Challenges in developing treatments for COVID-19: promising approaches for the future including RNA editing

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Doherty Institute

THE UNIVERSITY OF MELBOURNE

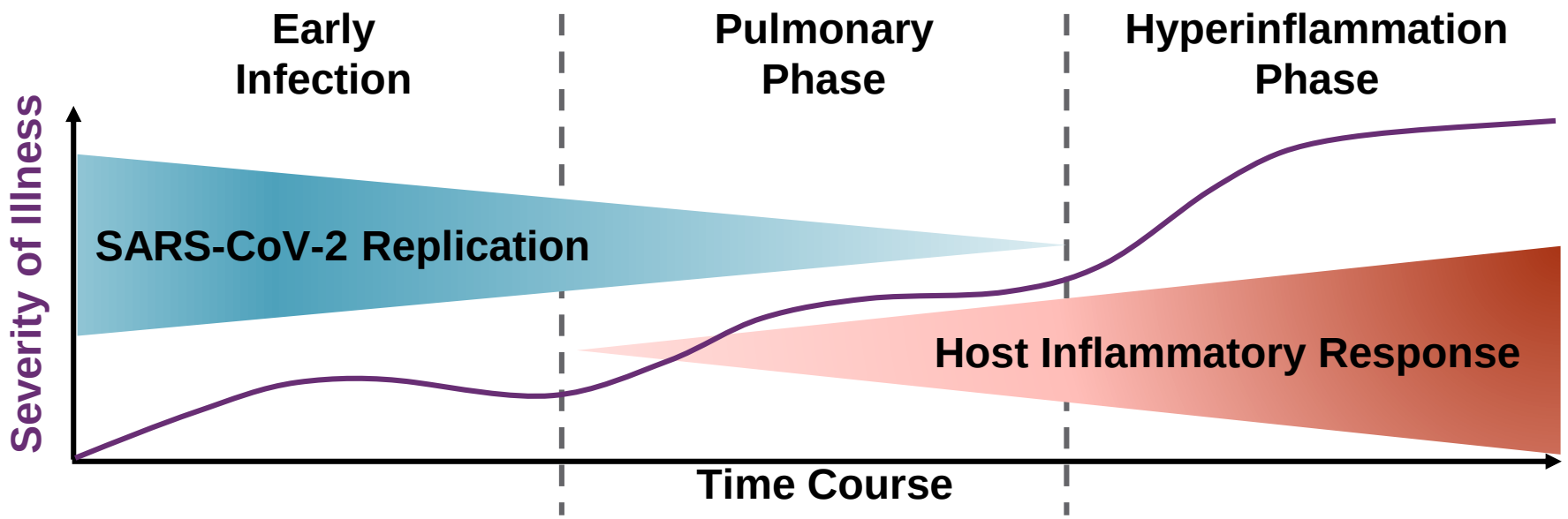
The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Outline

- Natural history of COVID-19 and opportunities for treatment
- Monoclonal antibodies and direct acting antivirals
- CRISPR Cas 13 as a direct acting antiviral
- Platform technologies for pandemic preparedness

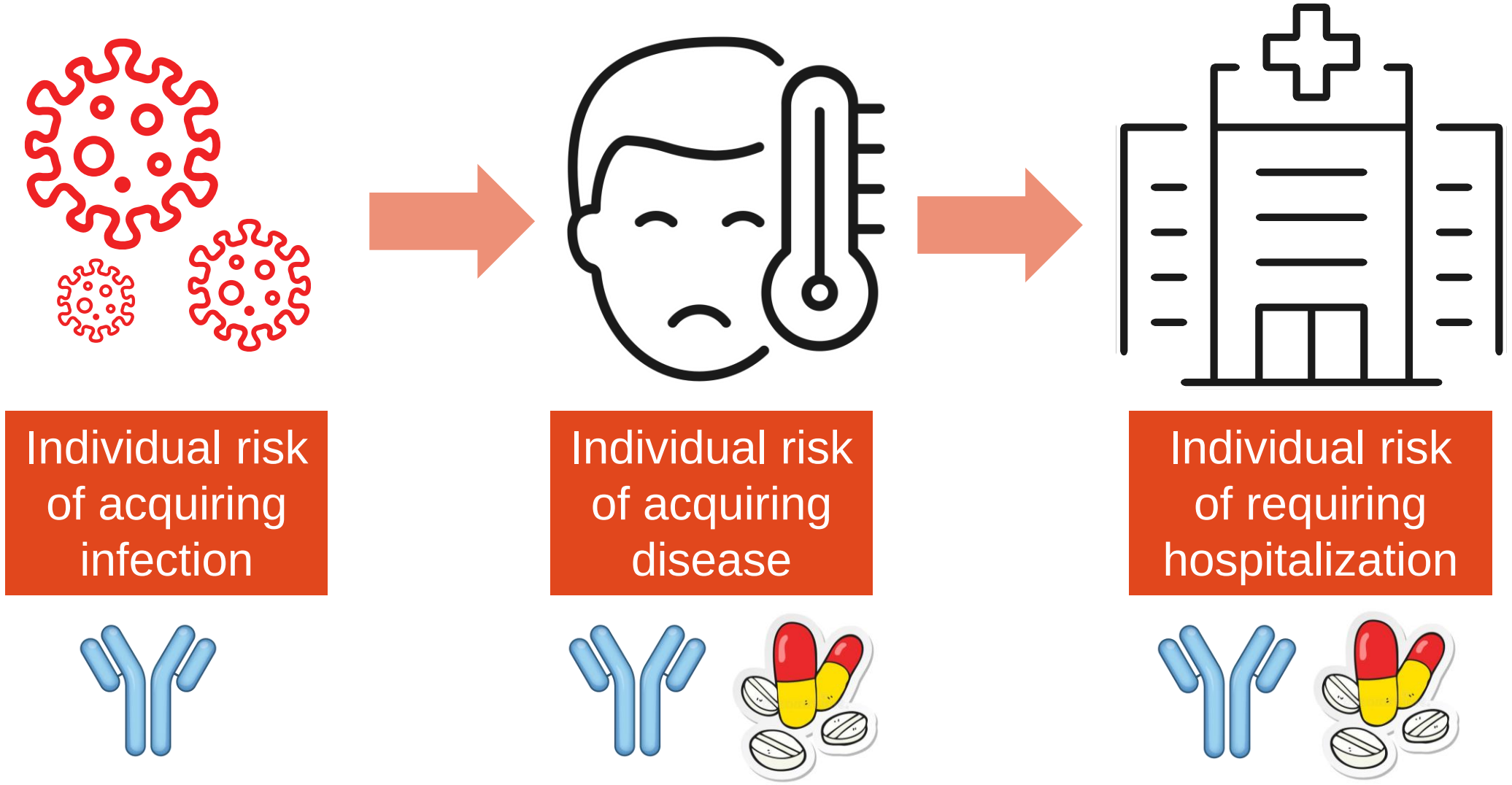
Natural history and treatment of COVID-19



NIH COVID-19 Treatment Guidelines. Clinical management summary. Last updated July 8, 2021. Siddiqi. J Heart Lung Transplant. 2020;39:405.

Slide credit: clinicaloptions.com

Multiple potential roles of antivirals in COVID-19



Effect of oral antivirals and antibodies on hospitalisation

	Proportion Progressed with Therapy (%)	Proportion Progressed with Placebo (%)	Relative Risk reduction (%)	Absolute Risk reduction (%)	Symptom onset
Sotrovimab	1.0	7.0	85	6.0	< 5 days
REGEN-COV	1.3	4.6	71	3.3	< 7 days
Molnupiravir	7.3	14.1	48	6.8	< 5 days
PF-07321332 / Ritonavir	1.0	6.7	85	5.7	< 5 days

- Antivirals and antibodies work in preventing disease progression and hospitalisation **in high risk patients** ie >50 years old or at least one co-morbidity but treatment must be early
- All studies to date performed in **unvaccinated participants**. Additional benefit in vaccinated will be lower
- Cost remains an issue for use as a public health intervention given the **number needed to treat** e.g., oral antivirals ($700 USD per course) and antibodies ($2,000 USD)

Gupta NEJM 2021, Weinreich NEJM 2021, merck.com, pfizer.com

Host targeted therapies can also reduce hospitalisation

Inhaled budesonide in the treatment of early COVID-19 (STOIC): a phase 2, open-label, randomised controlled trial

Sanjay Ramakrishnan, Dan V Nicolau Jr*, Beverly Langford, Mahdi Mahdi, Helen Jeffers, Christine Mwasuku, Karolina Krassowska, Robin Fox, Ian Binnian, Victoria Glover, Stephen Bright, Christopher Butler, Jennifer L Cane, Andreas Halner, Philippa C Matthews, Louise E Donnelly, Jodie L Simpson, Jonathan R Baker, Nabil T Fadai, Stefan Peterson, Thomas Bengtsson, Peter J Barnes, Richard E K Russell, Mona Bafadhel*

Effect of early treatment with fluvoxamine on risk of emergency care and hospitalisation among patients with COVID-19: the TOGETHER randomised, platform clinical trial

*Gilmar Reis, Eduardo Augusto dos Santos Moreira-Silva, Daniela Carla Medeiros Silva, Lehana Thabane, Aline Cruz Milagres, Thiago Santiago Ferreira, Castilho Vitor Quirino dos Santos, Vitoria Helena de Souza Campos, Ana Maria Ribeiro Nogueira, Ana Paula Figueiredo Guimaraes de Almeida, Eduardo Diniz Callegari, Adhemar Dias de Figueiredo Neto, Leonardo Cançado Monteiro Savassi, Maria Izabel Campos Simplicio, Luciene Barra Ribeiro, Rosemary Oliveira, Ofir Harari, Jamie I Forrest, Hinda Ruton, Sheila Sprague, Paula McKay, Alla V Glushchenko, Craig R Rayner, Eric J Lenze, Angela M Reiersen, Gordon H Guyatt, Edward J Mills, for the TOGETHER investigators**

Ramakrishnan et al., Lancet Resp Dis 2021; Reis et al., Lancet Global Health 2021

CRISPR Cas13 as a direct acting antiviral

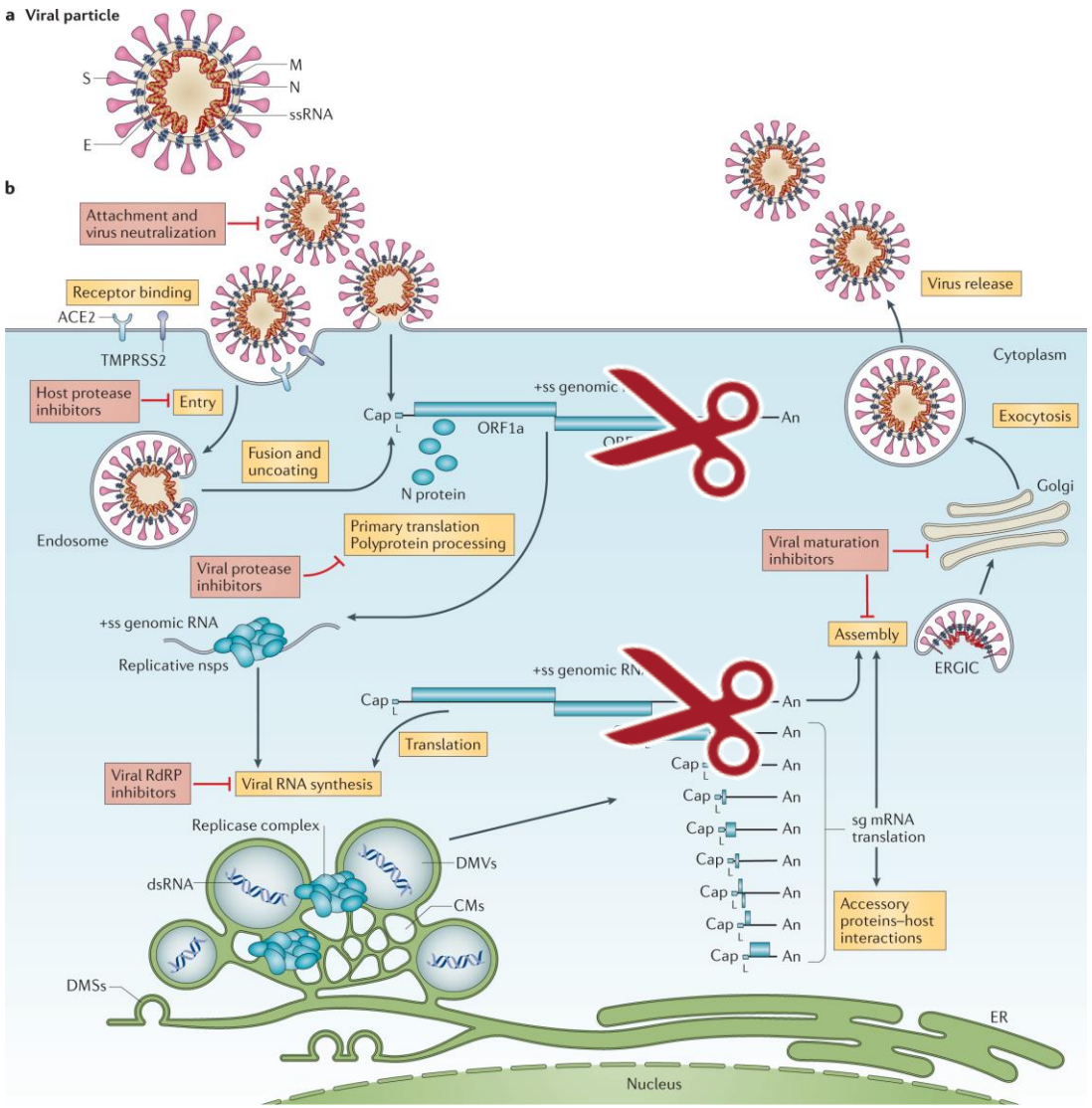
Multi-disciplinary research in a pandemic

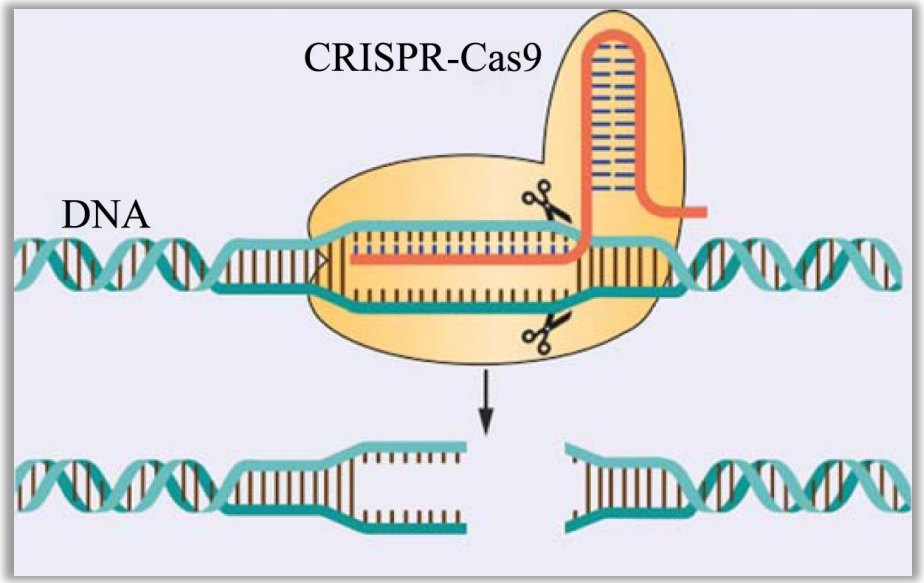
Dr Mohamed Fareh
Molecular Biologist
Peter Macallum Cancer Centre

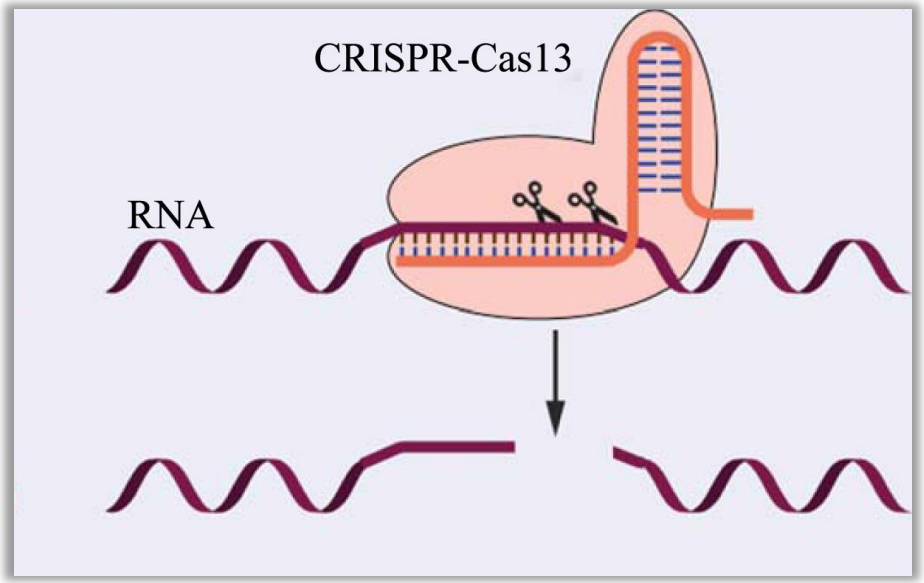
Professor Joe Trapani
Cancer Immunotherapy
Peter Macallum Cancer Centre

Wei Zhao
Post doctoral fellow
Doherty Institute

Direct acting antivirals: targeting viral RNA

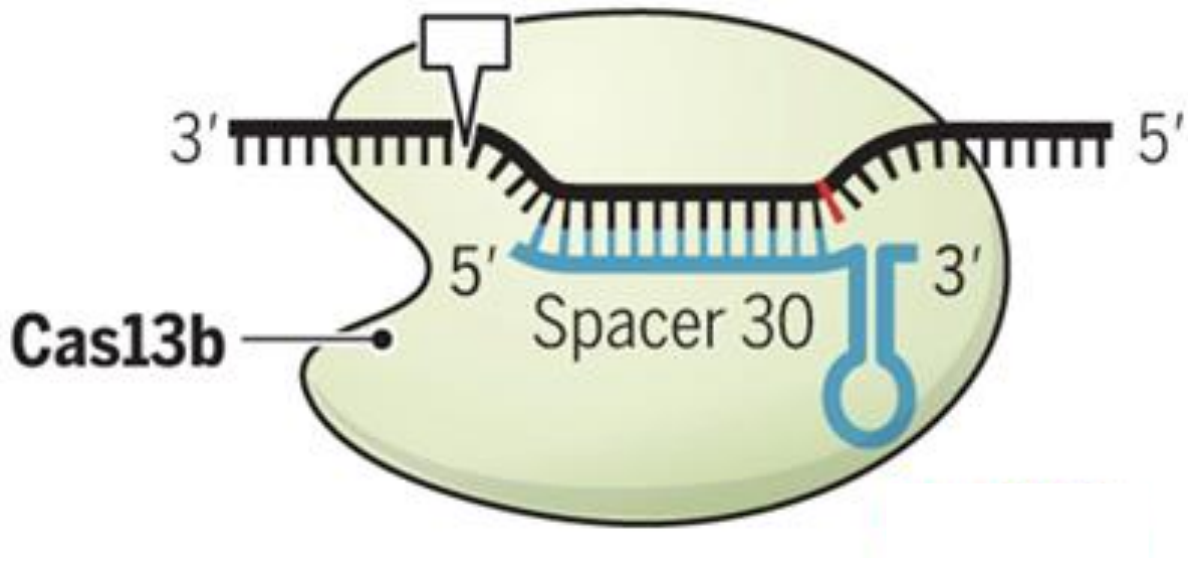






Fareh, Zhao et al Nat Comms 2021

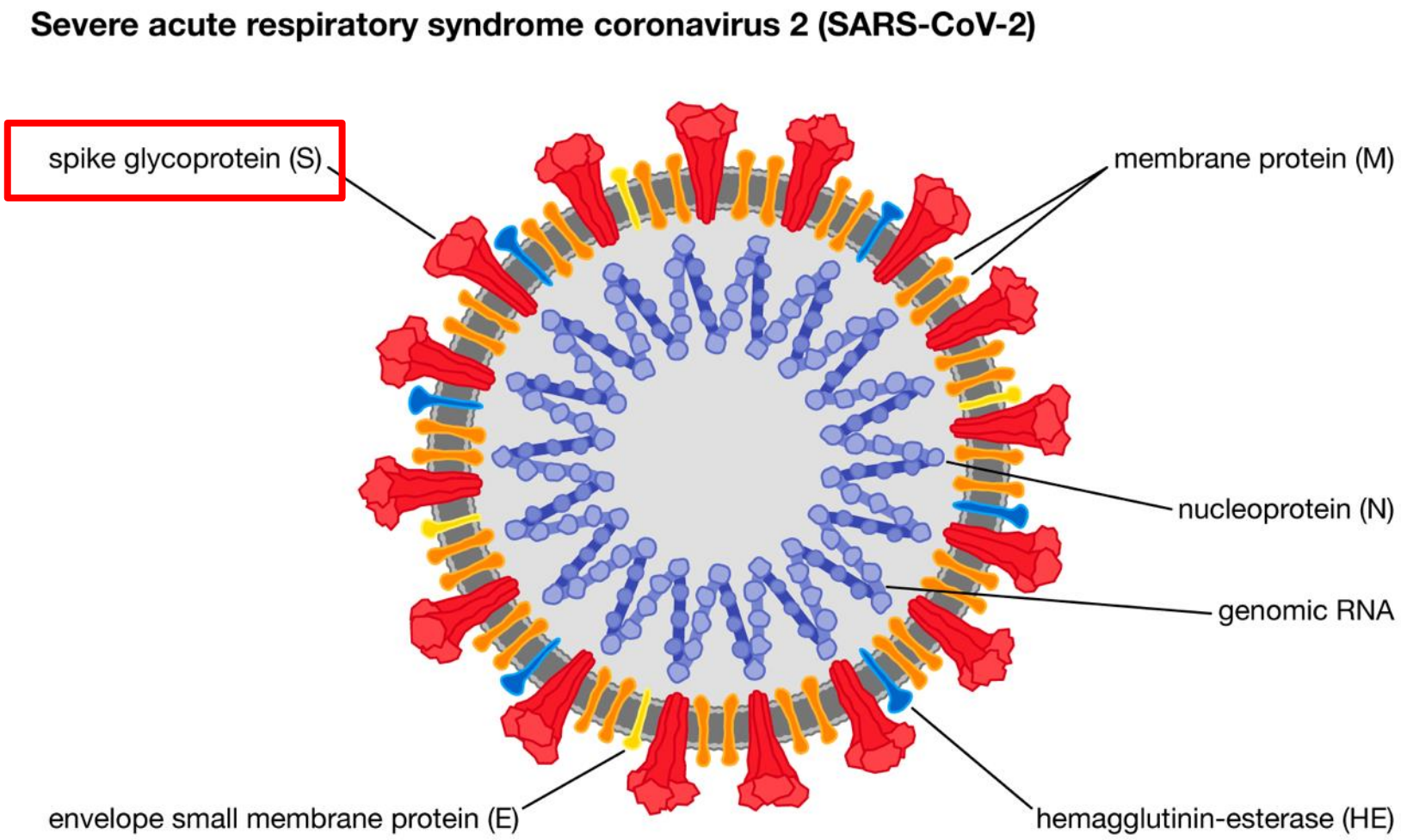
Sequence-specific RNA silencing with Cas13b



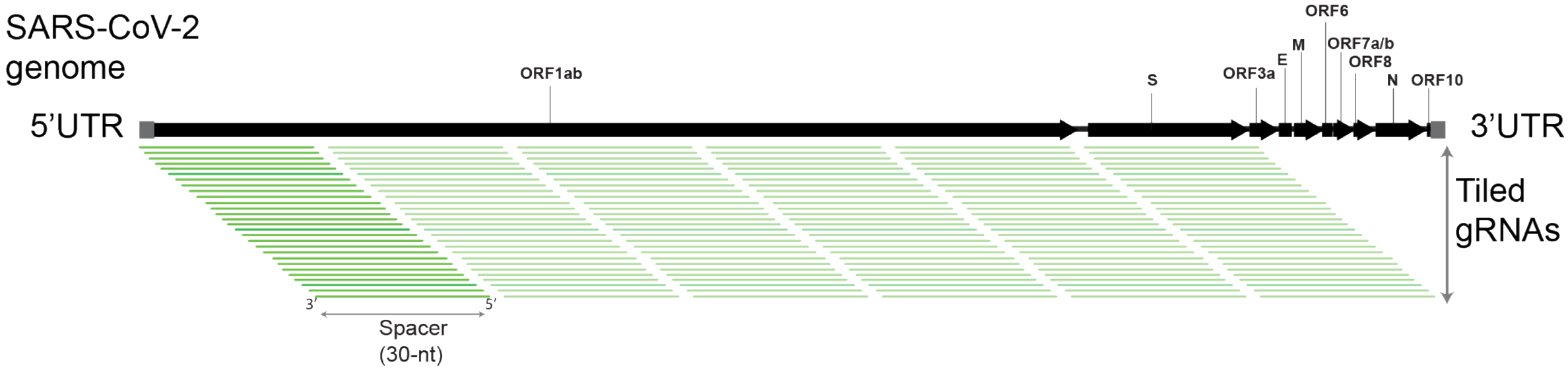
Omar O. Abudayyeh, **Science** 2016
East-Seletsky et al, **Nature**, 2016
Liang Liu et al, **Cell**, 2017
Lui et al, **Cell**, 2017
Cox et al, **Science**, 2017
Meeske et al, **Nature**, 2019

- Cas13 is an RNA-guided RNA-targeting CRISPR effector.
- Single protein with a single gRNA.
- Sequence-specific targeting through basepairing between the gRNA and target.
- High specificity (**30-nt Spacer**) with '**ZERO'** off-targeting probability.
- Programmable & multiplexable.

Targeting SARS-CoV-2 structural proteins



Bioinformatic pipeline for design of potent crRNAs



Single-nucleotide tiled gRNAs covering the entire genome of SARS-CoV-2 (29,874 gRNAs)

↓

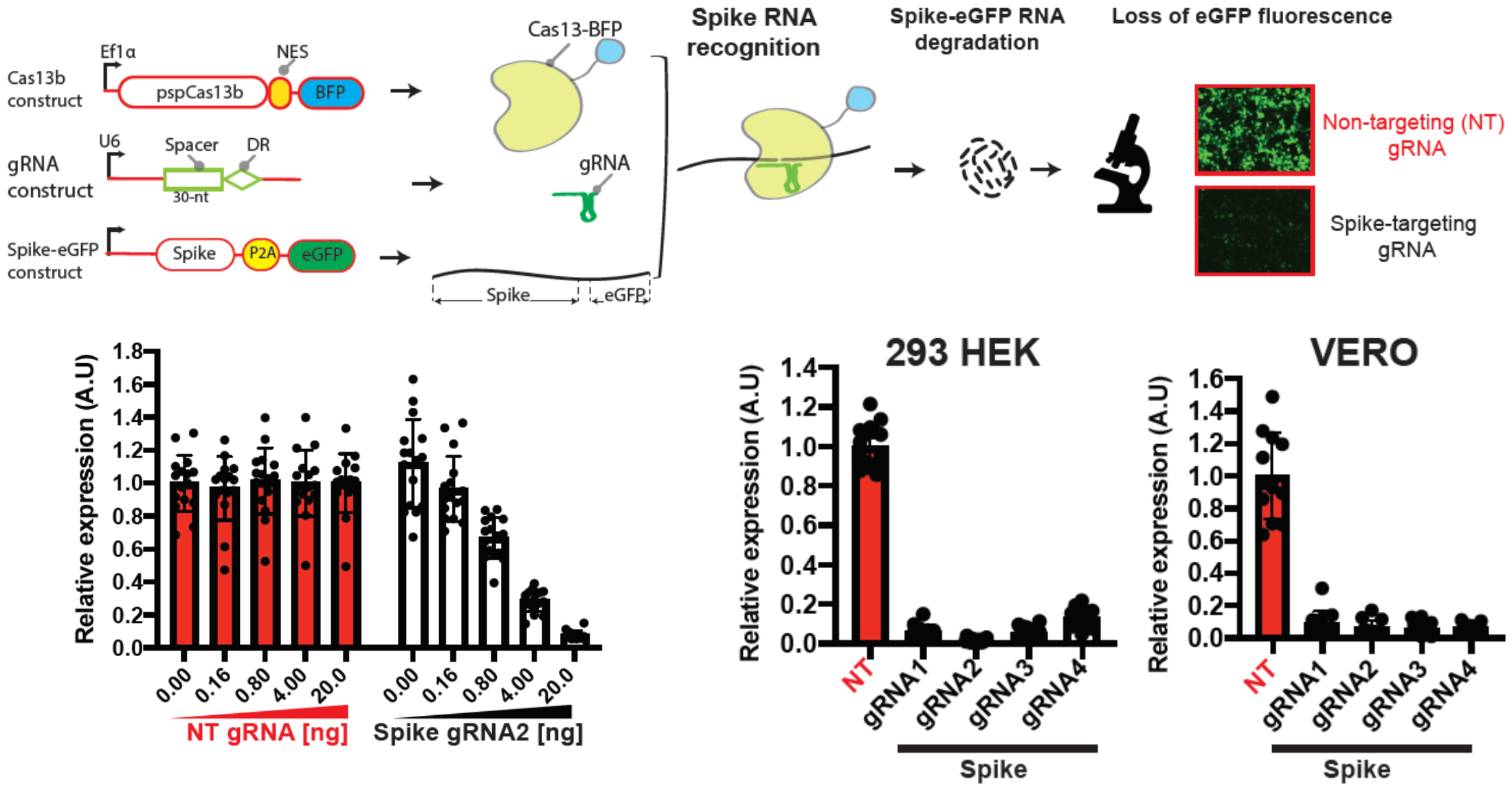
Spacer containing successive 4T repeats or more are removed

↓

Top gRNAs with predicted open secondary structure in the spacer and target sequence (839 gRNAs)

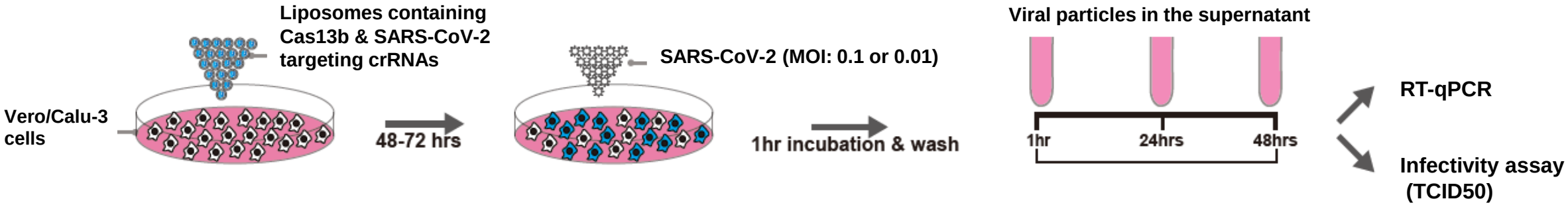
Fareh et al., Nat Comms 2021

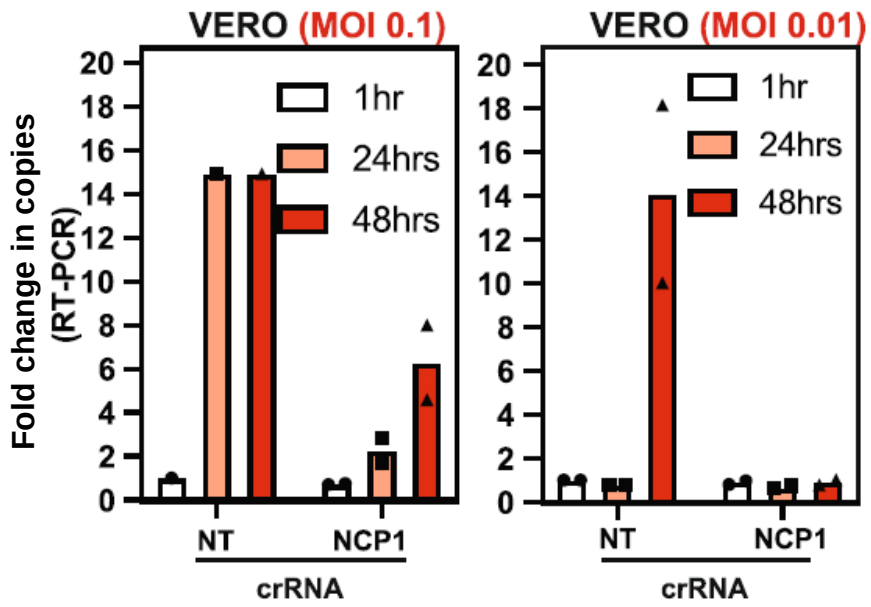
Cas13b eliminated SARS-CoV-2 spike RNA in 293 and Vero cells

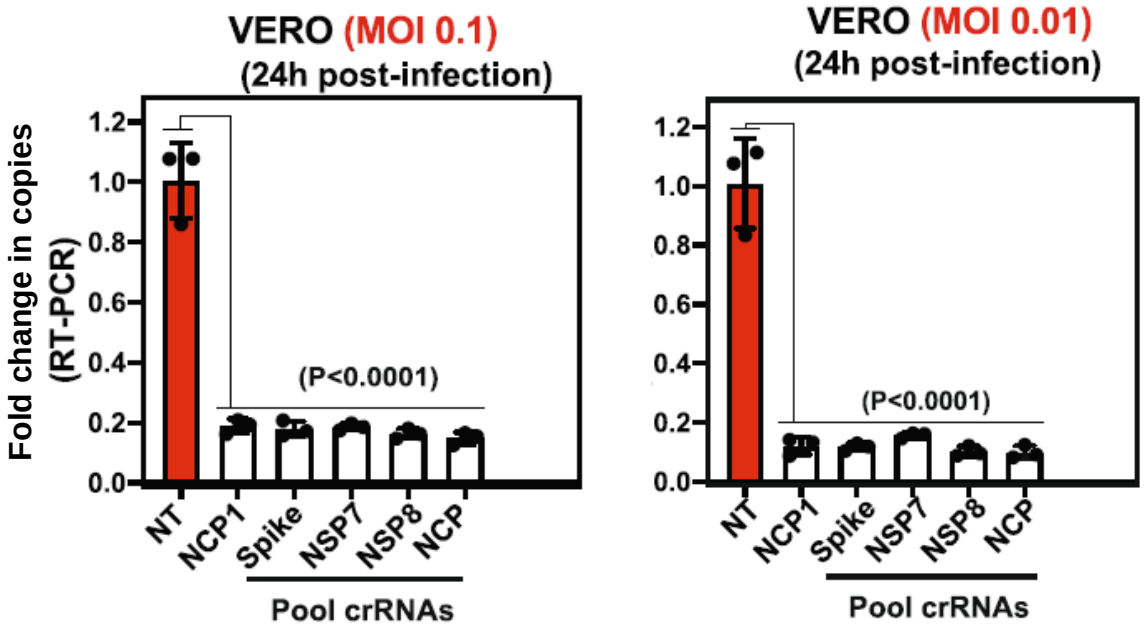


Fareh, Zhao et al., Nature Comms 2021

Cas13b suppresses SARS-CoV-2 replication in Vero / Calu-3 cells





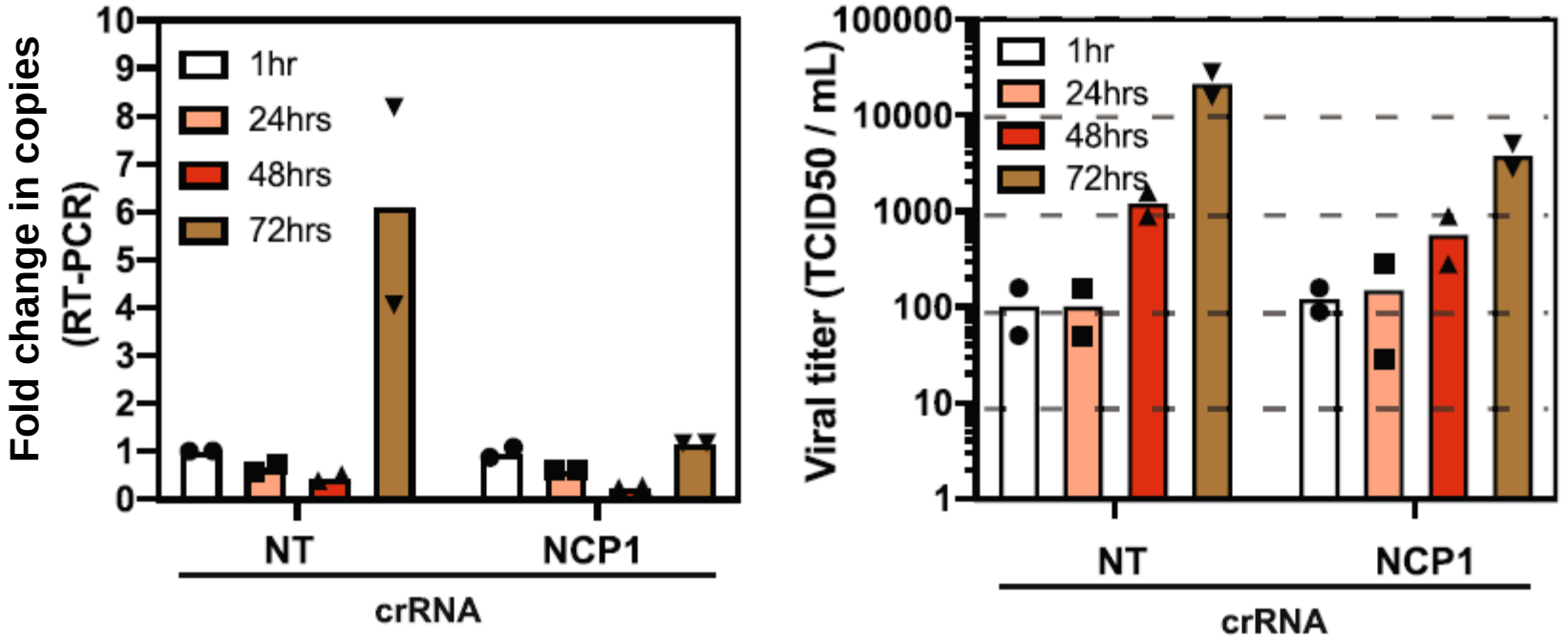


NSP = Non-Structural Protein; **NCP** = nucleocapsid protein

Fareh, Zhao et al., Nature Comms 2021

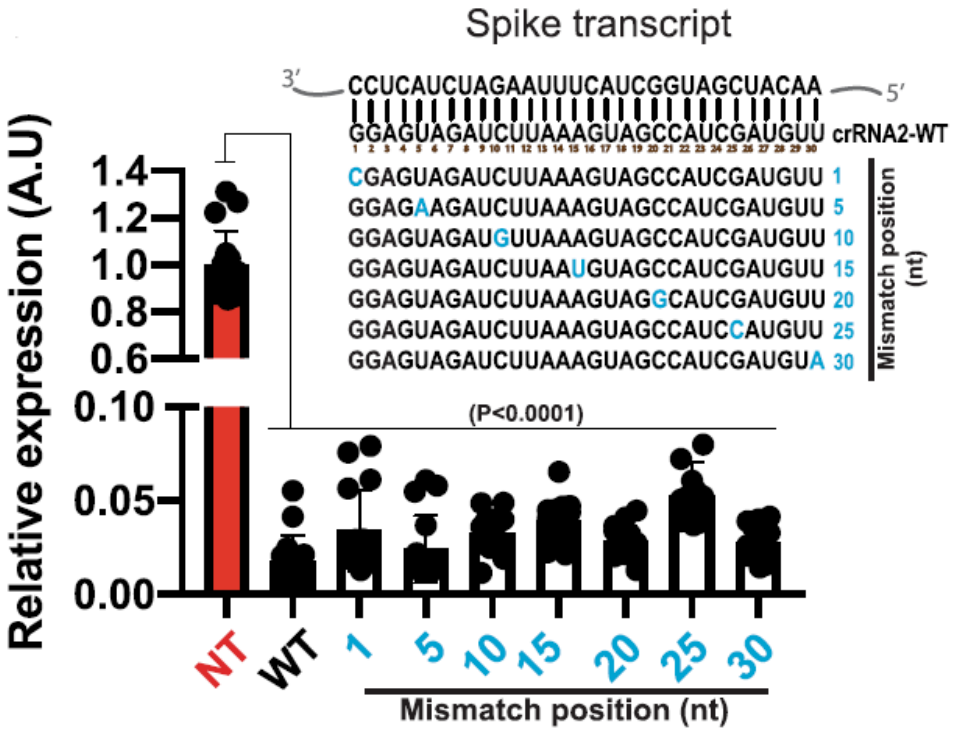
NCP-1 crRNA suppresses Alpha strain replication in Vero cells

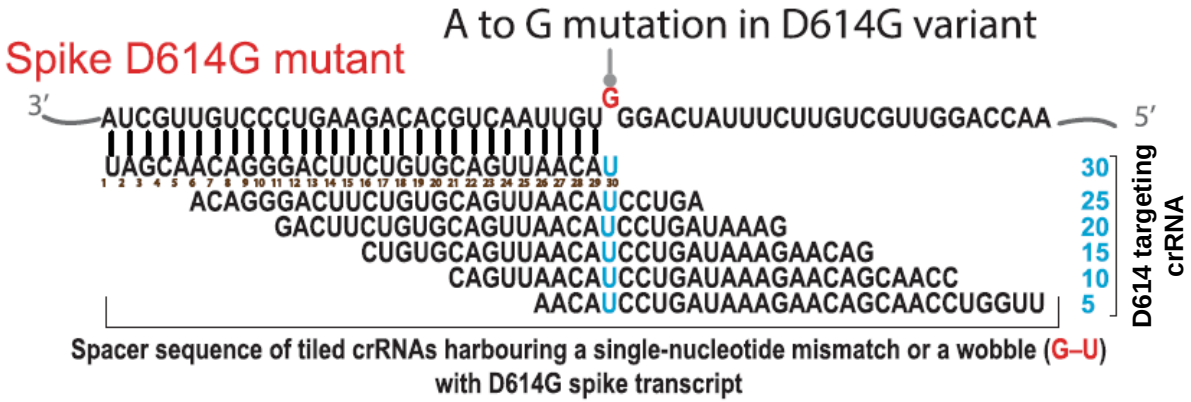
VERO (B.1.1.7 – Alpha strain, MOI 0.01)

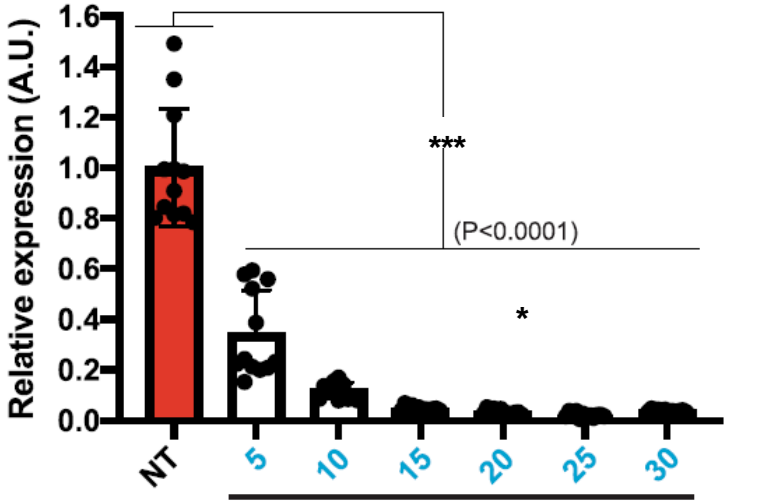


Fareh, Zhao et al., Nature Comms 2021

Cas13b silencing tolerates single-nucleotide mismatch (D614G)

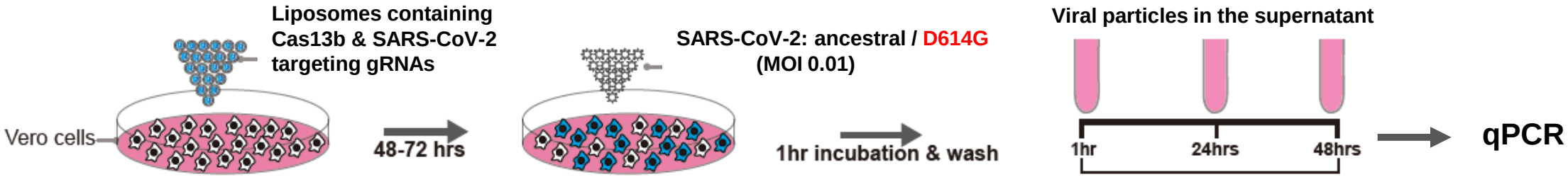


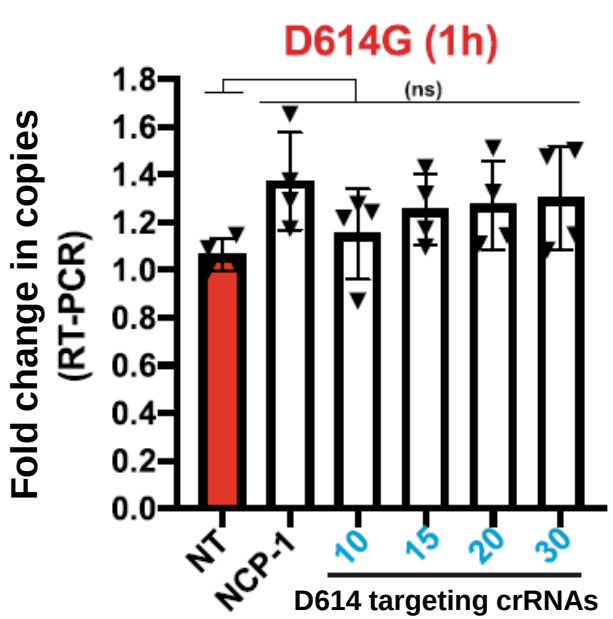


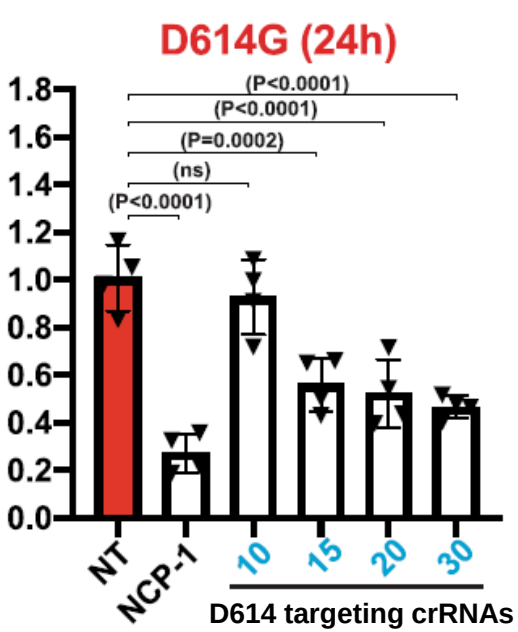


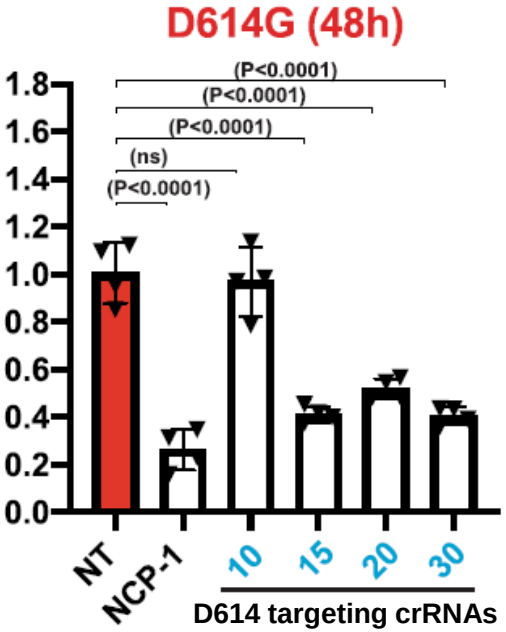
Fareh, Zhao et al., Nature Comms 2021

Cas13b suppressed replication of both ancestral and D614G mutant viruses





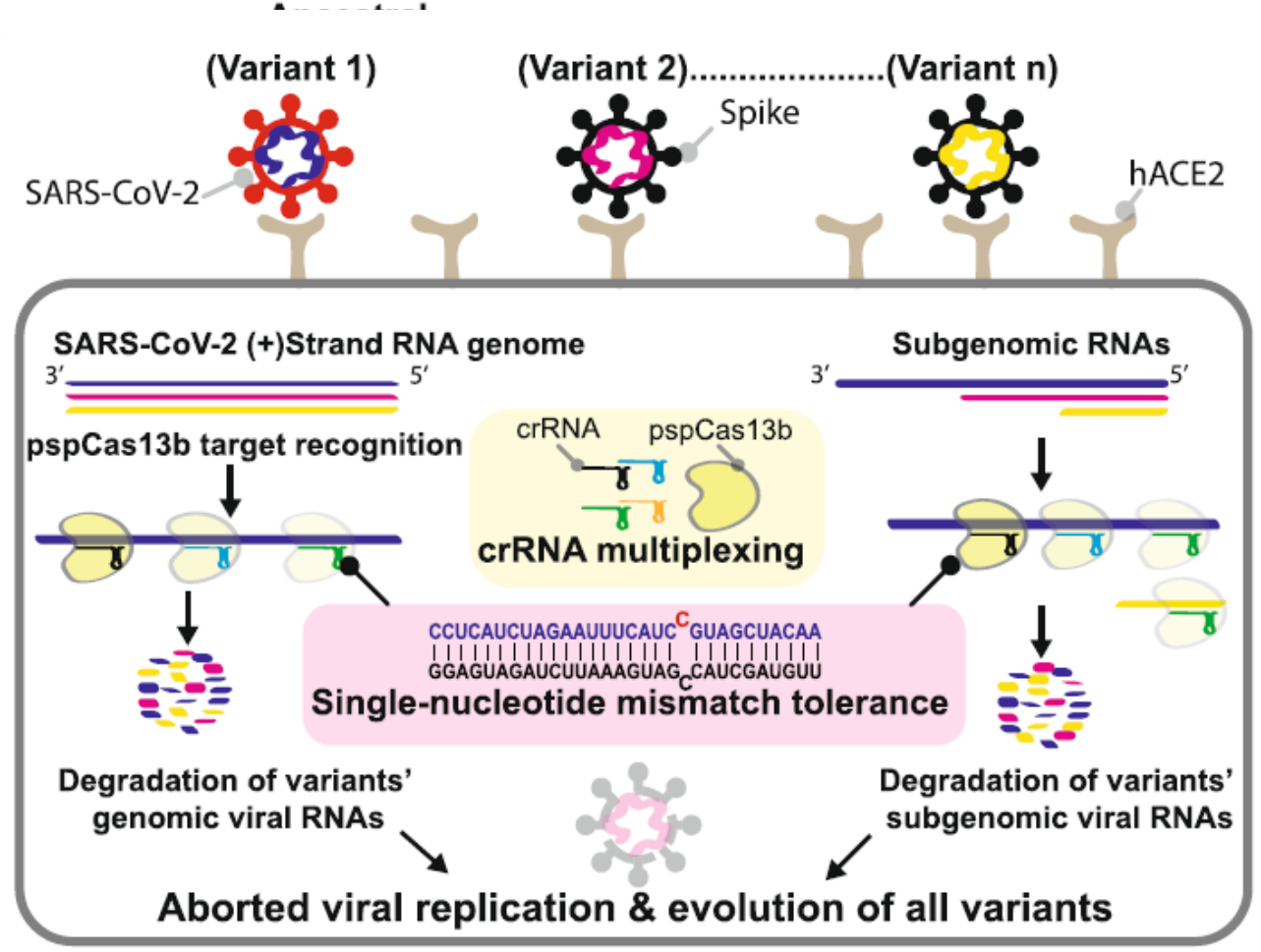




Fareh, Zhao et al., Nature Comms 2021

Summary

- Reprogrammed Cas13b effectors achieved >98% silencing efficiency in virus free-models
- crRNA multiplexing suppressed viral replication by up to 90% in mammalian cells
- Single-nucleotide mismatch with D614G did not dramatically reduce the capacity of a single crRNA to suppress SARS-CoV-2 replication
- This rapidly adaptable approach can be applied to any novel RNA virus but the major challenge being delivery



crRNA multiplexing & single-nucleotide mismatch tolerence enable the suppression of SARS-CoV-2 variants & their evolution potential

Targeted delivery of SARS-CoV-2 CRISPR/Cas13

LNPs can be modified and delivered by aerosolisation

pharmaceutics

MDPI

Article

Aerosolizable Li… Delivery of mRI…

Hairui Zhang †, Jasmim Leal…

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European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

A comparison of the lung clearance kinetics of solid lipid nanoparticles and liposomes by following the ^{3}H-labelled structural lipids after pulmonary delivery in rats

Shadabul Haque[a,b], Michael Whittaker[a,b], Michelle P. McIntosh[a], Colin W. Pouton[a], Simon Phipps[c], Lisa M. Kaminskas[a,c,*]

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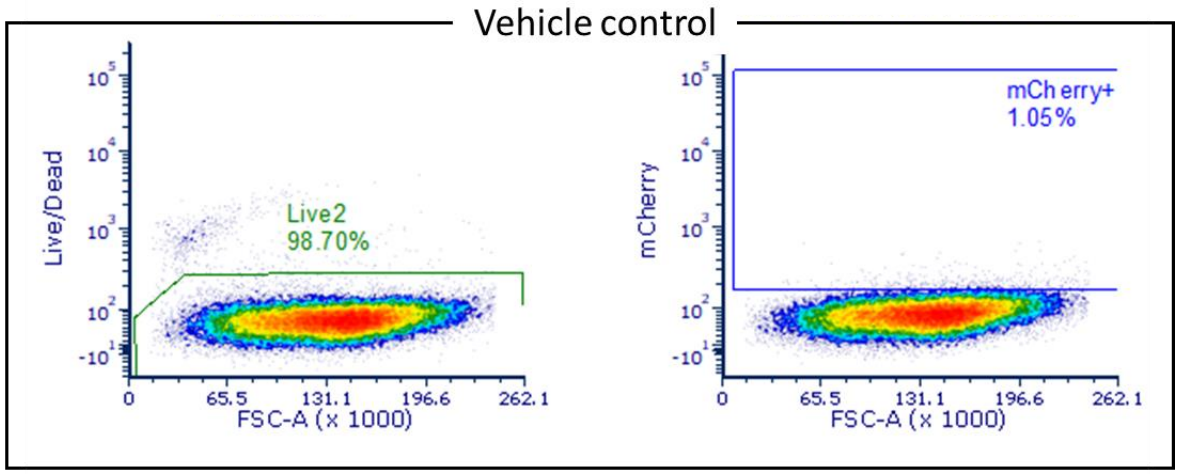
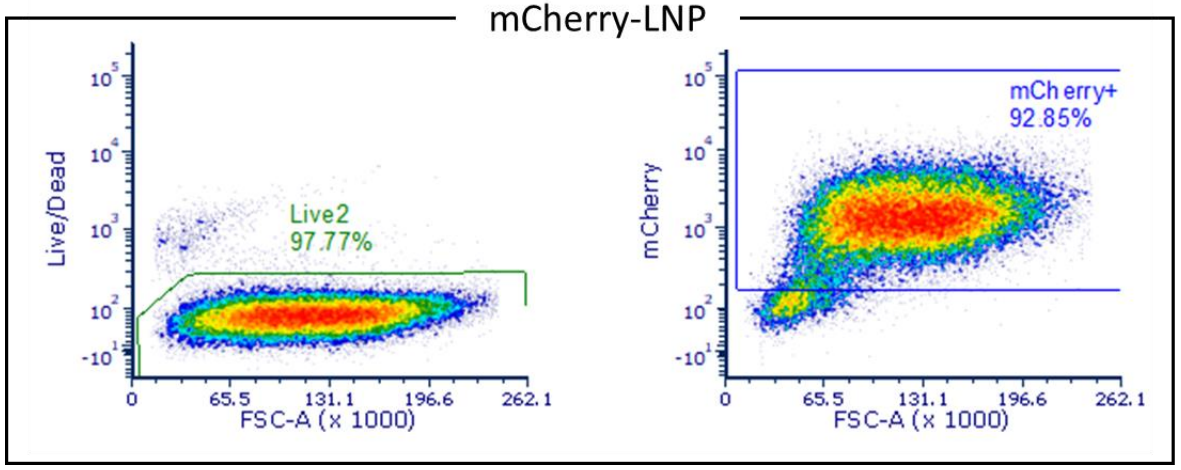
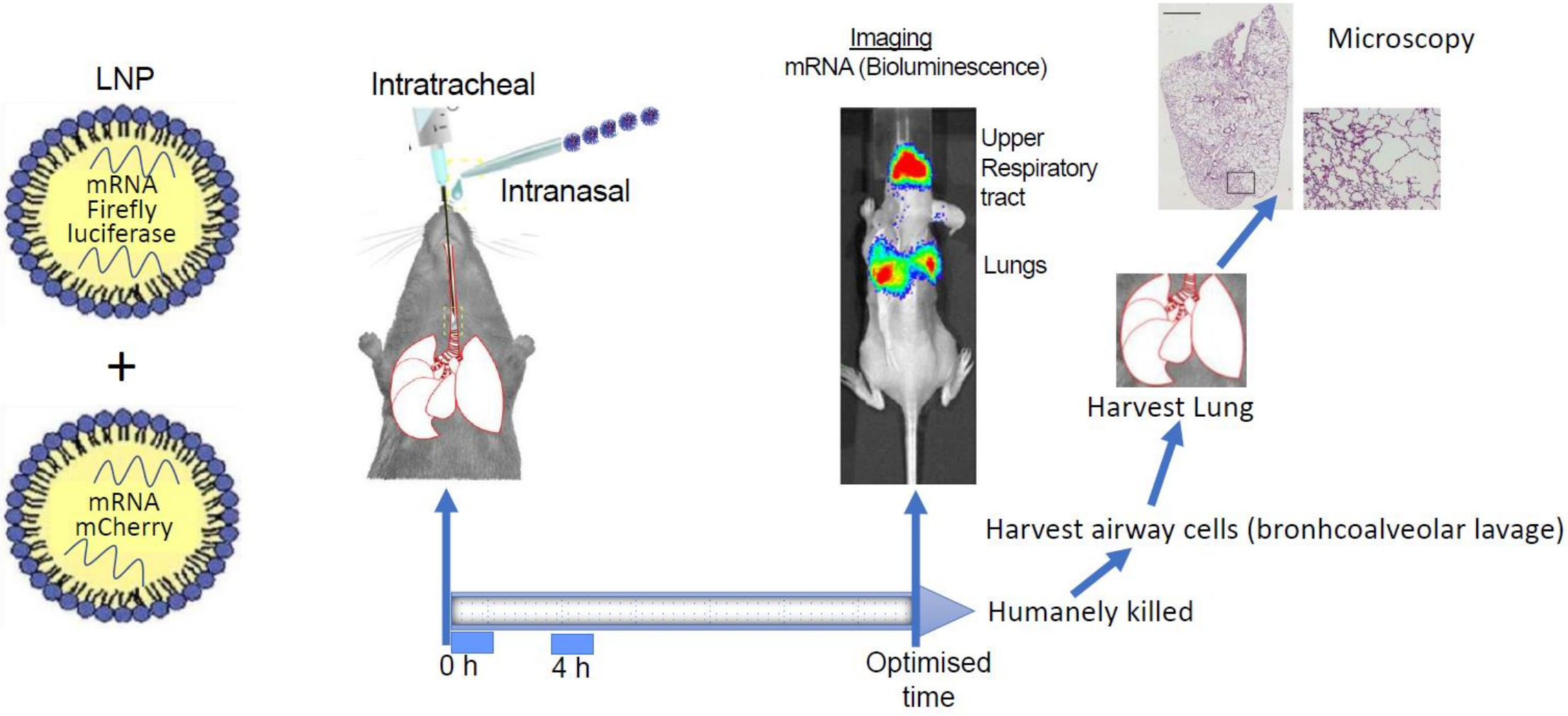
[c] *School of Biomedical Sciences, University of Queensland, St Lucia, QLD 4072, Australia*

Expression of mRNA in LNPs in vitro





Delivery of mRNA-LNP for respiratory infection

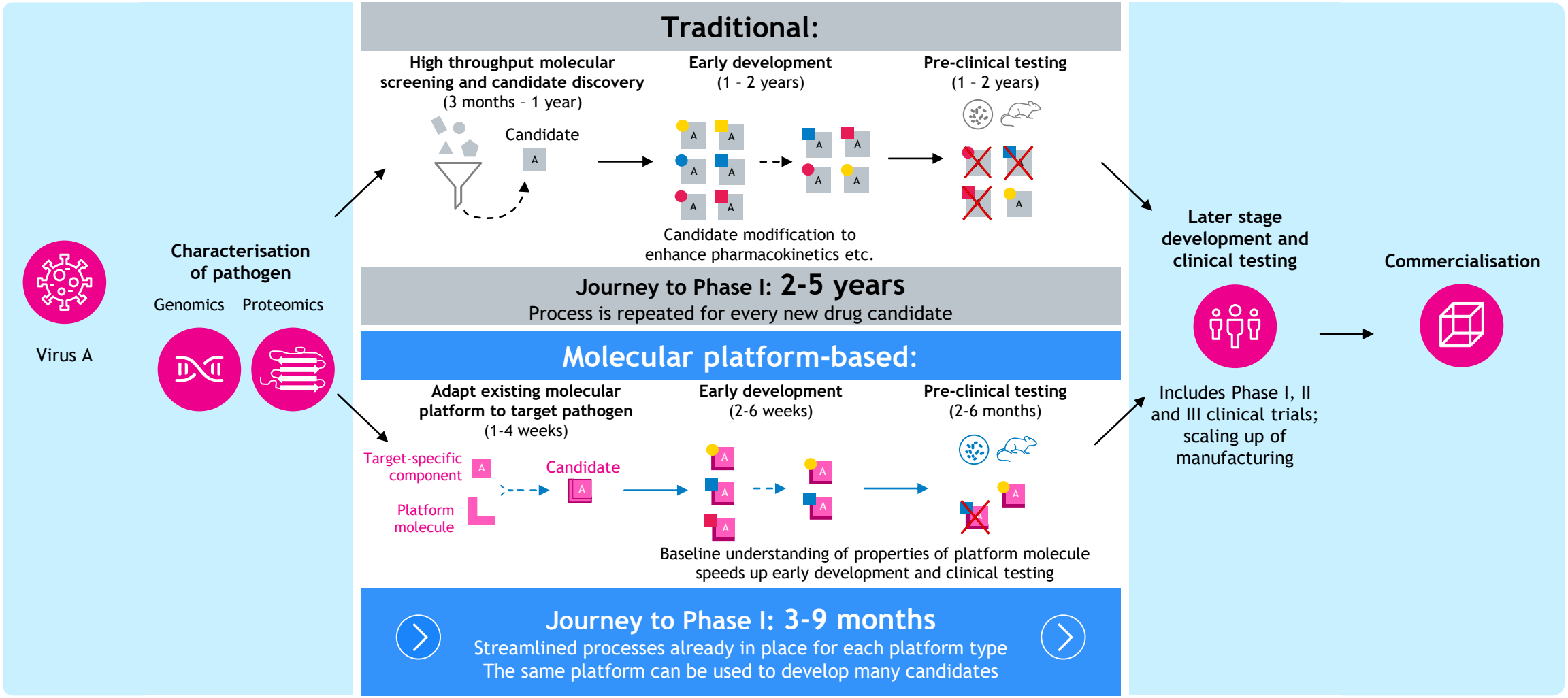


Platform technologies for pandemic preparedness

Being prepared paid off for vaccines

CEPI = Coalition for Preparedness Innovation. Fund established in 2017 by the Gates Foundation, Wellcome Trust and the Norwegian Government

Molecular platforms for antiviral therapeutics: antibodies and gene editing



Summary and implications

- Antivirals can play a **critically important role in pandemic response**, in addition to vaccines. For COVID-19, therapeutic advances have been slow given disease complexity and existing antiviral drug development approaches

- CRISPR-Cas13 RNA editing **has high specificity and potency** allowing for control of SARS-CoV2 replication in vitro, with limited tolerance for target sequence mismatch

- In vivo delivery of CRISPR-Cas therapeutics remains a major challenge but advances in **mRNA therapeutics** including lipid nanoparticles holds promise

- CRISPR-Cas therapeutics are **adaptable platform technologies** that only require the target sequence and therefore an ideal to tool for pandemic therapeutics

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