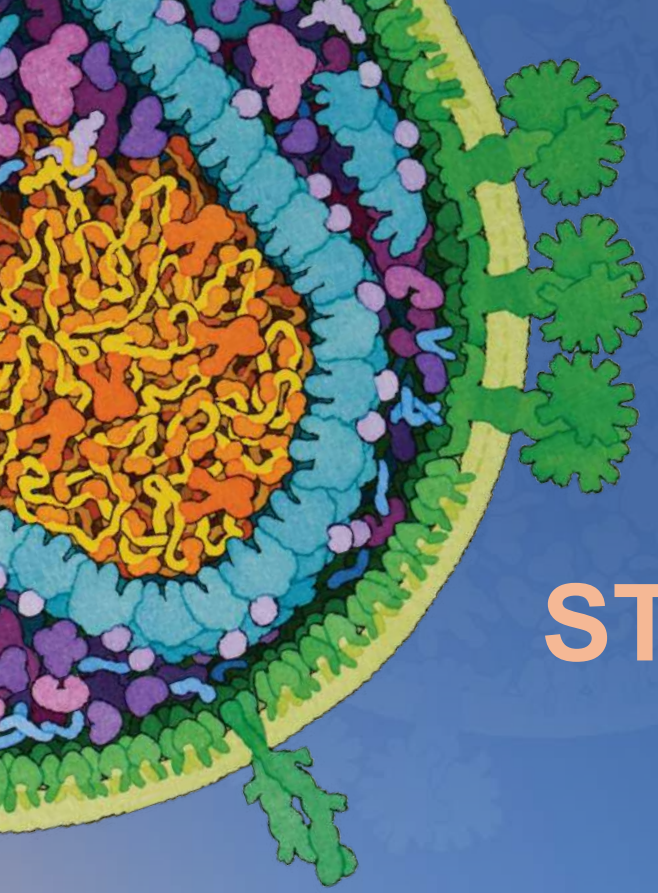

SCIENCE SPOTLIGHT™

A NOVEL COVID-19 CURE STRATEGY: HIJACKING SARS-COV-2 RNA-DEPENDENT RNA POLYMERASE

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Disclosure:
Enochian Biosciences, Stock and Salary
Frida Therapeutics, Stock
Pyrus Bio, Stock

CROI 2021

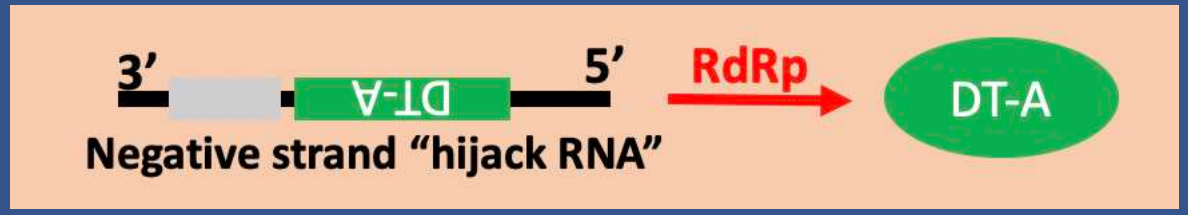
Introduction

Background

- SARS-CoV-2 is a single-stranded positive-sense RNA virus that utilizes negative-sense subgenomic (sg)RNA intermediates for viral protein synthesis.

Mechanism of Action

- We designed an oligonucleotide ("Hijack RNA"™) that contains negative strand of diphtheria toxin fragment A (DT-A) cDNA flanked between secondary structures of SARS-CoV-2 negative strand sgRNA.
- The Hijack RNA is recognized and translated into DT-A by SARS-CoV-2 RNA-dependent RNA polymerase (RdRp).



** DT-A is a segment of the diphtheria toxin that kills cells through inhibiting protein synthesis. When released from a dead cell, DTA is nontoxic and cannot enter other cells independently.

Methods

In Vitro Studies

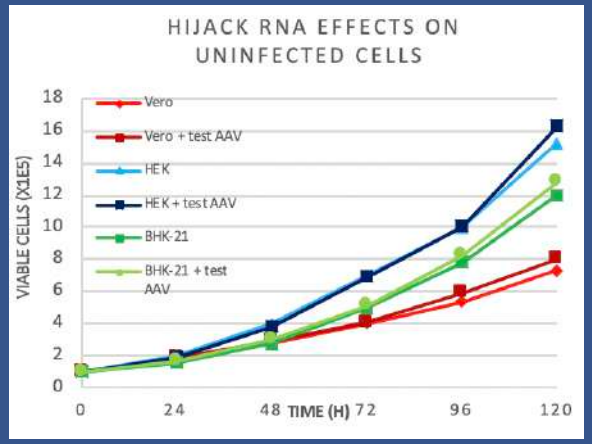
- Adeno-associated virus (AAV) particles were packaged with a novel vector expressing our SARS-CoV-2 Hijack RNA.
- Vero, Calu3 and HepG2 cells that were uninfected or infected with SARS-CoV-2 USA-WA1/2020 strain at 0.1 MOI, were transduced with test or GFP (control) AAVs.
- Uninfected Jurkat, HEK and BHK-21 cells were also transduced with test AAV to assess off-target effects of the Hijack RNA.
- Cell death and viability were evaluated daily by FACS and automated cell count.

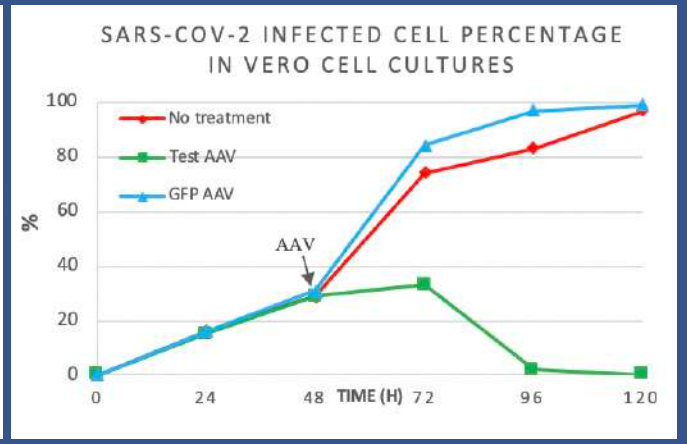
In Vivo Studies

- To establish an *in vivo* bioluminescent SARS-CoV-2 infection model SCID mice were subcutaneously injected with HepG2-SARS-CoV-2-Fluc cells, and hACE2 transgenic mice were infected with Luc reporter rSARS-CoV-2.
- Mice were treated with test AAV or control (GFP) AAV particles after a strong bioluminescence signal was established by *in vivo* imaging system (IVIS).
- Mice were monitored daily by IVIS and weekly for infection- and/or treatment-related toxicities.

Results

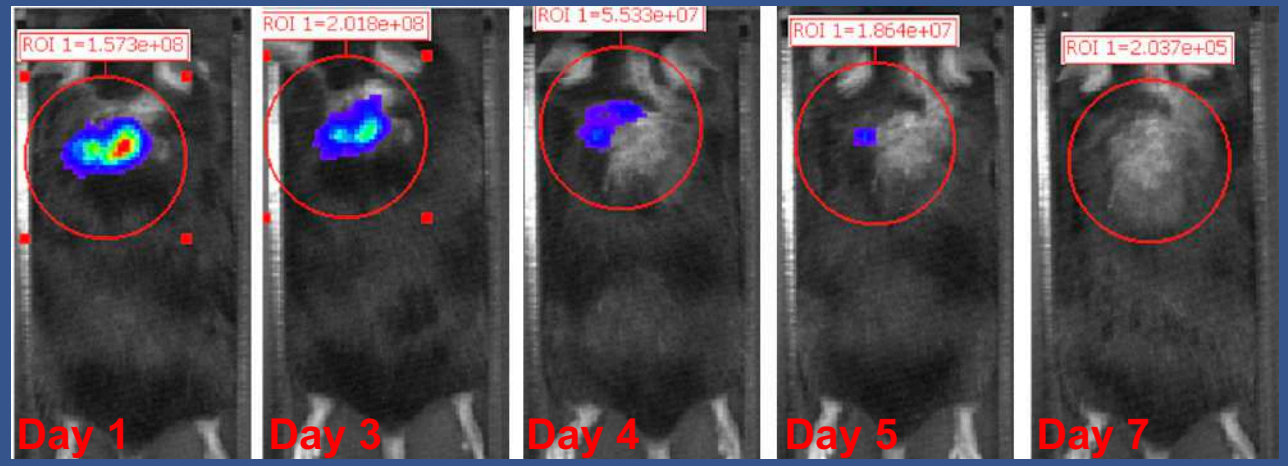
- Hijack RNA expression has no effect on uninfected cells.
- SARS-CoV-2 infection was eradicated from Vero cell cultures 48h after AAV transduction.



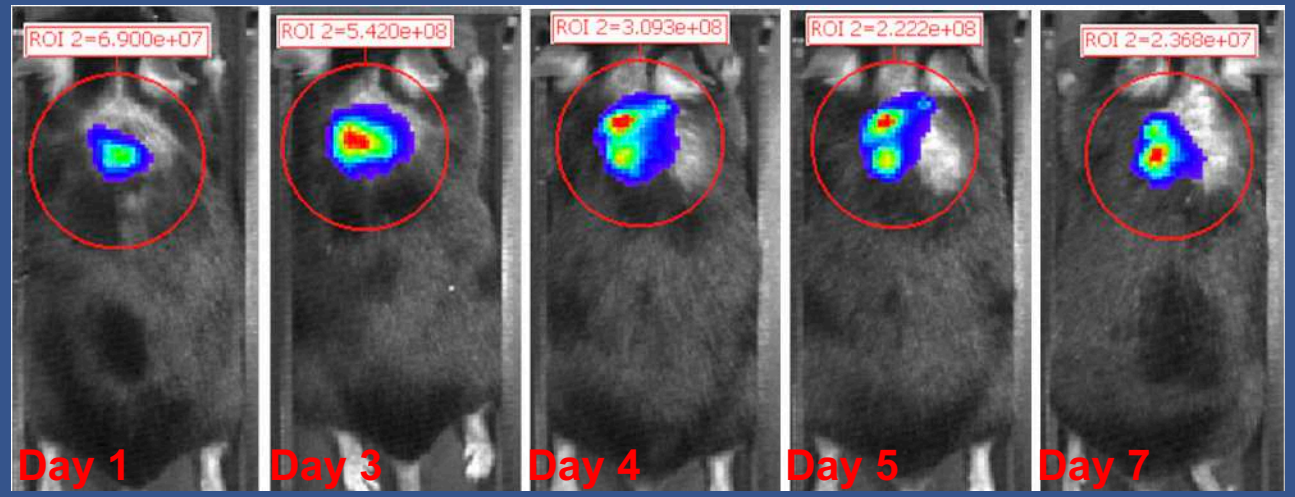


- SARS-CoV-2 was no longer visible in mice within 6 days after AAV administration.
- There was no treatment-related acute toxicity observed in mice.
- No toxicity or relapse of infection observed as of week 5 post-treatment.

"Hijack RNA" expressing AAV on Day 1



GFP expressing AAV on Day 1



Conclusion

- Novel Hijack RNA expression eradicated SARS-CoV-2 infection *in vitro* within 48h and *in vivo* within 6 days.
- Novel Hijack RNA had no effect on uninfected cells.
- Treatment had no acute toxicity in mice. No emergence of virus, or treatment-related toxicity were observed as of 5 weeks after virus clearance.
- **Because of AAV persistence in non-dividing cells, an aerosol treatment targeting respiratory epithelial cells could provide a long-term durable and easy-to-administer pre- or post-exposure prophylaxis,** simply by waiting in healthy cells, and effectively "ambushing" the virus immediately upon transmission.
- Effective protection could last up to one year, which is the approximate turnover of lung epithelial tissue.
- Given the conserved nature UTR regions of human coronaviruses, this approach would work against SARS-CoV and maybe some other common cold coronaviruses.
- Hijack RNA approach could be useful for other infections, e.g. HBV*, HIV (developed by Gumrukcu Lab & Enochian Biosciences collaboration), Influenza*. These projects are under development at the Gumrukcu Lab at Seraph Research Institute.

* A novel approach to induce HBV-infected cell death as a potential cure (Hep DART 2019), Hijacking HBV Pol to Selectively Induce Apoptosis in Infected Hepatocytes In Vivo: A Novel Approach for Potential Treatment or Cure (ASGCT 23rd Annual Meeting 2020), A Novel Approach to Potentially Treat Influenza: Selective Induction of Apoptosis in Infected Cells by Hijacking the Virus Machinery (ASGCT 23rd Annual Meeting 2020)

Acknowledgements

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RESEARCH INSTITUTE

Gumrukcu Lab

Serhat Gumrukcu, MD PhD
Phillip Musikanth, MD
Tung X Nguyen
Gregory Howell
Adiela Pineda
Kristen Terry

David Hardy, MD

Scripps Research

Gallay Lab

Philippe Gallay, PhD
Joseph Kuo, PhD
Michael Bobardt

IRCM
California Institute of Regenerative and Cellular Medicine

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