What did we learn about \textit{in vitro} models for COVID-19 that made a difference?

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Learning that makes a difference - propagation

Data sharing via working groups identified issues regarding propagation

Early lessons – some working stocks were genetically compromised

Why?
1. Serial passage in Vero cells (and others) permitted outgrowth of mutations/deletions
2. Mixed populations in samples? (Not yet proven)

Mitigation
1. Alternative host cell choices (e.g. Vero/hSLAMS better than Veros)
2. Deep sequencing of stocks to detect changes during production

Learning 1; RNA viruses adapt to new host cells with few exceptions – need deep sequencing verification
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A cautionary perspective regarding the isolation and serial propagation of SARS-CoV-2 in Vero cells


An array of SARS-CoV-2 virus variants have been isolated, propagated and used in in vitro assays, in vivo animal studies and human clinical trials. Observations of working stocks of SARS-CoV-2 suggest that sequential propagation in Vero cells leads to critical changes in the region of the furin cleavage site, which significantly reduce the value of the working stock for critical research studies. Serially propagating SARS-CoV-2 in Vero E6 cells leads to rapid increases in genetic variants while propagation in other cell lines (e.g., Vero/hSLAM) appears to mitigate this risk thereby improving the overall genetic stability of working stocks. From these observations, investigators are urged to monitor genetic variants carefully when propagating SARS-CoV-2 in Vero cells.

npj Vaccines (2021) 6:83; https://doi.org/10.1038/s41541-021-00346-z
Despite early murine and Vero data suggesting otherwise, Hydroxychloroquine was not effective in animal models of infection. Human organoid tissue culture systems supported the animal studies findings. Different labs with different organoid systems agreed on this outcome. This scenario of cell culture failure was almost identical for Imatinib.

Learning 2: Human organoid cultures (resp) provided an effective, reproducible screening tool.
Global data sharing provided a reliable signal

<table>
<thead>
<tr>
<th>Authors</th>
<th>Source</th>
<th>Test item</th>
<th>Test system</th>
<th>Dose</th>
<th>Antiviral</th>
<th>Symptomatic</th>
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<td>Frieman et al</td>
<td>NIH</td>
<td>CQ, HCQ</td>
<td>Vero cells + SARS-CoV</td>
<td>1.0E+05 PFU</td>
<td>Yes</td>
<td>Mild effect</td>
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<td>Frieman et al</td>
<td>NIH</td>
<td>CQ, HCQ</td>
<td>Mice + MA SARS-CoV</td>
<td>2.0E+06 TCID₅₀</td>
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<td>Minster et al</td>
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<td>Ingber et al</td>
<td>Wyss Inst</td>
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<td>Human respiratory Emulate + Pseudovirus</td>
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<td>Massonaise et al</td>
<td>INSERM</td>
<td>HCQ</td>
<td>Human respiratory Mucilair™ + SARS-CoV-2</td>
<td>6.3E+06 to 4.3E+07 TCID₅₀</td>
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</tr>
</tbody>
</table>

It is not viable or realistic to repeat all of these *in vivo* studies for all VOCs
But repeat human OoC studies may provide an acceptable bridging mechanism
Learning that makes a difference – Immunology needs identified

• Humoral immunity
  • Can be assessed for biological relevance using live virus neutralisation in vitro
  • Pseudovirus neutralisation does not always equal live virus neutralisation

• Cellular immunity
  • Difficult to standardise
  • Difficult to transfer the technology
  • Needs a better way to be assessed
  • Could be investigated as a research target using in vitro organoid cultures
  • We need methods to assess cellular and humoral immunity
The worst case scenario for Disease X = Disease X (Omega)

- It was not predicted as it is a “curve ball”
- Infectious, pathogenic, novel
- It has no known vaccine, therapeutic or drug treatment
- It will not infect any other species other than Homo sapien
- It will not grow in cell culture from cells derived from other animals
- It has an organ specificity

Human organoid culture (OoC or MPS, not cancer cells) would immediately be invaluable
Different organs have already developed (Liver, Brain, URT, LRT and others)
How does this help in preparedness for Disease X?

• No one *wants* to use animals in human medical research

• But animals are the best models we have for some disease efficacy testing

• Complex human culture systems may aid in the event of Disease X

• Such systems may also help to reduce other animal model dependency

• We cannot depend on animal models indefinitely
Learning that makes a difference - Summary

• RNA viruses adapt to most new host cells – we need deep sequencing verification

• Human organoid cultures (resp) provided an effective, reproducible screening tool

• Neutralisation of wild type virus *in vitro* infection has been an important tool

• We need better cellular immunity tools

• Human complex *in vitro* systems were shown to be reliable

• Human complex *in vitro* systems may offer long term 3Rs objective