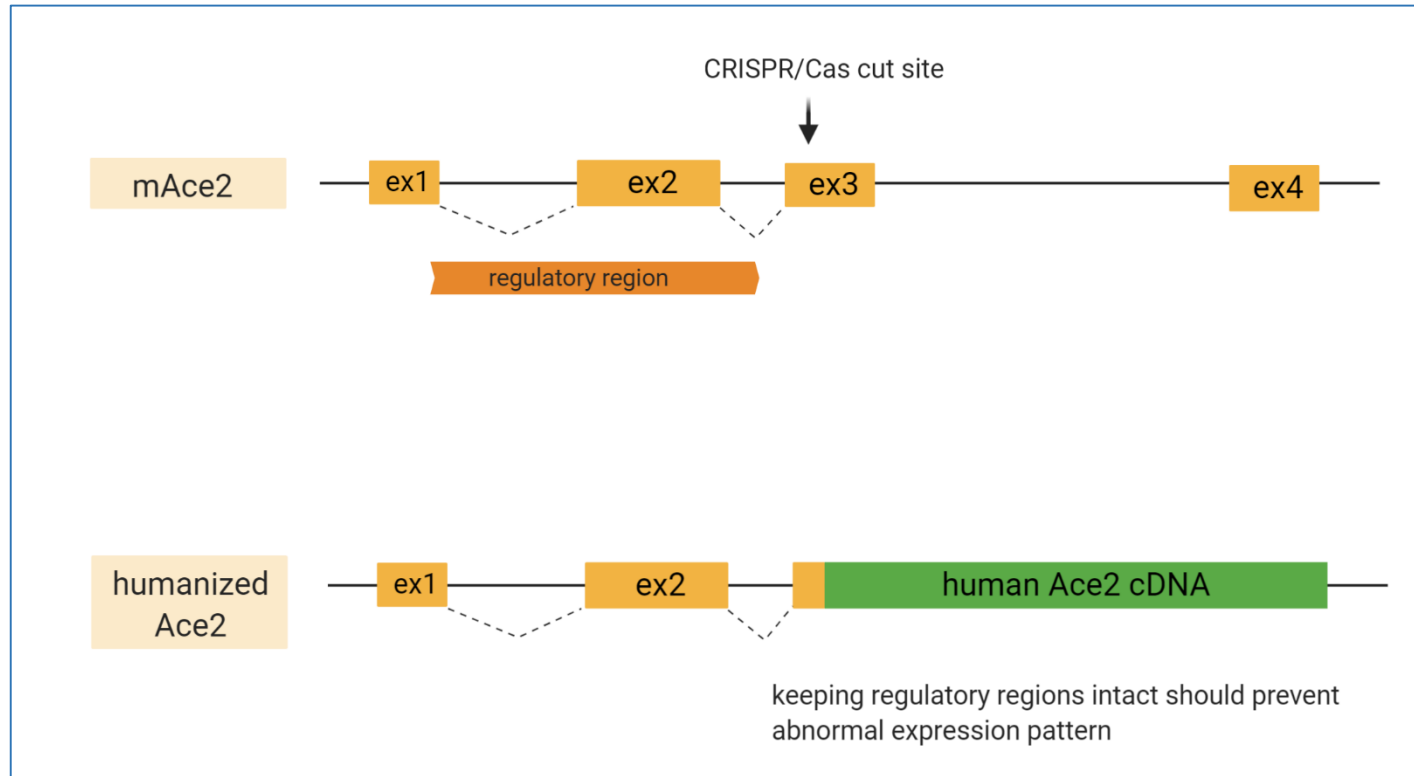
Summary

- AAV based humanized models can serve as great first line testing cohorts
- AAVs can fill the gap, before genetically engineered humanized models are developed and expanded
- AAV-based technology allows combination with genetically-engineered models
- **CCP development is now ready to use**

hACE2 genetically engineered mouse models:

humanized, variants, and tissues specific models

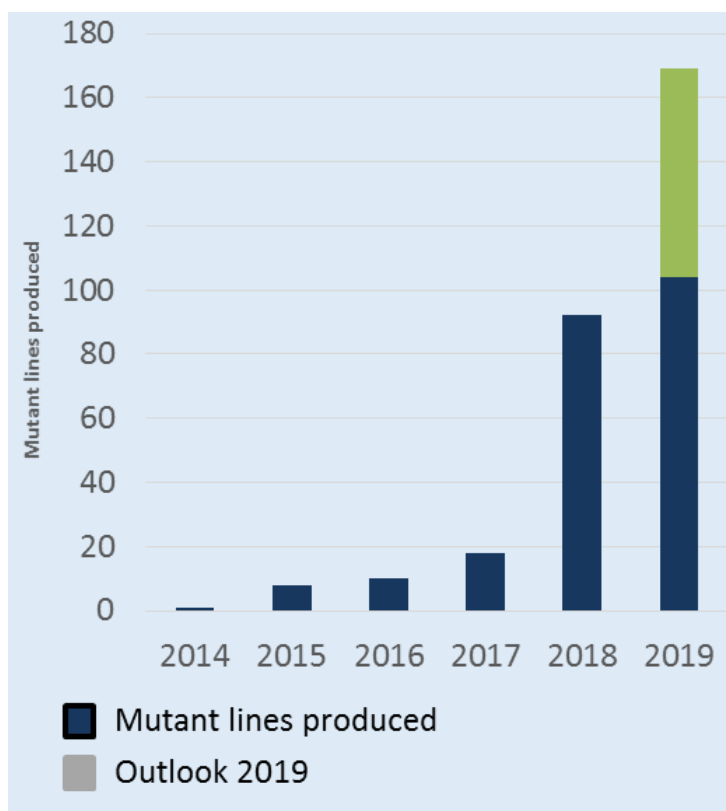
Generation of hAce2 mouse model by targeted insertion of hAce2 cDNA into mAce2 locus



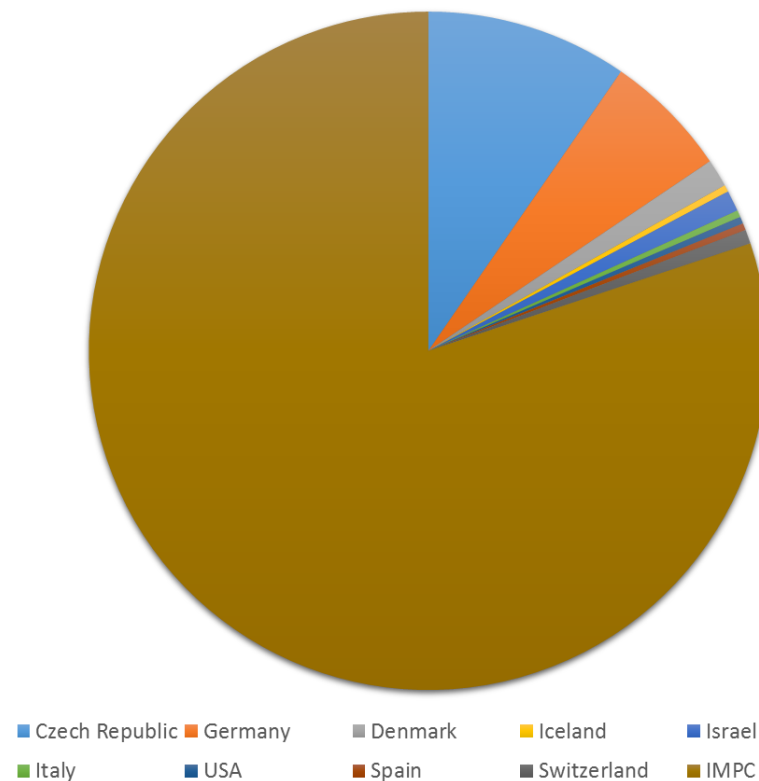
hACE2 genetically engineered mouse models:

humanized, variants, and tissues specific models

CRISPR/Cas genome-editing technology & generation mouse models at CCP



Model generation (requested by PI + IMPC)



ACE2 Variants and Ethnicity

13 variants were found as the interaction-booster between ACE2 and S1, the surface spike protein of SARS-CoV-2

13 ACE2 variants bind more efficiently to SARS-CoV-2. Importantly, two of these 13 ACE2 variants — H378R and S19P — are present specifically in Europeans and Africans, respectively

18 ACE2 variants were identified as interaction-inhibitors, meaning they bind less efficiently to SARS-CoV-2. And two of these — Q388L and M82I — are specific for Americans and Africans, respectively.

It is possible that in some individuals, if not all, the positive prognosis of the COVID-19 may be due to the existence of ACE2 variant

hACE2 genetically engineered mouse models:

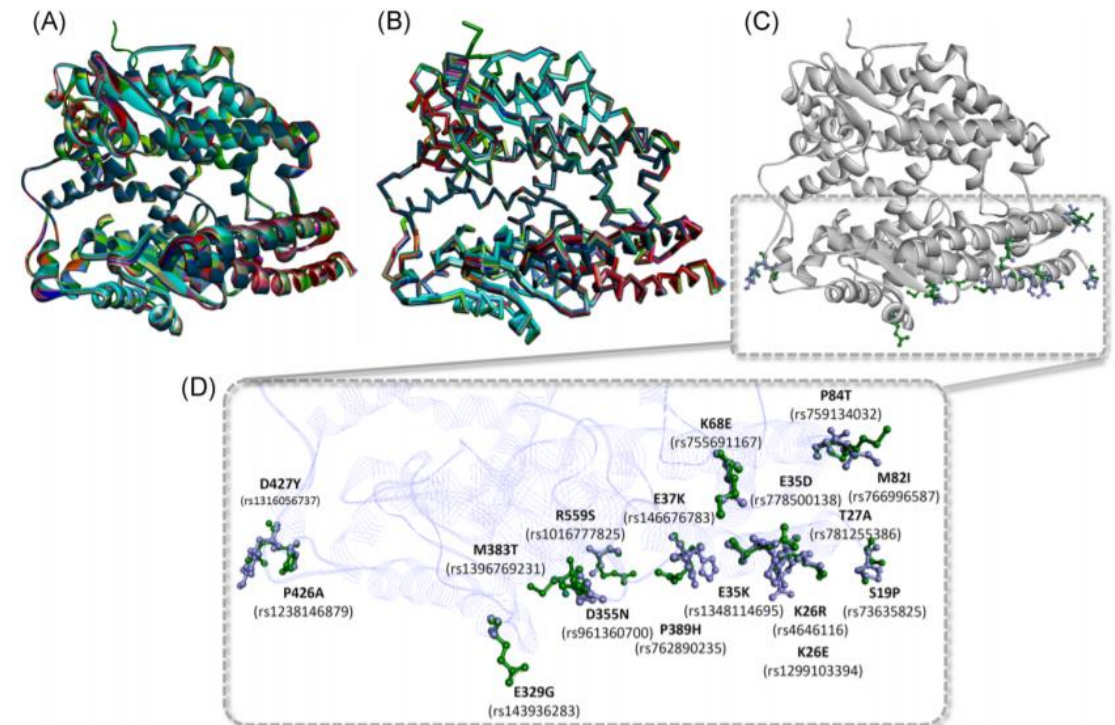
humanized, variants, and tissues specific models

genetic variants of ACE2

could affect its gene expression, protein conformation, and protein stability - the most questionable and important factors involving in [genetic predisposition to COVID-19 infection](#)

Meaning of genetic Variants

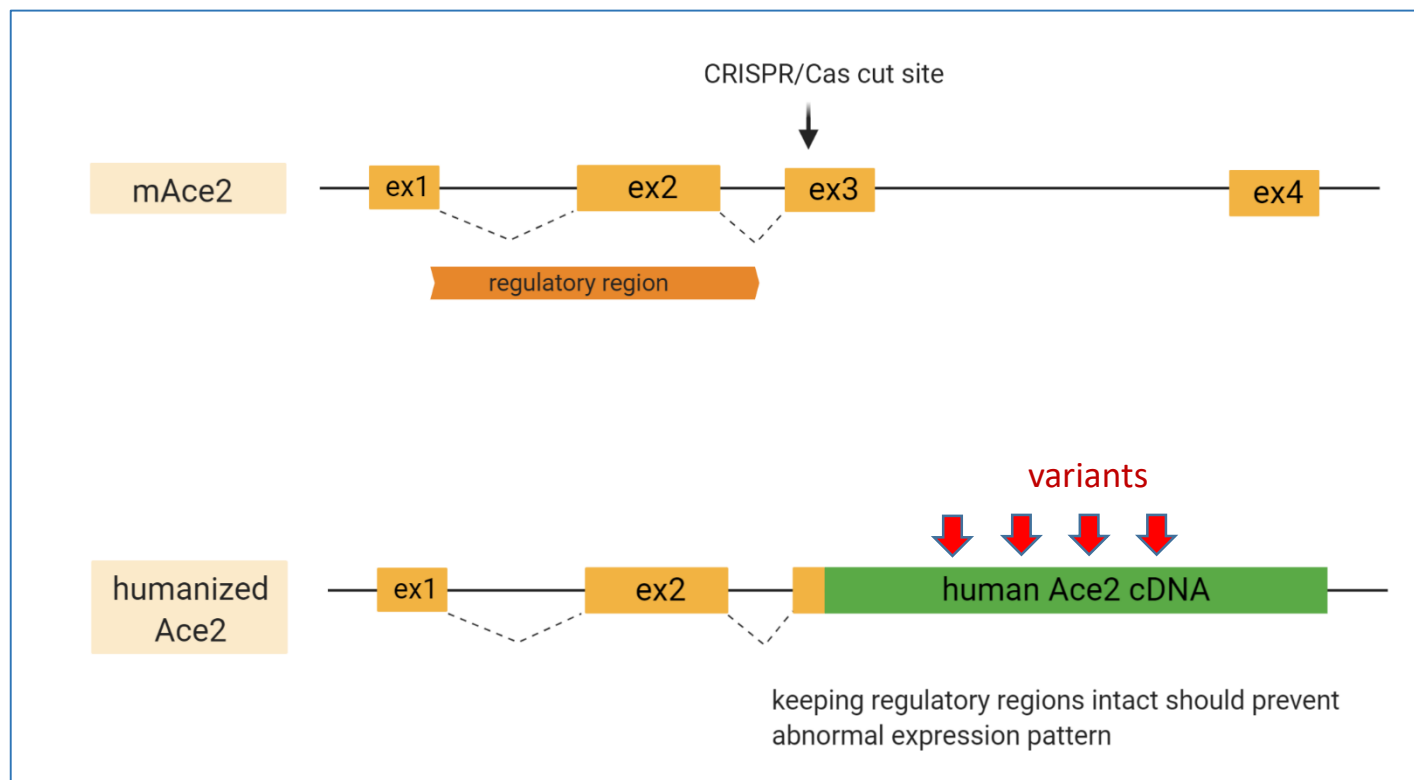
Genetic variants arise from changes in nucleotides, which is called single-nucleotide polymorphisms (SNPs). SNPs lead to different amino acid sequences, which then results in altered protein structure and function.



hACE2 genetically engineered mouse models:

humanized, variants, and tissues specific models

Generation of hAce2 mouse model by targeted insertion of hAce2 cDNA into mAce2 locus



hACE2 genetically engineered mouse models:

humanized, variants, and tissues specific models



Generation of mouse models to study biology of SARS-Cov-2

What we have prepared:

☐ AAV – hACE2 to infect mouse lung

GM – mouse models

☐ Ace2 – knockout

☐ transmembrane serine protease 2 (TMPRSS2) knockout

In preparation:

hACE2 – knockin – first mice born

hACE2 – 3-4 human variants (based on hACE2)

Planned:

Tissue specific expression: lung, intestine & endothelial cells

What we do not have - what is missing:

State-of-the-art animal facility

To study infection at Biosafety Level 2-3 laboratory (BSL2-3)

... cooperation with German and USA colleagues



Thank you for your attention