UK Health Security Agency

What did we learn about *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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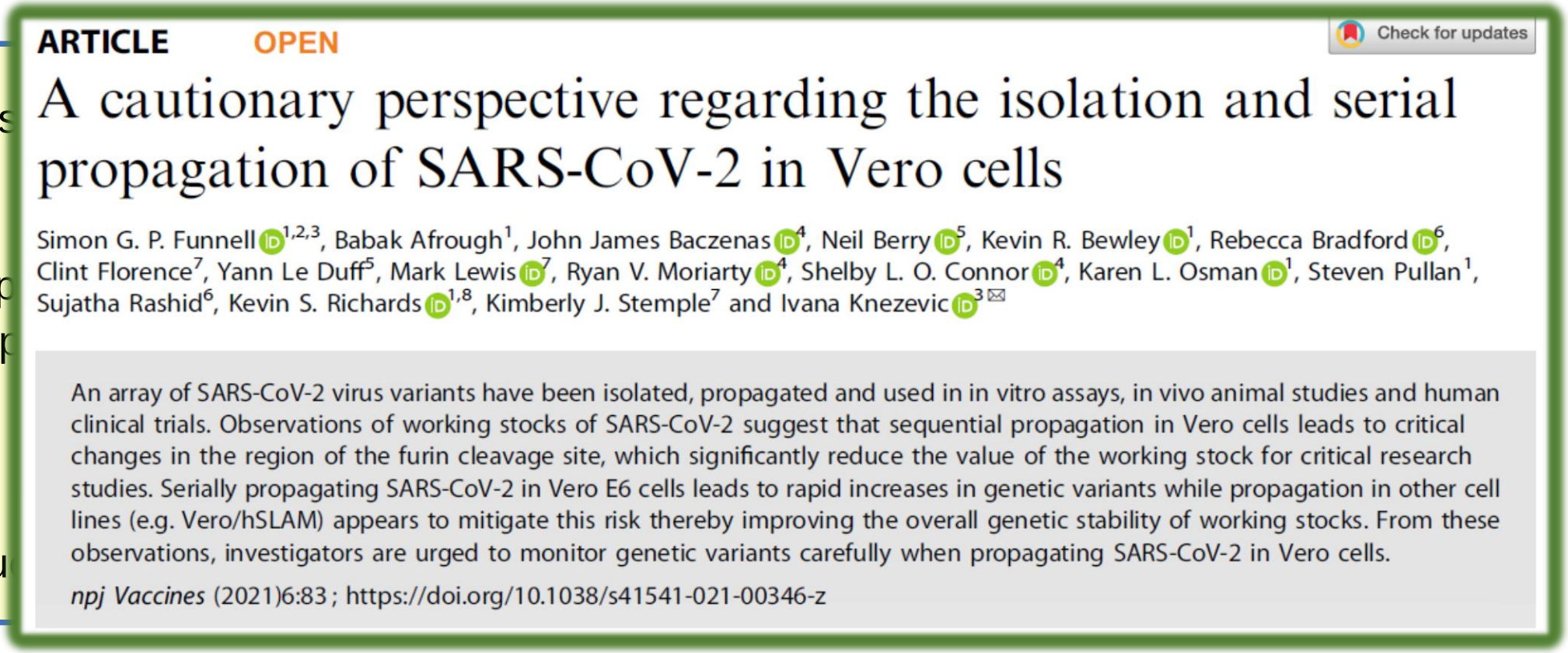
Early lessons – s

Why?

1. Serial p
2. Mixed p

Mitigation

1. Alternative
2. Deep sequ



ARTICLE OPEN

Check for updates

A cautionary perspective regarding the isolation and serial propagation of SARS-CoV-2 in Vero cells

Simon G. P. Funnell[1,2,3], Babak Afrough[1], John James Baczenas[4], Neil Berry[5], Kevin R. Bewley[1], Rebecca Bradford[6], Clint Florence[7], Yann Le Duff[5], Mark Lewis[7], Ryan V. Moriarty[4], Shelby L. O. Connor[4], Karen L. Osman[1], Steven Pullan[1], Sujatha Rashid[6], Kevin S. Richards[1,8], Kimberly J. Stemple[7] and Ivana Knezevic[3]✉

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

Learning 1; RNA viruses adapt to new host cells with few exceptions – need deep sequencing verification

Learning that makes a difference – human organoids don't deceive

Hydroxychloroquine and Imatinib

Despite early murine and Vero data suggesting otherwise,
Hydroxchloroquine 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

Emerging preclinical evidence does not support broad use of hydroxychloroquine in COVID-19 patients
S. G. P. Funnell , W. E. Dowling, C. Muñoz-Fontela, P.-S. Gsell, D. E. Ingber, G. A. Hamilton, L. Delang , J. Rocha-Pereira, S. Kaptein, K. H. Dallmeier, J. Neyts K. Rosenke, E. de Wit , H. Feldmann, P. Maisonnasse , R. Le Grand , M. B. Frieman & C. M. Coleman, Nature Communications **doi:** https://doi.org/10.1038/s41467-020-17907-w
Published 26AUG2020

Preclinical evaluation of Imatinib does not support its use as an antiviral drug against SARS-CoV-2
F Touret, J Driouich, M Cochin, P Rémi Petit, M Gilles, K Barthélémy, G Moureau, D Malvy, C Solas, X de Lamballerie, A Nougairède
doi: https://doi.org/10.1101/2020.11.17.386904

Learning that makes a difference – Data sharing helped

- Global data sharing provided a reliable signal

Authors	Source	Test item	Test system	Dose	Antiviral	Symptomatic
Frieman *et al*	NIH	CQ HCQ	Vero cells + SARS-CoV		Yes	Mild effect
Frieman *et al*	NIH	CQ HCQ	Mice + MA SARS-CoV	1.0E+05 PFU	None	Yes
Kaptein *et al*	KU Leuven	HCQ	Hamster + SARS-CoV-2	2.0E+06 $TCID_{50}$	None	None
Rosenke *et al*	RML NIAID	HCQ	Hamster + SARS-CoV-2	1.0E+04 $TCID_{50}$	None	None
Massonaise *et al*	Inserm	HCQ	Cyno + SARS-CoV-2	1.0E+06 PFU	None	None
Minster *et al* Rosenke *et al*	RML NIAID	HCQ	Rhesus + SARS-CoV-2	2.8E+06 $TCID_{50}$	None	None
Ingber *et al*.	Wyss Inst	HCQ	Human respiratory Emulate + Pseudovirus	-	None	None
Massonaise *et al*	INSERM	HCQ	Human respiratory Mucilair™ + SARS-CoV-2	6.3E+06 to 4.3E+07 $TCID_{50}$	None	None

- 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

How does this help in preparedness for Disease X?

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