NIAID Response to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Alan Embry, PhD

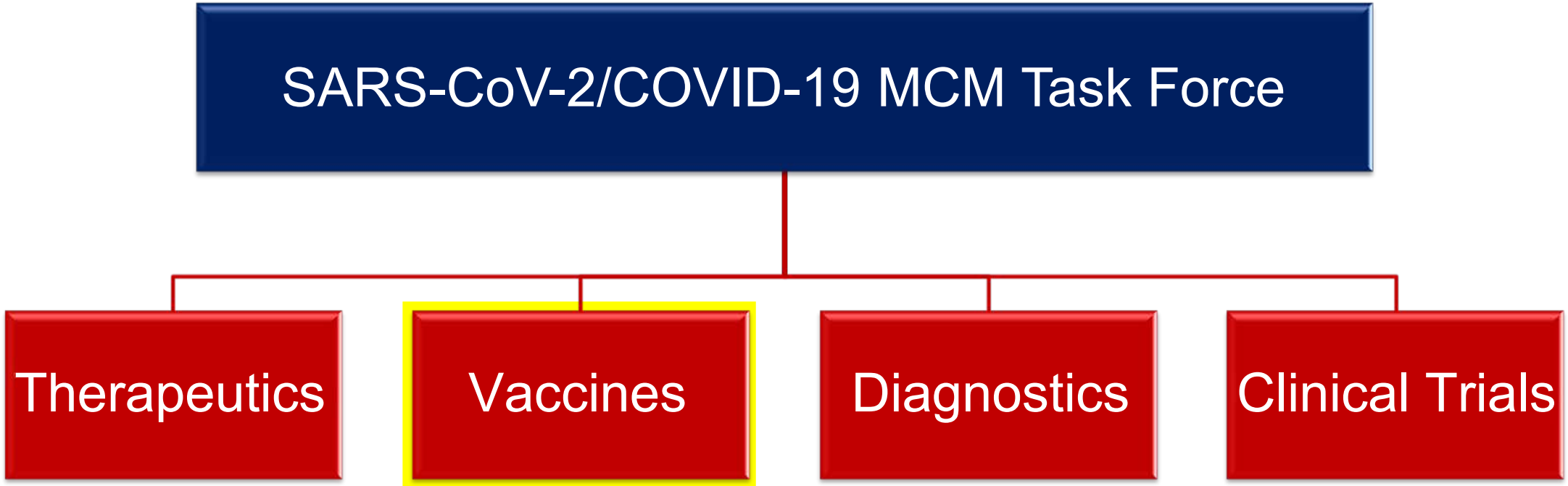
Chief, Respiratory Diseases Branch

Division of Microbiology & Infectious Diseases

NIAID, NIH, DHHS

NIH National Institute of Allergy and Infectious Diseases

SARS-CoV-2/COVID-19 Medical Countermeasures Task Force



NIAID Accelerating SARS-CoV-2 Research

- Improve understanding of SARS-CoV-2/COVID-19
- Evaluate potential cross-reactivity with existing SARS/MERS vaccine candidates (and antibodies)
- Develop SARS-CoV-2 vaccine candidates
- Provide resources to facilitate vaccine development

Current Funding Opportunities

- **Notice of Special Interest Regarding the Availability of Urgent Competitive Revisions for Research on the 2019 Novel Coronavirus (2019-nCoV)***
  - Improve understanding of 2019-nCoV
  - Development of medical countermeasures
  - Development of animal models

- **2020 NIAID Omnibus Broad Agency Announcement** solicits development of 2019-nCoV* vaccines, therapeutics and diagnostics

*SARS-CoV-2

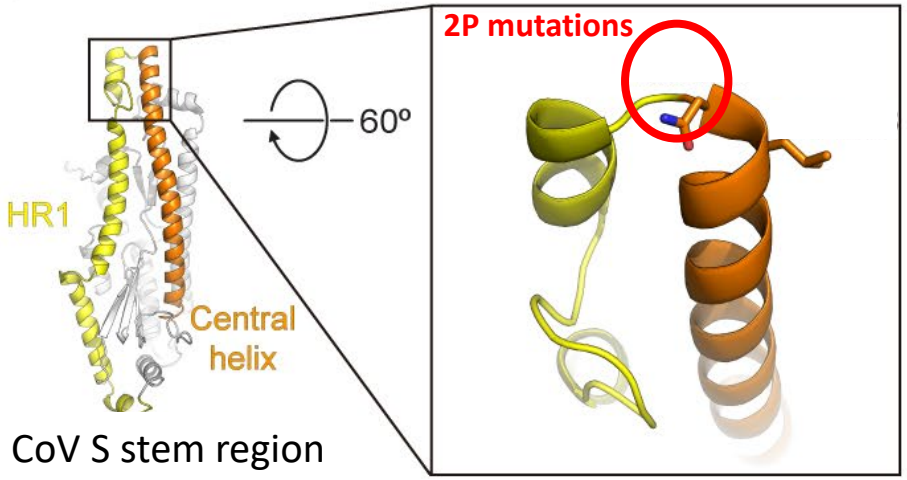
Sharing Samples and Reagents

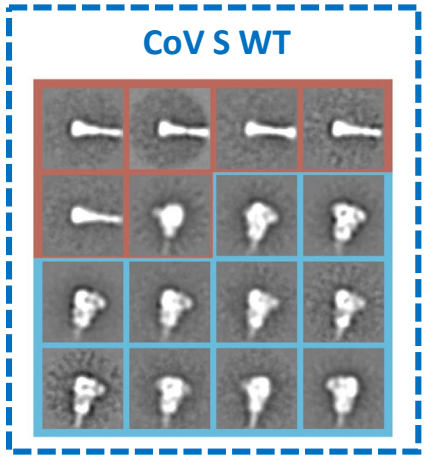
- Viral isolate from first U.S. patient available through BEI Resources (others soon)
- Patient samples as available (via USG sample sharing WG)
- Reagents including molecular clones, plasmids, pseudoviruses, recombinant protein in progress

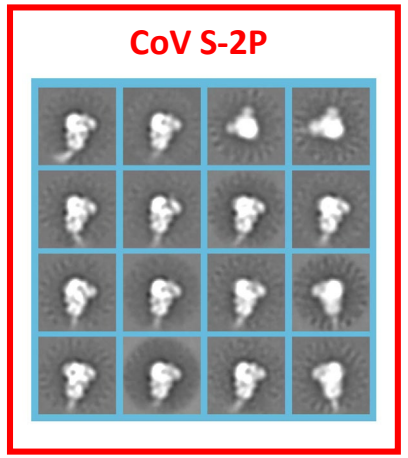
NIAID Vaccine Research Center
SARS-CoV-2 Vaccine Candidate

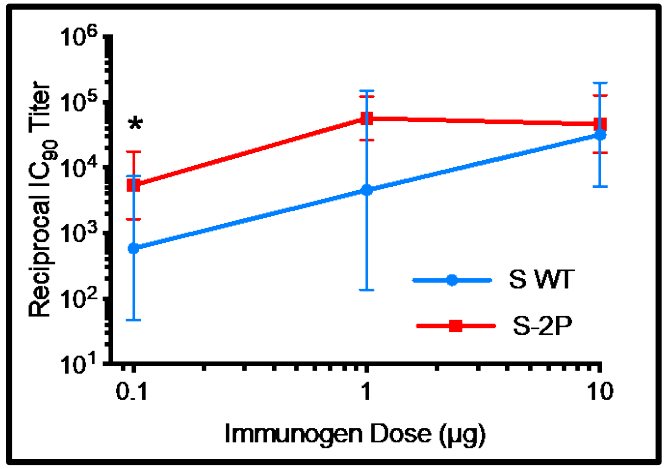
2 proline (2P) mutations at apex of central helix result in S protein locked in prefusion conformation.

Prefusion-stabilized CoV S-2P is more immunogenic than wild-type S.





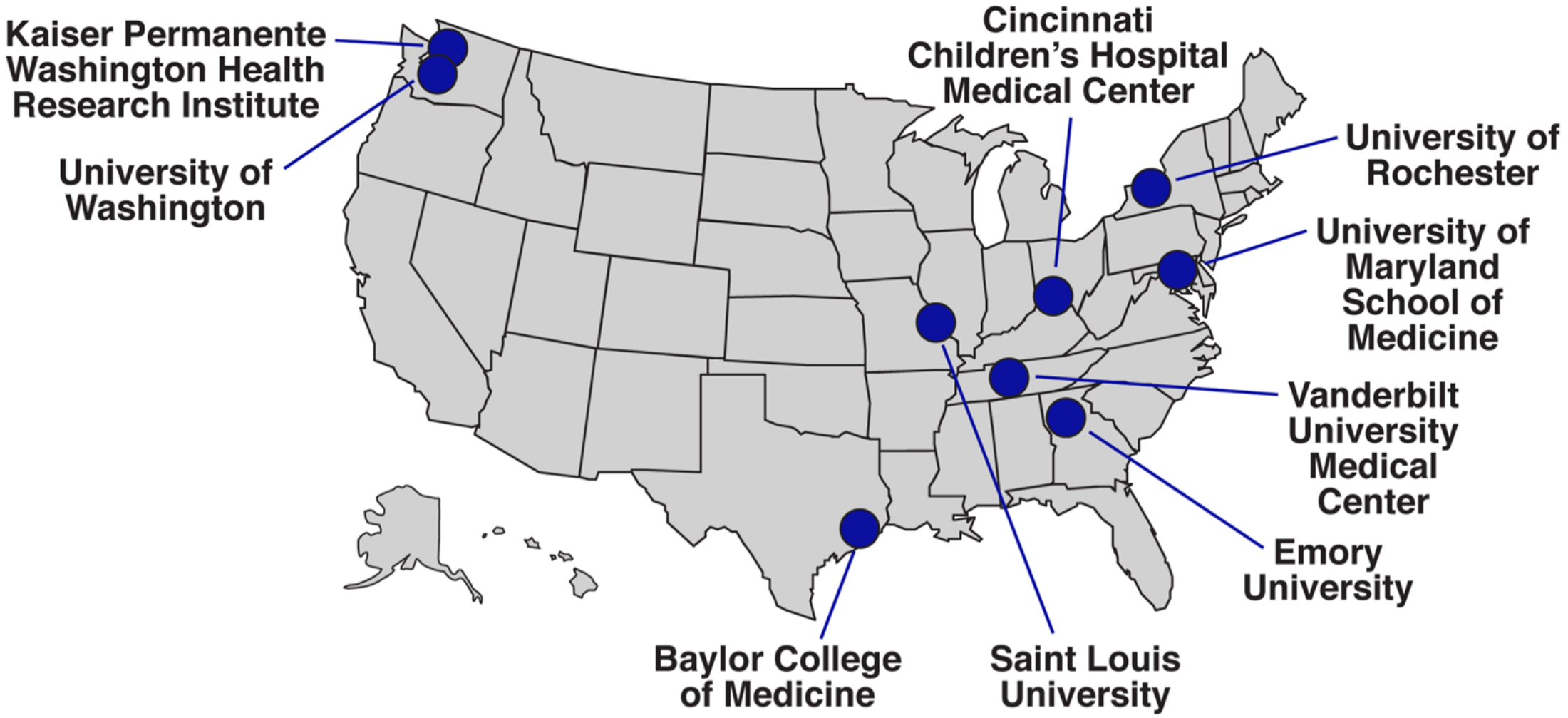




- **Partnership between the VRC/NIAID and Moderna**
- **GMP product expected in March 2020**

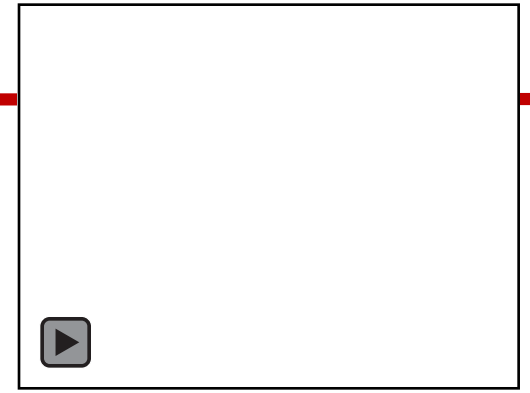
CoV S-2P designs in collaboration with Nianshuang Wang (McLellan Lab | UT Austin) | Wrapp, D*. Wang, N.*, et. al. submitted.
Pallesen, J*., Wang, N.*, Corbett, K*., et. al. PNAS. 2017.

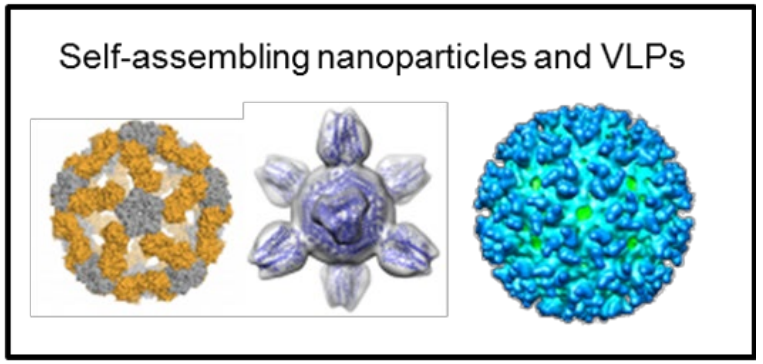
NIAID Infectious Disease Clinical Research Consortium

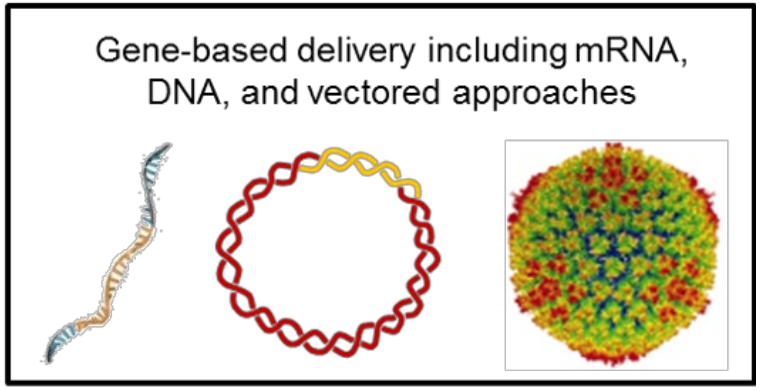


Ongoing Efforts Towards a Universal CoV Vaccine

- Optimize antigen design for potency and breadth
- Nanoparticles to display multiple CoV spike antigens and optimize immunogenicity
- Gene-based delivery for rapid response







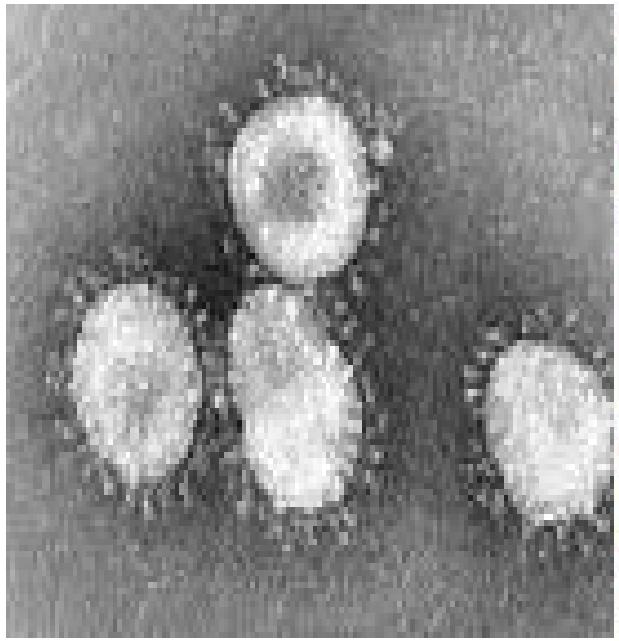
Slide Adapted from Barney Graham

Vaccine Development For Emerging Coronaviruses

- Coronaviruses have pandemic potential and novel coronaviruses will likely continue to emerge
- NIAID rapidly advancing development of SARS-CoV-2 vaccine candidates
- Global collaboration and transparency are critical

2019-nHCoV

Baric Laboratory
University of North Carolina



Outline

- **Introduction**
- **Emerging Coronaviruses**
  - SARS-CoV
  - Pre-pandemic SARS-like Bat-CoV
  - Drivers of Epidemic Disease Outbreaks
- **The Outbreak**
  - Origins
  - 2019-HCoV
    - Genome Organization and relatedness
  - Disease
- **Countermeasures**
  - Vaccines
- **Summary**

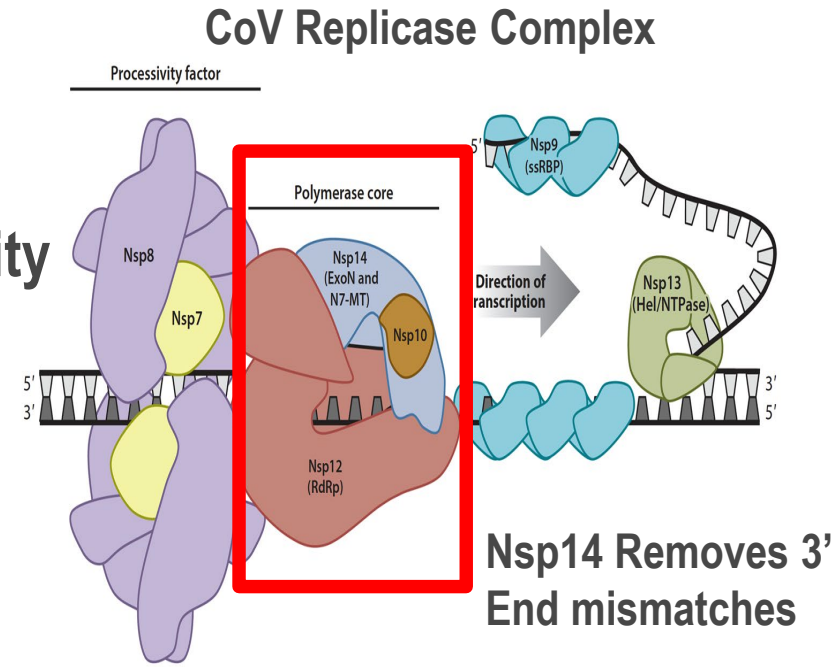
Timeline: Emerging Nidoviruses

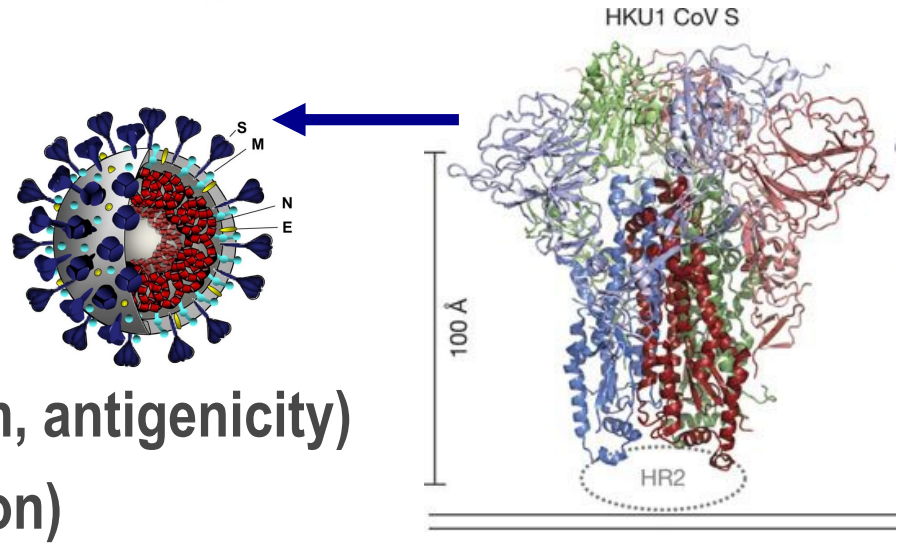
Virus	Species	Emergence	
HCoV-NL63	**Human**	500-800 years	
HCoV-229E	**Human**	200-300 years	
HCoV-OC43	**Human**	~120 years	
PEDV	Porcine	~25 years	← 2012 in US
PRRSV	Porcine	~25 years	
BCoV	Bovine	~20 years	
SARS-CoV	**Human**	~16 years	Accelerating Cross Species Movement
MERS-CoV	**Human**	~7 years	
SADS-CoV (HKU2)	Porcine	~2 years	
2019-nHCoV	Human	2 months	

Fu et al., 2018 Infect Genetic Evolution; Peiris JS et al., Lancet 2003, Huynh J et al., J.Virol 2012; Zaki AM et al., N Engl J Med. 2013, Mole B. Nature. 2013; Zhou P et al., Nature 2018

Drivers of CoV Evolution

- CoV Genome Size: 32Kb
- CoV Mutation Rate
  - 10^{-6} → Regulated Fidelity (nsp14: ExoN)
  - Environmental Change
    - Fidelity rates change
- High Rates RNA Recombination
  - 25% during mixed infections
  - Modular evolution
- Plastic Surface Glycoprotein
  - Tolerates high rates of mutation
  - Deletions and Insertions (tropism, antigenicity)
  - Recombination (modular evolution)

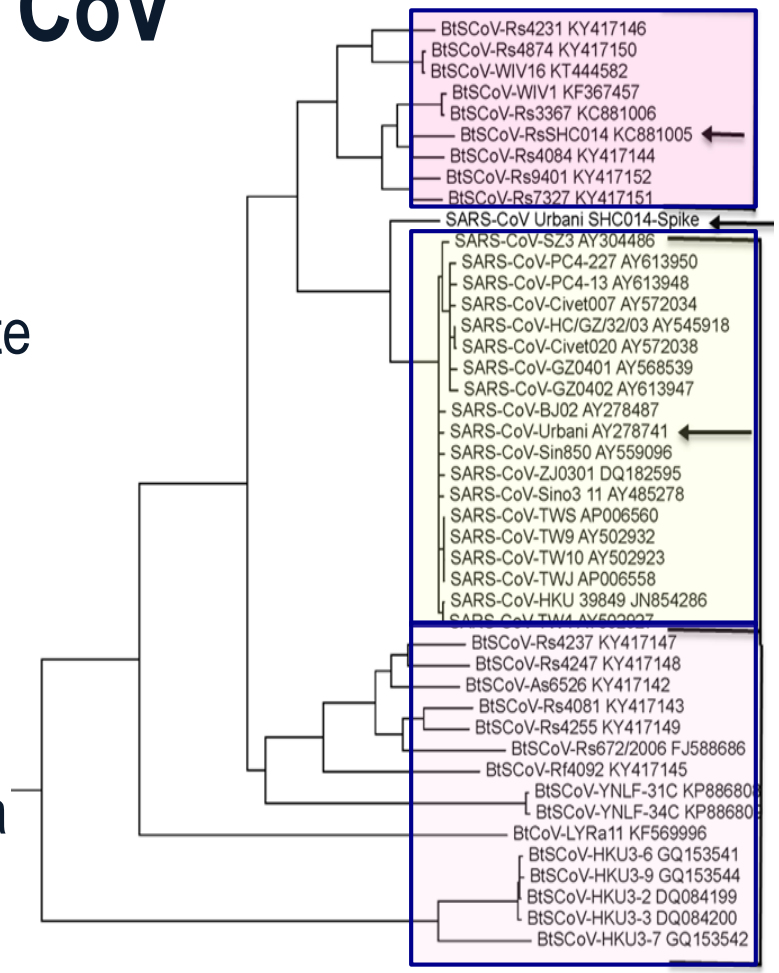




Position Piece: CoV: An RNA Proofreading Machine Regulates Replication and Fidelity (RNA Biol, 2011); Dudas G. Virus Evolution 2016; Eckerle et al., Plos Pathogens 2010; Graham et al., Nature Medicine 2012; Smith et al., Plos Path 2014; Kirchdoefer et al *Nature* 2016)

Origins of the Group 2B SARS and SARS-like CoV

- **SARS-CoV Origins (Yellow)**
  - bats
  - Open Markets and Civet Intermediate Hosts
- **SARS-like bat CoV (Pink)**
  - Pre-epidemic potential (high/low)
  - Bats, low level seroprevalence in people residing near bat hibernacula
- **2019-nHCoV**
  - Bats
  - Open Market Origins



Before Dec 2019

SARS-CoV Emergence in 2002 in China

8,096 cases, 774 deaths, in 32 countries, Nov 1 2002 - July 31 2003

Most Likely Model

Epidemic SARS-CoV

WIV-16 (98% Identical)

horseshoe bat

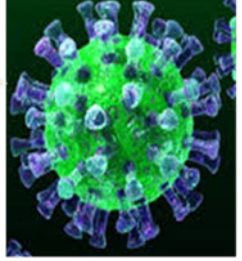
humans

palm civet

Intermediate host

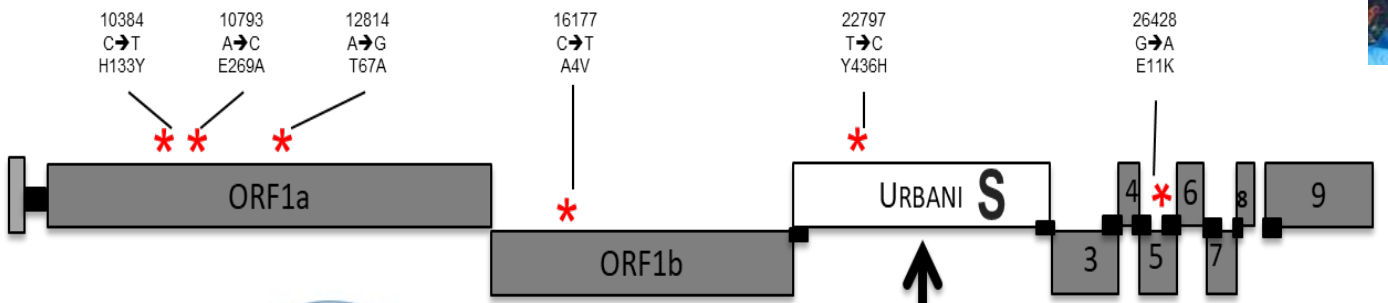
Bat to Human to Civet

Is SARS-CoV Extinct? ← **BtCoV** Bats Animals → **Threat Level?**

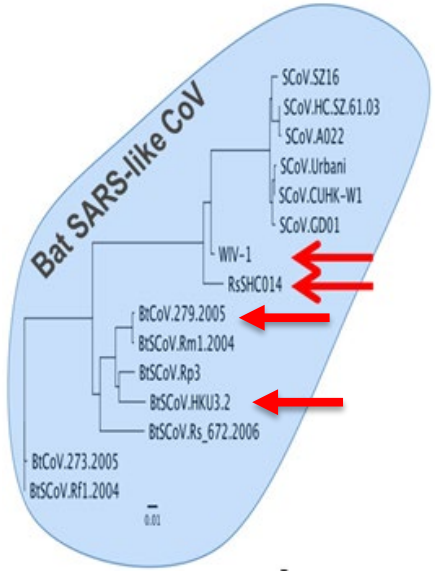


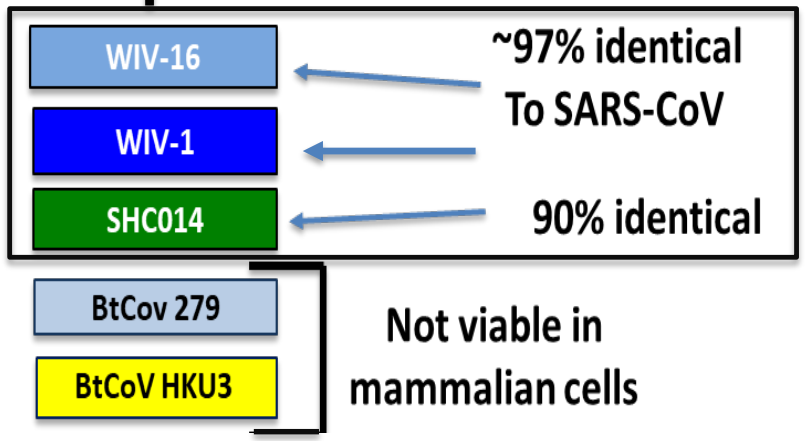
SARS MA15 Molecular Clone

Coronavirus Reverse Genetics



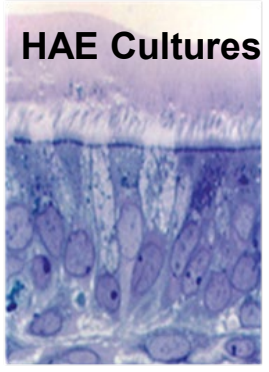
MA15 Molecular Clone





Bat CoV Spikes

Replicate like SARS-CoV on primary human airway epithelial cells
Use human receptor as well as SARS-CoV (if yes)
Synthesize full length genomes, recover full length virus



Rockx et al., JV 2007; Becker et al., PNAS 2008; Menachery et al., Nature Medicine, 2015; Menachery et al., PNAS 2017

Most Emerging Viruses

Zoonotic Reservoirs

SARS-CoV

2003-04

SARS-like bat CoV

SARS-CoV

MERS-CoV

0%

8,000 cases

774 deaths

10%

22%

Future Outbreak?

2019-nHCoV

Heterogeneous Pools of related viruses (0-35+%)

2019-nHCoV

PrePandemic Strains

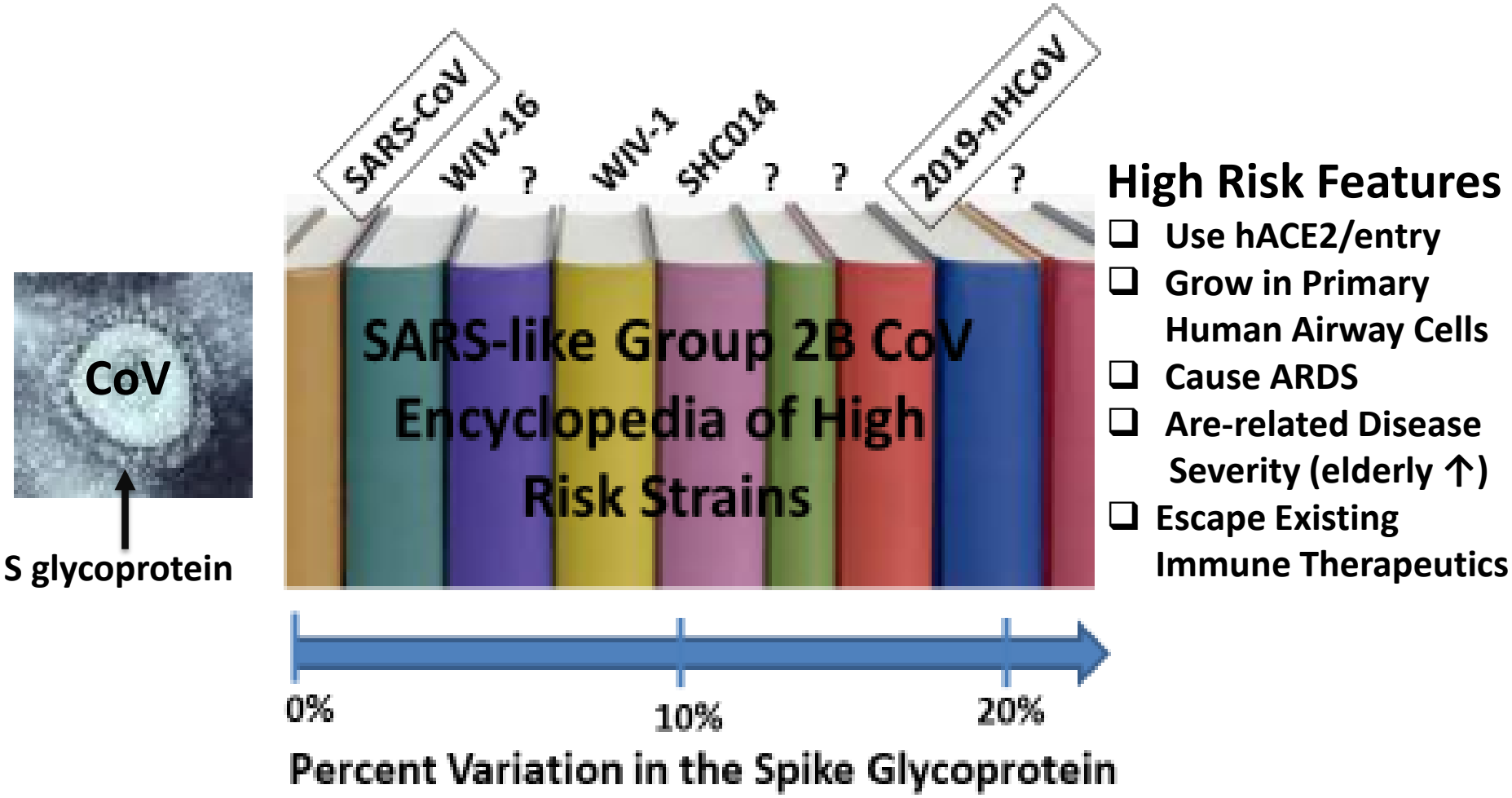
High Risk Emergence Strains

2019-

>60,000 cases 1360 deaths in 28 countries; Human transmission: Germany, US, Thailand, Japan, Vietnam, China

Z. Shi, Institute of Virology (Discovery Work on the SARS-like bat coronaviruses): Nature. 2013 Nov 28;503(7477):535-8.
Sheahan et al., JV 2008; Becker PNAS 2008; Menachery V et al., Nature Medicine 2015, Menachery PNAS 2016; Simon et al., mBIO 2017

Known Group 2B SARS-like CoV Poised for Human Emergence



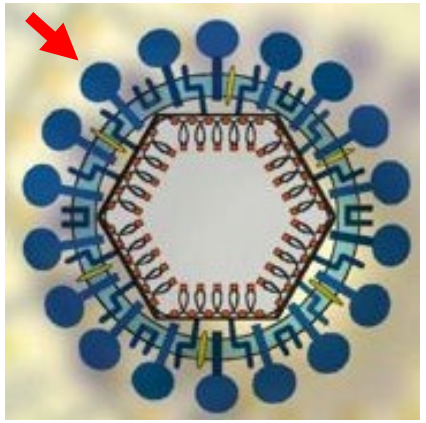
High Risk Features

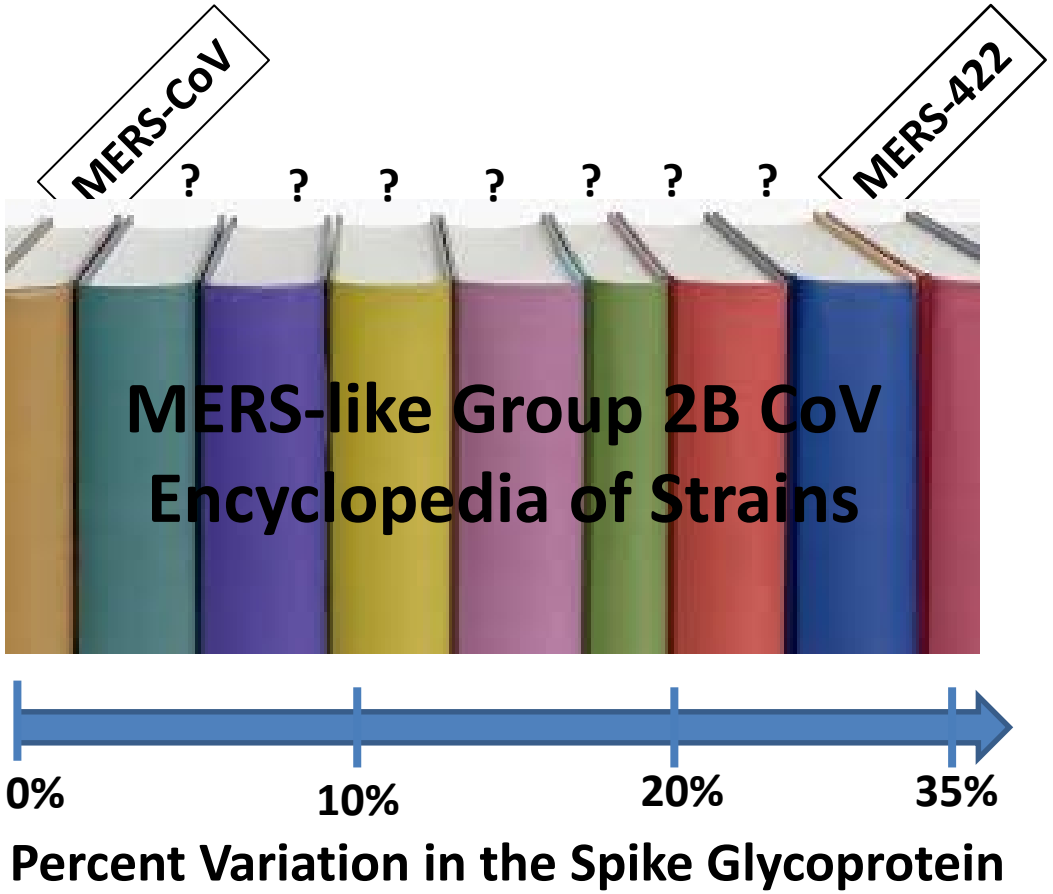
- ❑ Use hACE2/entry
- ❑ Grow in Primary Human Airway Cells
- ❑ Cause ARDS
- ❑ Are-related Disease Severity (elderly ↑)
- ❑ Escape Existing Immune Therapeutics

Platform to develop/test broad based vaccines, hmAB and antiviral drugs

Known Group 2C MERS-like CoV Poised for Human Emergence

MERS-Related Strains

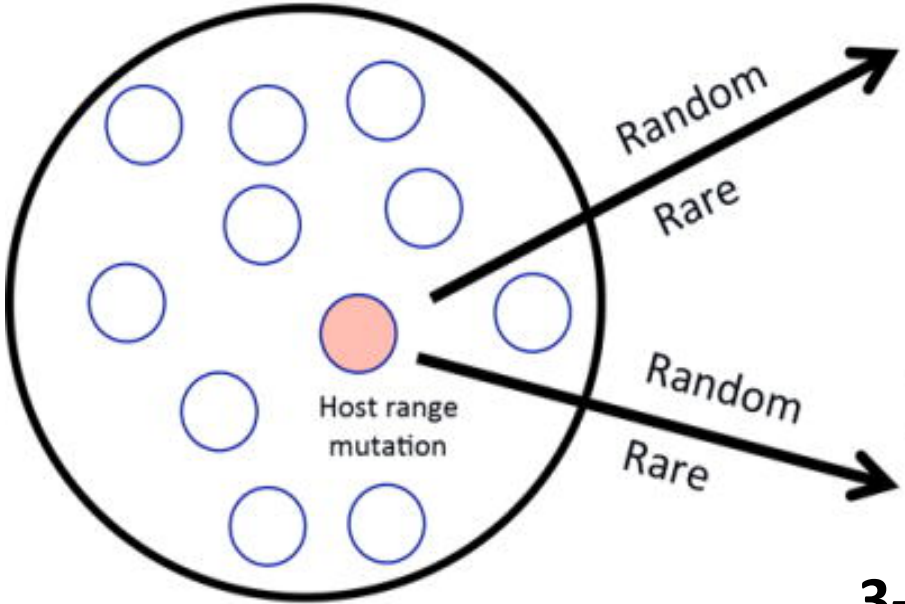




-MERS-like bat CoV (China) 65% Identity with MERS-CoV Spike
-Uses hDPP4 as a receptor for docking and entry
-Replicates efficiently in primary human airway epithelial cells

Zoonotic Virus Emergence Models

Classic Model: Mutation Driven



Zoonotic Virus Pools

a

Secondary host (reservoir) → Human infection

Adaptation

Adaptation

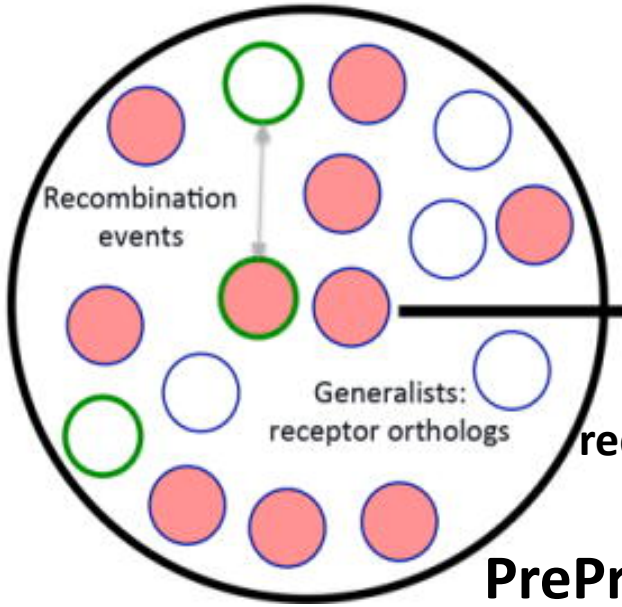
Epidemic strain

b

Adaptation

Direct human infection ↔ Secondary host (reservoir)

3-4 Step Model Requiring Mutations



May not require mutation-driven adaptation

c

Limited Mutation is Necessary

Direct human infection ↔ Secondary host (reservoir)

Epidemic strain

PrePrammed Viruses

2019-nHCoV

- **Emerged Early Dec in Wuhan China (Dec 1)**
- **Began as Cluster of Cases Associated with Open Markets (Dec 31)**
  - **No Evidence of Human to Human Transmission**
  - **Not Very Pathogenic**
  - **Not SARS-CoV, Likely a Novel Virus**

Lesson

Don't under-estimate epidemic potential of an emerging virus

- **Wuhan Open Fish Market Closed (Jan 1, 2020)**
- **Identified as a Coronavirus on Jan 7th, 2020**
  - **distant relative to the SARS-CoV (kissing cousin)**
- **Genome Length Sequence Reported (5 isolates) (~9-11th)**
- **15 HCW infected, China Confirms Person to Person Spread (~20th)**

UPDATE ON NEWLY DISCOVERED CORONAVIRUS

	SARS CoV	MERS CoV	2019 nCo-V (SARI)
Virion Structure	Enveloped RNA virus	Enveloped RNA virus	Enveloped RNA virus
Outbreak period	2003-2004	2012-present	2019-present
Initial site of isolation	Guangdong province, China	Saudi Arabia	Wuhan, China
No. of countries/cases	29	27	28
No. of cases (mortality)	8,096 (9.6%)	2,494 (~34%)	~60,000 (N=1367)(2%)* >8,243 critical (~16%)
No. of cases U.S.	8	2 (2014)	13 (WA, IL, CA, AZ, Mass, Wis)
Reservoir (intermediate host)	Bats (palm civet)	Bats (dromedary camels)	Bats (likely a zoonosis)
Incubation period	2-7 days (range, 2-21)	2-7 (range, 2-14 days)	2-14 days (mean 5-6)
Infectivity, rho	1.8-2.5	0.3-1.3	1.4-2.2 (WHO), 2.5-3.8*
Super spreaders	Yes	Yes (uncommon)	Yes (1 case infected 14 HCW)
Asymptomatic/mild Spread	No	Rare	Perhaps Yes?/Yes
Attack Rate	10.3% to 60%	4 to 20%	?, 80+% (one study)
Transmission (including to HCP)	Droplet/Direct, Airborne/Indirect?	Droplet/Direct, Airborne/Indirect?	Droplet/Direct, Airborne/Indirect?
Treatment (PEP)	Supportive (none)	Supportive (none)	Supportive (none)*
Infection Prevention^	Airborne, contact, face shield	Airborne, contact, face shield	Airborne, contact, face shield

*Wuhan is 4.1 percent and 2.8 percent in Hubei, compared to 0.17 percent elsewhere

Phylogenetic Relationships Between the Group 2B Coronaviruses

21st Century Emerging Human Coronaviruses

SARS-CoV 2003

MERS-CoV 2012

2019-nHCoV

Phylogenetic Relationships Between the Group 2B Coronaviruses

21st Century Emerging Human Coronaviruses

SARS-CoV 2003

MERS-CoV 2012

2019-nHCoV

High Risk SARS-like Bt CoV 3-10%

SARS/SHC014 Chimera

Spike

SARS-CoV 2003-04 (1-2%)

Spike

Low Risk SARS-like Bt CoV 15-25%

25% different

2019 nCoV

Differ by >5,000 nts

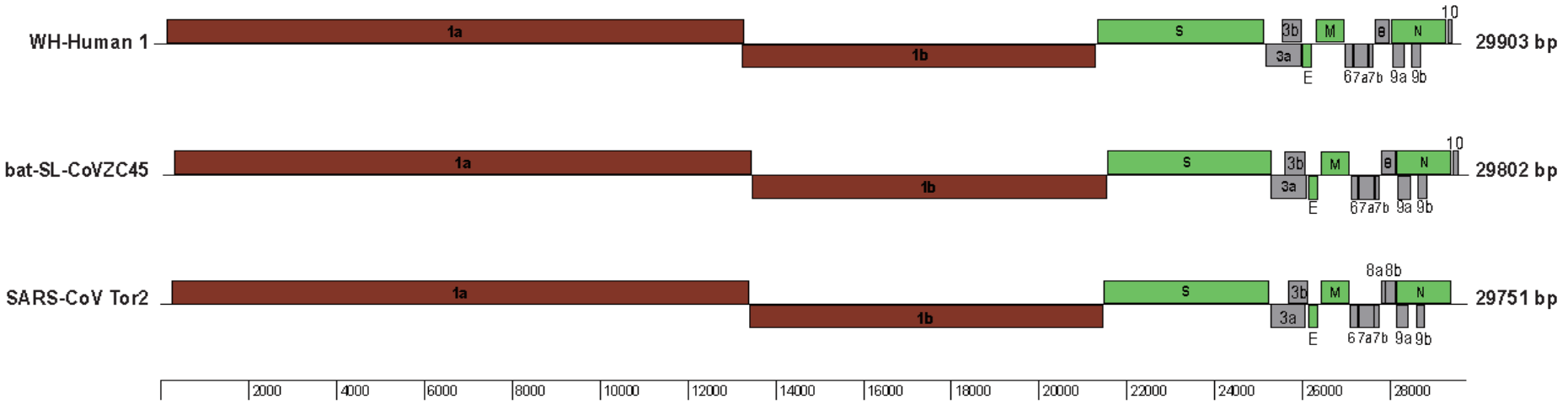
New Clade of SARS-like Viruses

Rooted with HCoV OC43

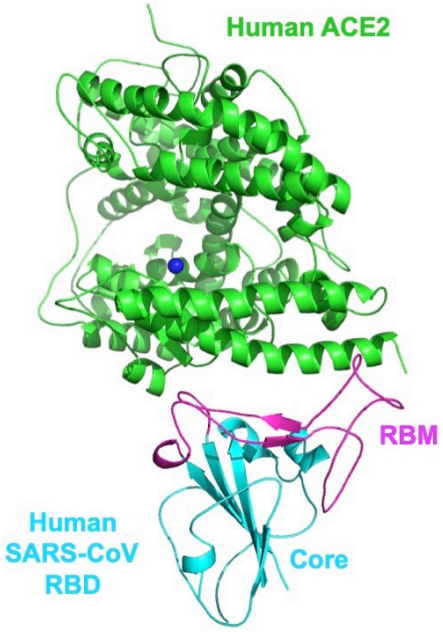
96% nt Identity (1200 nts)

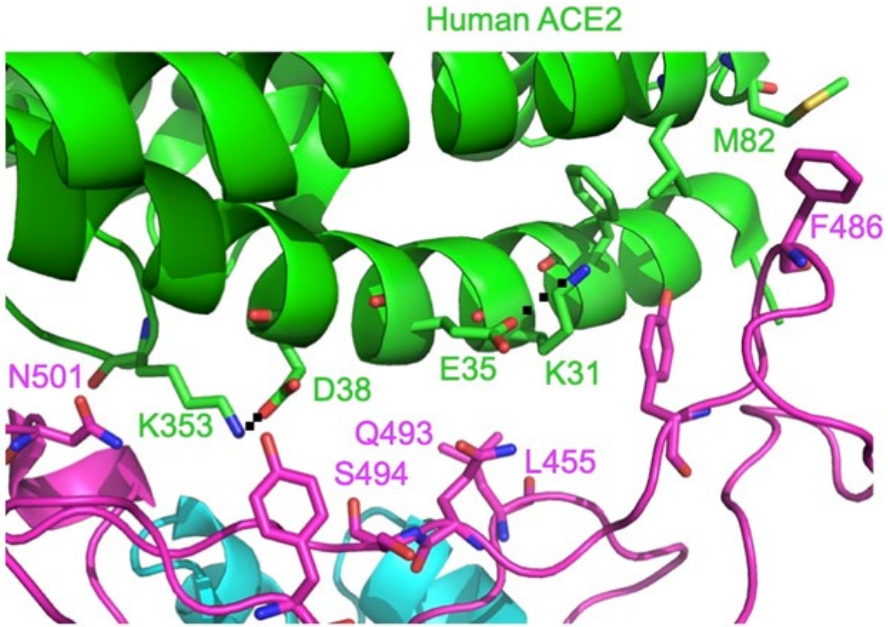
RaTG13 was sequenced from a bat in a cave from Yunnan Province in China

2019-nHCoV Genome Organization



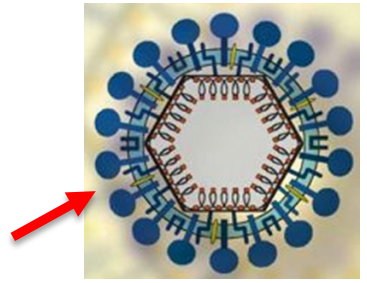
Uses hACE2 Receptor for Entry





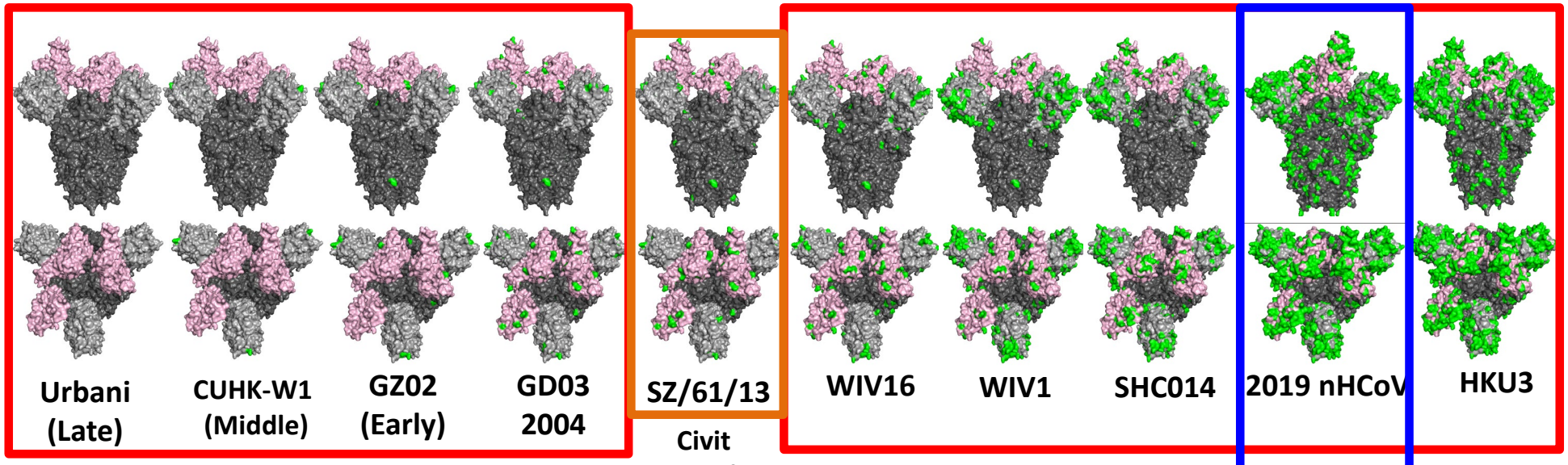
Zhou et al., bioRxiv 2020.01.22.914952

Immune Therapeutic Countermeasures



2003-2004 SARS-CoV Outbreak Strains

Group 2B SARS-like Bat Coronaviruses



All Are Poised for Human Emergence

Antigenic Distance is Large, SARS-CoV Immune Therapeutics (hmAB) and Vaccines likely Fail

variation

Broadly active drugs/vaccines are essential to control zoonotic CoV

Vaccine Targets

- **Spike is a major target for neutralizing antibodies, a principle target for vaccine design for emerging and animal coronaviruses**
  - SARS-CoV, SHC014, WIV1 and SARS-CoV 2.0
- **Produce broadly cross reactive vaccines that target group 2b SARS-like CoV**
  - Broadly cross neutralizing epitopes ill defined
- **Stem is more conserved than head domain of spike glycoprotein—target for broad nAB**
- **Potent Neutralizing Antibodies**
  - Globular Head

SARS Vaccine Complications

- **Vaccine efficacy in aged populations can reduce performance**
- **Heterogeneous group 2b SARS-like CoV pool may vary by as much as 35% (compared with SARS)**
- **Th2 Immune Pathology after Vaccination**
  - DIV SARS-CoV Vaccine + Alum Adjuvant (J Virol. 2011 Dec;85(23):12201-15; J Immunol. 2008 Nov 1;181(9):6337-48, J. Infect. Dis. 60:106–112)
  - Adjuvanted S glycoprotein Vaccines (JCI Insight. 2019 Feb 21;4(4). pii: 123158; J Virol. 2015 Mar;89(6):2995-3007)
- **Evidence for Enhancing Antibodies**
  - Primates (ACS Infect Dis. 2016 May 13;2(5):361-76)
  - Cell Culture (Biochem Biophys Res Commun. 2014 Aug 22;451(2):208-14, Clin Exp Immunol. 2005 Sep;141(3):500-8)

Acknowledgements

Baric Laboratory

Adam Cockrell
Emily Gallichotte
Rachel Graham
Lisa Gralinski
Lisa Lindesmith
Michael Mallory
Ande West
Kendra Gully
Ethan Fritch
Ariana Brown
Alexandra Schaefer
Trevor Scobey
Tommy Baric
Amy Sims
Sarah Leist
Jesica Swanstrom
Paul Brewer-Jensen
Boyd Yount
Ellen Young
Caitlin Edwards
Jenny Munt
Kenny Dinnon

UNC GILLINGS SCHOOL OF GLOBAL PUBLIC HEALTH
DEPARTMENT OF EPIDEMIOLOGY

Tim Sheahan Lab (UNC)

UNIVERSITY OF NORTH CAROLINA CHAPEL HILL

Mark Denison Lab

VANDERBILT UNIVERSITY

ADC3

Rich Whitley, UAB

National Institute of Allergy and Infectious Diseases

ORFEOME

CRDD
Center for Research in Diagnostics & Discovery

Developing Antivirals Against Coronaviruses

Denison Lab – Vanderbilt University Medical Center

Baric Lab – UNC Chapel Hill

Gilead Sciences

Emory University - DRIVE

The Coronavirus Antiviral Research Team

- **Vanderbilt University Medical Center:** Andrea Pruijssers, Jim Chappell, Maria Agostini, Laura Stevens, Xiaotao Lu, Tia Hughes, Amelia George, Mark Denison
- **University of North Carolia:** Tim Sheahan, Amy Sims, Rachel Graham, Boyd Yount, Ralph Baric
- **Gilead:** Joy Feng, Danielle Porter, Richard Mackman, Mike Clarke, Tomas Cihlar
- **Emory / EIDD / DRIVE:** Greg Bleumling, Mike Natchus, George Painter
- **NIH / NIAD – U19 (Whitley UAB) – CETR – AD3C**

Need for Antivirals against CoVs:

- Broad diversity of CoVs in bats with demonstrated capability to infect human cells animal models – “outbreak ready”
- Failure of antibodies to neutralize *“future”* zoonotic CoVs and loss of cross protection by vaccines
- Time to develop vaccines differs from trajectory of epidemic
- Universal vaccines across all CoV PPP groups will be difficult and potentially with gaps or not possible
- Potential for “off the shelf” use toward highly conserved functions

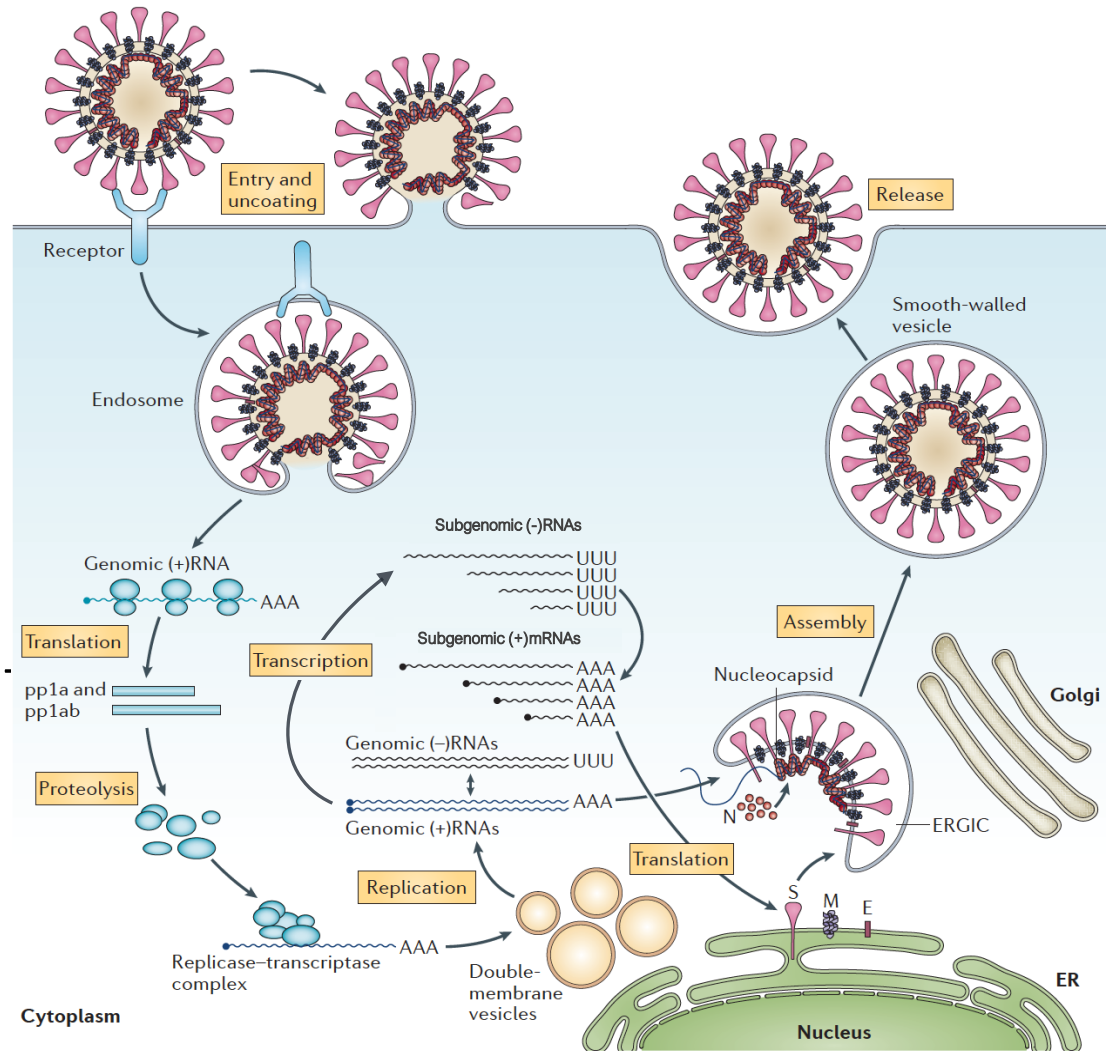
Goals for CoV antiviral development

- Broadly active against diverse coronaviruses
- High barrier to resistance - limited genetic paths, high fitness cost
- Extended therapeutic window for prevention, amelioration, treatment,
- Additional
  - decrease transmission,
  - oral administration

Coronavirus Replication

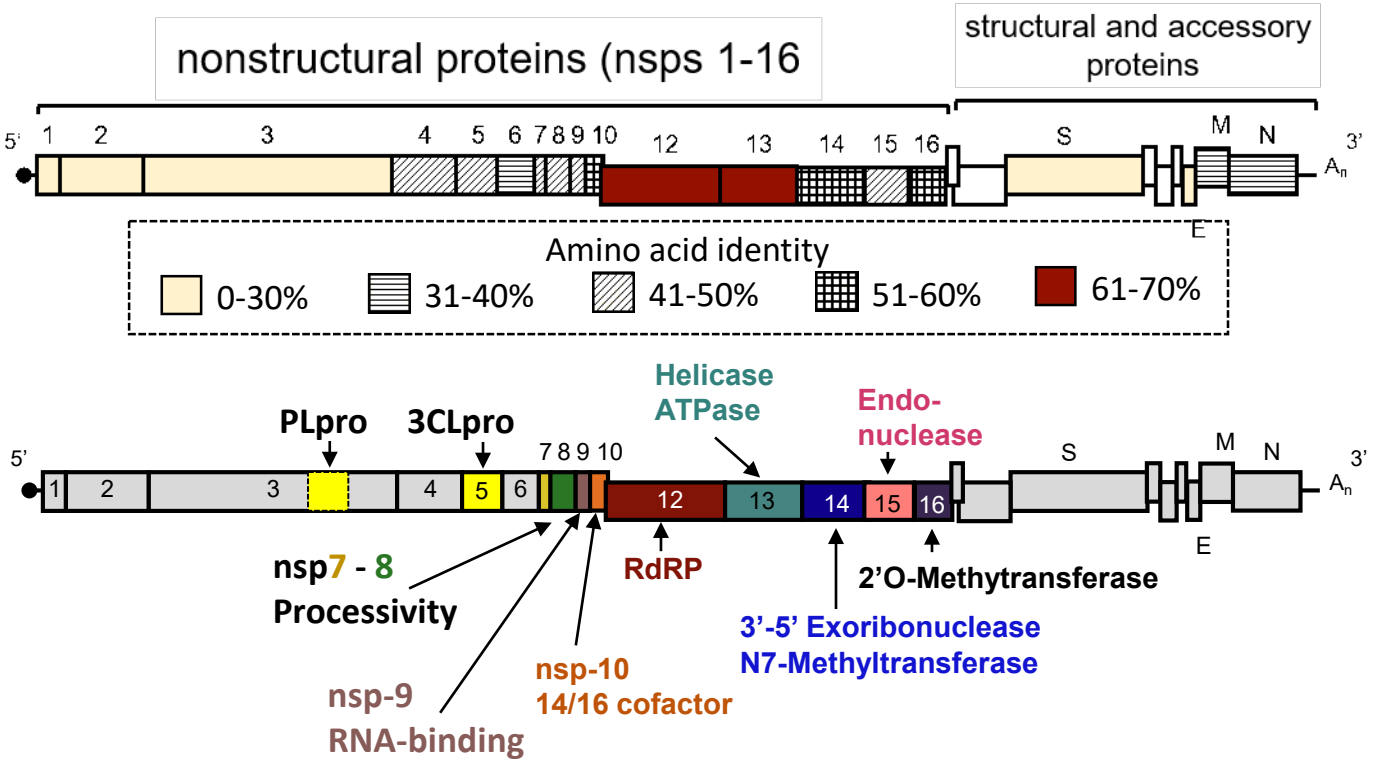
Essential functions and viral components:

- Entry - Spike
- Translation
- Proteolysis - nsp3 and nsp5
- Replication and Transcription - (nsp7-nsp14)
- Assembly and Release - structural proteins

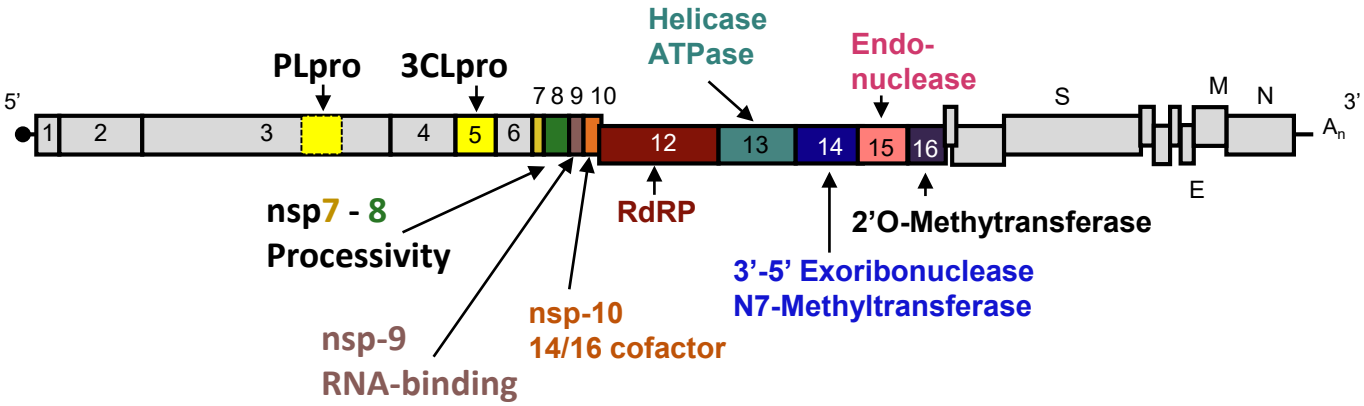


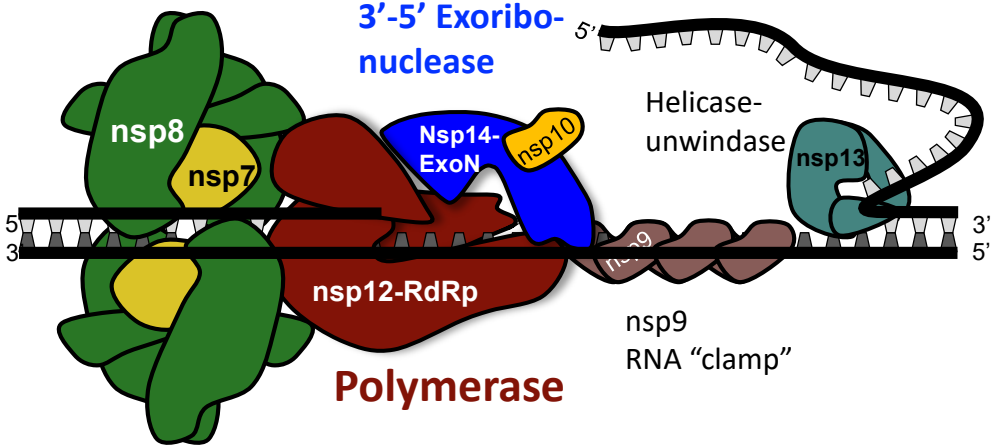
Adapted from de Wit et al. *Nat. Rev. Micro.* 2016.

Coronavirus amino acid and function is highly conserved in the core replicase proteins

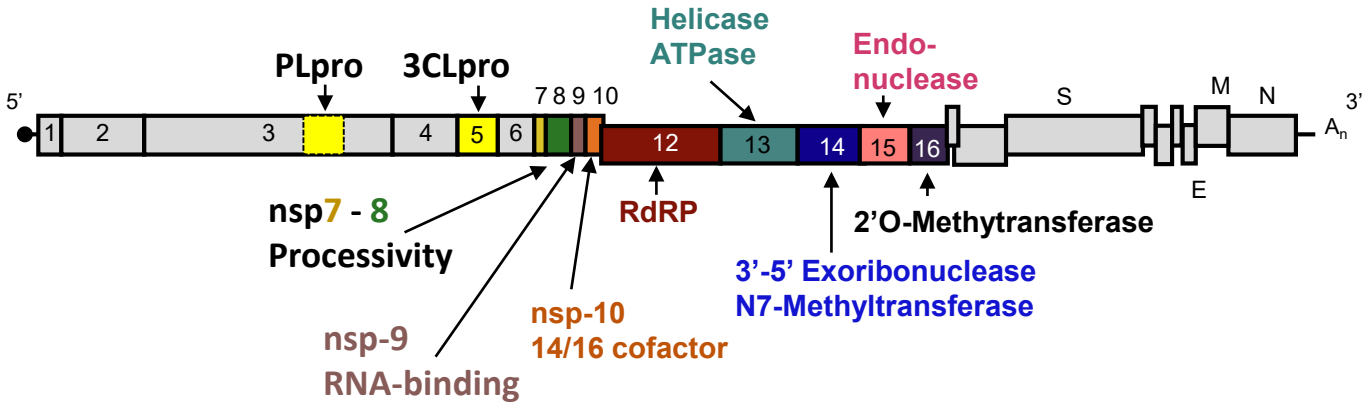


Coronaviruses assemble a multiprotein replicase complex

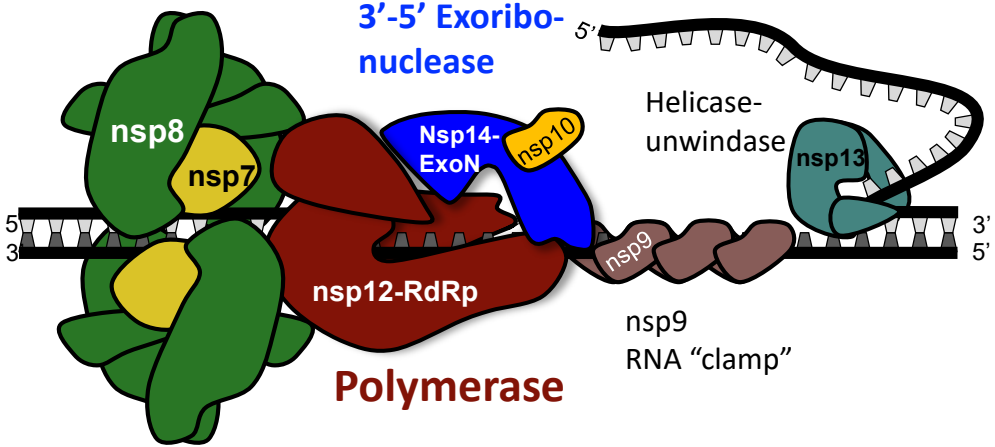




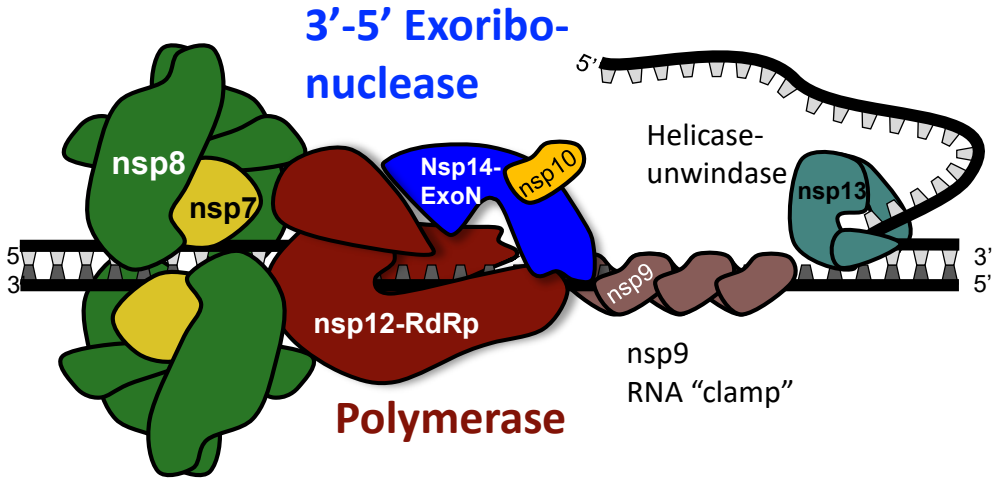
Coronaviruses assemble a multiprotein replicase complex



- Only RNA virus order (nidovirales) to encode proofreading ExoN
- Removes mis-incorporated nucleotides
- Confers high fidelity replication (up to 20-fold)

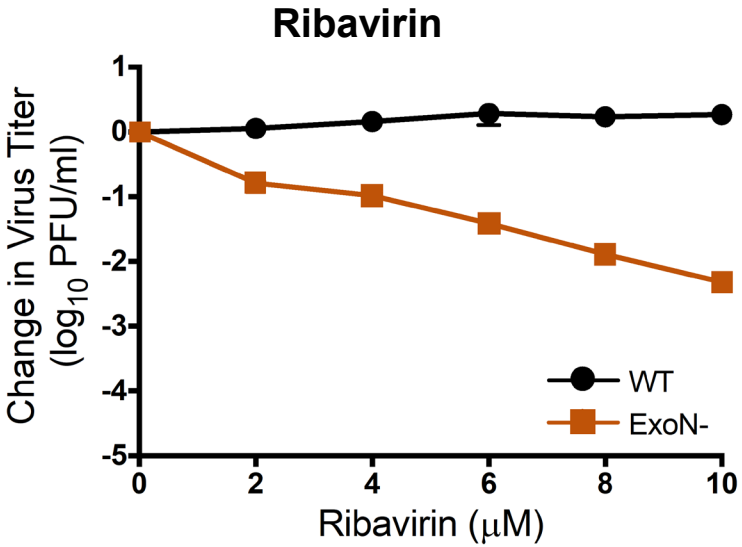


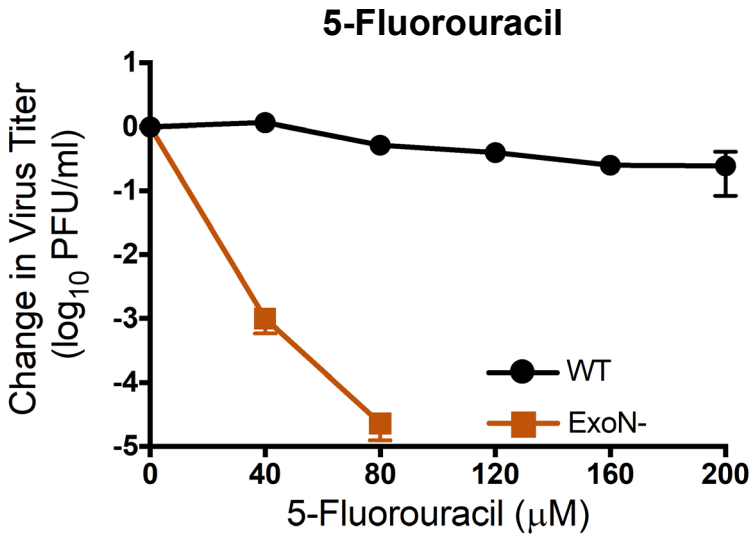
Coronaviruses encode a proofreading exoribonuclease (nsp14-ExoN)



- Only RNA virus to encode a proofreading exonuclease
- Removes mis-incorporated nucleotides
- Confers high fidelity replication (up to 20-fold)

Native resistance of coronaviruses to nucleoside analogues is due to ExoN-proofreading

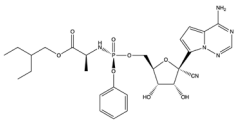


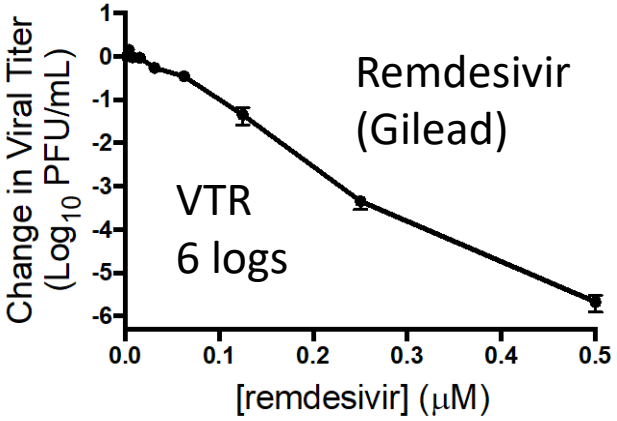


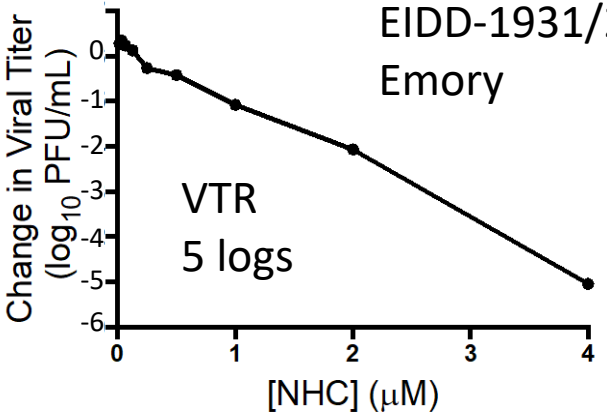
MOI = 0.01 PFU/cell
24 h p.i.

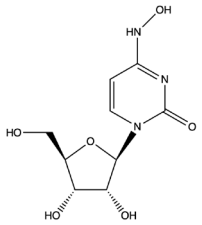
Adapted from Smith et al. *PLOS Path.* 2013.

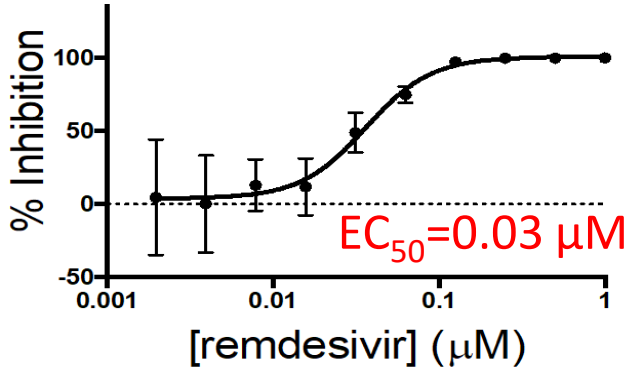
Remdesivir and β-D-*N*⁴-Hydroxycytidine (EIDD-1931/2801, NHC) inhibit CoV replication

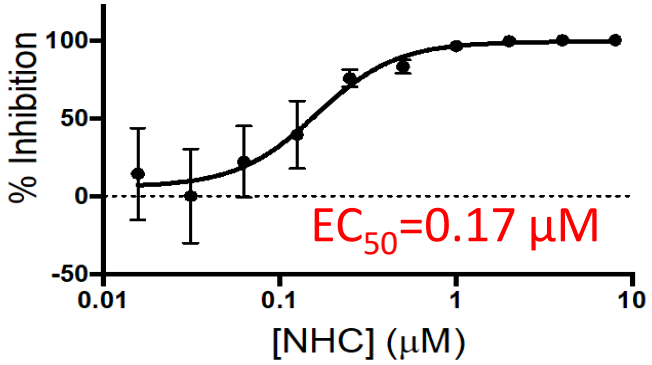










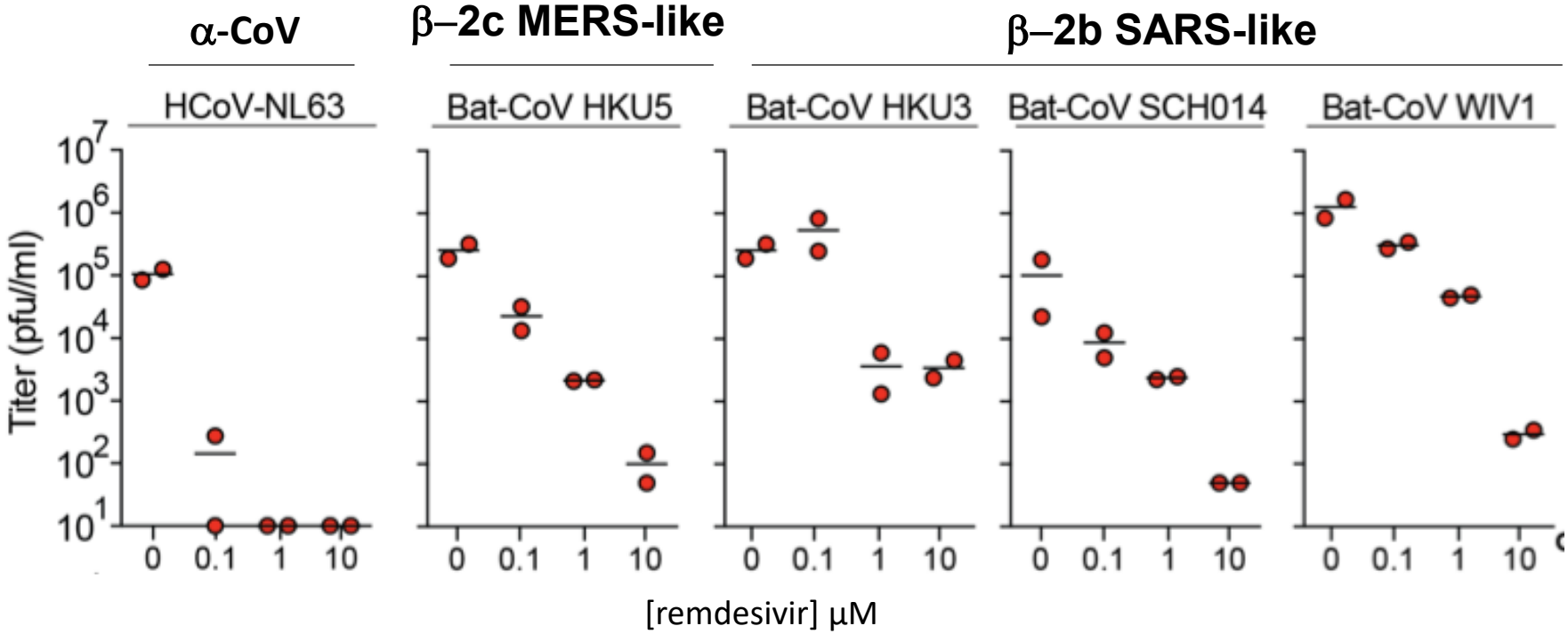


MOI = 0.01 PFU/cell
24 h p.i.

Agostini et al mBio 2017

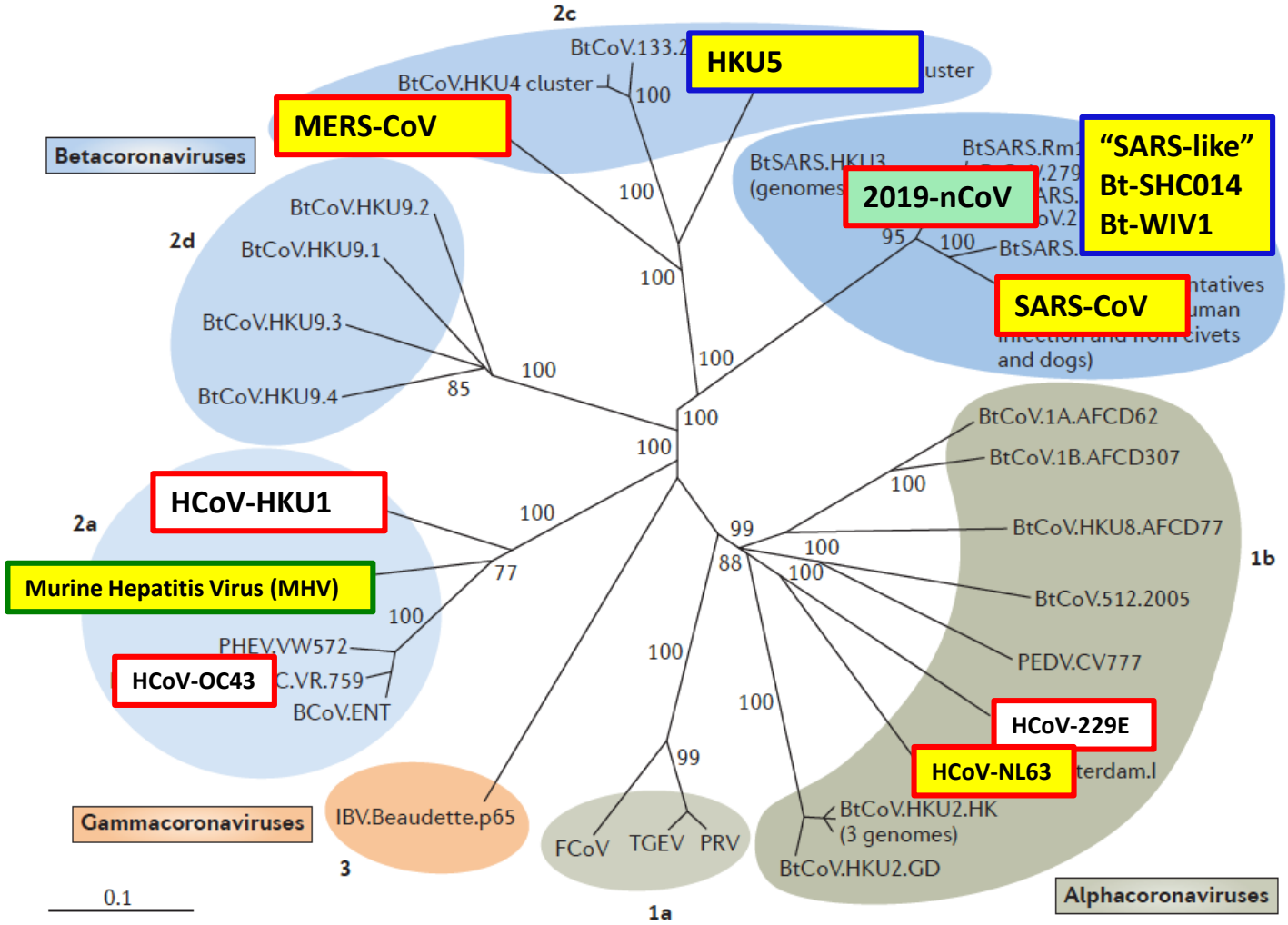
Agostini et al J Virol 2019

Remdesivir inhibits other human CoVs and potential zoonotic CoVs

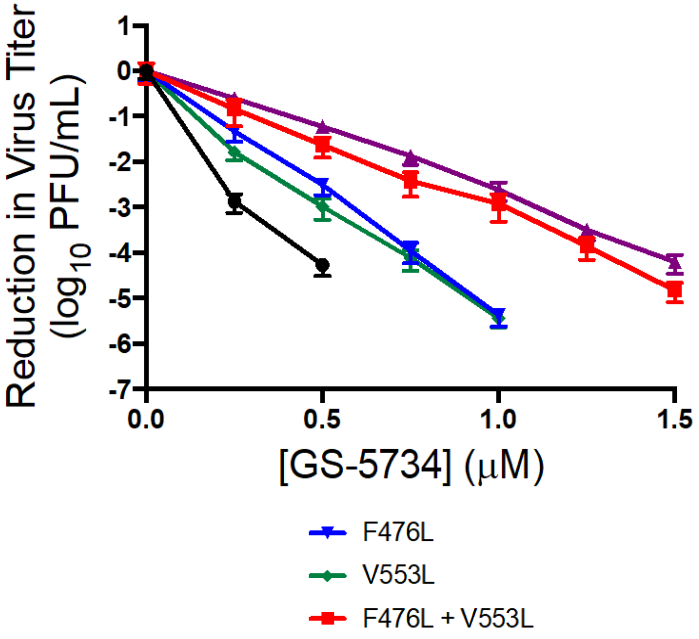


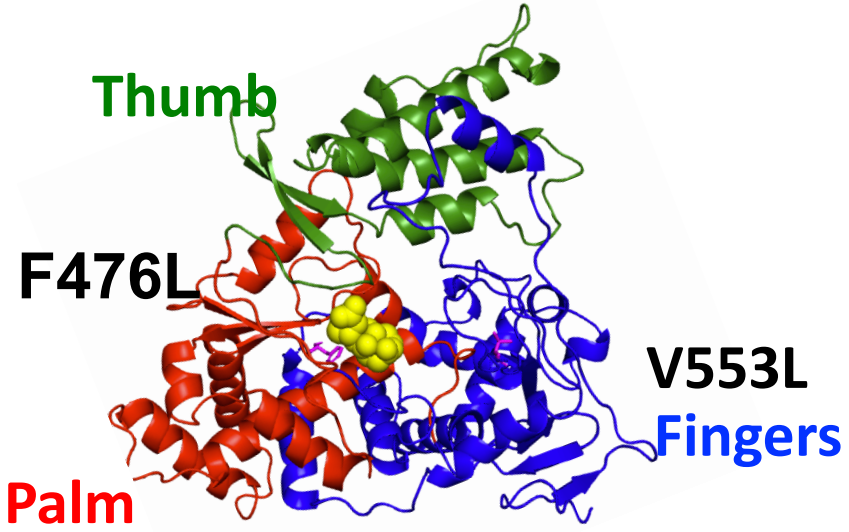
MOI = 0.5 PFU/cell
48 h p.i.

Sheahan et al. *Sci. Trans. Med.* 2017.


2c
BtCoV.133.2
BtCoV.HKU4 cluster
100
HKU5
MERS-CoV
Betacoronaviruses
100
100
BtSARS.HKU3
(genomes
BtSARS.Rm1
2019-nCoV
95
100
BtSARS.
SARS-CoV
"SARS-like"
Bt-SHC014
Bt-WIV1
and dogs)
2d
BtCoV.HKU9.2
BtCoV.HKU9.1
BtCoV.HKU9.3
BtCoV.HKU9.4
100
85
100
100
100
BtCoV.1A.AFCD62
BtCoV.1B.AFCD307
100
HCoV-HKU1
2a
100
99
BtCoV.HKU8.AFCD77
100
88
100
1b
77
Murine Hepatitis Virus (MHV)
BtCoV.512.2005
100
PHEV.VW572
PEDV.CV777
HCoV-OC43
C.VR.759
BCoV.ENT
100
HCoV-229E
100
HCoV-NL63
terdam.I
99
IBV.Beaudette.p65
Gammacoronaviruses
FCoV
TGEV
PRV
BtCoV.HKU2.HK
(3 genomes)
BtCoV.HKU2.GD
3
1a
Alphacoronaviruses
0.1

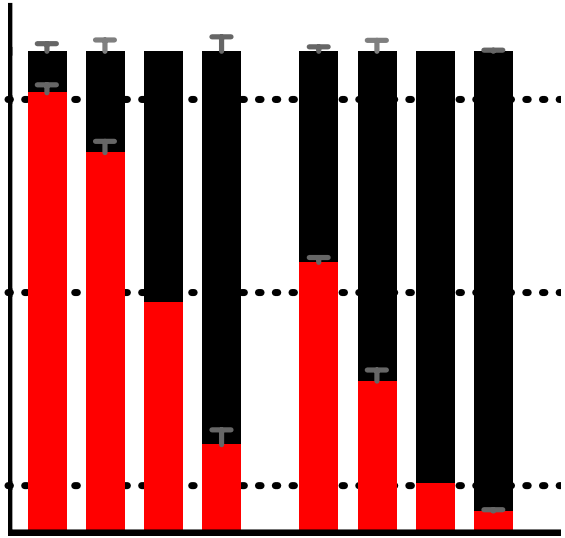
Two mutations (F476L and V553L) selected in the nsp12-RdRp after 23 passages in the presence of Remdesivir





6 fold resistance
In SARS-CoV

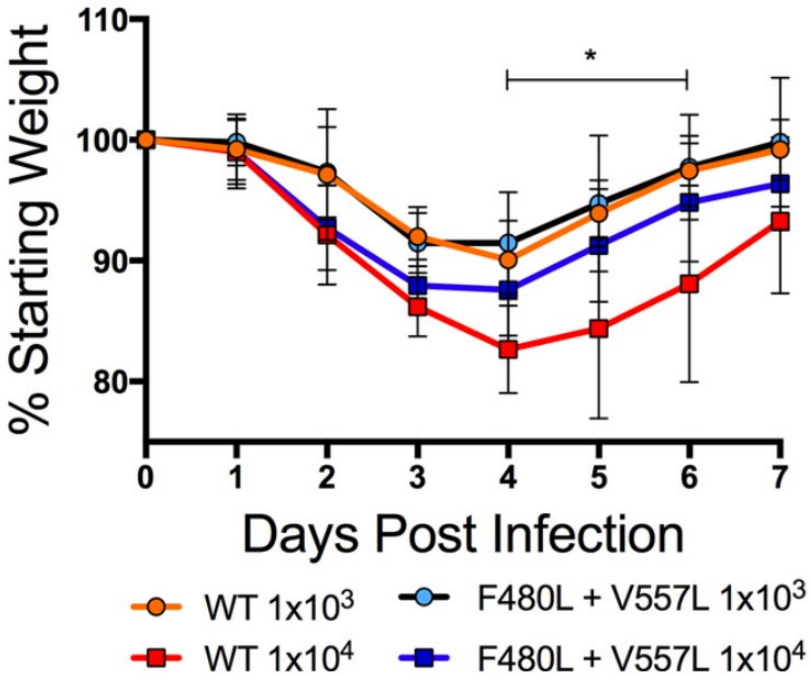
Remdesivir resistance mutations are less fit than WT in vitro and attenuated in vivo



WT F476L V553L

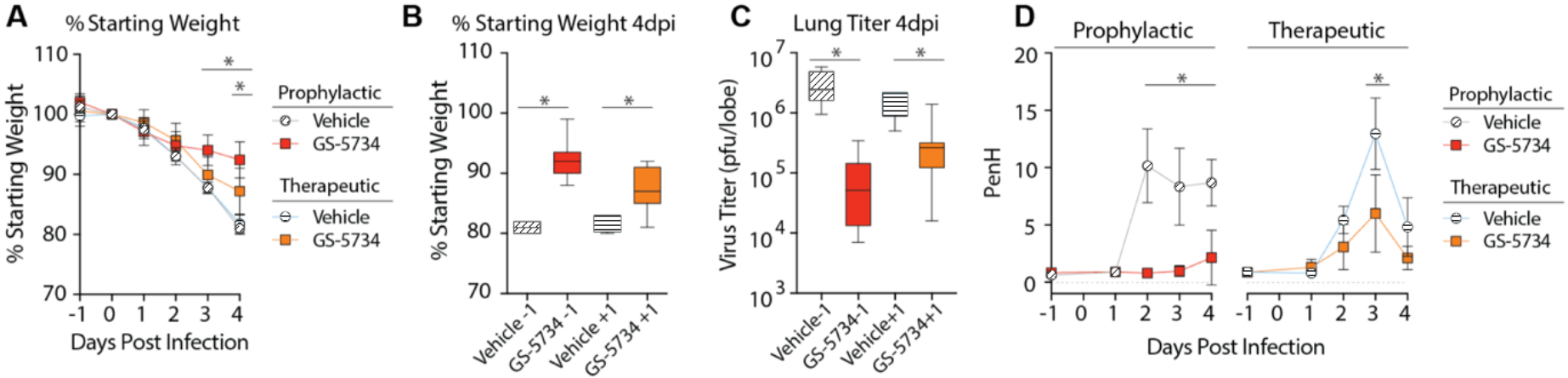
WT F476L V553L

MOI = 0.01 PFU/cell
20 h.p.i



Adapted from Agostini et al. *mBio.* 2018.

Remdesivir given before or 1 day post exposure mitigates disease in a mouse model of Lethal SARS-CoV infection



Remdesivir - IV

- Potently inhibits multiple divergent CoVs
- Mechanism includes RNA chain termination
- Resistance has high barrier – difficult to achieve
- Resistance mutations associated with fitness loss in vitro and attenuation in vivo.
- Efficacious for prophylaxis in mouse model of lethal SARS-CoV
- Decreases disease and virus titer when administered early in infection

Remdesivir - IV

EIDD-2801- NHC

oral

- Potently inhibits multiple divergent CoVs ✓
- Mechanism includes RNA chain termination **Mutagenesis**
- Resistance has high barrier – difficult to achieve ✓
- Resistance mutations associated with fitness loss in vitro and attenuation in vivo. ✓
- Efficacious for prophylaxis in mouse model of lethal SARS-CoV ✓
- Decreases disease and virus titer when administered early in infection ✓

Coronavirus Countermeasures

Direct acting antivirals (DAA's) - for treatment, prophylaxis, and decreasing transmission

Monoclonal antibodies -to block infection and act as "passive immunization" during an epidemic

Host Directed therapy - inhibitors or immunomodulators –modify disease – extend therapeutic window for DAA's and mAbs

Combinations

- **DAA's + DAA's**: increase potency and efficacy, prevent resistance
- **DAAs + mAbs:** block infection and stop virus replication
- **DAA's + Host Directed Rx:** target disease and extend therapeutic window

VANDERBILT UNIVERSITY MEDICAL CENTER

- CoV replication fidelity
- CoV reverse genetics
- CoV resistance mutants
- *In vitro* synergy testing

UNC SCHOOL OF MEDICINE

- CoV efficacy spectrum
- Human lung cell cultures
- CoV reverse genetics
- Primer ID sequencing
- *In vivo* pathogenesis

GILEAD

Remdesivir

- Formulation
- *In vivo* PK
- Drug synergy
- Regulatory expertise

EMORY UNIVERSITY

EIDD-1931 (NHC)

- Formulation
- *In vivo* PK
- Drug synergy
- Regulatory expertise

SR SOUTHERN RESEARCH

SR-36097, SR-35293

- Lead optimization
- Medicinal chemistry
- *In vivo* PK