PAASE Webinar Series

SARS-CoV2: knowns and unknowns in this COVID-19 pandemic

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Image Credit: NIAID - RML

Conflicts of Interest: None

Disclaimer:

Views are my own and should not be construed as an official statement from NCI/NIH.

Goals of this webinar

- Why is this COVID-19 pandemic difficult to contain?
- What makes SARS-CoV2 different? What can we do about it?
- Questions for further research

“To know that we know *what we know*,
and that we do not know *what we do not know*,
that is true knowledge.”
-Copernicus

Epidemiological Comparison of Respiratory Viral Infections

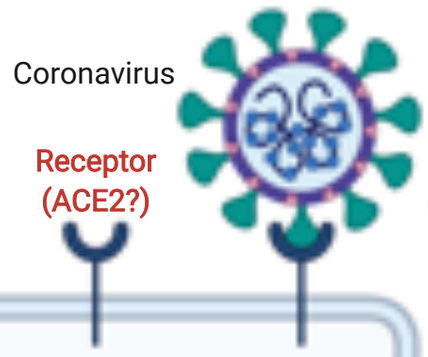
Disease	Flu	COVID-19	SARS	MERS
Disease Causing Pathogen	 Influenza virus	 SARS-CoV-2	 SARS-CoV	 MERS-CoV
R_0 Basic Reproductive Number	1.3	2.0 - 2.5 *	3	0.3 - 0.8
CFR Case Fatality Rate	0.05 - 0.1%	~3.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	4 - 14 days *	2 - 7 days	6 days
Hospitalization Rate	2%	~19% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%
Annual Infected (global)	~ 1 billion	N/A (ongoing)	8098 (in 2003)	420
Annual Infected (US)	10 - 45 million	N/A (ongoing)	8 (in 2003)	2 (in 2014)
Annual Deaths (US)	10,000 - 61,000	N/A (ongoing)	None (since 2003)	None (since 2014)

* COVID-19 data as of March 2020.

SARS-CoV2: Severe Acute Respiratory Syndrome - Coronavirus 2
4 other CoVs endemic to humans (common cold)

SARS-CoV2

single strand RNA virus
positive-sense

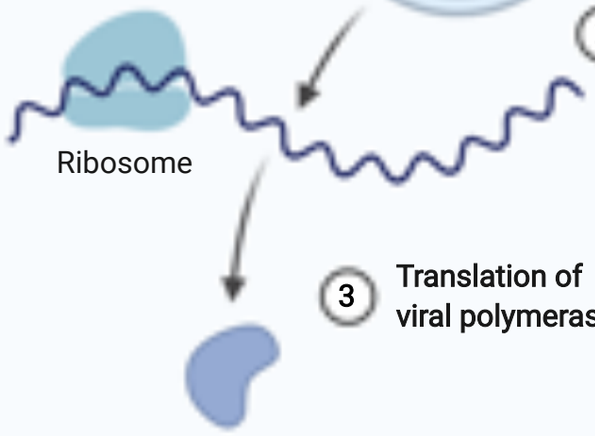


Requires protease (TMPRSS)
to prime S protein for entry

1 Binding and viral entry

Cytoplasm

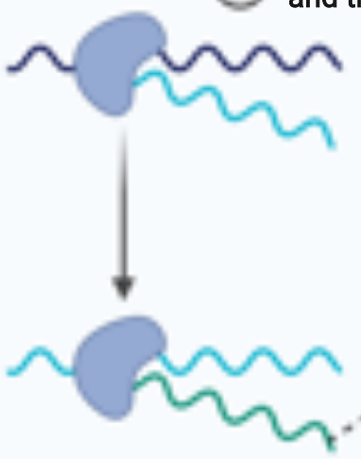
2 Release of viral genome



3 Translation of
viral polymerase

4 RNA replication
and transcription

ExoN for proof-reading



Replicated genomic RNA

Subgenomic transcripts

Nucleocapsid (N)

Spike (S)

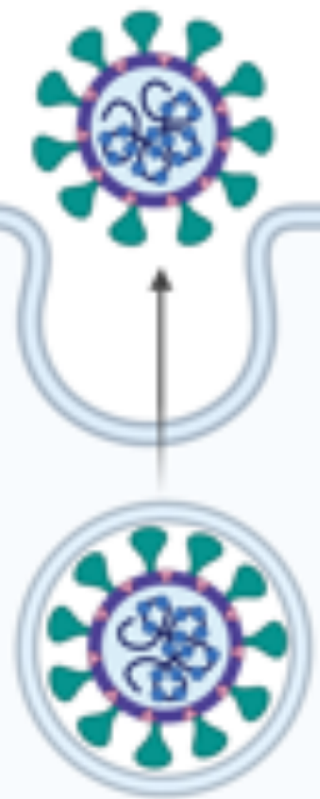
Membrane (M)

Envelope (E)

5 Translation of
viral proteins

6 Structural proteins combine
with nucleocapsid

7 Virion formation



8 Exocytosis

Viral shedding

Host Cells

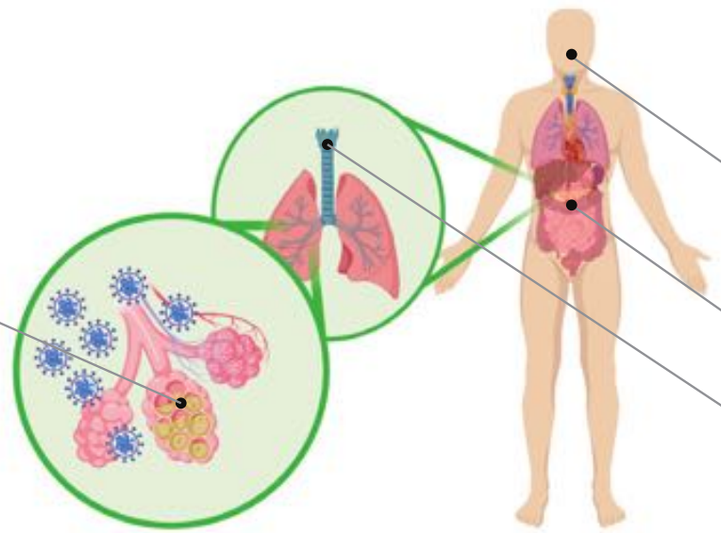
(tentative list; number of cells per person)

Type I & II pneumocytes (~10^{11} cells)

Alveolar macrophage (~10^{10} cells)

Mucous cell in nasal cavity (~10^{9} cells)

Host cell volume: ~10^{3} μm^3 = 10^{3} fL



virions not to scale

Concentration

maximal observed values following diagnosis

(Woelfel et al. 2020; Kim et al. 2020; Pan et al. 2020)

Nasopharynx: 10^{6}-10^{9} RNAs/swab

Throat: 10^{4}-10^{8} RNAs/swab

Stool: 10^{4}-10^{8} RNAs/g

Sputum: 10^{6}-10^{11} RNAs/mL

RNA counts can markedly overestimate infectious virions

YM Bar-On et al. eLife 2020

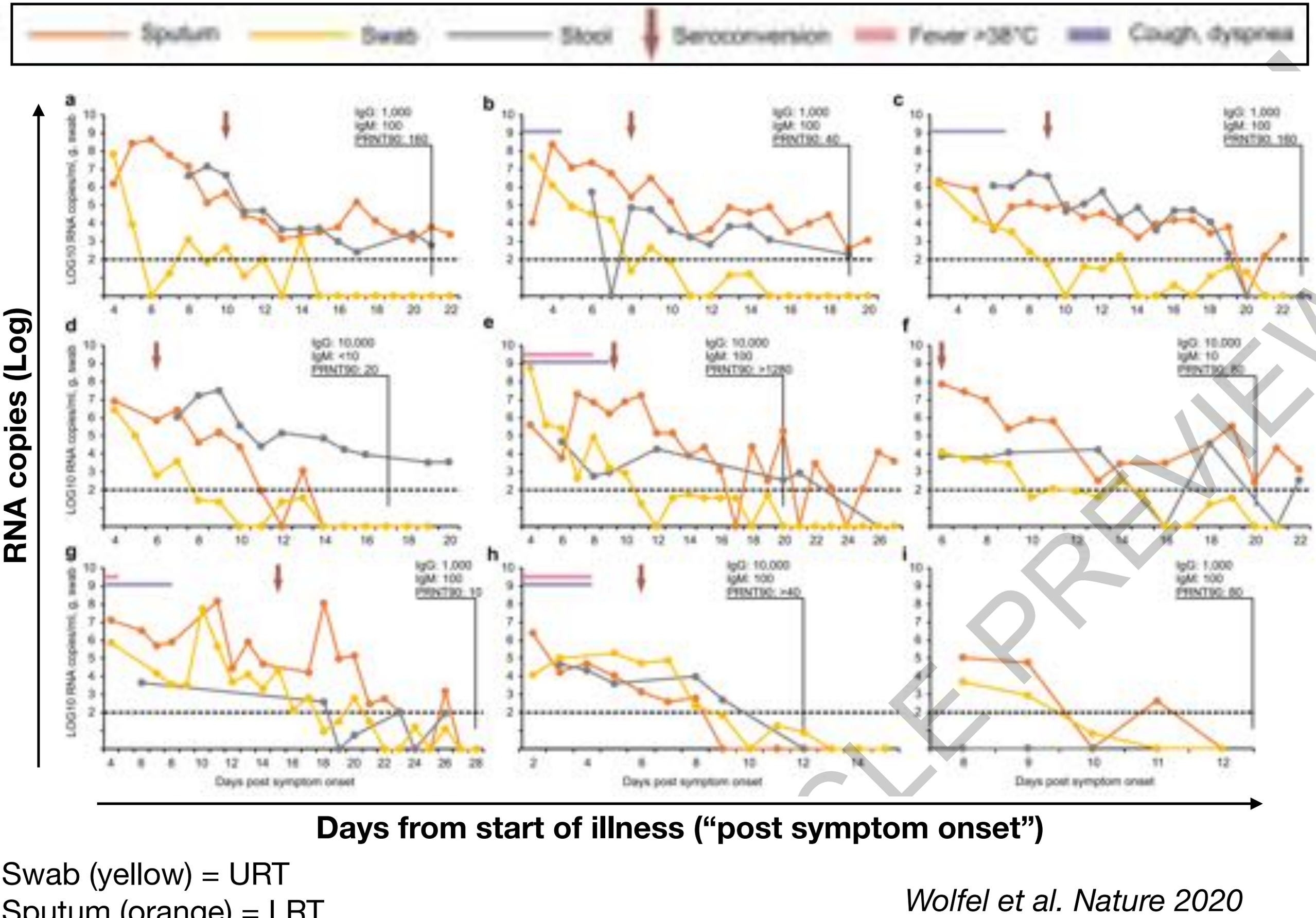
Death of Type II pneumocytes during COVID-19
—> Loss of air exchange and fluid leakage into lungs

URT = upper respiratory tract (throat, nasopharynx)
LRT = lower respiratory tract (lungs)

Natural History

- From exposure to onset of symptoms: 4-14 days
- Illness duration
  - Mild cases: 2 weeks
  - Severe cases: 3-6 weeks
  - Fatalities: 2-8 weeks
- ***What is the viral load kinetics (replication, shedding) during the course of the illness?***

Viral Load Kinetics during Mild COVID-19 Illness



Swab (yellow) = URT
Sputum (orange) = LRT

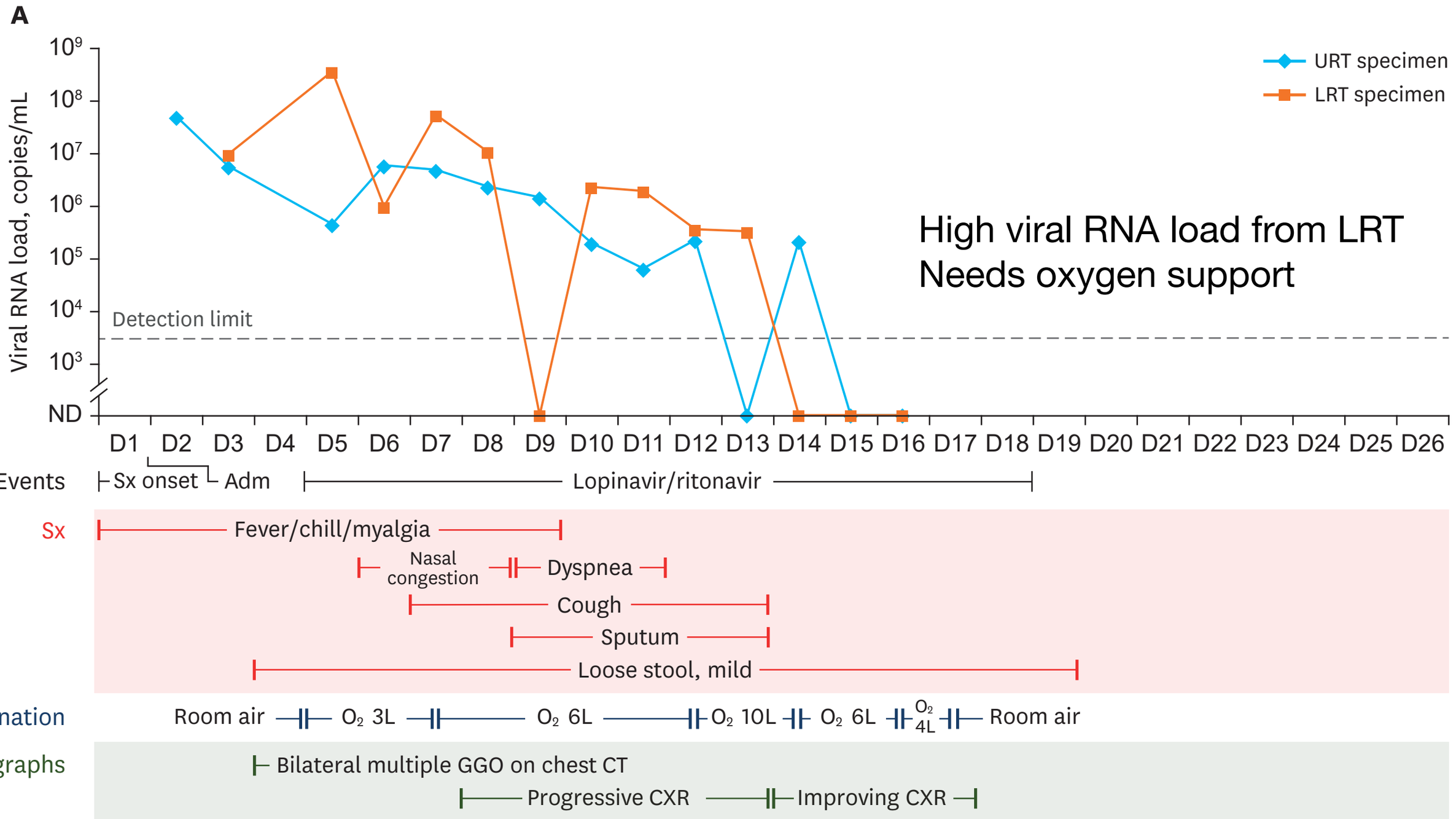
Wolfel et al. Nature 2020

Mild COVID-19 Illness

(Wolfel et al. Nature 2020)

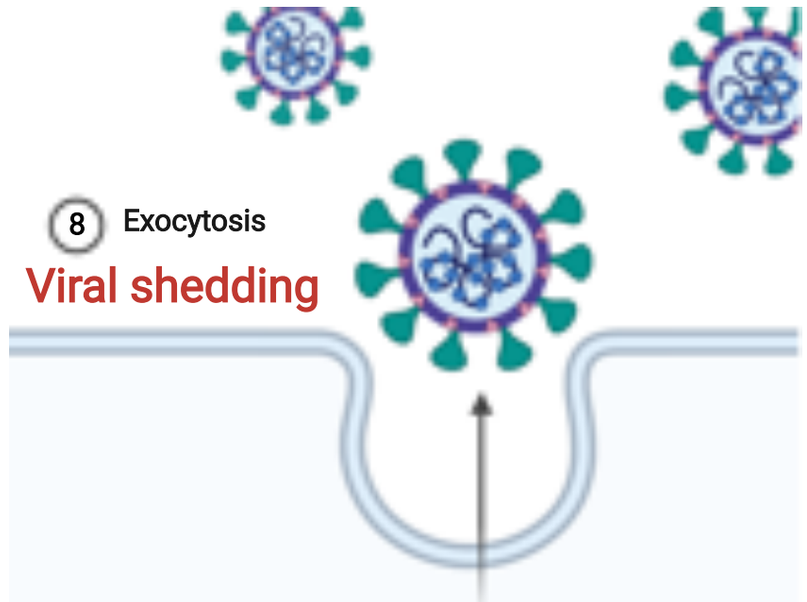
- RT-PCR (reverse transcription and polymerase reaction)
    - All swabs day 1-5 were positive
    - After day 5, ~40% detection rate
    - Last positive swab @ day 28
        - none of urine and serum samples were positive
- Isolation of infectious virus (can grow on cells): no virus isolated after day 7
- ***Majority of patients are beyond shedding peak in URT at time of 1st testing***
- Seroconversion in 50% of patients by day 7, all by day 14
- All patients showed neutralizing Ab; titer did not correlate with clinical course
- Neutralizing Ab - cross-reactivity with 4 endemic CoVs

Viral Load Kinetics during Moderate COVID-19 Illness



JY Kim et al. J Korean Med Sci 2020

Viral shedding



- Patient is spreading virus that can still grow
  - in contrast to detecting bits of virus that have been cleared/non-viable ex. RNA genome

- Mild/moderate: 7-12 days (day 7 for mild, Wolfel paper)

- Severe: > 2 weeks

	SARS-CoV2	SARS-CoV (2003)
Peak viral RNA	before day 5	day 7-10
Copy #	~7 x10^8 copies/swab	5 x10^5 copies
Sites of replication	Throat, Lung	Lung

Asymptomatic & Presymptomatic Viral Shedding

- True asymptomatic infection rate can only be known if serology is done in population

- Spreading virus 2-8 days before onset of symptoms

Asymptomatic & Presymptomatic Viral Shedding

- “Cluster F: A woman aged 58 years (patient F1) attended a singing class on February 27, where she was exposed to a patient with confirmed COVID-19. She attended a church service on March 1, where she likely infected a woman aged 26 years (patient F2) and a man aged 29 years (patient F3), both of whom sat one row behind her. Patient F1 developed symptoms on March 3, and patients F2 and F3 developed symptoms on March 3 and March 5, respectively.”

CDC MMWR,“Presymptomatic Transmission of SARS-CoV-2 — Singapore, January 23–March 16, 2020” , published April 1, 2020

	Dates of likely transmission, symptom onset, and other exposure								
	Feb			Mar					
Cluster F	27	28	29	1	2	3	4	5	Symptoms
Patient F1 ★									Sore throat, blocked nose
Patient F2									Cough
Patient F3									Cough, runny nose, sore throat, myalgia

Day of exposure

COVID-19 in Children

	Age <18
Total population of USA	22%
COVID-19 cases Feb 12-Apr 2, 2020	1.7%

- **Milder symptoms**
- **Less hospitalizations /ICU**
- **Except: Infants, children with underlying conditions (asthma, etc.)**

CDC MMWR April 6, 2020

The conundrum of pediatric patients

- Do recent immunizations protect against COVID-19?

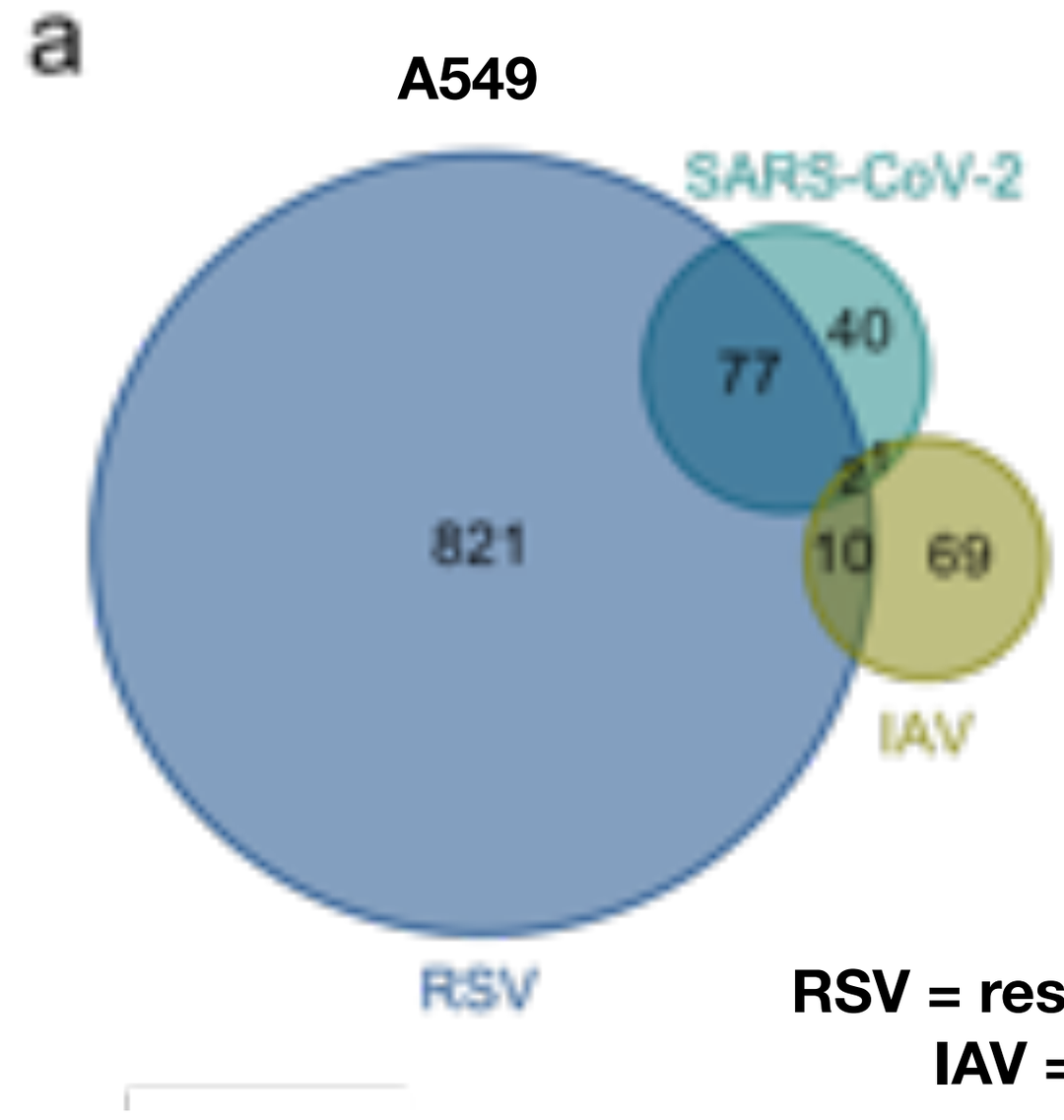
- Children and the common cold - does recent infection with CoVs causing colds have a protective effect?

- Exception: Children born prematurely have worse outcomes (lung development?)

Why is COVID-19 so deadly in some patients?

- Host response to virus

Transcriptome analysis of infected lung cells *in vitro* and *in vivo* (ferrets)

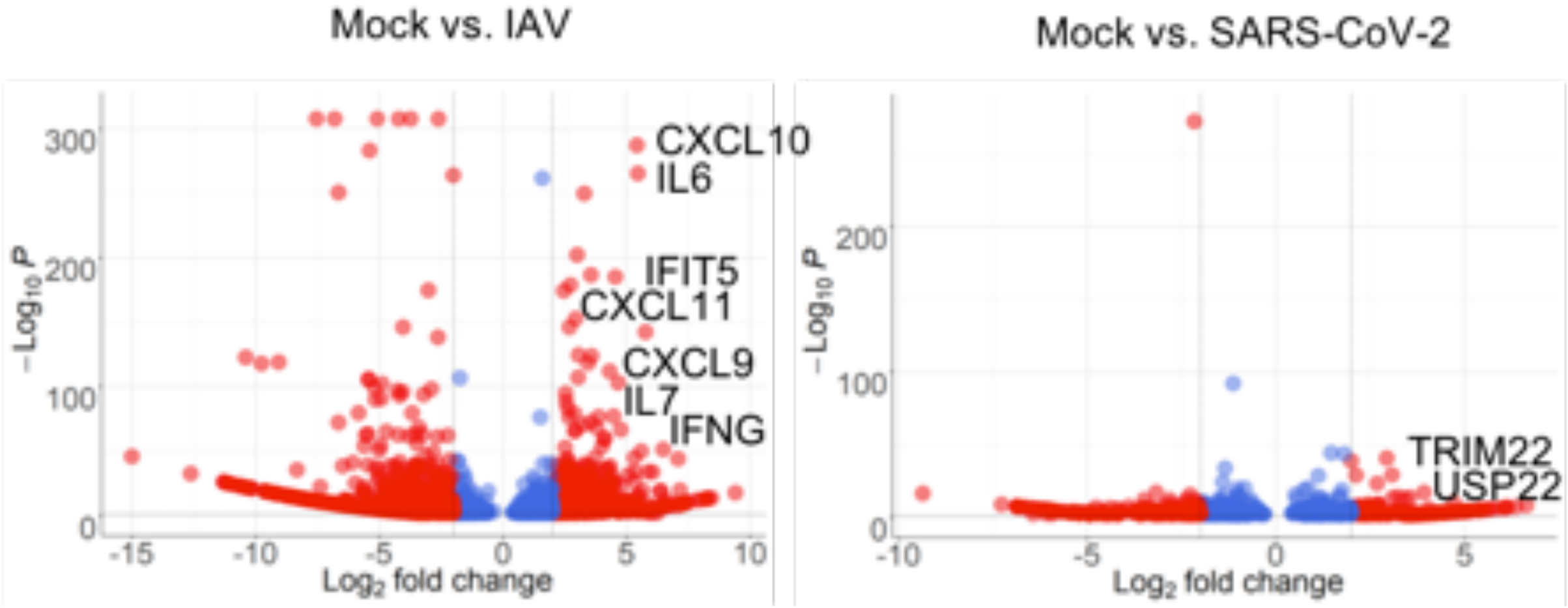


RSV = respiratory syncytial virus
IAV = influenza A virus

Also done on NHBE cells
(normal human bronchial epithelial cells from 79 yo Caucasian female)

Blanco-Melo D, et al. (tenOever Lab, Mt. Sinai, NY), preprint
https://www.biorxiv.org/content/10.1101/2020.03.24.004655v1

Transcriptome analysis of infected lung cells *in vitro* and *in vivo* (ferrets)



Trachea samples from ferrets
For RNA-Seq

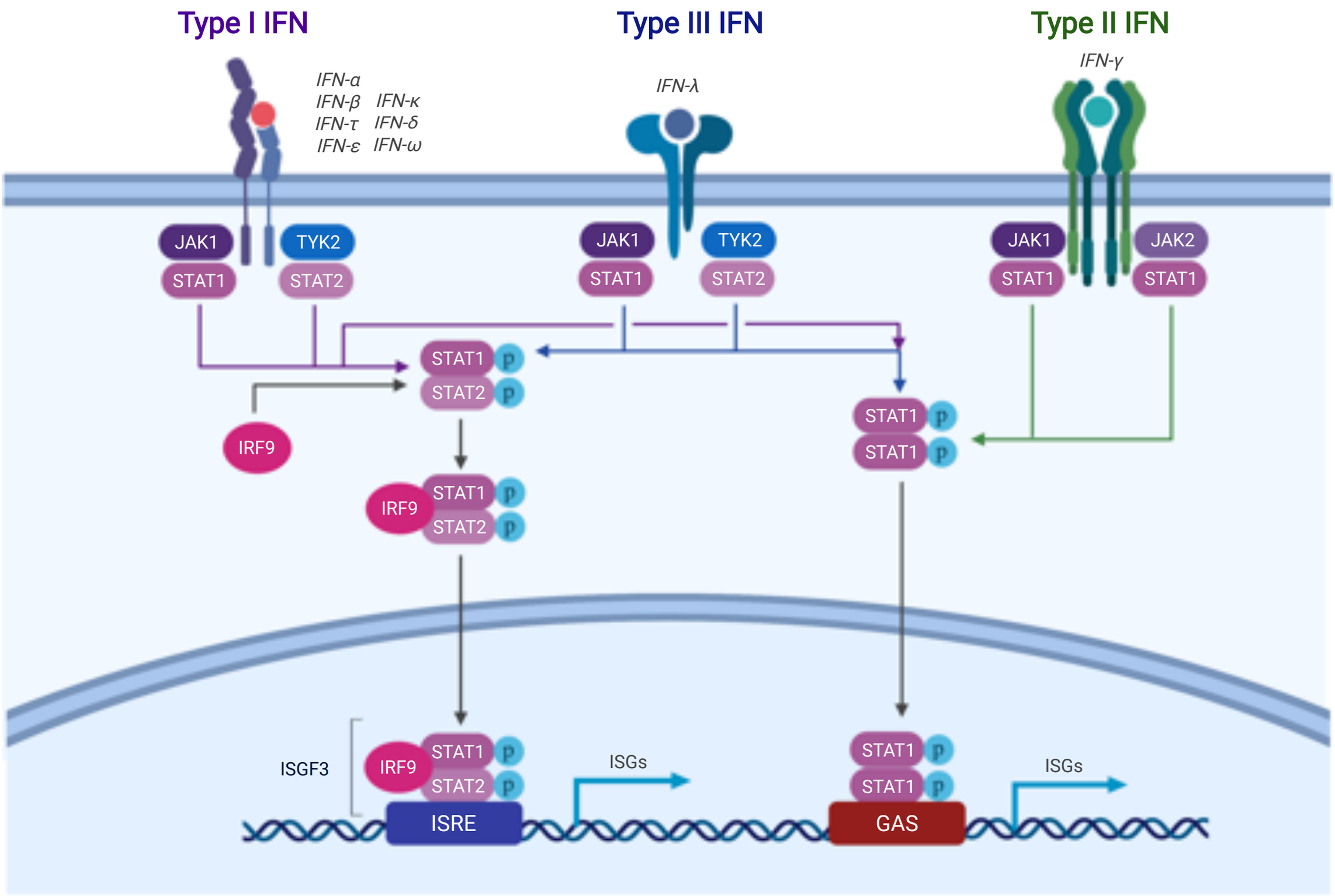
Also induction of the following cytokines

- **EDN1 (Endothelin 1)**
  - **Also increased in children with asthma**
  - **Increased during cigarette smoking**
- **TNFSF15**

Muted immune response, including absence of induction of Type I and III interferons in SARS-CoV2

Blanco-Melo D, et al. (tenOever Lab, Mt. Sinai, NY), preprint
https://www.biorxiv.org/content/10.1101/2020.03.24.004655v1

Interferons activate signaling cascades to mount an antiviral response



Experimental therapies and Clinical Trials

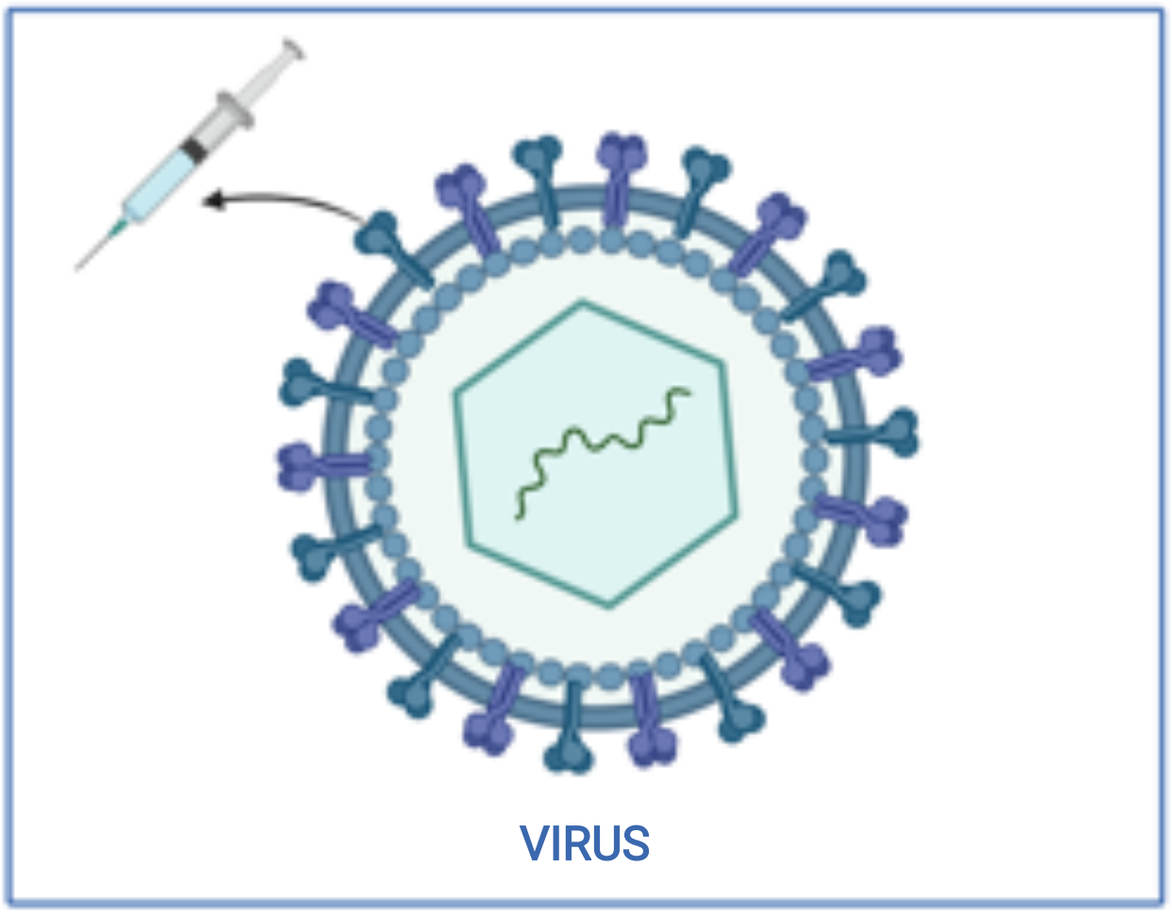
- Hydroxychloroquine + Azithromycin
    - Anecdotes of efficacy
    - Mixed results in small trials
    - Need large, randomized, controlled trials
- Remdesivir (Gilead and NIAID/NIH)
    - Promising preclinical data (Baric Lab, Denison Lab)
- Lopinavir-ritonavir
    - Not effective?
- Anti-IL6 (Tocilizumab)
- Convalescent Plasma
    - From patients who have developed immunity after illness
- Etc.

www.ClinicalTrials.gov
WHO Solidarity Trial

Approaches to Viral Vaccine Development

a. Live attenuated

b. Whole inactivated



i. Recombinant viral vectors

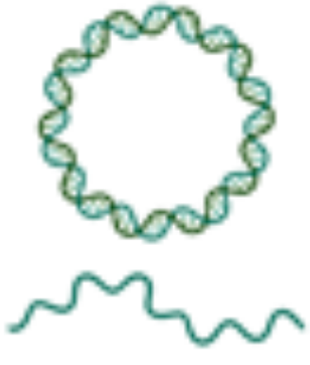
h. Recombinant bacterial vectors

c. Split inactivated

d. Synthetic peptides

e. Virus-like particles

f. DNA or RNA



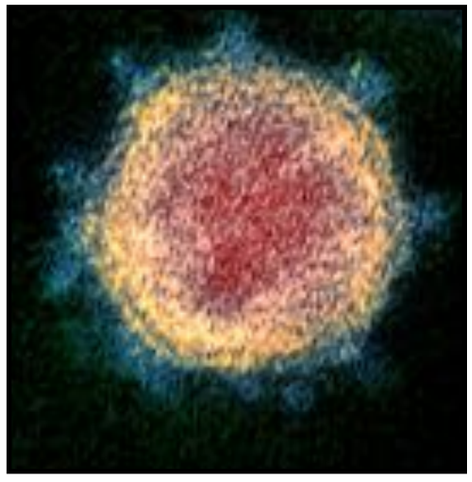
g. Recombinant subunits

e. Novavax starting Phase I in mid-May, results by end of June 2020
f. Moderna and NIAID/NIH started Phase I in March 2020

Why is SARS-CoV2 difficult to contain?

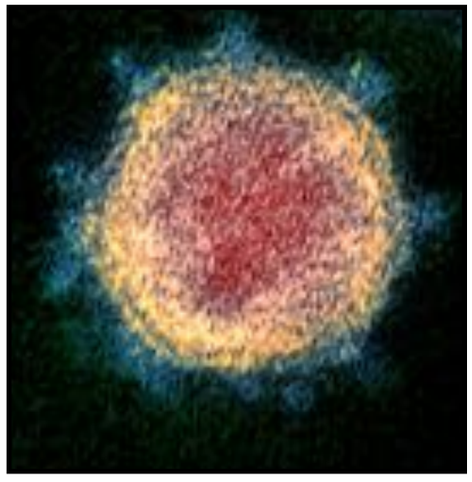
It is highly transmissible and replicates efficiently.

- Clues:
  - ◉ efficient viral replication in throat (1,000x more than SARS-CoV) and lungs, then expelled via droplets through sneezing, coughing, talking, singing…); evidence for airborne
  - ◉ peak shedding prior to day 5 (including 2-8 days presymptomatic); also asymptomatic spreaders
  - ◉ persistence of viral particles in air, surfaces, etc. (3 hours half-life)
  - ◉ insertion of poly basic furin-type cleavage site -> faster entry into cell (?)
  - ◉ no/weak interferon response to virus -> host unable to clear virus



Why is SARS-CoV2 difficult to contain?
It is highly transmissible and efficient.

- Implications:
  - ◉ Diagnostic testing within first 3 days of symptoms (peak of shedding)
  - ◉ PPE for everyone caring for COVID+ patients; mask for patients
  - ◉ After discharge, patients need to continue self-isolation (moderately ill patients may continue to shed virus)
  - ◉ Disinfection of hospitals, nursing facilities, etc.
  - ◉ Assume everyone is COVID+ (asymptomatic)
    - ▸ wear mask, social distancing (6 ft vs. 25 ft.)



So many questions…

- Why do children have milder COVID-19?
  - Endothelin 1 gene expression in children?
- What conditions or co-morbidities predispose host to weak interferon response? (elderly, hypertension, diabetes, obesity/metabolic syndrome?)
  - Ability to mount antiviral response
- What factors lead to COVID-19 complications? (lung damage, encephalitis, myocarditis?)
  - Inability to clear the virus?
  - Destructive inflammation?
- What is the intermediate host? Can we vaccinate them? (ex. in MERS, camels were vaccinated to mitigate transmission to humans)
- Animal models for continuing research to understand how virus works (ferrets? mouse model?)
- How can we end this pandemic? How do we break the transmission chain?
  - Dr. Jomar Rabajante's webinar from last week

What can we do about COVID-19?

- More diagnostic testing at earlier time point
- Support the front-liners (more PPE, please!)
- Accelerated pace of research: clinical trials, vaccine studies
    - Pivot one’s research/expertise to SARS-CoV2
    - Volunteer (study participant, etc.)
- Open access to reliable, reproducible, validated data

“No way of thinking or doing, however ancient, can be trusted without proof”

–Henry David Thoreau, “Walden”