
# Pre-clinical Models for Downselecting Candidates

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# COVID-19 prevention and treatment: a 3-tier race

1. **Short term (now!)**

   Treatment of severe patients and epidemic containment (damage control with what we have at hand)

2. **Medium term (+12 months)**

   Specific SARS-CoV-2/COVID19 antivirals/treatments

3. **Long term (+18 months)**

   Vaccine(s) to prevent further outbreaks/epidemics

# COVID-19 prevention and treatment: a 3-tier race

1. **Short term (now!)**

   Treatment of severe patients and epidemic containment (damage control with what we have at hand)

   - ❑ broad spectrum antivirals: **Remdesivir, Favipiravir**
   - ❑ immonomodulators: **Interferons, anti-IL6, Dxm**
   - ❑ drug repurposing: **Kaletra, (H)chloroquine, h-t**
   - ❑ Ab cocktails, convalescent plasma

   } + symptomatic treatment +/- vital support (ex.: ventilation)

2. **Medium term (+12 months)**

   Specific SARS-CoV-2/COVID19 antivirals/treatments

3. **Long term (+18 months)**

   Vaccine(s) to prevent further outbreaks/epidemics

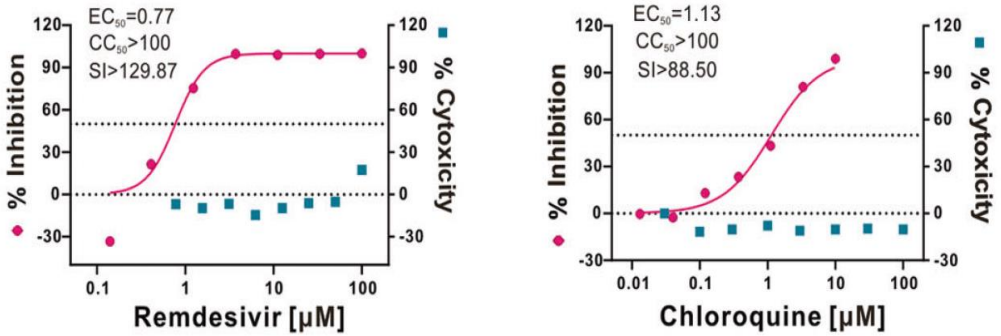
# The importance (and limits) of pre-clinical models to improve clinical management

LETTER TO THE EDITOR OPEN

## Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro

February

*Cell Research* (2020) 30:269–271; https://doi.org/10.1038/s41422-020-0282-0



# The importance (and limits) of pre-clinical models to improve clinical management

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March

STAT Topics Coronavirus Opinion Podcast Newsletters Reports Events

HEALTH

## WHO to launch multinational trial to jumpstart search for coronavirus drugs

By HELEN BRANSWELL @HelenBranswell / MARCH 18, 2020 Reprints

August

## COVID-19 Update: FDA Broadens Emergency Use Authorization for Veklury (remdesivir) to Include All Hospitalized Patients for Treatment of COVID-19

July

Health Topics Countries Newsroom Emergenci

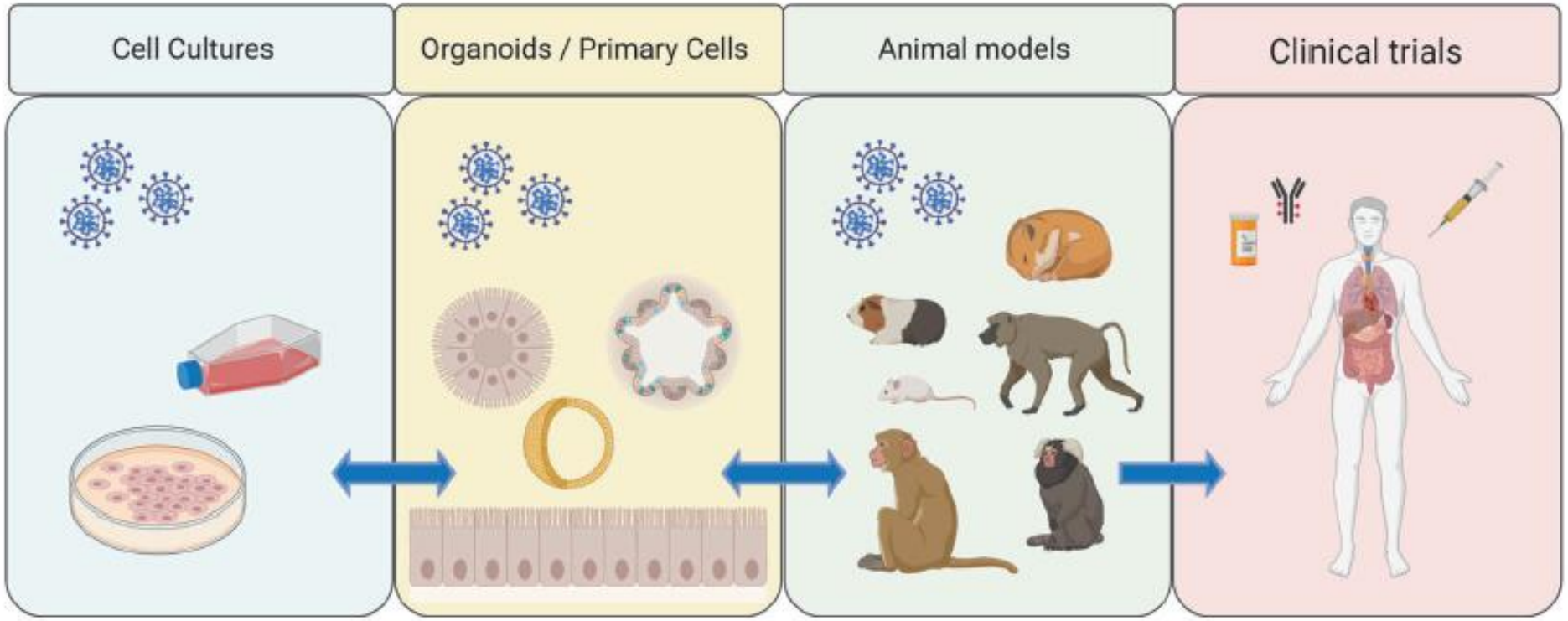
sroom / Detail / WHO discontinues hydroxychloroquine and lopinavir/ritonavir treatment arms

## WHO discontinues hydroxychloroquine and lopinavir/ritonavir treatment arms for COVID-19

(Hydroxy)chloroquine – previous viruses

| | *In vitro* effect | Clinical efficacy |
|---|---|---|
| Chikungunya | ✓ | ✗ RCT: deleterious |
| Dengue | ✓ | ✗ RCT: negative |
| Influenza | ✓ | ✗ RCT: negative |
| SARS | ✓ | ✗ |
| Zika | ✓ | ✗ |

# Three major classes of pre-clinical experimental models for SARS-CoV-2



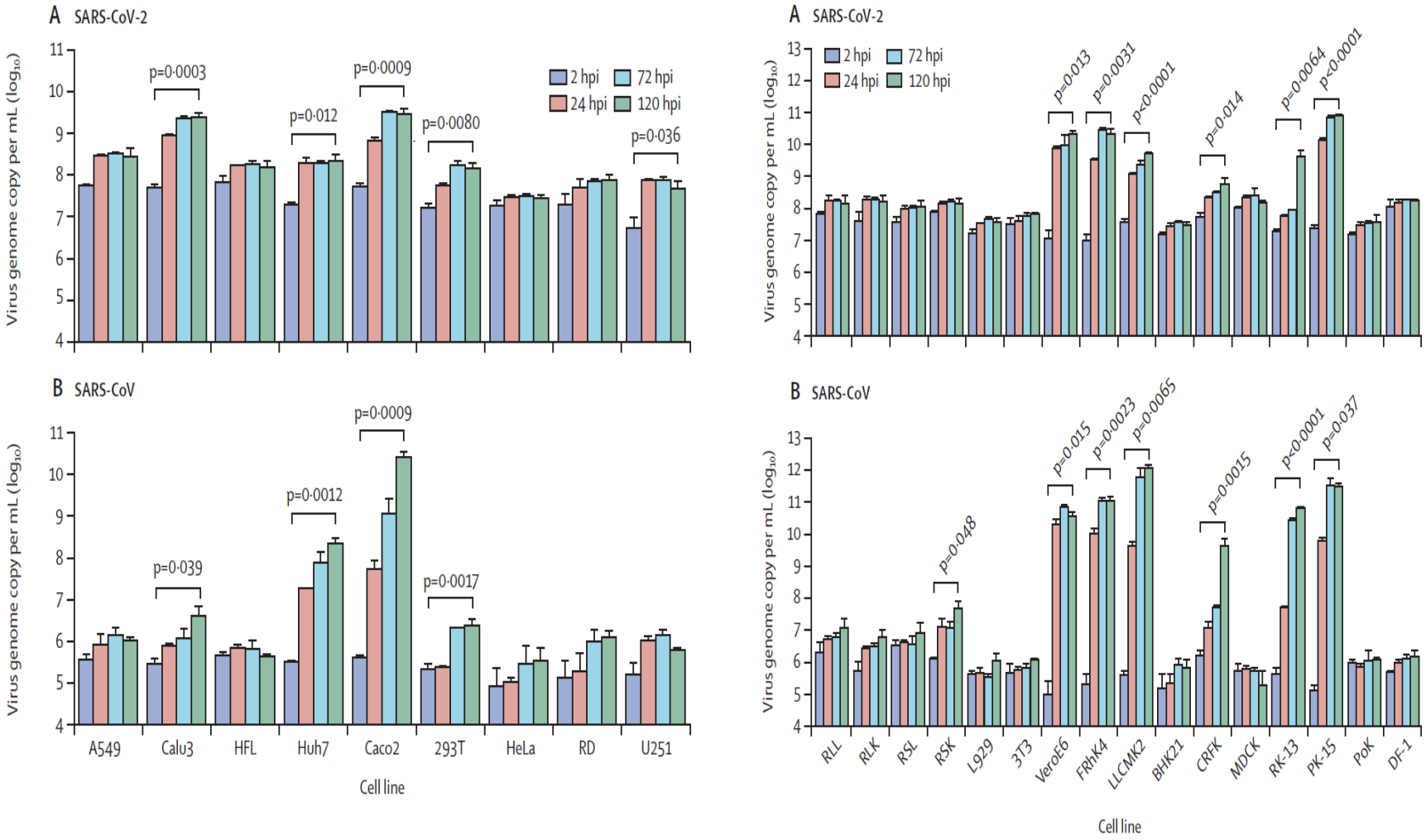

Johansen MD et al *Mucosal Immunology* 2020

# 1. “Classic” 2D cultured cell lines

| Pros | Cons |
| --- | --- |
| ❑ Suitable for viral isolation and propagation | ❑ Transformed cell lines (immortalization) |
| ❑ Suitable for viral replication and pathogenesis | ❑ Limited receptor repertoire |
| ❑ Suitable for early HT candidate screening | ❑ “Artificial” infection and treatment conditions |
| ❑ Panoply of reagents available | ❑ Limited value for “omics”-based approaches |
| ❑ Easy to manipulate | ❑ Limited value for host-targeted candidates |
| ❑ Cost $ | ❑ Pro-drug vs metabolite? |
| ❑ Rapidly available in pandemic context | ❑ **High rate of false positive “hits”!** |

# 1. “Classic” 2D cultured cell lines

## Differential SARS-CoV-2 vs SARS-CoV replication depending on the cell line







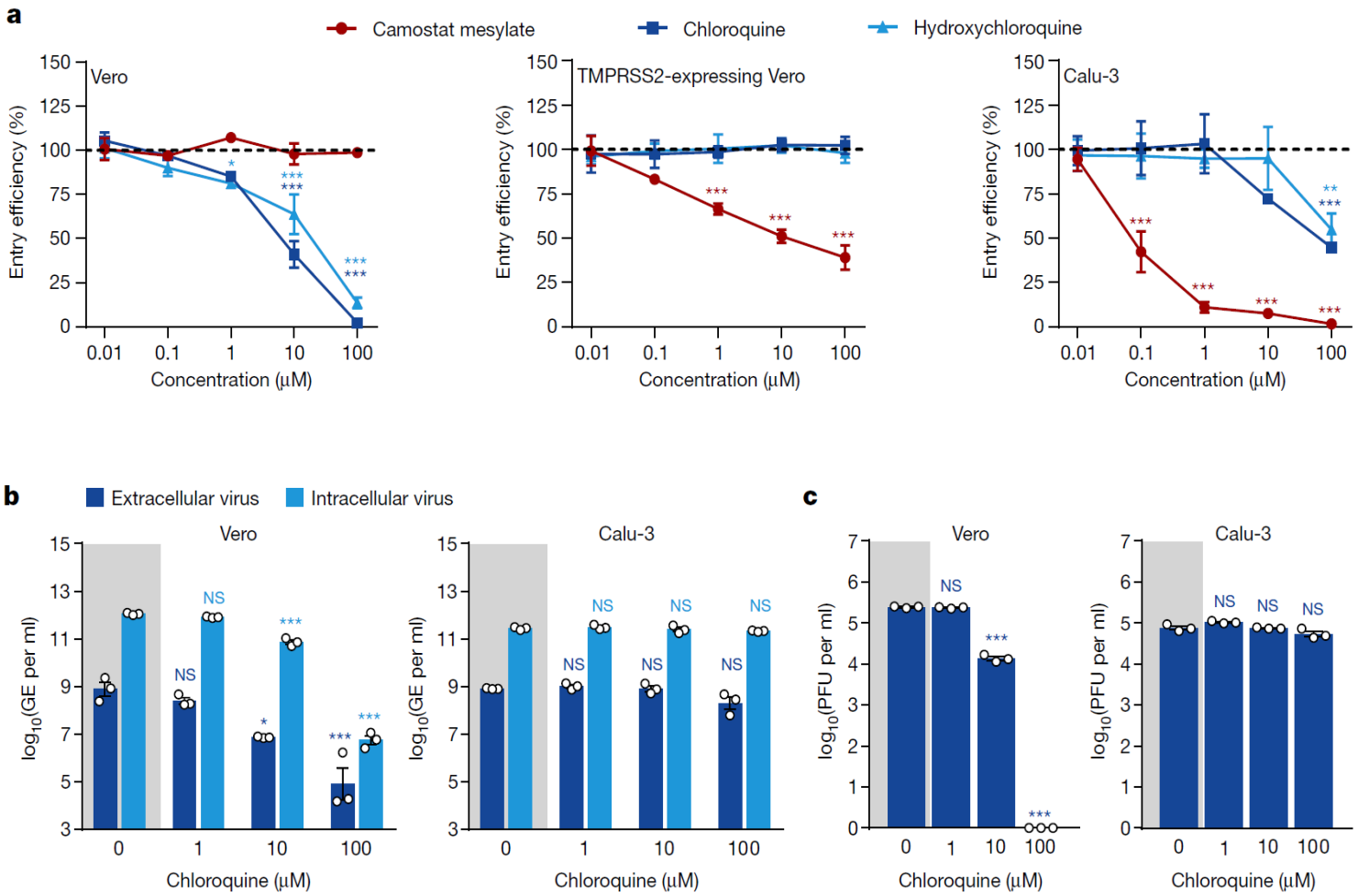
**Robust SARS-CoV-2 replication:**

- ❑ Vero/E6 (AGM kidney)
- ❑ Calu-3 (pulmonary)
- ❑ Caco2 (intestinal)
- ❑ Huh7 (hepatic)
- ❑ ACE2++ A549
- ❑ TMPRSS2++ Vero

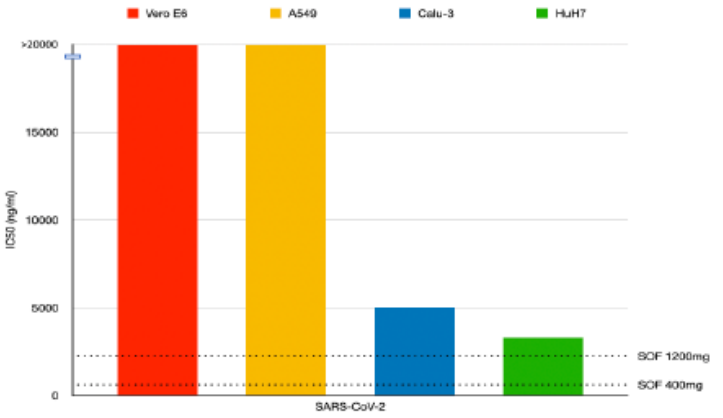
Chu H et al *The Lancet Microbe* 2020

# 1. “Classic” 2D cultured cell lines

## Differential candidate inhibition of SARS-CoV-2 depending on the cell line



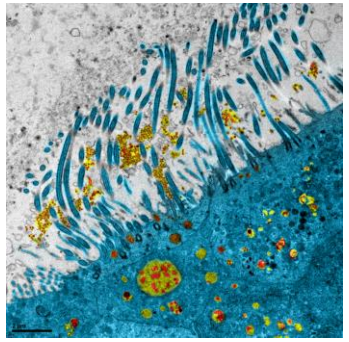

Hoffmann M et al *Nature* 2020



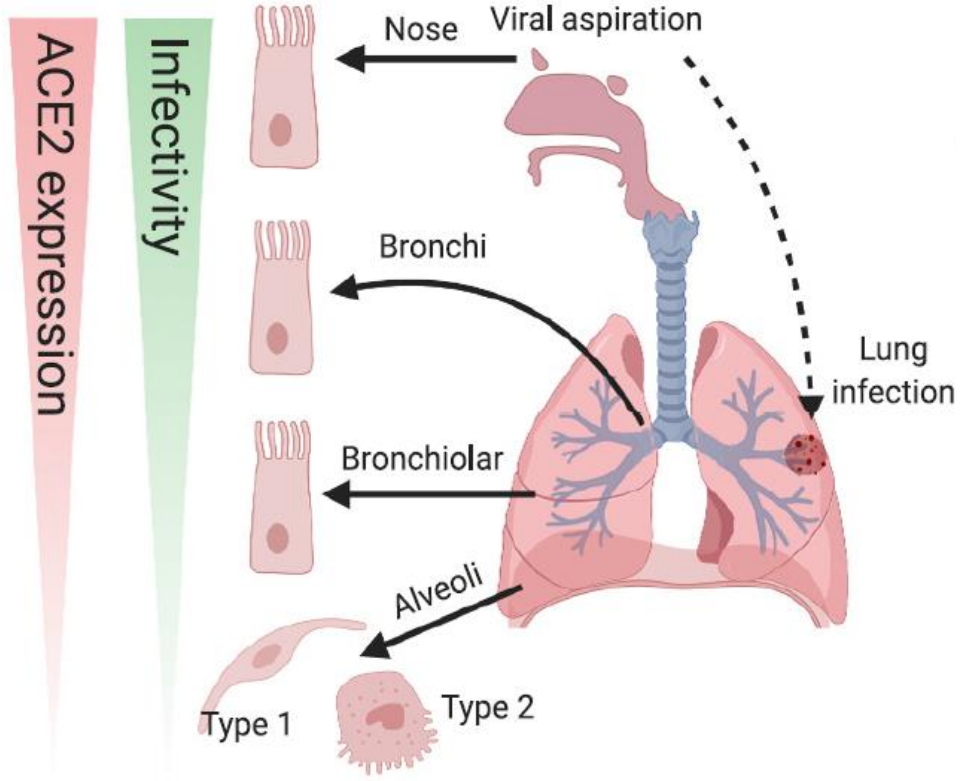

**Differential sofosbuvir IC50 against SARS-CoV-2 depending on the cell line**

Levi J et al **poster #19**

# 2. Complex 3D *in vitro/ex vivo* models



- ❑ Issued from primary human respiratory cultures or pluripotent cells
- ❑ Differentiated, (pseudo)stratified 3D architecture, air-liquid interface
- ❑ Physiologically relevant (receptors, TJ, mucus, cilia, etc)
- ❑ Suitable for viral infection, pathogenesis, “omics” and innate immune responses
- ❑ Suitable for unbiased and host-targeted approaches
- ❑ Multiple proxys of infection/treatment efficacy
- ❑ Possibility of adding immune cells
- ❑ Rapidly available in pandemic context




Pizzorno A et al *Cell Reports Medicine* 2020; Hou YJ et al *Cell* 2020

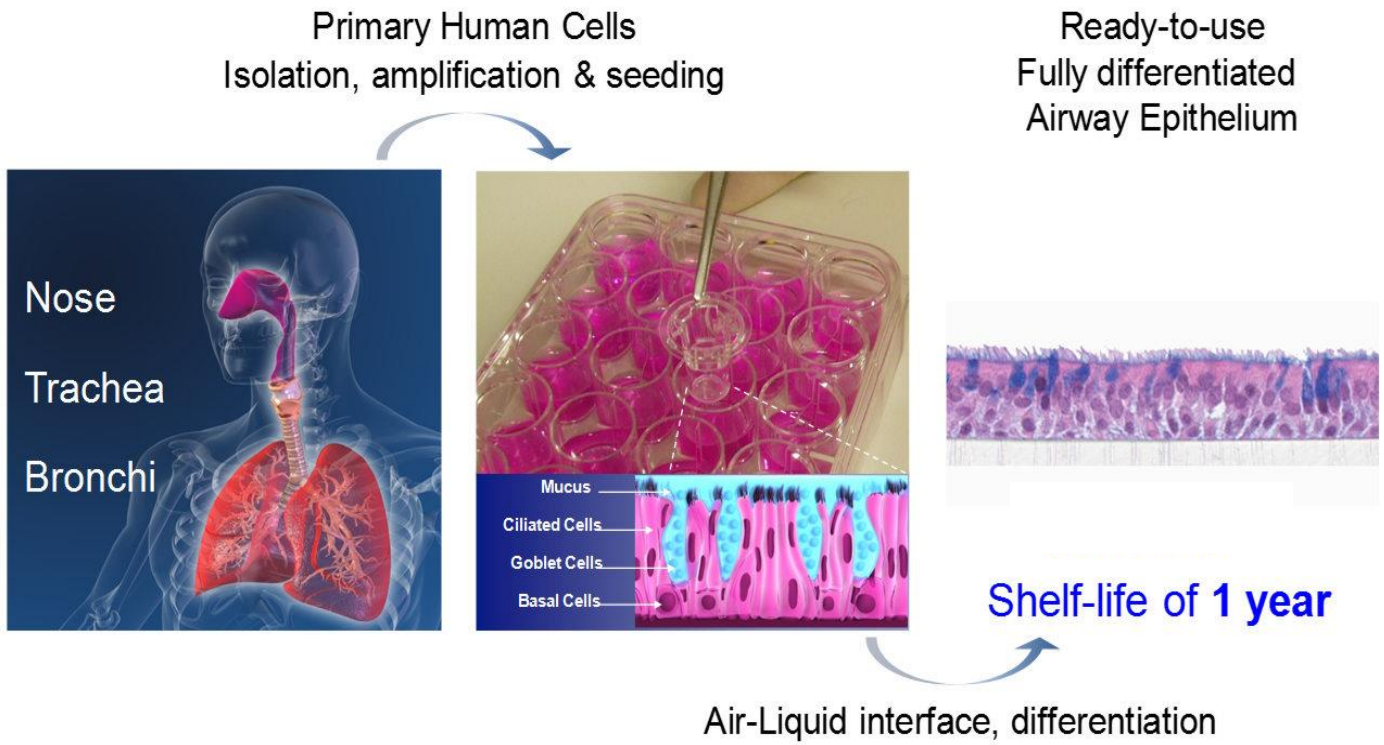
# 2. Complex 3D *in vitro/ex vivo* models

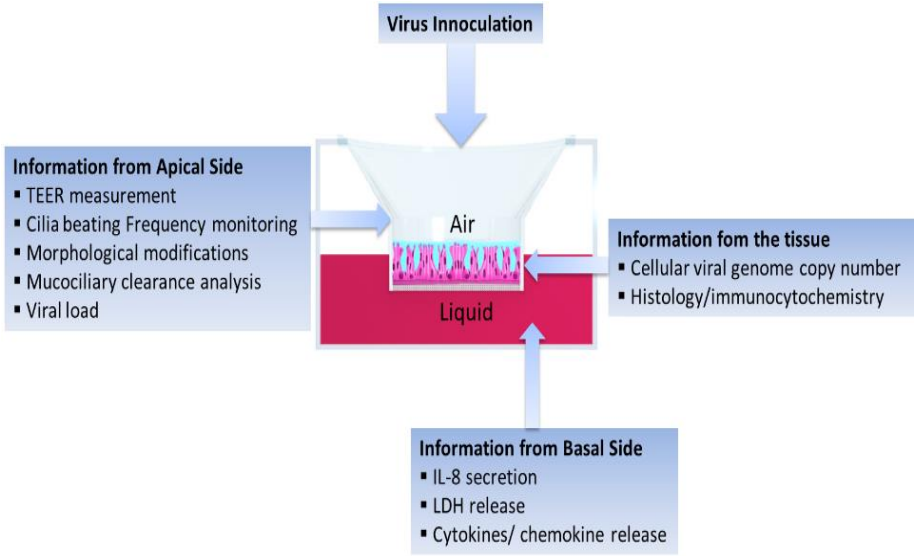
## Limits of complex in *vitro/ex vivo* models

- ❑ Unsuitable for studying complex (innate or adaptive) immune responses
- ❑ Unsuitable for PK/PD evaluation
- ❑ Unsuitable for “off organ” or systemic drug effects
- ❑ Require access to human cell/tissue samples
- ❑ Short lifespan (organoids)
- ❑ Cost $$

# 2. Complex 3D *in vitro/ex vivo* models

## 2.1 Reconstituted HAEs

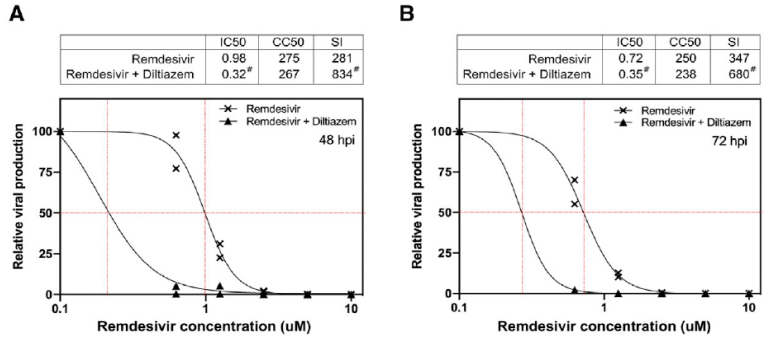



Huang S et al *Eur J of Pharm and Biopharm* 2017

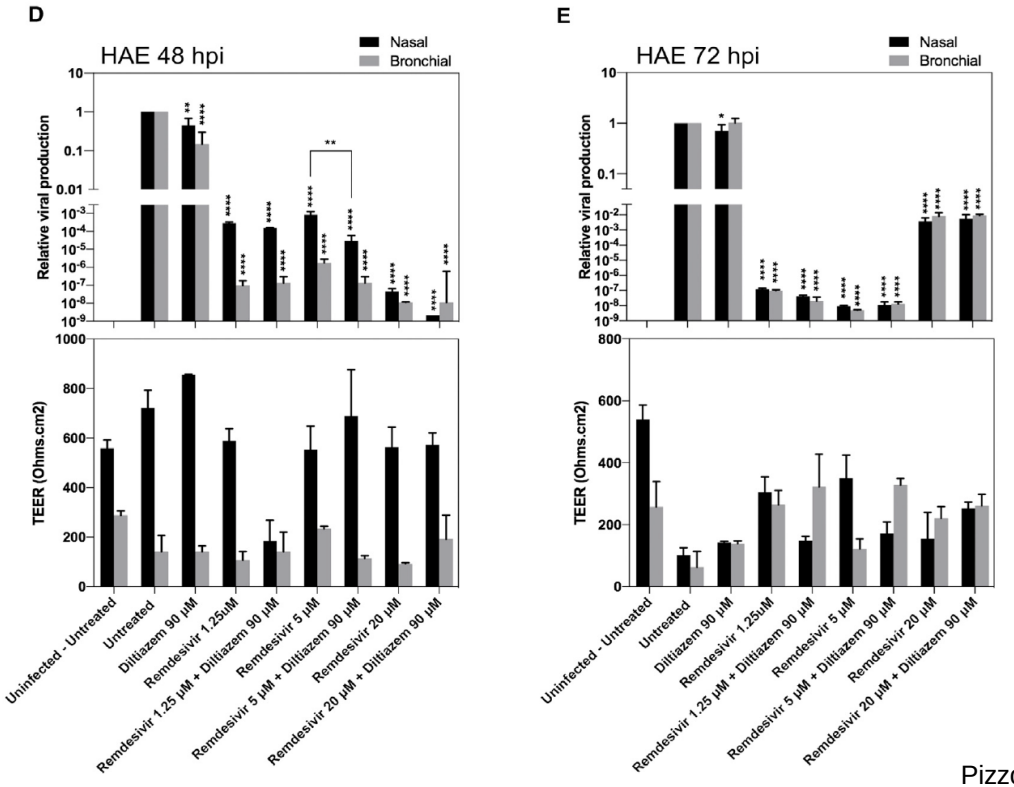
# 2. Complex 3D *in vitro/ex vivo* models

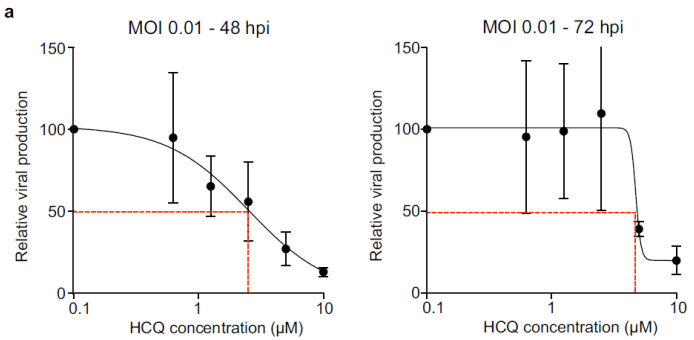
## 2.1 Predictive value of HAEs for SARS-CoV-2 candidate downselection



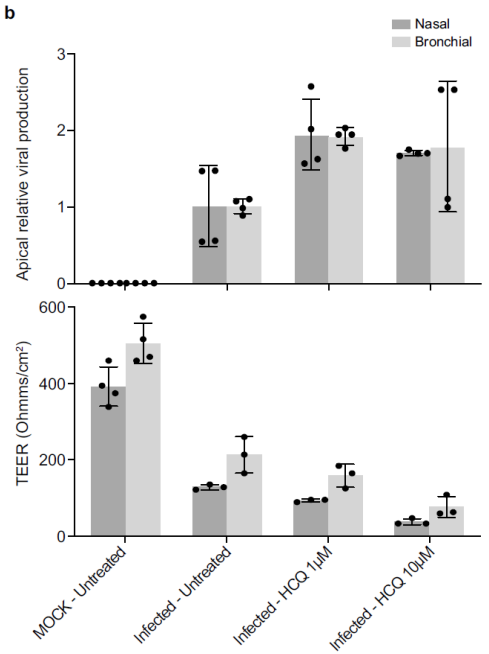

| | IC50 | CC50 | SI |
|---|---|---|---|
| Remdesivir | 0.98 | 275 | 281 |
| Remdesivir + Diltiazem | 0.32# | 267 | 834# |

| | IC50 | CC50 | SI |
|---|---|---|---|
| Remdesivir | 0.72 | 250 | 347 |
| Remdesivir + Diltiazem | 0.35# | 238 | 680# |




| HCQ | $IC_{50}$ (µM) | $CC_{50}$ (µM) | SI |
|---|---|---|---|
| MOI 0.01 48 hpi | 2.189 | 215 | 98.218 |
| MOI 0.01 72 hpi | 4.394 | 161 | 36.640 |

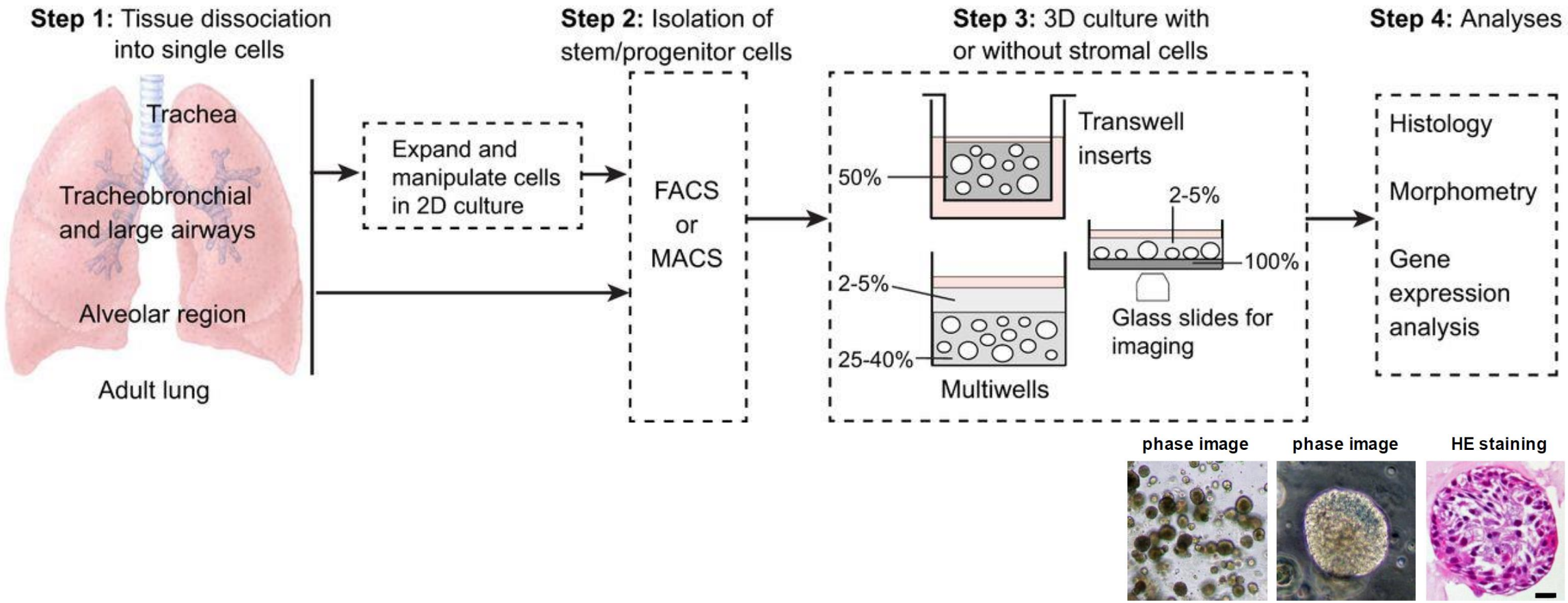



| Treatment post-infection | | Apical Relative viral production | Infectious titer ($log_{10}$ $TCID_{50}$/mL) |
|---|---|---|---|
| Nasal Epithelium MOI 0.1 48hpi | Untreated | 1 +/- 0.53 | 6.8 |
| | HCQ - 1 µM | 1.92 +/- 0.46 | 7.3 |
| | HCQ - 10 µM | 1.70 +/- 0.03 | 6.97 |
| Bronchial Epithelium MOI 0.1 48hpi | Untreated | 1 +/- 0.09 | 7.63 |
| | HCQ - 1 µM | 1.90 +/- 0.11 | 7.3 |
| | HCQ - 10 µM | 1.77 +/-0.85 | 7.63 |

Pizzorno A et al *Cell Reports Medicine* 2020; Maisonnasse P et al *Nature* 2020; Huang J et al *Cell Stem Cell* 2020

# 2. Complex 3D *in vitro/ex vivo* models

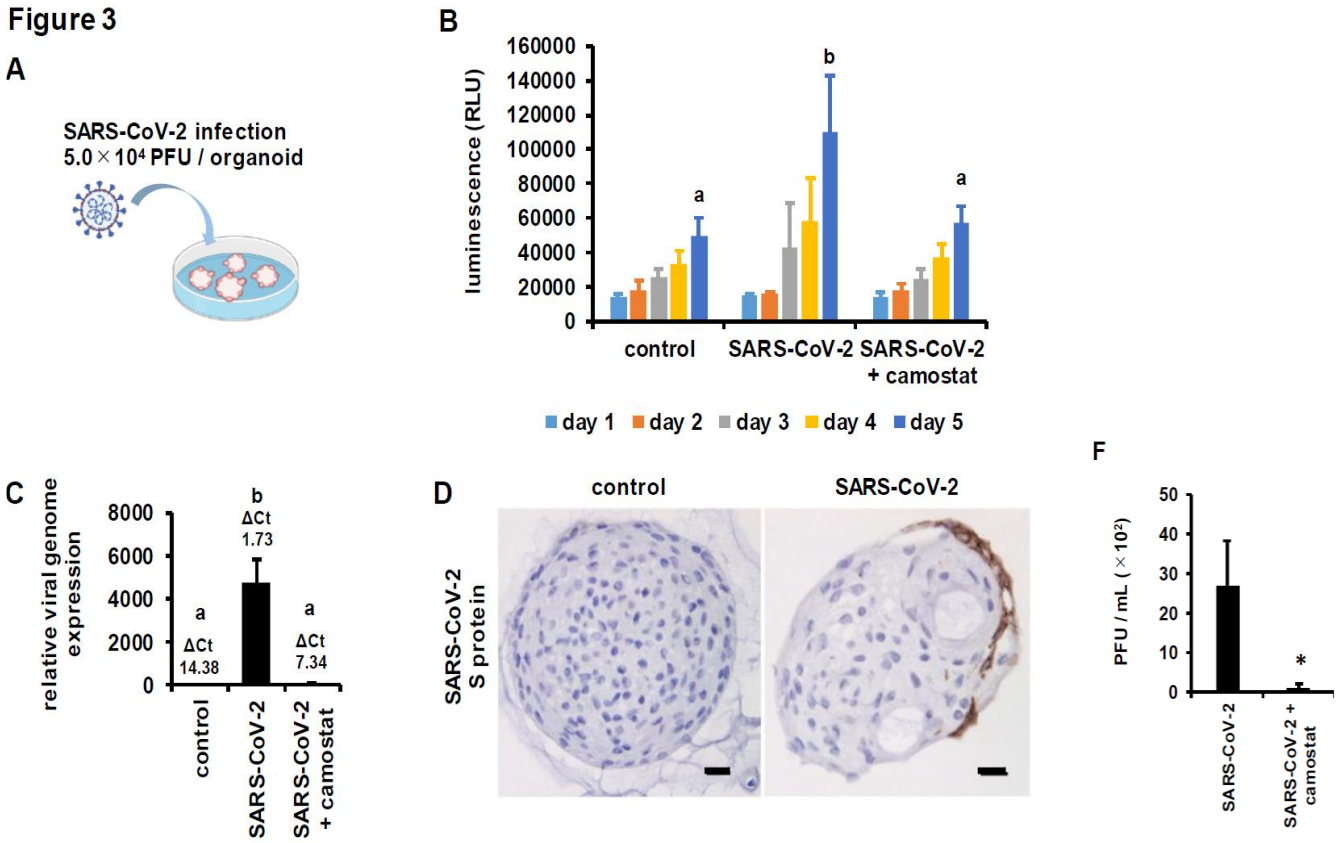
## 2.2 Bronchial/lung organoids




Barkauskas CE et al *Development* 2017, Suzuki T et al biorXiv 2020

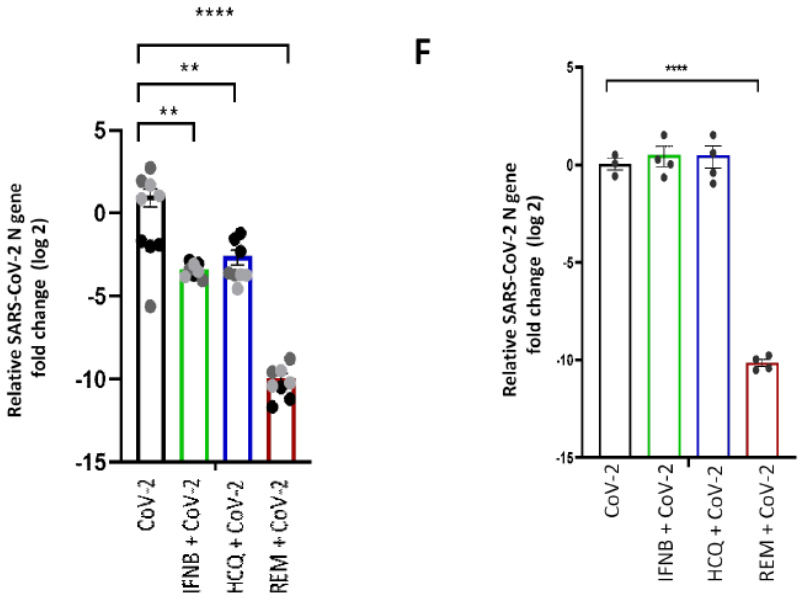
# 2. Complex 3D *in vitro/ex vivo* models

## 2.2 Predictive value of bronchial organoids for SARS-CoV-2 candidate downselection




Suzuki T et al biorXiv 2020

Alveolar organoid vs organoid-derived ALI

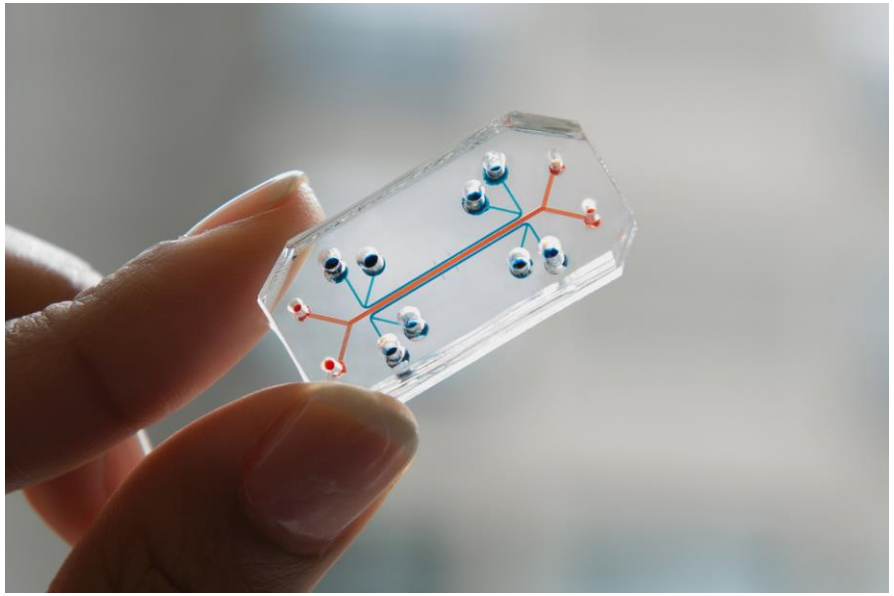


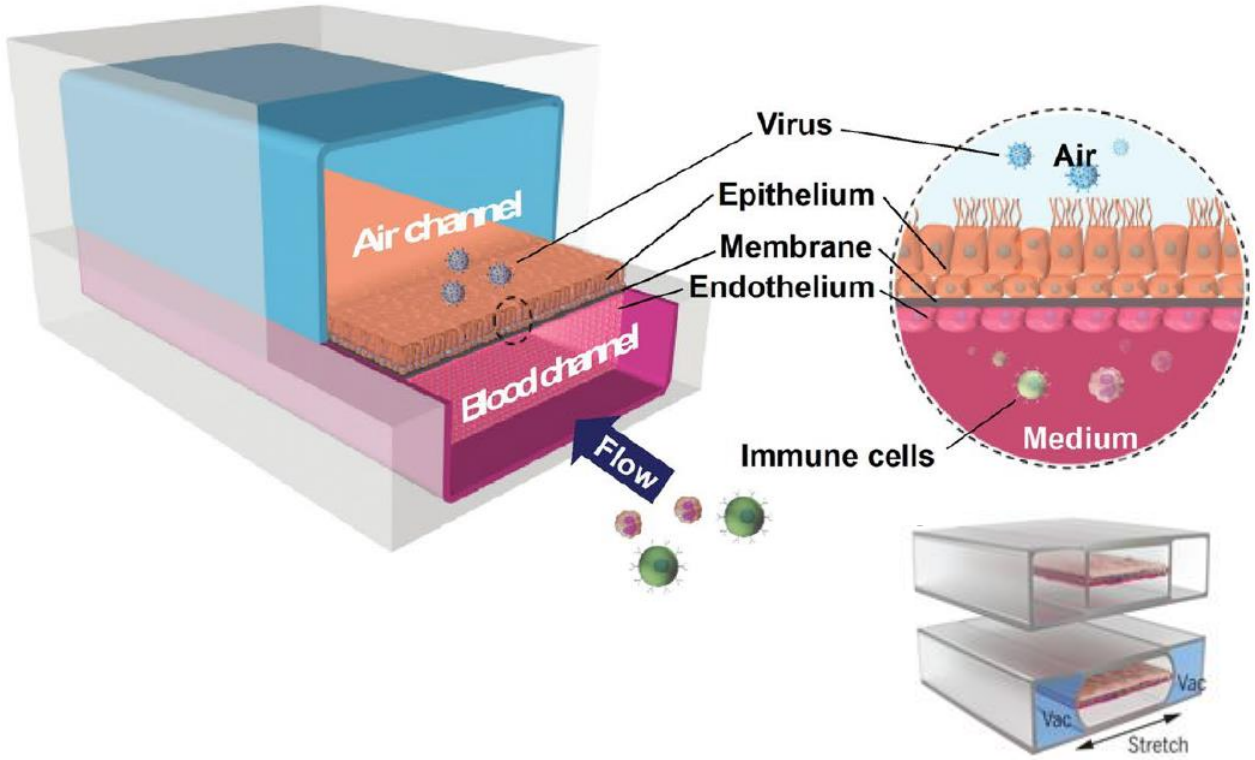

Mulay A and Konda B et al biorXiv 2020

**To be further validated for SARS-CoV-2**

# 2. Complex 3D *in vitro/ex vivo* models

## 2.3 Organ-on-a-chip






**To be validated for SARS-CoV-2**

Park SE et al *Science* 2019; Si L and Bai H et al biorXiv 2020

# 3. Animal models

- ❑ The most complex pre-clinical models available
- ❑ Suitable for viral infection, pathogenesis and transmission
- ❑ Suitable for systemic effects and complex immune responses
- ❑ Suitable for PK/PD evaluation
- ❑ Insight on clinical signs of infection
- ❑ Multiple proxys of infection/treatment efficacy
- ❑ Last candidate efficacy predictive go/no-go before clinical evaluation

**Reviewed in**: Takayama K *Trends in Pharmacological Sciences* 2020; Johansen MD et al *Mucosal Immunology* 2020; Muñoz-Fontela C et al *Nature* 2020

# 3. Animal models

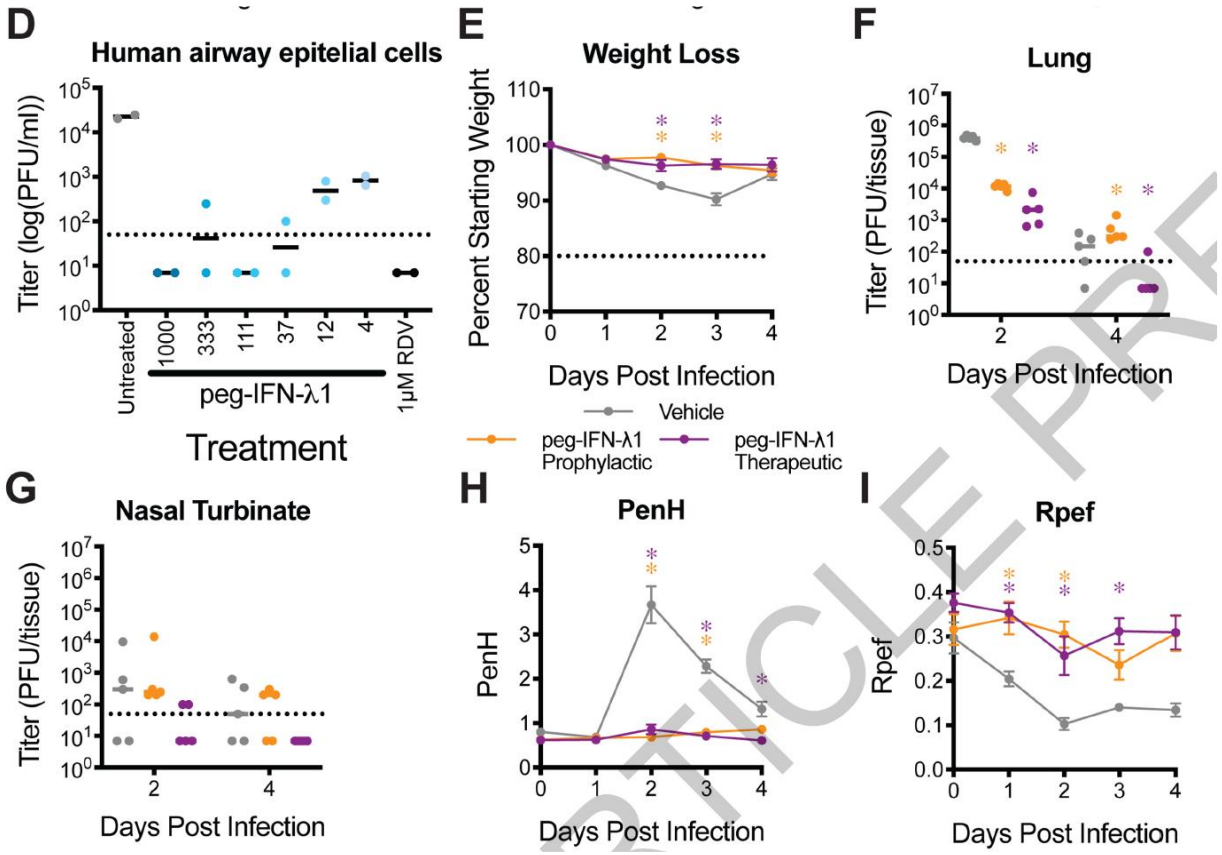
## Limits of *in vivo* models

- ❑ Might require genetic modification and/or animal adapted viral strains (permissiveness)
- ❑ Some host-responses different from human
- ❑ Limited offer of reagents (except mice)
- ❑ Need dedicated facilities and complex logistics
- ❑ Cost $$$
- ❑ Not immediately available in pandemic context

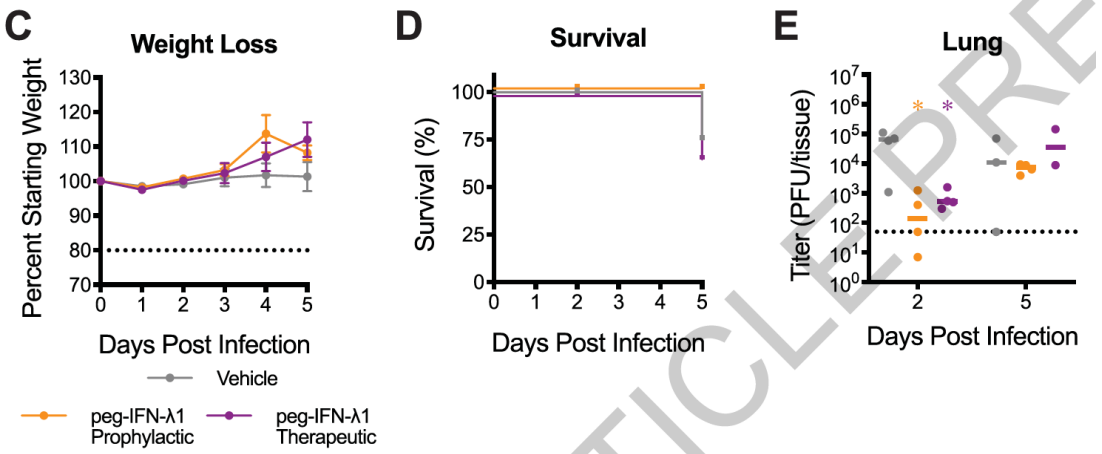
# 3. Animal models

## 3.1 Mice

### peg-IFN-λ1 treatment of BALB/c mice vs hACE2-mice infected with a MA-SARS-CoV-2




BALB/c mice




HFH4-hACE2 mice

Dinnon III KH et al *Nature* 2020

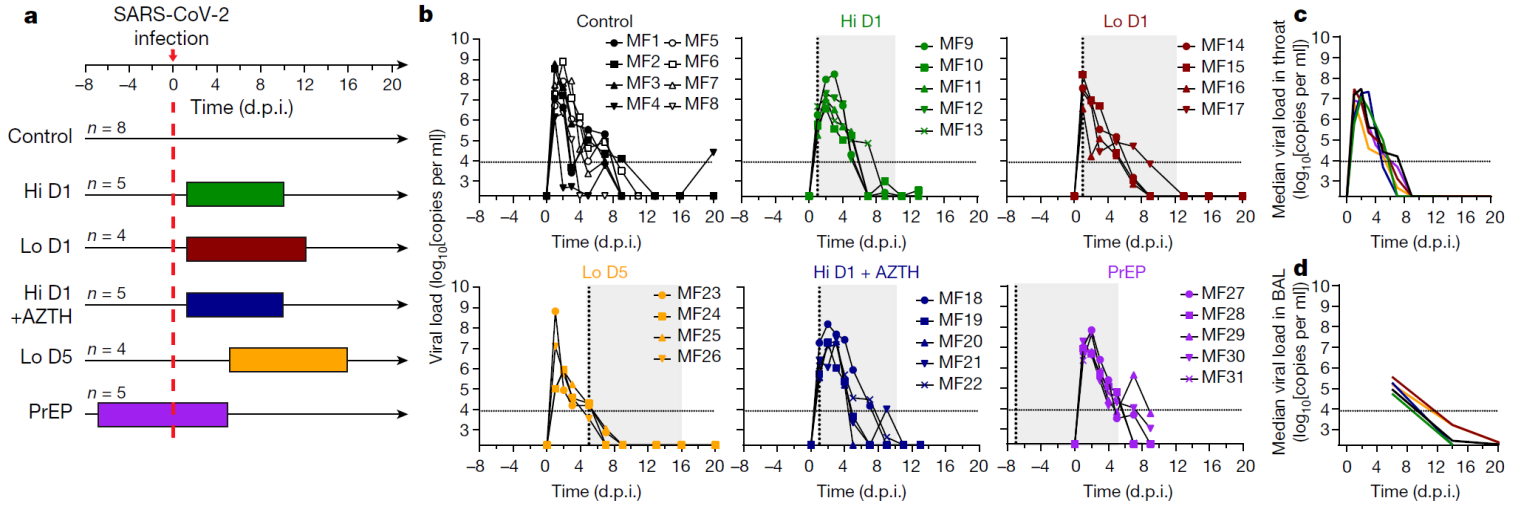
# 3. Animal models

## 3.2 Non-human primates

# Hydroxychloroquine use against SARS-CoV-2 infection in non-human primates

https://doi.org/10.1038/s41586-020-2558-4
Received: 30 April 2020
Accepted: 10 July 2020
Published online: 22 July 2020

Pauline Maisonnasse[1,11], Jérémie Guedj[2,11], Vanessa Contreras[1,11], Sylvie Behillil[3,4,11], Caroline Solas[5,11], Romain Marlin[1,11], Thibaut Naninck[1], Andres Pizzorno[6], Julien Lemaitre[1], Antonio Gonçalves[2], Nidhal Kahlaoui[1], Olivier Terrier[6], Raphael Ho Tsong Fang[1], Vincent Enouf[3,4,7], Nathalie Dereuddre-Bosquet[1], Angela Brisebarre[3,4], Franck Touret[8], Catherine Chapon[1], Bruno Hoen[9], Bruno Lina[6,10], Manuel Rosa Calatrava[6], Sylvie van der Werf[3,4], Xavier de Lamballerie[8] & Roger Le Grand[1]✉



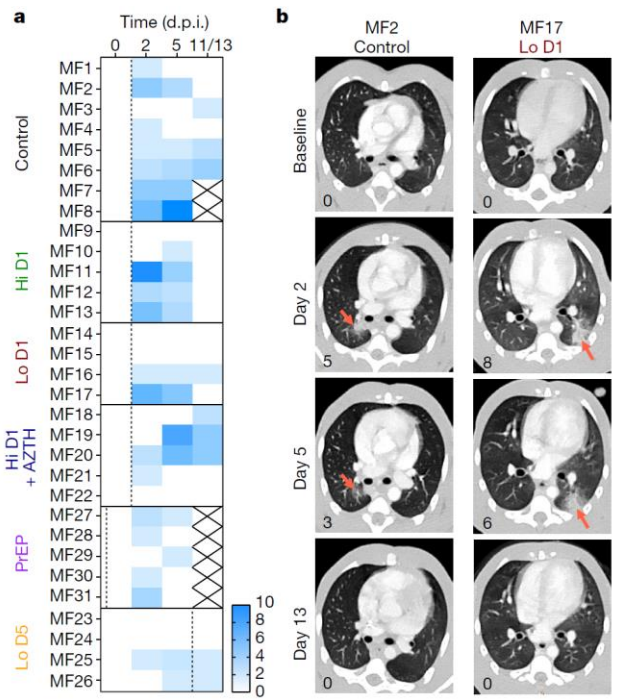




**Fig. 1 | Study design and viral loads in the respiratory tract of SARS-CoV-2-infected cynomolgus macaques treated with HCQ and AZTH. a**, Study design. The red dotted line indicates infection with $10^6$ PFU of SARS-CoV-2 by the combined intranasal and intratracheal routes. Coloured areas indicate HCQ treatment periods. Each group received either a high (Hi) or a low (Lo) dose of HCQ according to the regimens described in the Methods. The treatment started 1 d.p.i. (D1) or 5 d.p.i. (D5), or 7 days before viral challenge for the pre-exposure prophylaxis (PrEP) group. One group received AZTH in combination with a high dose of HCQ. The control group received vehicle (water) as placebo. **b**–**d**, Viral loads were analysed by PCR in throat swabs (**b**, **c**) and bronchoalveolar lavages (BAL) (**d**). The limit of detection was estimated to be 2.3 $\log_{10}$ copies of SARS-CoV-2 RNA per ml and the limit of quantification was estimated to be 3.9 $\log_{10}$ copies per ml (dotted horizontal line). **b**, Shaded zones indicate treatment periods and each symbol and line combination represents one NHP. Dotted vertical lines indicate day of treatment initiation. **c**, **d**, Data are represented as medians of each group as described in **a**.

# 3. Animal models

## 3.2 Non-human primates

# Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2

https://doi.org/10.1038/s41586-020-2423-5
Received: 23 April 2020
Accepted: 2 June 2020
Published online: 9 June 2020

Brandi N. Williamson[1], Friederike Feldmann[2], Benjamin Schwarz[3], Kimberly Meade-White[1], Danielle P. Porter[4], Jonathan Schulz[1], Neeltje van Doremalen[1], Ian Leighton[3], Claude Kwe Yinda[1], Lizzette Pérez-Pérez[1], Atsushi Okumura[1], Jamie Lovaglio[2], Patrick W. Hanley[2], Greg Saturday[2], Catharine M. Bosio[3], Sarah Anzick[5], Kent Barbian[5], Tomas Cihlar[4], Craig Martens[5], Dana P. Scott[2], Vincent J. Munster[1] & Emmie de Wit[1]✉

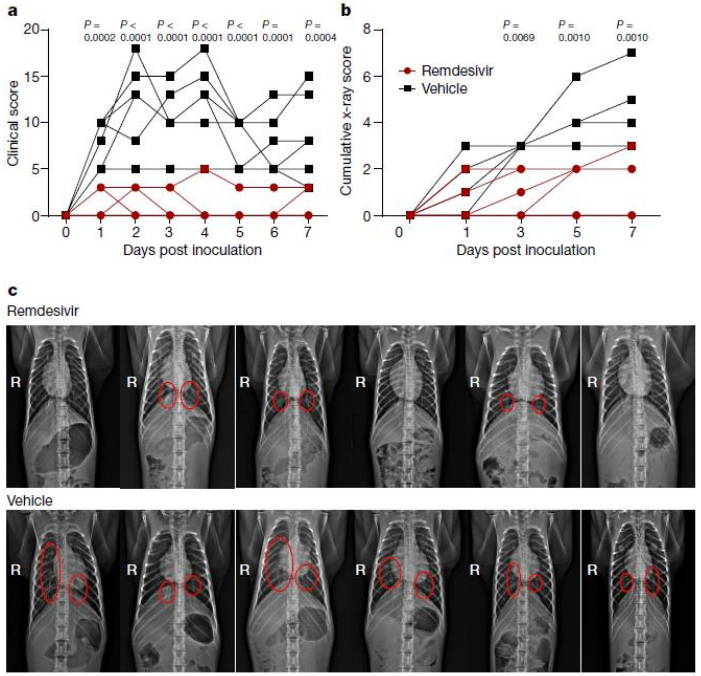
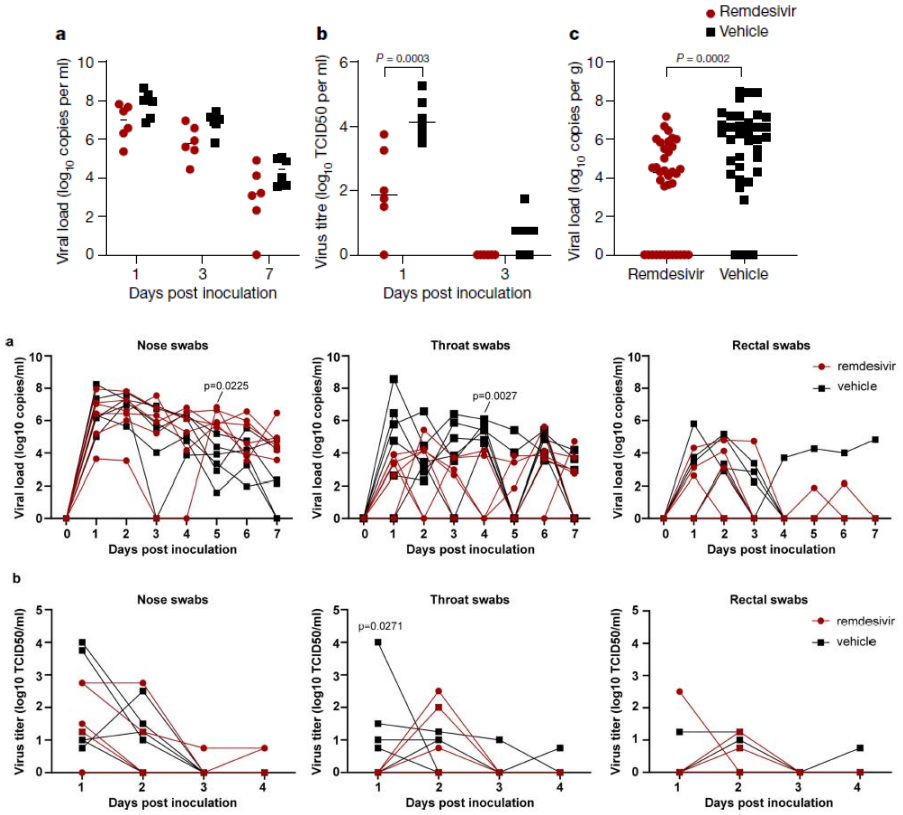



**Fig. 1 | Reduced respiratory disease in rhesus macaques infected with SARS-CoV-2 and treated with remdesivir.** **a**, Daily clinical scores for animals infected with SARS-CoV-2 and treated with remdesivir (red circles, $n = 6$) or vehicle solution (black squares, $n = 6$). **b**, Cumulative radiograph scores. Ventrodorsal and lateral radiographs were scored for the presence of pulmonary infiltrates by a clinical veterinarian according to a standard scoring system (0, normal; 1, mild interstitial pulmonary infiltrates; 2, moderate pulmonary infiltrates perhaps with partial cardiac border effacement and small areas of pulmonary consolidation; 3, severe interstitial infiltrates, large areas of pulmonary consolidation, alveolar patterns and air bronchograms). Individual lobes were scored and scores per animal per day were totalled and displayed. **c**, Ventrodorsal radiographs for each animal taken on 7 dpi. Areas of pulmonary infiltration are circled. Statistical analysis was performed using a two-way ANOVA with Sidak's multiple comparisons test.




**Extended Data Fig. 2 | Viral loads and virus titres in swabs collected from rhesus macaques infected with SARS-CoV-2 and treated with remdesivir.** **a**, Viral loads; **b**, infectious virus titers in nose, throat and rectal swabs collected daily from animals treated with remdesivir ($n = 6$) or vehicle solution ($n = 6$). Statistical analysis was performed using a 2-way ANOVA with Sidak's multiple comparisons test.

Remdesivir i.v. 12 hpi and 1/day x6

# 3. Animal models: recap

**Table 1 | SARS-CoV-2 infection in humans and in animal models**

| Trait | Organism |
|---|---|
| **Virus replication** | |
| Upper respiratory tract | Humans, mice, ferrets, non-human primates, mink, cats, bats |
| Lower respiratory tract | Humans, mice, hamsters, ferrets, non-human primates |
| Other organs | Humans (GI tract, CNS and kidney), hACE2 mice (CNS), hamsters, ferrets and non-human primates (GI tract) |
| **Clinical signs** | |
| Fever | Human, ferrets |
| Nasal discharge | Humans, ferrets |
| Laboured breathing | Humans, hamsters |
| **Pneumonia** | |
| Bilateral lung involvement | Humans, hamsters, non-human primates |
| Ground-glass opacities | Humans, hamsters, non-human primates |
| Focal oedema and inflammation | Humans, hamsters, ferrets, non-human primates |
| ARDS | Humans |
| **Transmission** | Humans, hamsters, ferrets, cats, bats |
| **Immunology** | |
| Seroconversion | Humans, hamsters, non-human primates, ferrets, bats, mice |
| Neutralizing antibody titres | Humans, hamsters, non-human primates, ferrets, mice |
| T cell immunity | Humans, non-human primates, ferrets, mice |
| Pro-inflammatory cytokines | Humans, non-human primates, mice |

| Animal species | | Key points |
|---|---|---|
| Mice | Wild type mice | SARS-CoV-2 cannot invade cells through mouse Ace2. |
| | Human ACE2 transgenic mice | After SARS-CoV-2 infection, the mice show weight loss, virus replication in the lungs, and interstitial pneumonia. |
| Syrian hamster | | After SARS-CoV-2 infection, the hamsters show rapid breathing, weight loss, and diffuse alveolar damage with extensive apoptosis. |
| Ferrets | | After SARS-CoV-2 infection, acute bronchiolitis was observed in the lungs. |
| Cats | | After SARS-CoV-2 infection, intra-alveolar edema and congestion in the interalveolar septa were observed. Abnormal arrangement of the epithelium with loss of cilia and lymphocytic infiltration into the lamina propria were also observed. |
| Cynomolgus macaques | | SARS-CoV-2 can infect both type I and type II pneumocytes. After SARS-CoV-2 infection, pulmonary consolidation, pneumonia, and edema fluid in alveolar lumina were observed. |
| Rhesus macaques | | Infected macaques had high viral loads in the upper and lower respiratory tract, humoral and cellular immune responses, and pathologic evidence of viral pneumonia. The therapeutic effects of adenovirus-vectored vaccine, DNA vaccine candidates expressing S protein, and remdesivir treatment could be evaluated. |

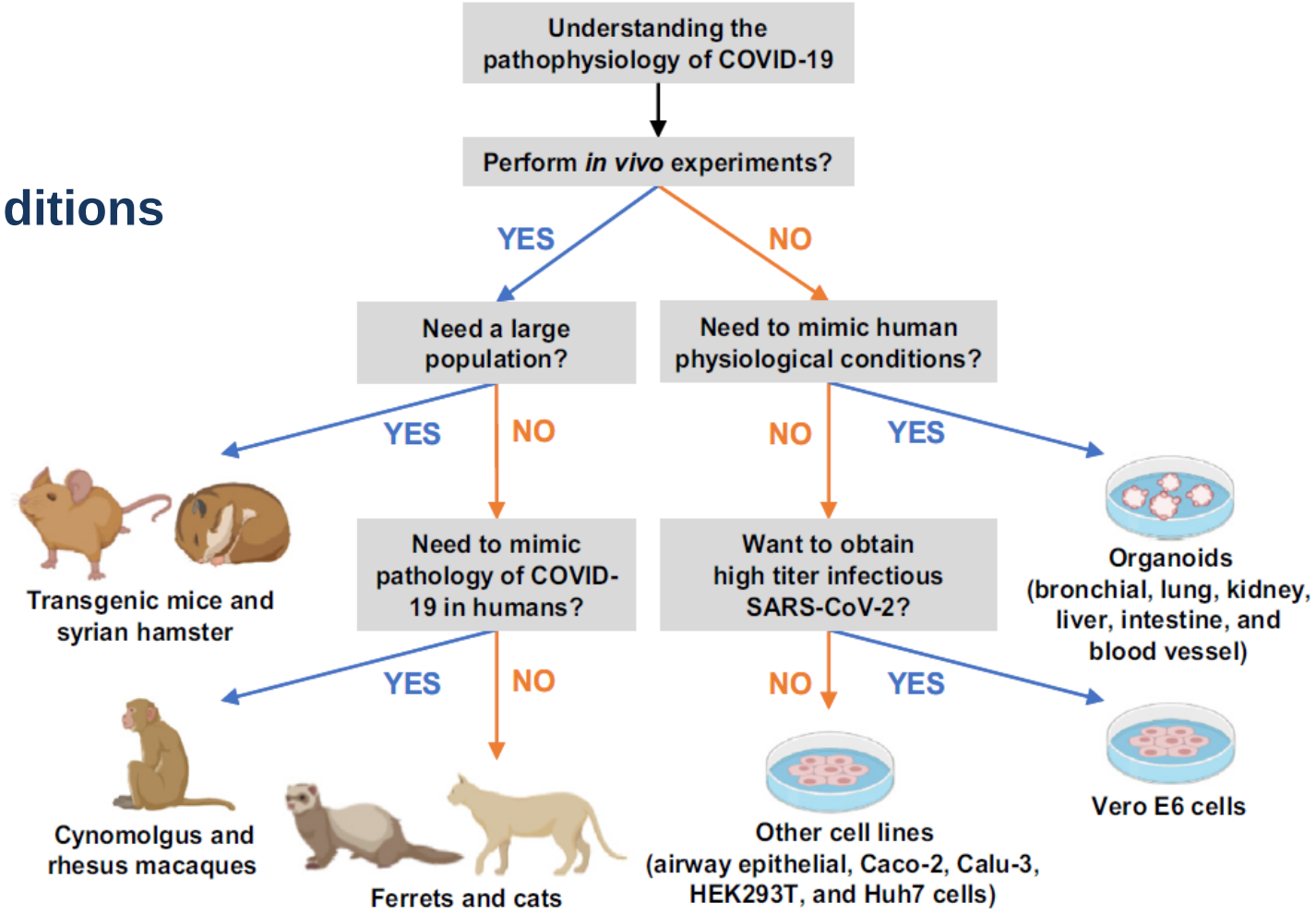
Muñoz-Fontela C et al *Nature* 2020; Takayama K *Trends in Pharmacological Sciences* 2020

# Conclusions and future directions

- ❑ Thorough **model characterization** is mandatory (know their **limits** and clearly **disclose** them)
- ❑ No “one size fits all” solution for candidate downselection, **integrated approach**
- ❑ Combination of **cells (x2) + HAE + NHP** → collaborative consortia?
- ❑ Improve pre-clinical **data reporting**: exp model, viral strain, treatment regimen and assay readout strongly impact IC50 results → ARRIVE-like?

# Conclusions and future directions

- ❑ High **quality pre-clinical evaluation** is worth the investment
- ❑ Prioritize **physiologically relevant conditions** whenever possible
- ❑ **RCT** to ultimately separate the wheat from the chaff




Takayama K *Trends in Pharmacological Sciences* 2020,figure created with Biorender


# Thank you for your attention

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