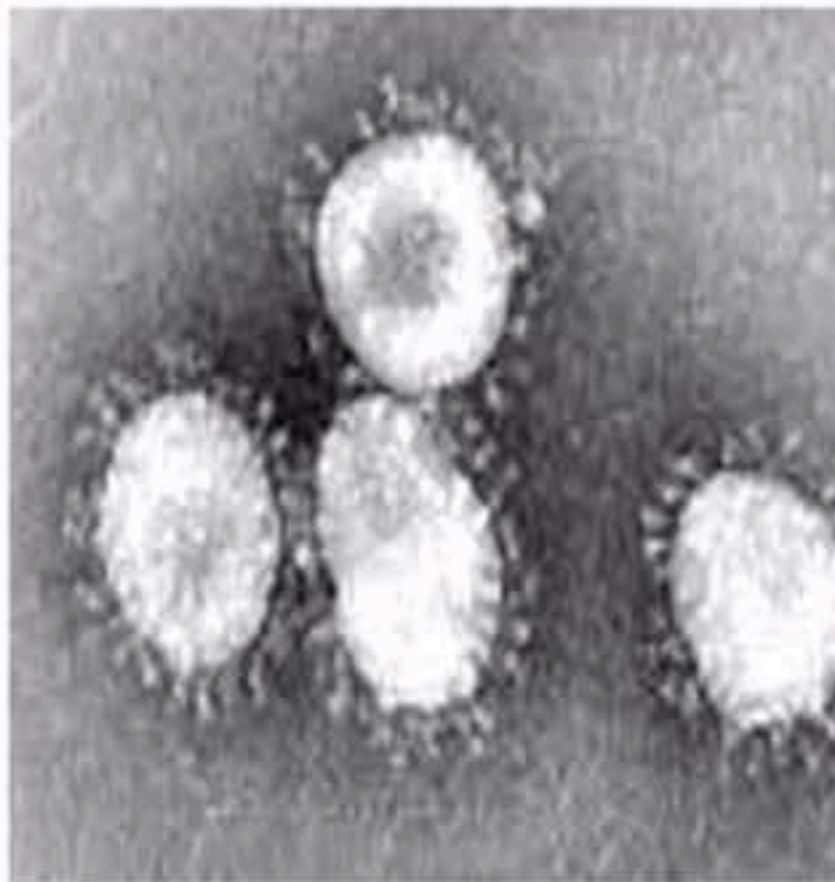




SARS-CoV-2 Update (2019-nCoV)

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
 - SARS-CoV 2
 - Genome Organization and relatedness
 - COVID-19 Disease
- Countermeasures
 - Vaccines
 - Broad based CoV nucleoside inhibitors
- Summary



Timeline: Emerging Nidoviruses

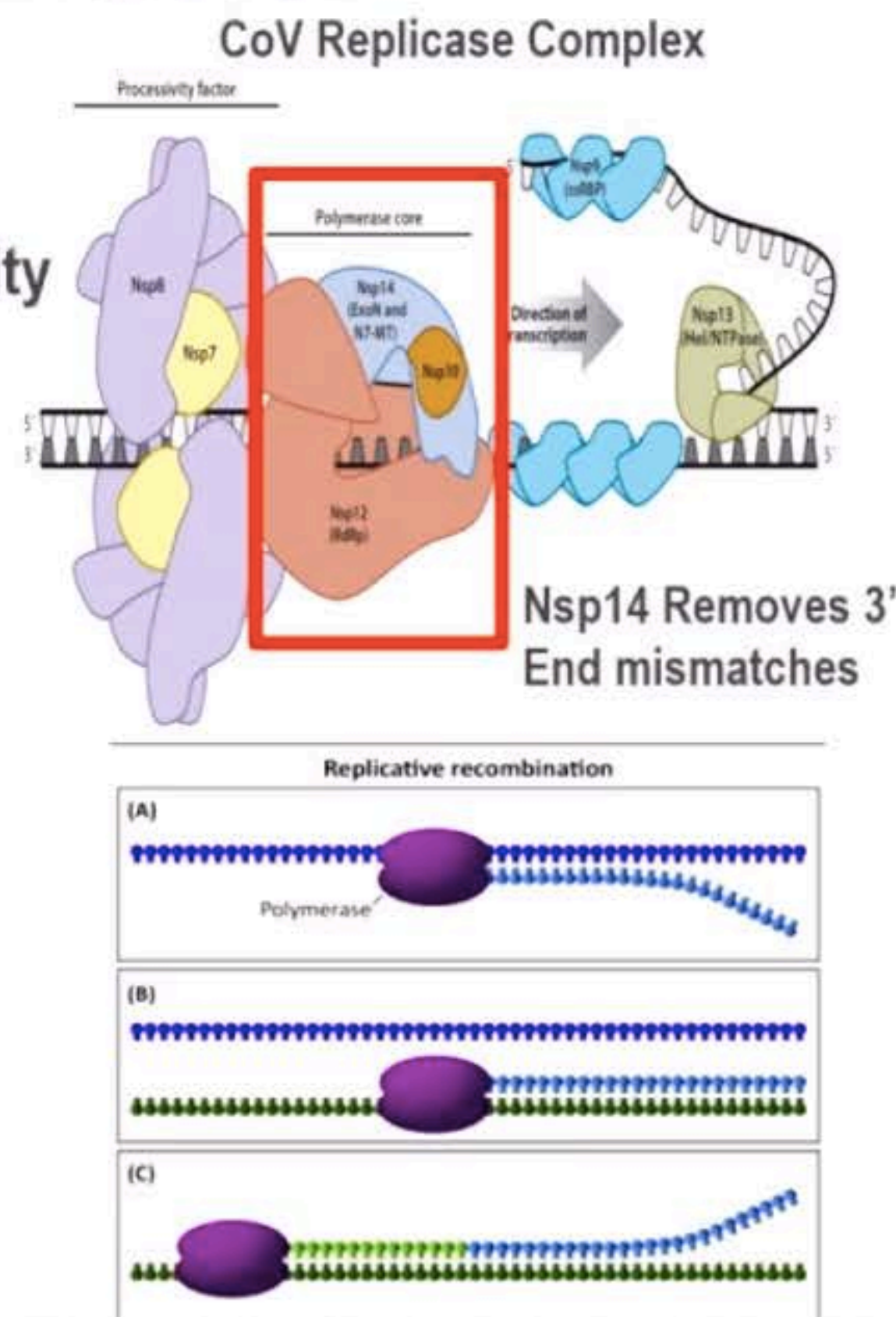
<u>Virus</u>	<u>Species</u>	<u>Emergence</u>
HCoV-NL63	Human	500-800 years
HCoV-229E	Human	200-300 years
HCoV-OC43	Human	~120 years
PEDV	Porcine	~25 years ← 2012 in US
PRRSV	Porcine	~25 years
rBCoV	Bovine	~25 years
SARS-CoV	Human	~16 years
MERS-CoV	Human	~7 years
SADS-CoV (HKU2)	Porcine	~2 years
SARS-CoV 2	Human	3 months

Accelerating
Cross Species
Movement
21st Century

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

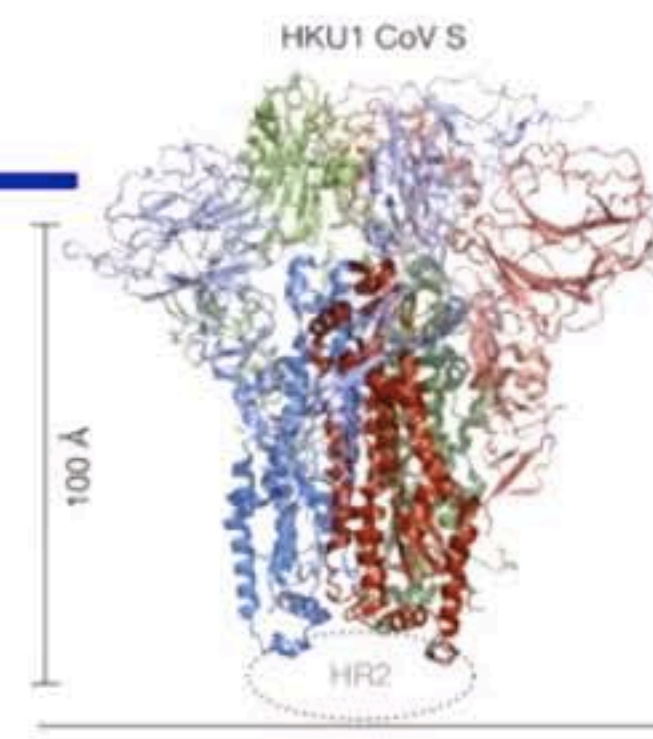
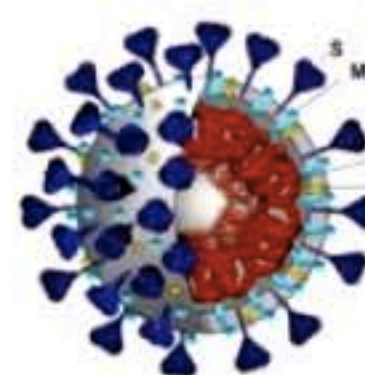
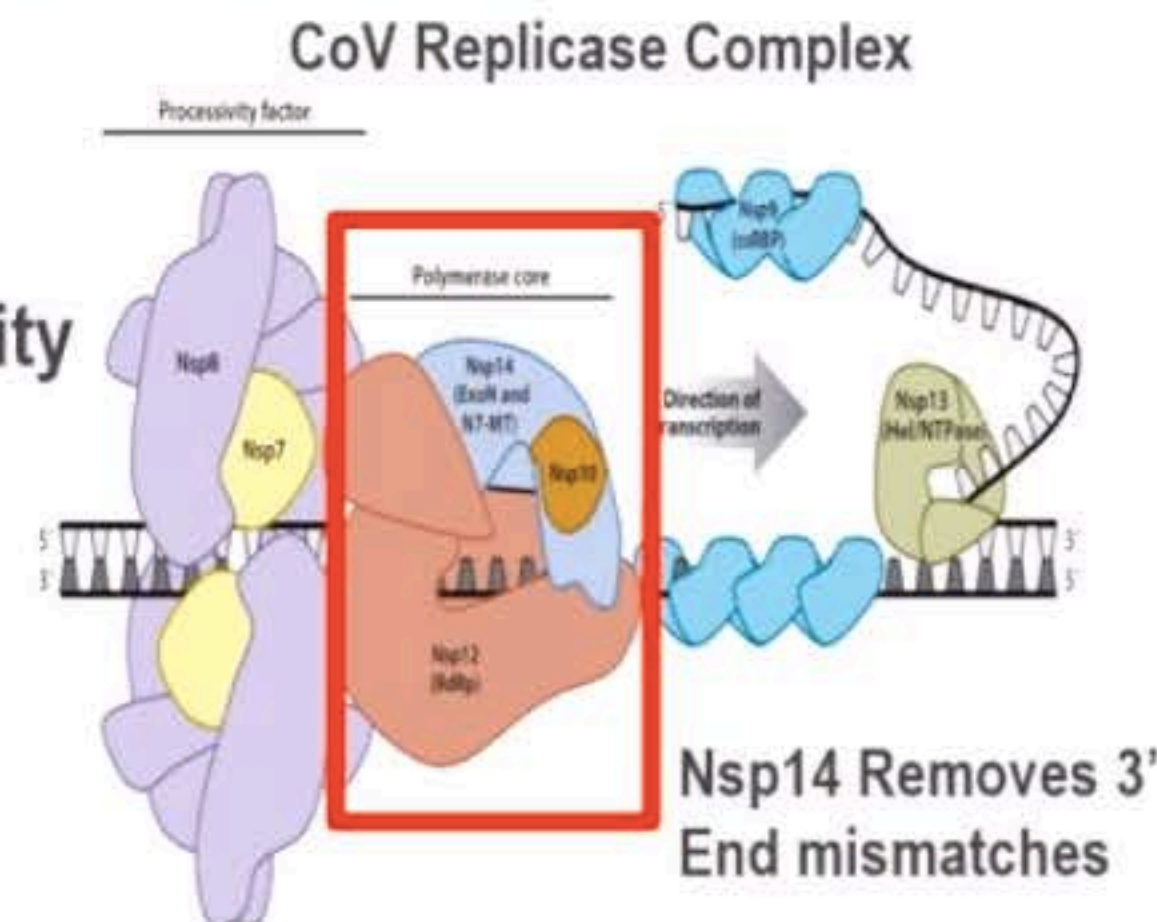


Position Piece: CoV: An RNA Proofreading Machine Regulates Replication and Fidelity (RNA Biol, 2011), Dudas G. Virus Evolution 2016; Eckerle et al., Plos Pathogens 2010; Graham et al., Nature Medicine 2012; Smith et al., Plos Path 2014

Drivers of CoV Evolution

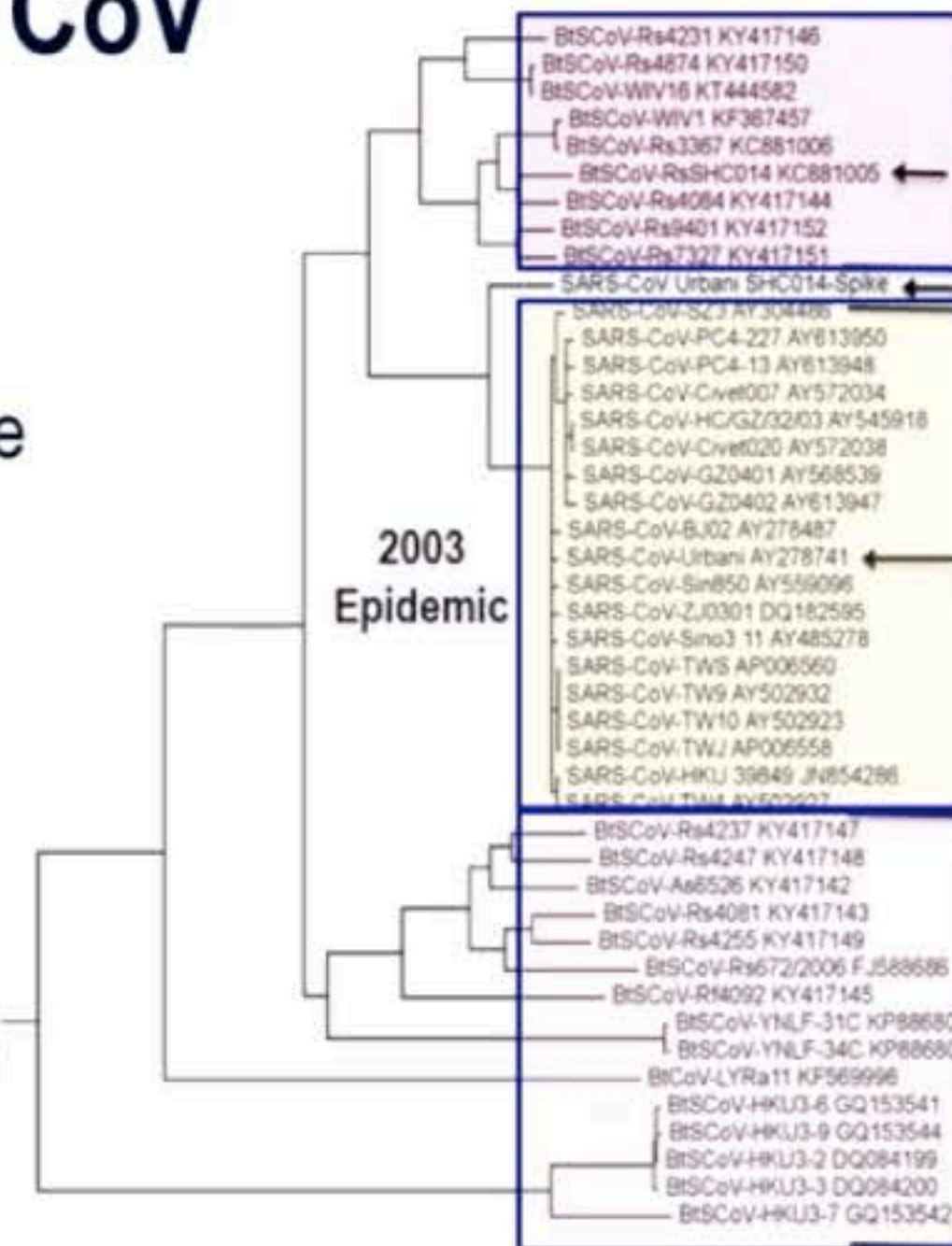
- CoV Genome Size: 32Kb
- CoV Mutation Rate
 - 10^{-6}
 - Environmental Change
 - ◆ Fidelity rates change
- High Rates RNA Recombination
 - 25% during mixed infections
 - Modular evolution
- Plastic Surface Glycoprotein
 - Tolerates high rates of mutation
 - Deletions and Insertions (tropism, antigenicity)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

→ Regulated Fidelity
(nsp14: ExoN)



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

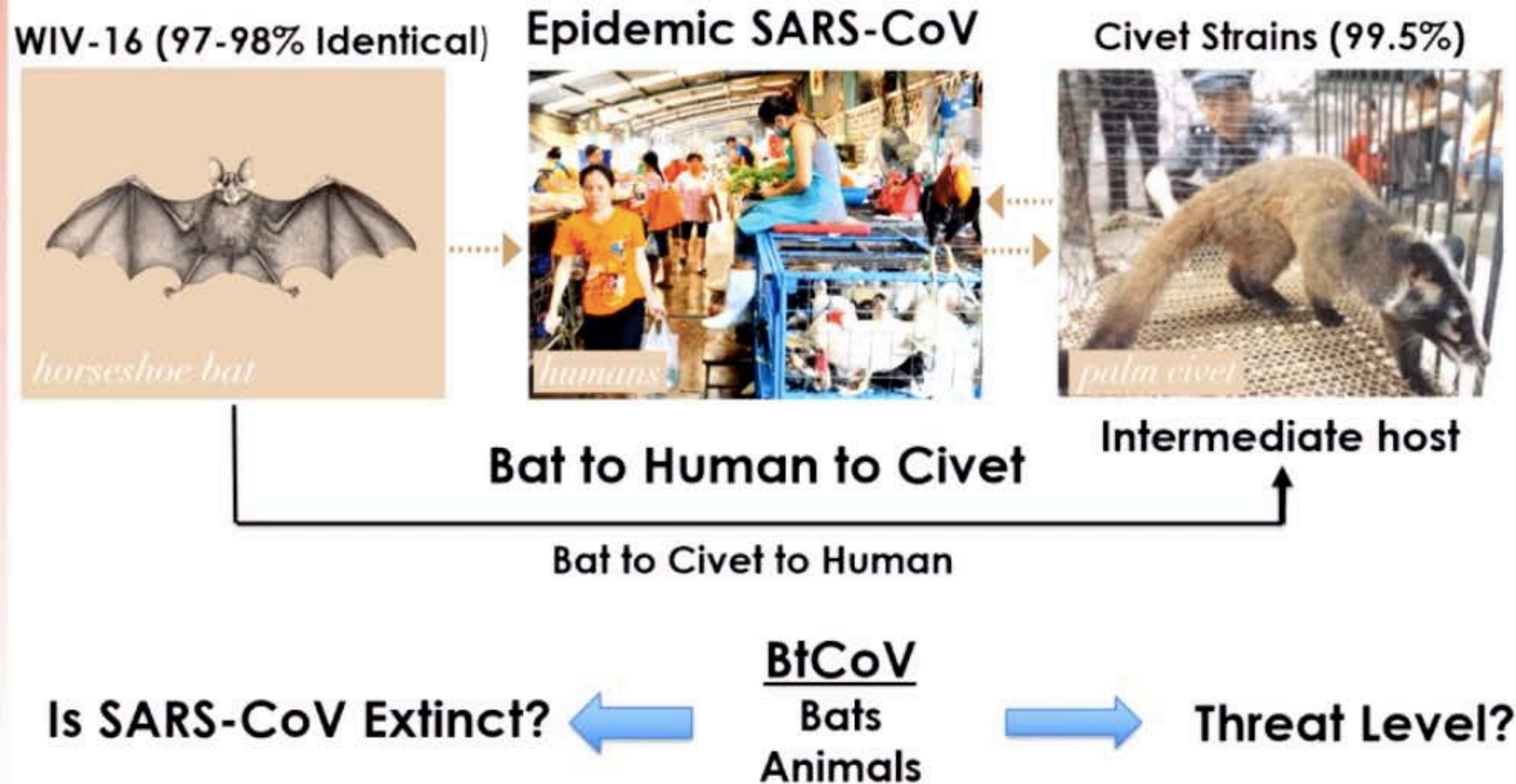
- **SARS-CoV Origins (Yellow)**
 - bats
 - Open Markets and Civet Intermediate Hosts
- **SARS-like bat CoV (Pink)**
 - Pre-epidemic potential (high/low)
 - Bats, low level seroprevalence in people residing near bat hibernacula
- **SARS-CoV 2**
 - Bats
 - Open Market Origins?

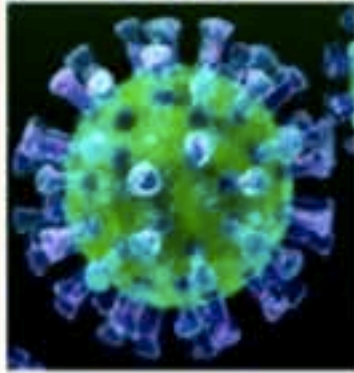


SARS-CoV Emergence in 2002 in China

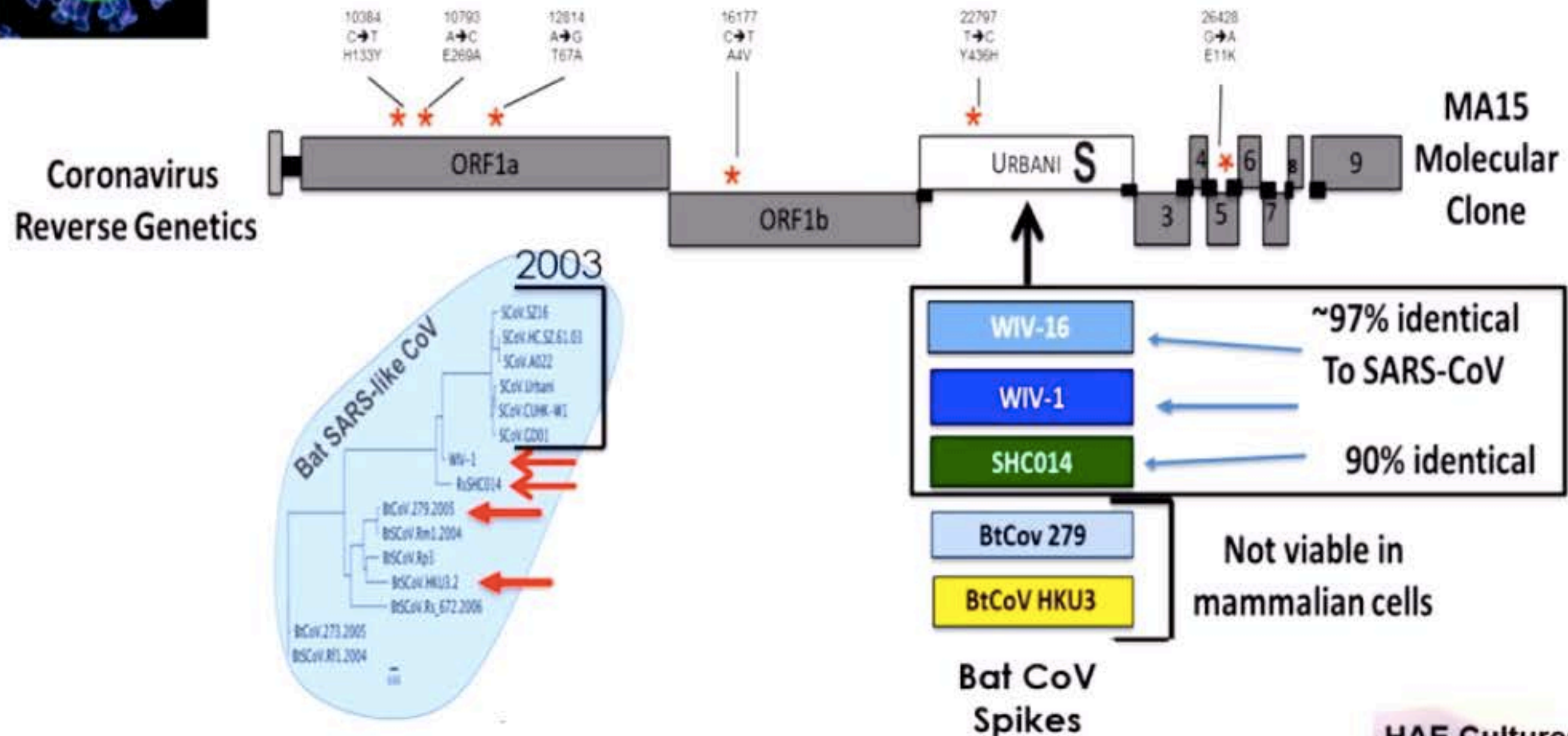
8,096 cases, 774 deaths, in 32 countries, Nov 1 2002 - July 31 2003

Most Likely Model



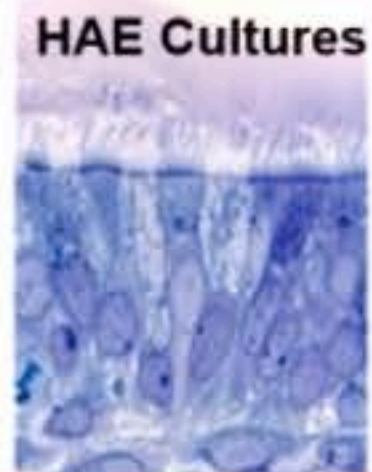


SARS MA15 Molecular Clone



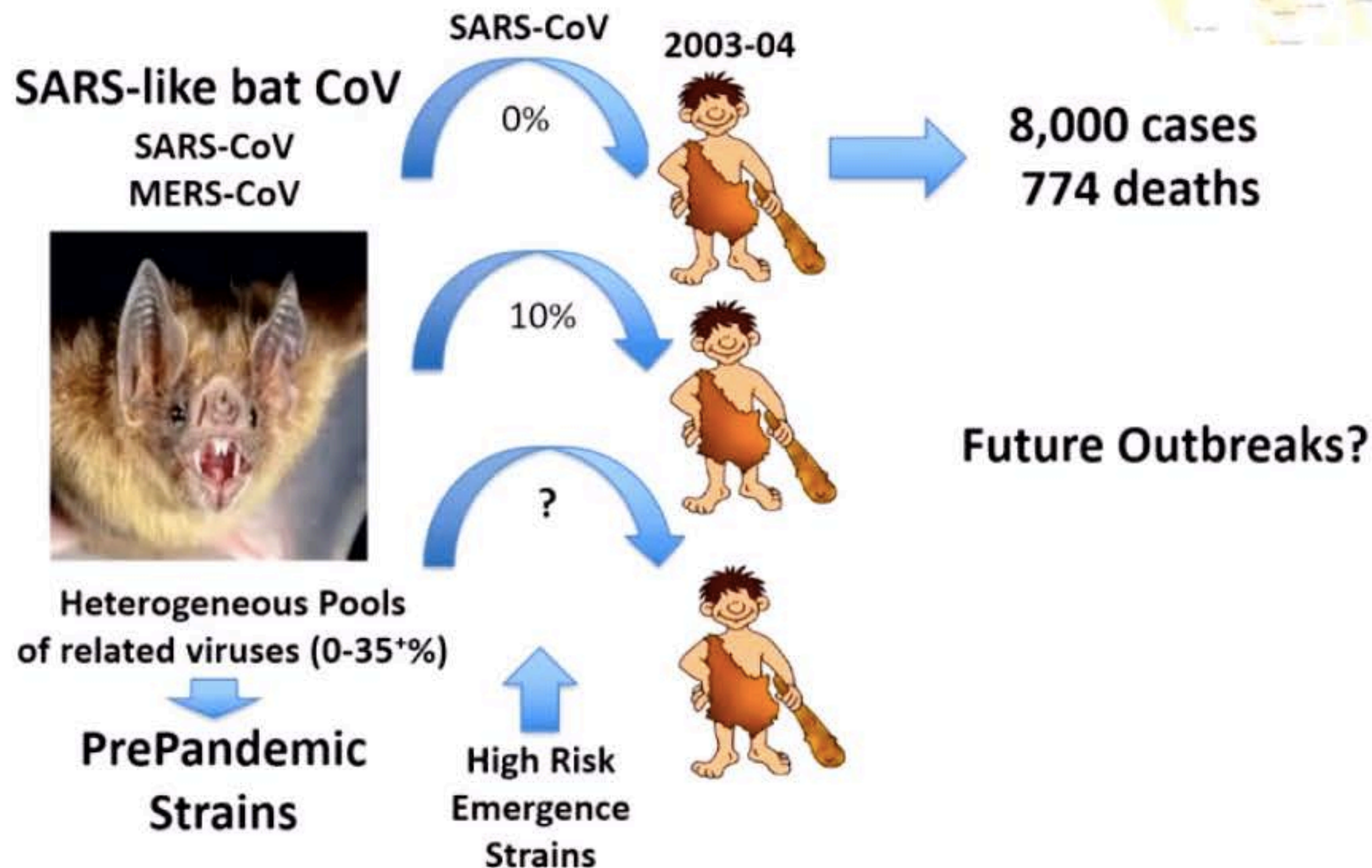
Replicate like SARS-CoV on primary human airway epithelial cells
 Use human receptor as well as SARS-CoV (if yes)
 Synthesize full length genomes, recover full length virus

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

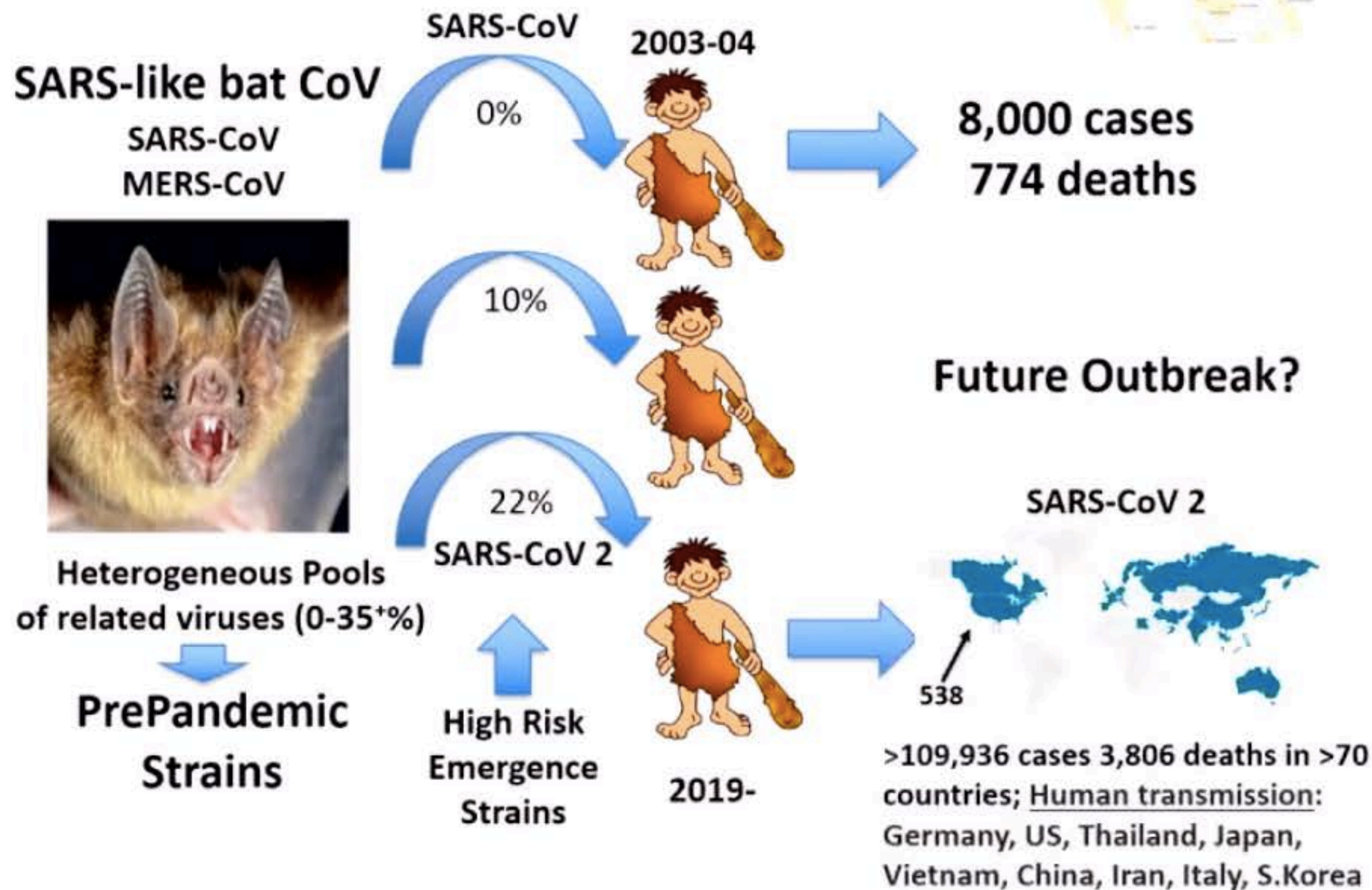


Z. Shi, Nature. 2013 Nov 28;503(7477):535-8.

Sheahan et al., JV 2008; Becker PNAS 2008; Menachery V et al., Nature Medicine 2015, Menachery PNAS 2016; Simon et al., mBIO 2017

Most Emerging Viruses

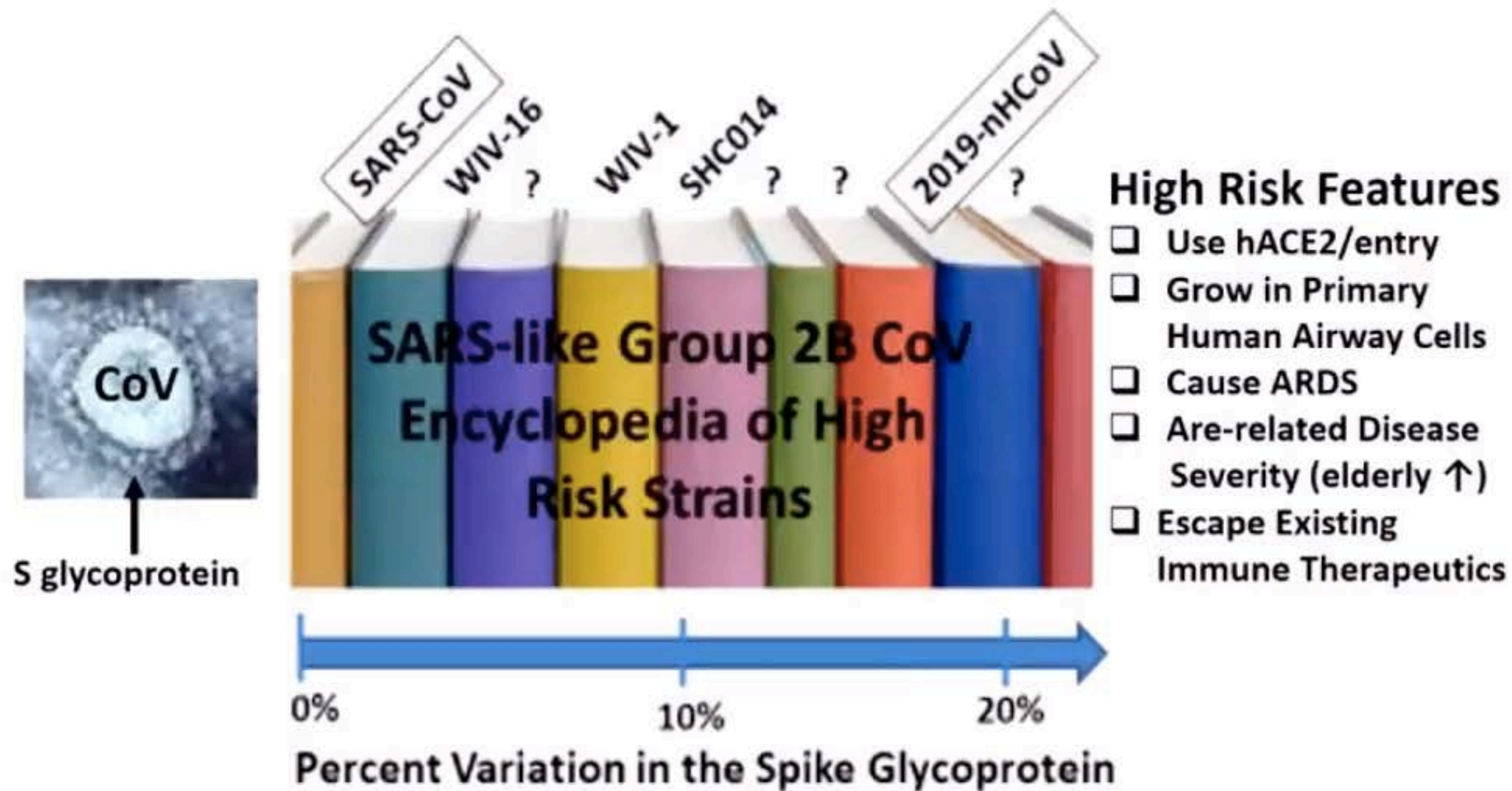
Zoonotic Reservoirs



Z. Shi, Nature. 2013 Nov 28;503(7477):535-8.

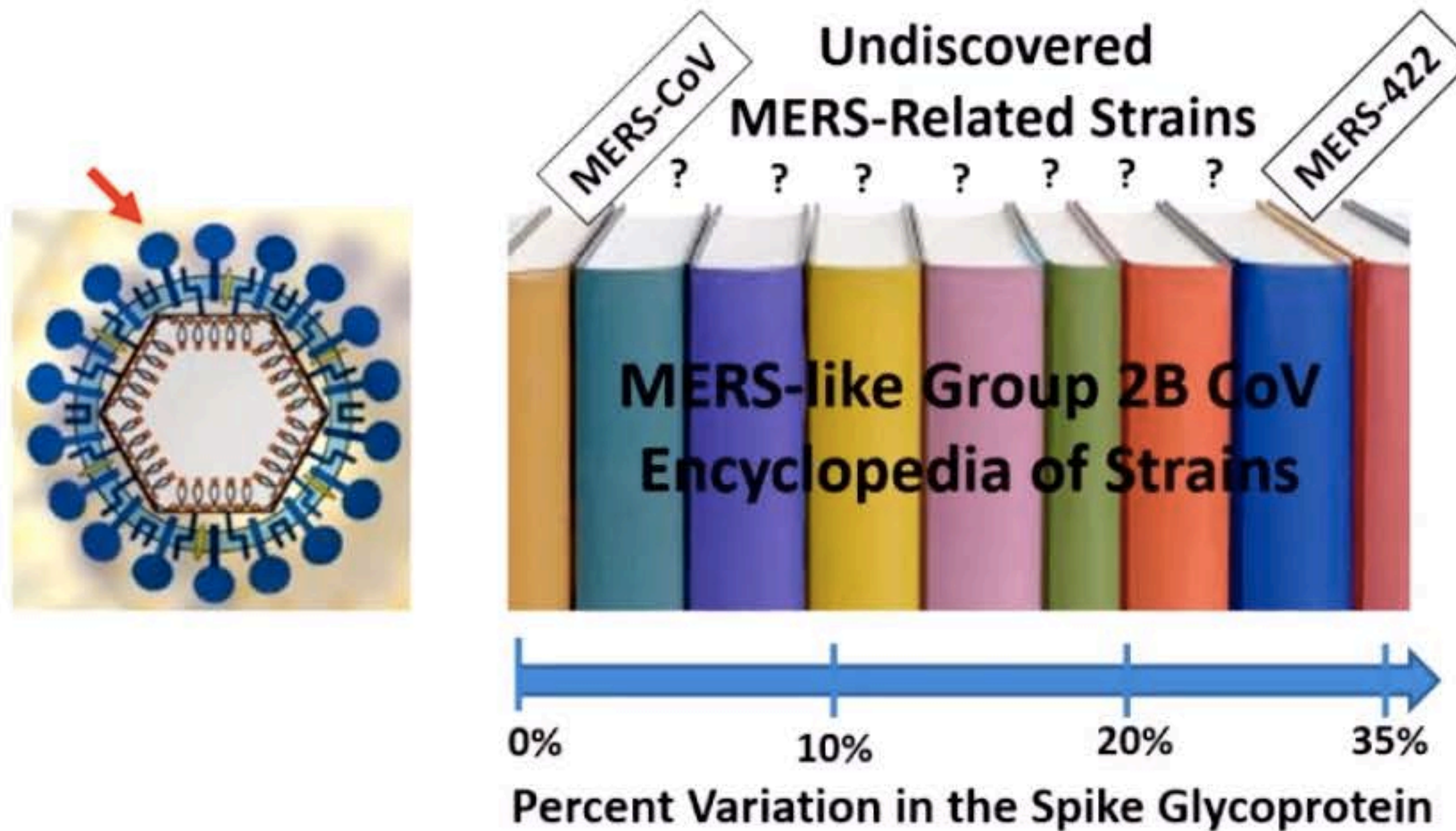
Sheahan et al., JV 2008; Becker PNAS 2008; Menachery V et al., Nature Medicine 2015; Menachery PNAS 2016; Simon et al., mBIO 2017

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



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

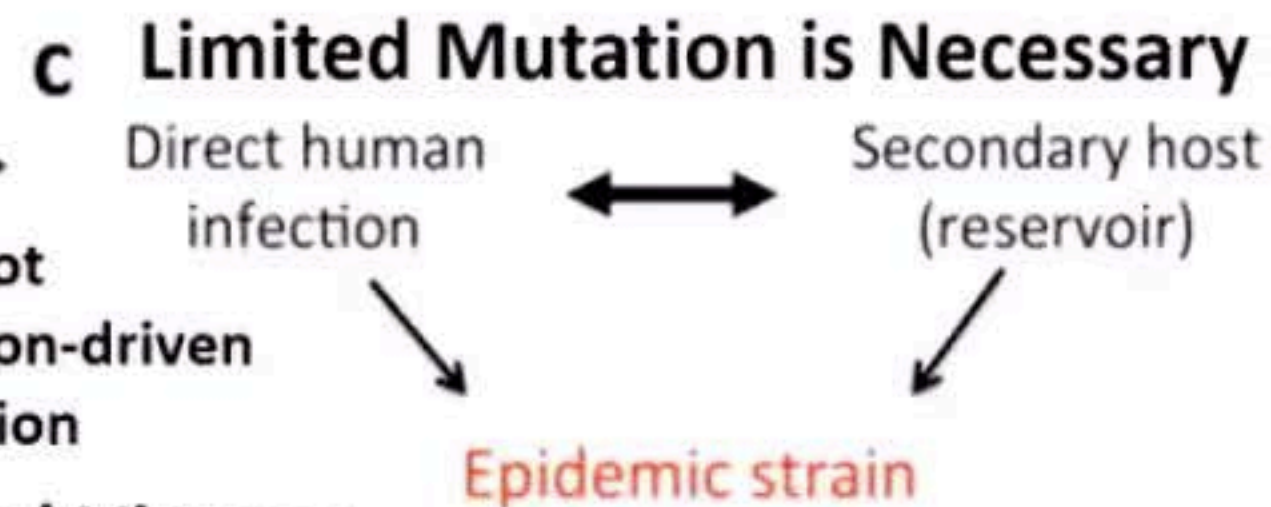
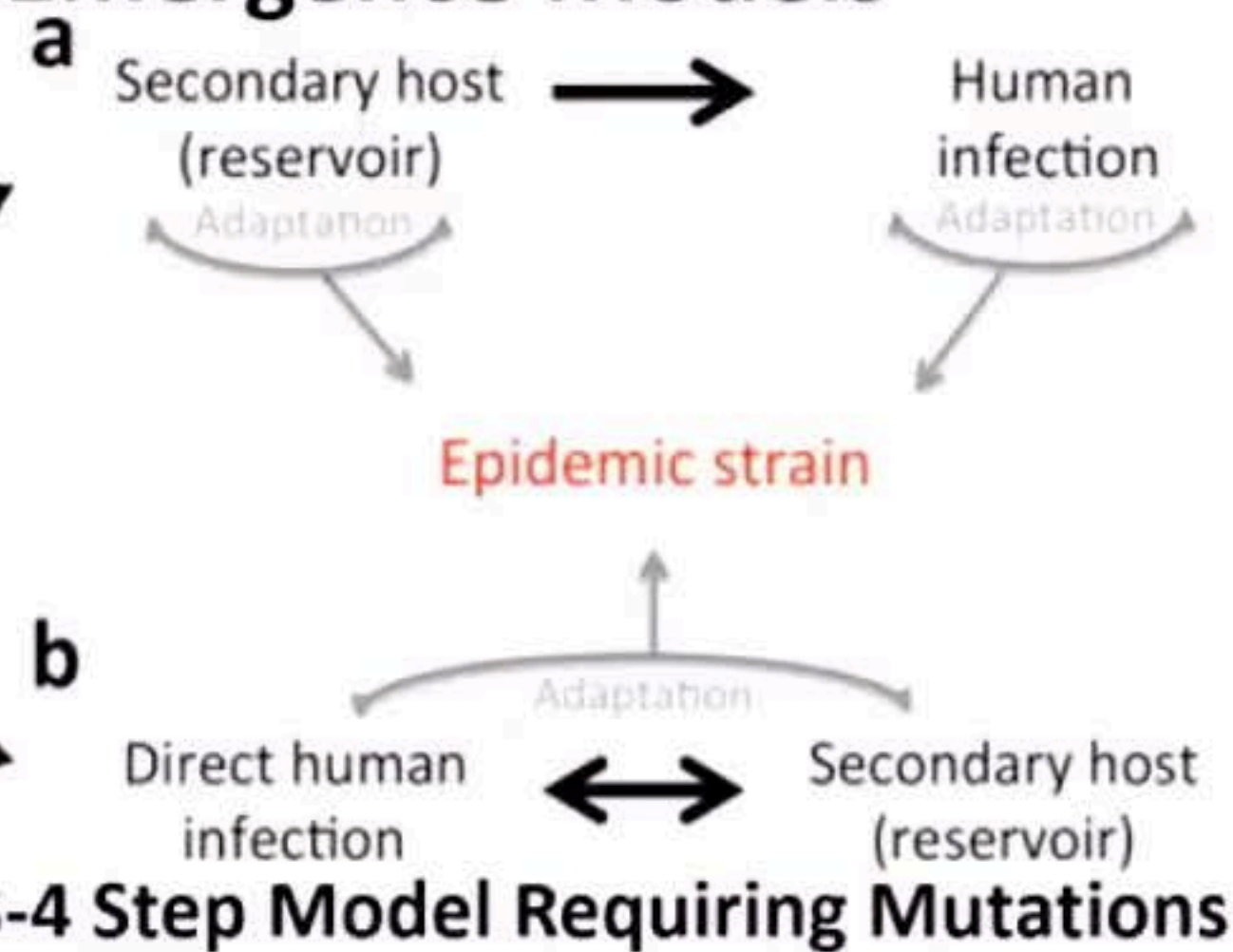
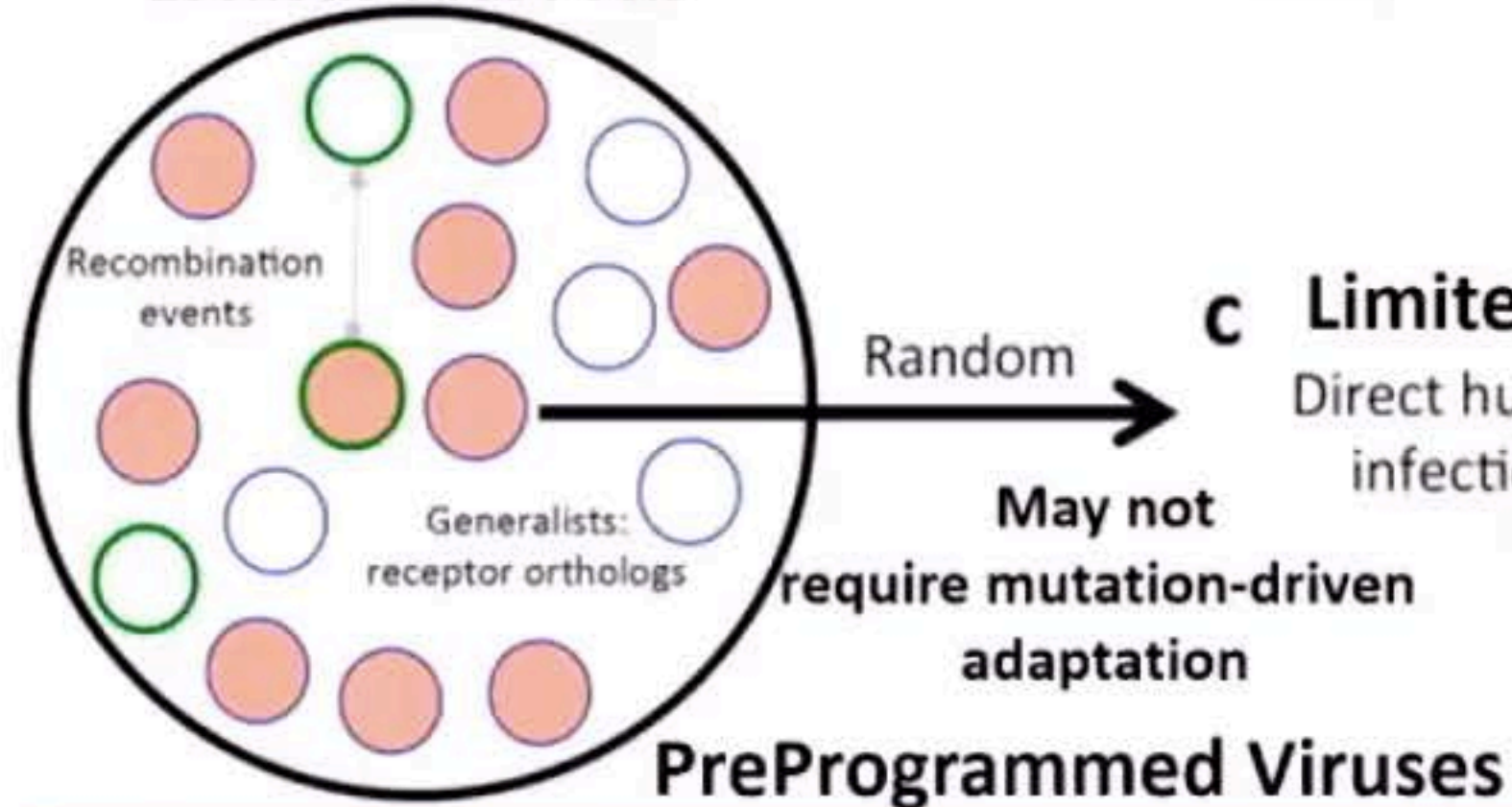
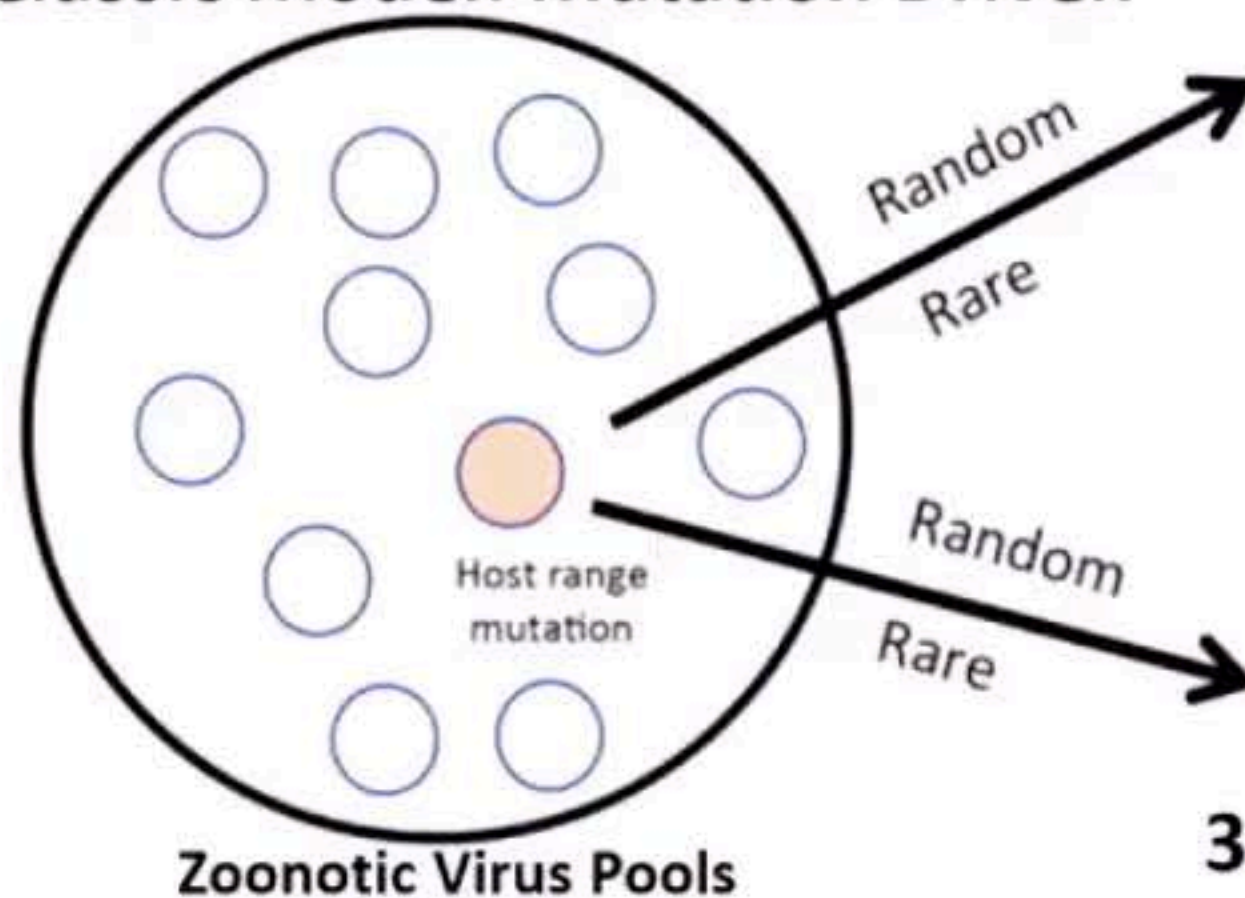
Known Group 2C MERS-like CoV Poised for Human Emergence



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

Zoonotic Virus Emergence Models

Classic Model: Mutation Driven



SARS-CoV 2

- 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/Message**
Never under-estimate epidemic potential of an emerging virus
- Wuhan Open Fish Market Closed (Jan 1, 2020)
 - Identified as a SARS-like 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	SARS-CoV 2
Virion Structure	Enveloped RNA virus	Enveloped RNA virus	Enveloped RNA virus
Outbreak period	2003-2004	2012-present	Dec 2019-present
Initial site of isolation	Guangdong province, China	Saudi Arabia	Wuhan, China
No. of countries/cases	29	27	>70
No. of cases (mortality)	8,096 (9.6%)	2,494 (~34%)	~109,936 (N=3,806)(3.4%)* >6,129 critical (~14%)
No. of cases U.S.	8	2 (2014)	538 (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	~3 (2.4-3.8)*
Super spreaders	Yes	Yes (common)	Yes (many examples)
Asymptomatic/mild Spread	No	Rare	Yes/Yes
Attack Rate	10.3% to 60%	4 to 20%	20-30%, 80% (early study)?
Transmission (including to HCP)	Droplet/Direct, Airborne/Indirect?	Droplet/Direct, Airborne/Indirect?	Droplet/Direct, Airborne/Indirect/Fecal
Treatment (PEP)	Supportive (none)	Supportive (none)	Supportive (drugs CU)
Infection Prevention	Airborne, contact, face shield	Airborne, contact, face shield	Airborne, contact, face shield

*About 83% of cases are mild or asymptomatic, Mortality Rates are age Stratified:

80+=14.8%, 70-79=8%; 60-69=3.6%, 50-59=1.3%, 40-49=0.4%, <40=0.2%, less than 15=0%.

Cause of Death: ARDS

- Emerging CoV and IAV

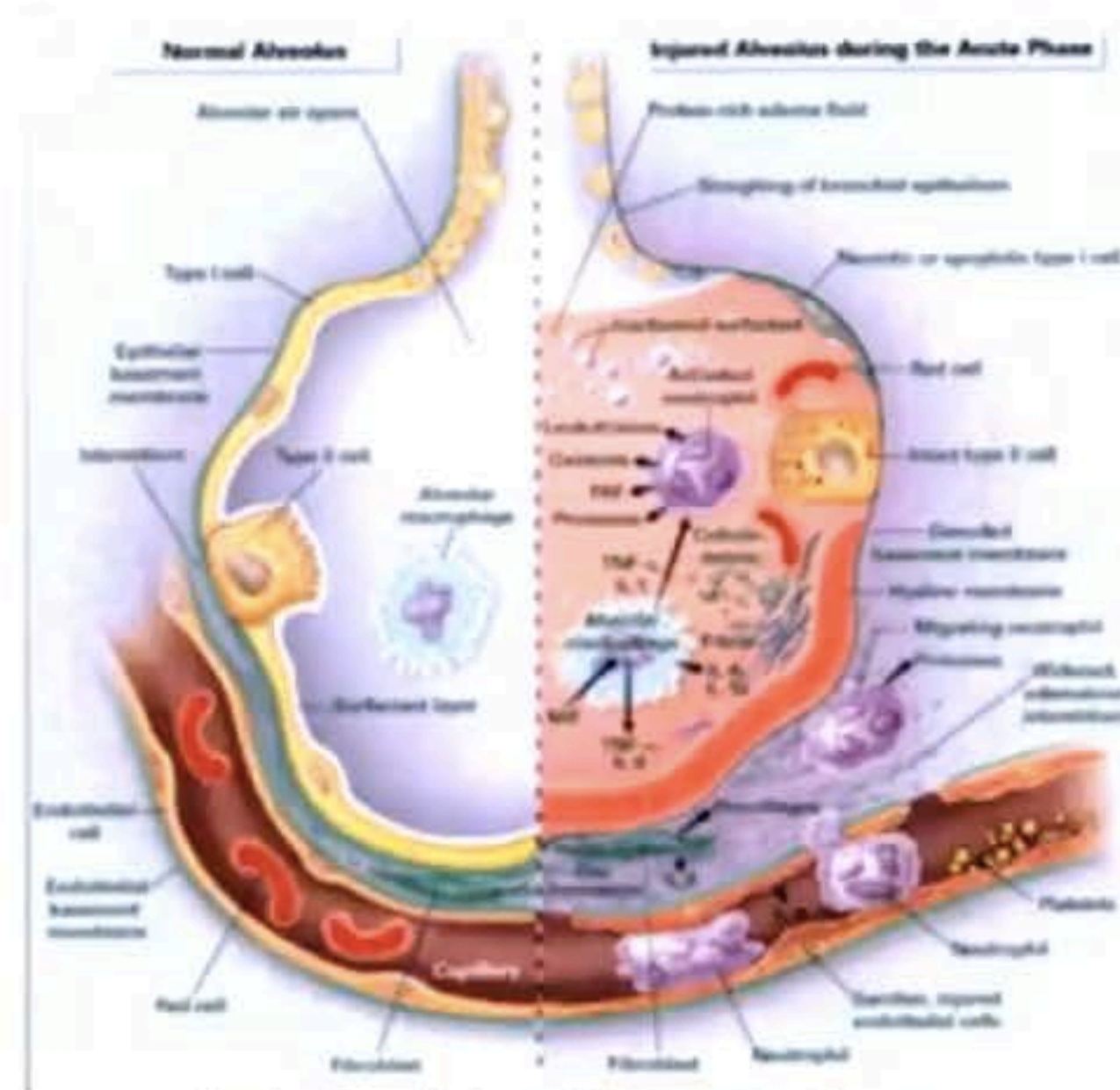
- | | | |
|------------|-----------|-----------|
| • SARS-CoV | H7N9 | 2019-nCoV |
| | H1N1-2009 | 1918 H1N1 |
| • MERS-CoV | H5N1 | |

- Acute Respiratory Distress Syndrome (ARDS) (SARS, MERS, 2019-nCoV)

- End stage lung disease
- ~30% mortality, ~17% require respiratory assistance (13% invasive ventilators)
- Little evidence 2nd bacterial infections

- ARDS:

- ~75,000 deaths in US
- 1 million deaths worldwide
- Progress: Pulmonary Fibrosis
- 5 million deaths/worldwide



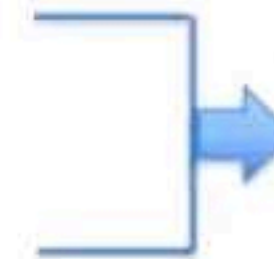
Acute Respiratory Distress Syndrome
(hypoxia, oxygen insufficiency, organ failure, death)

Infections in children generally mild, males generally develop more serious disease

Wang et al., 2020, JAMA Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China

SARS-CoV Outbreak Drivers

- **Animal Markets-Civets**



civet cats were culled
their sale and
consumption banned

- **Hospitals: Epicenters for Disease Expansion**



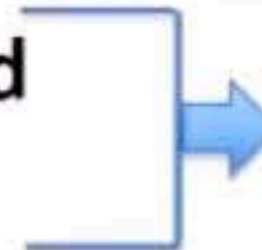
Health Care Workers
Super-spreader Events



Barrier
Nursing

- **Transmission occurs 24-36 hrs after Disease Onset**

- Community spread limited
- Few asymptomatic cases



Vulnerable to quarantine
Contact Tracing

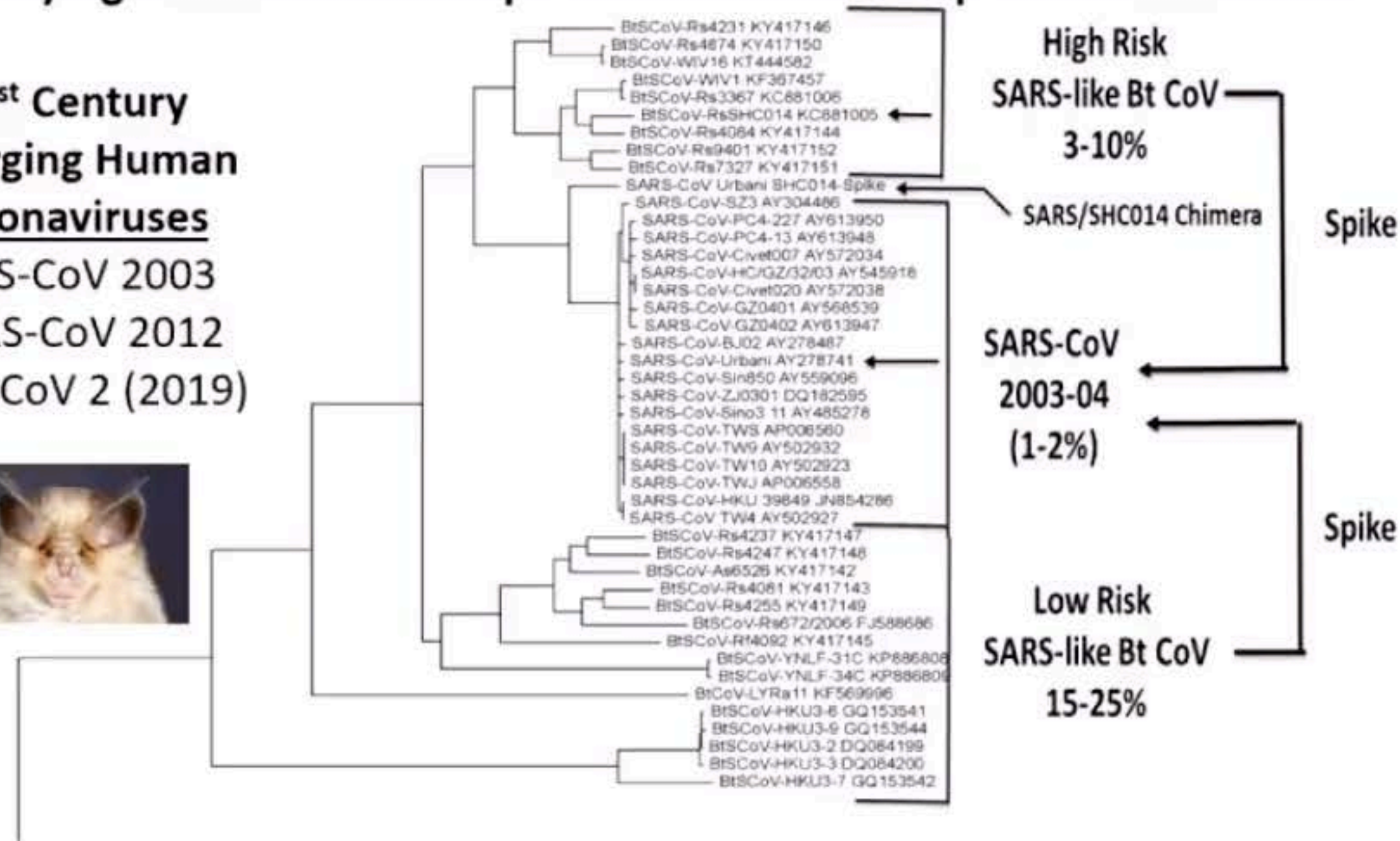
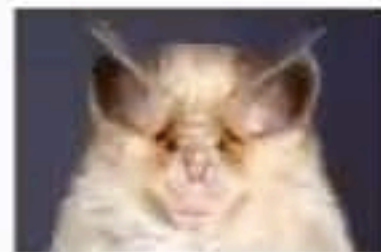
$R_0 < 1.0$ (epidemic goes extinct)

Public Health Response

We not so fortunate with SARS-CoV 2 (difficult)

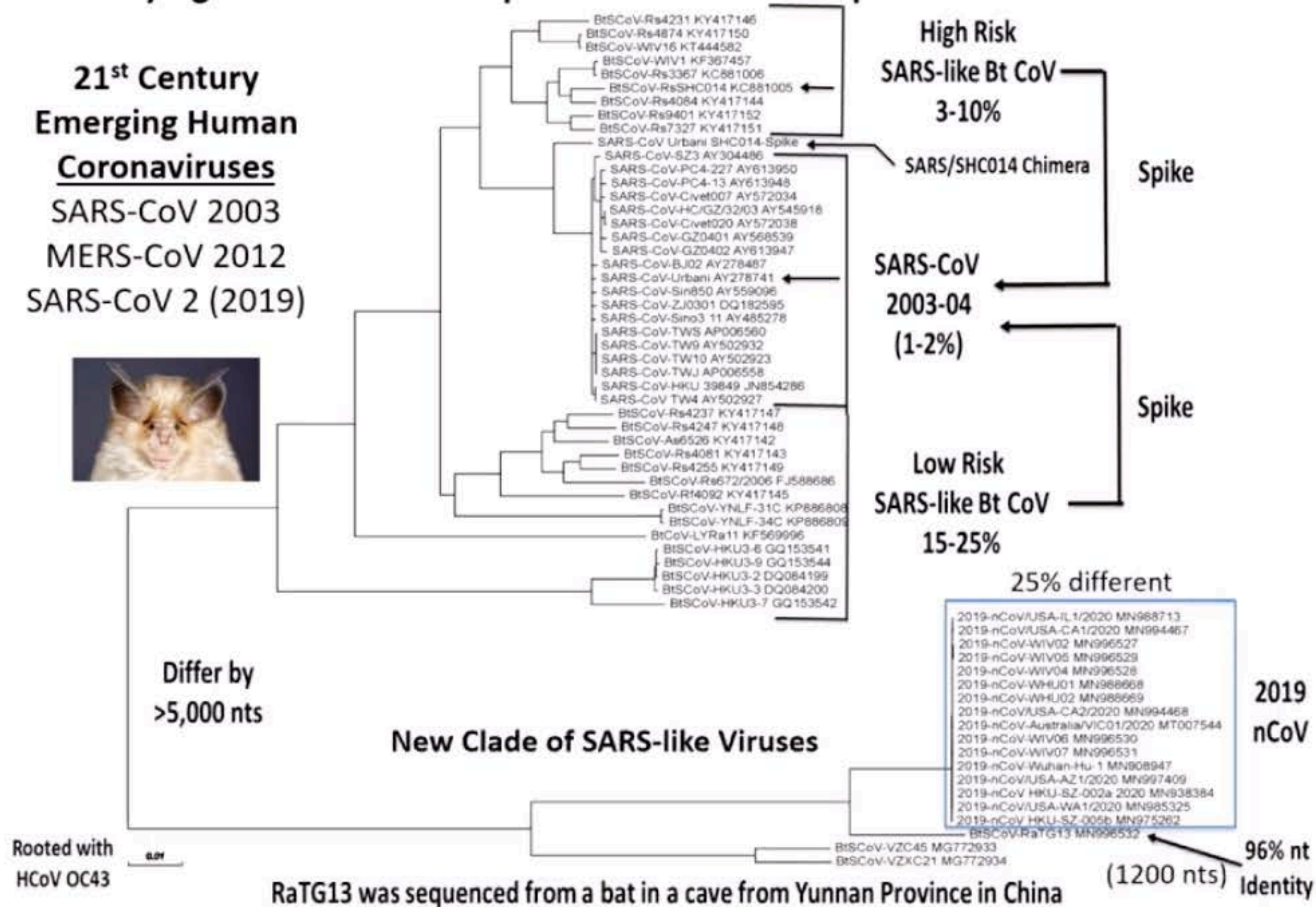
Phylogenetic Relationships Between the Group 2B Coronaviruses

**21st Century
Emerging Human
Coronaviruses**
SARS-CoV 2003
MERS-CoV 2012
SARS-CoV 2 (2019)

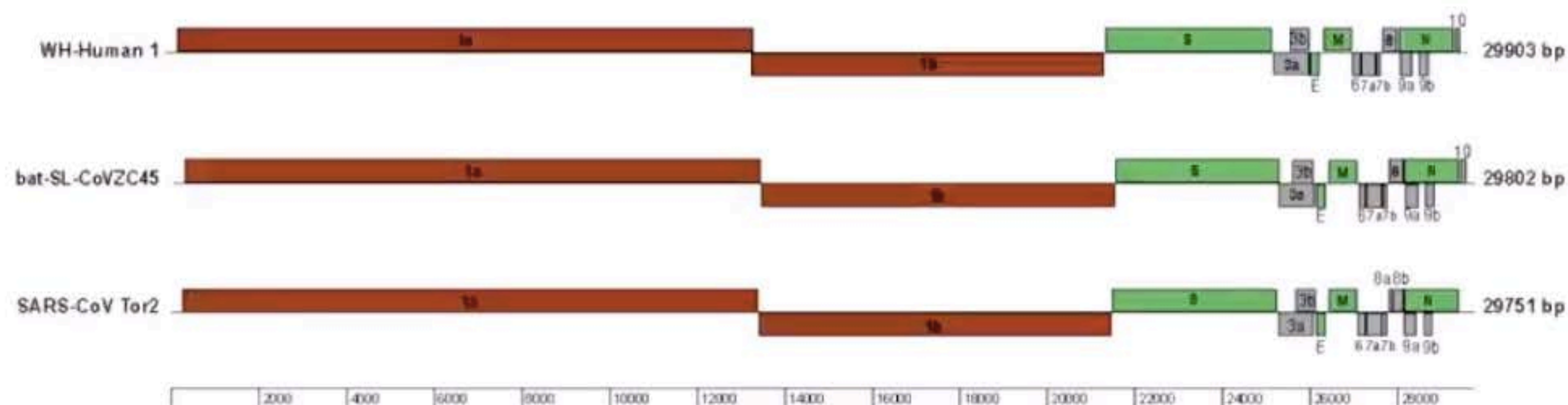


Phylogenetic Relationships Between the Group 2B Coronaviruses

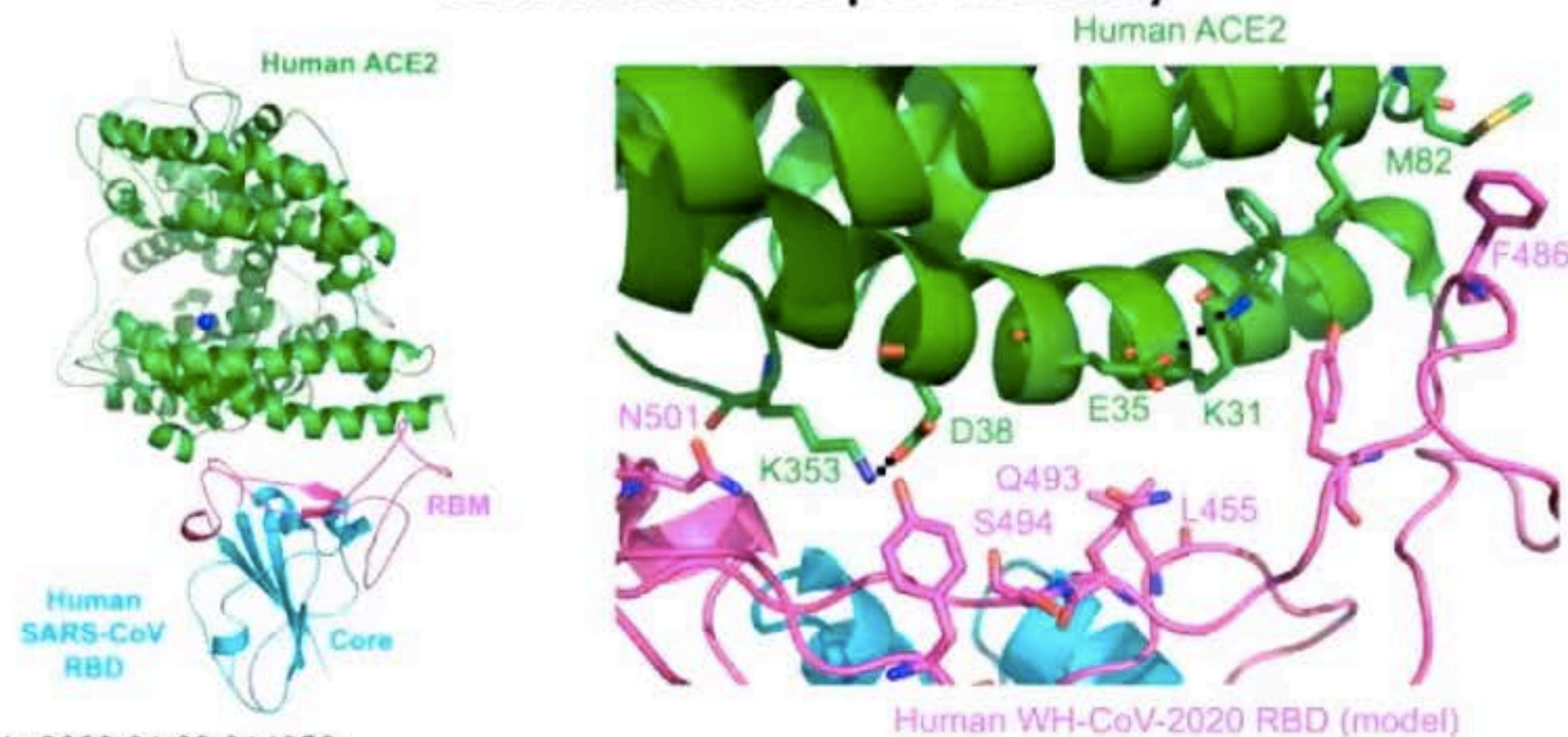
**21st Century
Emerging Human
Coronaviruses**
SARS-CoV 2003
MERS-CoV 2012
SARS-CoV 2 (2019)



SARS-CoV 2 Genome Organization



Uses hACE2 Receptor for Entry



Zhou et al., bioRxiv 2020.01.22.914952

SARS-like CoV Group 2B S-RBD ACE2 Interface Sites*

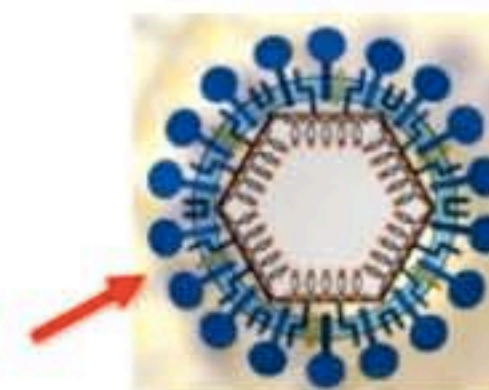
	Virus	402	426	436	440	442	472	473	475	479	484	486	487	488	491	
High Risk Strains	SARS-CoV	T	R	Y	Y	Y	L	N	Y	N	Y	T	T	G	Y	Use hACE2 cACE2 bACE2 mACE2 7/14
	CUHK-W1	T	R	Y	Y	Y	L	N	Y	N	Y	T	T	G	Y	
	GD03	T	R	Y	Y	Y	P	N	Y	N	Y	T	S	G	Y	
	HC/SZ/61/03	T	R	Y	Y	Y	P	N	Y	R	Y	T	S	G	Y	
	WIV16	T	R	Y	Y	S	F	N	Y	N	Y	T	N	G	Y	
	Rs3367	T	R	Y	Y	S	F	N	Y	N	Y	T	N	G	Y	
	WIV1	T	R	Y	Y	S	F	N	Y	N	Y	T	N	G	Y	
	SHC014	T	N	Y	Y	W	P	N	Y	R	F	T	A	G	H	
Low Risk Strains	PDF2386	T	N		Y	L	L	G	Y	K	T	T	V	G	Y	?
	ZXC45	T	A		Y	S				S	N	N	V	P	Y	
	ZXC21	T	A		Y	S				S	N	N	V	P	Y	
2019 nCoV	RaTG13 BtCoV	T	K	F	Y	L	L	N	Y	Y	Y	T	D	G	H	8/14
	WUH Original	T	N	Y	Y	L	F	N	Y	Q	Q	T	N	G	Y	
	WUH 402121	T	N	Y	Y	L	F	N	Y	Q	Q	T	N	G	Y	
Low Risk Strains	Rp3	T	A		Y	S				S	Y	S	V	P	Y	
	HKU3	T	A		Y	S				S	N	N	V	P	Y	
	Rm1	T	A		Y	S				S	Y	S		P	Y	
	279	T	A		Y	S				S	Y	S		P	Y	
	Rf1	T	A		Y	S				S	N	N	V	P	Y	
	273	T	A		Y	S				S	N	N	V	P	Y	

14 Contact Interface Sites that Bind the ACE2 Receptor

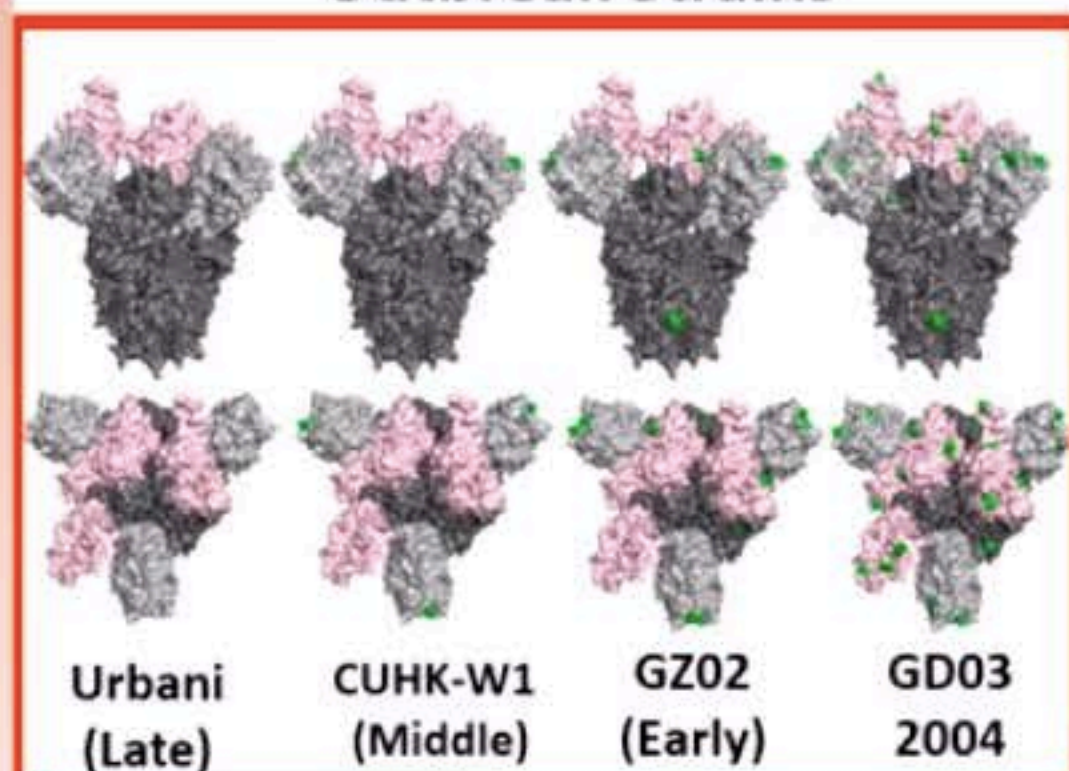
Variation across Interface Site Can Alter Orthologue Species ACE2 Receptor Usage

*Conservations based on BLOSUM62 Matrix

Immune Therapeutic Countermeasures



2003-2004 SARS-CoV
Outbreak Strains

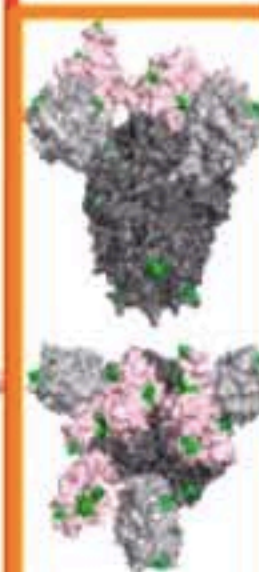


Urbani
(Late)

CUHK-W1
(Middle)

GZ02
(Early)

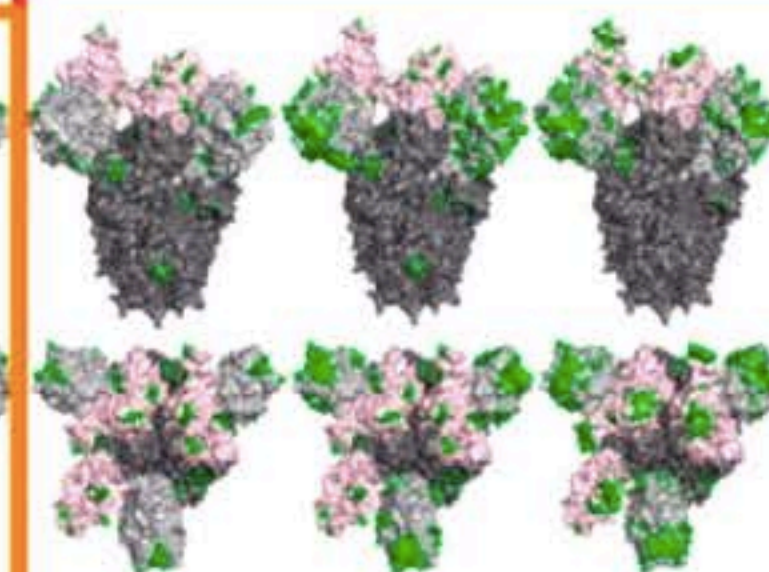
GD03
2004



SZ/61/13

Civit
Intermediate

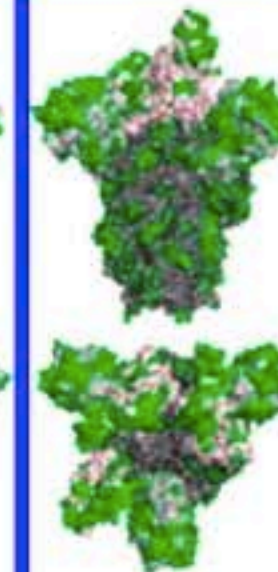
Group 2B SARS-like Bat Coronaviruses



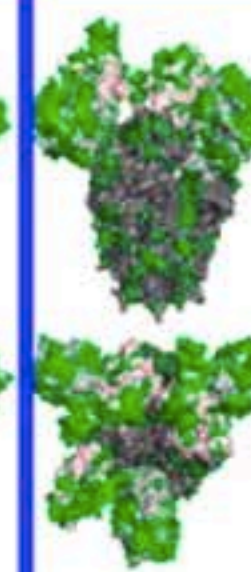
WIV16

WIV1

SHC014



2019 nHCoV



HKU3

All Are Poised for Human Emergence

2019-2020
Outbreak

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

Broadly active drugs/vaccines are essential to control zoonotic CoV

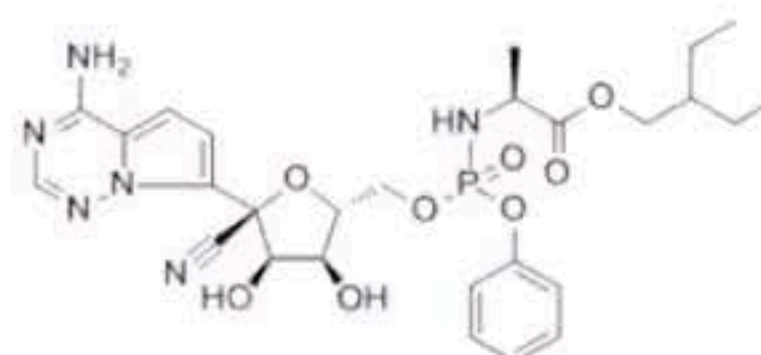
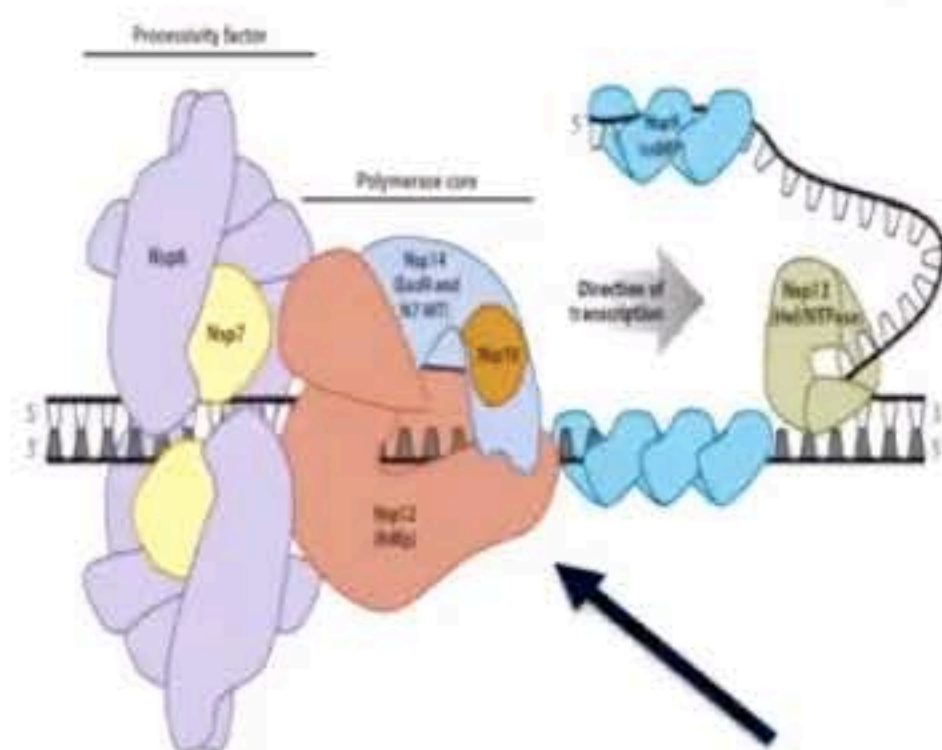
● variation

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
 - Adjuvanted S glycoprotein Vaccines
- **Evidence for Enhancing Antibodies (controversial)**
 - Primates
 - Cell Culture
- **SARS-CoV 2 ??—we don't know (right adjuvant)**

Therapeutic Interventions

- No approved drugs, immune therapeutics and vaccines against any group 2b coronavirus
- **Experimental Drugs (nsp12-RdRp target)**
 - Remdesivir-ribonucleoside inhibitor



monophosphoramidate prodrug
of an adenosine analog, GS-5734

Chain Termination

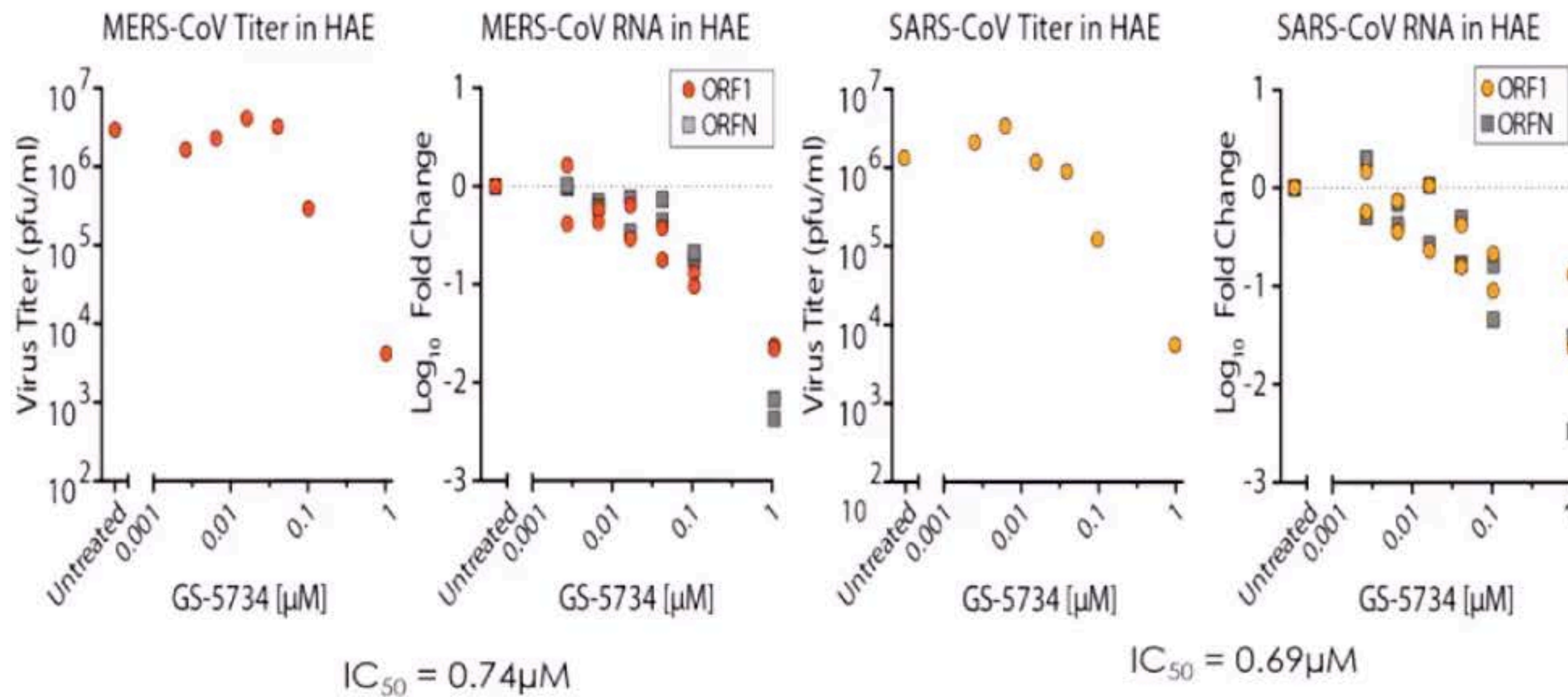
- Combination lopinavir, ritonavir, and interferon beta tested in China?
- Therapeutic antibodies (MERS, likely soon for SARS-CoV 2)

Sheahan et al., *Nature Communications* 11, 222 (2020)

Sheahan et al., *Sci Transl Med*. 2017 Jun 28;9(396).

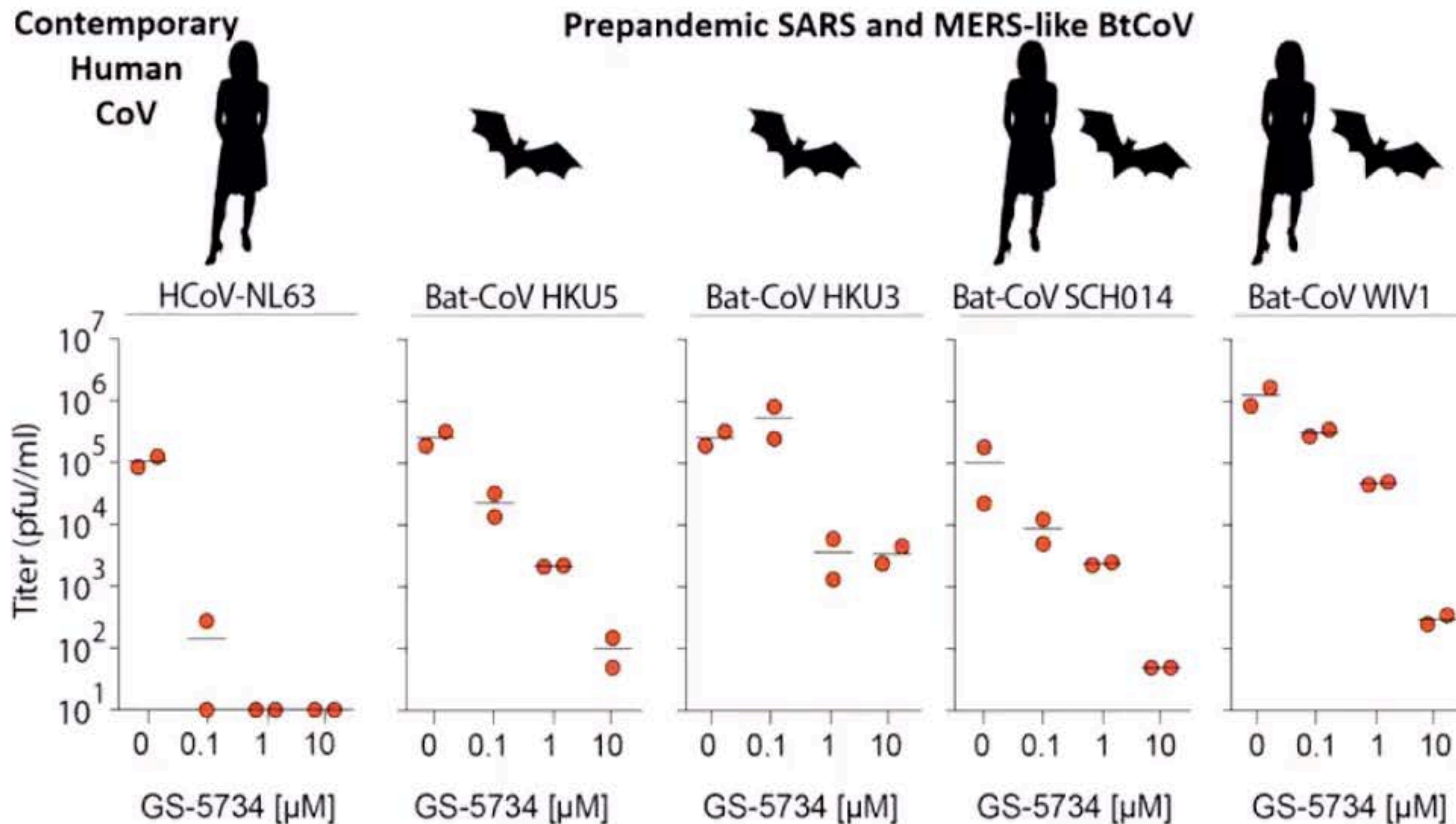


Antiviral effect in primary human cells



Sheahan et. al 2017 Science Translational Medicine 2017 Jun 28;9(396)

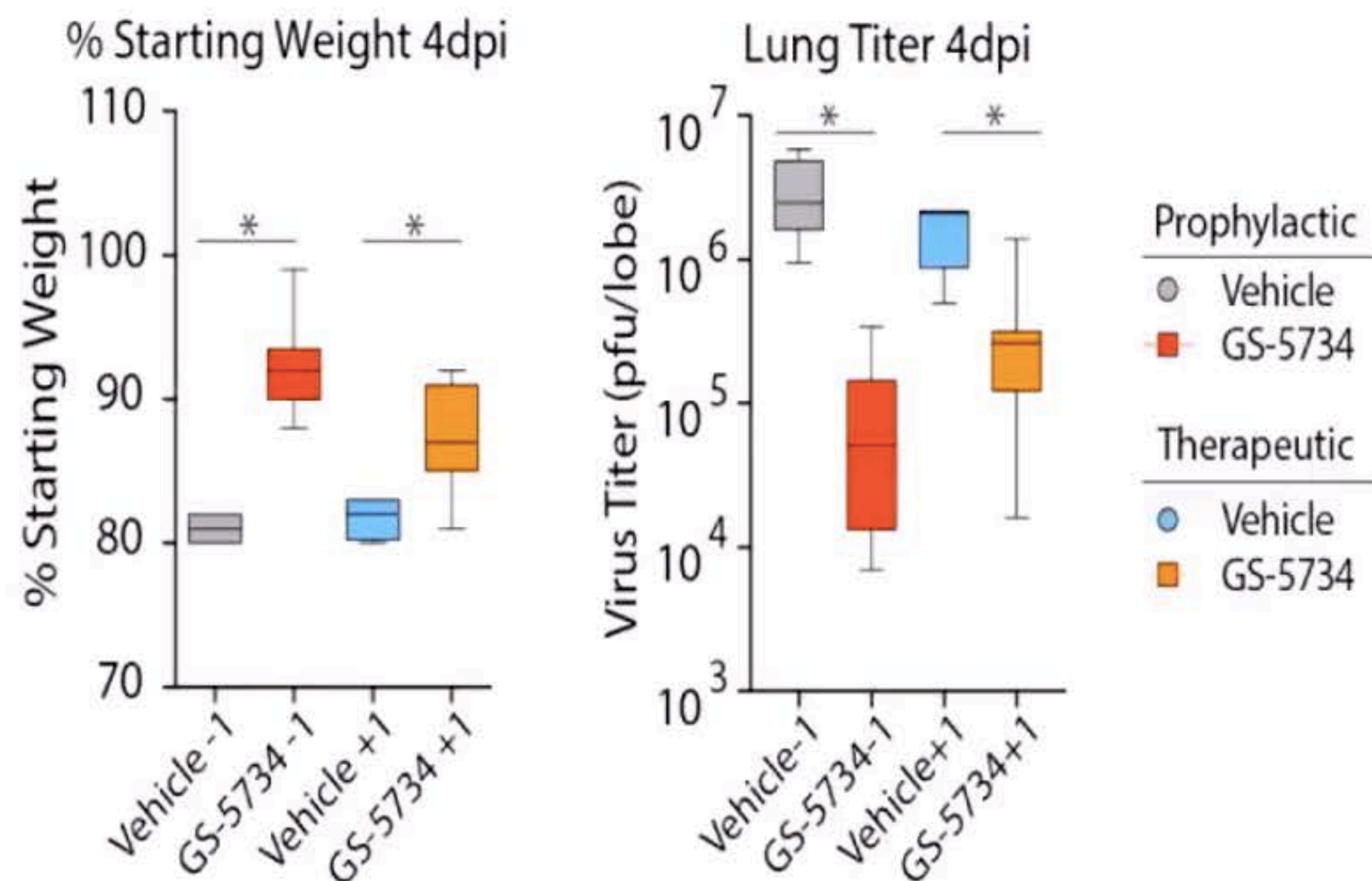
Efficacy of GS-5734 against diverse CoV



GS5734 Also Inhibits SARS-CoV 2 replication in cells in culture

Sheahan et. al 2017 Science Translational Medicine 2017

GS-5734 diminishes SARS-CoV Disease



Enhances Respiratory Function
Effective against SARS-like HKU3 in Aged animals
Predict efficacy against SARS-CoV 2

Sheahan et. al 2017 Science Translational Medicine 2017

Clinical Testing of Remdesivir for Treatment of COVID-19

- Five randomized controlled trials in hospitalized patients with diagnosed COVID-19

COVID-19 Study Design	Location	Sponsor	Study size (randomization)	First patient enrolled	Primary endpoint
Severe Double-blind Placebo-controlled	Wuhan, China	Capital Medical University, China	N = 453 (2:1) 10d RDV:Placebo	Feb 6, 2020	Time to clinical improvement by Day 28
Mild/Moderate Double-blind Placebo-controlled	Wuhan, China	Capital Medical University, China	N = 308 (1:1) 10d RDV:Placebo	Feb 13, 2020	Time to clinical recovery by Day 28
All hospitalized* Double-blind Placebo-controlled	Global	NIAID	N = 394 (1:1) 10d RDV:Placebo	Feb 21, 2020	Clinical status at Day 15 based on 7-point ordinal scale
Severe Open-label	Global	Gilead	N = 400 (1:1) 10d RDV:5d RDV	Enrollment not yet started	Normalization of fever and O ₂ saturation by Day 14
Moderate Open-label	Global	Gilead	N = 600 (1:1:1) 10d:5d RDV: Placebo	Enrollment not yet started	Hospital discharge by Day 14

* Stratified by disease severity at enrollment

Future: 2019 nHCoV

- **Another nucleoside inhibitor drug, EIDD 1931 is under FDA review**
 - Manuscript is under review
- **We have tested both drugs in vitro against 2019 nCoV, in vivo studies are in process**

Baric Laboratory

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National Institute
of Allergy and
Infectious Diseases

