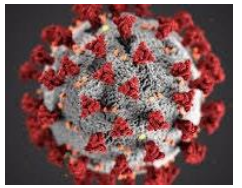
UNIVERSITÀ DEGLI STUDI DI BRESCIA

Antivirali e resistenza: HIV e SARS-COV-2 a confronto

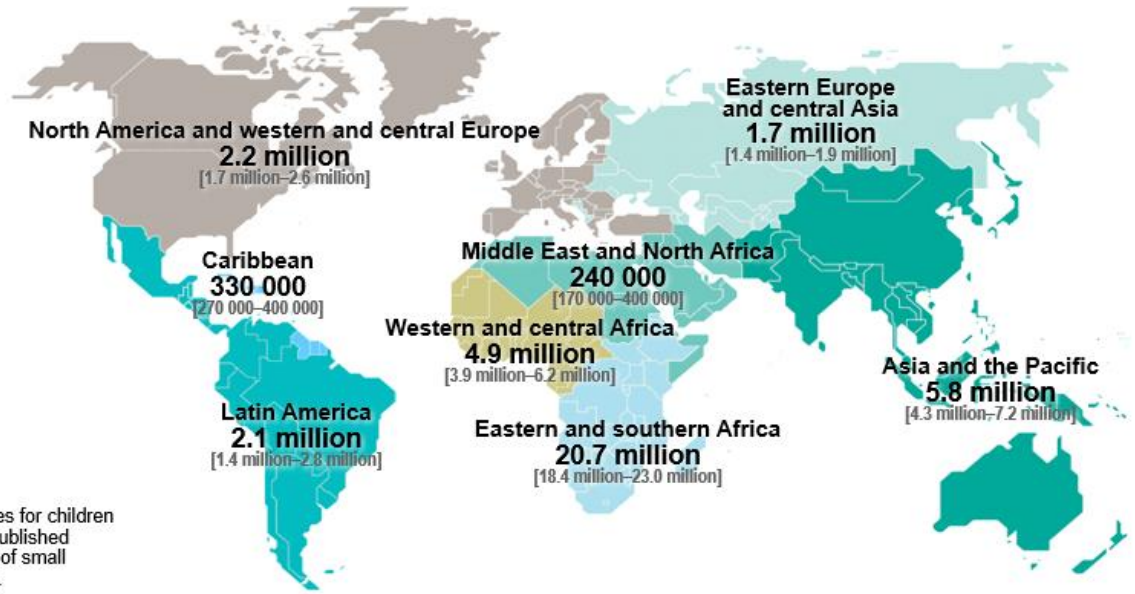
Eugenia Quiros Roldan



The two great viral pandemics of our days



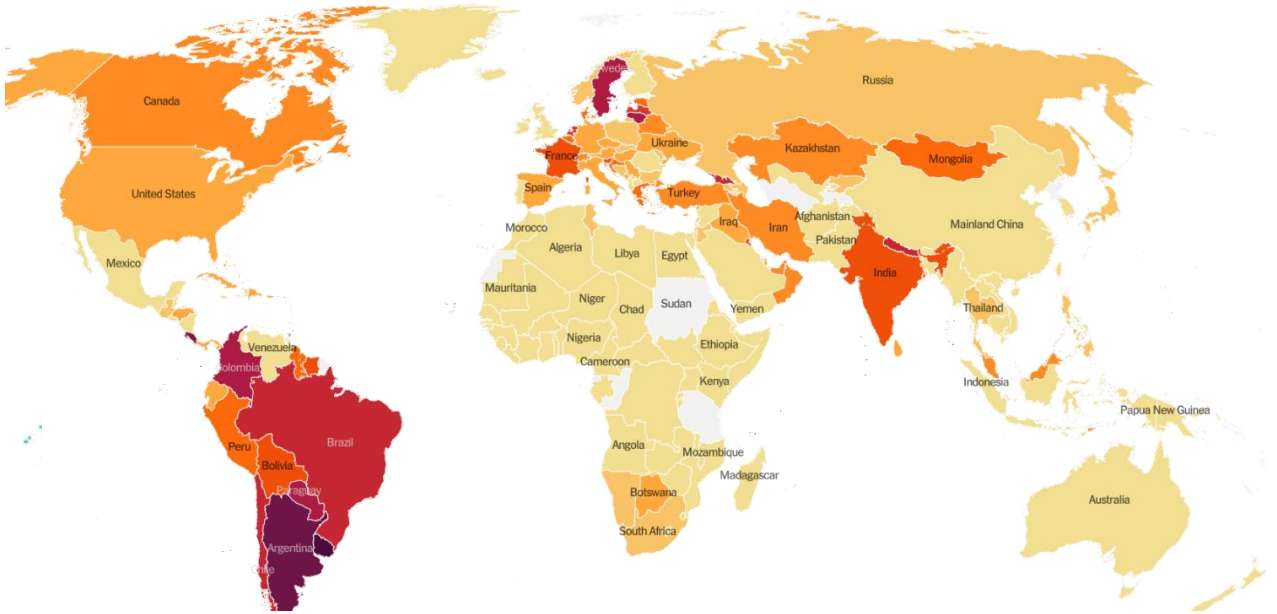
Adults and children estimated to be living with HIV | 2019



Since beginning of pandemic
HIV Infected= 84.2 million [64.0–113.0 million]
Deaths = 40.1 million [33.6–48.6 million]

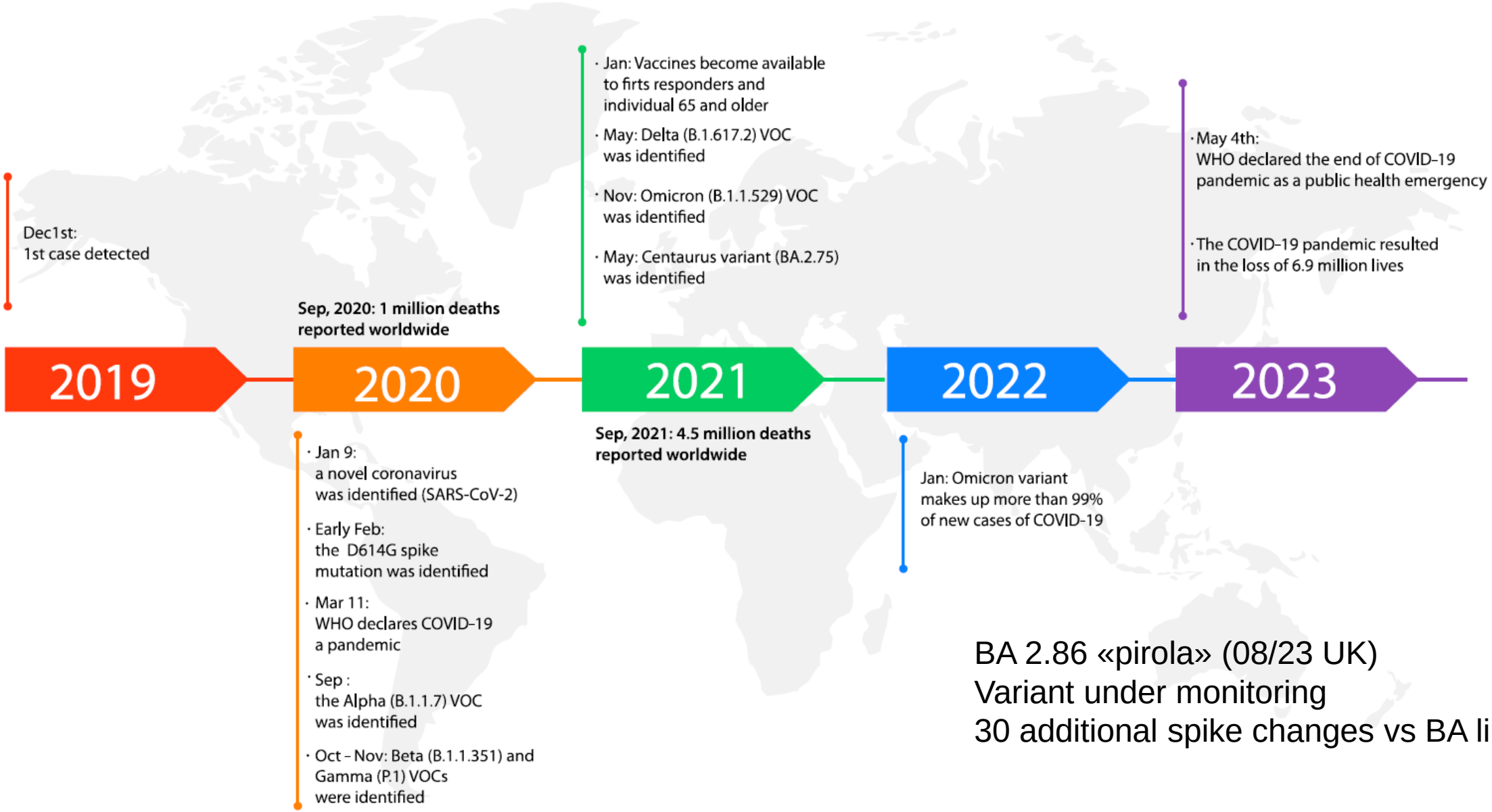
Although first reported June 5, 1981, declared an epidemic in 1985

COVID-19 cases around the world (september 6, 2023)



Since beginning of pandemic
Infected= 768 million
Deaths = 7 million

First reported December 1st, 2019
Pandemic declared March 11, 2020
Ended May 5, 2023



BA 2.86 «pirola» (08/23 UK)
Variant under monitoring
30 additional spike changes vs BA lineage

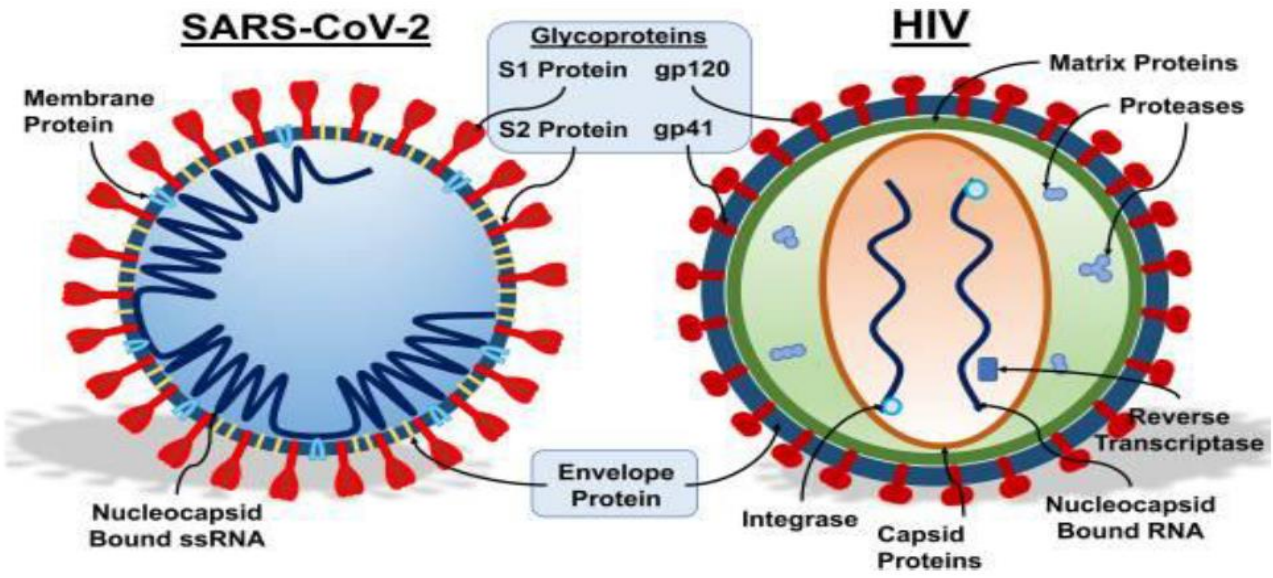
Comparing viruses side by side…

sRNA 27-32 Kd (longest known)

ssRNA 9.3Kd

J Biotechnol Biomed 2021; 4 (4): 147-158

DOI: 10.26502/jbb.2642-91280041



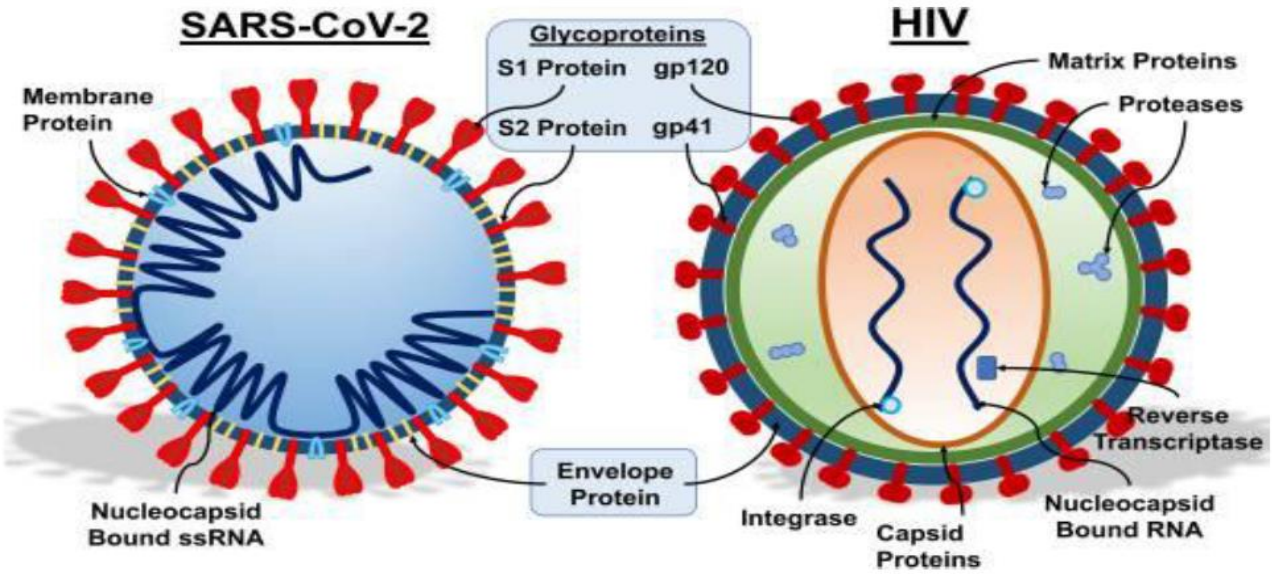
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

- Coronavirus family
- >95% homology bat coronavirus
- Likely made the transfer to humans 2019 (market Huan)
- SARS-Cov-2 is transmitted by respitarory dropless, airbone
- No leads to a chronic infection (?)
- Results in respiratory insufficiency and death in frailty people
- RNA-directed RNA polymerase (RdRp) with proof reading activity.

Human Immunodeficiency Virus (HIV)

- Retroviridae virus family,
- 50% homology with SIV found in wild chimpanzees
- Likely made the transfer to humans early in the 20th
- HIV-1 is transmitted sexually or through body fluids,
- Leads to a chronic infection
- Results in immunodeficiency and death by OI.
- Reverse Transcriptase without proof reading

J Biotechnol Biomed 2021; 4 (4): 147-158 DOI: 10.26502/jbb.2642-91280041



Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

- Target: ACE2 receptor (lung, heart, blood vessel, kidney)
- Symptoms: difficult breathing, fever, pneumonia
- Percentage death without treatment 1-4%.

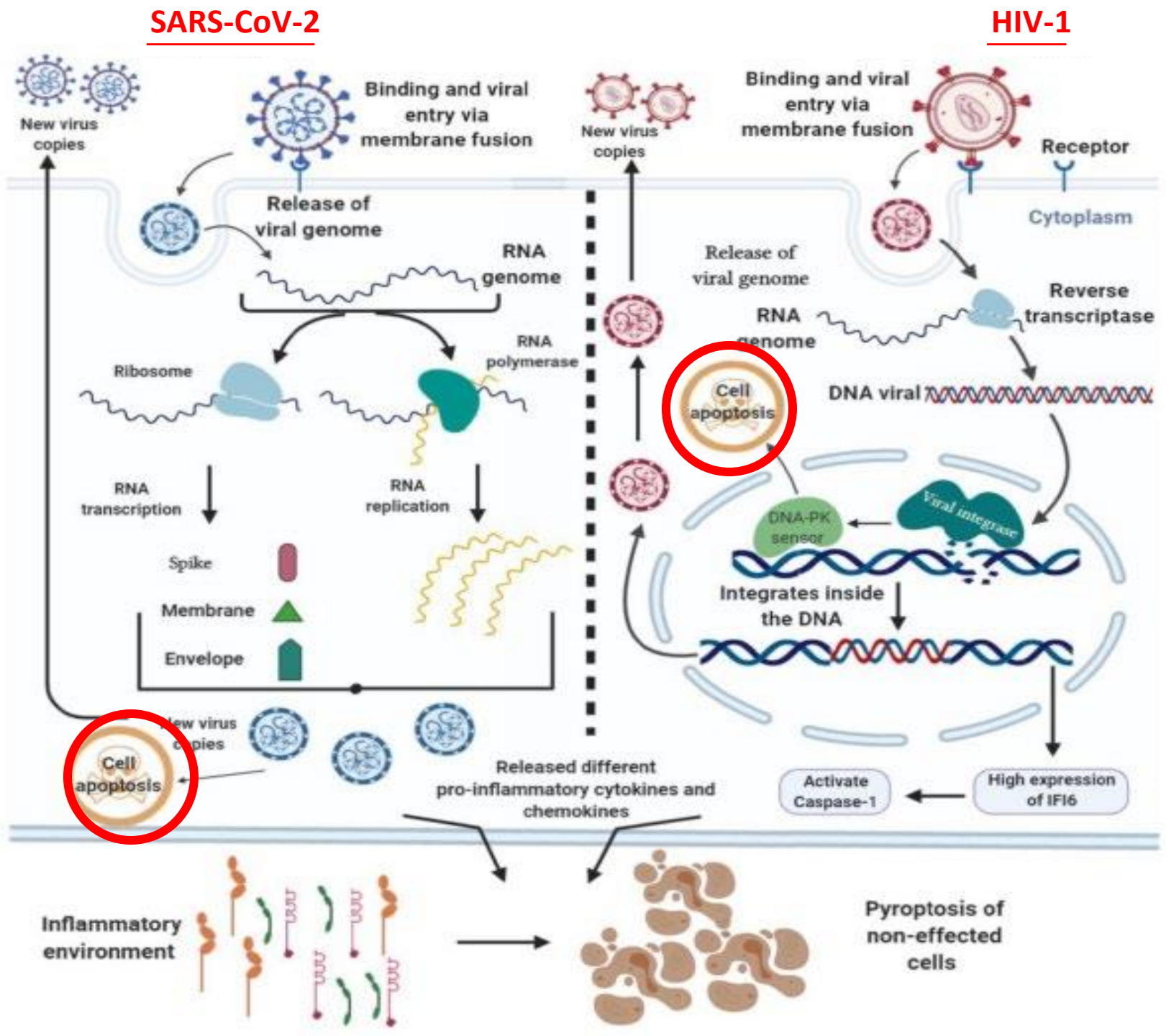
Human Immunodeficiency Virus (HIV)

- Target: CD4 T lymphocyte, macrophage, dendritic cells CCR5, CCR4-coreceptor
- Symptoms: flu-like, mononucleosis like (acute phase)
- Percentage death without treatment >95%.

Similarities

a) Fear in the population
b) Existence of animal reservoirs
c) Increased synthesis of proinflammatory cytokines
d) Modifications of the intestinal microbiota.
e) No or mild symtoms in animal reservoirs but severe when infect humans

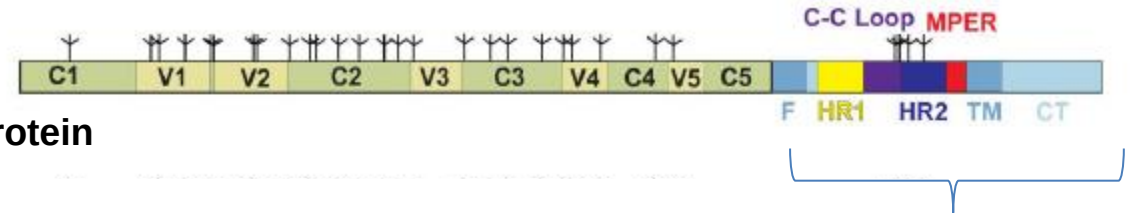
Replicative cycle



Saleemi et al., Infection, Genetics and Evolution 2020

ENV in HIV and S in SARS-CoV-2: similar function and different structure…

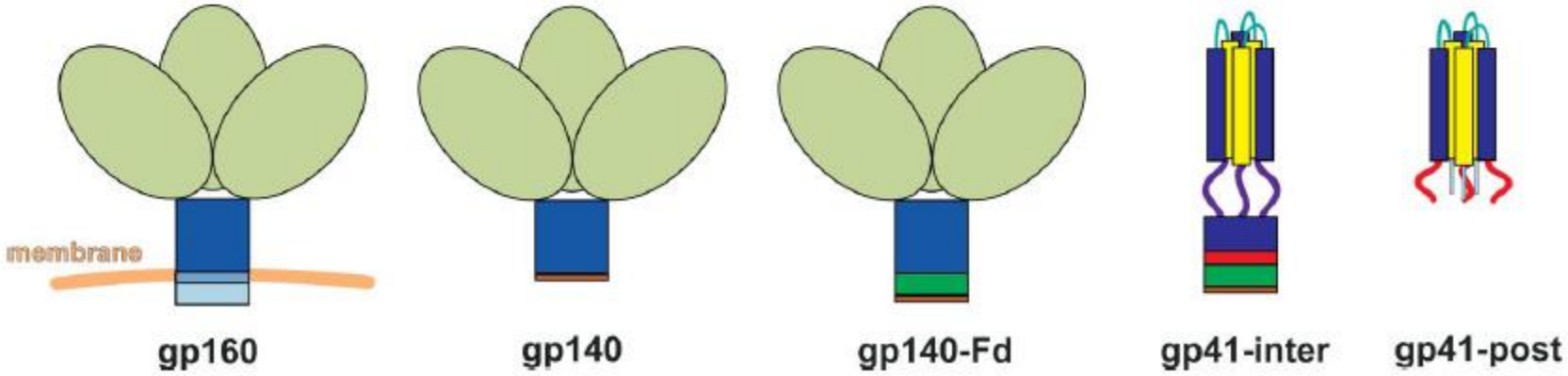
HIV envelope glycoprotein (gp120–gp41)



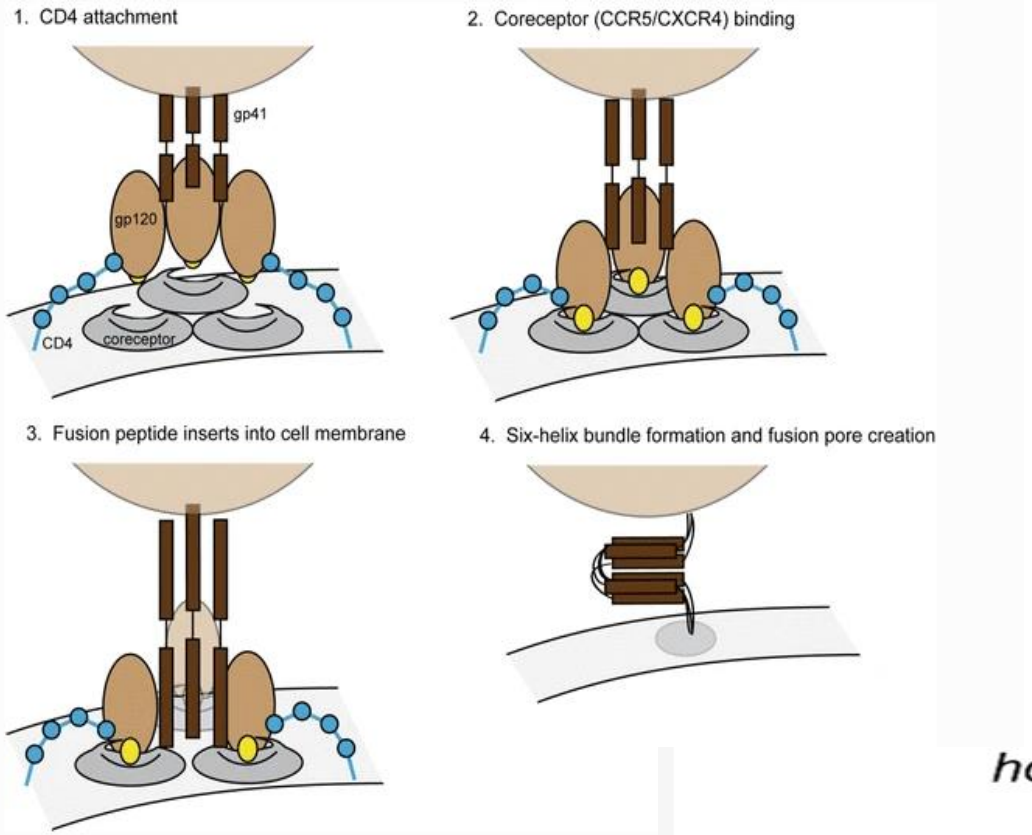
Surface glycoprotein

C1-C5: Conserved region
V1-V35: Variable region
F: Fusion peptide
HR: Eptad repeat

Gp41
transmembrane glycoprotein



Model of HIV entry

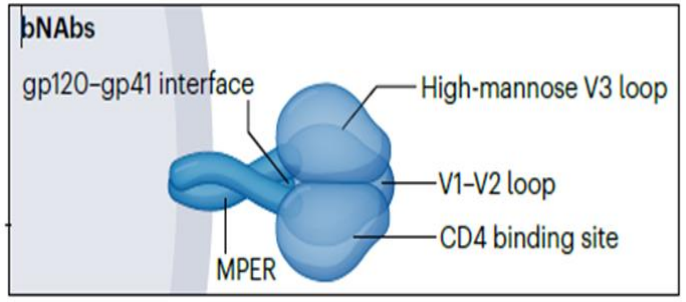


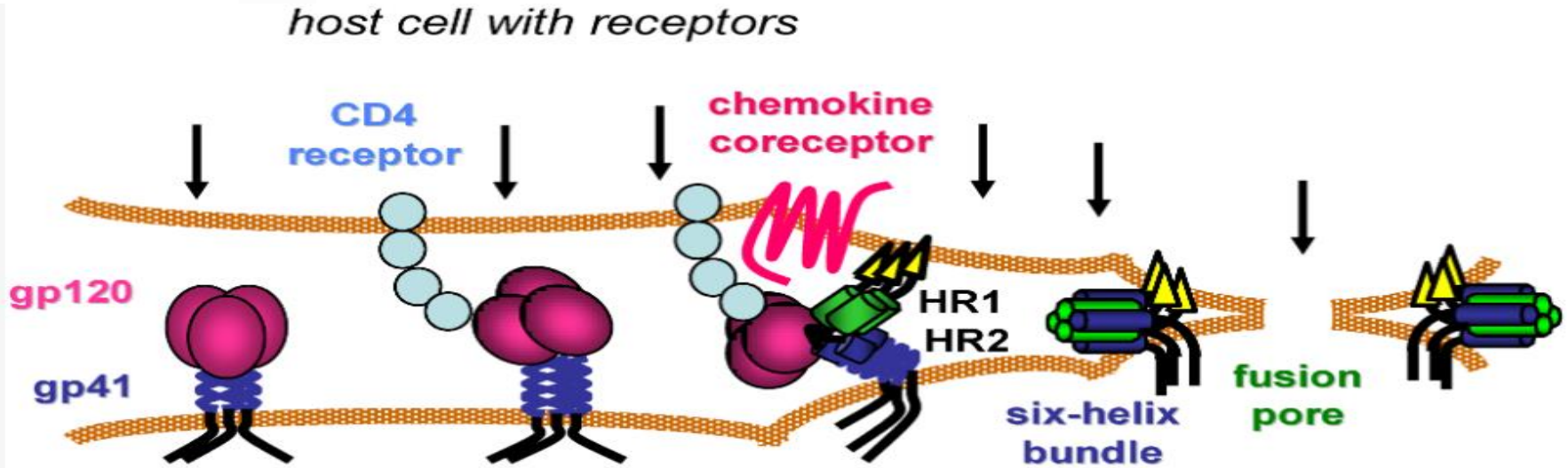
1- gp120 binds to CD4 (D1-D2), and with the chemokine co-receptor

2. conformational changes in the gp41 (V1-v2) that expose HR1 and HR2 (v3)

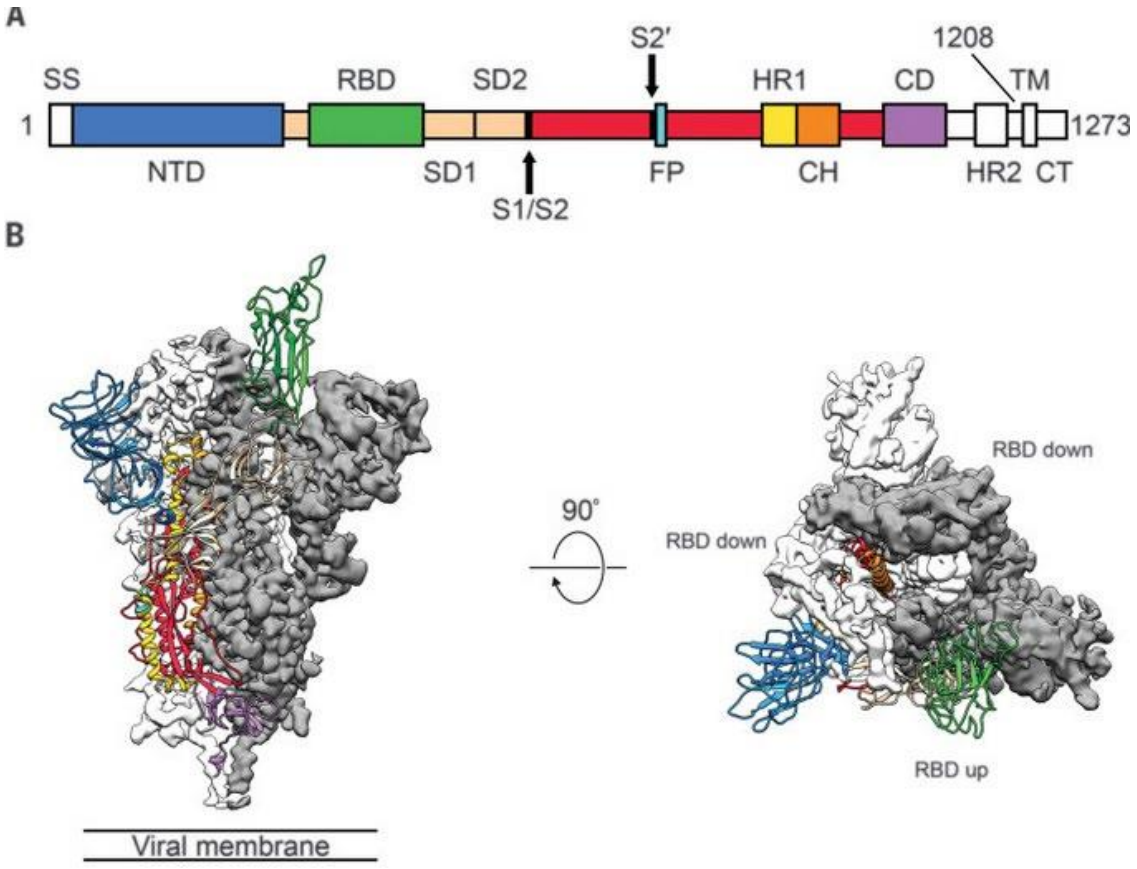
3- form a six-helix bundle structure that bring the host and viral membranes together for fusion (fusion pore)

The envelope glycoprotein (gp120–gp41) is the major immunogen protein of HIV to which neutralizing antibodies are directed



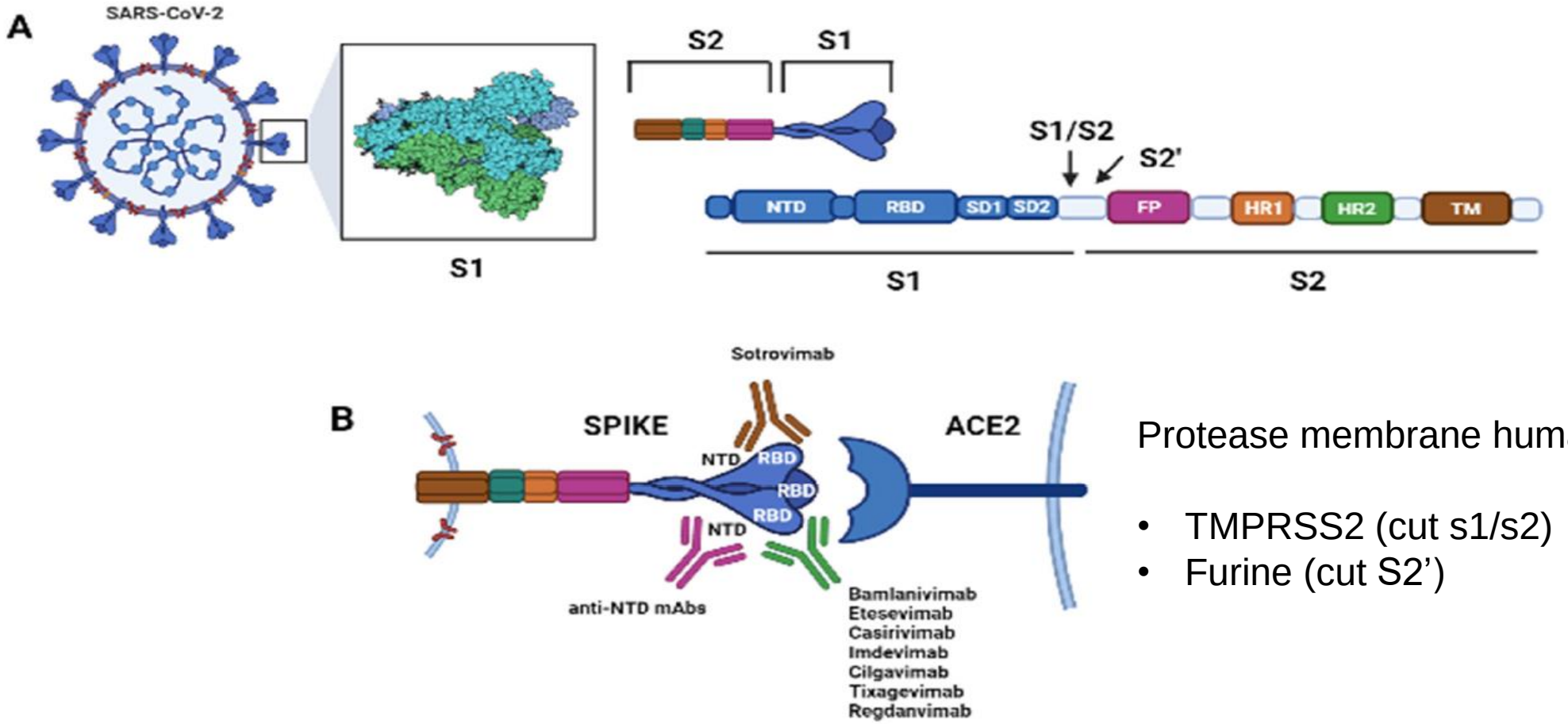


The Spike (S) protein (of about 150kDa) is the major antigen presented on the surface of SARS-CoV-2



- The S protein forms a protruding from the viral surface to attach to the host cellular receptor **ACE2.**

Wrapp D et al. Science. 2020

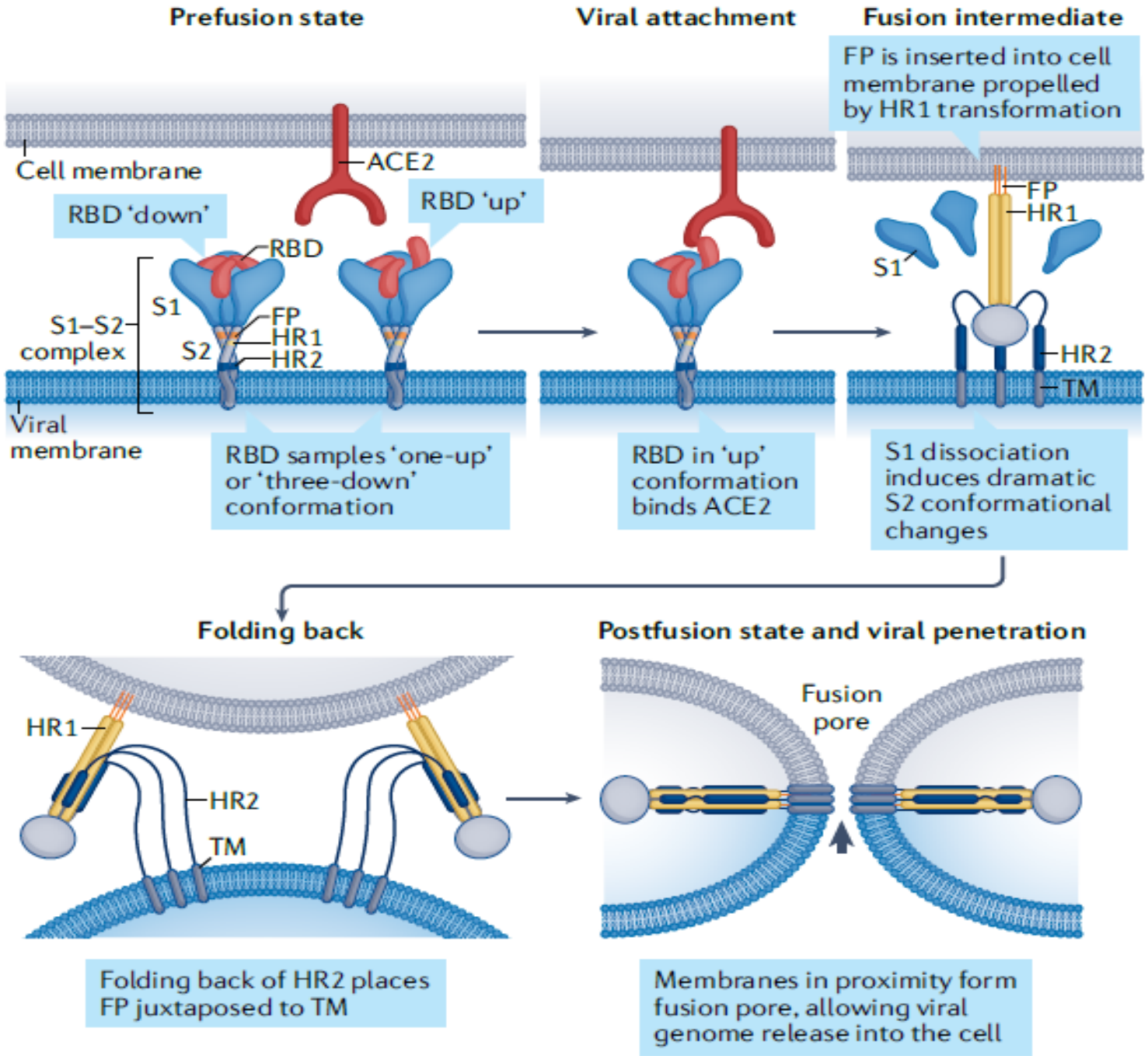


Protease membrane human cells:

- TMPRSS2 (cut s1/s2)
- Furine (cut S2')

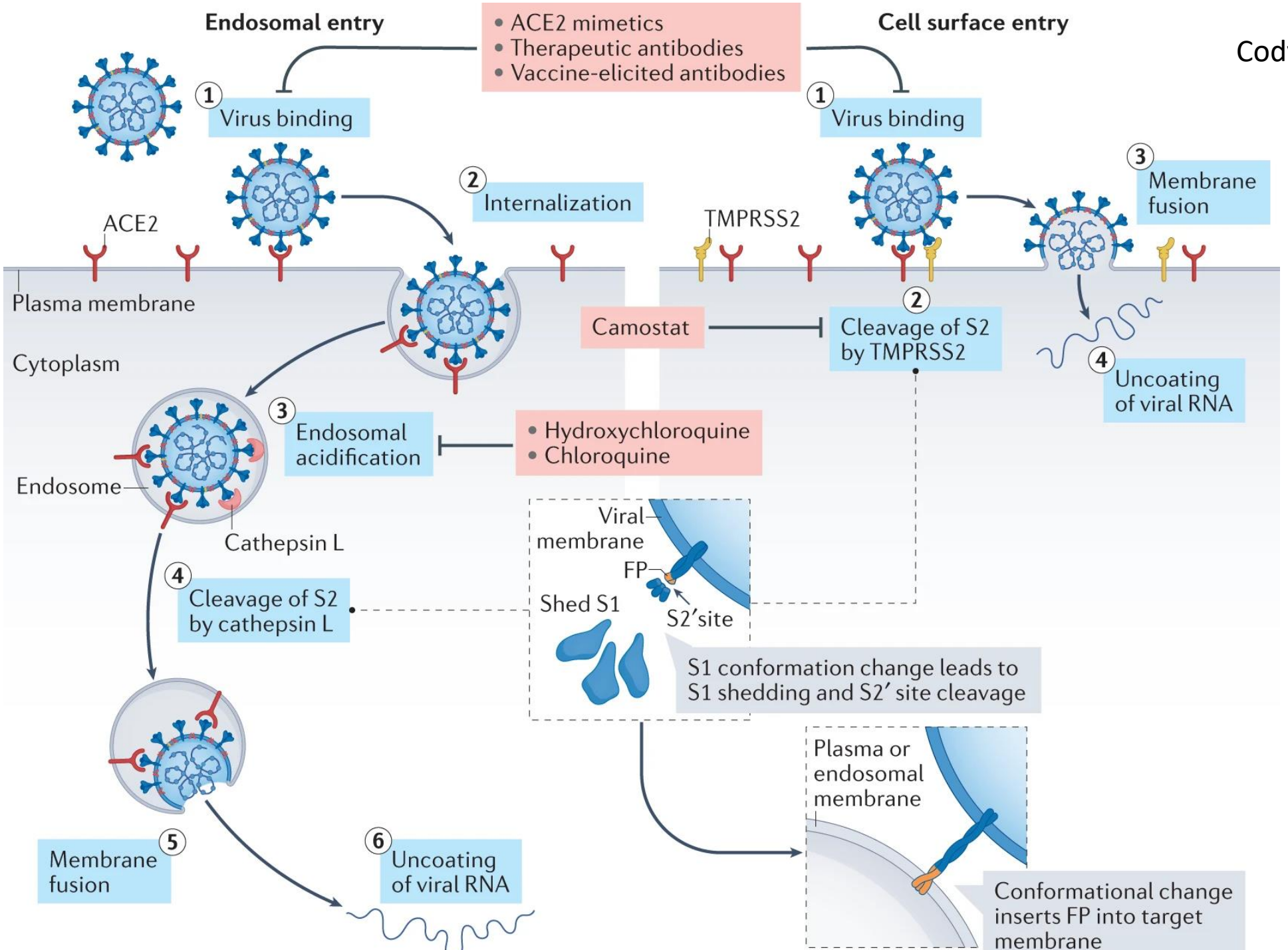
The **S protein** is a trimer, and each monomer comprises an N-terminal S1 subunit and a C-terminal S2 subunit.
S1 subunit further separates into the N-terminal domain (NTD), subdomain 1 (SD1), subdomain 2 (SD2) and RBD.
S2 subunit further divides into the fusion peptide (FP), the heptad repeats 1 (HR1), and heptad repeat 2 (HR2)

During the virus entry phase, S1 is responsible for the receptor binding, while S2 is involved in the membrane fusion.



TMPRSS2/Furine host
S1/S2 dissociation

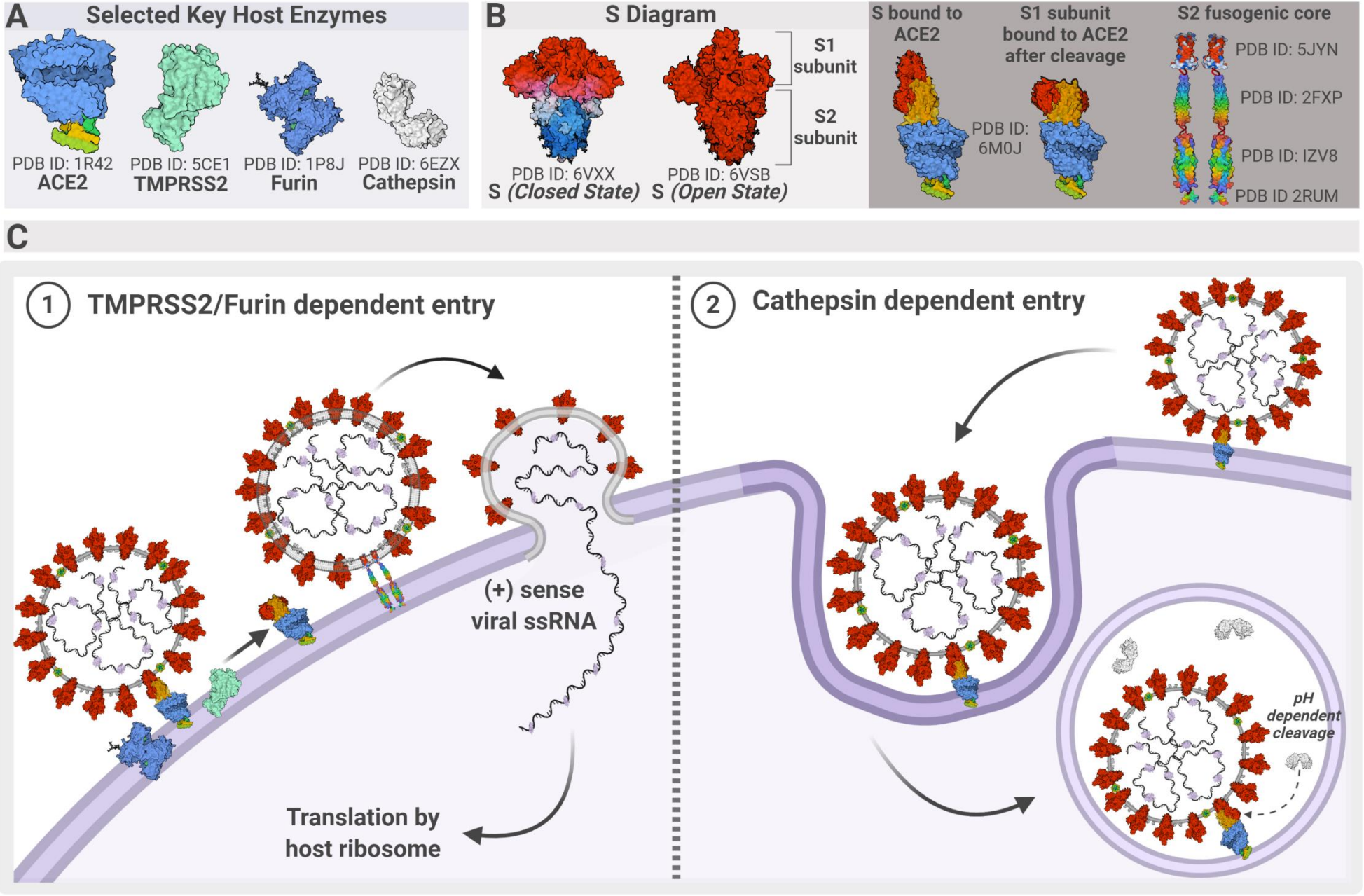
Cody Nature 2022



Cody et al. Nature 2022

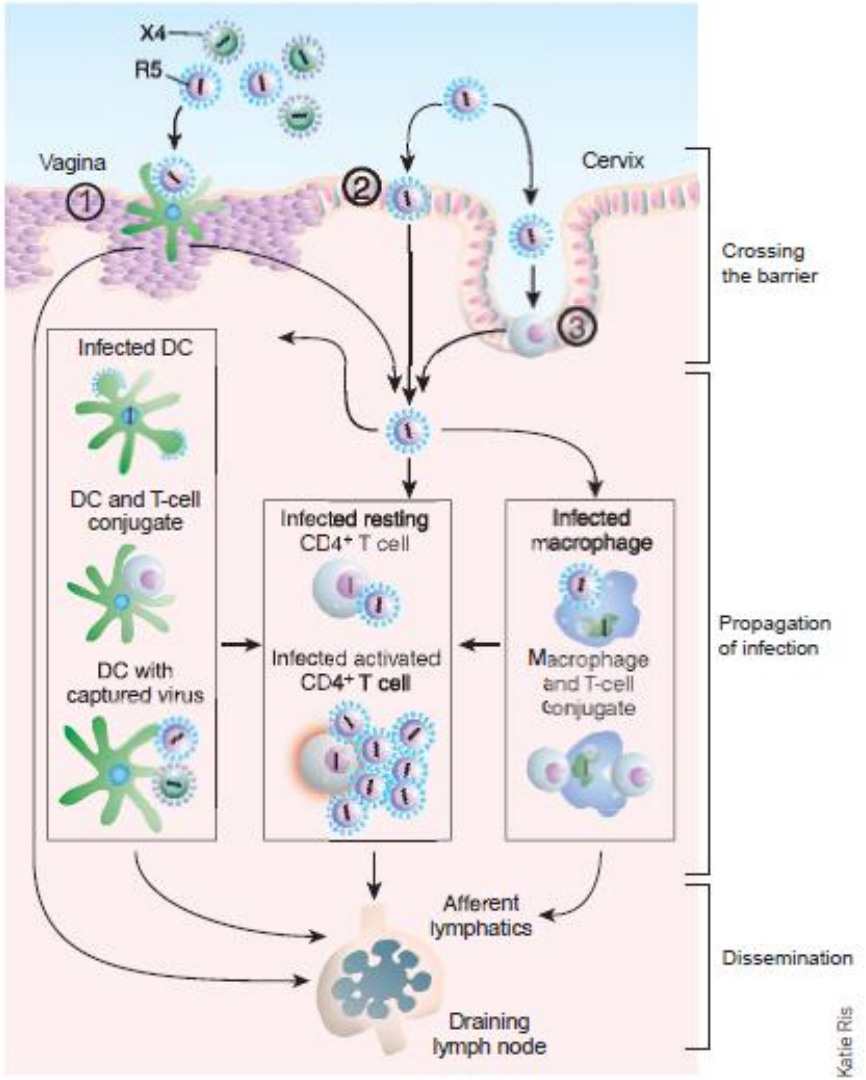
Cody Nature 2022

SARS-CoV-2 Viral Entry Mechanisms

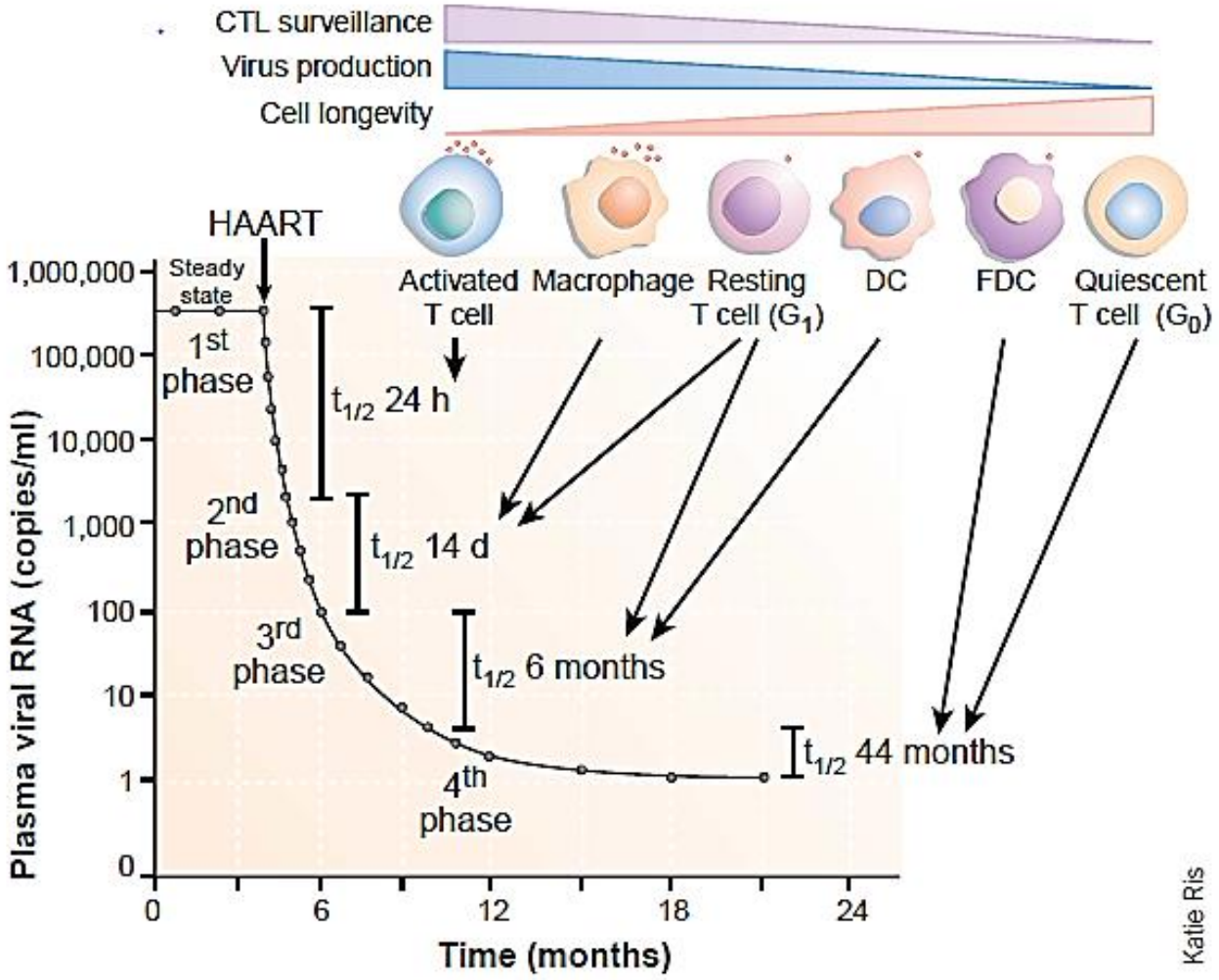


Infection dynamics and eradication

HIV rapid spread and dissemination are favoured by the presence of different target cells in multiple organs

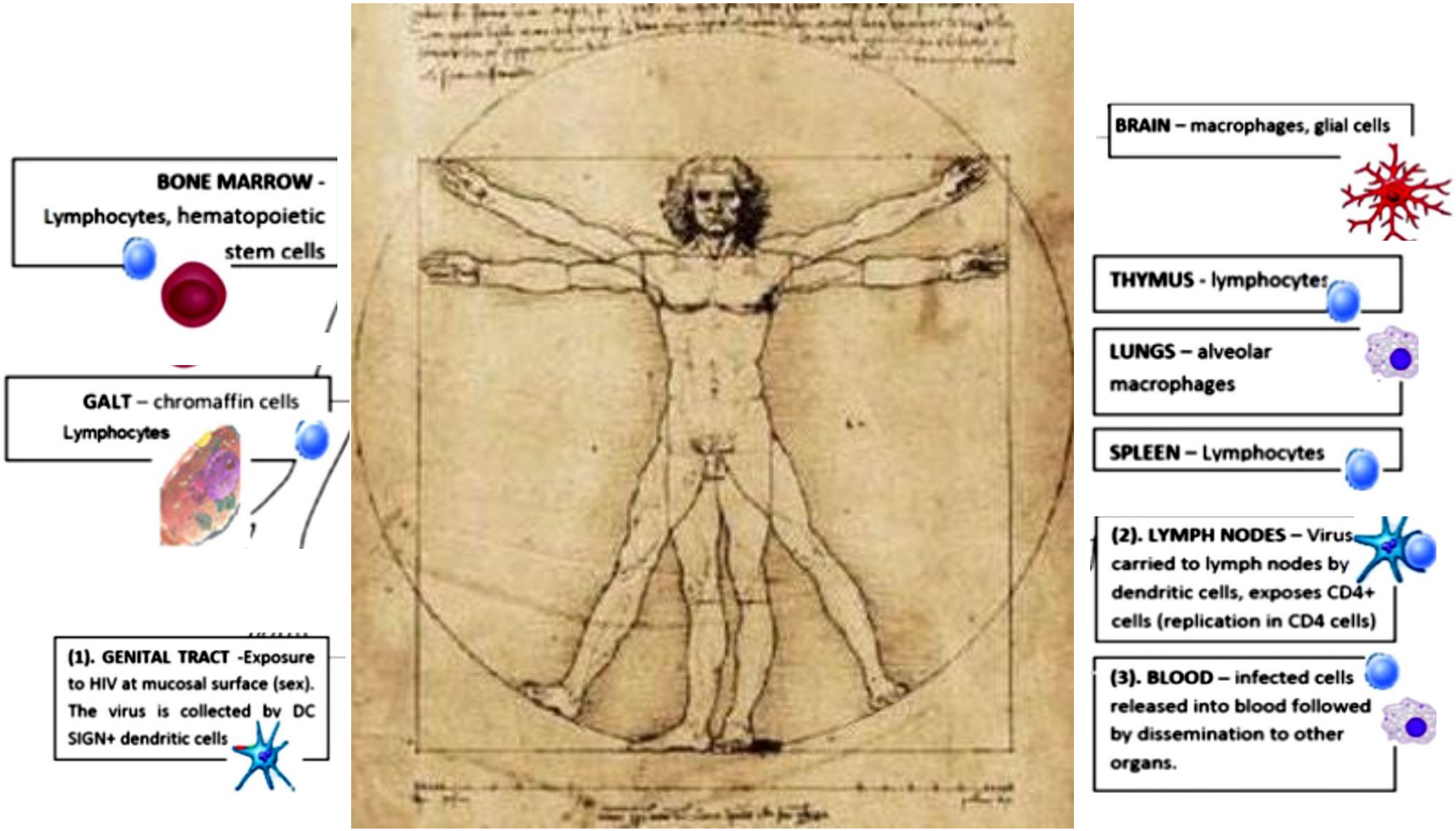


Melissa Pope & Ashely Haase Nature Medicine, 2003



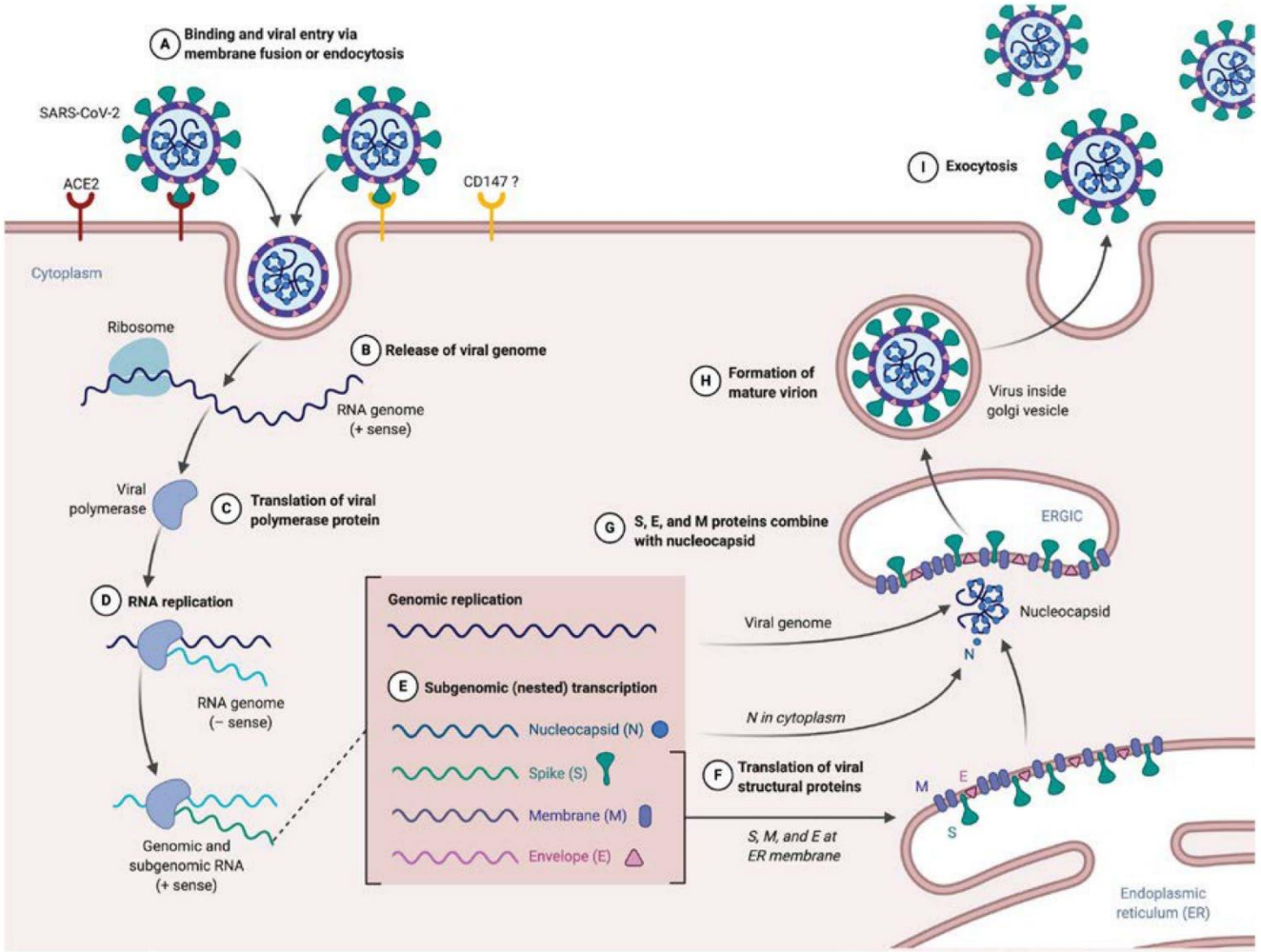
Mario Stevenson Nature Medicine, 2003

HIV hiding places:

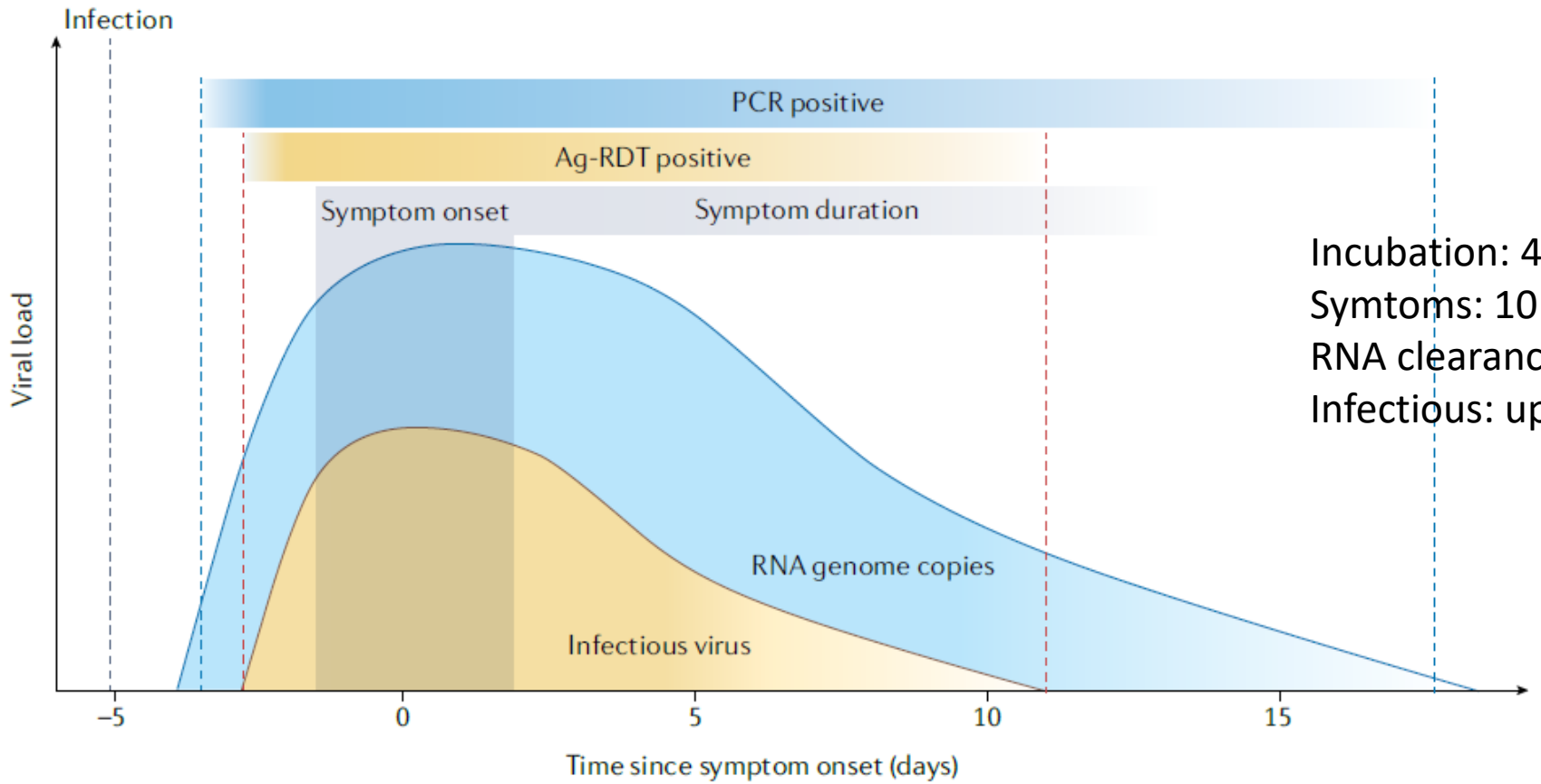


HIV's hiding places. After exposure at mucosal surfaces (1), the virus is carried to the local lymph nodes (2) by dendritic cells. Fusion of dendritic cells with CD4+ T lymphocytes results in infection of the lymphocytes and viral replication in these cells. Infected CD4+ T lymphocytes are released into the blood stream (3) and disseminated to anatomical reservoirs in other organs (4) including the brain, CNS, spleen, bone marrow, thymus, lungs, kidneys, lymph nodes, and GALT with infection of associated cellular reservoirs in these organs. DC-SIGN indicates dendritic cell–specific intercellular adhesion molecule-3-grabbing nonintegrin; GALT, gut-associated lymphoid tissue

SARS-CoV-2 cytoplasmic localization allows for viral clearance



Lebeau et al., IJMS 2020



Incubation: 4.6-6.4 d
Symtoms: 10 d
RNA clearance : 16 d
Infectious: up to 8-10 d post onset

Fig. 2 | Kinetics of RNA viral loads and infectious virus for ancestral SARS-CoV-2 in patients with mild-to-moderate disease. According to different studies, the incubation period for ancestral SARS-CoV-2 was estimated to lie between 4.6 and 6.4 days. On average, symptoms continue to persist for 10 days. RNA can already be detected before the onset of symptoms; RNA levels peak around the onset of symptoms and then gradually decline. Median clearance for RNA viral load is 16 days post-onset of symptoms. Infectious virus titres are highest around symptom onset, and infectious virus can be isolated up to 8 or 10 days post-onset of symptoms. RNA can be detected for prolonged periods by real-time PCR, when infectious virus is no longer detectable, whereas virus detection by antigen-detecting (rapid) diagnostic tests (Ag-RDTs) was shown to be a better correlate for infectiousness. Gradients reflect variability between individuals (lighter shade towards the end of infection shows that viral loads continue to be detected in some but not all individuals). The grey dashed line marks the initial infection, the blue dashed lines mark the PCR-positive period and the red dashed lines mark Ag-RDT positivity. Details of the underlying studies used to generate Fig. 2 can be found in Supplementary Table 1.

Puhach Nature 2023

RNA clearance faster in vaccinated
Infectious viral load lower in vaccinated and faster decline

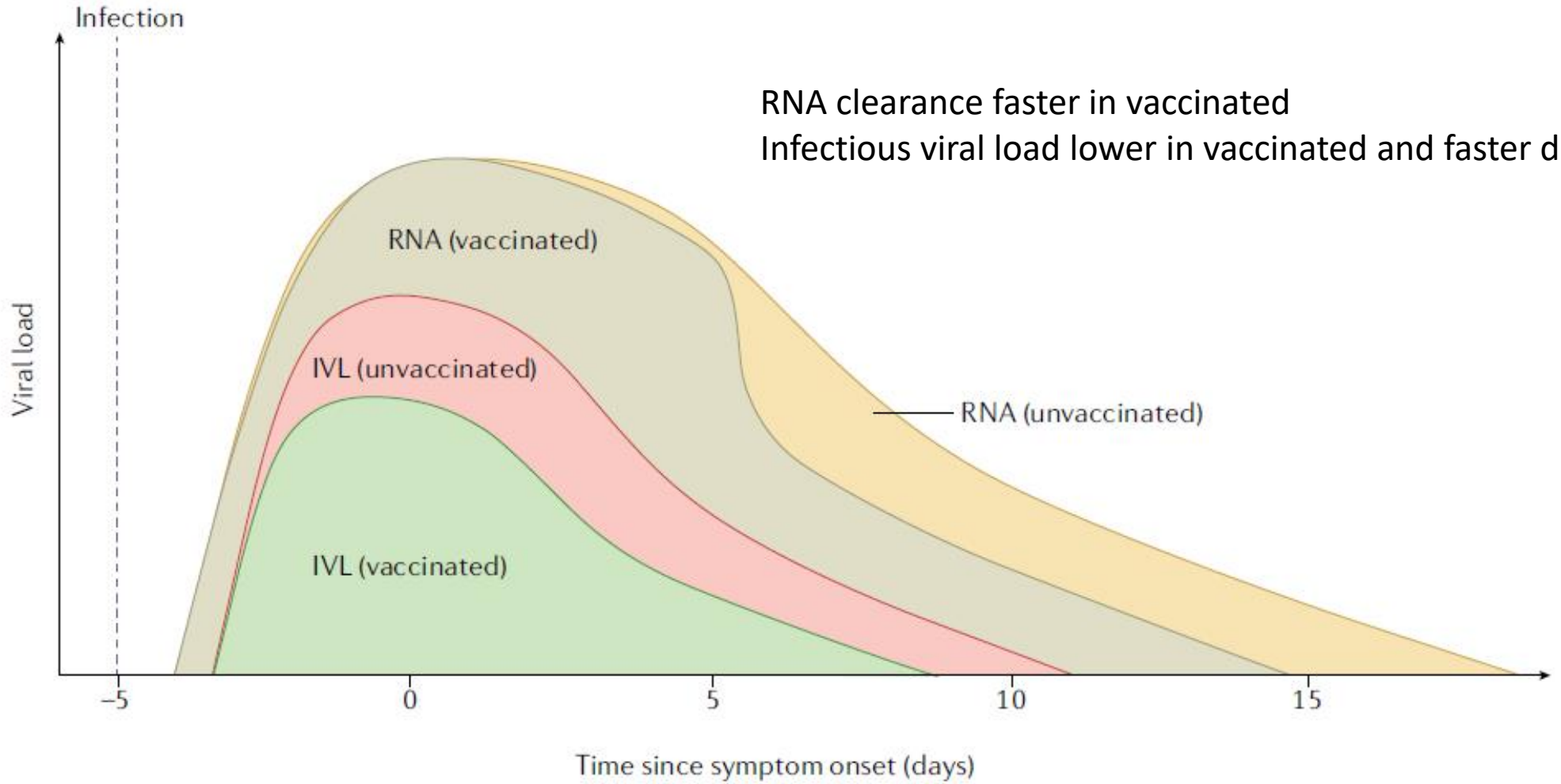


Fig. 4 | Influence of vaccination on viral load. Similar RNA viral loads were detected in vaccinated and unvaccinated patients infected with the Delta variant of concern during the first 5 days post-onset of symptoms. However, faster clearance of viral RNA was shown in vaccinated patients. Infectious viral loads (IVLs) were significantly lower in vaccinated individuals and declined faster than in unvaccinated individuals infected with Delta. Dynamics of viral loads in vaccinated individuals may vary widely in case of infection with another variant. Details of the underlying studies used to generate Fig. 4 can be found in Supplementary Table 3.

Puhach Nature 2023

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

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

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

Proal et al Nat Immunol 2023

Extremely different variability

OPEN ACCESS Freely available online

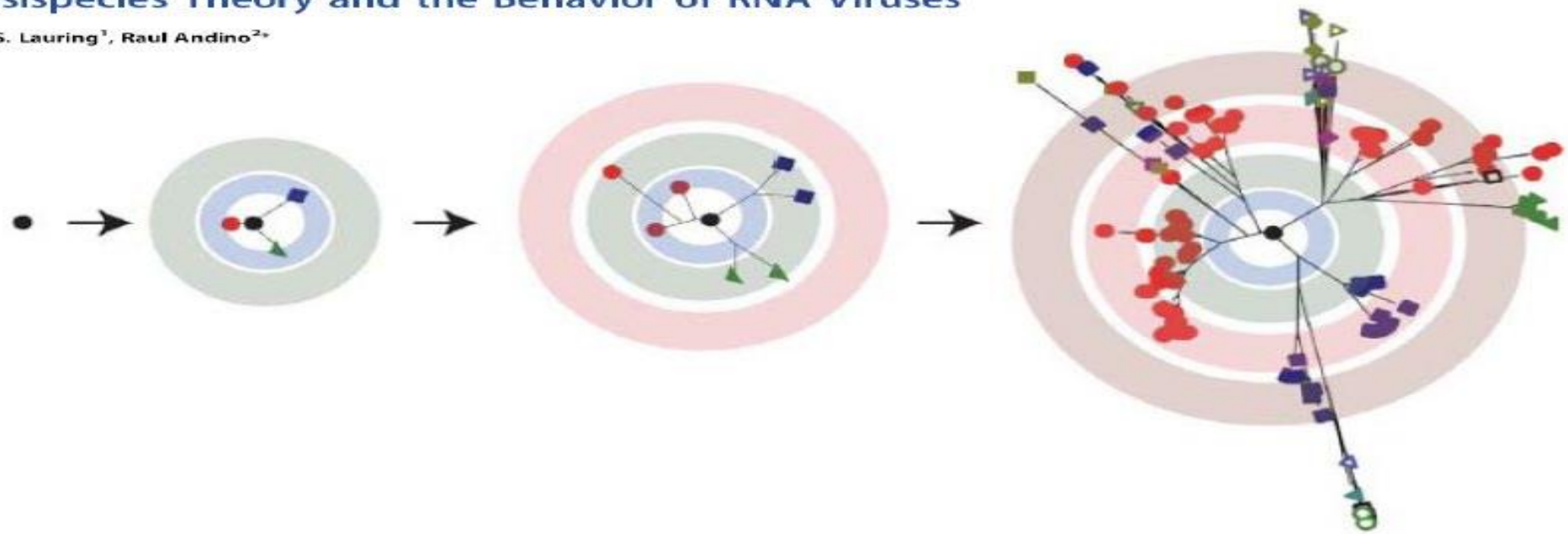
PLoS PATHOGENS

Review

July 2010 | Volume 6 | Issue 7 | e1001005

Quasispecies Theory and the Behavior of RNA Viruses

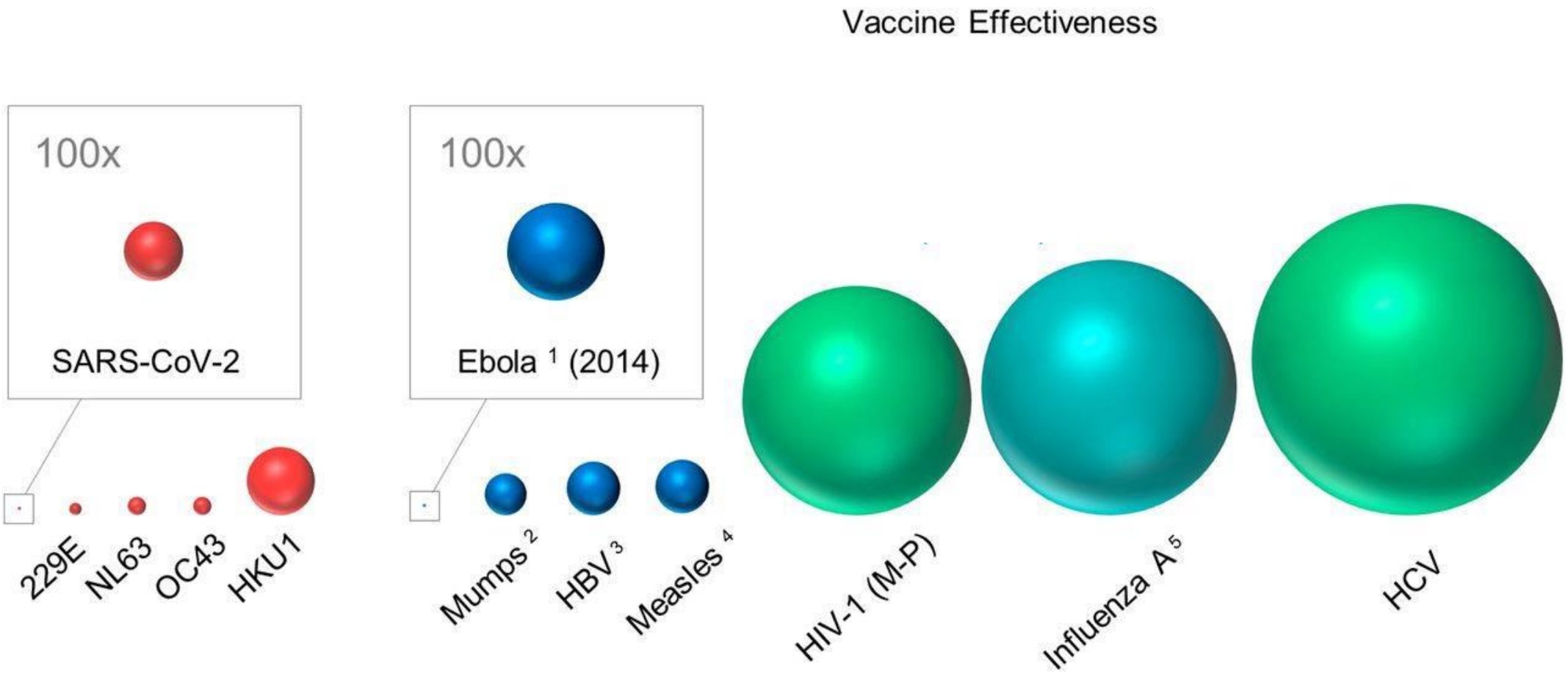
Adam S. Lauring[1], Raul Andino[2]*



RNA viruses: error prone nature of the RNA-dependent RNA polymerase,
7×10^{-4} to 5.4×10^{-3} mutations per site per infectious cycle

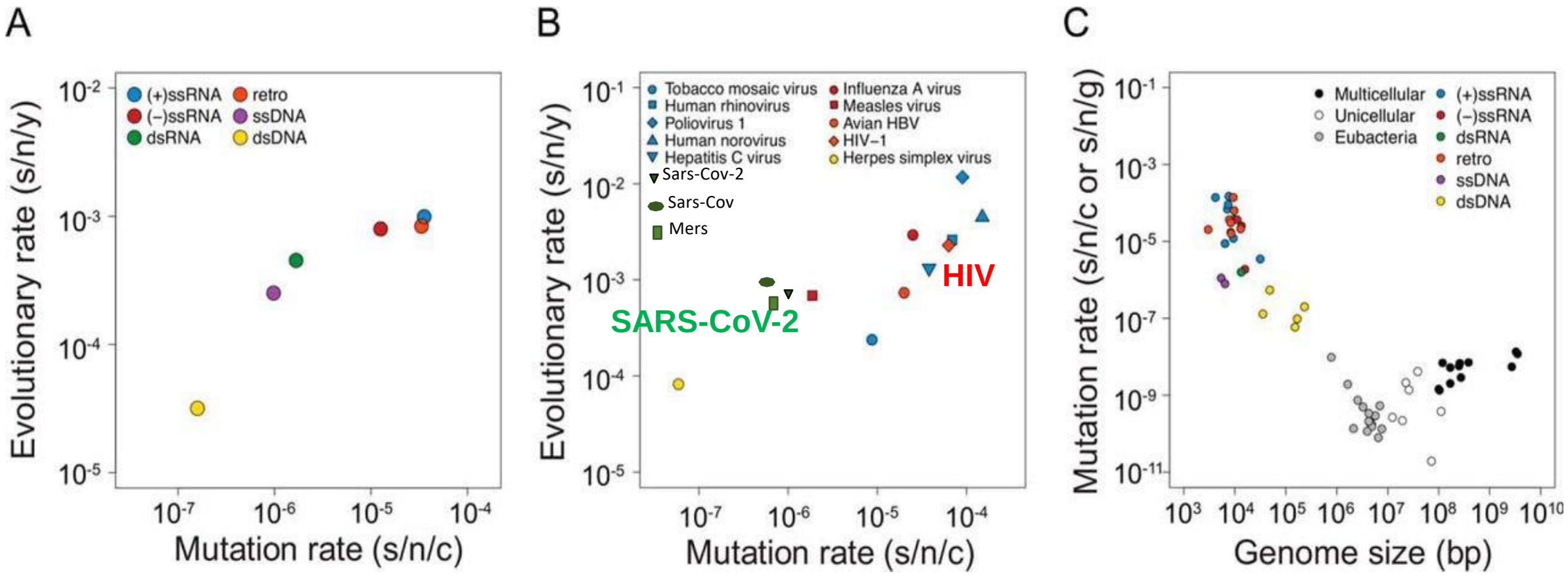
This does not occur in SARS-CoV-2: its exonuclease-based proofreader increases replication fidelity by removing mismatched nucleotides to maintain the viral genome integrity

SARS-CoV-2 exhibits an extremely low variability respect to others RNA-based viruses



Rausch et al., 2020

Comparative genetic diversity among coronaviruses and select viral pathogens

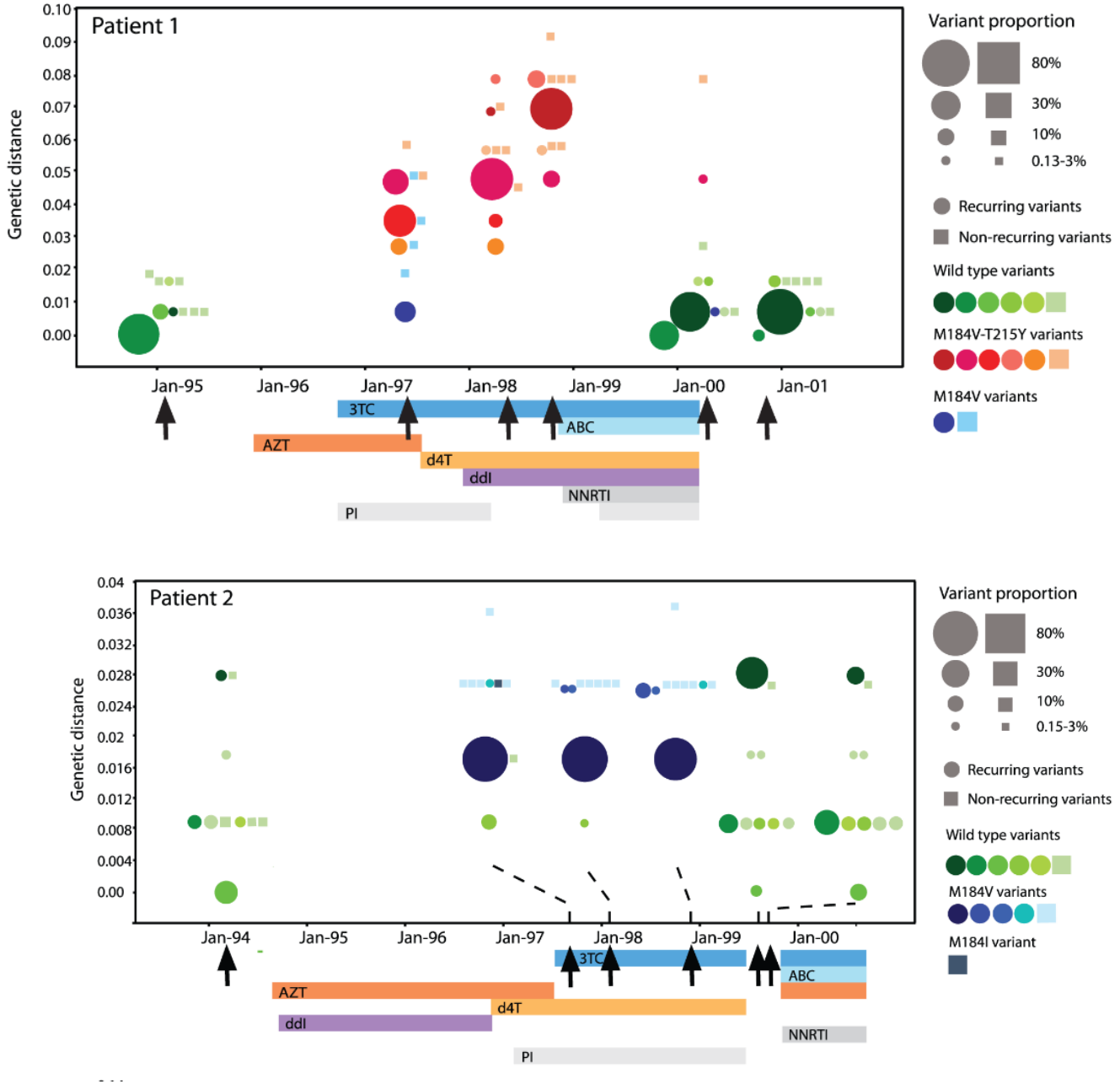


Evolutionary rates (substitutions per nucleotide site per year [s/n/y])
Mutation rate (substitutions per nucleotide site per cell infection [s/n/c])

Modified accordig to Peck et al., 2017

Within an HIV-1 infected individual, selective pressures, such as the host immune response and ART, influence the evolution of the virus.

This leads to the formation of a diverse pool of closely related virus variants **(quasispecies)**.



Hedskog et al., PLoS One. 2010

Adaptive evolution of SARS-CoV-2

Table 1
Main characteristics of SARS-CoV-2 and its different variants.

SARS-CoV-2 strain	Declaration as VOC	Majority extension	Epidemic period in Spain[a]	Transmissibility	Severity and lethality	Escape to immune response	Mutations found in the S protein gene
Original	Wuhan (China), 07/01/2020	Worldwide	First and second.	R_0 = 2.5. Incubation period: 2–14 days, median 5.1 days. SAR: 0.7–75%.	81% mild[b], 14% severe[b], 5% critical[b], 2.3% death.	PVE 95% for symptomatic infection.	
Alpha	United Kingdom, 29/12/2020	Europe, Oceania and North America	Dominant in Spain. Third, fourth and fifth.	↑ **Transmissibility** (50% higher). SAR: 25.1%, 1.43–1.82 times higher.	↑ **Severity and lethality.** 1.55–1.73 times more lethality.	↑ **Immune escape.** PVE 89% for symptomatic infection, and 95% for hospitalization or death.	**Del69-70, del144, N501Y,** A570D, D614G, **P681H,** T716I, S982A, D1118H.
Beta	South Africa, 29/12/2020	Africa	Minority in Spain. Third, fourth and fifth.	↑ **Transmissibility** (50% higher, 2.5 times higher). SAR higher.	↑ **Severity and lethality.**	↑ **Immune escape.** PVE 84% for symptomatic infection, and 95% for hospitalization or death.	L18F, D80A, D215G, R246I, **K417N, E484K, N501Y,** D614G, A701V.
Gamma	Brazil, 29/12/2020	Latin America	Minority in Spain. Third, fourth and fifth.	↑ **Transmissibility** (1.7–2.4 times higher). R_0 = 3.4. SAR higher.	↑ **Severity and lethality.**	↑ **Immune escape.** PVE 84% for symptomatic infection, and 95% for hospitalization or death.	L18F, T20N, P26S, D138Y, R190S, K417T, **E484K, N501Y,** D614G, **H655Y,** T1027I, V1176F.
Delta	India, 11/05/2021	Worldwide	Fifth and sixth.	↑↑ **Transmissibility** (1.97 times higher). R_0 = 7. Intradomiciliary delta SAR (10.3–21%) 1.70 times higher than intradomiciliary alpha SAR. Shorter incubation period (median 4.5 days). Higher viral load, 2.5 times more in nasopharyngeal exudate and 15 times more in saliva.	↑↑ **Severity and lethality.** 2.20 times more hospitalization. 3.87 times more ICU admission. 2.37 times more lethality.	↑↑ **Immune escape.** PVE 87% for symptomatic infection, and 93% for hospitalization or death.	T19R, G142D, del156-157, R158G, K417N (delta plus), **L452R, T478K,** D614G, **P681R.**
Omicron	South Africa and Botswana, 26/11/2021	Worldwide	Sixth.	↑↑↑↑ **Transmissibility** (36.5% higher than delta). R_0 = 10. Intradomiciliary omicron SAR 15.8%-31% versus delta SAR 10.3–21%. Extradomiciliary omicron SAR 8.7% versus delta SAR 3.0%. 70-fold higher respiratory viral load at 24 hours in omicron than in original and delta strains. **Shorter incubation period** (median 3 days).	↓ **Severity and lethality.** 0.71 times less (29% less) hospitalization. 10-fold lower viral load in lung tissue at 24 hours in omicron than in original strain.	↑↑↑↑ **Immune escape.** PVE 10% for symptomatic infection, 49% if third dose. PVE 70% for hospitalization. 2.4–5.4 times higher risk of **reinfection.**	**A67V, del69-70,** T95I, **G142D, del143-145,** Y145D, del211, L212I, ins214EPE, **G339D, S371L, S373P, S375F, K417N, N440K,** G446S, **S477N, T478K, E484A,** Q493R, G496S, **Q498R, N501Y,** Y505H, T547K, D614G, **H655Y, N679K, P681H,** N764K, D796Y, N856K, Q954H, N969K, L981F.

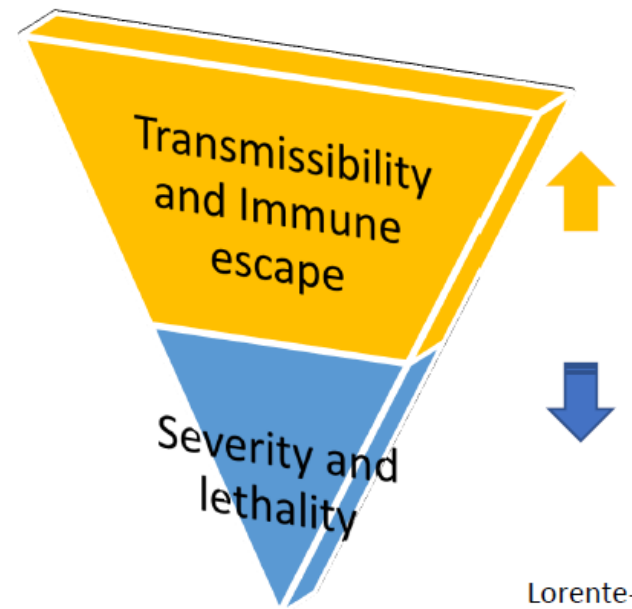
VOC: variant of public health concern.
R_0: basic reproductive number: average number of new cases generated (by contagion) from a single case.
SAR: secondary attack rate: number of new cases of a disease among the total number of exposed susceptible people within a specific group (i.e., household or close contacts), that is, the proportion of contacts of a primary case who become ill.
PVE: preventive vaccine effectiveness, in all cases after two doses with Comirnaty vaccine (based on messenger RNA technology).

The objective of SARS-CoV-2 is:

TO REPLICATE AND PERPETUATE ITSELF

To do this has generated a large number of mutations

Several of these mutations have caused SARS-CoV-2 to adapt better to its hosts, which explains:



Lorente-Gonzalez et al., ORA, 2022

SARS-COV-2: acute infection = low immune presion, short therapy (vaccine ??)

HIV: chronic infection = high immune presion, chronic therapy

TABLE 2 Lineage-Specific Mutations in SARS-CoV-2 Variants of Concern (VOCs).

Variants	Spike	NTD	RBD	Pathological effects
Alpha (B.1.1.7)	ΔH69, ΔV70, ΔY144, N501Y, A570D, D614G, E484K, T716I, S982A, P681H, D1118H	ΔH69, ΔV70, ΔY144	E484K, S494P, N501Y	Increased transmissibility; increased binding affinity between RBD and ACE-2.
Beta (B.1.351)	L18F, D80A, E84K, D215G, Δ241/4, Δ242, Δ243, R246I, N501Y, D614G, K417N, A701V	L18F, D80A, E84K, D215G, R246I, Δ241/4, Δ242, Δ243	K417N, E484K, N501Y	Increased transmissibility; resistance to neutralization by NTD/RBD specific mAbs, convalescent plasma and vaccine sera.
Gamma (P1)	L18F, T20N, P26S, D138Y, R190S, D614G, E484K, N501Y, K417T, H655Y, T1027I	L18F, T20N, P26S, D138Y, R190S,	K417N, E484K, N501Y	Reduction of neutralizing antibody effectiveness in convalescent plasma and vaccine sera; enhanced viral entry through endosomal pathways.
Delta (B.1.617.2)	T19R, G142D, Δ156, Δ157, R158G, L452R, T478K, E484Q, D614G, P681R, D950N	T19R, G142D, Δ156, Δ157, R158G	L452R, E484Q	High transmissibility and pathogenicity alongside enhanced immune escape; increase in hospitalization.
Omicron BA.1 (B.1.1.529)	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211-212, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	A67V, Δ69-70, T95I, G142D Δ143-145, Δ211-212	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H	Higher infectivity (by strengthening the binding with host ACE2 receptors); reduced neutralization by antibodies (mAb, T-cell, or convalescent or vaccine plasma).

Signal Transduction and Targeted Therapy, 2022

RESEARCH HIGHLIGHT OPEN

Omicron: increased transmissibility and decreased pathogenicity

Gábor Bálint[1], Barbara Vörös-Horváth[1] and Aleksandar Széchenyi[1]

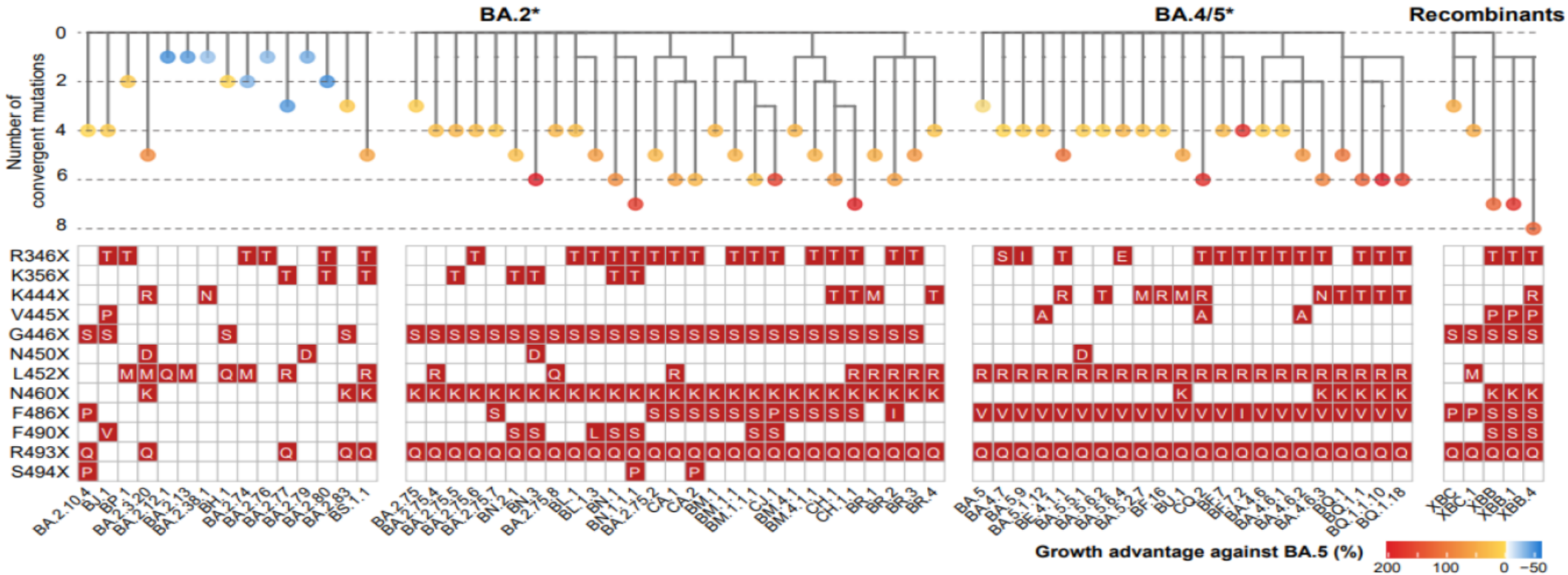
- Omicron variant have **three mutations in the furin cleavage** site region (P681H, H655Y, N679K) **decrease S1/S2 cleavage, fusogenicity, and syncytia formation associated with pathogenesis.**

- The multiple amino acid substitutions presumably **cause the increased ACE2 binding** affinity of Omicron in its S-protein, including Q493R, Q489R, and S477N.

- In vivo studies using the hamster model confirmed that **Omicron possesses decreased pathogenicity, investigating lung function parameters**: enhanced pause, total expiratory time, and subcutaneous oxygen saturation.

Omicron is continuously evolving, leading to various new subvariants

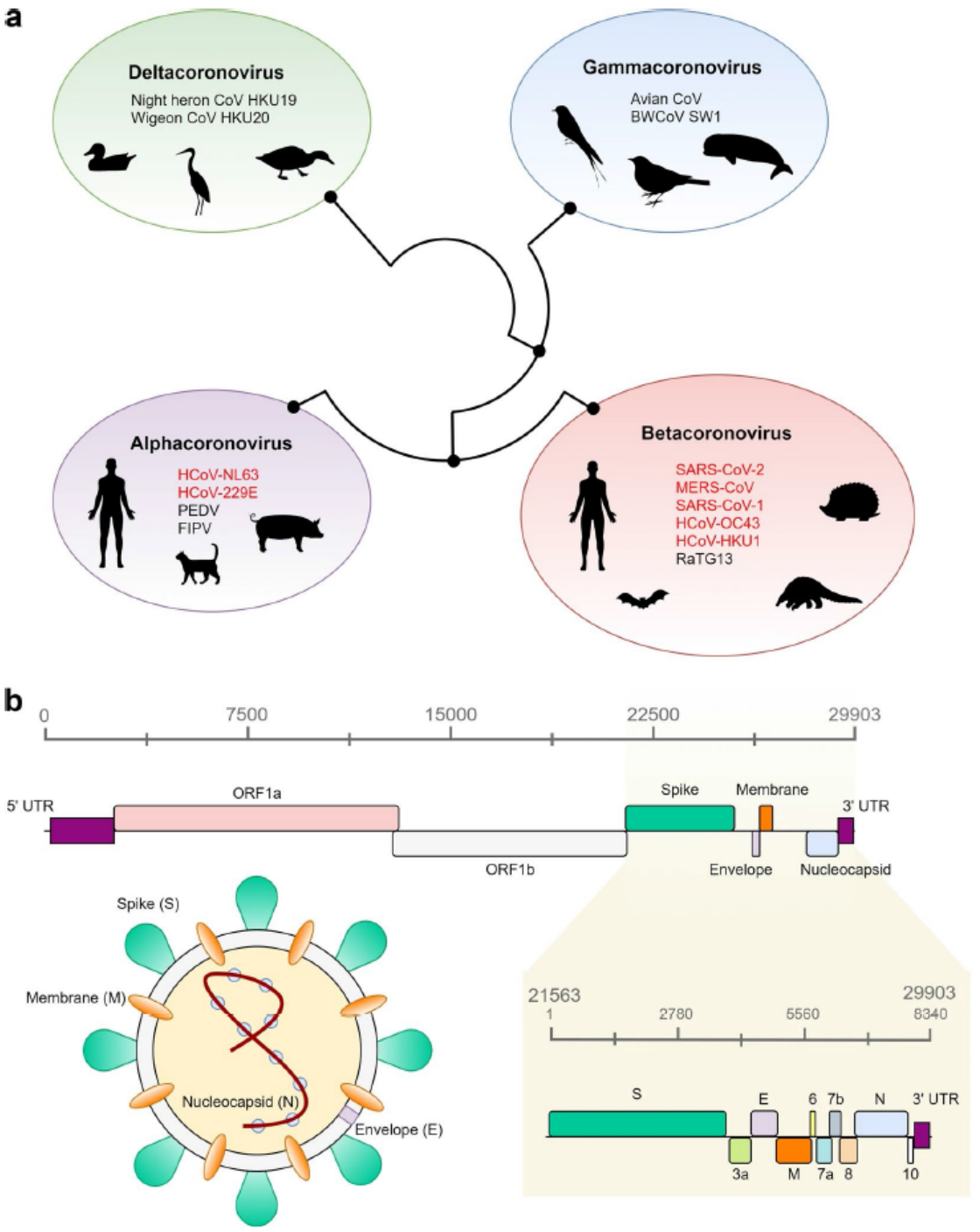
- A high proportion of these emerging variants display significant growth advantages over BA.5, such as BA.2.3.20, BA.2.75.2, BQ.1.1, and **especially XBB, a recombinant of BJ.1 and BM.1.1.1**
- It's crucial to examine the impact of these convergent mutations on ***antibody-escaping capability***, receptor binding affinity, and the ***efficacy of vaccines and antibody therapeutics***



Coronaviruses undergo frequent recombination.

If animals harboring different coronaviruses come in close contact and exchange viruses, then recombination can occur among the different strains, leading to diversification.

Unfortunately, it appears that such events during the evolutionary history of SARS-CoV-2 have led to the evolution of a potent strain capable of easily infecting human cells



Singh D, Yi SV. *Exp Mol Med*. 2021

The XA lineage: One of the first reported cases of inter-lineage SARS-CoV-2 recombinants was the XA lineage, first detected in the United Kingdom. Tentative evidence of recombination between VOCs (Alpha and Delta) has also been reported in a small cluster of cases in Japan.

The XB lineage: A recombinant B.1.631/B.1.634 lineage (designated as lineage XB by the Pango nomenclature) in North America.

Later studies found three recombinant lineages, which were given Pango designation.

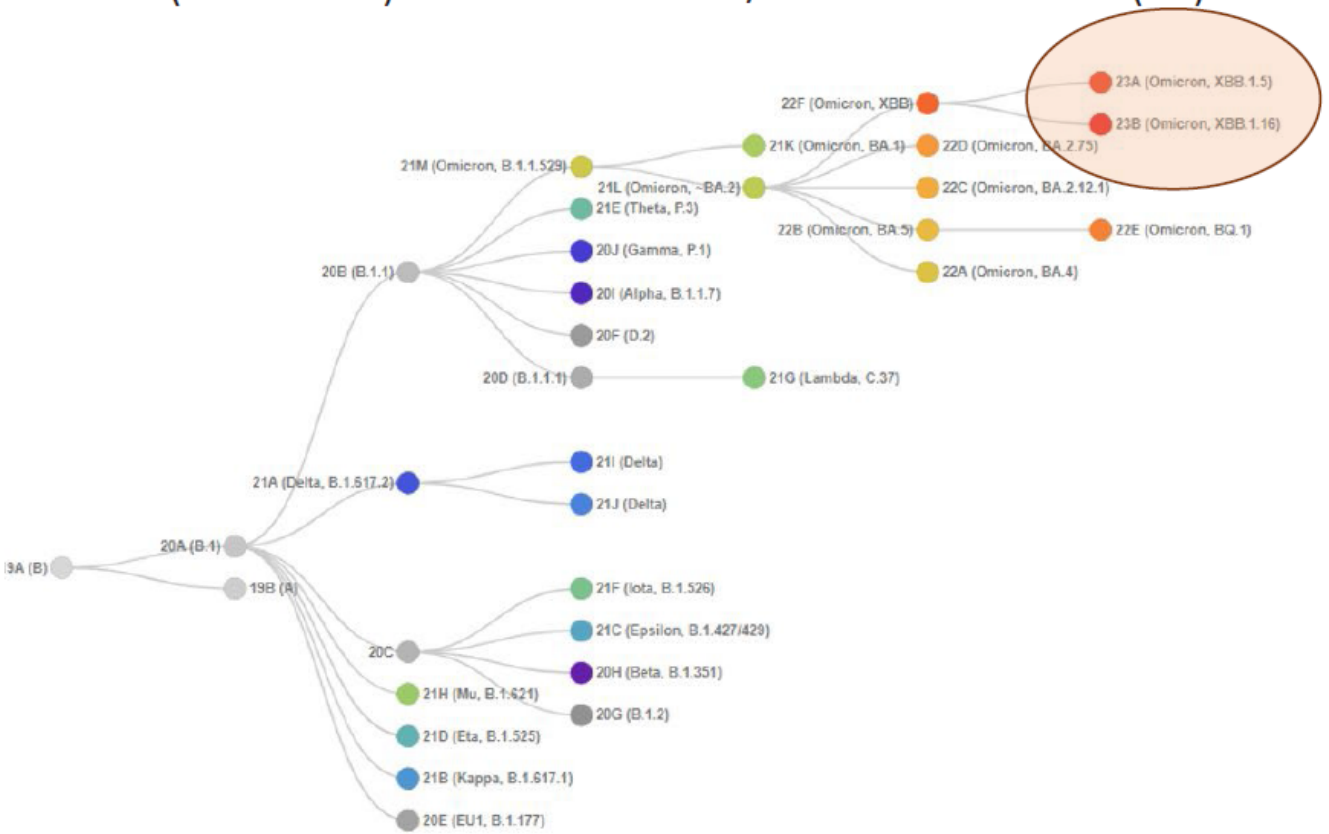
The XD, XF, XE lineage: Two are a combination of Delta and BA.1 (XD and XF) and one is a BA.1/BA.2 recombinant (XE).

SARS-CoV-2 Recombinant Lineages

Notable SARS-CoV-2 Recombinant Lineages

Recombinant	Hybrid Lineage
XC	Alpha (B.1.1.7) and Delta (AY.29)
XD "Deltacron"	Delta (AY.4) and Omicron (BA.1)
XE	BA.1 and BA.2
XF	Delta (AY.4) and Omcrion (BA.1)
XG	BA.1 and BA.2
XAY	AY.45 (Delta) & BA.4/BA.5 (Omicron)
XBB "Gryphon"	BJ.1 (BA.2.10.1.1) and BM.1.1.1 (BA.2.75.3.1.1.1)
XBC	B.1.617 (Delta) & BA.2 (Omicron)
XBF	BA.5.2.3 and CJ.1
XBL*	XBB.1 and BA.2.75

**XBL (XBB.1 and BA.2.75) is the first recombinant of a recombinant!!*



CoVariants

CoVariants

Markov PV, et al. Nat Rev Microbiol. 2023

HIV-1

GRUPPI M (major) con 9 sottotipi: A, B, C, D, F, G, H, J, K
e > 40 forme circolanti ricombinanti (CRFs)

O (outlier)

N (non M-non O)

Variazione tra cladi 20-30%

Variazione intraclade 10-15%

AIDS 2004, Vol 18 (suppl 3)

Wainberg M.A

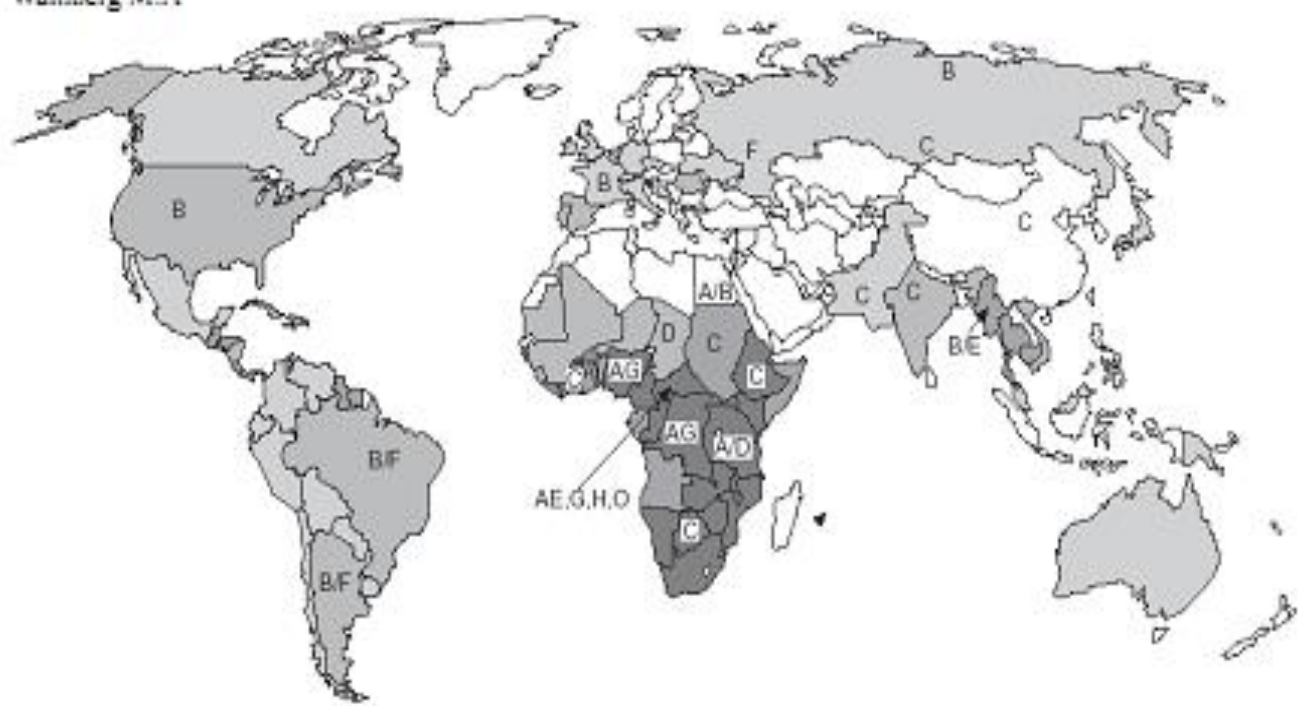


Fig. 1. Geographical distribution of HIV-1 clades. Letters indicate the predominant HIV-1 clade circulating in selected countries. A/B, B/E, and B/F indicate co-circulation of two dominant clades; AE and AG indicate circulating recombinant forms.

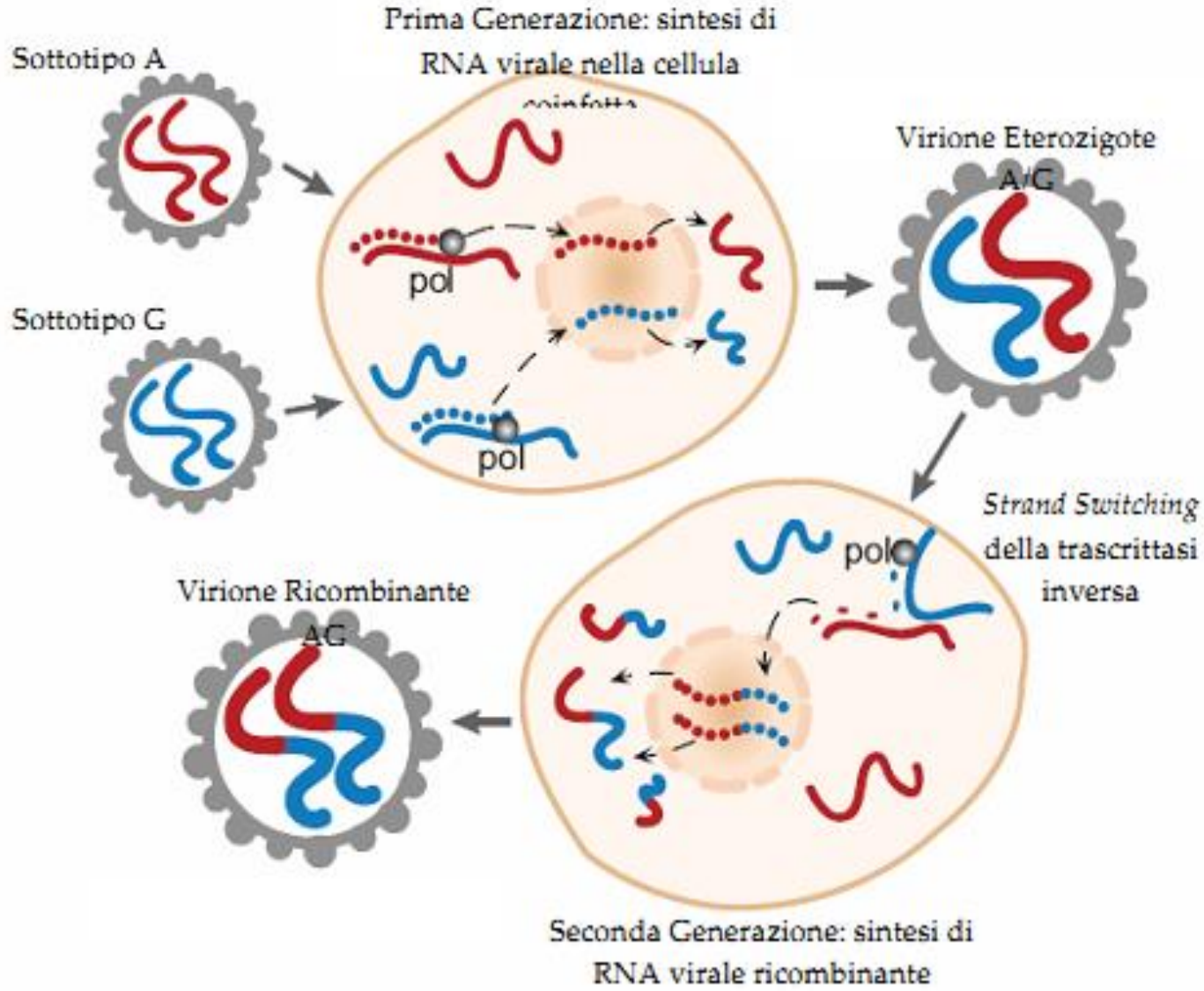
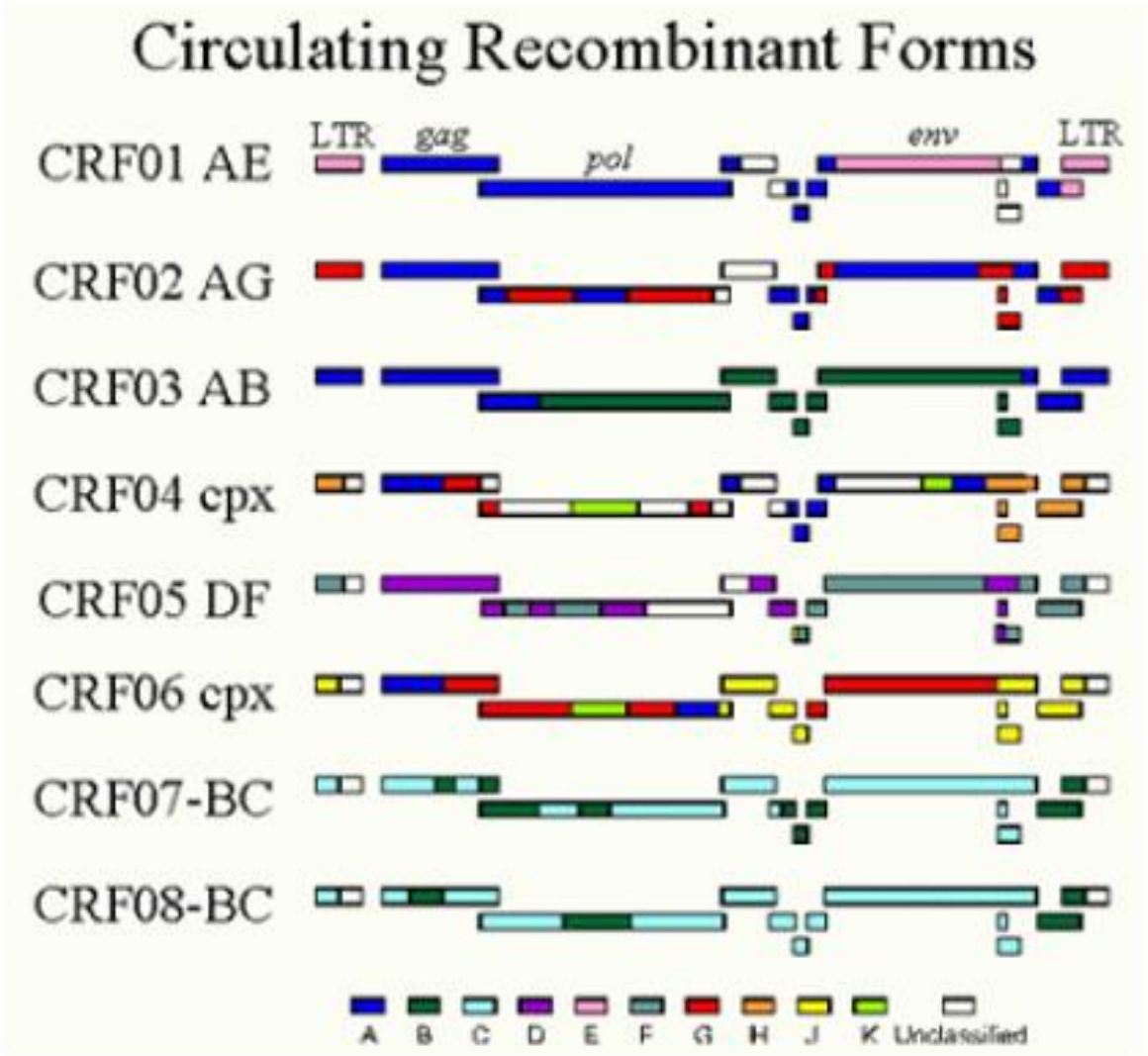
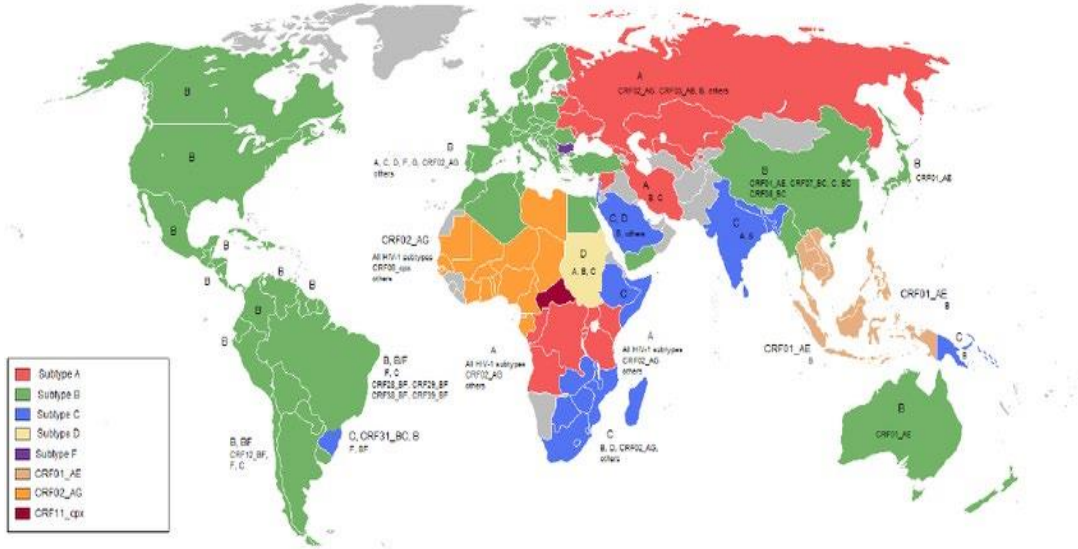


Figura 5. Modello di formazione delle forme ricombinanti di HIV-1.





HIV-1 distribution wordlwide 2019

HIV variability and antiviral drugs

Immunological pression:
HIV-1 quasispecies co-existence and subvariant selection

Longitudinal analysis of HIV-1 proviral env sequences obtained from subjects PEC52 (a,

2000–2013, 2000–2012 and 2000–2 0 11 , respectively.

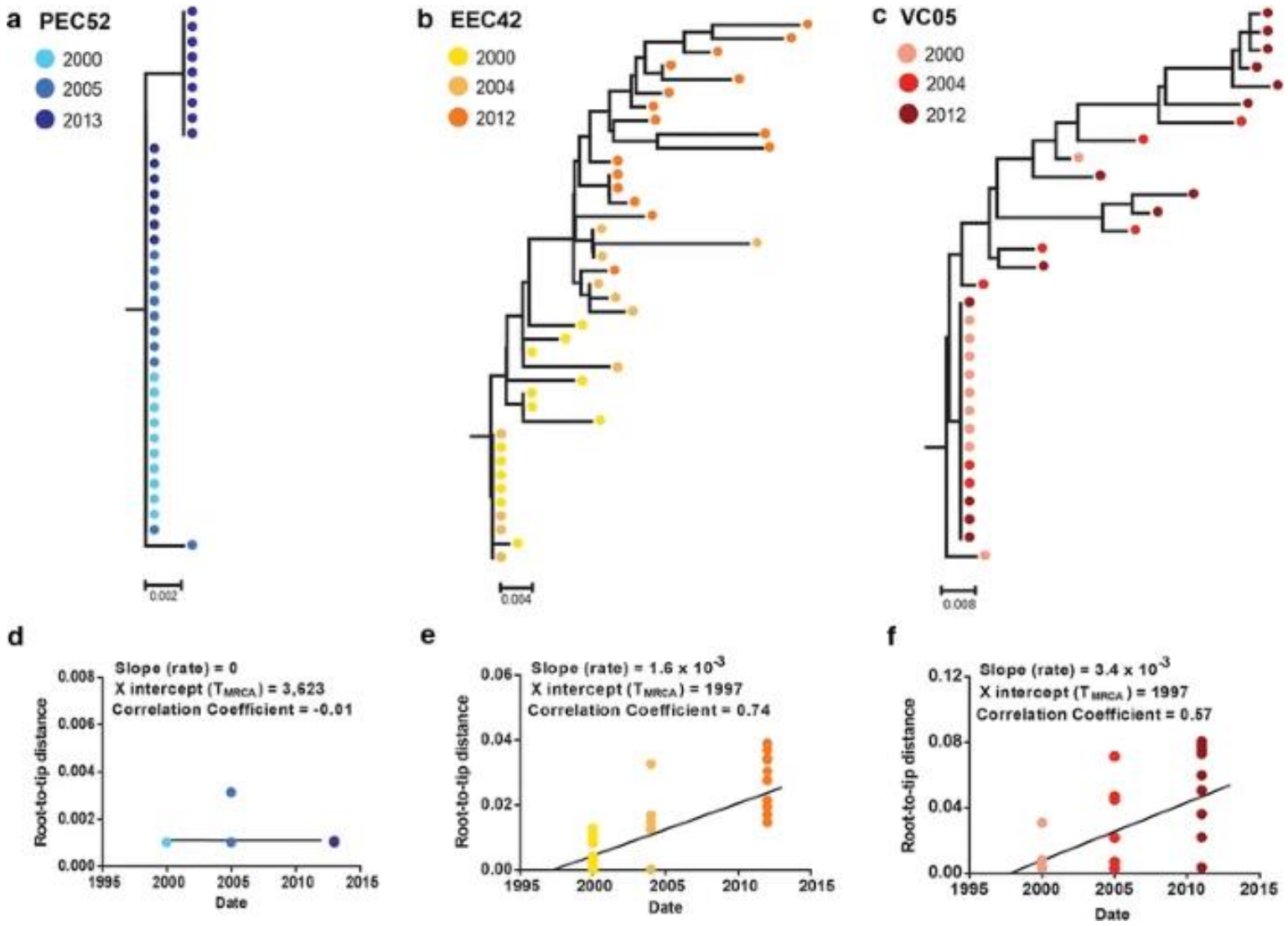
a–c ML phylogenetic trees for each individual are shown, in which horizontal branch lengths

indicating nucleotide substitutions per site.

d–f Plots of the root-to-tip distance against sequence sampling time are shown below

regression and X intercept of linear regression analysis is indicated. The colors of the circles

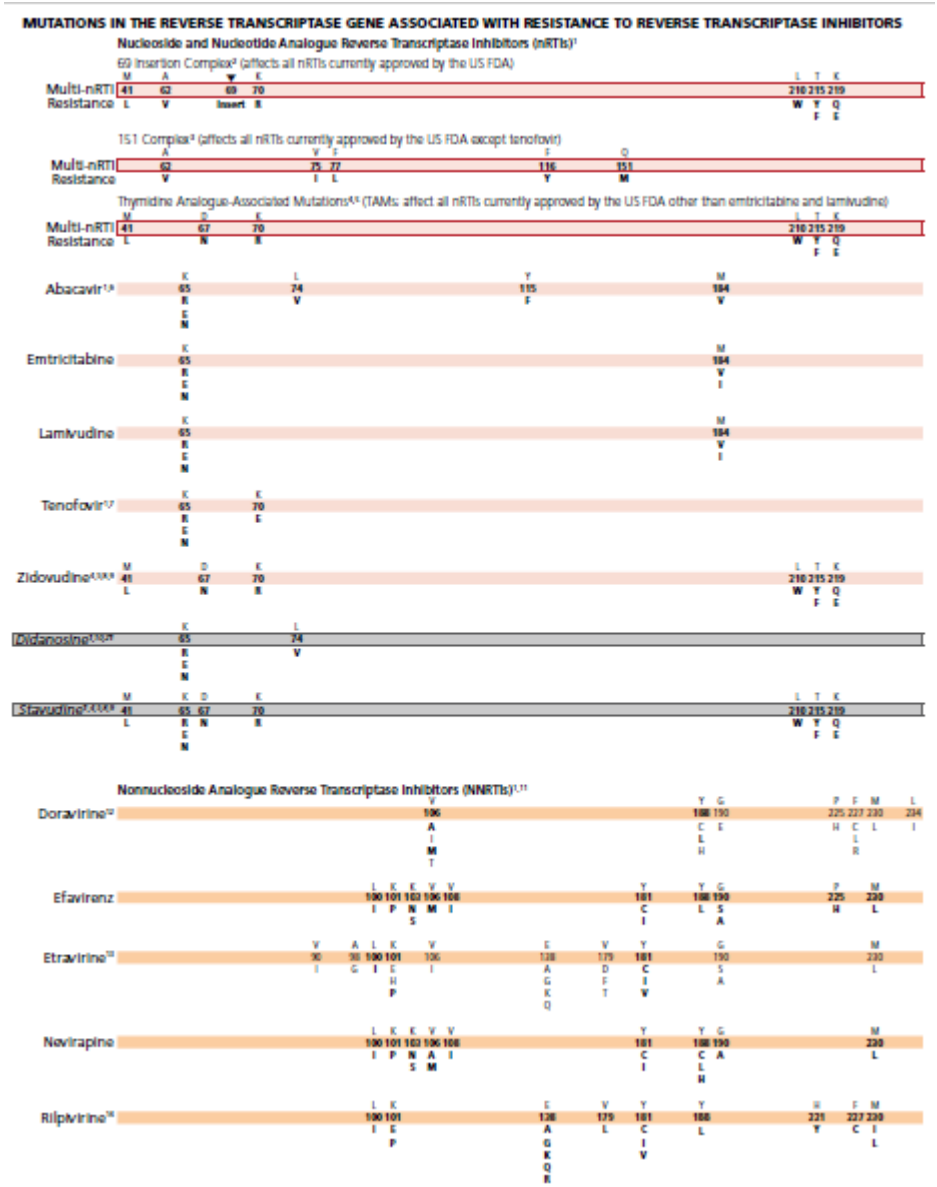
sampling year at which sequences were obtained

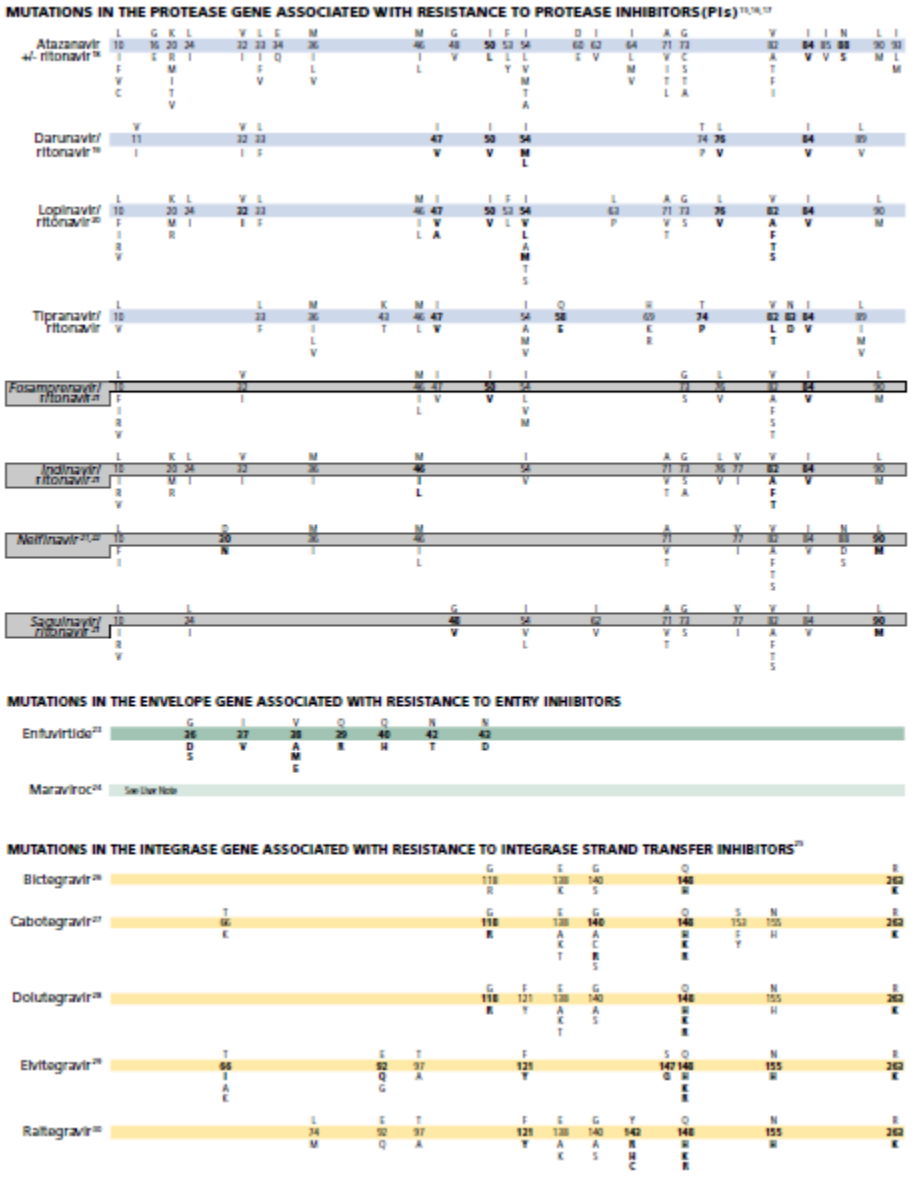


https://retrovirology.biomedcentral.com/articles/10.1186/s12977-017-0354-5

Actually HIV-1 cumulated more than 100 mutations have been associated with ART resistance

Wensing AM, et al. Top HIV Medicine 2019





Journal of NeuroVirology
https://doi.org/10.1007/s13365-018-0633-5 2018

Ultradeep single-molecule real-time sequencing of HIV envelope reveals complete compartmentalization of highly macrophage-tropic R5 proviral variants in brain and CXCR4-using variants in immune and peripheral tissues

Robin L. Brese[1] · Maria Paz Gonzalez-Perez[1] · Matthew Koch[1] · Olivia O'Connell[1] · Katherine Luzuriaga[1] · Mohan Somasundaran[1] · Paul R. Clapham[1] · James Jarad Dollar[2] · David J Nolan[2] · Rebecca Rose[2] · Susanna L. Lamers[2]

1. Brain derived-viruses were compartmentalized from virus in tissue outside the brain. Lymph nodes, blood, lung and colon

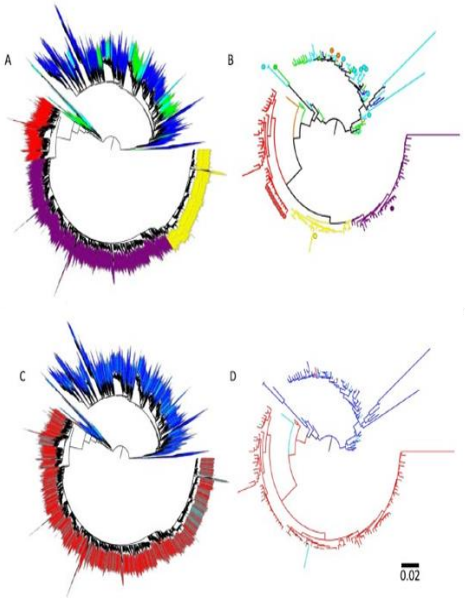
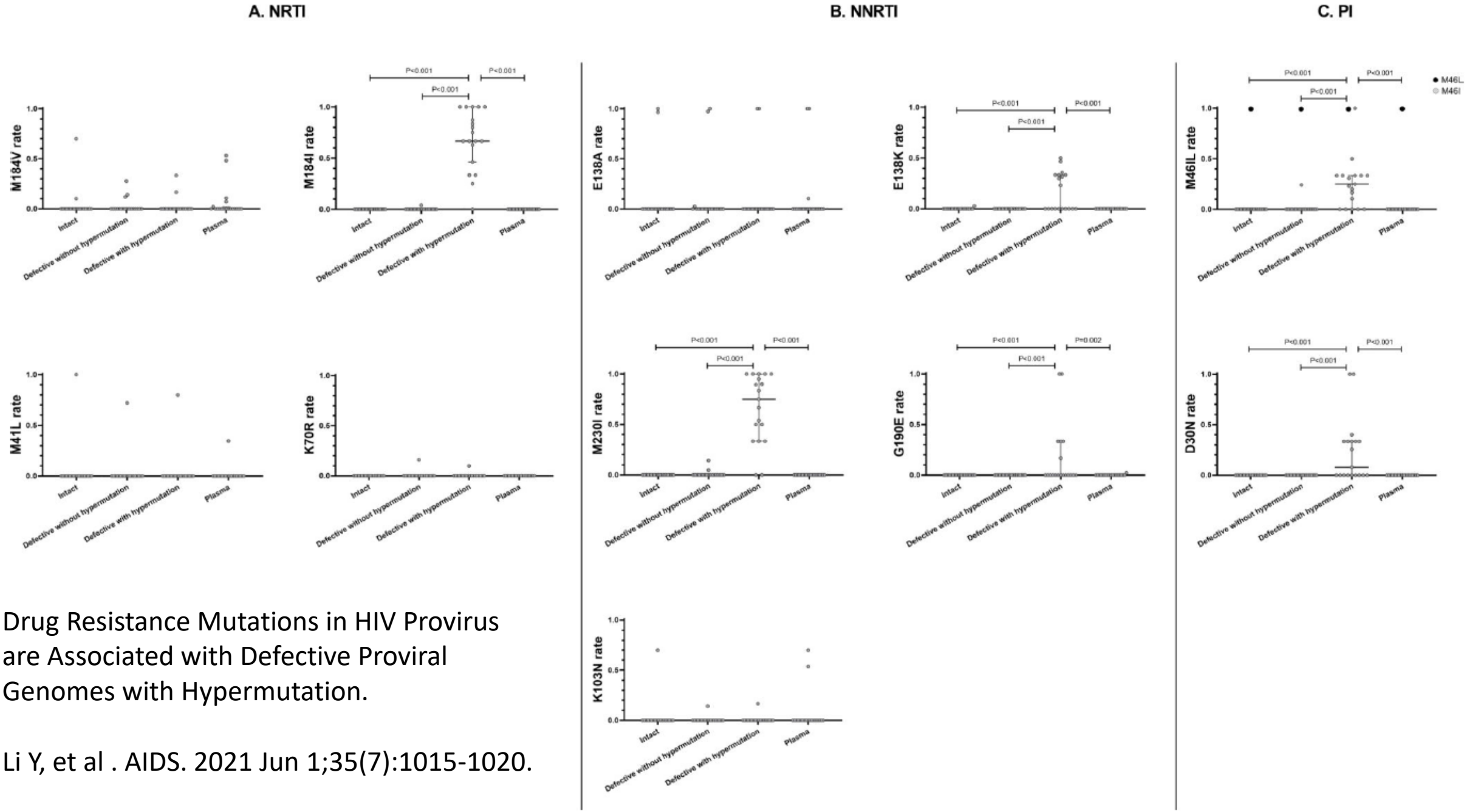


Fig. 1 Maximum-likelihood tree using the HQCS (a, c) and HQCS10 (b) datasets. Branches are scaled in substitutions/site according to the bar at the bottom of each tree. a, b Branches are colored to indicate the tissue of origin as follows: dark blue = blood, orange = colon, aqua = lung, green = lymph node, red = frontal lobe, yellow = occipital lobe, purple = parietal lobe. b Molecular clones are indicated with circles. c R5 and X4 variants estimated using WebPSSM x4r5 algorithm. Branches are colored as follows: blue = R5, red = X4, aqua = undetermined



Drug Resistance Mutations in HIV Provirus are Associated with Defective Proviral Genomes with Hypermutation.

Li Y, et al . AIDS. 2021 Jun 1;35(7):1015-1020.

communications biology

ARTICLE

https://doi.org/10.1038/s42003-023-05177-3 OPEN

Bacteria exposed to antiviral drugs develop antibiotic cross-resistance and unique resistance profiles

Veronica J. Wallace[1], Eric G. Sakowski[1,2], Sarah P. Preheim[1] & Carsten Prasse[1]✉

Our results establish the previously unrecognized capacity of antivirals to broadly alter the phenotypic antimicrobial resistance profiles of both gram-negative and gram-positive bacteria Escherichia coli and Bacillus cereus.

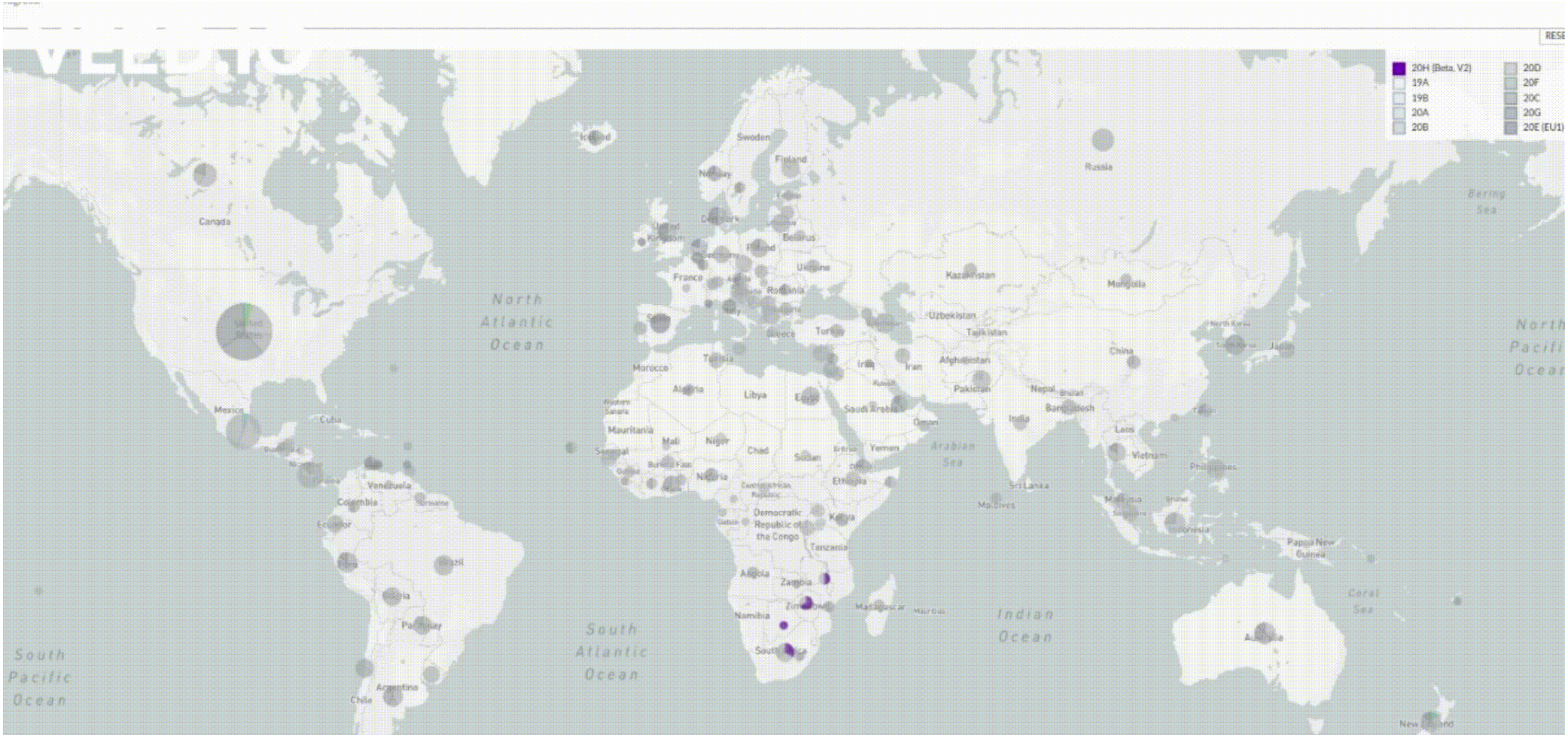
Bacteria exposed to antivirals including zidovudine, dolutegravir and raltegravir developed cross-resistance to commonly used antibiotics including trimethoprim, tetracycline, clarithromycin, erythromycin, and amoxicillin

August 2023

SARS-COV-2 variability and antiviral drugs

Low variability is balanced by high transmission and worldwide diffusion:

SARS-CoV-2 mutates and evolves during the course of adaptation in humans

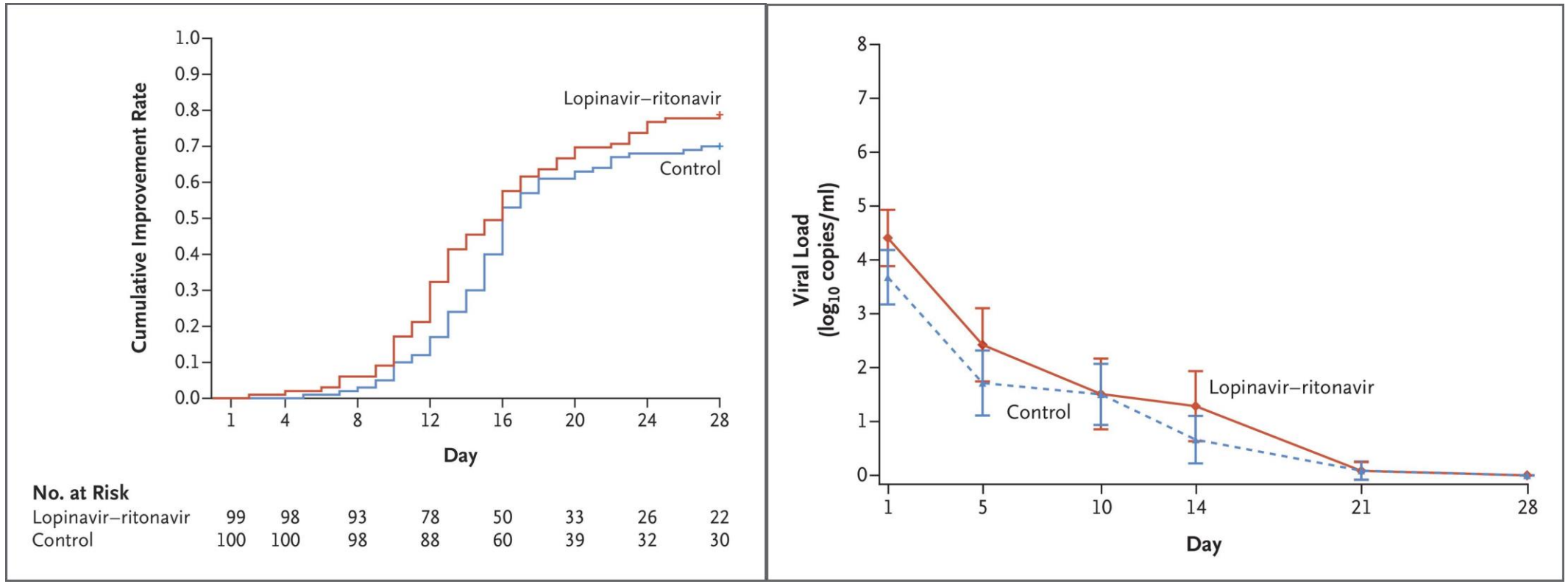


https://nextstrain.org/ncov/gisaid/global/all-time

SARS-CoV-2 RNA protease as target therapy

The Lopinavir-ritonavir combination is one the first repurposed drugs used in the treatment of COVID-19.

- They did not improve virological cure, radiological findings, mortality in COVID-19 patients



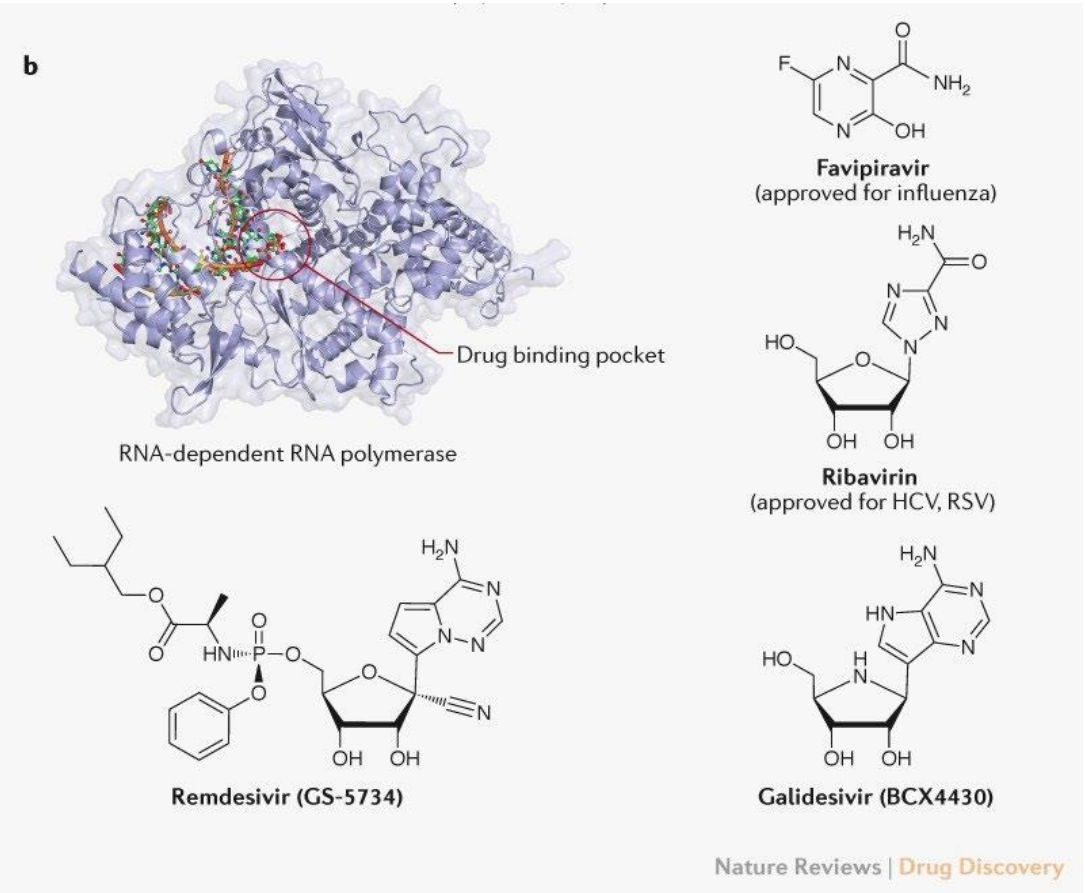
In hospitalized adult patients with severe Covid-19, no benefit was observed with lopinavir–ritonavir treatment beyond standard care

Cao et al., NEJM 2020

SARS-CoV-2 RNA-dependent RNA-polymerase as first target therapy

Initial analyses indicate that the catalytic sites of SARS-CoV-2 RNA polymerase,

that could represent antiviral targets, are highly conserved.



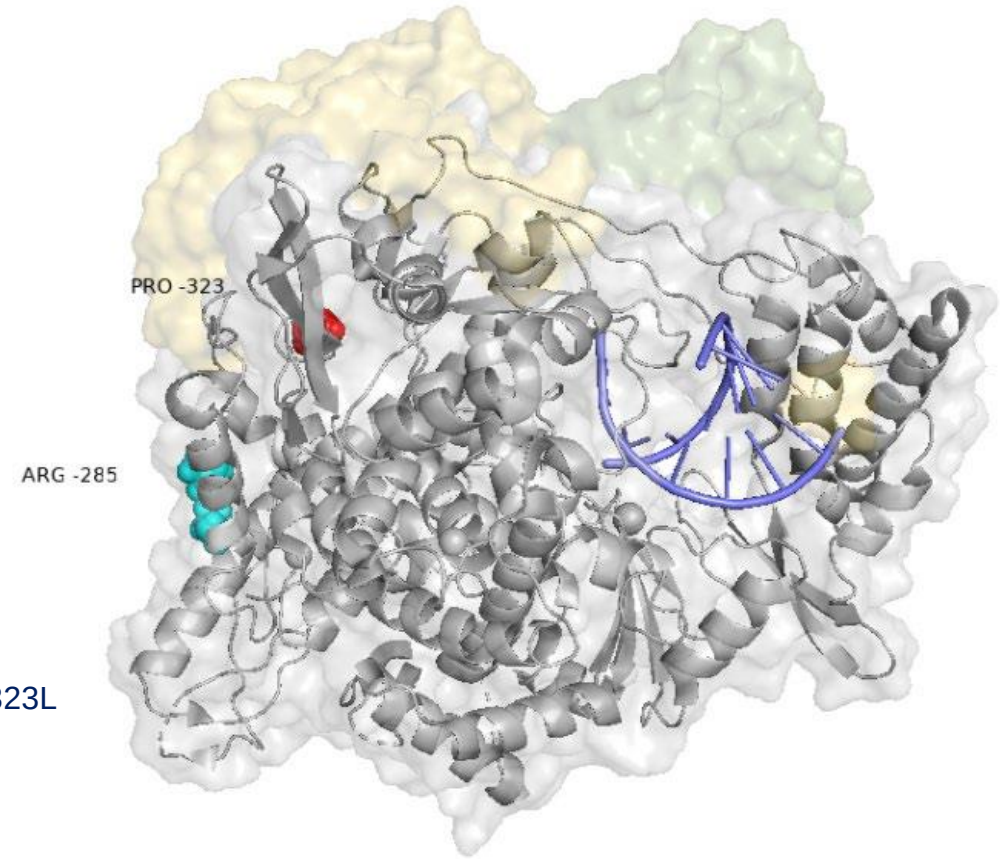
BUT…..

https://www.nature.com/articles/d41573-020-00016-0

Focusing on RdRp variability of Omicron clade

- RdRp is characterized by 2 mutations: P323L mutated in 100% of BA sublineages and sublineages.

- No mutations are localized in palm (RNA template and NTP binding site) and thumb (template



The structure of SARS-CoV-2 RdRp showing the residues coloured against different mutational rate

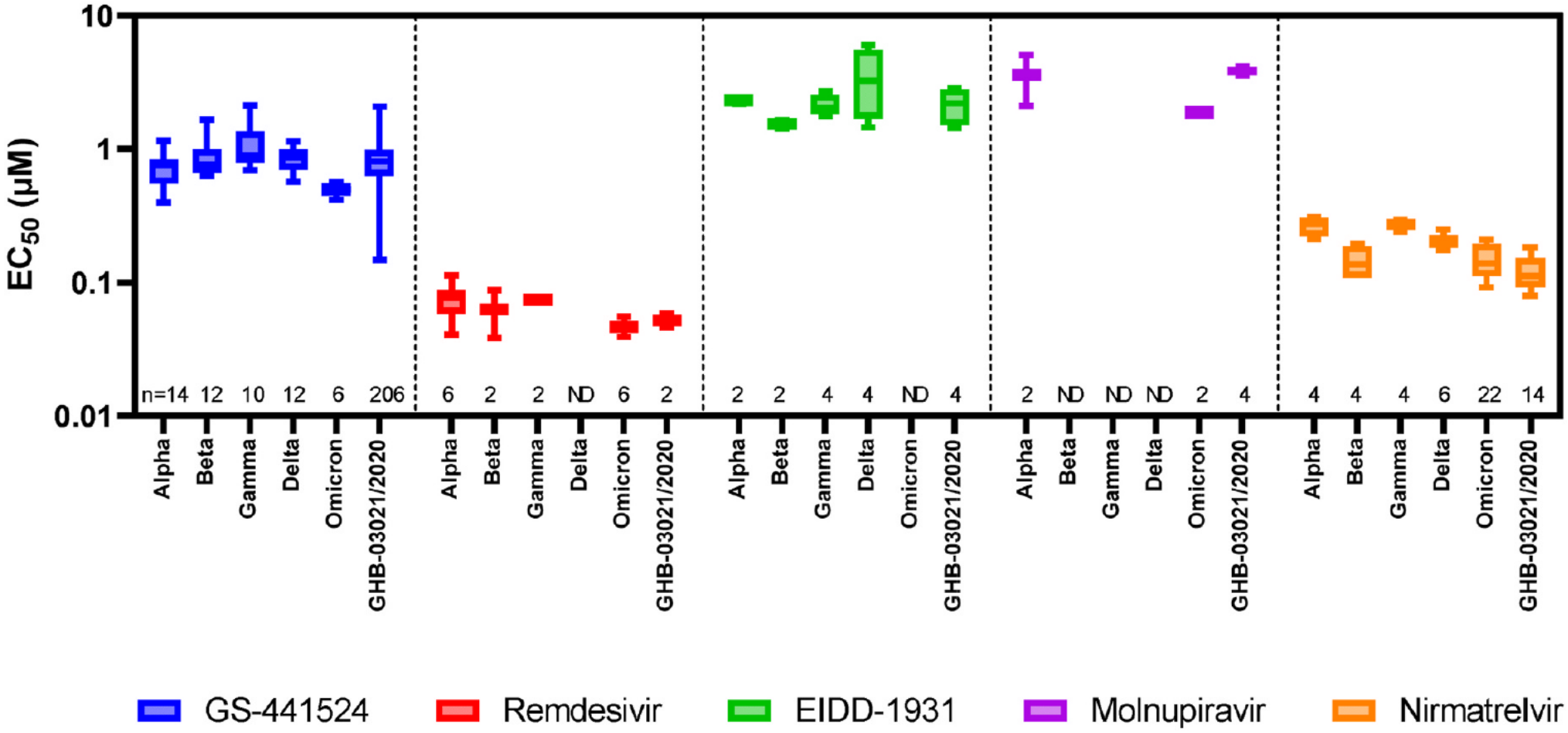
(dark green).

TABLE 1 Mutations occurring in the RNA-dependent RNA polymerase (RdRp) region and main protease (M^{pro}) region in the main dominant variants* since the beginning of the pandemic, and *in vitro* impact on antiviral activity.

Variant	Mutations in the RdRp region	Impact on remdesivir *in vitro* activity	Impact on molnupiravir *in vitro* activity	Mutations in the M^{pro} region	Impact on nirmatrelvir *in vitro* activity
Alpha	P323L	No significant change; Vangeel et al. (2022)	No significant change; Vangeel et al. (2022)		No significant change; Vangeel et al. (2022)
Delta	P323L, G671S	No significant change; Vangeel et al. (2022)	No significant change; Vangeel et al. (2022)		No significant change; Vangeel et al. (2022)
Omicron BA.1	P323L	No significant change (Vangeel et al., 2022; Veklury, 2022)	No significant change (Vangeel et al., 2022; Uraki et al., 2022b)	P132H	No significant change (Joyce et al., 2022; Vangeel et al., 2022)
Omicron BA.2	P323L	No significant change (Veklury, 2022; Takashita et al., 2022a)	No significant change (Takashita et al., 2022a; Uraki et al., 2022a)	P132H	No significant change (Takashita et al., 2022a; Uraki et al., 2022a)
Omicron BA.5	P323L	No significant change (Veklury, 2022; Takashita et al., 2022b)	No significant change (Takashita et al., 2022b)	P132H	No significant change (Takashita et al., 2022b)
Omicron BQ.1.1	Y273H, P323L	No significant change (Imai et al., 2022)	No significant change (Imai et al., 2022)	P132H	No significant change (Imai et al., 2022)
Omicron XBB	P323L, G671S	No significant change (Imai et al., 2022)	No significant change (Imai et al., 2022)	P132H	No significant change (Imai et al., 2022)

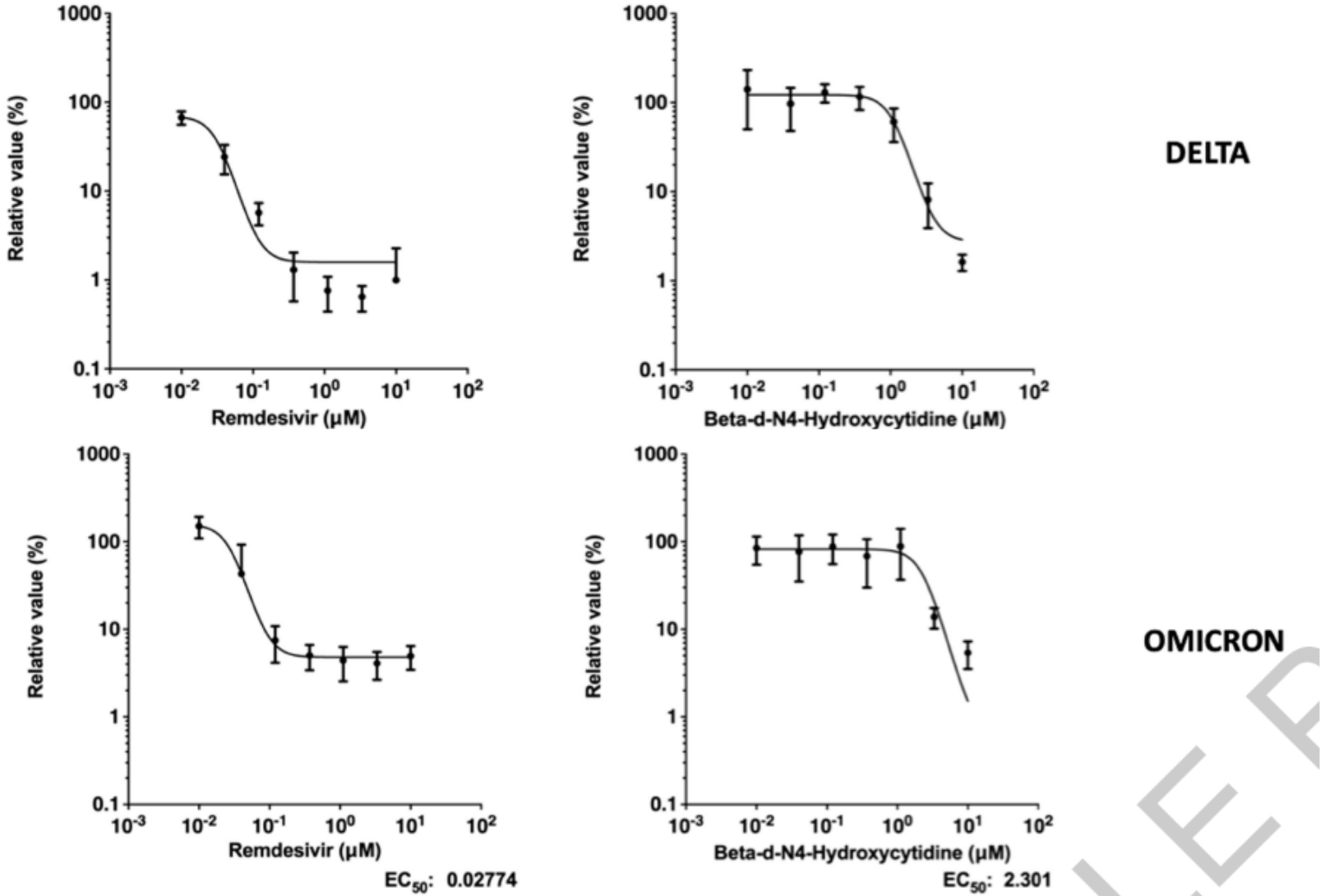
*Mutations are presented compared to the index Wuhan-HU-1 strain, according to Stanford University Coronavirus Antiviral & Resistance Database (Stanford University, 2022a).

Remdesivir, Molnupiravir and Nirmatrelvir remain active against SARS-CoV-2 Omicron and other variants of concern



Vangeel et al., Antiviral research 2022

Importantly, antiviral drugs remdesivir and molnupiravir retain efficacy against Omicron and the newest BA.B.2.86



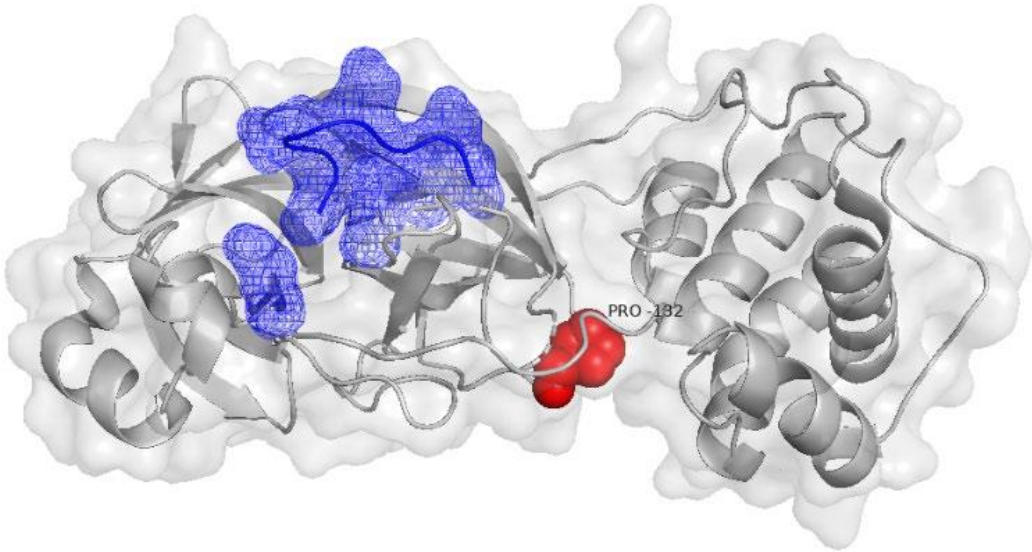
Meng, B. et al. *Nature 2022*

Focusing on Omicron Nsp5- 3CLpro…

• Nsp5-3CLpro is characterized by one mutation (P132H)

involved in the 3CLpro–PF complex.

• No resistance



The structure of SARS-CoV-2 Nsp5-3CLpro showing the active site in blu and the residues coloured against

nature communications

Article

https://doi.org/10.1038/s41467-023-39704-x

In vitro and in vivo characterization of SARS-CoV-2 strains resistant to nirmatrelvir

Received: 8 January 2023

Accepted: 20 June 2023

Published online: 04 July 2023

Maki Kiso[1], Yuri Furusawa[1,2], Ryuta Uraki[1,2], Masaki Imai[1,2,3], Seiya Yamayoshi[1,2,3] ✉ & Yoshihiro Kawaoka[1,2,4,5] ✉

- Nsp5-L50F/E166V and Nsp5-L50F/E166A/L167F mutant viruses showed decreased susceptibility to nirmatrelvir
- Both mutants are dominated by the wild-type virus, therefore they do not become dominant in nature
- However, it is important to closely monitor the emergence of nirmatrelvir-resistant SARS-CoV-2 variants because resistant viruses with additional compensatory mutations could emerge, outcompete the wild-type virus, and become dominant.

Monoclonal Antibodies (mAb)

Escape

pharmaceuticals-14-01272.pdf - Adobe Acrobat Reader DC
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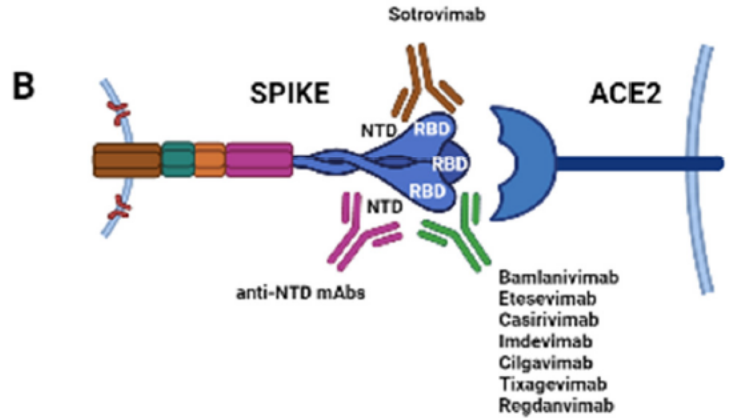


Figure 1. Schematic of the spike protein (S) of Sars-CoV-2 and its interactions with its cellular receptor, the angiotensin converting enzyme 2 (ACE2), and with therapeutic monoclonal antibodies (mAbs). The S protein is a trimer. (**A**) Each monomer of the S protein consists of a N-terminal S1 subunit [comprising the N-terminal domain (NTD), the receptor-binding domain (RBD), subdomain 1 (SD1), and subdomain 2 (SD2)] and a C-terminal S2 subunit [comprising the fusion peptide (FP), the heptad repeat 1 (HR1), the heptad repeat 2 (HR2) and the transmembrane domain (TM)]. The S1 subunit binds the ACE2 receptor, while the S2 subunit is involved in membrane fusion during cell entry. Upon binding of the trimer to the host cell receptor through the RBD, the S1 and S2 subunits are cleaved by the host transmembrane protease serine 2 (TMPRSS2) at the S1/S2 junction; then, a second site within the S2 subunit, termed the S2′ site, is cleaved by serine proteases or cathepsins and viral-host membranes fusion is initiated. (**B**) Interaction between the S protein and the host cell receptor ACE2. Most therapeutic mAb targets the RBD of the S protein at positions required for the interaction with ACE2 (Bamlanivimab, Etesevimab, Casirivimab, Imdevimab, Cilgavimab, Tixagevimab, Regdanvimab) while Sotrovimab targets the RBD, but does not compete with human ACE2 receptor binding. mAbs binding the NTD have been demonstrated to neutralize SARS-CoV-2, and these could be developed for therapeutic purposes [15,19–26] (Created with BioRender.com).

As expected, mutations causing the strongest resistance were found within the binding footprint of the mAb at cognate epitope positions in the spike RBD

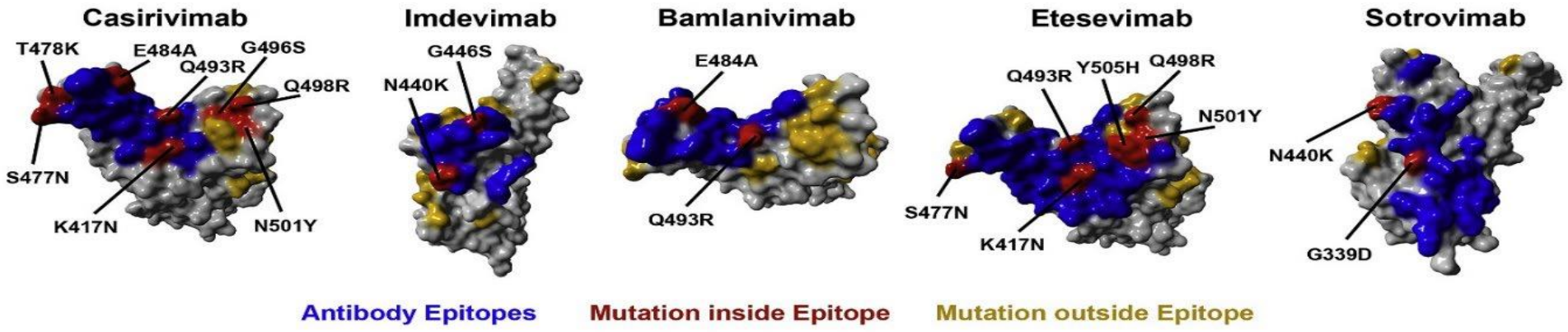
Cerca 10:06 26/09/2023



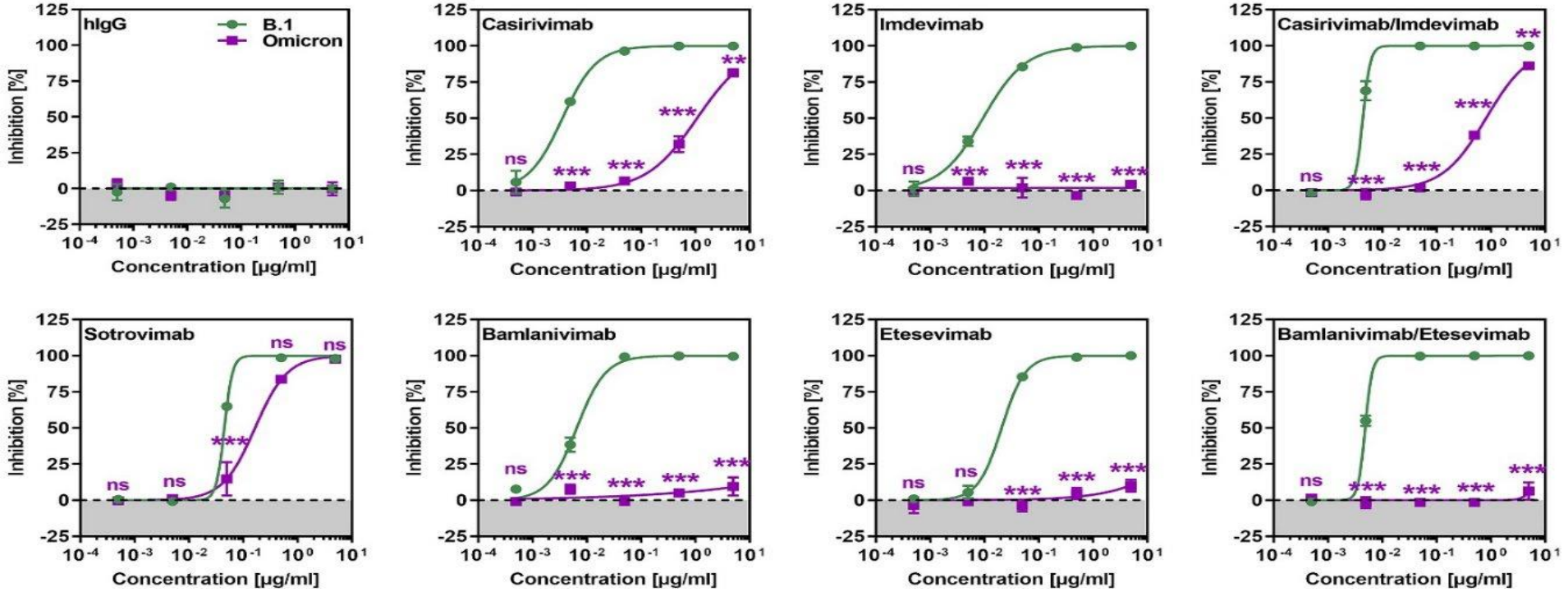
Variante SARS-CoV-2	Bamlanivimab	Etesevimab	Bamlanivimab / Etesevimab	Casirivimab	Imdevimab	Ronapreve	Cilgavimab	Tixagevimab	Evusheld	Sotrovimab	Bebtelovimab
Delta	>1000	0.4	1	0.7	1.5	1.3	2.4	1	1	1.1	1
Omicron/BA.1	>1000	459	980	>1000	>1000	>1000	263	264	63	3.8	1
Omicron/BA.2	>1000	515	744	>1000	219	387	1.8	928	8	21	1
Omicron/BA.2.12.1	>1000	515	794	>1000	125	250	2.8	410	9.1	20	1
Omicron/BA.2.75	>1000	383	554	477	>1000	>1000	14	29	24	12	3.1
Omicron/BA.2.75.2	556	489	>1000	589	588	>1000	>1000	>1000	>1000	8	2.8
Omicron/XBB	>1000	>1000	-	589	588	200	>1000	>1000	738	14	>1000
Omicron/XBB.1	>1000	>1000	>1000	880	>1000	887	>1000	>1000	>1000	15	900
Omicron/XBB.1.5	-	-	-	650	572	751	433	>1000	867	18	475
Omicron/XBB.1.16	-	-	-	420	143	615	127	448	488	5.3	149
Omicron/BA.4/5	>1000	504	588	>1000	143	387	8.1	>1000	25	22	1
Omicron/BA.4.6	556	489	>1000	589	173	738	527	819	738	44	1
Omicron/BQ.1	-	-	-	177	175	200	>1000	>1000	>1000	26	900
Omicron/BQ.1.1	>1000	972	>1000	>1000	>1000	>1000	>1000	>1000	>1000	118	>1000
Omicron/BF.7	-	-	-	>1000	383	878	>1000	>1000	>1000	48	1.3
Omicron/CH.1.1	-	-	-	>1000	>1000	>1000	>1000	>1000	>1000	16	>1000

Figura 3. La riduzione della capacità neutralizzante degli anticorpi monoclonali nei confronti del clade Delta e dei diversi lignaggi del clade Omicron. La cella non colorata indica una riduzione della capacità neutralizzante inferiore a 5-fold; il blu chiaro indica una riduzione della capacità neutralizzante tra -5 e 29.4-fold; il blu scuro indica una riduzione della capacità neutralizzante maggiore o uguale a 25-fold. https://covdb.stanford.edu/susceptibility-data/table-mab-susc/, ultimo accesso, 8 Giugno 2023

Omicron is resistant against neutralization by several therapeutic antibodies



C



Hoffman et al., Cell 2022

Resistance mutations in SARS-CoV-2 omicron variant in patients treated with sotrovimab

Sotrovimab-resistant spike mutations were observed in half of the patients who remained SARS-CoV-2 RNA positive 7 to 21 days after infusion.

Table 1
Clinical characteristics and viral evolution of sotrovimab-treated patients with resistant spike mutations

Patient number	Clinical characteristics	Vaccination status[a]	Variant	Days after sotrovimab[b]	Mutation acquired	Number of haplotypes	Mutation, % (in the quasispecies)	Viral load evolution[c]
#1	Liver SOT	Boosted	BA.1	14	P337H	1	100	Rebound
#2	IS treatment	Unvaccinated	BA.1.1	21	E340D	1	100	Rebound
#3	Renal SOT	Boosted	BA.1.1	14	E340G	1	100	Rebound
#4	IS treatment	Full	BA.1.1	21	E340K	1	100	Rebound
#5	IS treatment	Boosted	BA.2	7	E340A	1	100	Rebound
#6	IS treatment	Full	BA.1.1	7	P337S	2	86	Slight decline
#7	Renal SOT	Single dose	BA.1.1	14	P337L	1	100	Slight decline
#8	Renal SOT	Boosted	BA.1	14	E340D	1	100	Slight decline
#9	Pulmonary SOT	Boosted	BA.1	14	E340D	1	100	Slight decline
#10	Primary immunode-ficiency	Boosted	BA.1.1	7	E340D	2	20	Slight decline
#11	IS treatment	Boosted	BA.1.1	7	E340K	1	100	Slight decline
#12	Liver SOT	Boosted	BA.1.1	7	E340K	2	100	Slight decline
#13	Renal SOT	Full	BA.1.1	7	S371F	3	16	Slight decline
#14	Chronic kidney disease	Full	BA.1.1	7	P337R	2	57	Decline
#15	Chronic kidney disease	Boosted	BA.1	7	E340D	3	100	Decline
#16	IS treatment	Boosted	BA.1.1	7	E340D	2	44	Decline
#17	IS treatment	Full	BA.1.1	7	E340K	1	100	Decline
#18	IS treatment	Boosted	BA.1	7	K356T	2	27	Decline

Abbreviations: IS, immunosuppressive; SOT, solid organ transplant.
[a] Doses of SARS-CoV-2 vaccine received (single dose, full: two doses, boosted: three doses).
[b] Time after sotrovimab infusion when the mutation was first detected.
[c] Rebound of the viral load after mutation emergence—slight decline means viral load decline <1 $\log_{10}$ copies/mL, decline means viral load decline >1 $\log_{10}$ copies/mL.

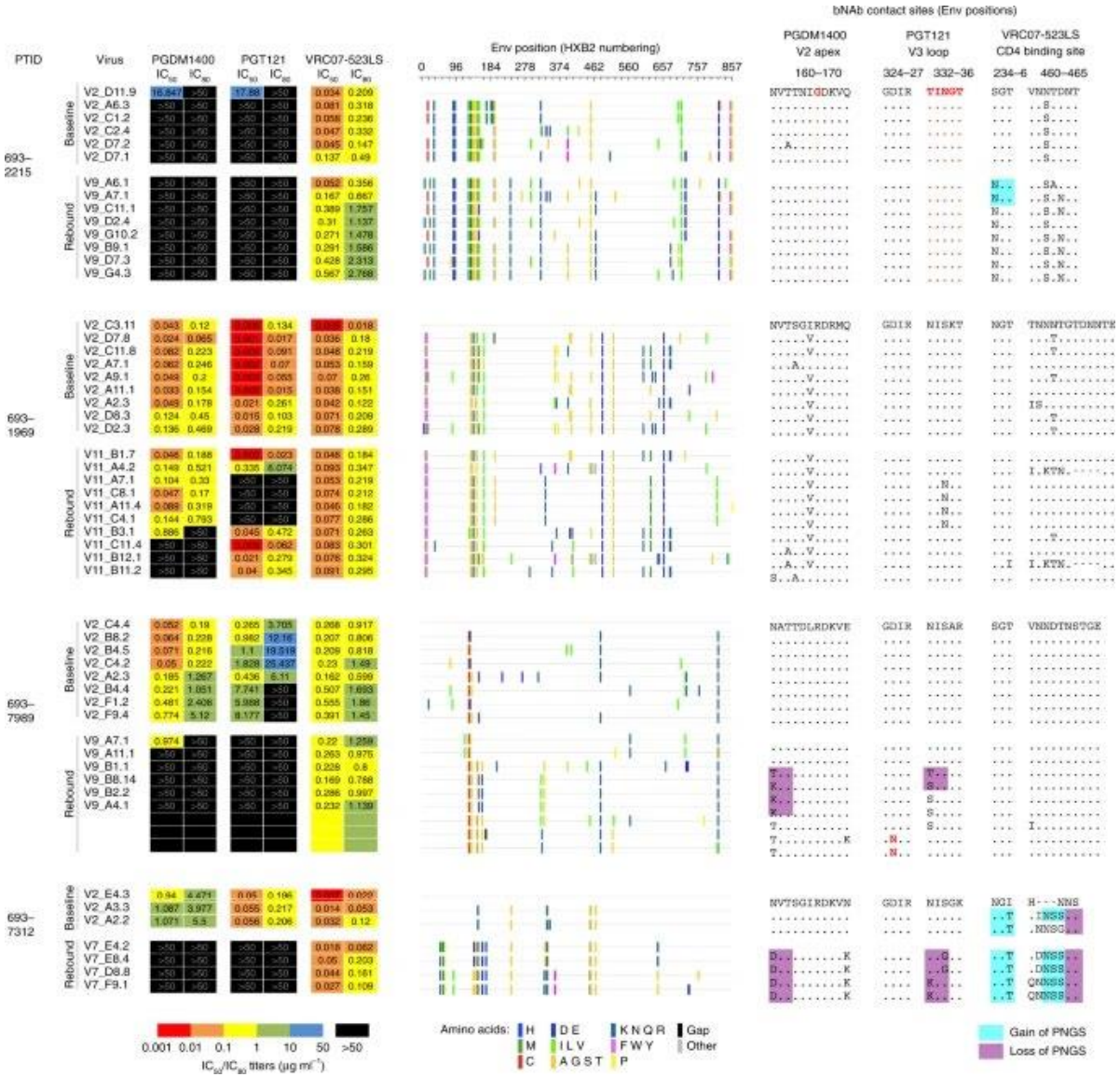
Vellas C et al., CMI 2022

Table 1 | Monoclonal antibodies approved for clinical use and SARS-CoV-2 variant evasion of neutralization

Generic name	Other name	Manufacturer	mAb class	Spike epitope[a]	VOC resistance[b]
Amubarvimab+ romlusevimab[85,86]	BRII-196+BRII-198, P2C-1F11+P2B-1G5	Brii Biosciences	Amubarvimab: class 1 Romlusevimab: no data	Amubarivimab: 403, 415, 416, 417, 420, 421, 453, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487, 489, 493, 502, 505 Romlusevimab: no data	Strong: BA.1.1, BA.4/5 Moderate: BA.1, BA.2, BA.2.12.1
Bamlanivimab[10] (withdrawn)	LYCoV555, LY3819253	Eli Lilly	Class 2	351, 449, 450, 452, 455, 456, 470, 472, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 492, 493, 494	Strong: Beta, Gamma, Delta, BA.1, BA.1.1, BA.2, BA.2.12.1, BA.4/5
Bamlanivimab+ etesevimab[13,87,88]	Etesevimab: LY3832479, LY-CoV016 (see bamlanivimab)	Eli Lilly	Etesevimab: class 1 Bamlanivimab: class 2	Etesevimab: 403, 405, 406, 408, 409, 415, 416, 417, 420, 421, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487, 489, 493, 494, 495, 500, 501, 502, 504, 505 (see bamlanivimab)	Strong: Beta, Gamma, BA.1, BA.1.1, BA.2, BA.2.12.1, BA.4/5
Bebtelovimab[89]	LY-CoV1404, LY3853113	Eli Lilly	Class 3	346, 439, 440, 441, 443, 444, 445, 446, 447, 448, 449, 450, 498, 499, 500, 501, 502, 506, 509	
DXP-604 (ref.[90])	NA	Singlomics+ BeiGene	Class 1	403, 415, 416, 417, 420, 421, 453, 455, 456, 457, 458, 459, 460, 473, 474, 475, 476, 477, 486, 487, 489, 493, 496, 498, 500, 501, 502, 503, 505	Moderate: BA.1 Mild: Beta
Regdanvimab[91]	Regkirona, CT-P59	Celltrion Healthcare	Class 1	351, 403, 417, 446, 449, 450, 452, 453, 455, 456, 470, 483, 484, 485, 486, 489, 490, 492, 493, 494, 495, 496, 498, 505	Strong: BA.1, BA.2, BA.1.1 Moderate: Beta, Delta, Gamma
Ronapreve[12,92]	REGEN-CoV2, casirivimab+imdevimab	Regeneron	Casirivimab: class 1 Imdevimab: class 3	Casirivimab: 403, 417, 421, 453, 455, 456, 475, 476, 484, 485, 486, 487, 488, 489, 493 Imdevimab: 346, 439, 440, 441, 444, 445, 446, 447, 448, 449, 450, 498, 500	Strong: BA.1, BA.1.1, BA.2, BA.2.12.1, BA.4/5
Sotrovimab[11,93,94]	Xevudy, VIR-7831, GSK4182136 Parental antibody: S309	GlaxoSmith-Kline	Class 3	333, 334, 335, 337, 339, 340, 343, 344, 345, 346, 354, 356, 357, 359, 360, 361, 441, 509	Moderate: BA.2, BA.2.12.1, BA.4/5 Mild: BA.1
Cilgavimab+ tixagevimab[95–97]	Evusheld AZD7442, AZD1061/AZD8895 Parental antibodies: COV2-2130, COV2-2196	AstraZeneca	Tixagevimab: class 1 Cilgavimab: class 2	Tixagevimab: 455, 456, 458, 475, 476, 477, 478, 479, 484, 485, 486, 487, 488, 489, 493 Cilgavimab: 345, 346, 439, 440, 441, 443, 444, 445, 446, 447, 448, 449, 450, 452, 484, 490, 492, 493, 494, 499	Strong: BA.1.1 Moderate: BA.1, BA.2.12.1, BA.4/5 Mild: BA.2

mAb, monoclonal antibody; mFRN, geometric mean fold reduction in neutralization; NA, not available; VOC, variant of concern. [a]Epitope positions are defined as those within 4.5 Å of the mAb when it is bound to the spike protein. [b]VOC strength of resistance is reported as strong (mFRN>100), moderate (mFRN=10–100) or mild (mFRN=3–10), based on the data reported in Fig. 1. Although these designators are useful to the extent that they assign a qualitative judgement to the level of resistance, it must be emphasized that resistance of variants occurs across a continuum.

HIV-1 extreme genetic variability behind mAb treatment failure

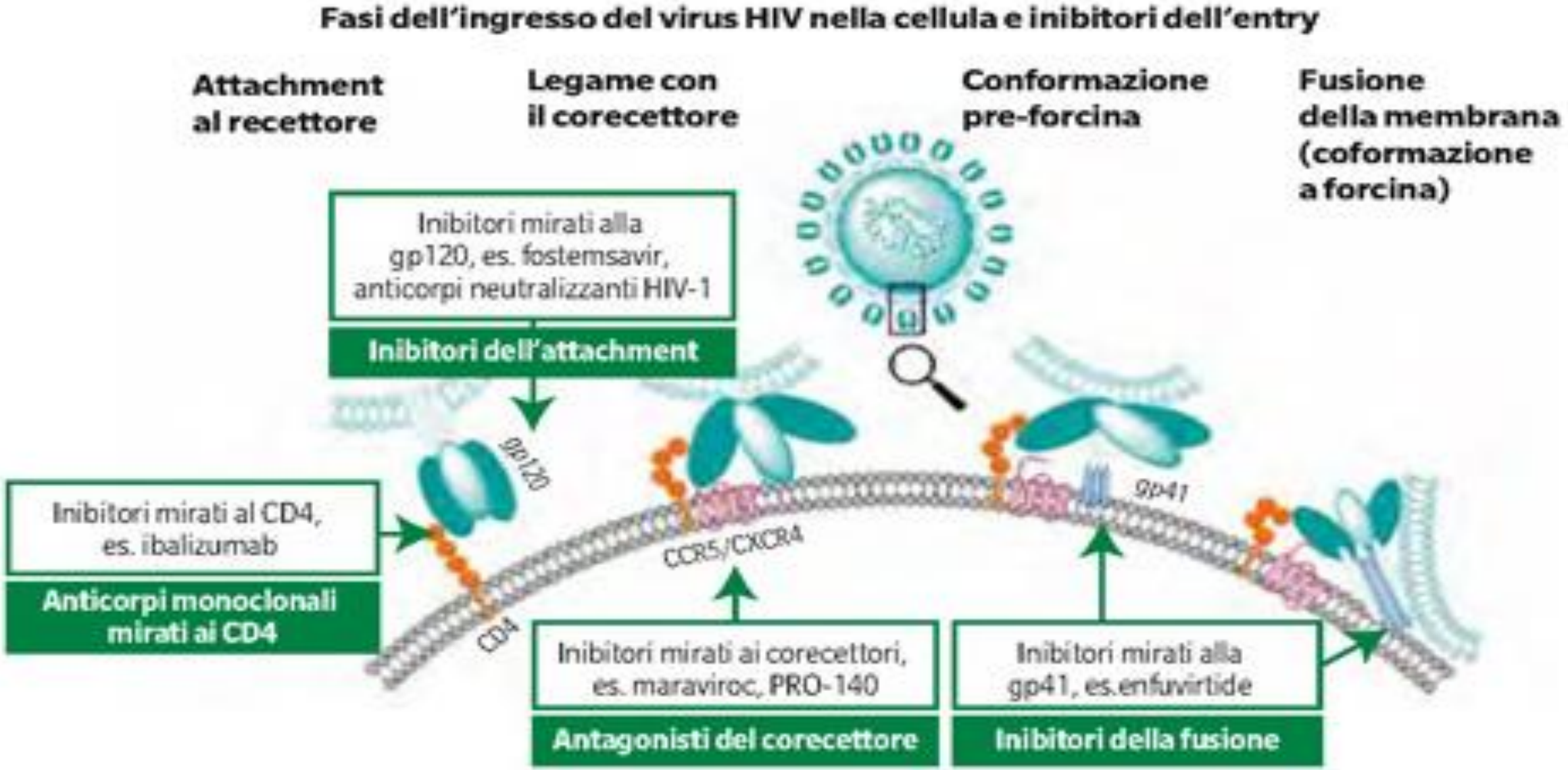


All participants either showed resistance to PGDM1400 and PGT121 at baseline or showed viral escape at rebound.

By analyzing *Env* sequences from participant isolates with the matched neutralization data they identified the mutations underlying the resistance patterns

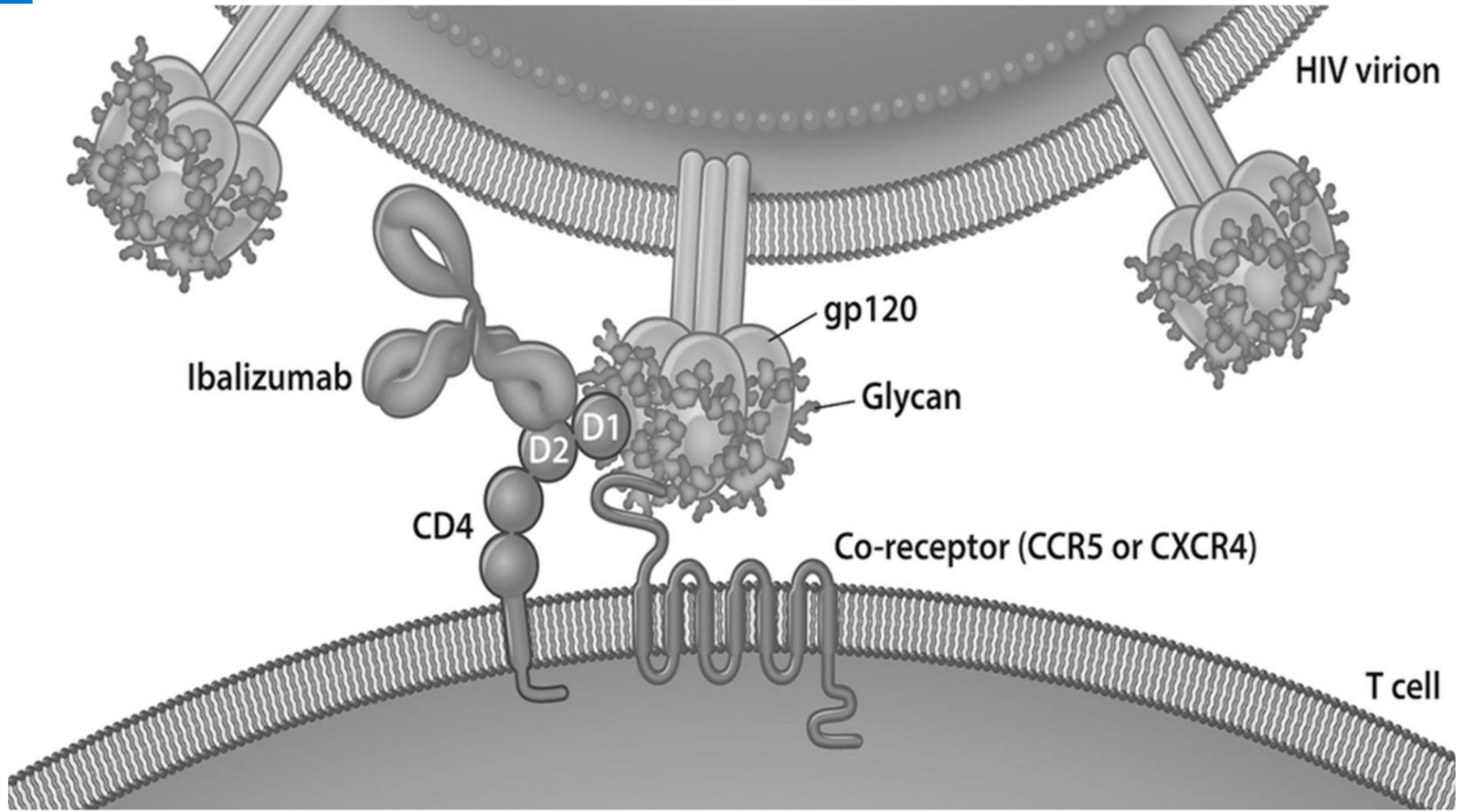
Julg, Nature Med 2022

FIG. 1 Meccanismo di azione (MoA)



Bruxelle JF et al. Microorganisms 2021

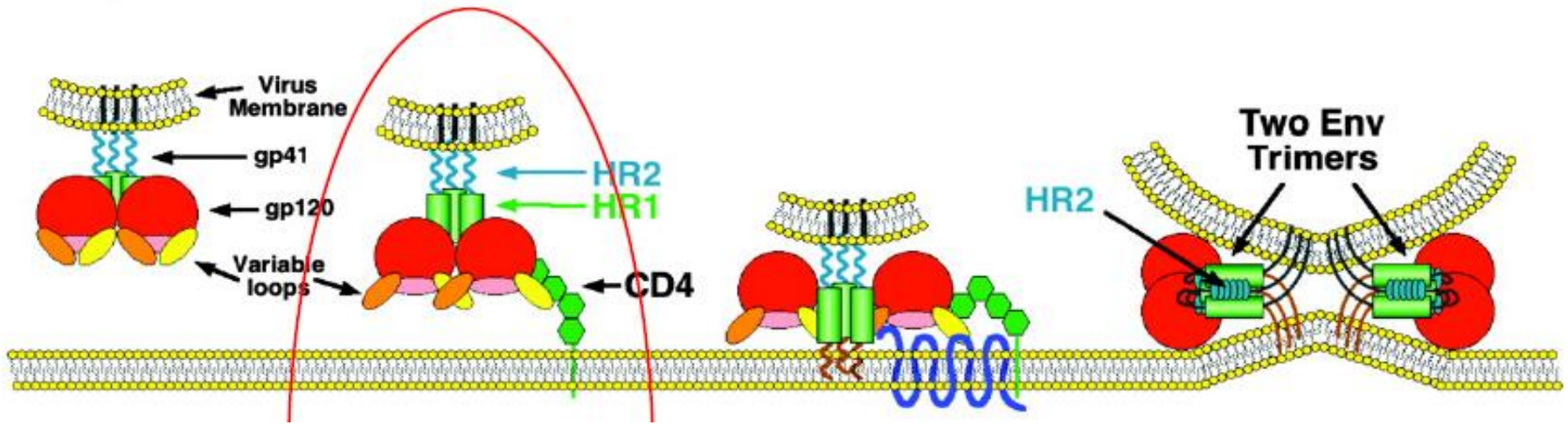
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INTRODUCCIÓN II (IBALIZUMAB)

- Ibalizumab es un anticuerpo monoclonal que se une al receptor CD4 de manera no competitiva bloqueando así la entrada del VIH-1



Mutations resistance in V5 region

Resistance has been observed to develop within 1–2 weeks in patients receiving ibalizumab monotherapy

TAB. 1 Nuove opzioni per l'infezione da HIV multiresistente: interpretazione del test genotipico

Farmaco	Obiettivo	Interpretazione genotipica	Commenti
Leronlimab	CCR5 (ospite)	Disponibile (geno2feno)	Non è stata definita la resistenza mediante il CCR5 legato a leronlimab, simile a maraviroc
Ibalizumab	CD4 (ospite)	Non disponibile	La maggior parte dei ceppi sviluppano resistenza mediante uso di CD4 legati a ibalizumab dopo la perdita del sito di N-glicosilazione nel dominio V5 della gp120
Fostemsavir	CD4 (ospite)	Non disponibile	Le signature RAMs devono essere definite ulteriormente, genotipo e fenotipo sono scarsamente predittivi della risposta alla terapia

- Longer virus replication (duration)---- Higher chance that mutated/recombinant virus occur
- Higher risk of SARS-COV-2 evolution in immunocompromised host

Immunocompromised patients:
weak immune pression therefore virus tend to increase infectivity with lower pathogenicity

Is conceivable that in those patients virus evolution produce strains more infectious but less pathogenic and probably without immunoscape properties

CONCLUSIONS

SARS-CoV-2 and HIV share some functions and replication steps, but the difference among them is greater than the similarities:

- Lifetime persistence (HIV) vs natural elimination (SARS-COV-2)
- High (HIV) versus intermediate rate of variability (SARS-COV-2)
- Intermediate rate of replication and diffusion (HIV) vs extremely high rate of diffusion (SARS-COV-2)

Final result: **Relatively similar rate of variability**

- Abundant presence of quasispecies (HIV) vs absence (SARS-COV-2)
- High resistance to drugs (HIV) vs low resistance (SARS-COV-2)
- Available mAb againts host (HIV) or virus (SARS-COV-2)