Clinical Trials For Multisystem Inflammatory Syndrome In Children (MIS-C) And Long COVID

Alessio Fasano, M.D.
W. Allan Walker Chair in Pediatric Gastroenterology and Nutrition
Professor of Pediatrics Harvard Medical School
Professor of Nutrition Harvard T.H. Chan School of Public Health
Mucosal Biology and Immunology Research Center
And Center for Celiac Research
Massachusetts General Hospital for Children

MassGeneral Hospital for Children

EBRIS European Biomedical Research Institute of Salerno

Harvard Medical School

Overview

1. Description of the target population (for inclusion & exclusion criteria)

2. Overview on pathogenesis to identify mechanistic targets for patients' stratification (precision medicine) and therapeutic interventions

3. Study design and execution

Prevalence Of Symptoms Following COVID In Children And Adolescents

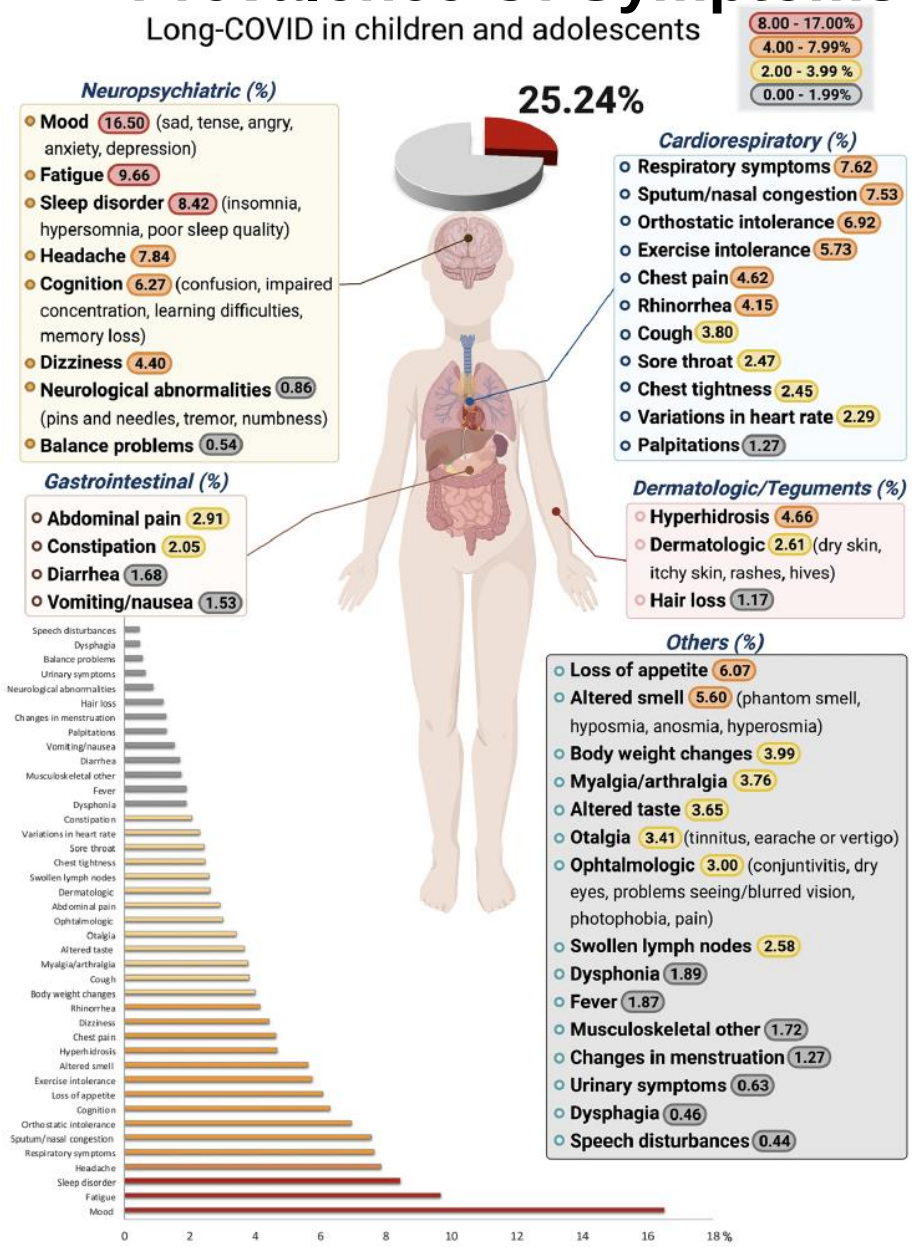
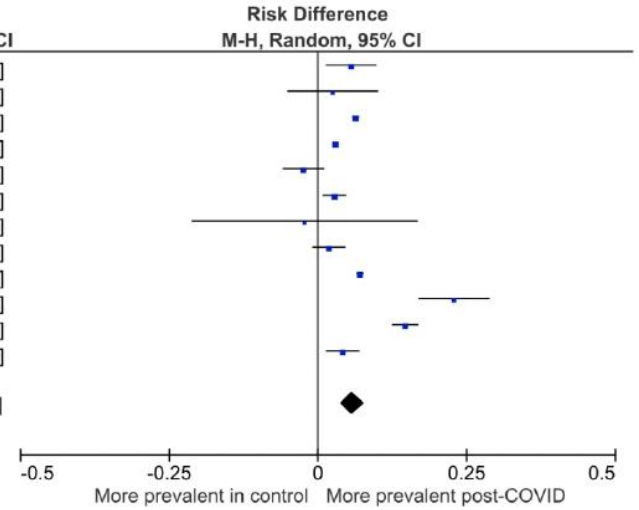


Figure 2. The pooled prevalence of long-COVID by symptoms in children and adolescents. Meta-analyses revealed that the prevalence of more than 40 long-COVID symptoms in children and adolescents. The presence of one or more symptoms following a SARS-CoV-2 infection was 25.24%.

Reported symptom: fatigue

Study or Subgroup	Post-COVID Events	Post-COVID Total	Controls Events	Controls Total	Weight	Risk Difference M-H, Random, 95% CI
Bergia 2022	39	451	3	98	7.6%	0.06 [0.01, 0.10]
Blankenberg 2021	71	178	496	1327	4.4%	0.03 [-0.05, 0.10]
Borch 2022	1578	14883	654	15234	11.2%	0.06 [0.06, 0.07]
Ewan Donnachie 2022	3211	43903	3226	73873	11.3%	0.03 [0.03, 0.03]
Kikkenborg Berg 2022a	19	242	542	5261	8.5%	-0.02 [-0.06, 0.01]
Kikkenborg Berg 2022b	12	333	78	9408	10.2%	0.03 [0.01, 0.05]
Molteni 2021	65	77	13	15	1.1%	-0.02 [-0.21, 0.17]
Radtke 2021	2	109	0	1246	9.4%	0.02 [-0.01, 0.05]
Roessler 2021	1503	11950	3292	59750	11.2%	0.07 [0.06, 0.08]
Roge 2021	59	236	3	142	5.7%	0.23 [0.17, 0.29]
Stephenson 2021	1196	3065	911	3739	10.0%	0.15 [0.12, 0.17]
Zavala 2021	33	472	11	387	9.3%	0.04 [0.01, 0.07]
Total (95% CI)		**75899**		**170480**	**100.0%**	**0.06 [0.04, 0.08]**
Total events	7788		9229			

Heterogeneity: Tau² = 0.00; Chi² = 372.72, df = 11 (P < 0.00001); I² = 97%
Test for overall effect: Z = 5.50 (P < 0.00001)



Reported symptom: anxiety

Study	Post-COVID Yes	Post-COVID No	Control Yes	Control No	Risk Difference with 95% CI	Weight (%)
Bergia	22	27	8	6	-0.12 [-0.42, 0.17]	2.66
Roessler	1,996	9,954	6,495	53,255	0.06 [0.05, 0.07]	36.15
Roge	31	205	0	142	0.13 [0.09, 0.17]	28.62
Zavala	33	439	11	376	0.04 [0.01, 0.07]	32.58
Overall					0.07 [0.02, 0.12]	

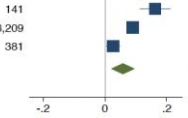
Heterogeneity: τ^2 = 0.00, I^2 = 88.39%, H^2 = 8.62

Rar

Reported symptom: headache

Study	Post-COVID Yes	Post-COVID No	Control Yes	Control No	Risk Difference with 95% CI	Weight (%)
Bergia	31	18	5	9	0.28 [-0.01, 0.56]	1.53
Blankenburg	109	69	728	598	0.06 [-0.01, 0.14]	7.91
Borch	1,033	13,850	911	14,323	0.01 [0.00, 0.02]	11.67
Kikkkenborg Berg A	15	227	309	4,952	0.00 [-0.03, 0.03]	10.84
Kikkkenborg Berg B	7	326	49	9,359	0.02 [0.00, 0.03]	11.47
Molteni	60	17	12	3	-0.02 [-0.24, 0.20]	2.31
Radtke	5	104	39	1,207	0.01 [-0.03, 0.06]	10.31
Roessler	4,382	7,568	13,886	45,864	0.13 [0.13, 0.14]	11.62
Roge	40	196	1	141	0.16 [0.11, 0.21]	9.72
Stephenson	710	2,355	530	3,209	0.09 [0.07, 0.11]	11.37
Zavala	20	452	6	381	0.03 [0.00, 0.05]	11.25
Overall					0.06 [0.02, 0.10]	

Heterogeneity: τ^2 = 0.00, I^2 = 97.60%, H^2 = 41.75

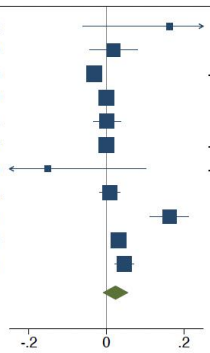


Random-effects REML model

Reported symptom: cognitive difficulties

Study	Post-COVID Yes	Post-COVID No	Control Yes	Control No	Risk Difference with 95% CI	Weight (%)
Bergia	15	34	2	12	0.16 [-0.06, 0.39]	1.81
Blankenburg	144	34	1,049	278	0.02 [-0.04, 0.08]	8.32
Borch	910	13,973	1,408	13,826	-0.03 [-0.04, -0.03]	11.75
Ewan	58	43,845	43	73,830	0.00 [0.00, 0.00]	11.80
Kikkkenborg Berg A	20	222	426	4,835	0.00 [-0.03, 0.04]	10.37
Kikkkenborg Berg B	7	326	201	9,207	-0.00 [-0.02, 0.02]	11.49
Molteni	14	63	5	10	-0.15 [-0.41, 0.10]	1.46
Radtke	2	107	12	1,234	0.01 [-0.02, 0.03]	11.00
Roge	40	196	1	141	0.16 [0.11, 0.21]	9.28
Stephenson	198	2,867	123	3,616	0.03 [0.02, 0.04]	11.66
Zavala	29	443	6	381	0.05 [0.02, 0.07]	11.05
Overall					0.02 [-0.01, 0.06]	

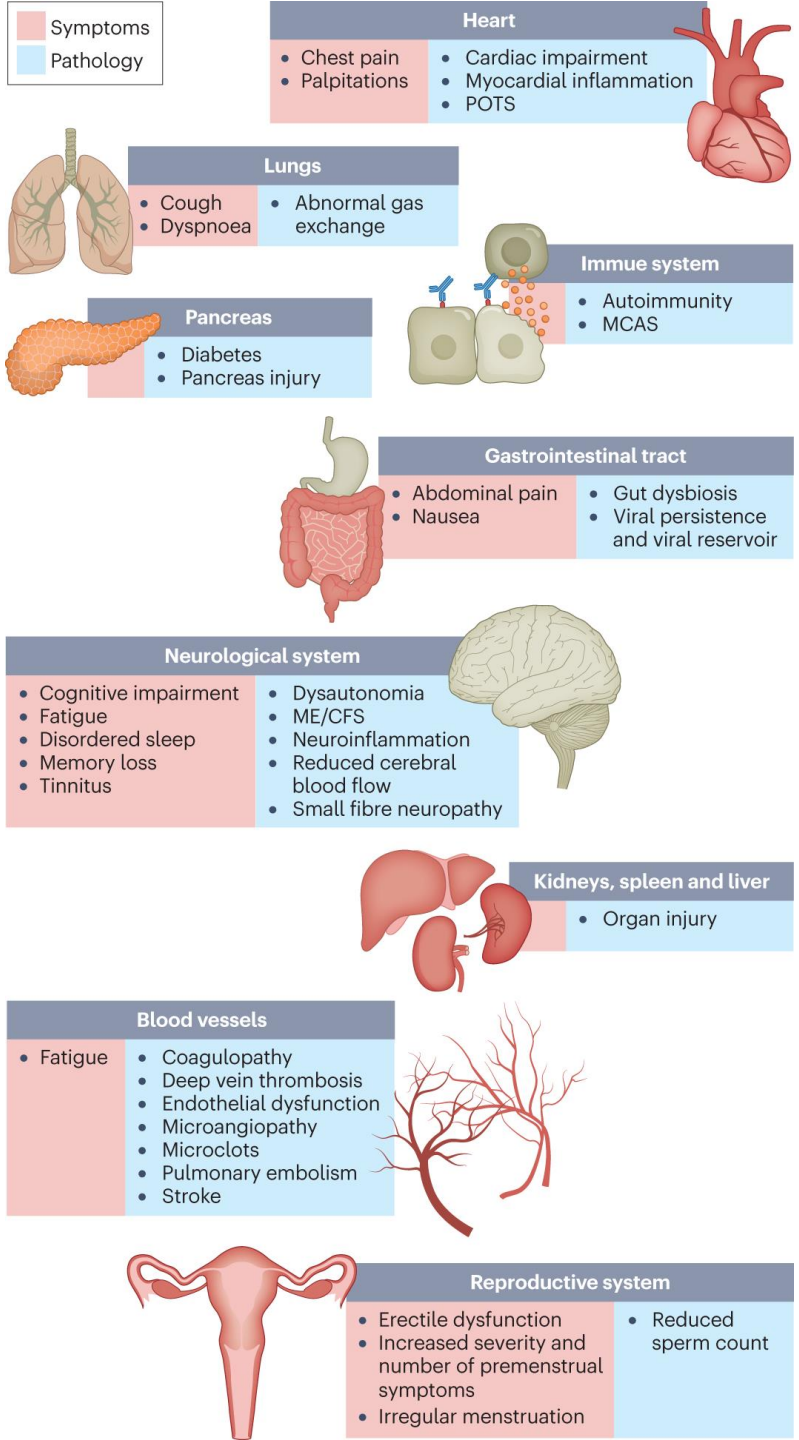
Heterogeneity: τ^2 = 0.00, I^2 = 98.81%, H^2 = 83.91

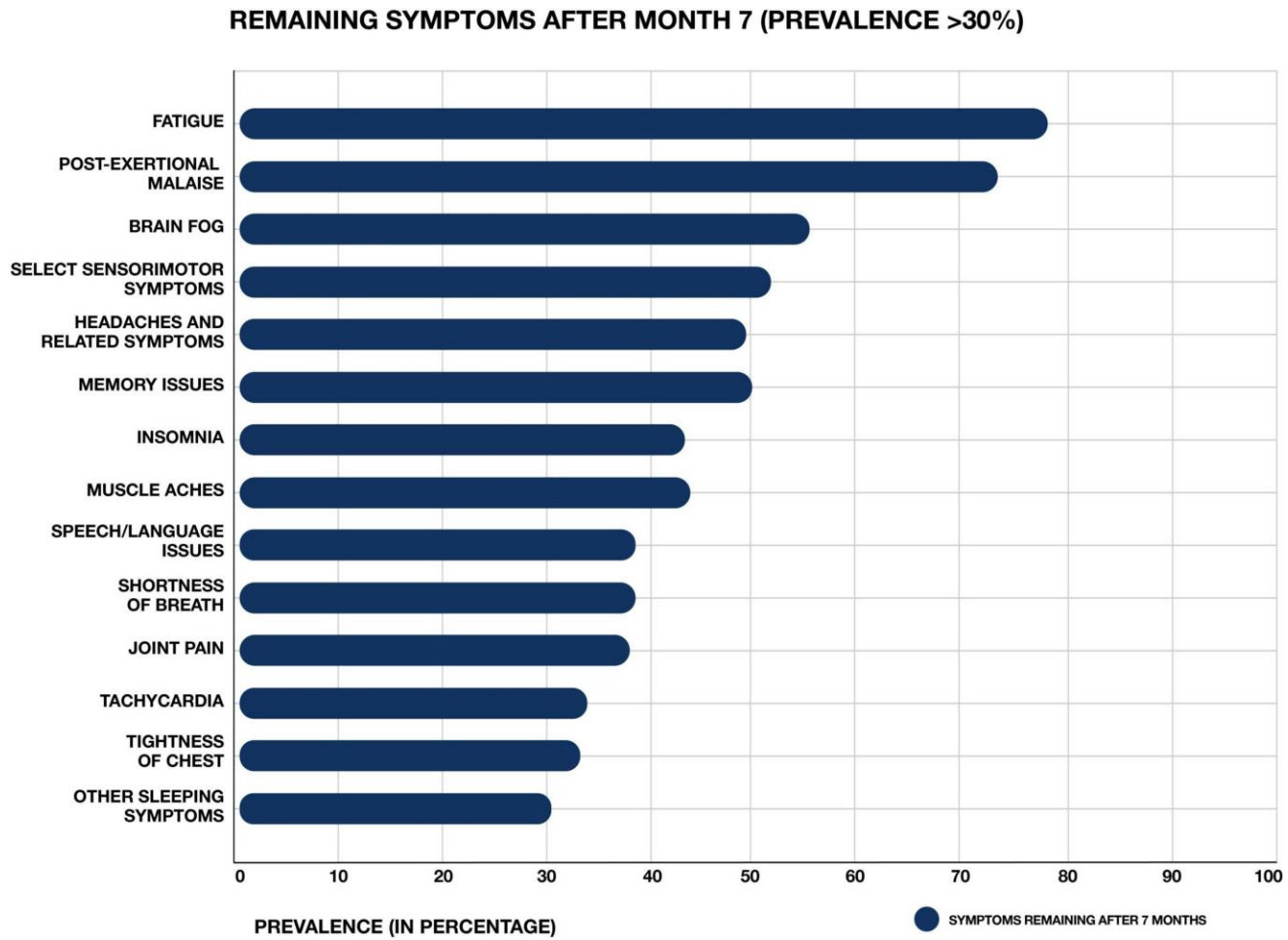


Random-effects REML model

Lopez-Leon, et al *Sci Rep* **12**, 9950 (2022).

Prevalence Of Symptoms Following COVID In Adults

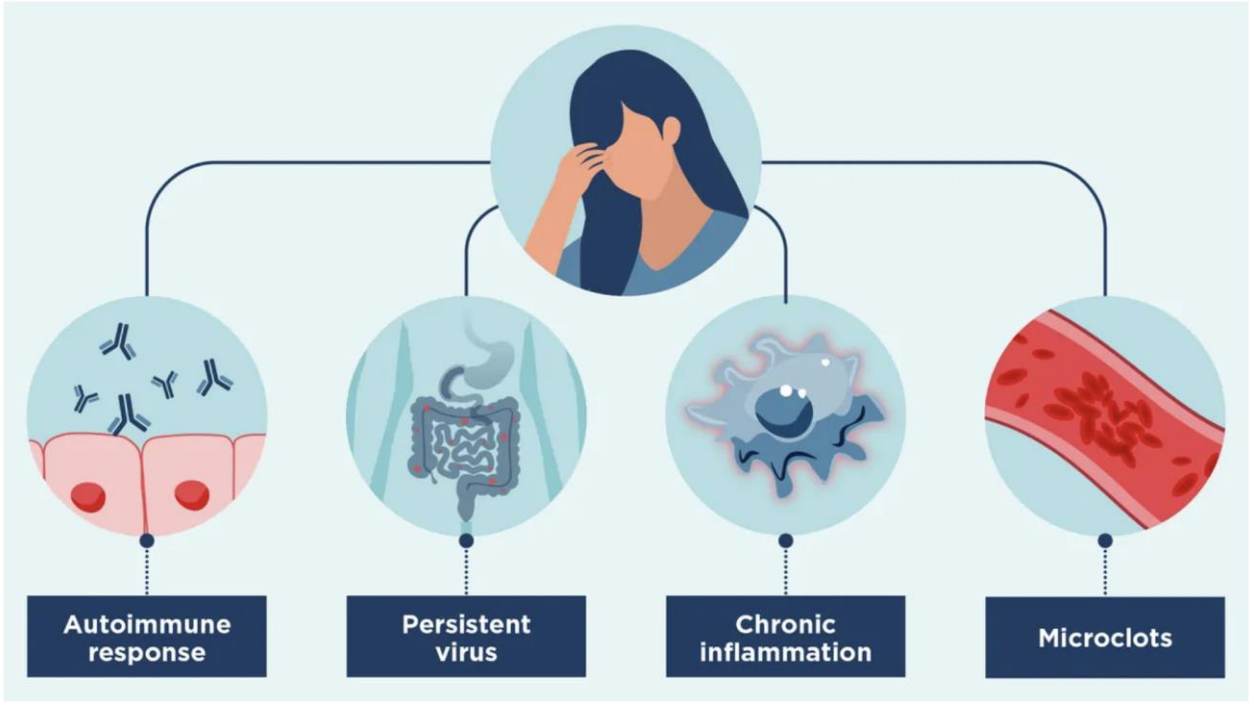




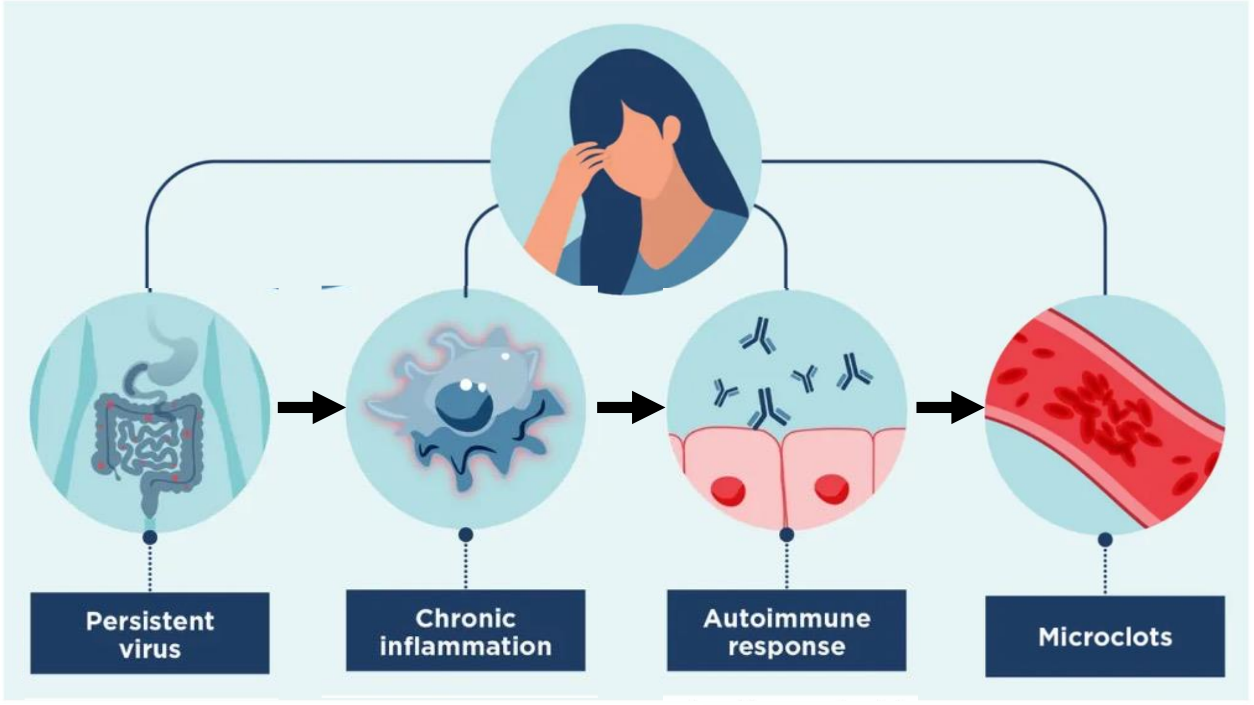
Davis HE, et al *Nat Rev Microbiol* **21**, 133–146 (2023)

What Causes Long COVID?

Four Hypothesis As To What Might Be Causing Long COVID Symptoms

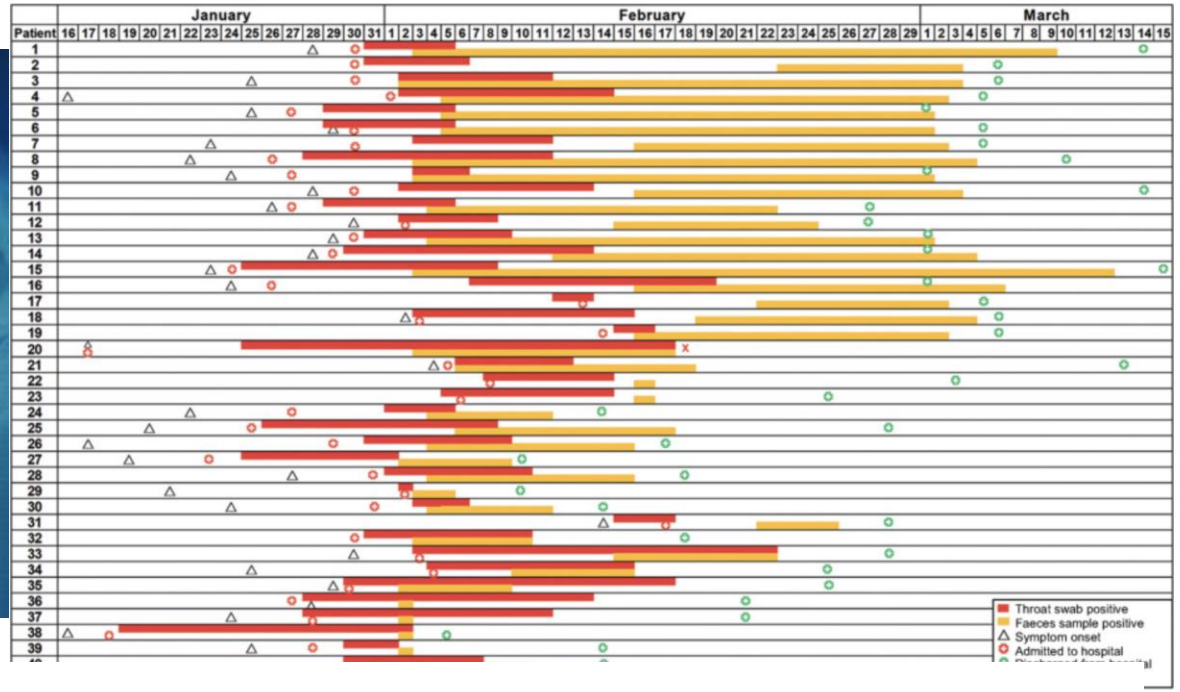


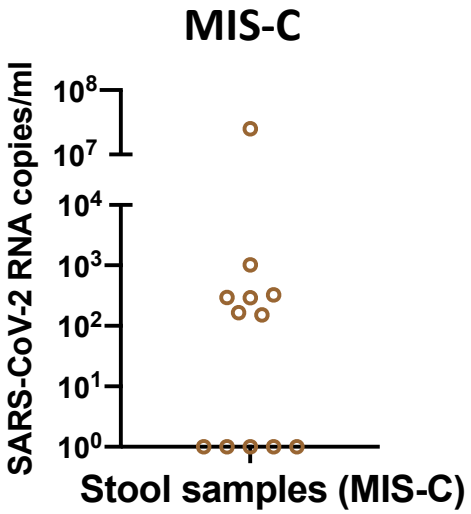
Four Interconnected Steps Leading To Long COVID Symptoms

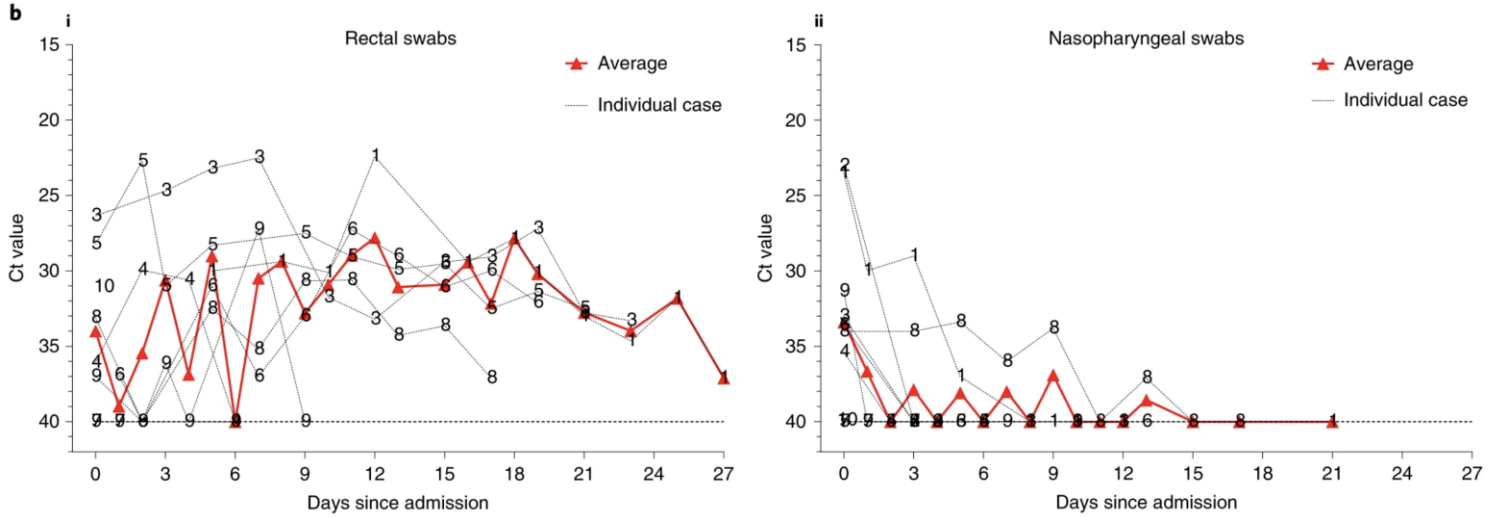


1. Persistent Virus:
The Role of The Gut in COVID-19

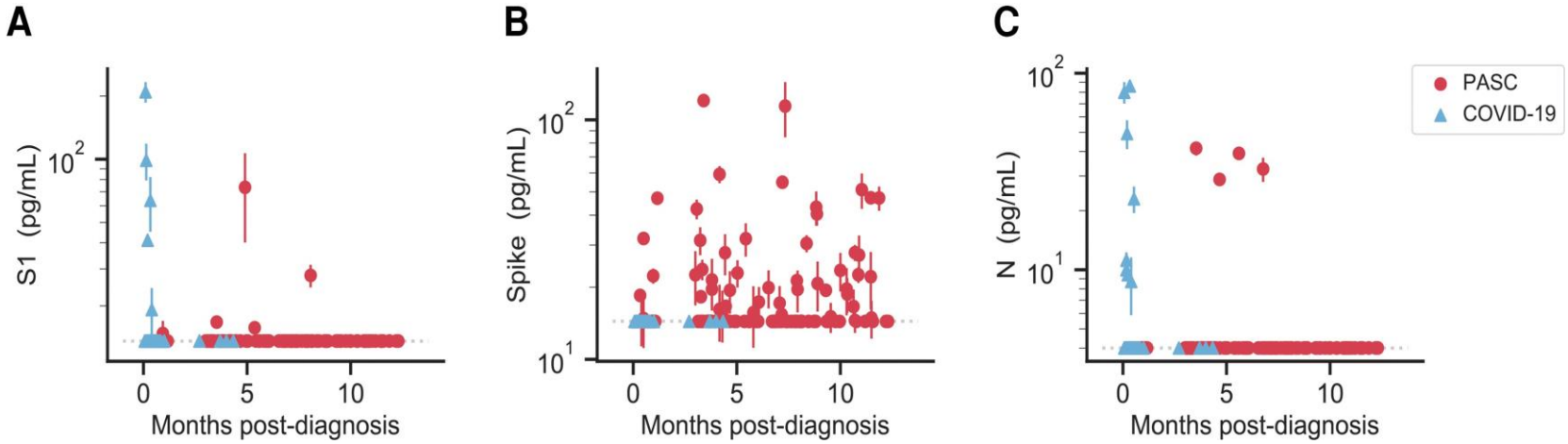
- Prolonged shedding of virus in stools
- Role in acute illness (?)
- Role in long COVID
- Role in MIS

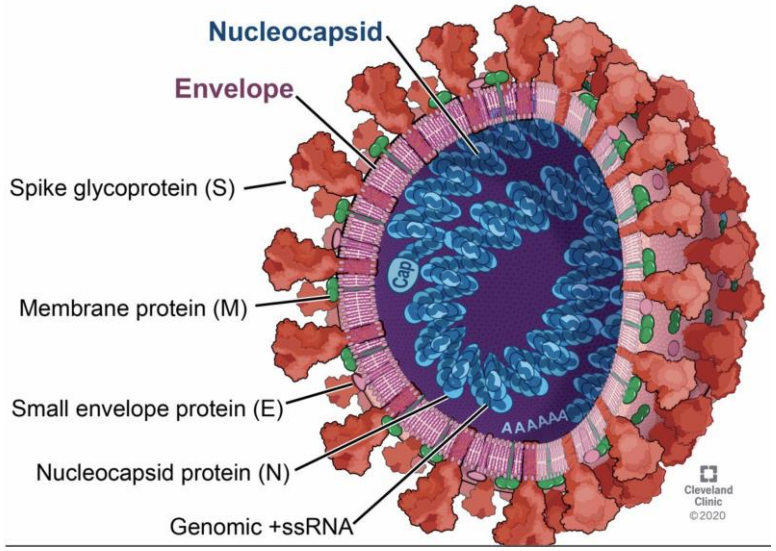






Spike protein present in the serum of long COVID patients despite no virions are present in circulation

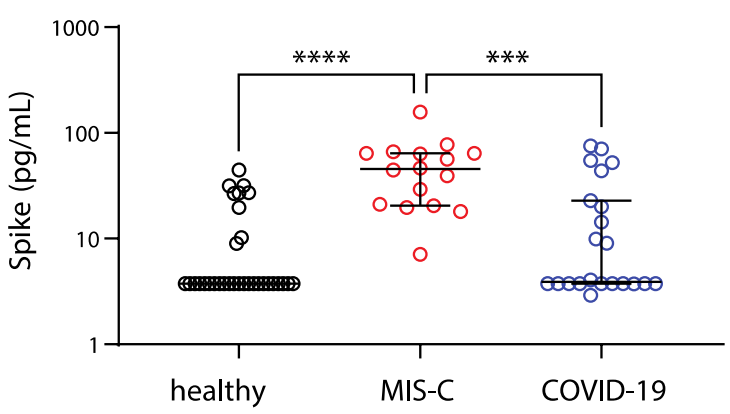


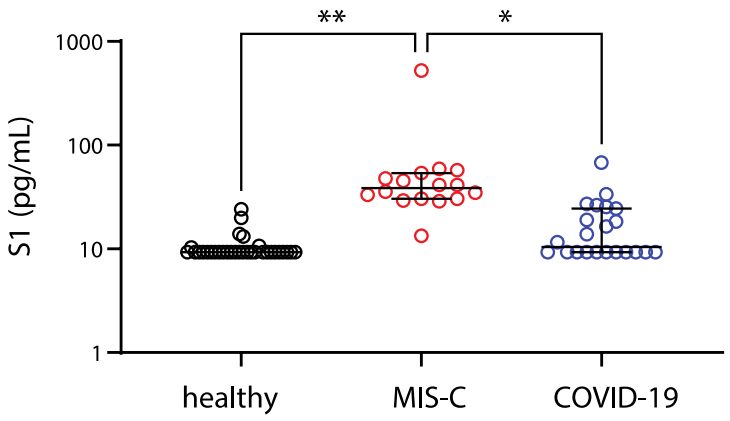


Clin Infect Dis, Volume 76, Issue 3, 1 February 2023, Pages e487–e490, https://doi.org/10.1093/cid/ciac722

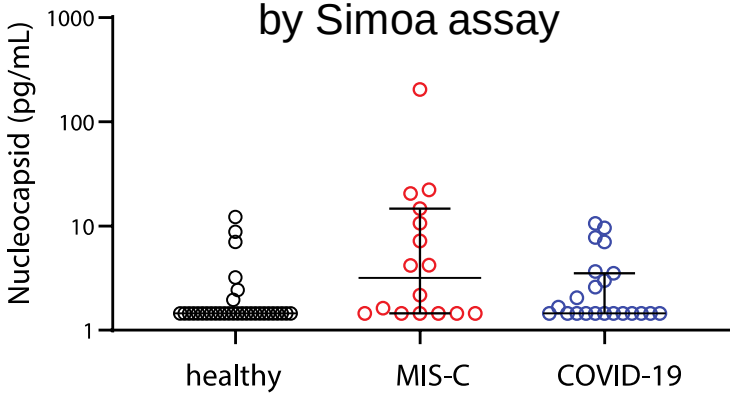
Why? How?

Spike protein is present in the serum of MIS-C patients despite no virions are present in circulation





SARS-CoV2 Ags profile measured by Simoa assay



Yonker, Gilboa, Ogata et al, JCI, July 2021

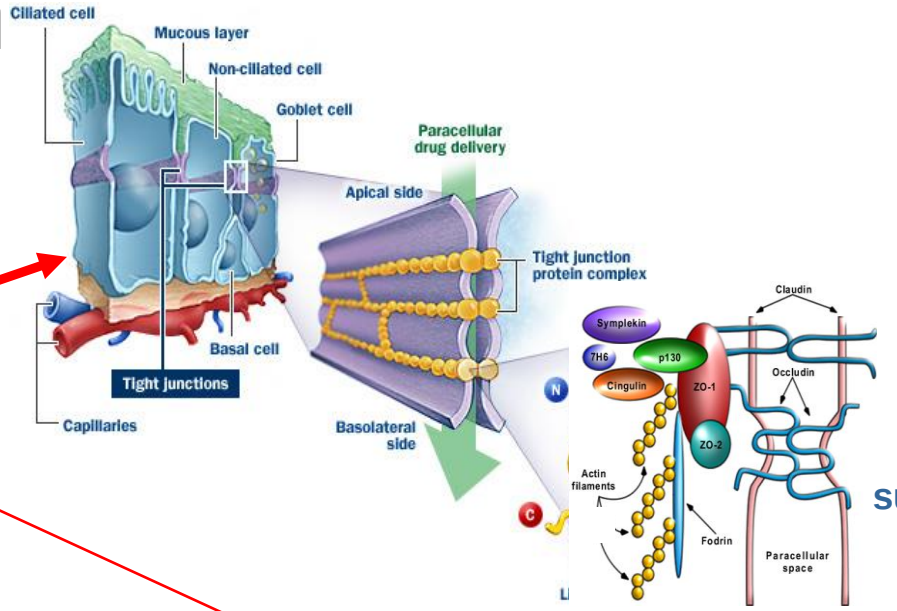
2. Chronic Inflammation

Loss of Mucosal Immune Homeostasis

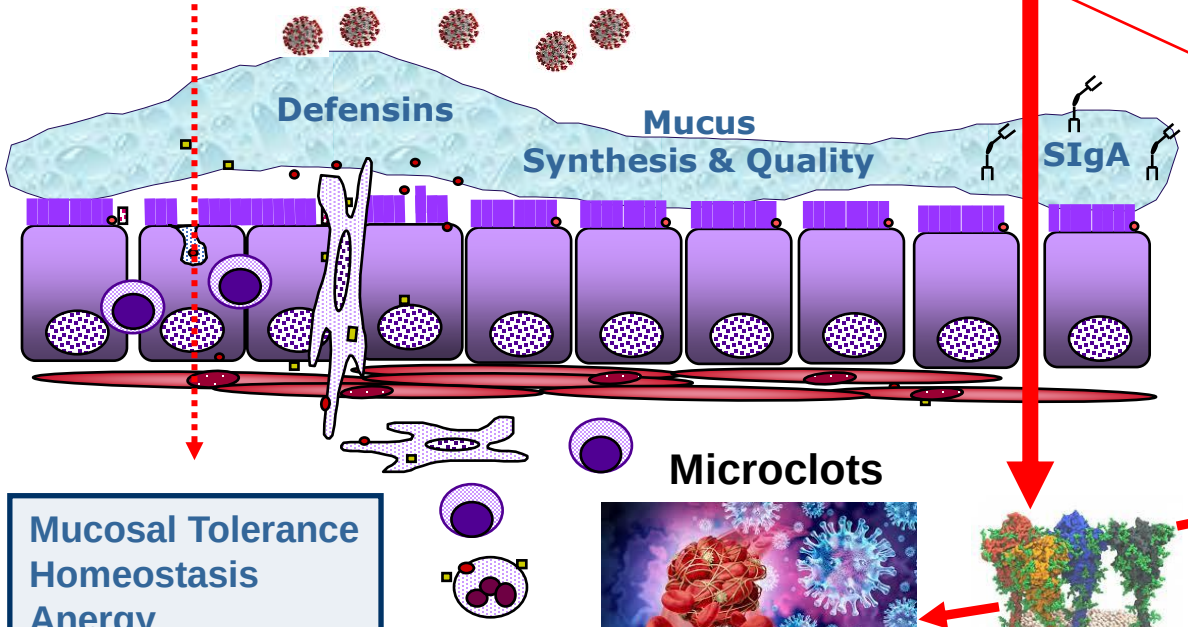
❶ Normal/physiologically controlled permeability

❷ SARS-CoV2 in the GI tract causes dysbiosis and Increased zonulin release

❸ Zonulin-dependent barrier defect leads to SARS-CoV2 spike influx



❹ SARS-CoV2 superantigen triggers T cell proliferation, cytokines storm, and microclots



Zonulin Inhibitor
AT1001 (Larazotide)

Mucosal Tolerance
Homeostasis
Anergy

Regulatory DC's
Macrophages
Tregs
IL-10/TGF-β

Microclots

Spike

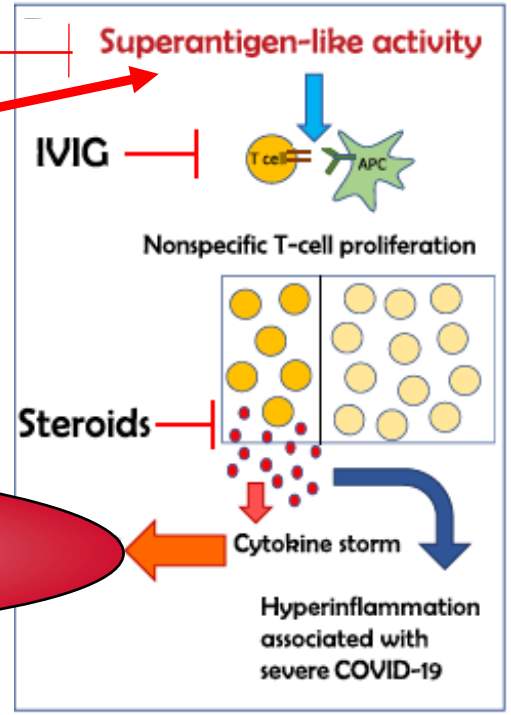
Endothelial vessels

SIgA

C3 & C4 activation

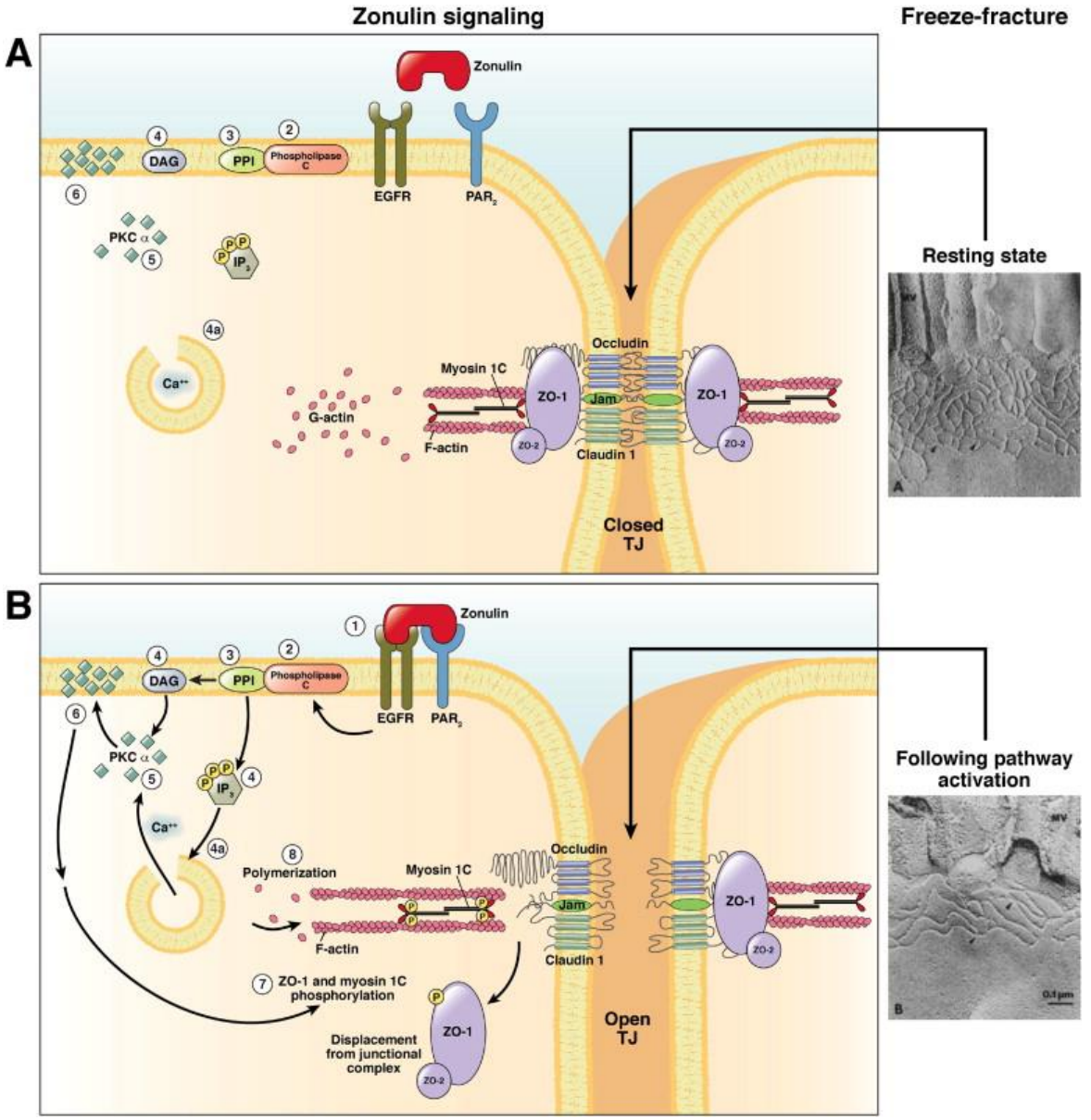
IL-1β, IL-6, IL-8, IL-10, IP-10, IFN-γ, IL-17, TNF-α

MIS-C
Long COVID

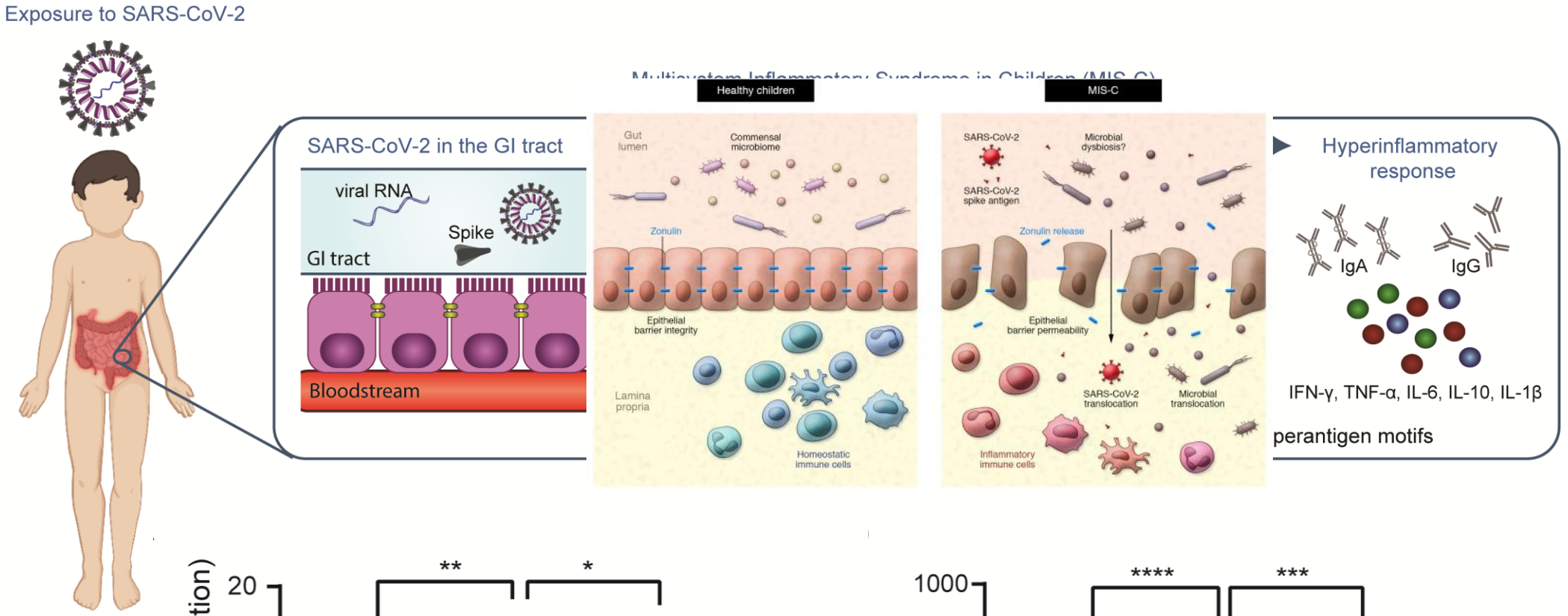


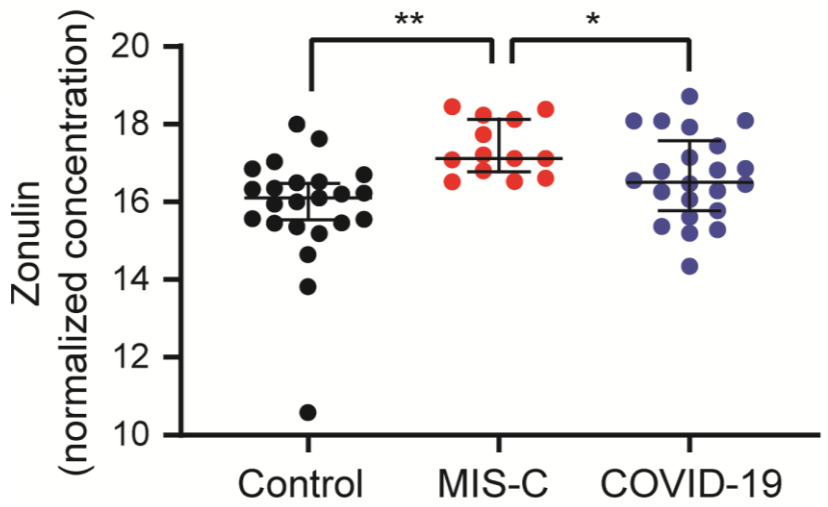
Literature Report On Zonulin Association With CID

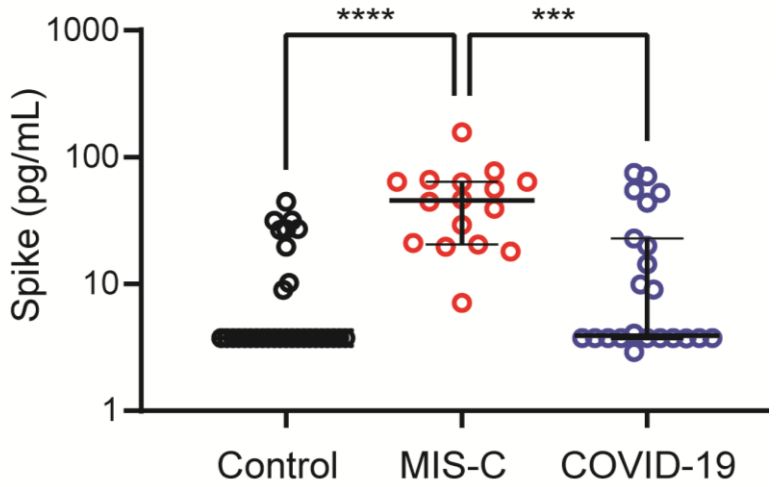
Disease	Model	PMID: Reference
ADHD	Human	36786182
Aging	Human	29896420
Ankylosis spondylitis	Human	28069576
Autism	Human	36447452
Bipolar Disorders	Human	37098666
Celiac Disease	Human	32162764
Colitis/IBD (Crohn's disease)	Human	34979917
Colitis	Mouse	28423466
Depressive Disorders	Human	34320451
Gestational diabetes	Human	35994108
Glioma	Human	19701495
Glioma	Cells	23637756
Irritable bowel syndrome	Human	31210949
HIV	Human	29762690
Long COVID	Human	1182544
MIS-C	Human	34032635
ME/CFS	Human	35946099
Multiple sclerosis	Cells	30449598
Multiple sclerosis	Mouse	25184418
Multiple sclerosis	Human	31317818
Necrotizing Enterocolitis (NEC)	Human	35279661
Nonalcoholic fatty liver disease	Human	32255299
Non-Celiac Gluten Sensitivity	Human	32060130
Obesity/Insulin resistance	Human	35666025
Sepsis	Human	23457771
Type 1 diabetes	Human	16644703
Type 2 diabetes	Human	24347174



MIS-C is driven by zonulin-dependent loss of gut mucosal barrier







Yonker, Gilboa, Ogata et al, JCI, July 2021

Pathogenesis of MIS-C

- SARS-CoV-2 detected in the stool weeks-months after COVID-19 first encounter.
- SARS-CoV2 presence in GI tract causes microbiome imbalance (dysbiosis)
- Zonulin release detected in MIS-C plasma.
- Highly inflammatory viral particles leak into circulation.
- Current treatments target immune hyperactivation, not mucosal barrier integrity.

Increase In Zonulin And Oxidized LDL Is Associated With Post-Acute Sequelae of SARS-CoV-2

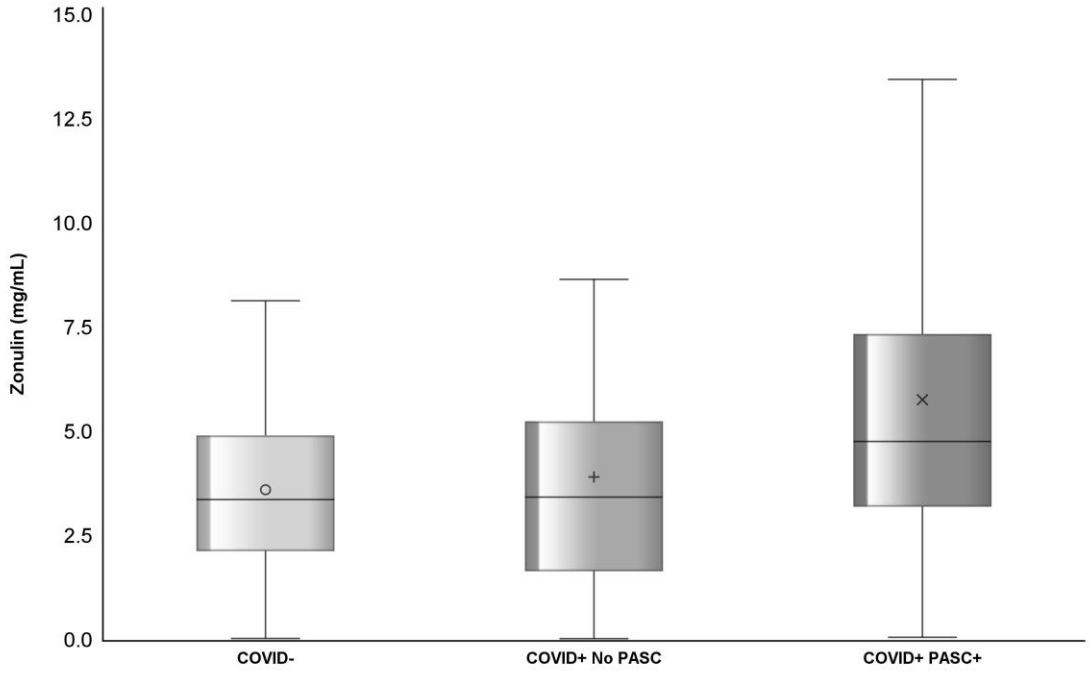
	COVID- (n=258)	COVID+		p-value
		No PASC (n=72)	PASC+ (n=85)	
	n (%) or median (IQR) / mean ± std			
Age (years)	43.68 ± 13.69	44.85 ± 13.31	47.81 ± 13.49	0.05
Female Sex	101 (24.34)	35 (8.43)	50 (12.05)	**0.01**
Non-white Race*	108 (26.02)	25 (6.02)	26 (6.27)	0.14
BMI (kg/m2)	27.91 ± 6.05	30.68 ± 9.49	31.82 ± 8.63	**0.0002**
Current Smoker	158 (38.35)	19 (4.61)	9 (2.18)	**<.0001**
Median number of days from infection	- -	292 (IQR: 172, 518)	229 (IQR: 147, 478)	0.22
Comorbidities				
Hypertension	27 (10.47)	14 (19.44)	23 (27.06)	**0.001**
Diabetes	5 (1.94)	5 (6.94)	9 (10.59)	**0.0002**
HIV infection	98 (37.98)	22 (30.56)	22 (25.88)	0.1
Medications				
Statin	5 (1.94)	11 (14.1)	10 (9.17)	0.28
Ox-LDL (U/L)	47.02 (35.52, 62.77)	57.24 (40.7, 75.37)	76.75 (59.95, 103.28)	**<.0001**
IL-6 (pg/ml)	2.65 (1.62, 4.25)	2.21 (1.44, 3.63)	2.46 (1.77, 4.01)	0.25
D-dimer (ng/mL)	423.7 (256.02, 705.09)	429.96 (243.58, 677.41)	451.57 (266.36, 626.74)	0.93
hs-CRP (ng/ml)	2720.07 (941.82, 7205.25)	2764.87 (1188.0, 7241.84)	3182.65 (1423.9, 8931.79)	0.35
sTNF-RI (pg/ml)	1103.11 (887.11, 1375.03)	966.99 (755.06, 1331.06)	1099.98 (886.19, 1316.1)	0.08
Zonulin (mg/mL)	3.37 (2.13, 4.91)	3.43 (1.65, 5.25)	4.76 (3.2, 7.35)	**<.0001**
LBP (ng/mL)	16657.37 (11899.67, 21686.01)	14441.98 (9919.42, 23111.83)	16438.56 (10640.08, 22563.73)	0.66

*Includes African American, Asian, Hispanic, and Other Numbers in Bold indicates statistical significance ($P < 0.05$).

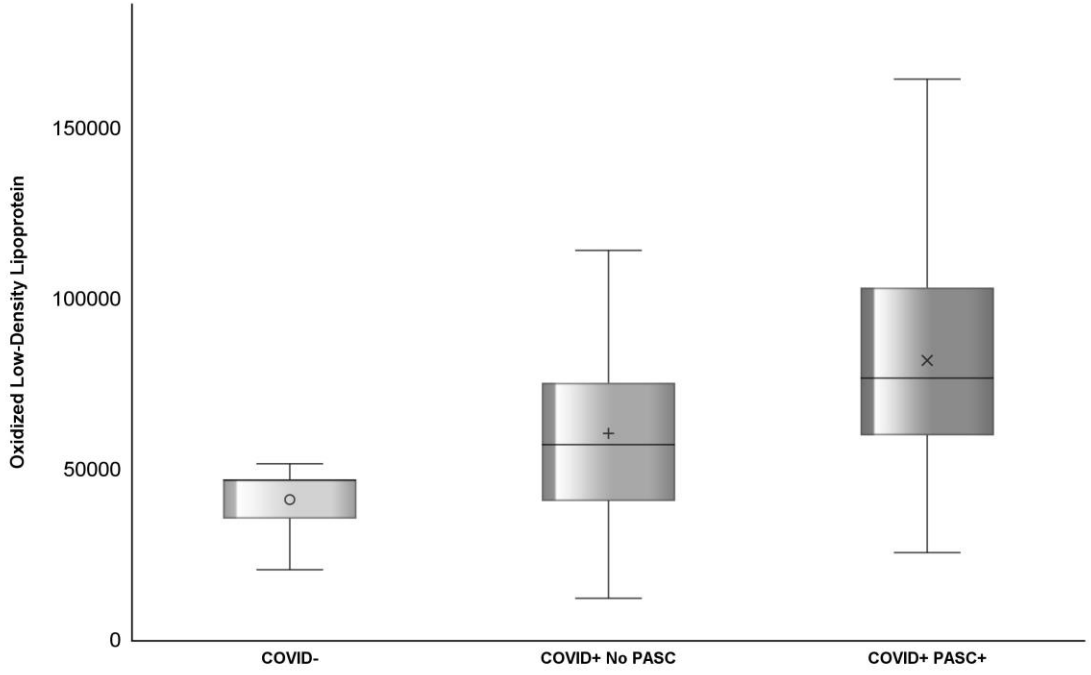
Characteristics of participants by COVID and PASC (Long COVID) Status

Mouchati C et al, Front Immunol, 2023

Distribution of Zonulin by COVID And PASC Status

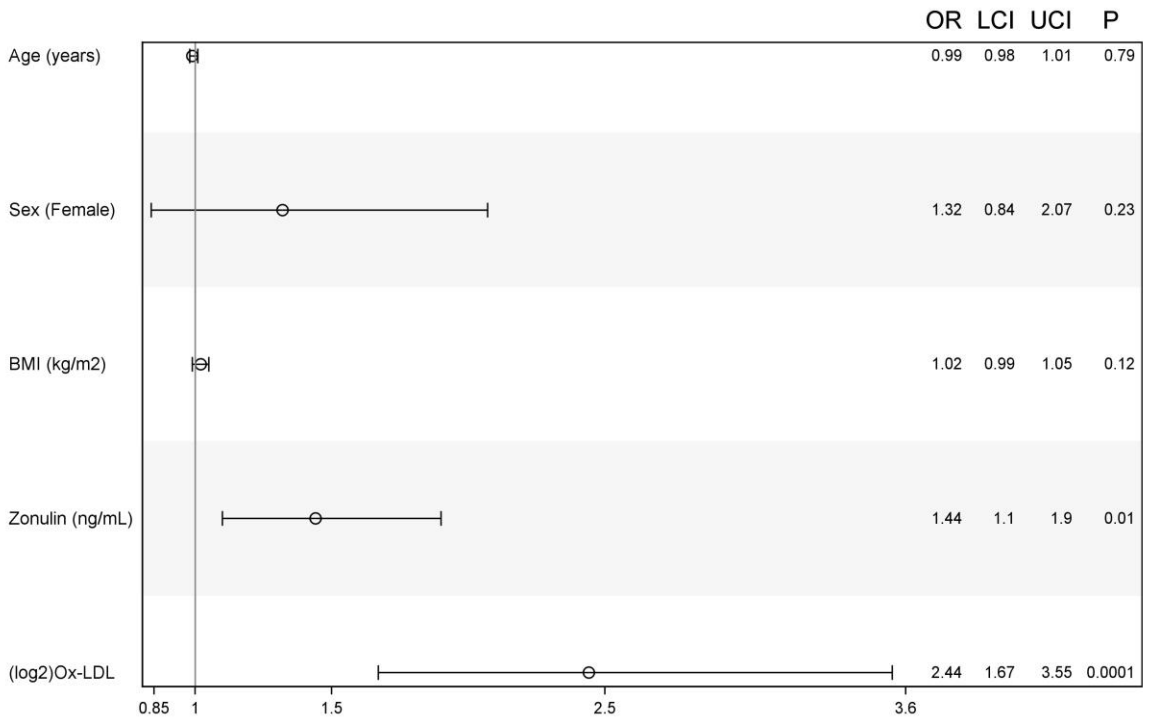


Distribution of Oxidative-LDL by COVID And PASC Status



Mouchati C et al, Front Immunol, 2023

Association of Gut Permeability And Oxidated-LDL with PASC



*Race*Smoke[aOR:3.85(95%CI:1.41,10.51; p=0.01)]
*LCL = lower confidence limit; UCL = upper confidence limit

Independent Associations with COVID+ PASC+

	uOR (95% CI)*	p-value
Age	1.02 (1.002, 1.03)	**0.02**
Female *vs.* Male Sex	1.88 (1.2[illegible] 2.78)	**0.001**
non-White *vs.* White Race	[illegible] (0.44, 0.99)	**0.04**
BMI	1.06 (1.03, 1.09)	**<.0001**
Current Smoker	0.13 (0.08, 0.22)	**<.0001**
IL-6	0.84 (0.65, 1.09)	0.2
hs-CRP	1.09 (0.95, 1.26)	0.23
D-dimer	0.86 (0.68, 1.07)	0.18
Ox-LDL	6.38 (3.01[illegible])	**<.0001**
Zonulin	1.48 (1.14, 1.91)	**0.003**
LBP	0.8 (0.61, 1.06)	0.12

Patients' stratification

Potential Biomarkers

*uOR=Unadjusted Odds Ratio and 95% Confidence Intervals.
Numbers in Bold indicates statistical significance (P < 0.05).

Mouchati C et al, Front Immunol, 2023

3. Autoimmune Response

Post-COVID-19 Autoimmunity Reports

1. Immune thrombocytopenic purpura – ITP

2. Guilliam Barrè Syndrome (GBS)

3. Miller Fisher Syndrome (MFS)

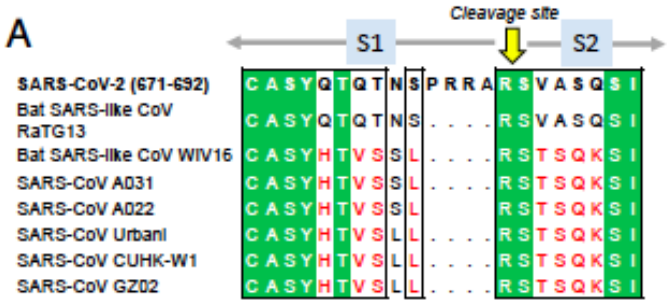
4. Antiphospholipid antibodies and thrombosis

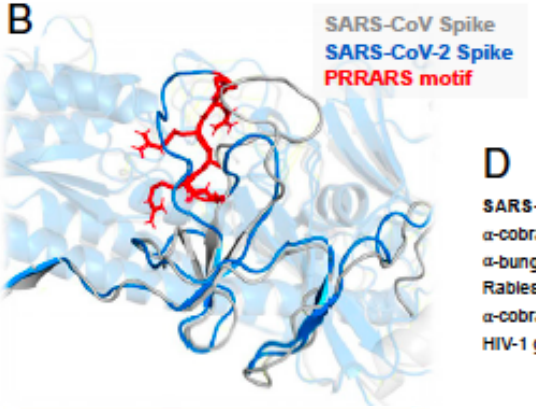
5. Multisystem inflammatory syndrome in children (MIS-C) (?)

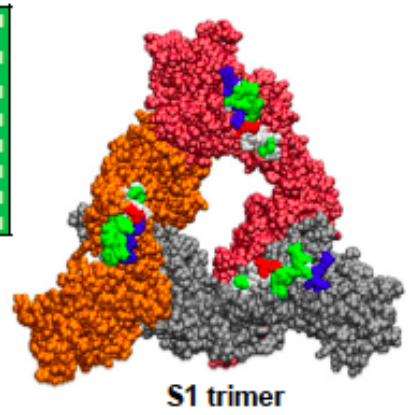
6. Long COVID (?)

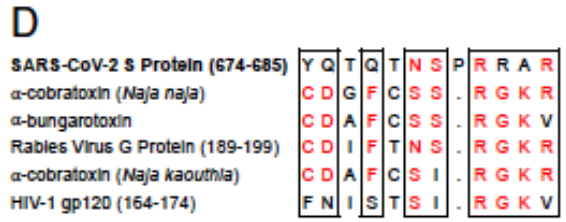
Ehrenfelda M et al , Autoimmunity Reviewers 19 (2020) 102597

Spike Superantigen Structural Signature Supported by Skewed TCR Repertoire in Children Affected By MIS-C

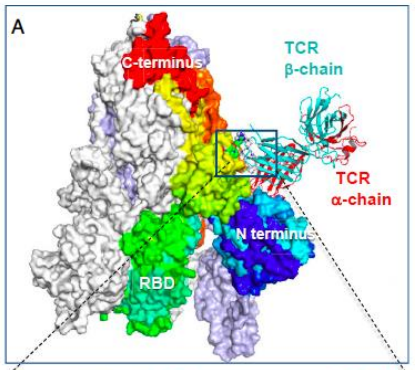


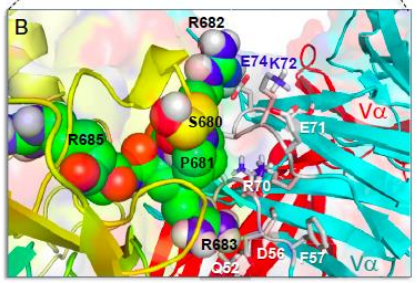




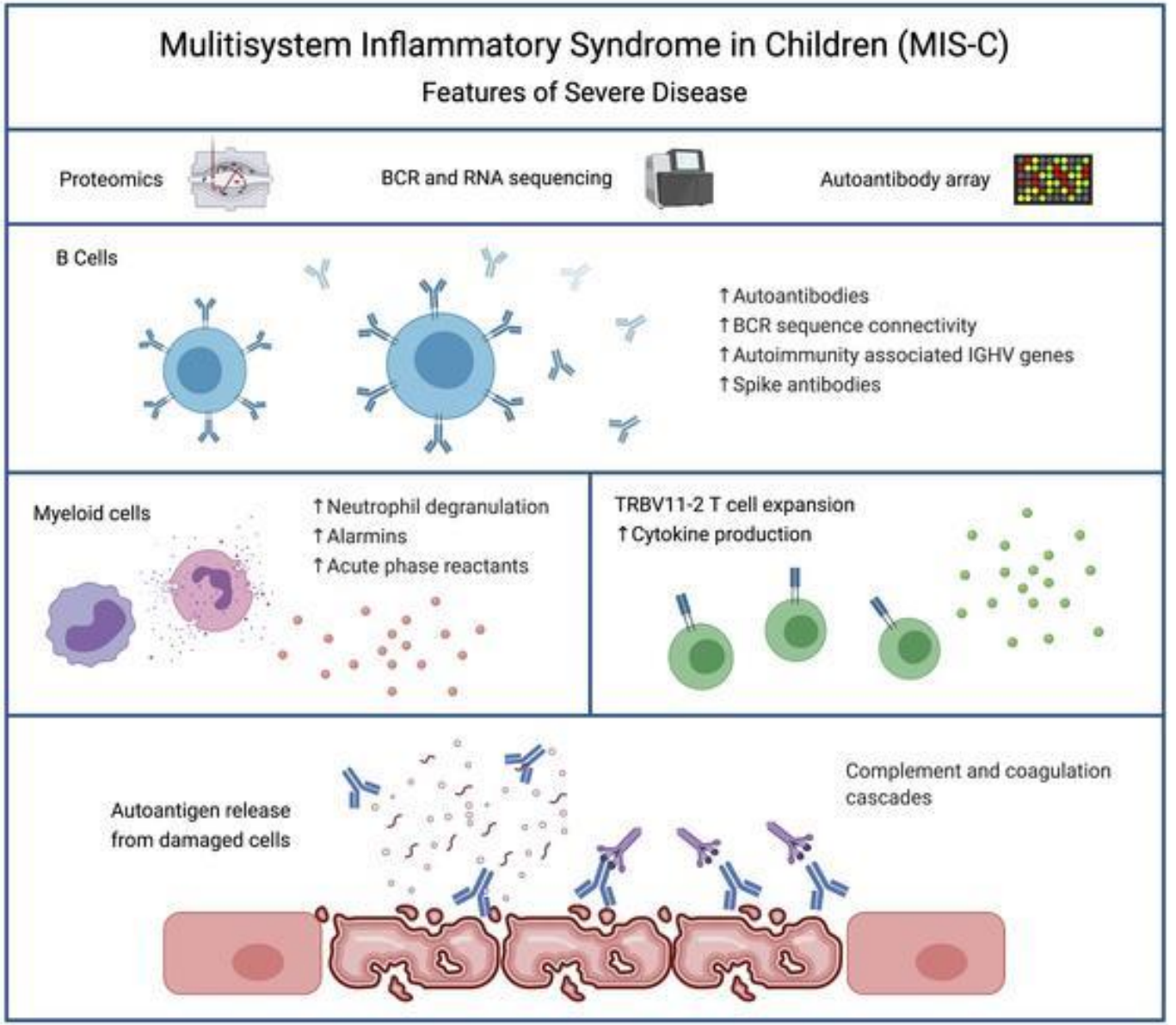


Sequence and structural properties of the insert PRRA. (A and B) SARS-CoV-2 encodes both a cleavage site and SAg-like motifs (20) near the insertion PRRA that distinguishes it from all SARS-related β-CoVs



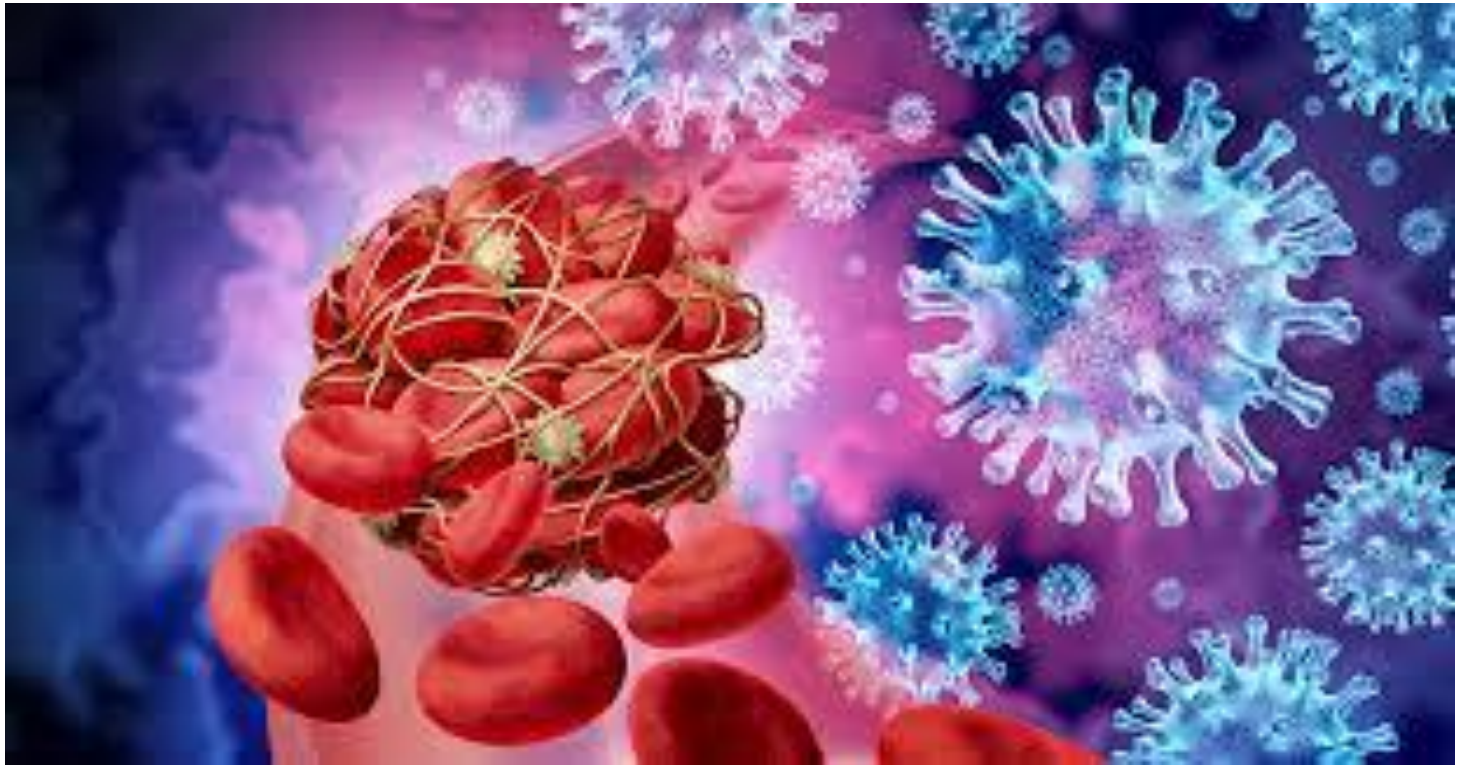


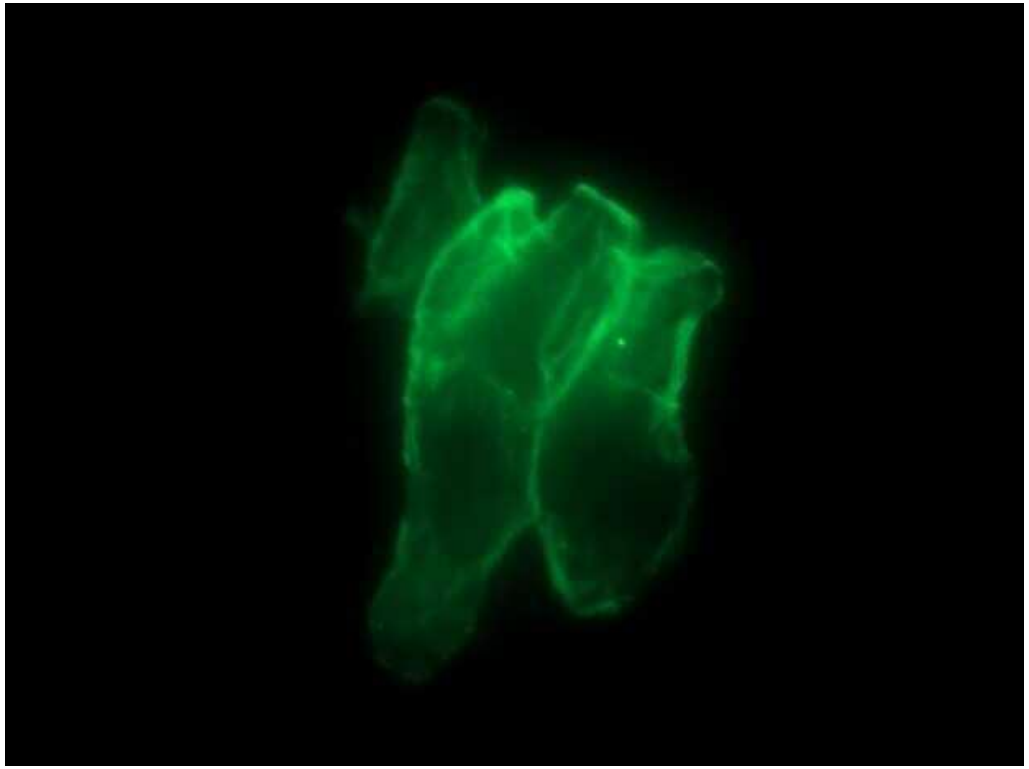
Binding of TCR to SARS-CoV-2 spike trimer near the "PRRA" insert



Cheng M.H. et al PNAS 2020
Porritt R.A. et al , J Clin Invest 2021

4. Microclots

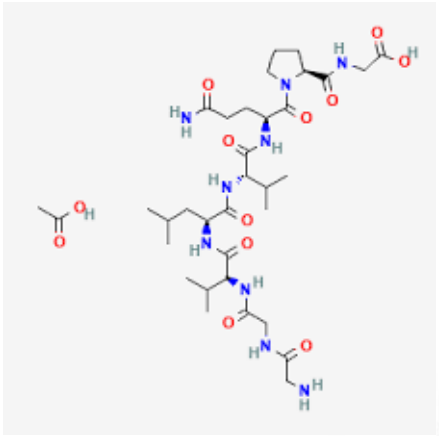


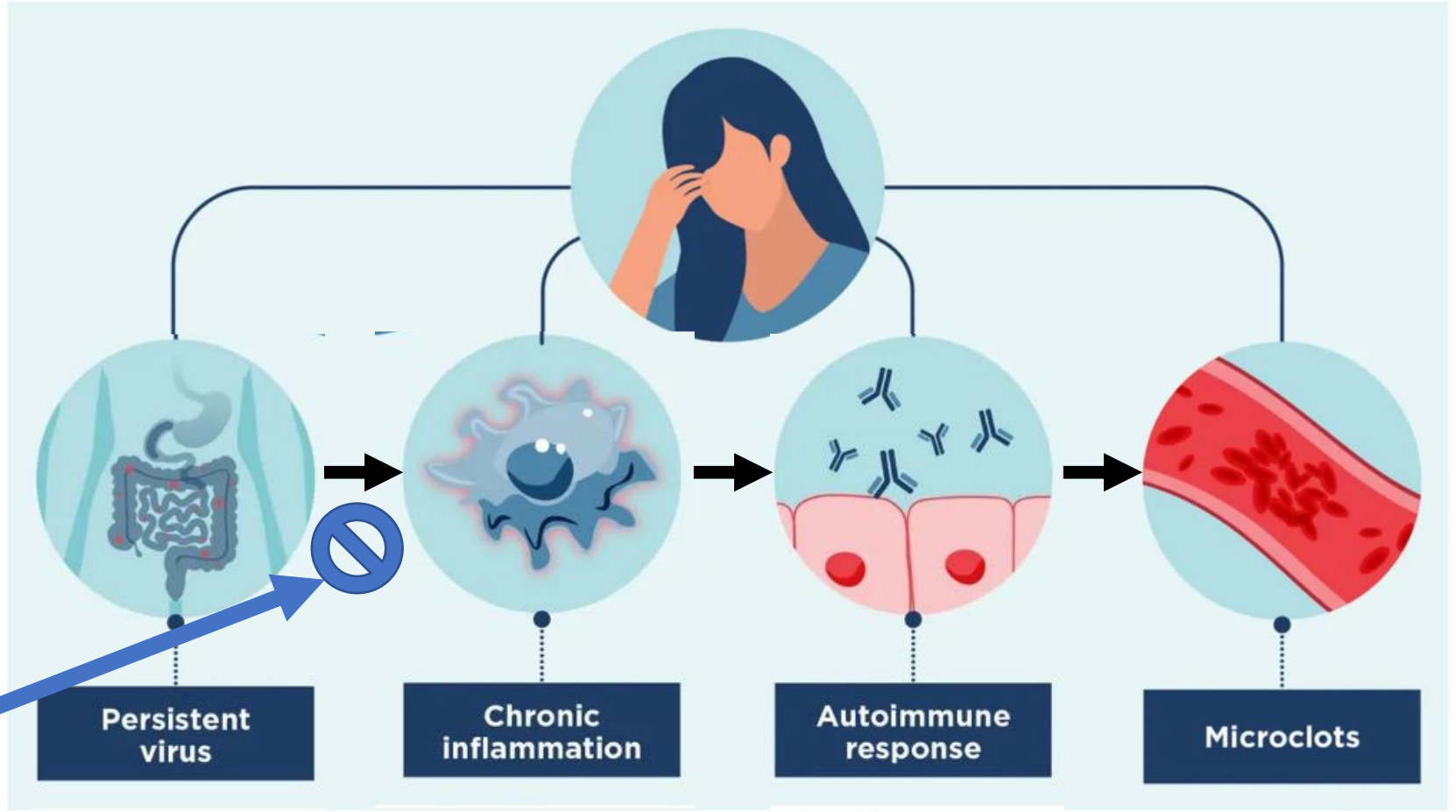


The spike protein has the capability to change soluble clotting proteins to insoluble little microclots

Blocking Zonulin to Stop The March Toward Chronic Inflammation Onset (Experimental Medicine At Work)

Zonulin Inhibitor
Larazotide Acetate
(AKA AT1001)





Larazotide Expedites GI Symptoms Resolution and Spike Clearance in MIS-C

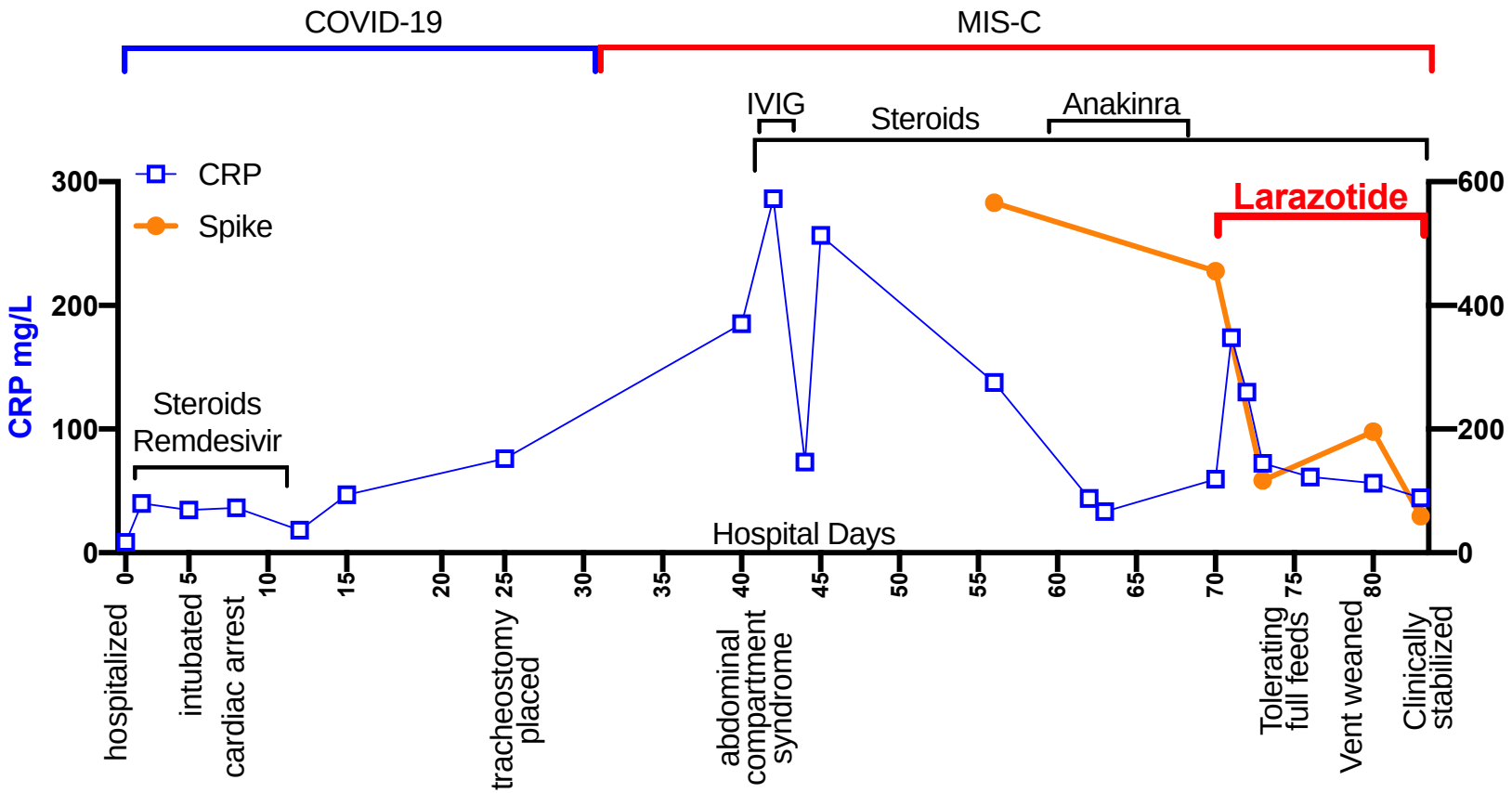
- FDA approval for compassionate use
- IRB approval
- Clinical team approval
- Patient/family consent
- Larazotide 10mcg/kg (max 500mcg/dose) four times daily x 21 days

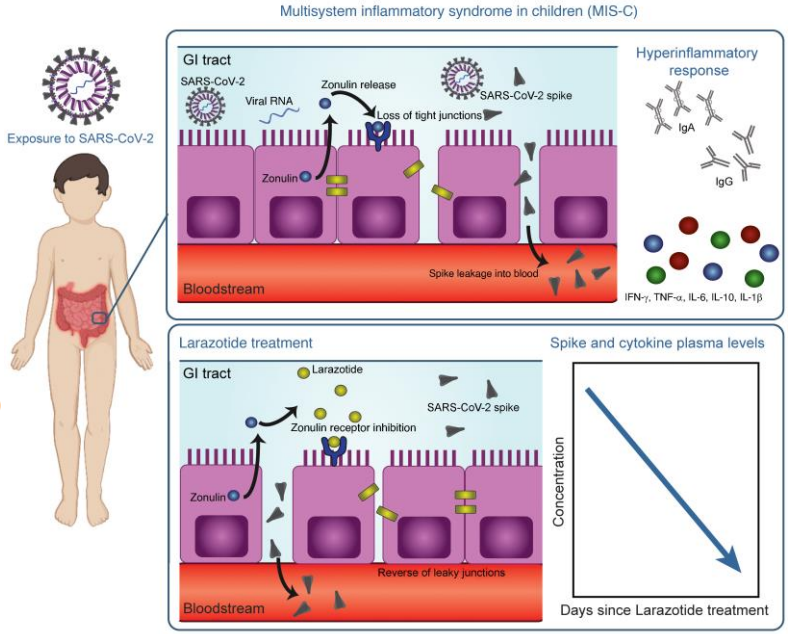
- Larazotide treatment associated with:
- Significantly shorter time to resolution of GI symptoms
- Significantly faster clearance of Spike antigenemia
- *Trend* toward improvement in:
  - Length of stay
  - Fever duration
- No escalation of care in larazotide-treated group
- **Currently in phase 2 DBPC trial**

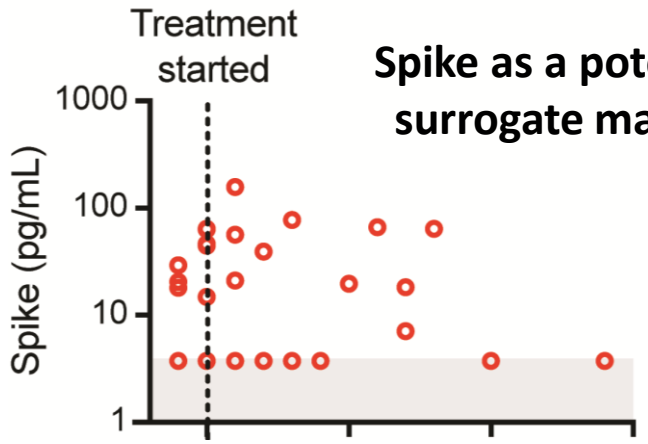
Patient characteristics	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**
Age (years)	17	3	6	9
Sex	F	F	F	M
Race, Ethnicity	White Non-Hispanic	Asian Non-Hispanic	Black Non-Hispanic	White Non-Hispanic
SARS-CoV-2 RT-PCR or antibody positive	Yes	Yes	Yes	Yes
SARS-CoV-2 Spike antigenemia	Yes	Yes	Yes	Yes
Cardiac involvement	None	None	coronary aneurysm	Mild dilation of coronary artery
Gastrointestinal involvement	adbominal pain, diarrhea	abdominal pain, vomiting, diarrhea	abdominal pain, vomiting	abdominal pain, vomiting, diarrhea
Highest level of care	Ward	Ward	PICU	Ward
Treatment with approved, expanded use of larazotide	Yes	Yes	Yes	Yes

MIS-C with larazotide add-on vs historic controls	Larazotide treated (n=4)	Historic controls (n=22)	*P value*
Age, mean years (range)	8.8 (3-17)	9.1 (1-21)	*ns*
LOS, mean days (range)	4.5 (3-7)	7 (2-16)	*ns*
Escalation of care, number	0	3	*ns*
Fever duration, post steroids/IVIG, mean days (range)	0.8 (0-2)	1.6 (0-6)	*ns*
Time to resolution of GI symptoms, mean days (range)	**2.3 (1-3)**	**6.7 (1-17)**	***0.03***
Time to first clearance of Spike, median days (95% CI)	**1 (1-12)**	**10 (6-190)**	***0.04***

Spike As A Potential Surrogate Marker For Larazotide Efficacy Signal







Yonker, Gilboa, Ogata et al, JCI, July 2021

AT1001 for the Treatment of Long COVID

ClinicalTrials.gov ID NCT05747534
Sponsor Massachusetts General Hospital
Information provided by Lael Yonker, M.D., Massachusetts General Hospital (Responsible Party)
Last Update Posted 2023-06-22

Study Overview

Brief Summary:
The primary objective of this study is to evaluate the safety and efficacy of **Larazotide** (AT1001) versus placebo in children and young adults 7 to ≤21 years of age who present with symptoms of Long COVID in the presence of SARS-CoV-2 antigenemia. AT1001 (n=32) or placebo (n=16) will be administered orally four times a day (QID) for 21 days.

Detailed Description:
This is a Phase 2a randomized, double-blind, placebo-controlled clinical trial to evaluate the safety and efficacy of AT1001 for use in children and young adults with symptoms of Long COVID in the setting of SARS-CoV-2 antigenemia. Eligible participants (N= 48) will be treated with AT1001 (n= 32) or matching placebo (n= 16) orally four times a day (QID) for 21 days. The study will consist of three phases:

I. Baseline Screening Visit

After obtaining informed consent and before starting treatment with Larazotide or placebo, an initial study visit will be conducted in person to confirm subject eligibility. Subjects will be asked complete a...

+ Show more

OFFICIAL TITLE

Phase 2a Randomized, Double-Blind, Placebo-Controlled, Multi-Center Study to Evaluate the Safety and Efficacy of **Larazotide** (AT1001) for the Treatment of **Long COVID** in Children and Young Adults

STUDY START (ACTUAL)
2023-05-31

PRIMARY COMPLETION (ESTIMATED)
2025-03-31

STUDY COMPLETION (ESTIMATED)
2026-03-31

ENROLLMENT (ESTIMATED)
48

STUDY TYPE
Interventional

PHASE
Phase 2

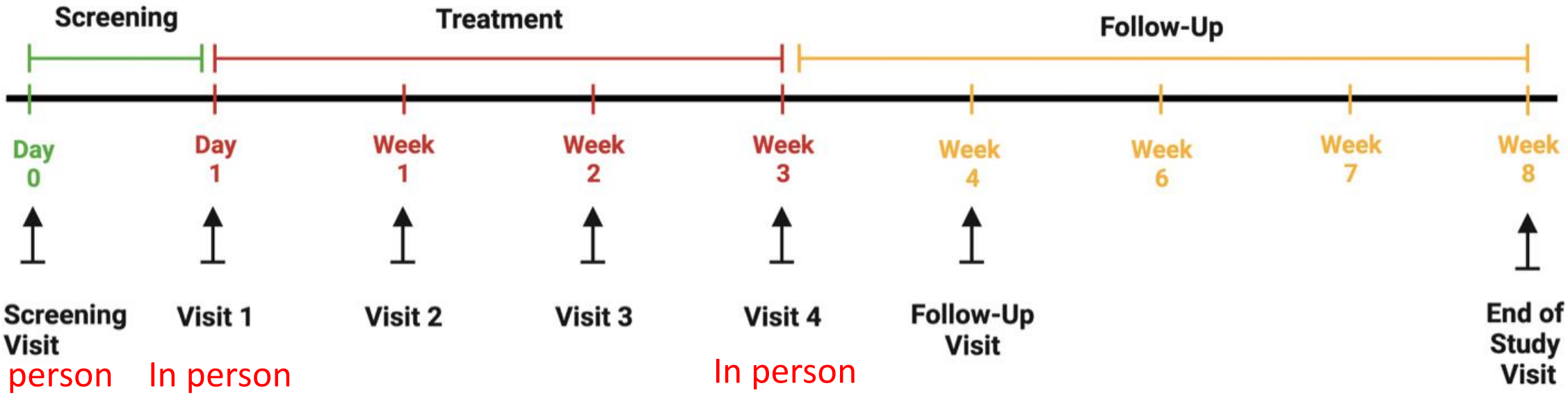
OTHER STUDY ID NUMBERS

Long COVID Clinical Trial: Targeted Population

Spike/S1 biomarkers for patients' stratification and targeted intervention

Inclusion Criteria	Exclusion Criteria
Age 7 to ≤21 years	Age ≤6 years or >22 years at time of enrollment
History of SARS-CoV-2 infection, documented by positive PCR and/or antigen test	Pregnancy and/or lactation
SARS-CoV-2 Antigenemia, defined as any detectable presence of full-length spike protein and/or Spike S1 subunit in plasma	Female participant of childbearing age unwilling to use an acceptable method of birth control for the duration of the study
Ongoing, worsening, new, or recurrent symptoms present ≥4 weeks after SARS-CoV-2 infection. Symptoms include but are not limited to fatigue, malaise, headache, cognitive impairment, neuropsychiatric symptoms, decreased exercise tolerance, post exertional malaise, dyspnea, cough, chest pain, palpitations, tachycardia, gastrointestinal symptoms, musculoskeletal symptoms, fever, lightheadedness, insomnia and other sleep disturbances, anosmia or dysgeusia, pain, paresthesia, menstrual cycle irregularities, erectile dysfunction.	Inability to tolerate drug
	Unstable medical conditions or significant co-morbid disease that, by the investigator's determination would make the participant unsuitable for enrollment
	Participation in any other clinical investigation using an experimental drug within 30 days prior to screening
	Intent to participate in another clinical study while participating in this clinical trial
	Blood/plasma donation and or blood loss greater than 400 mL within 90 days, or greater than 200 mL within 30 days prior to screening
	Known hypersensitivity to any of the formulation components of AT1001.
	Abnormal baseline liver function as indicated by AST or ALT ≥3 times the upper limit of normal (ULN), or direct bilirubin ≥2x ULN for age
	Abnormal baseline renal function, defined as glomerular filtration rate ≤50 mL/min/1.73m^2

Long COVID Clinical Trial: Study Design



- 48 participants (N= 48) will be enrolled: study drug 4 times/day x 21 days
- 32 treated with Larazotide
- 16 placebo

Acknowledgments

MGH Cidar Sinai Wyss Institute

- Lael Yonker
- Rachel Bloom
- Brittany Boribong
- Abigail Kane
- Serena Rossi
- Aisha Seardf

• Moshe Arditi's Lab • David Walt's Lab

Funding

Mass General Brigham
Mass General for Children

POLYBIO

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA
NIH National Heart, Lung, and Blood Institute

Executive Committee on RESEARCH | Fostering Innovation at MGH