Targeting viral persistence in post-COVID syndromes

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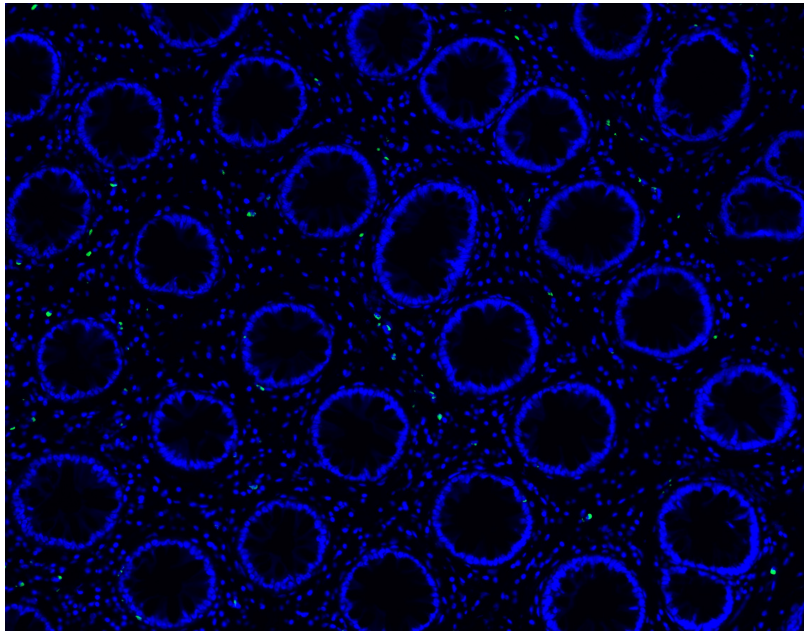


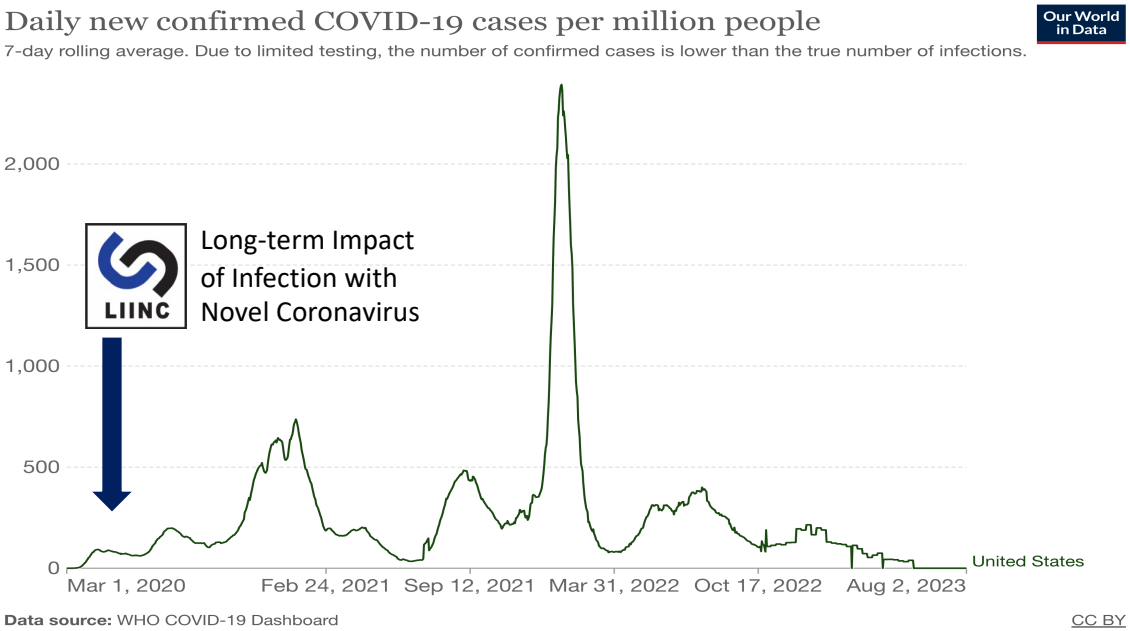
UCSF University of California San Francisco

ZUCKERBERG SAN FRANCISCO GENERAL Hospital and Trauma Center

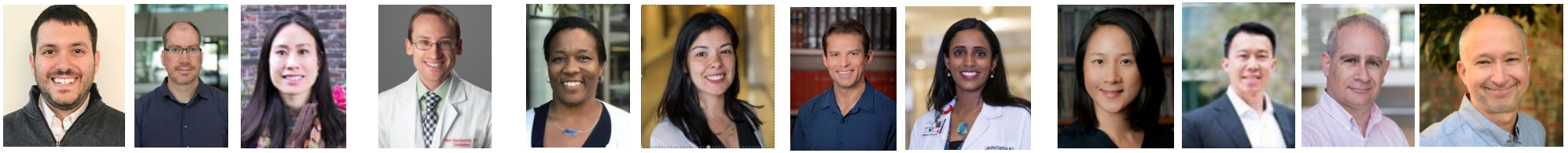
LIINC

LIINC: Long-term Impact of Infection With Novel Coronavirus

Launched April 2020, supporting Long COVID pathogenesis research



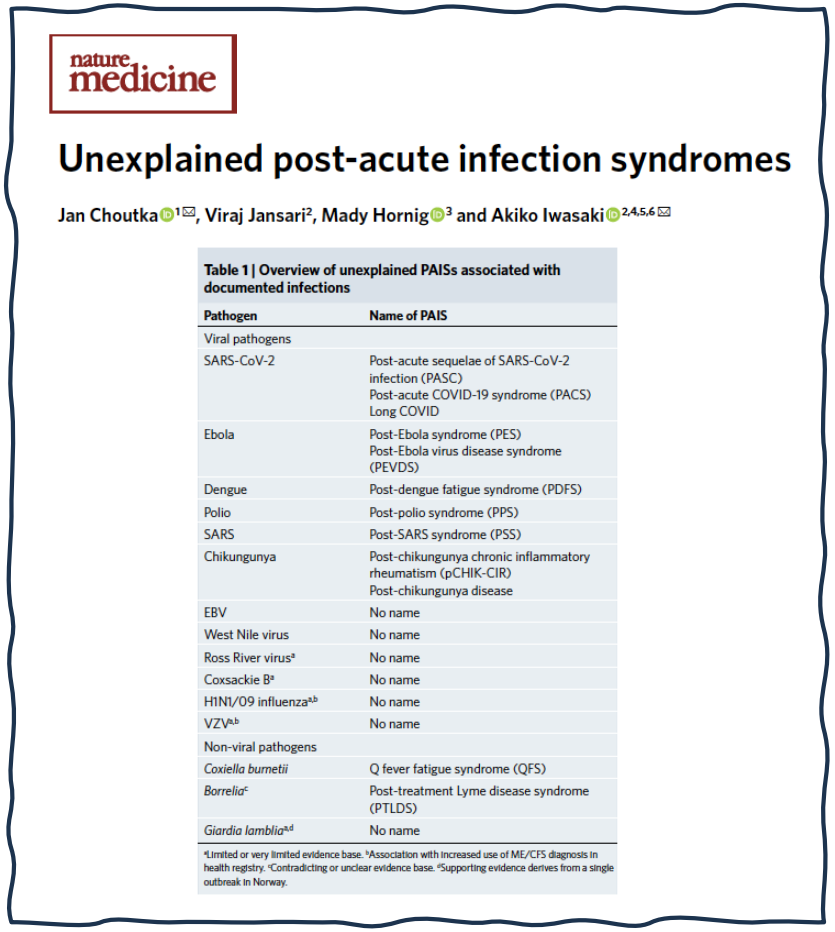
- > 1300 participants
- > 6500 visits
- > 70K specimens banked
- > 75 collaborations
- Tissue program
- Imaging program
- Clinical trials program
- Expansion to other IACCs



Michael Peluso | Tim Henrich | Priscilla Hsue | Matt Durstenfeld | Kim Rhoads | Carina Marquez | Dan Kelly | Lekshmi Santhosh | Nadia Roan | Ma Somsouk | Jeff Martin | Steven Deeks

Evidence of Pathogen Persistence in Many IACCs

- ME/CFS (enteroviruses?)
- Post-Ebola syndrome
- Post-Lyme syndromes
- DNA viruses (EBV)



nature medicine

Unexplained post-acute infection syndromes

Jan Choutka[1], Viraj Jansari[2], Mady Hornig[3] and Akiko Iwasaki[2,4,5,6]

Table 1 | Overview of unexplained PAISs associated with documented infections

Pathogen	Name of PAIS
Viral pathogens	
SARS-CoV-2	Post-acute sequelae of SARS-CoV-2 infection (PASC) Post-acute COVID-19 syndrome (PACS) Long COVID
Ebola	Post-Ebola syndrome (PES) Post-Ebola virus disease syndrome (PEVDS)
Dengue	Post-dengue fatigue syndrome (PDFS)
Polio	Post-polio syndrome (PPS)
SARS	Post-SARS syndrome (PSS)
Chikungunya	Post-chikungunya chronic inflammatory rheumatism (pCHIK-CIR) Post-chikungunya disease
EBV	No name
West Nile virus	No name
Ross River virus[a]	No name
Coxsackie B[a]	No name
H1N1/09 influenza[a,b]	No name
VZV[a,b]	No name
Non-viral pathogens	
Coxiella burnetii	Q fever fatigue syndrome (QFS)
Borrelia[c]	Post-treatment Lyme disease syndrome (PTLDS)
Giardia lamblia[a,d]	No name

[a]Limited or very limited evidence base. [b]Association with increased use of ME/CFS diagnosis in health registry. [c]Contradicting or unclear evidence base. [d]Supporting evidence derives from a single outbreak in Norway.

There is precedent for persistence of RNA viruses

PLOS BIOLOGY

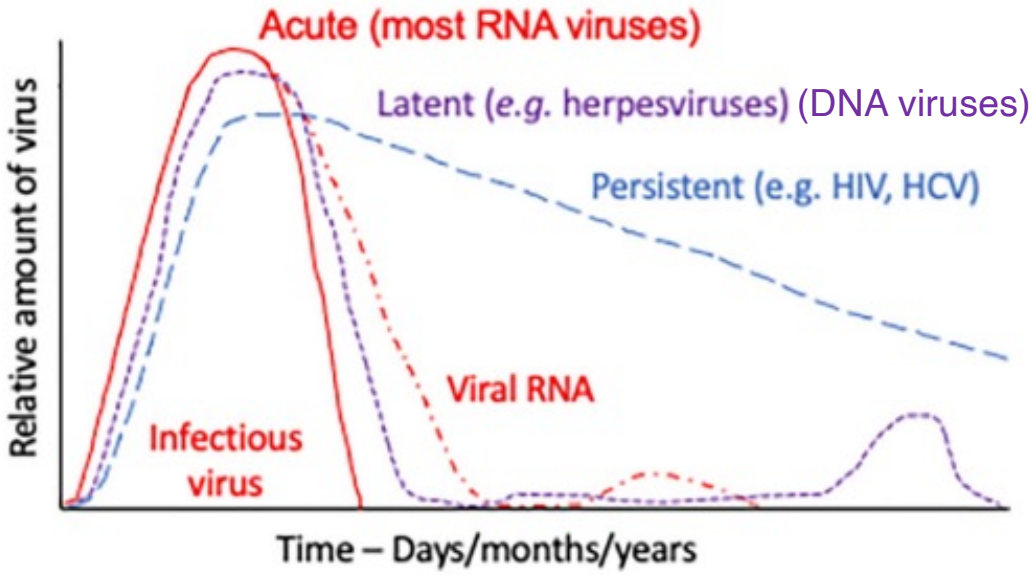
UNSOLVED MYSTERY

Why does viral RNA sometimes persist after recovery from acute infections?

Diane E. Griffin*

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States of America

Diane Griffin, MD, PhD
1940-2024



- Viral factors
  - Expression of factors that limit activity and evade immunity
  - Escape mutations
- Host factors
  - Immune dysfunction

Viral Persistence in LC: Where We Are

1. We know that SARS-CoV-2 persistence occurred in the first few years of the pandemic (minimum immunity, severe infections)

2. We think it is still occurring with modern infections, but the science is still catching up with the evolving pandemic

3. We don't know if viral persistence is a cause of Long COVID, or a consequence of something else that causes Long COVID

SCV2 persistence occurred (at least early on)

nature immunology

Review article https://doi.org/10.1038/s41590-023-01601-2

SARS-CoV-2 reservoir in post-acute sequelae of COVID-19 (PASC)

Amy D. Proal[1], Michael B. VanElzakker[1,2], Soo Aleman[3], Katie Bach[1,4], Brittany P. Boribong[5,6,7], Marcus Buggert[8], Sara Cherry[9], Daniel S. Chertow[10,11], Helen E. Davies[12], Christopher L. Dupont[13], Steven G. Deeks[14], William Eimer[7,15,16,17], E. Wesley Ely[18], Alessio Fasano[5,6,7], Marcelo Freire[19], Linda N. Geng[20], Diane E. Griffin[21], Timothy J. Henrich[22], Akiko Iwasaki[23,24,25], David Izquierdo-Garcia[26,27], Michela Locci[28], Saurabh Mehandru[29,30], Mark M. Painter[31], Michael J. Peluso[14], Etheresia Pretorius[32,33], David A. Price[34,35], David Putrino[36], Richard H. Scheuermann[37,38,39], Gene S. Tan[13,40], Rudolph E. Tanzi[7,15,16,17], Henry F. VanBrocklin[41], Lael M. Yonker[5,6,7] & E. John Wherry[31]

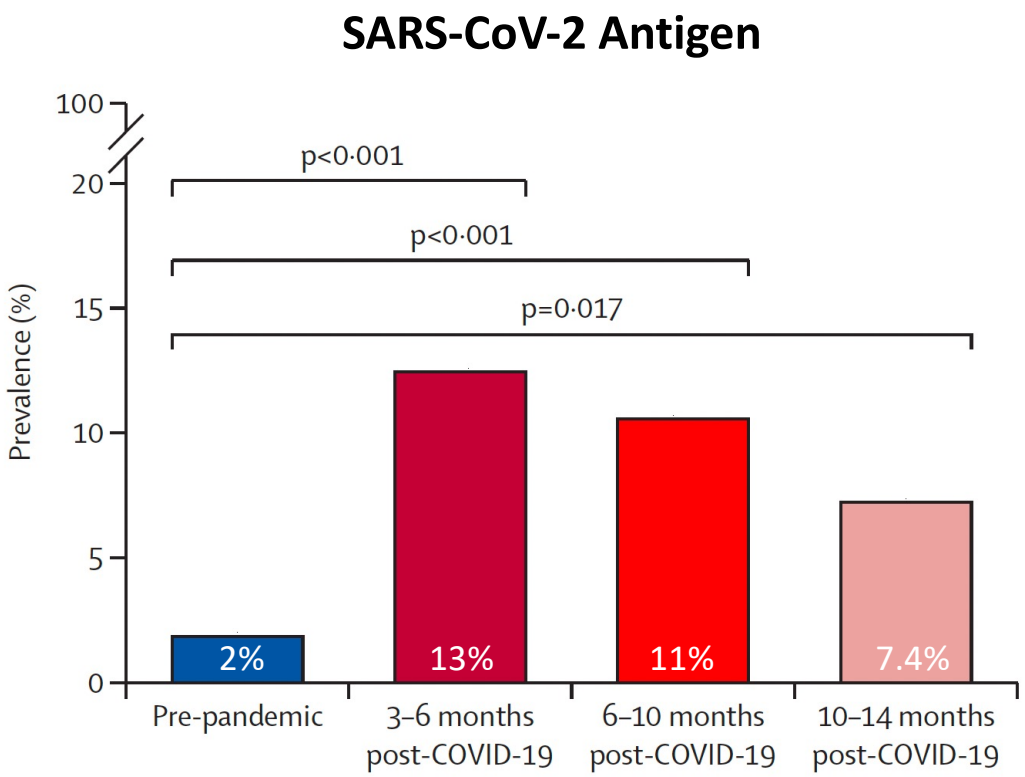
- Dozens of studies of blood and tissue
- Most small
- Similar findings across multiple orthogonal assays
- Most pre-Omicron/early Omicron

Table 1 | Identification of SARS-CoV-2 RNA and protein after COVID-19

	RNA	Protein	PASC symptoms	Location
Tissue (biopsy)				
Goh et al.[39]	√	S, N	√	Appendix, skin and breast tissues 163 and 426 d after COVID-19
Zollner et al.[38]	√	N	√	Gut mucosa/epithelium tissue ~7 months after COVID-19
deMelo et al.[27]	√	N	√	Olfactory neuroepithelium tissue 110–196 d after COVID-19
Gaebler et al.[33]	√	N	No	Intestinal tissue ~4 months after COVID-19
Cheung et al.[114]	√	S, N	NM	Colon, appendix, ileum, hemorrhoid, liver, gallbladder and lymph nodes 9–180 d after COVID-19
Hany et al.[29]	NM	N	NM	Gastric and gallbladder tissues 274–380 d after COVID-19
Miura et al.[30]	√	N	No	Adenoid tonsil, adenoid tissue, nasal cytobrush and nasal wash from children with no documented COVID-19 or upper airway infection in the month before collection
Xu et al.[37]	√	NM	No	Child adenoid and tonsil tissue up to 303 d after COVID-19
Peluso et al.[24]	√	NM	√	Colorectal lamina propria tissue 158–676 d after COVID-19
Yao et al.[25]	√	S,N	√	Fungiform papillae tongue tissue 6–63 weeks after COVID-19
Tissue (autopsy)				
Stein et al.[31]	√	N	NM	Dozens of human body and brain tissue types at least 31 d and up to 230 d after COVID-19
Roden et al.[32]	√	NM	NM	Lung tissue up to 174 d after COVID-19
Rendiero et al.[26]	NM	S	NM	Lung tissue up to 359 d after COVID-19
Stool				
Natarajan et al.[115]	√	NM	√	Stool up to 230 d after COVID-19
Yonker et al.[84]	√	S, N	√	RNA in stool of children with MIS-C 13–62 d after COVID-19, S and N protein in plasma
Jin et al.[116]	√	S	NM	Neonatal stool in infants born to mothers whose COVID-19 symptoms resolved more than 10 weeks before delivery
Blood				
Schultheiß et al.[40]	NM	S1	√	Plasma at a median time of 8 months after COVID-19
Swank et al.[41]	NM	S, S1, N	√	Plasma up to 12 months after COVID-19
Peluso et al.[44]	NM	S1, N	√	Plasma neuron-derived EVs 35–84 d after COVID-19
Peluso et al.[42]	NM	S1, S, N	√	Plasma up to 16 months after COVID-19
Craddock et al.[45]	√	S	√	Spike linked to EVs in samples obtained at least 8–12 weeks (up to 1 year) after COVID-19
Tejerina et al.[117]	√	NM	√	Plasma at a median time of 55 d after COVID-19 (also found in stool/urine at the same median time point)

√, identified; No, not present; NM, not measured; S and S1, spike protein.

Post-Acute SCV2 Antigen in Blood



Associated with COVID-19 severity

- 2x prevalence among previously hospitalized compared to non-hospitalized, p=0.03
- 3x prevalence among non-hospitalized with self-reported severe COVID symptoms, p=0.14
- Lends biological plausibility to finding
- Now extended to 24+ months

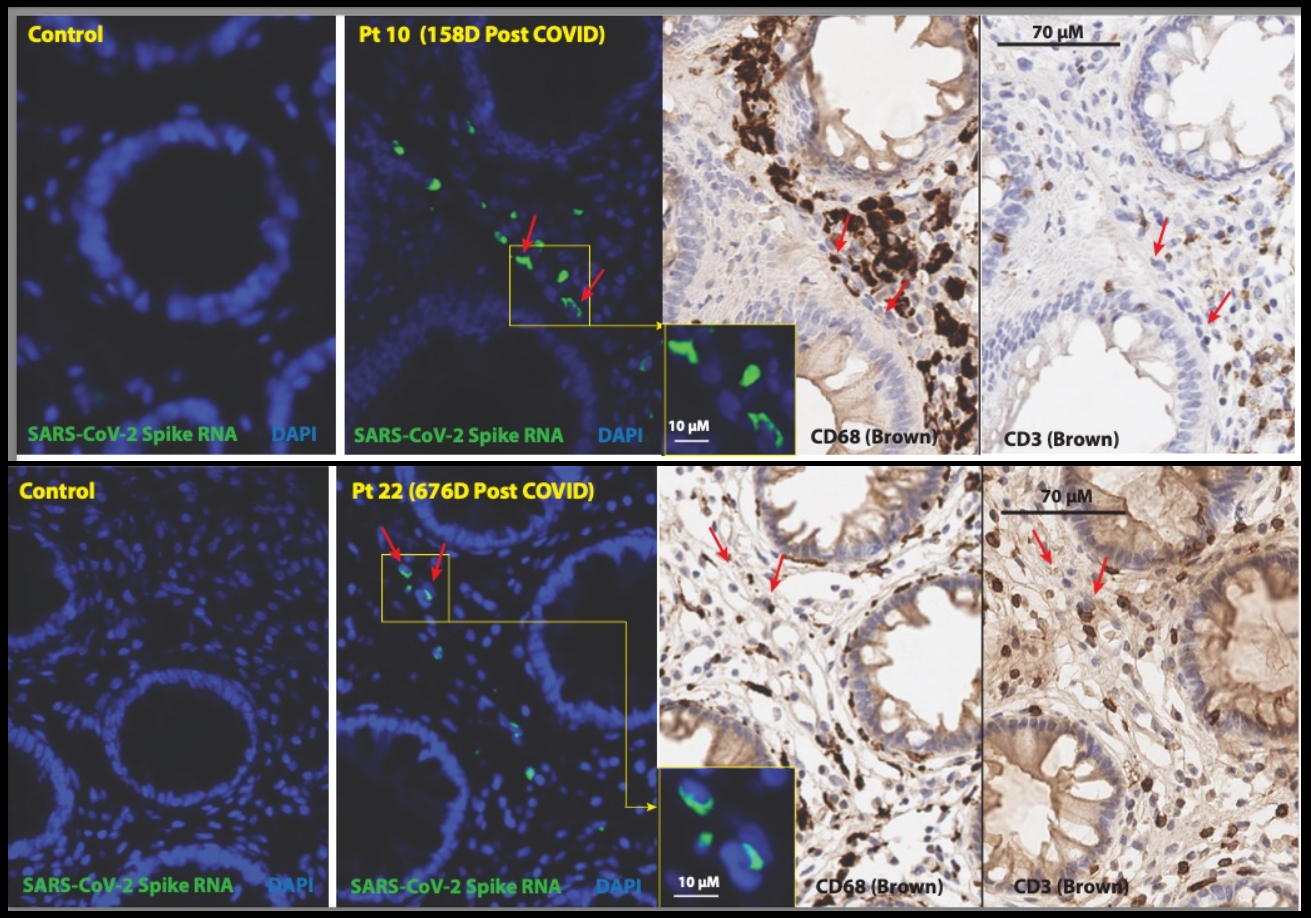
Peluso, Swank, Goldberg…Walt, Martin, Lancet ID 2024

Spike RNA on Gut Biopsy Up to 2 Years Post-COVID

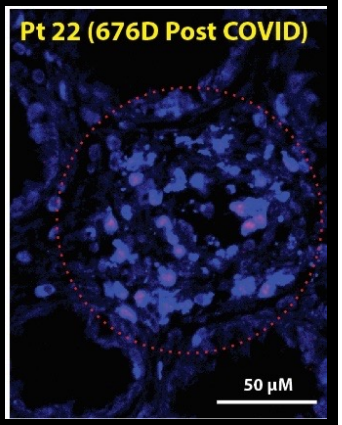
Tim Henrich

Ma Somsouk

Minimal Background | Spike RNA detection | CD68+ cells and dense areas



Double-stranded RNA



Peluso et al *Science Translational Medicine* 2024

Does Viral Persistence Cause Long COVID?

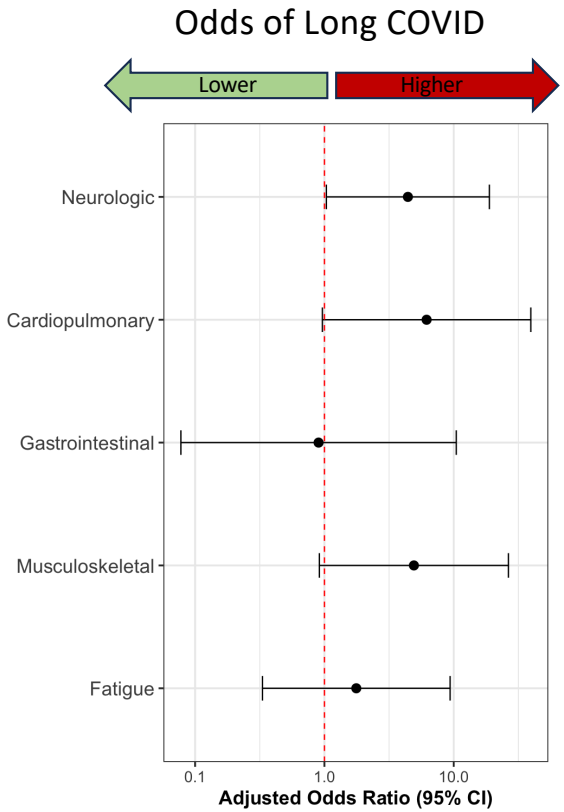
CMI CLINICAL MICROBIOLOGY AND INFECTION

Measurement of circulating viral antigens post-SARS-CoV-2 infection in a multicohort study

Zoe Swank[1,2,3±], Ella Borberg[1,2,3±], Yulu Chen[3,4], Yasmeen Senussi[1,2,3], Sujata Chalise[1,2,3], Zachary Manickas-Hill[5], Xu G. Yu[3,5,6], Jonathan Z. Li[3,6], Galit Alter[5,7], Timothy J. Henrich[8], J. Daniel Kelly[9,10,11], Rebecca Hoh[12], Sarah A. Goldberg[9], Steven G. Deeks[12], Jeffrey N. Martin[9], Michael J. Peluso[12], Aarthi Talla[13], Xiaojun Li[13], Peter Skene[13], Thomas F. Bumol[13], Troy R. Torgerson[13], Julie L. Czartoski[14], M. Juliana McElrath[14], RECOVER Consortium Authors, Elizabeth W. Karlson[3,4], and David R. Walt[#1,2,3]

PASC definition	≥ 1 PASC symptom	≥ 2 PASC symptoms	≥ 3 PASC symptoms	≥ 2 symptom groups
Risk factors	**OR (95% CI)**			
Antigen	1.8 (1.4-2.2)	2.0 (1.7-2.3)	2.1 (1.8-2.4)	1.5 (1.1-1.9)

Strongest relationships with neurocognitive, cardiopulmonary, and musculoskeletal symptoms



RECOVER Researching COVID to Enhance Recovery

LIINC

Viral Persistence in LC: What We Need

1. Studies aimed at preventing viral persistence from happening in the first place (would have been nice to have these a few years ago)

2. Studies aimed at targeting viral persistence once it has occurred (and let's not make big claims from a few small imperfect studies)

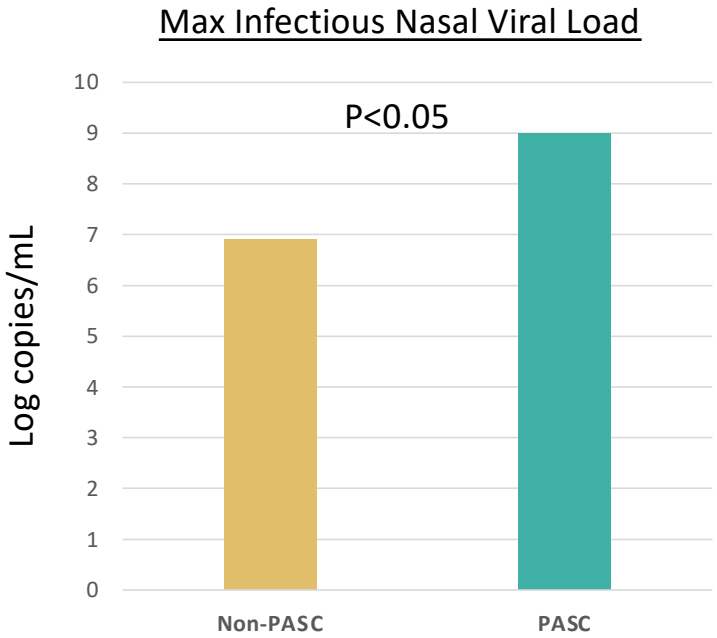
3. A dedicated biomarker development program to figure out how to measure viral persistence in a scalable way (and to admit the limitations of our current biomarkers and stop saying that we don't need biomarkers)

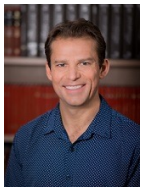
4. An open mind about whether or not this is the right target

How Does Acute Infection Influence Viral Persistence?

nature communications

Early biological markers of post-acute sequelae of SARS-CoV-2 infection





Dan Kelly

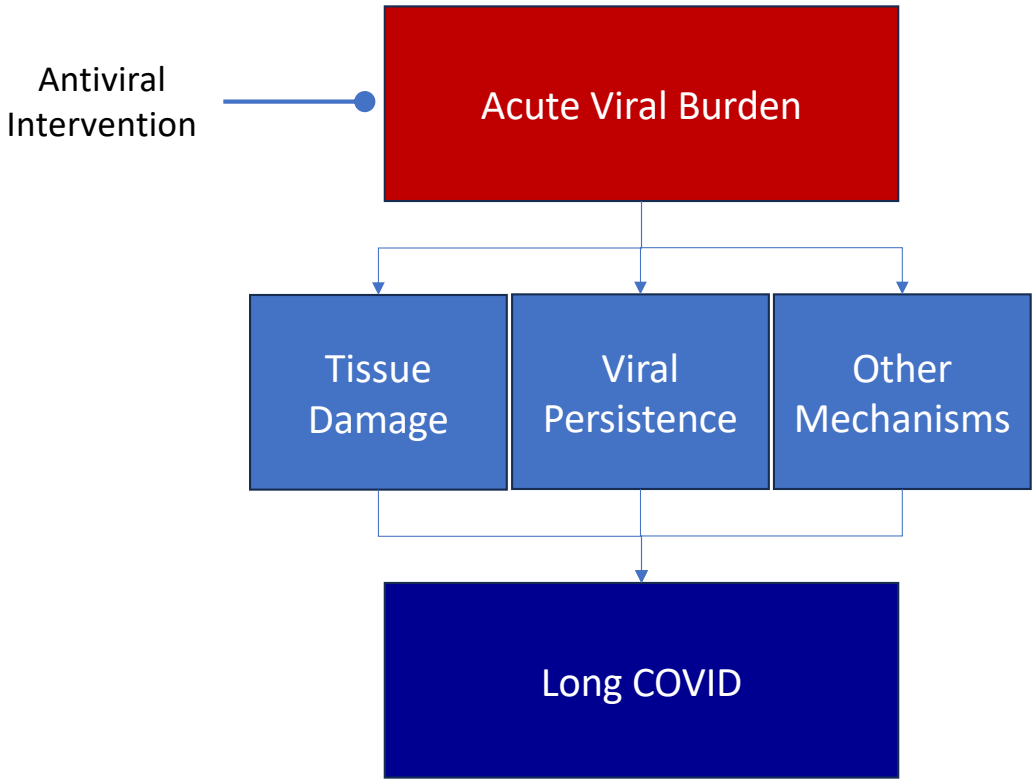
FIND COVID

LIINC

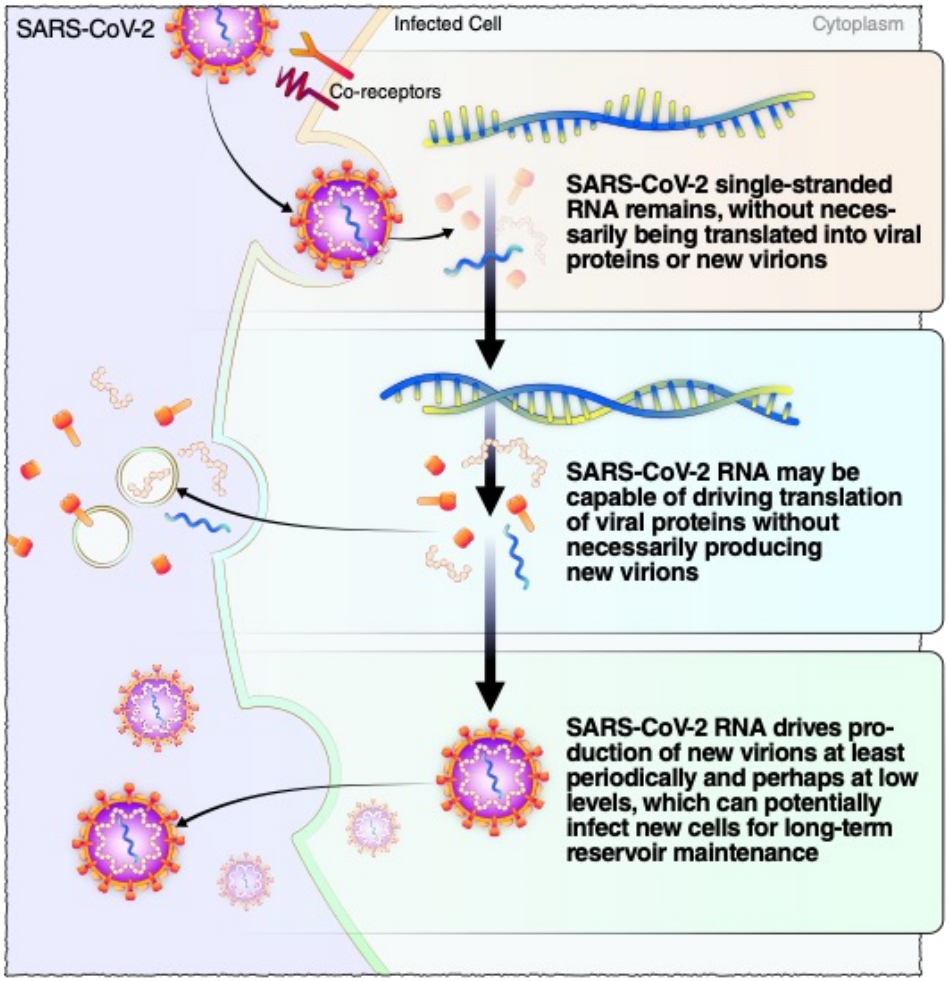
Do acute events determine who develops viral persistence?
Does early treatment prevent viral persistence?

Preventing Long COVID

- Can sustained treatment with a tissue-penetrant, well-tolerated antiviral in the first 2-4 weeks prevent the downstream cascade that leads to Long COVID?
- Could this work for other IACCs?
- Currently, 0 U.S.-based clinical trials examining these questions
- We should be studying this!



Does Treating Viral Persistence Matter?



Immunotherapy?

Monoclonals?

Antivirals?

Proal,…Peluso et al Lancet ID 2025

Treating Viral Persistence: Case Reports

Antivirals

PATHOGENS AND IMMUNITY

Effect of Oral Nirmatrelvir on Long COVID Symptoms: 4 Cases and Rationale for Systematic Studies

Research Square

Case Report of Breakthrough Long COVID and the Use of Nirmatrelvir-Ritonavir

Impact of extended-course oral nirmatrelvir/ritonavir (Paxlovid) in established Long COVID: Case series and research considerations

Monoclonal Antibodies

American Journal of Emergency Medicine

Remission of severe forms of long COVID following monoclonal antibody (MCA) infusions: A report of signal index cases and call for targeted research

Does Treating Viral Persistence Matter?

JAMA Internal Medicine | Original Investigation

Nirmatrelvir-Ritonavir and Symptoms in Adults With Postacute Sequelae of SARS-CoV-2 Infection
The STOP-PASC Randomized Clinical Trial

Linda N. Geng, MD, PhD; Hector Bonilla, MD; Haley Hedlin, PhD; Karen B. Jacobson, MD; Lu Tian, DSc; Prasanna Jagannathan, MD; Phillip C. Yang, MD; Aruna K. Subramanian, MD; Jane W. Liang, PhD; Sa Shen, PhD; Yaowei Deng, MA; Blake J. Shaw, MS; Bren Botzheim, MS; Manisha Desai, PhD; Divya Pathak, MS; Yasmin Jazayeri, MPH; Daniel Thai, BS; Andrew O'Donnell, MA; Sukanya Mohaptra, BS; Zenita Leang, BS; Gabriella Z. M. Reynolds, BS; Erin F. Brooks, MS; Ami S. Bhatt, MD, PhD; Robert W. Shafer, MD; Mitchell G. Miglis, MD; Tom Quach; Anushri Tiwari; Anindita Banerjee, PhD; Rene N. Lopez, MPH; Magdia De Jesus, PhD; Lawrence R. Charnas, MD, PhD; Paul J. Utz, MD; Upinder Singh, MD

THE LANCET
Infectious Diseases

Nirmatrelvir–ritonavir versus placebo–ritonavir in individuals with long COVID in the USA (PAX LC): a double-blind, randomised, placebo-controlled, phase 2, decentralised trial

Mitsuaki Sawano, Bornali Bhattacharjee*, César Caraballo, Rohan Khera, Shu-Xia Li, Jeph Herrin, Dany Christian, Andreas Coppi, Frederick Warner, Julie Holub, Yashira Henriquez, Maria A Johnson, Theresa B Goddard, Erica Rocco, Amy C Hummel, Mohammad AL Mouslmani, William Brenham Hooper, David F Putrino, Kevin D Carr, Lawrence Charnas, Magdia De Jesus, Dale Nepert, Paula Abreu, Frank W Ziegler 3rd, John A Spertus, Akiko Iwasaki†, Harlan M Krumholz†*

No benefit in 2 studies of Long COVID, but:

- Could not confirm persistence (? ~25%)
- Tissue penetration may be limited
- 2 weeks may be too short (12 weeks for feline coronaviruses that persist – no tox data for such long courses)
- Biomarkers not yet reported

RECOVER-VITAL (n=900, 3 phenotypes) will be be hugely informative

→ even if negative there remains a lot to be done

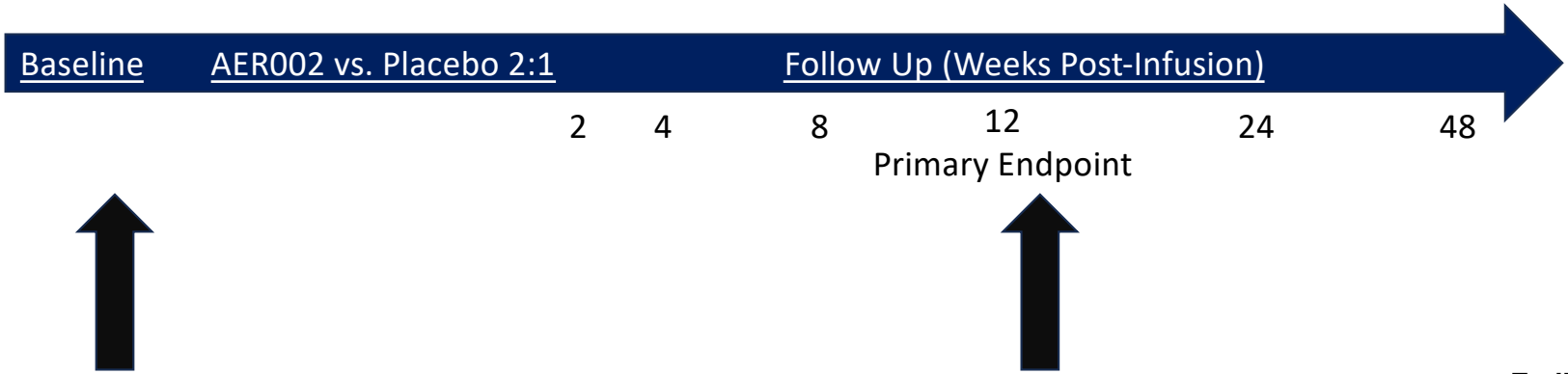
outSMART-LC: Monoclonals for Long COVID

PATIENT-LED RESEARCH COLLABORATIVE

AERIUM THERAPEUTICS

outSMART-LC (NCT05877507): Anti-SARS-CoV-2 Monoclonal Antibodies for Long COVID

Standard Assessments: Symptoms, Quality of Life, Physical Function, Neurocognitive Function, Dysautonomia, Biomarkers



Optional Tissue Procedures

Optional Tissue Procedures

Over half completed pre-post gut biopsies and imaging

Emily Fehrman

Top Line Results Do Not Always Tell the Whole Story


THE LANCET
Infectious Diseases

A synbiotic preparation (SIM01) for post-acute COVID-19 syndrome in Hong Kong (RECOVERY): a randomised, double-blind, placebo-controlled trial

Raphaela I Lau, Qi Su*, Ivan S F Lau, Jessica Y L Ching, Martin C S Wong, Louis H S Lau, Hein M Tun, Chris K P Mok, Steven W H Chau, Yee Kit Tse, Chun Pan Cheung, Moses K T Li, Gianni T Y Yeung, Pui Kuan Cheong, Francis K L Chan†, Siew C Ng†*


Clinical Infectious Diseases

MAJOR ARTICLE

Assessment of the Impact of RNase in Patients With Severe Fatigue Related to Post-Acute Sequelae of SARS-CoV-2 Infection: A Randomized Phase 2 Trial of RSLV-132

James S. Andrews,[1] Jim B. Boonyaratanakornkit,[2,3] Eva Krusinska,[4] Suzanne Allen,[4] and James A. Posada[4,©]

RCT of 463 people with LC to receive a combo pre/probiotic vs placebo

- Significant reduction in Long COVID symptoms, but:
- No effect on overall quality of life

RCT of 105 people with LC to receive an RNase1 molecule vs placebo

- No difference in overall study
- Different pattern in women vs men with potential response in women only

Viral Persistence: We Need Better Biomarkers

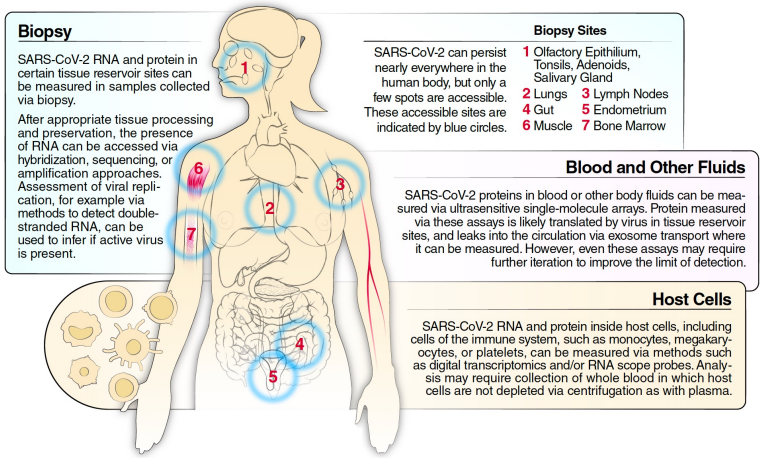
Industry: "We want to help…but we need a biomarker first."

Some Advocates/Stakeholders: "Do not limit who can enroll in the studies."

1) **Tissue-based markers not available/accessible (need accessible biomarkers)**

2) **Assay sensitivity/specificity**

3) **Within-person variability**

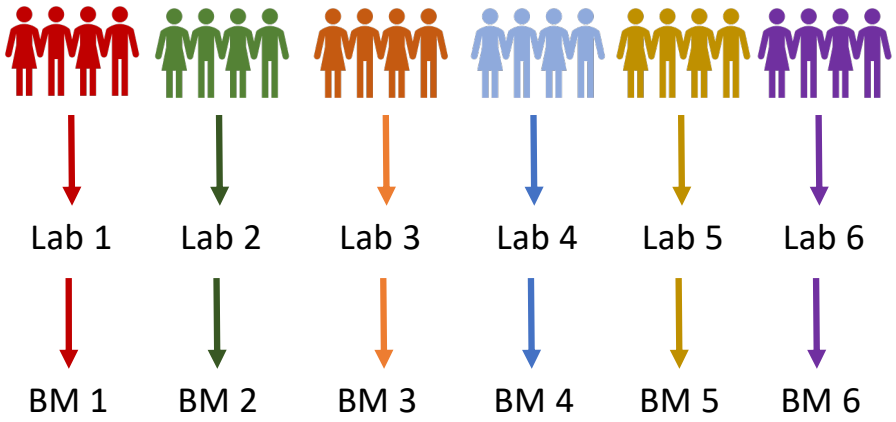


Proal,…Peluso et al Lancet ID 2025

<u>We need a large, multi-institution, dedicated biomarker program focused on viral persistence and other causes of Long COVID</u>

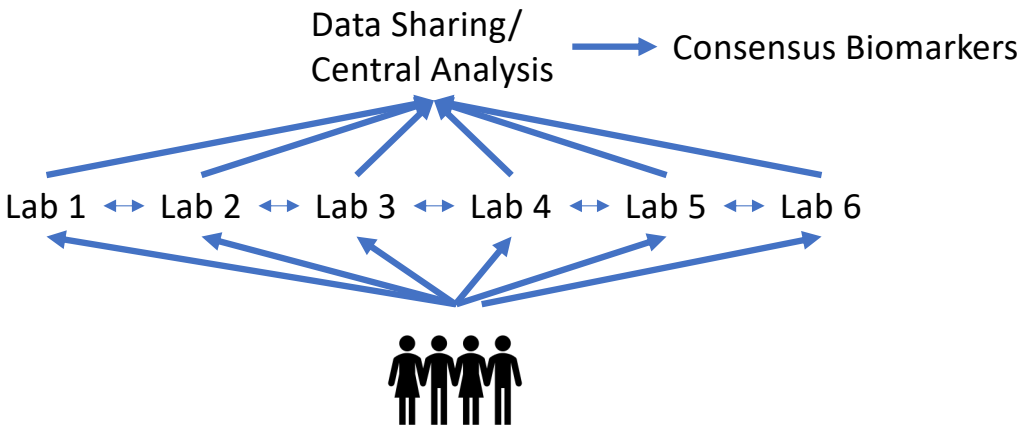
Tackling the Biomarker Problem

Current Situation



No consensus on best performing biomarker

What We Need



Central Cohort: Deep phenotyping, repeat measures

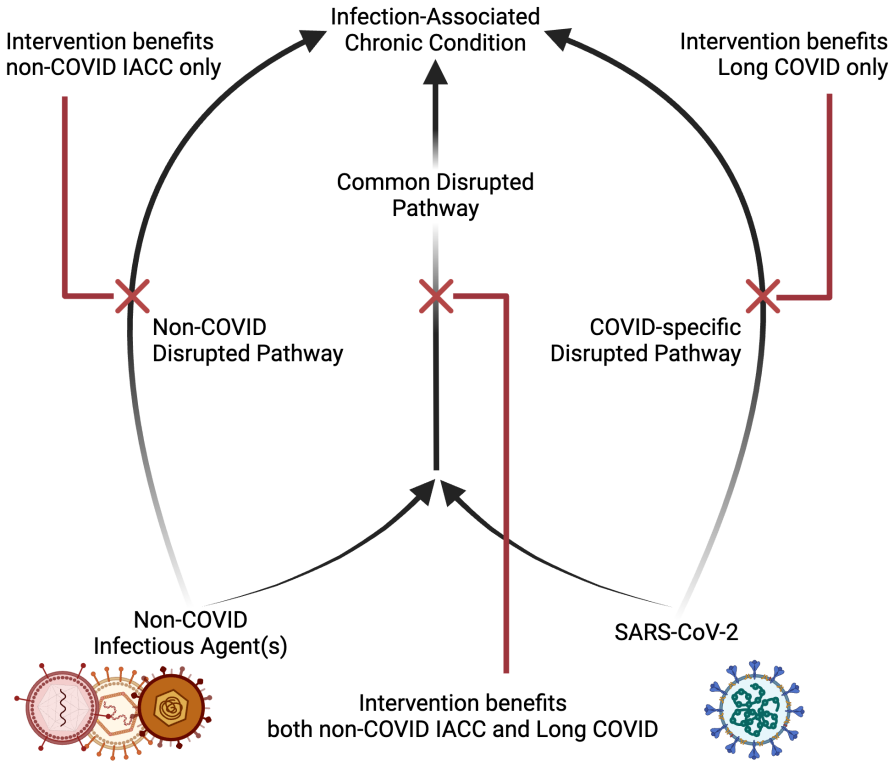
Built on experience from tackling the same problem in HIV (RAVEN Program)
Initially proposed to focus on viral persistence, but design allows for expansion to any potential mechanism

Long COVID as a model for other IACCs

SARS-CoV-2 and LC

The synchronicity of the inciting infection, universality of the exposure, and visibility resulted in the ideal environment for a coordinated effort to understand this new IACC.

If successful, this can be translated to multiple other conditions that currently lack treatments or cures.



Peluso, Hanson, and Deeks, Sci Trans Med 2024

Summary

Where We Are

1. We know that SARS-CoV-2 persistence occurred in the first few years of the pandemic (minimum immunity, severe infections)

2. We think it is still occurring with modern infections, but the science is still catching up with the evolving pandemic

3. We don't know if viral persistence is a cause of Long COVID, or a consequence of something else that causes Long COVID

What We Need

1. Studies aimed at preventing viral persistence from happening in the first place (would have been nice to have these a few years ago)

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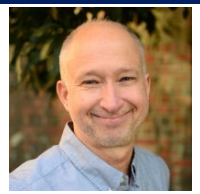
4. An open mind about whether or not this is the right target

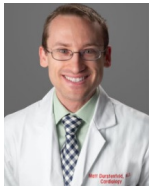
UCSF LIINC/RECOVER/AHRQ Principal Investigators


Michael Peluso

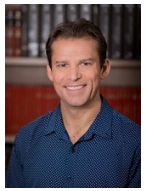

Steven Deeks


Tim Henrich

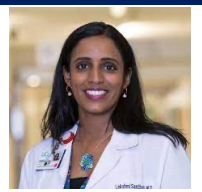

Priscilla Hsue


Matt Durstenfeld


Jeff Martin


Dan Kelly

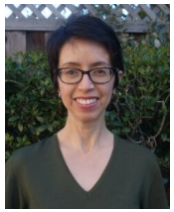

Ma Somsouk


Lekshmi Santhosh


Vivek Jain


Edwin Charlebois

LIINC/RECOVER/AHRQ Program Managers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LIINC RECOVER Researching COVID to Enhance Recovery

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NIH National Institute of Allergy and Infectious Diseases
K23 AI157875
R01 AI158013
R21 AI167648
10T2HL156812

NIH National Heart, Lung, and Blood Institute
OTHL161847
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NIH National Institute of Neurological Disorders and Stroke
1R01NS136197

NIH National Institute on Aging
1R01AG085873

AHRQ Agency for Healthcare Research and Quality
1U18HS029951