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
   - immunomodulators: 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
Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro
The importance (and limits) of pre-clinical models to improve clinical management

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

WHO to launch multinational trial to jumpstart search for coronavirus drugs

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

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

(Hydroxy)chloroquine – previous viruses

<table>
<thead>
<tr>
<th>In vitro effect</th>
<th>Clinical efficacy</th>
</tr>
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<tbody>
<tr>
<td>Chikungunya</td>
<td>RCT: deleterious</td>
</tr>
<tr>
<td>Dengue</td>
<td>RCT: negative</td>
</tr>
<tr>
<td>Influenza</td>
<td>RCT: negative</td>
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<tr>
<td>SARS</td>
<td></td>
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<tr>
<td>Zika</td>
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</tbody>
</table>
Three major classes of pre-clinical experimental models for SARS-CoV-2
1. “Classic” 2D cultured cell lines

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>❑ Suitable for viral isolation and propagation</td>
<td>❑ Transformed cell lines (immortalization)</td>
</tr>
<tr>
<td>❑ Suitable for viral replication and pathogenesis</td>
<td>❑ Limited receptor repertoire</td>
</tr>
<tr>
<td>❑ Suitable for early HT candidate screening</td>
<td>❑ “Artificial” infection and treatment conditions</td>
</tr>
<tr>
<td>❑ Panoply of reagents available</td>
<td>❑ Limited value for “omics”-based approaches</td>
</tr>
<tr>
<td>❑ Easy to manipulate</td>
<td>❑ Limited value for host-targeted candidates</td>
</tr>
<tr>
<td>❑ Cost $</td>
<td>❑ Pro-drug vs metabolite?</td>
</tr>
<tr>
<td>❑ Rapidly available in pandemic context</td>
<td>❑ High rate of false positive “hits”!</td>
</tr>
</tbody>
</table>
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
Differential candidate inhibition of SARS-CoV-2 depending on the cell line

Levi J et al poster #19

Hoffmann M et al Nature 2020
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

- Primary Human Cells
  - Isolation, amplification & seeding

- Ready-to-use
  - Fully differentiated Airway Epithelium

- Shelf-life of 1 year

- Air-Liquid interface, differentiation

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

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

### 2.2 Bronchial/lung organoids

**Step 1:** Tissue dissociation into single cells

- Trachea
- Tracheobronchial and large airways
- Alveolar region
- Adult lung

**Step 2:** Isolation of stem/progenitor cells

- Expand and manipulate cells in 2D culture
- FACS or MACS

**Step 3:** 3D culture with or without stromal cells

- Transwell inserts
- Glass slides for imaging
- Multiwells

**Step 4:** Analyses

- Histology
- Morphometry
- Gene expression analysis

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

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

**Figure 3**

- **A** SARS-CoV-2 infection. 5.0 × 10⁴ PFU/organoid
- **B** Luminescence (RLU) of *SARS-CoV-2* infection.
- **C** Relative viral genome expression with *SARS-CoV-2* infection.
- **D** *SARS-CoV-2* S protein expression.

**Figure 4**

- **F** Alveolar organoid vs organoid-derived ALI.

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

Suzuki T et al biorXiv 2020

Mulay A and Konda B et al biorXiv 2020
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 proxies of infection/treatment efficacy
- Last candidate efficacy predictive go/no-go before clinical evaluation

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

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

Pauline Maisonnasse(10), Jerémie Guedj(10), Vanessa Contreras(10), Sylvie Behillii(1,11), Carcine Solas(1,10), Romain Marlin(10), Thibaut Naninck(1), Andres Pizzorno(1), Julien Lemaître(1), Antomico Gonçalves(1), Nidhal Khaled(1), Olivier Terrier(1), Raphael Ho Tsong Fang(1), Vincent Euny(1,11), Nathalie Dormay-Buscaglia(1), Angela Brindis(2), François Touzet(1), Catherine Chapon(1), Bruno Hoen(1), Bruno Lina(1), Manuel Rosa Calatrava(1), Sylvie van der Werf(1), Xavier de Lamballerie(1) & Roger Le Grand(1)

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Published online: 22 July 2020

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 10^7 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 1d.p.i. (D1) or 5d.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

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Brandi N. Williamson, Friederike Feldmann, Benjamin Schwarz, Kimberly Meade-White, Daniele P. Porter, Jonathan Schulte, Neeltje van Doremalen, lan Leighton, Claude Kow Yinda, Lizzette Perez-Perez, Atsushi Okumura, Jamie Lovaglio, Patricia W. Hanley, Greg Saturday, Catharine M. Besio, Sarah Anzick, Kent Barbarin, Tomas Cihlar, Craig Martin, Dana P. Scott, Vincent J. Munster & Emanuella H. Wth1

Fig. 1A: 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=4) or vehicle solution (black squares, n=6). b. Cumulative radiographs showing ventrodorsal and lateral radiographs 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, 3: severe interstitial pulmonary infiltrates, and 4: consolidation in all lung areas). c. Representative radiographs for each animal per day are included and displayed. 

Fig. 2A: Remdesivir treatment reduces nasal, throat, and rectal swabs collected daily from animals treated with remdesivir (green line) or vehicle solution (blue line). Statistical analysis was performed using a two-way ANOVA with Sidák's multiple comparison 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

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

Animal species | Key points
--- | ---
Mice | Wild type mice  SARS-CoV-2 cannot invade cells through mouse Acs2.

  | 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 alveolar lumina 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 oedema 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.

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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?
High quality pre-clinical evaluation is worth the investment.

Prioritize physiologically relevant conditions whenever possible.

RCT to ultimately separate the wheat from the chaff.
Thank you for your attention

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