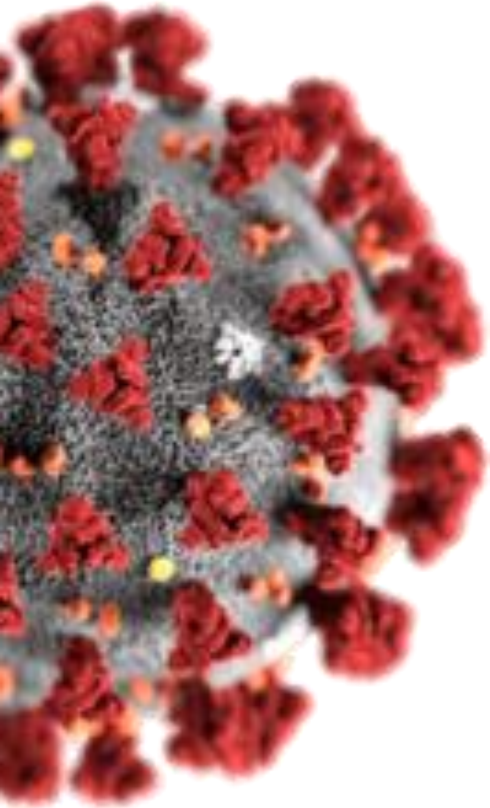
Drug design pro COVID-19

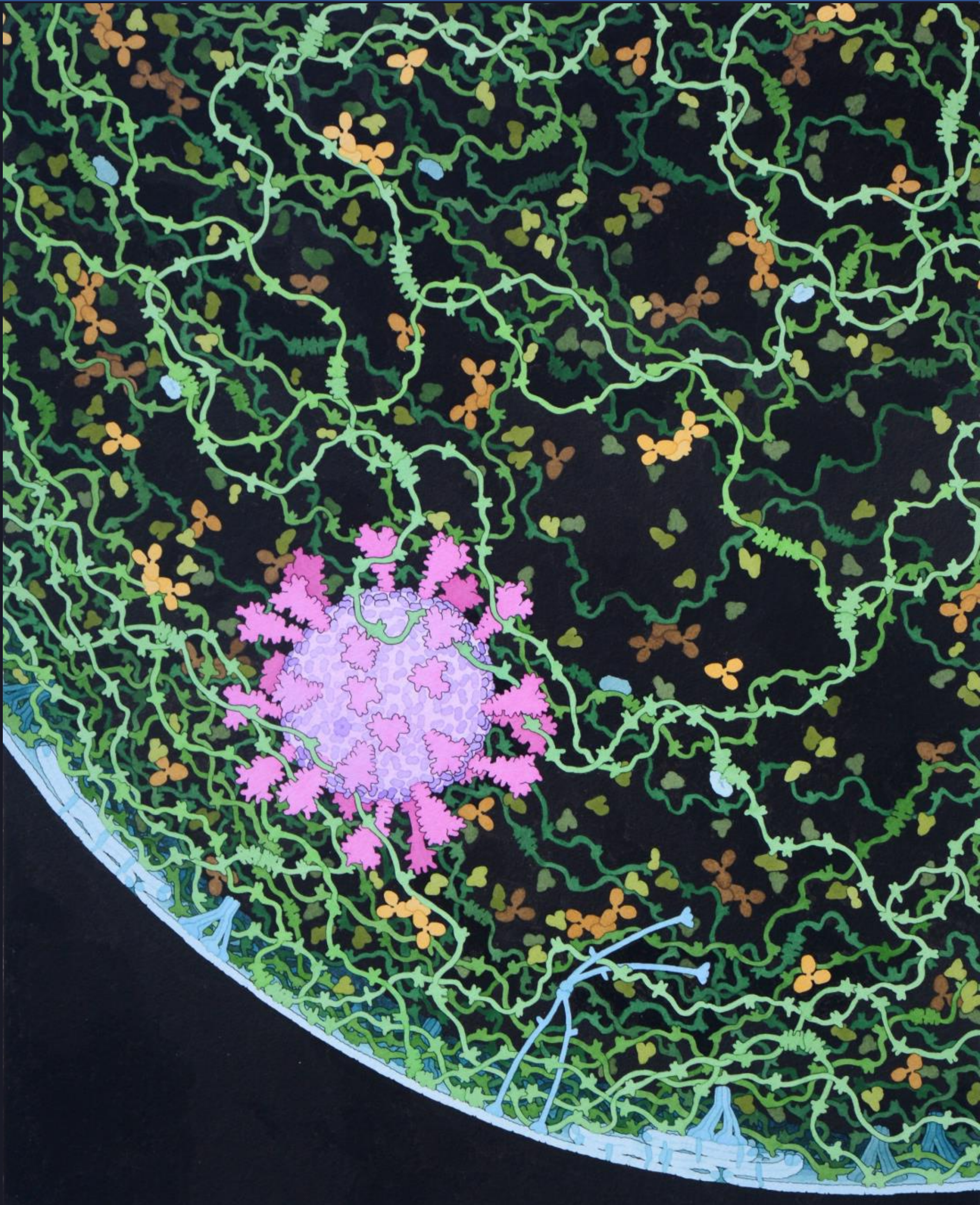


Karel Berka

Osnova

SARS-CoV-2

- Genom
- Proteiny
  - Viru
  - Hostitele
- Virová kapsle
- Life cycle viru
- Léčba
- Vakcíny



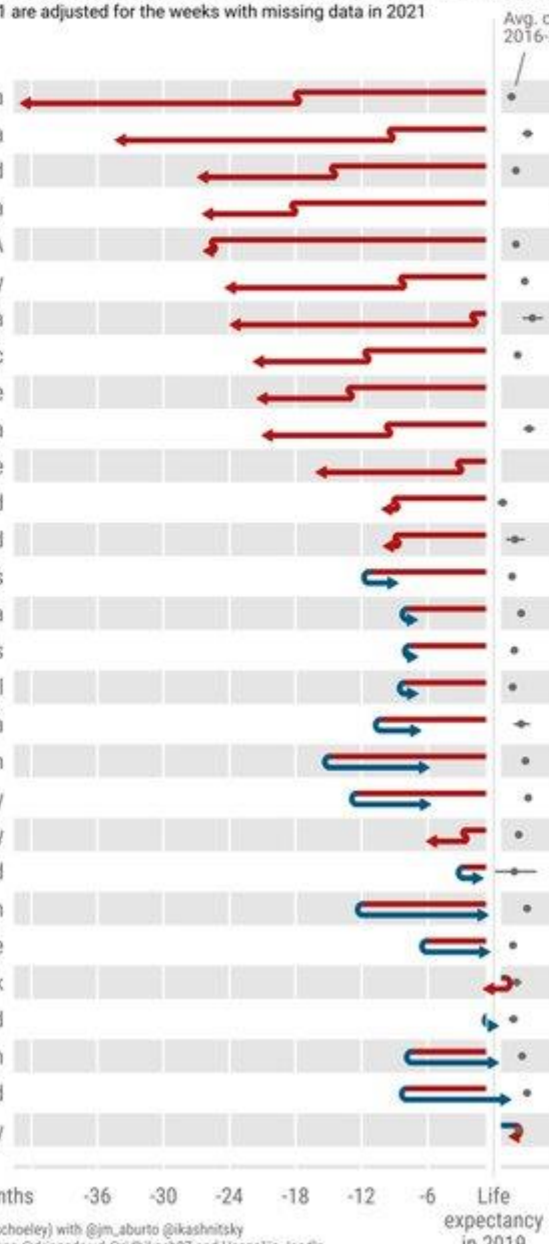
http://pdb101.rcsb.org/sci-art/goodsell-gallery/respiratory-droplet

Illustration by David S. Goodsell, RCSB Protein Data Bank; doi: 10.2210/rcsb_pdb/goodsell-gallery-024

Life expectancy bounce-backs amid continued losses

Life expectancy changes since the start of the COVID-19 pandemic

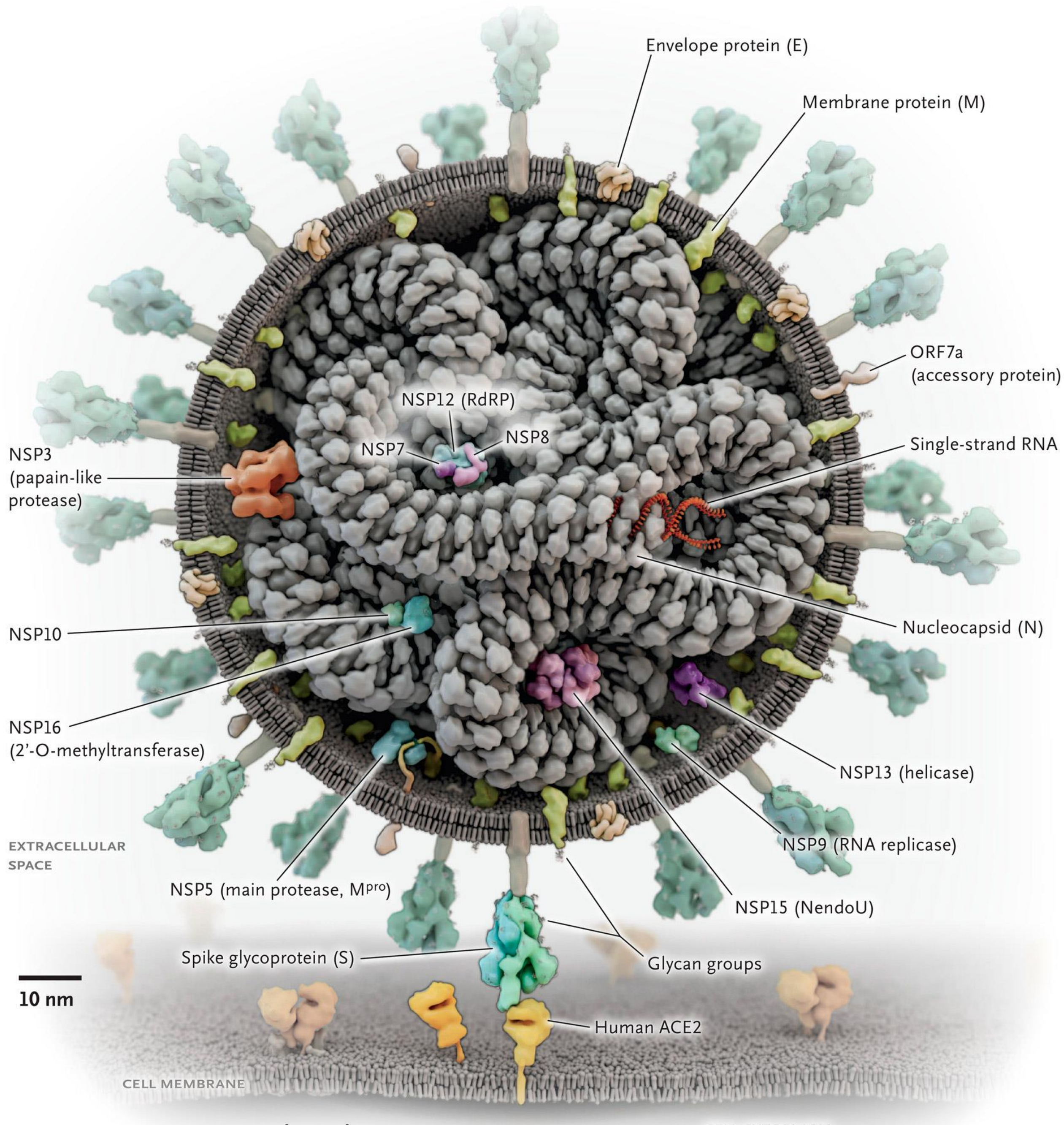
Estimates for 2021 are adjusted for the weeks with missing data in 2021



Country	Δ e_0 19/21	19/20	20/21	Av_{16-19}	As of week
Bulgaria	-42.4	-17.8	-24.6	+1.6	52
Slovakia	-33.8	-9.3	-24.5	+3.1	51
Poland	-26.3	-14.5	-11.7	+2.0	52
Lithuania	-25.8	-18.2	-7.7	+5.9	52
USA	-25.8	-25.5	-0.3	+2.0	52
Hungary	-23.8	-8.3	-15.5	+2.8	52
Estonia	-23.4	-1.8	-21.5	+3.5	52
Czech Republic	-21.2	-11.5	-9.7	+2.2	51
Chile	-20.9	-13.0	-7.9	·	52
Croatia	-20.4	-9.6	-10.9	+3.2	52
Greece	-15.5	-3.1	-12.5	·	52
Scotland	-9.6	-8.9	-0.7	+0.8	52
Northern Ireland	-9.4	-8.8	-0.7	+1.9	52
England and Wales	-9.3	-11.4	+2.1	+1.7	52
Austria	-7.5	-8.0	+0.4	+2.5	52
Netherlands	-7.5	-7.7	+0.2	+1.9	52
Portugal	-7.5	-8.2	+0.7	+1.7	52
Slovenia	-7.2	-10.4	+3.2	+2.5	52
Spain	-6.4	-15.0	+8.6	+2.9	52
Italy	-6.3	-12.6	+6.2	+3.1	47
Germany	-5.5	-2.6	-2.9	+2.3	52
Iceland	-1.6	-2.9	+1.3	+1.9	52
Belgium	-1.1	-12.0	+10.9	+3.0	52
France	-1.0	-6.2	+5.2	+1.7	52
Denmark	-0.3	+1.1	-1.5	+2.1	52
Finland	-0.2	-0.5	+0.3	+1.8	51
Sweden	-0.2	-7.6	+7.4	+2.6	52
Switzerland	+0.9	-8.0	+9.0	+3.0	52
Norway	+2.0	+2.1	-0.1	+2.2	52

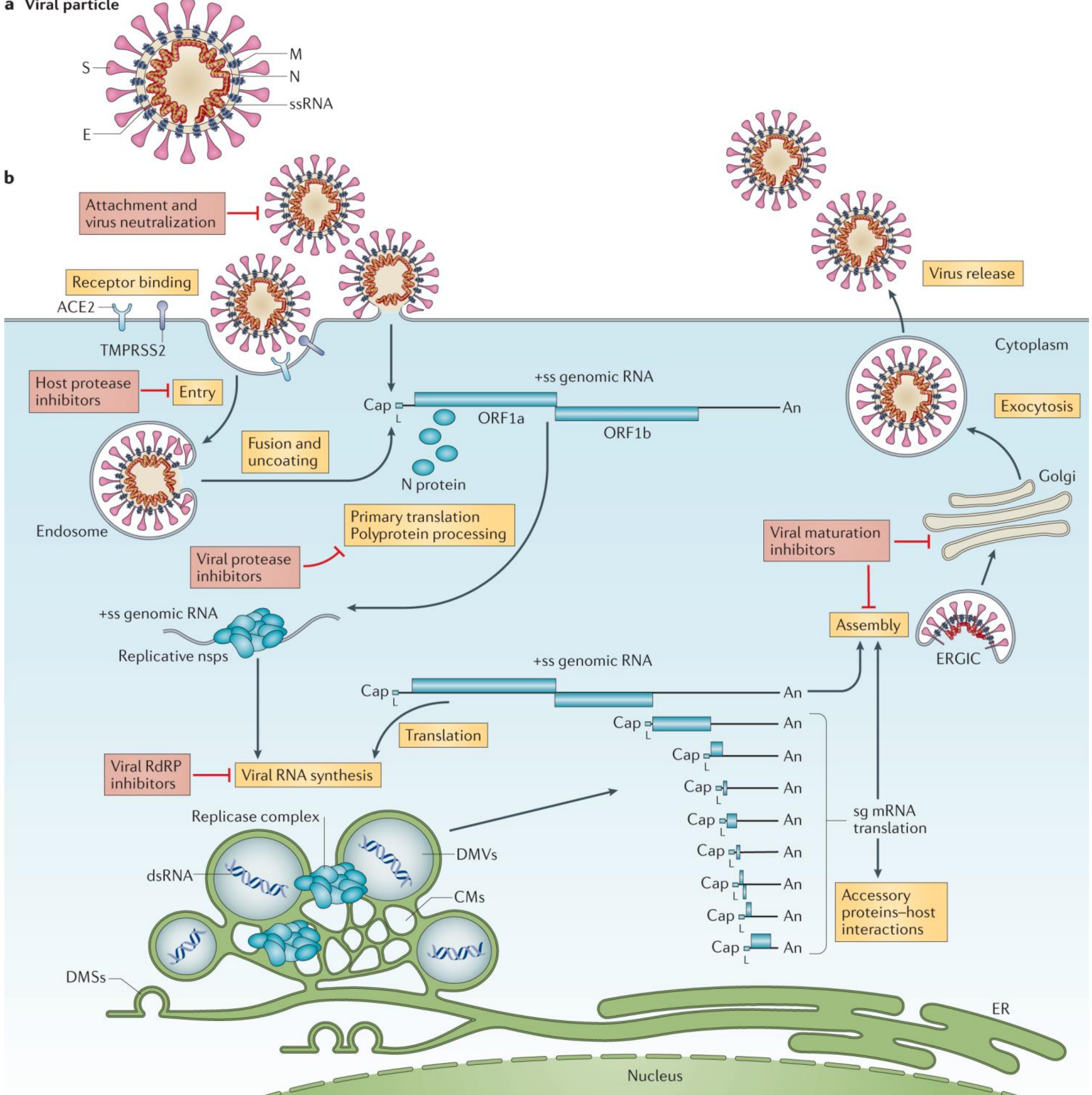
Life Cycle of SARS-CoV-2

https://www.youtube.com/watch?v=k2GIafQ9YhY



http://3dbionotes-ws.cnb.csic.es/ws/covid19

Life Cycle of SARS-CoV-2



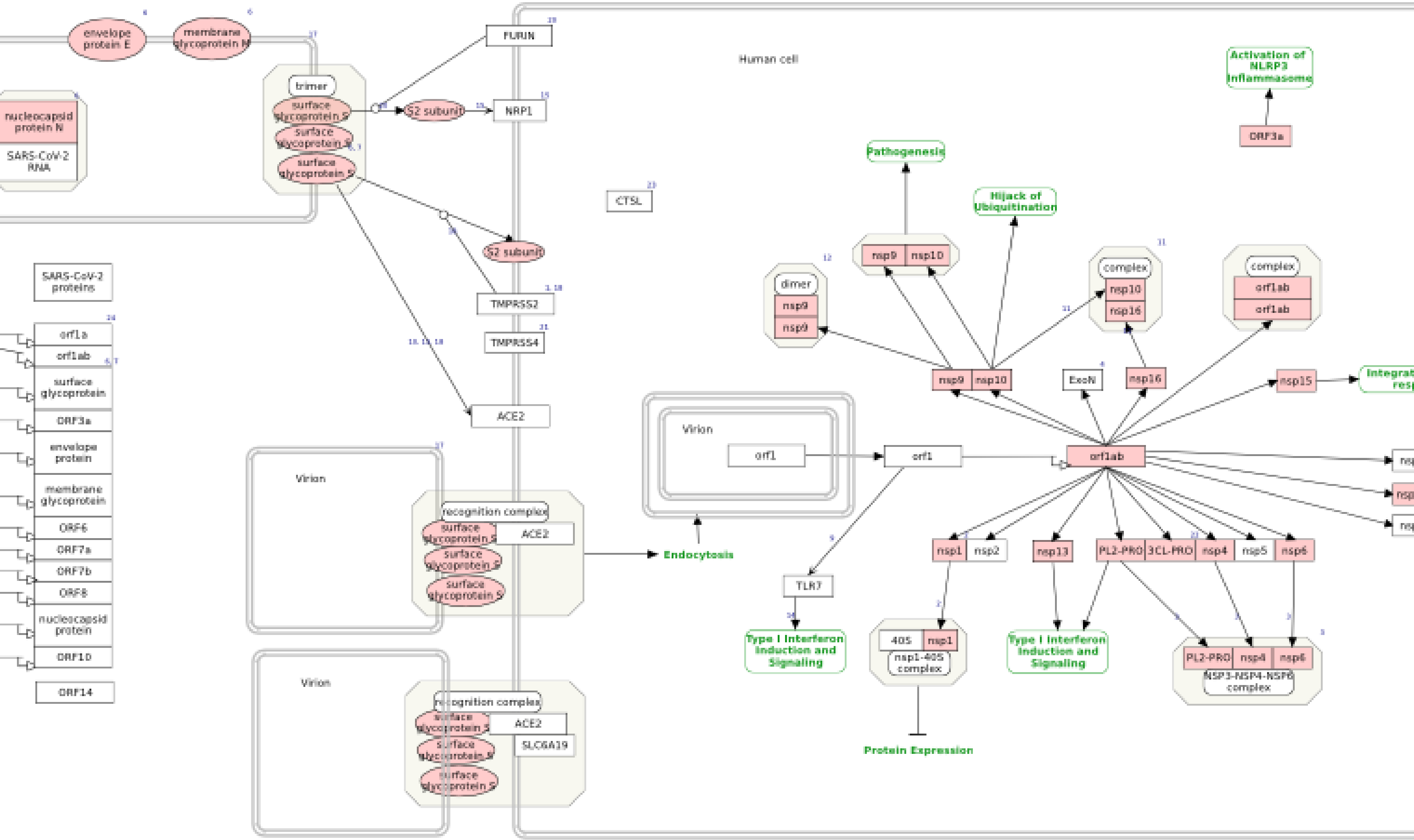
https://www.nature.com/articles/s41579-020-00468-6/figures/1

Reakce organismu

- **Replikace viru** - WP4846
- **Viral subversion of host defence:**
  - ER stress and unfolded protein response - **WP4861**
  - Autophagy and protein degradation **-**WP4860, WP4936, WP4863
  - Apoptosis - WP4864
- **Integrative stress response:**
  - Renin-angiotensin **-** WP4883, WP4965
  - Coagulopathy **-** WP4927
- **Innate Immune Response:**
  - PAMP signalling - WP4912
  - Induction of interferons and the cytokine storm - WP4868, WP4880, WP4876
  - Altered host metabolism - WP4853

https://covid.pages.uni.lu/map_contents

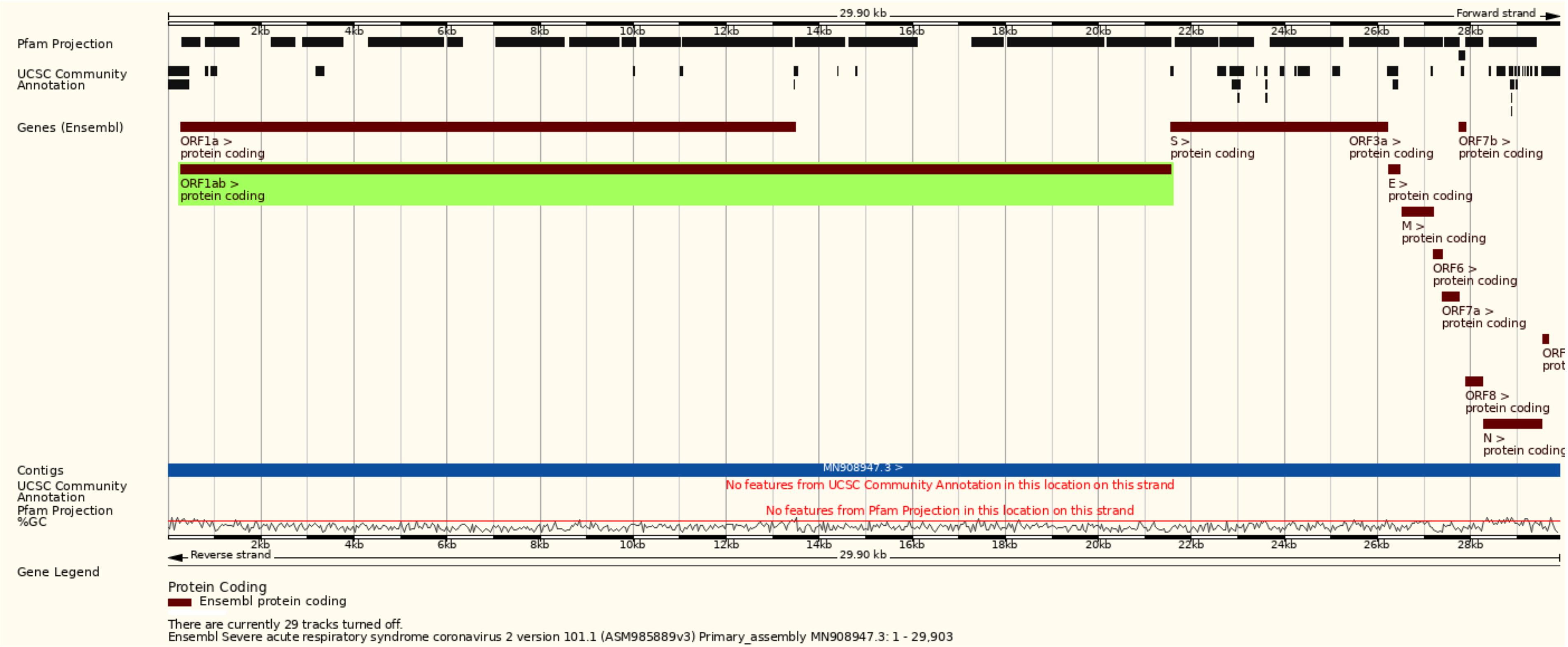
Wikipathways



https://www.wikipathways.org/index.php/Pathway:WP4846#nogo2

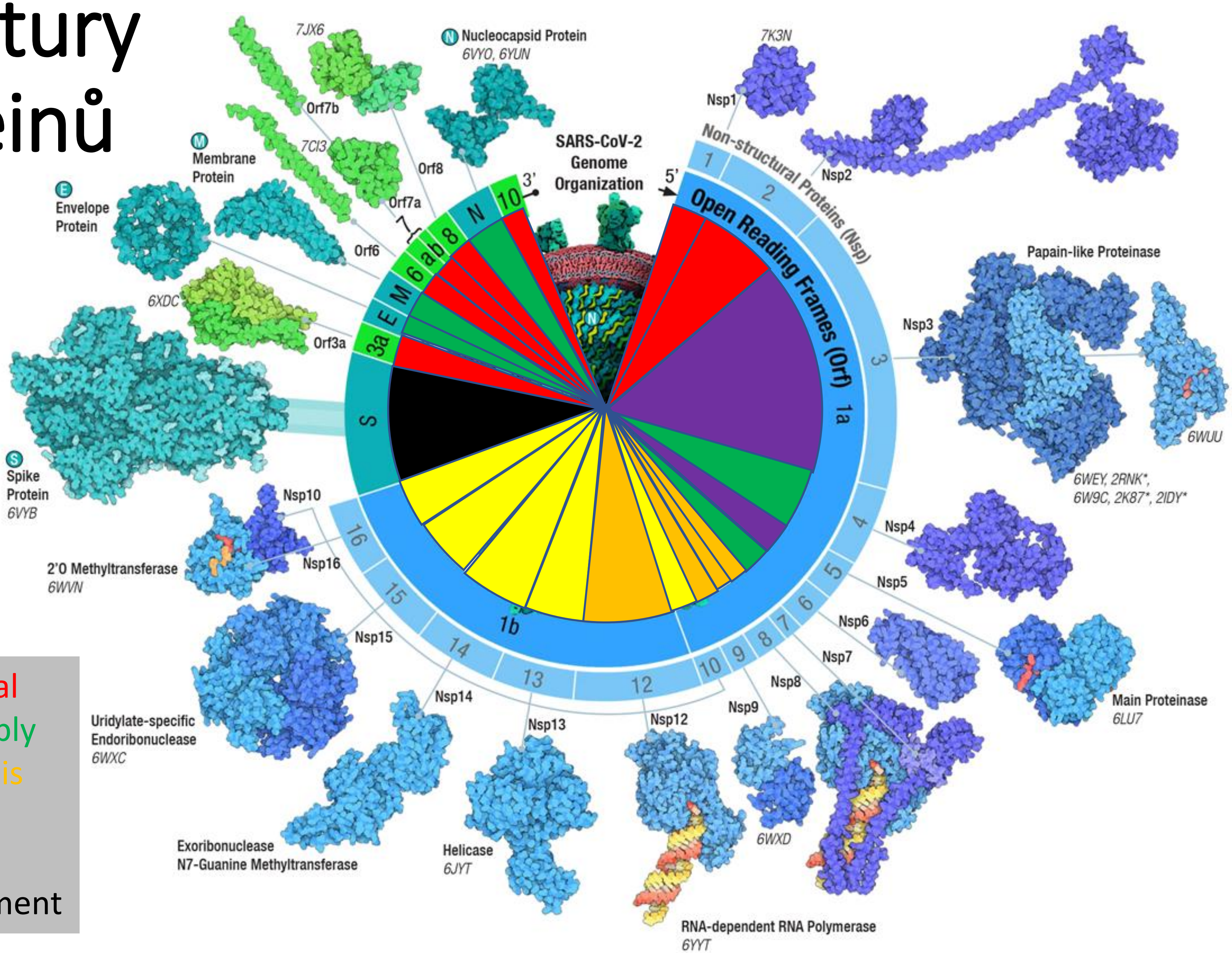
Genom SARS-Cov-2

RNA+ virus
29,903 b
12 coding genes – ORF1ab v sobě obsahuje 16 nsp proteinů



https://covid-19.ensembl.org/Sars_cov_2/Location/View?r=MN908947.3:1-29903

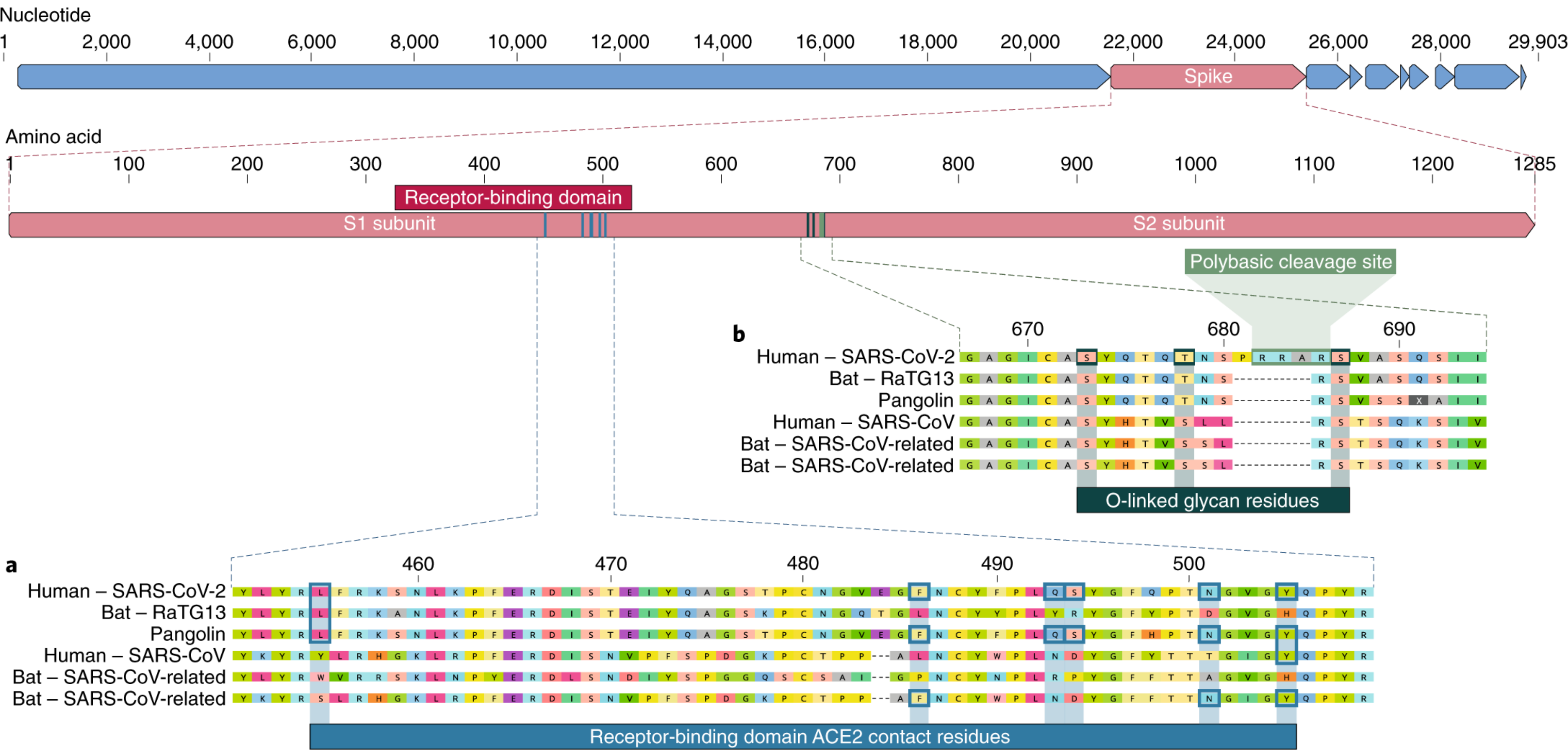
Struktury proteinů



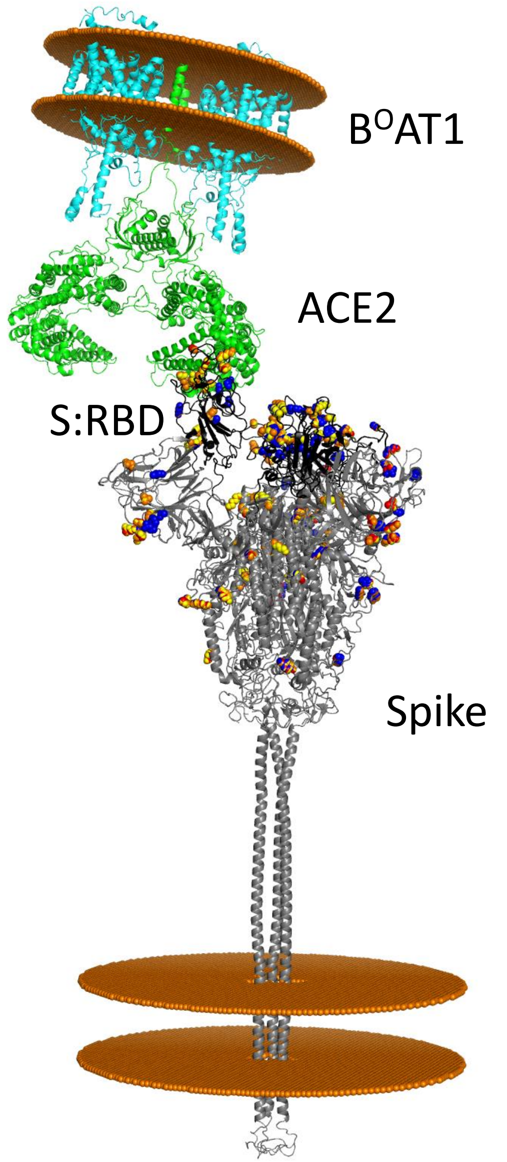
X Cell survival
Virus assembly
RNA synthesis
RNA editing
Proteases
Host attachment

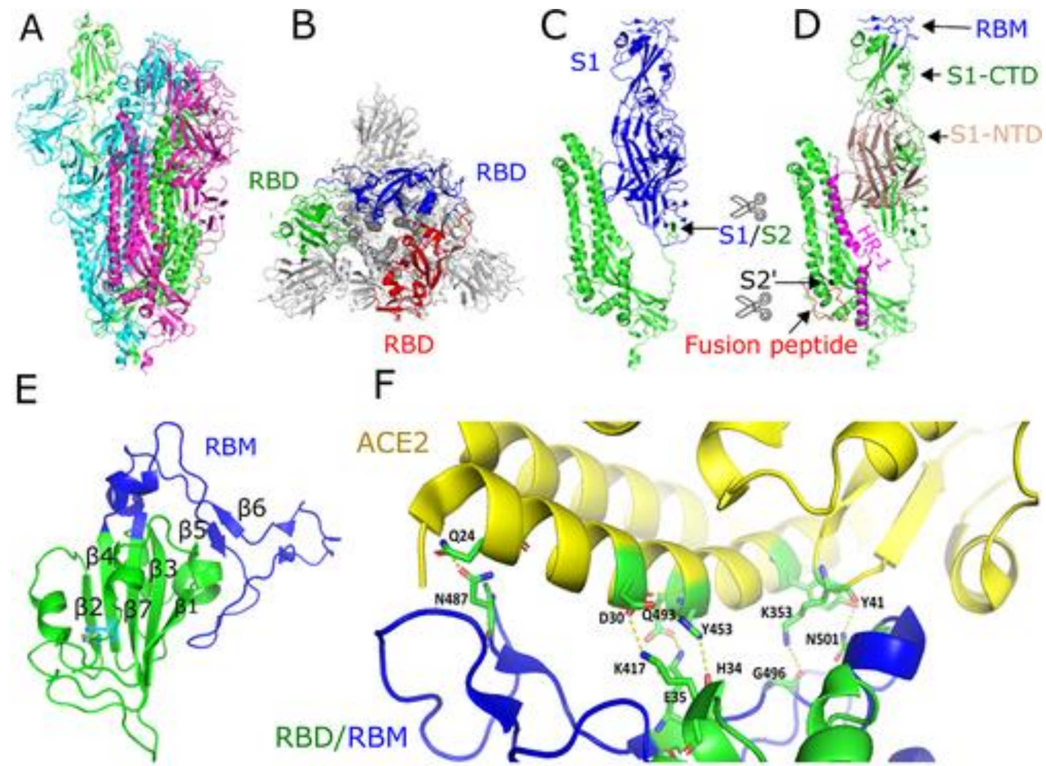
- https://cdn.rcsb.org/pdb101/learn/resources/flyers/covid-genome/covid-genome-prots.png

Spike Protein



SARS-CoV-2 S protein + ACE2



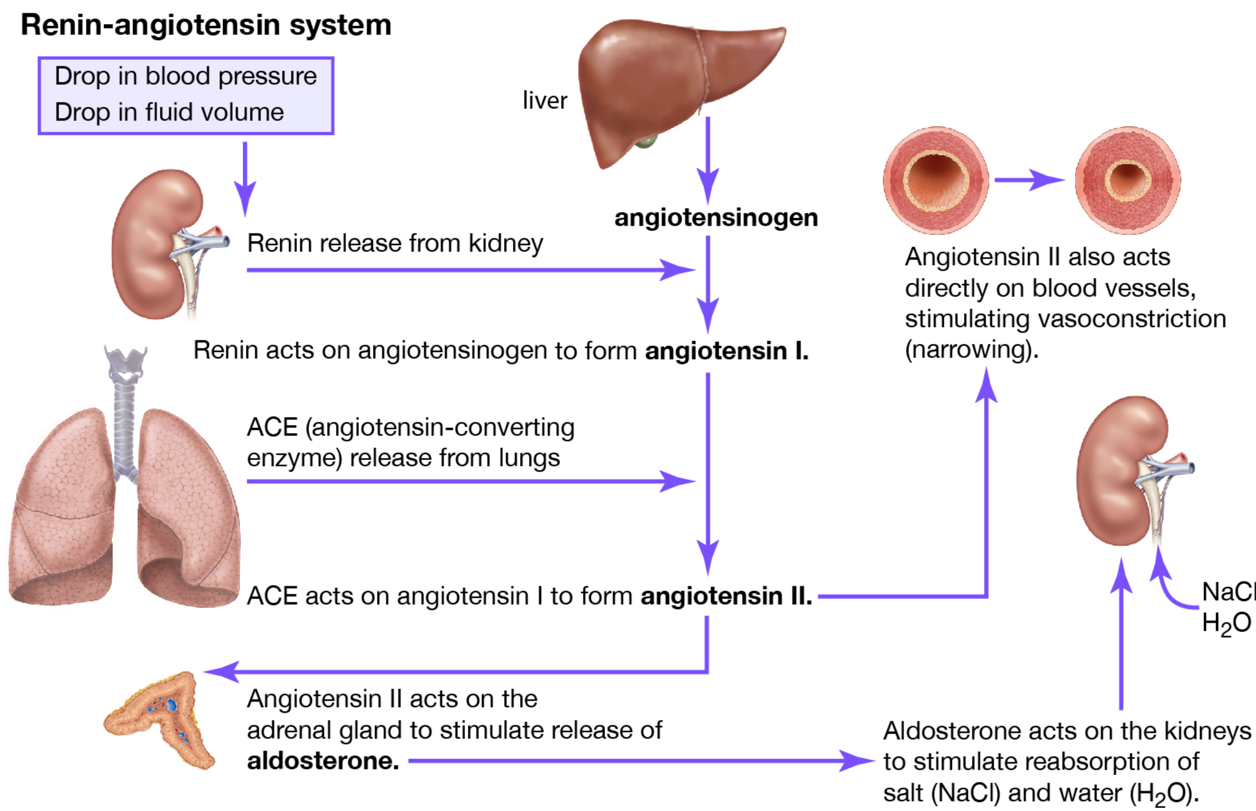


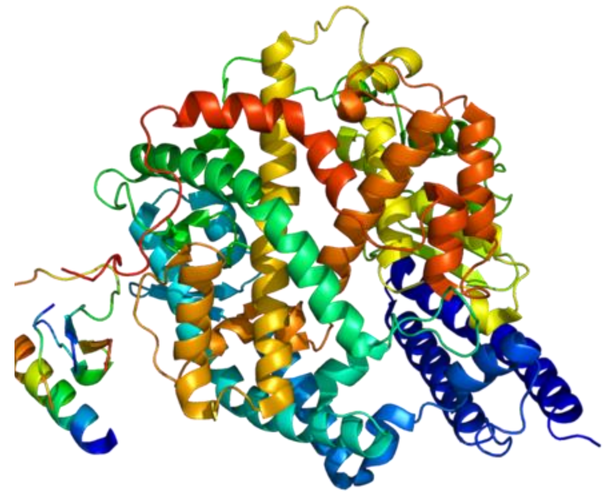
- Hlavní kontakt s buňkami

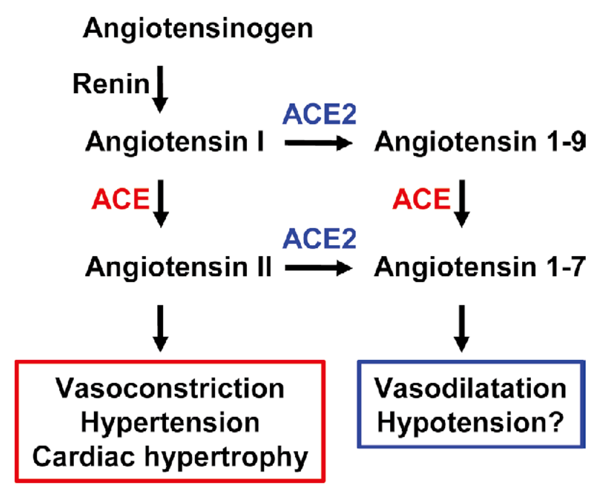
[Berka K (2022) https://virus.img.cas.cz/](https://virus.img.cas.cz/)

Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, et al. (2020) COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. PLOS Pathogens 16(8): e1008762. https://doi.org/10.1371/journal.ppat.1008762
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008762

ACE2 – what is it? Why do we care?







Receptor binding domain (RBD) antibodies contribute more to SARS-CoV-2 neutralization when target cells express high levels of ACE2

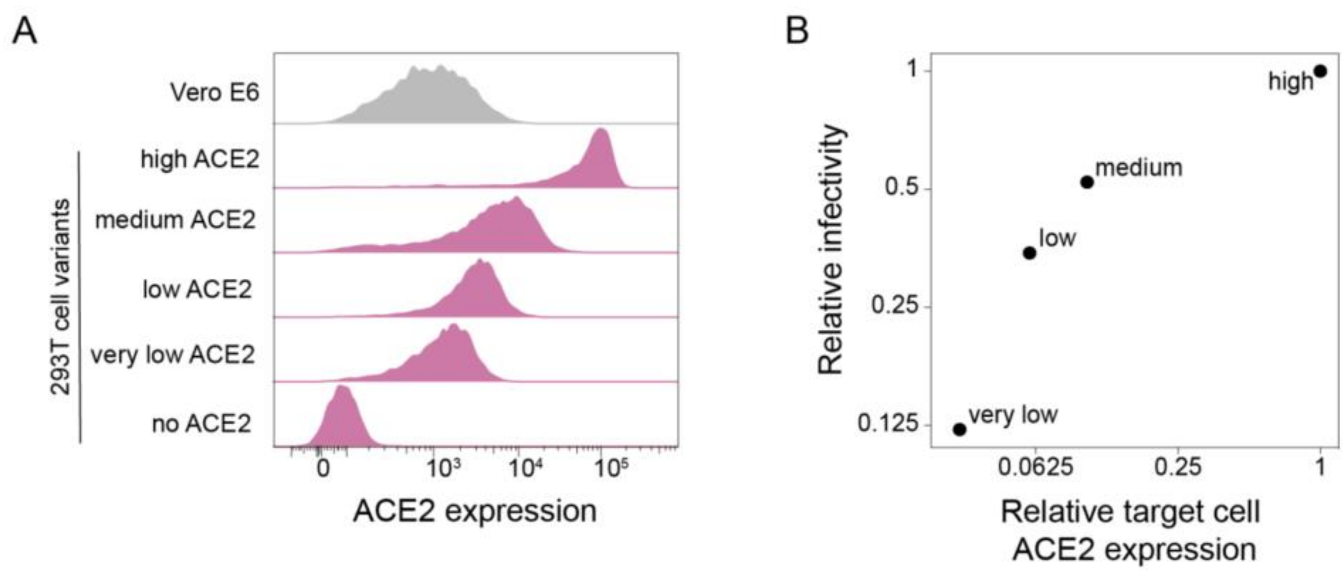
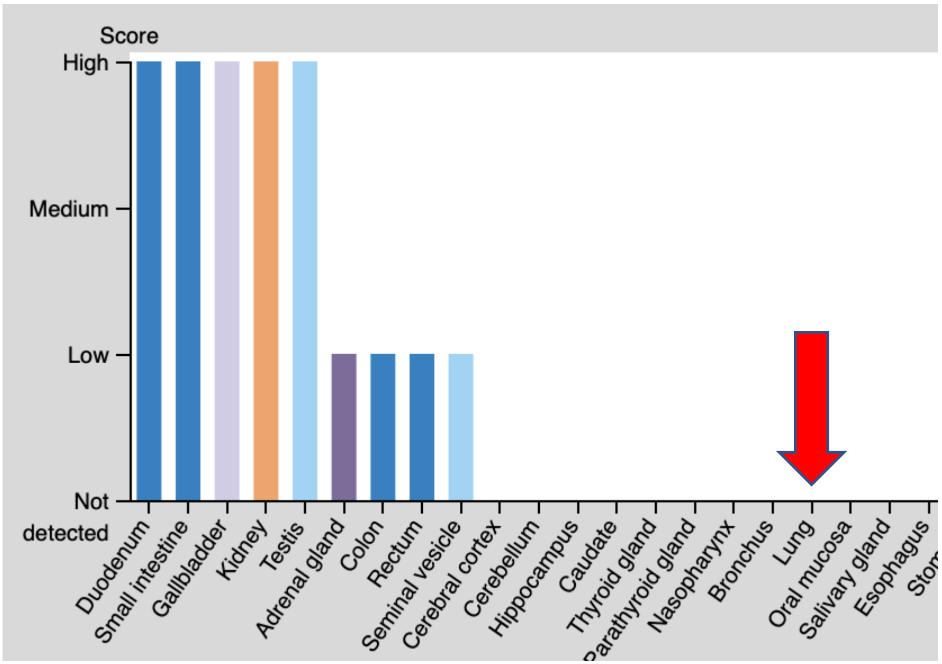
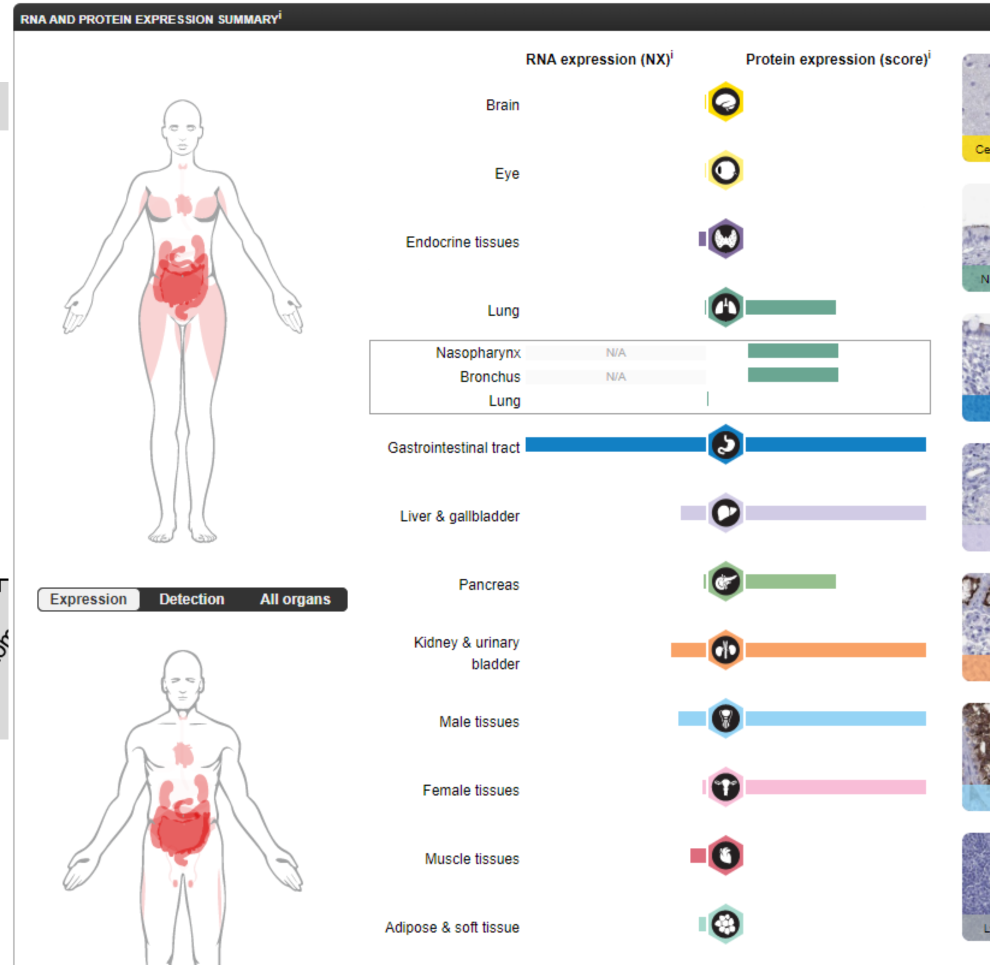


Fig. 1. 293T cell clones expressing ACE2 at different levels. (A) ACE2 expression in 293T cells engineered to express different levels of ACE2. ACE2 surface expression was measured by flow cytometry, and the histograms show the distribution of expression levels over a population of cells. Vero E6 cells are included for comparison. **(B)** Relationship between ACE2 expression in the four 293T target cell clones and infection by lentiviral particles pseudotyped with the SARS-CoV-2 D614G spike.

https://www.biorxiv.org/content/10.1101/2022.08.29.505713v1

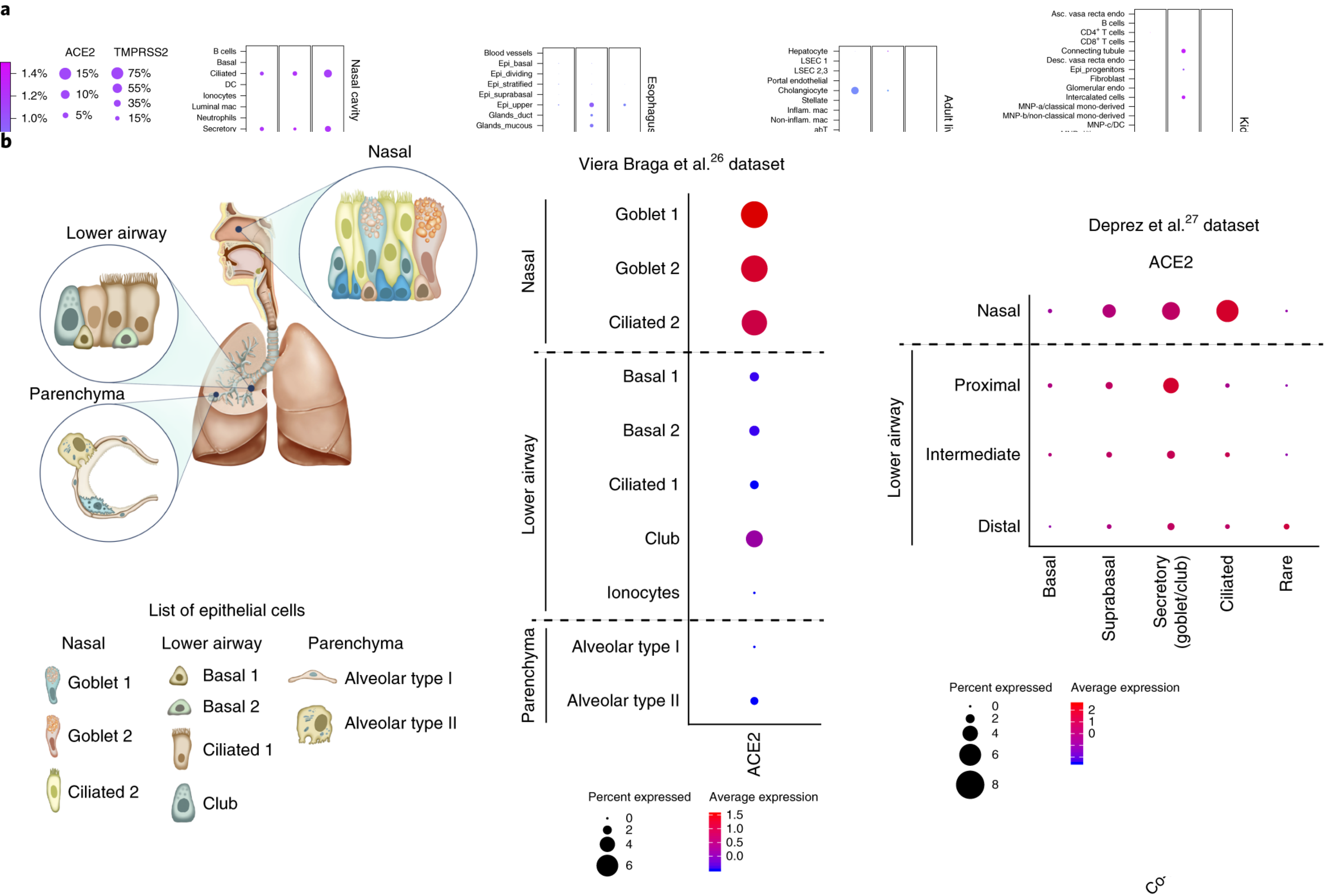
Exprese ACE2





Trochu zvláštní, že?

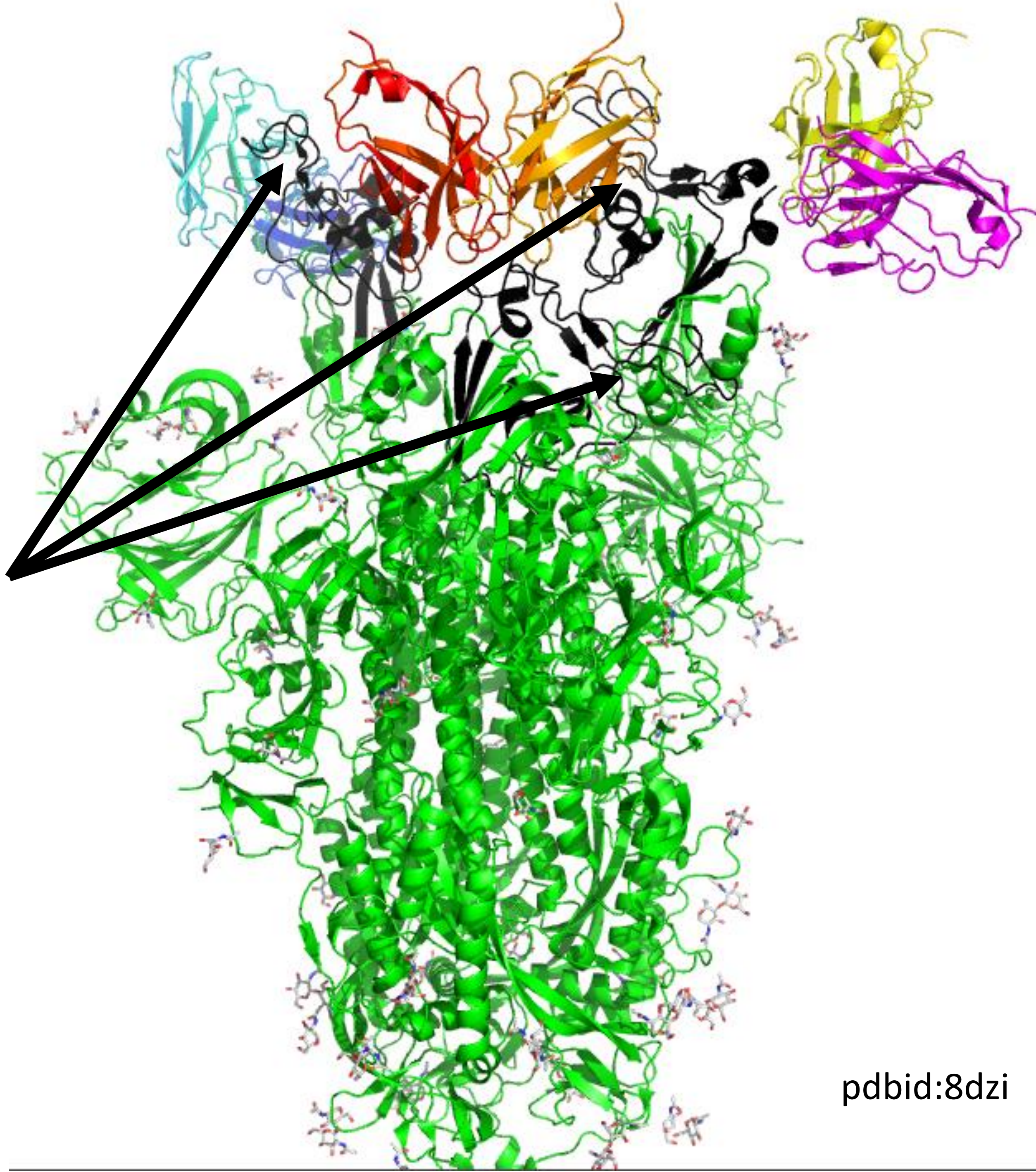
https://www.proteinatlas.org/ENSG00000130234-ACE2/tissue



https://www.nature.com/articles/s41591-020-0868-6

Interaction with antibodies

- Neutralizing antibodies binds with RBD domain – blocking ACE2 interaction

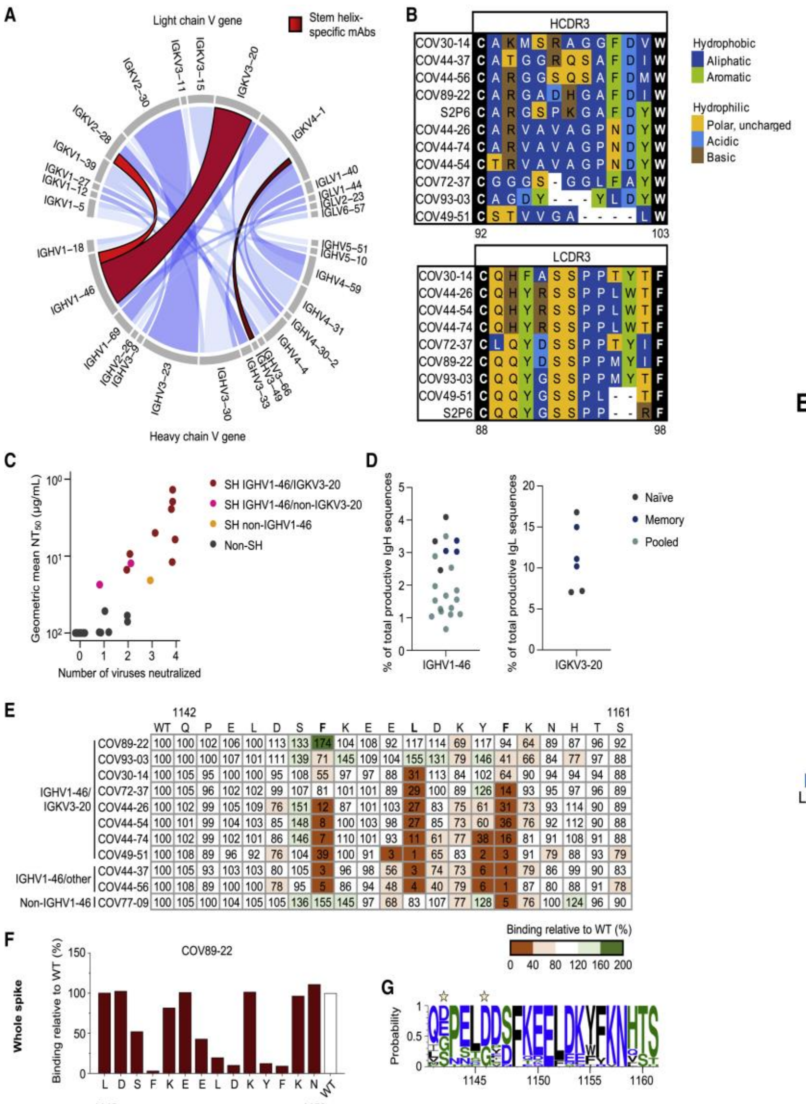


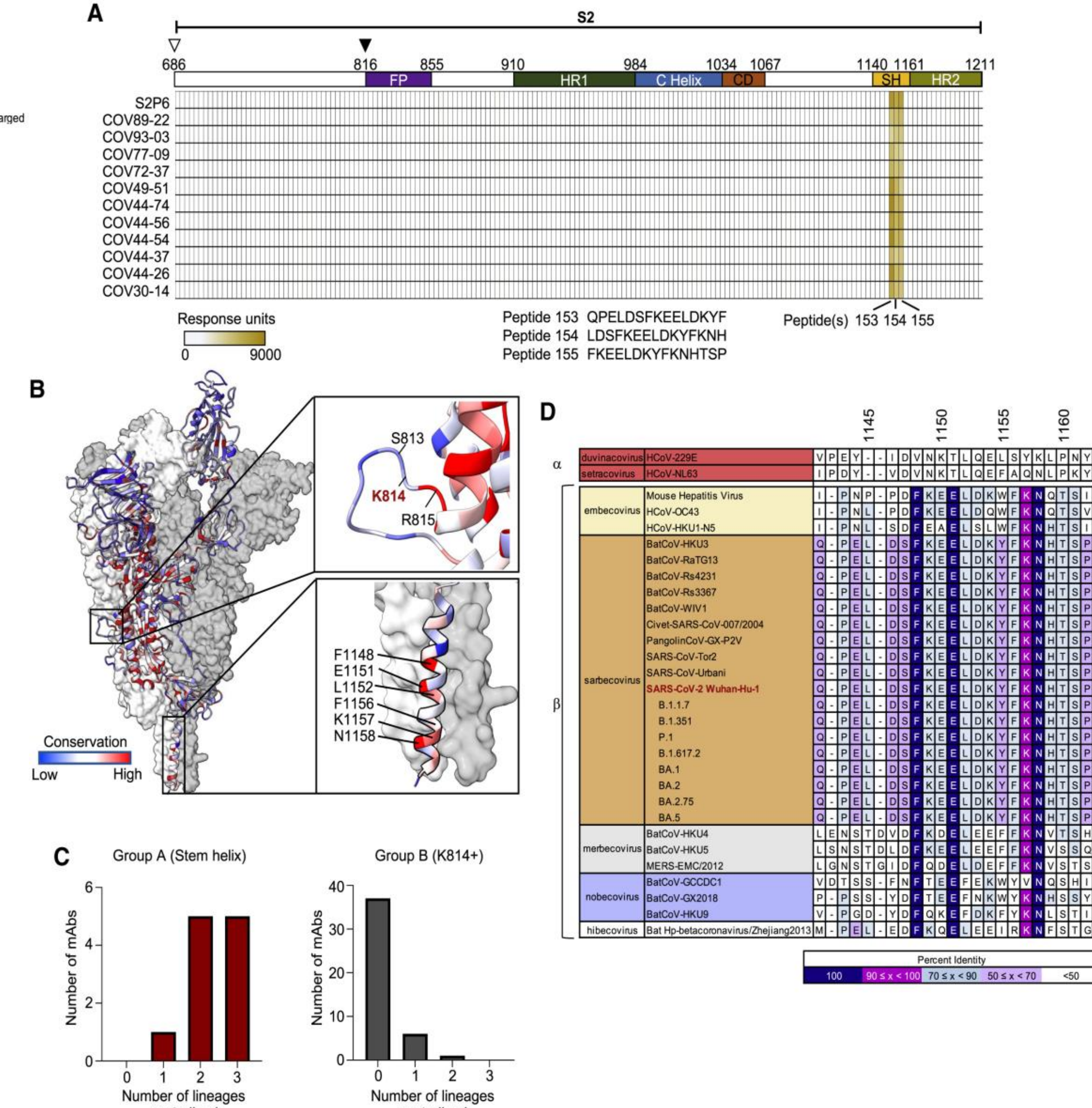
Protilátky

CoV-AbDab
The Coronavirus Antibody Database

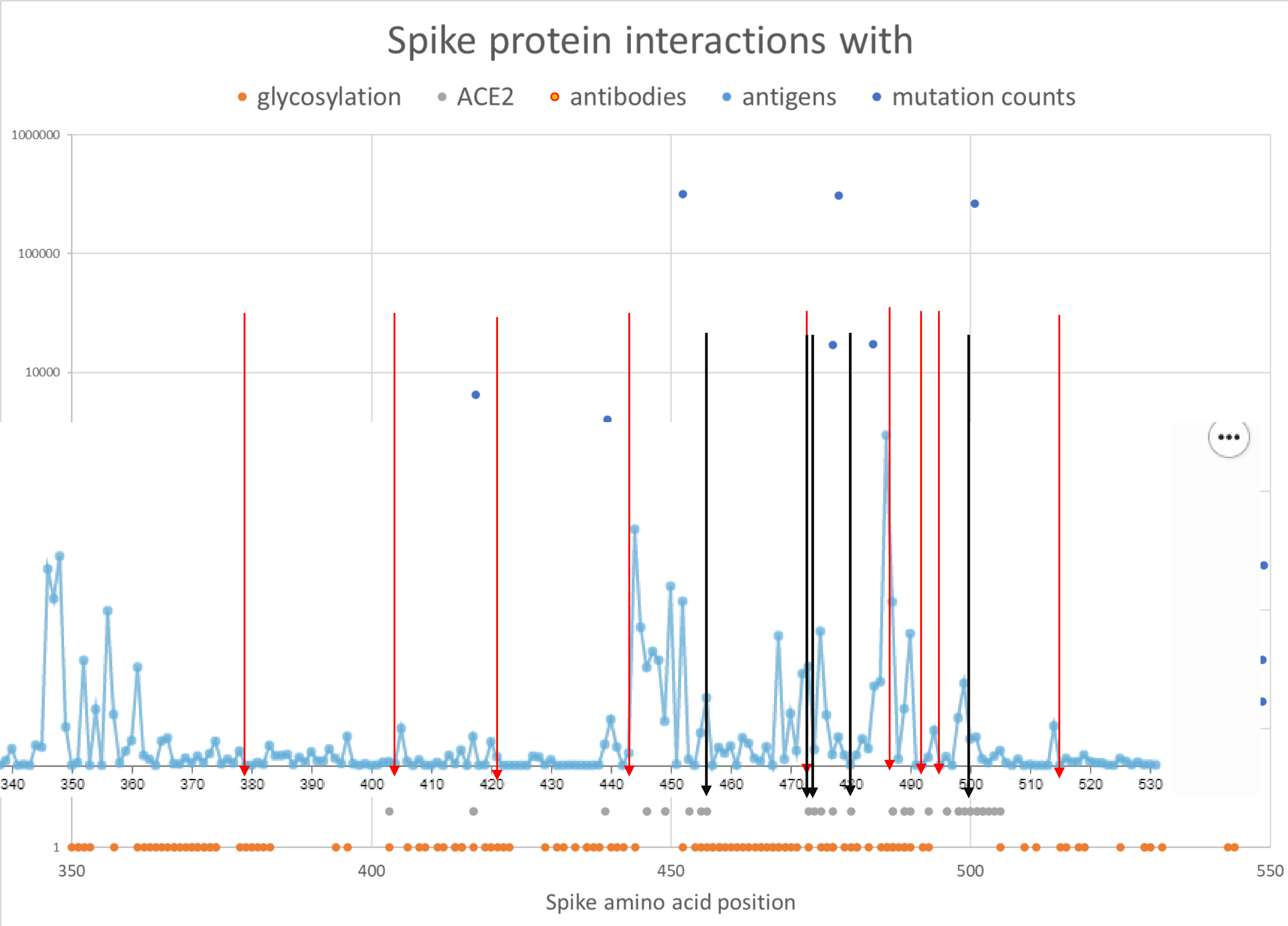
http://opig.stats.ox.ac.uk/webapps/covabdab/

Rare, convergent antibodies targeting the stem helix broadly neutralize diverse betacoronaviruses



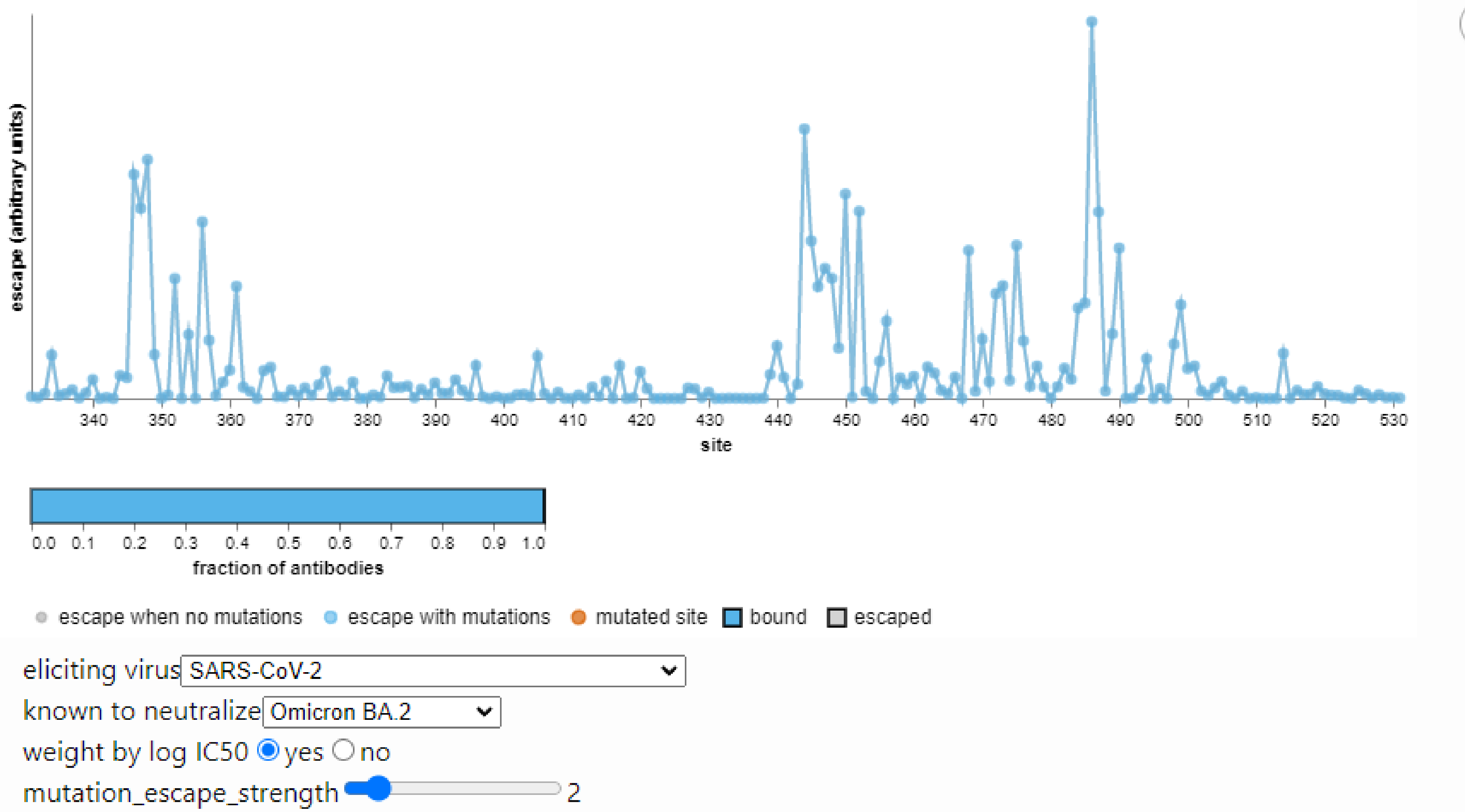


Dacon et al. Rare, convergent antibodies targeting the stem helix broadly neutralize diverse betacoronaviruses.
Cell Host & Microbe DOI: 10.1016/j.chom.2022.10.010



Spike protein interactions with
glycosylation
ACE2
antibodies
antigens
mutation counts
1000000
100000
10000
1
340
350
360
370
380
390
400
410
420
430
440
450
460
470
490
510
520
530
350
400
450
500
550
Spike amino acid position

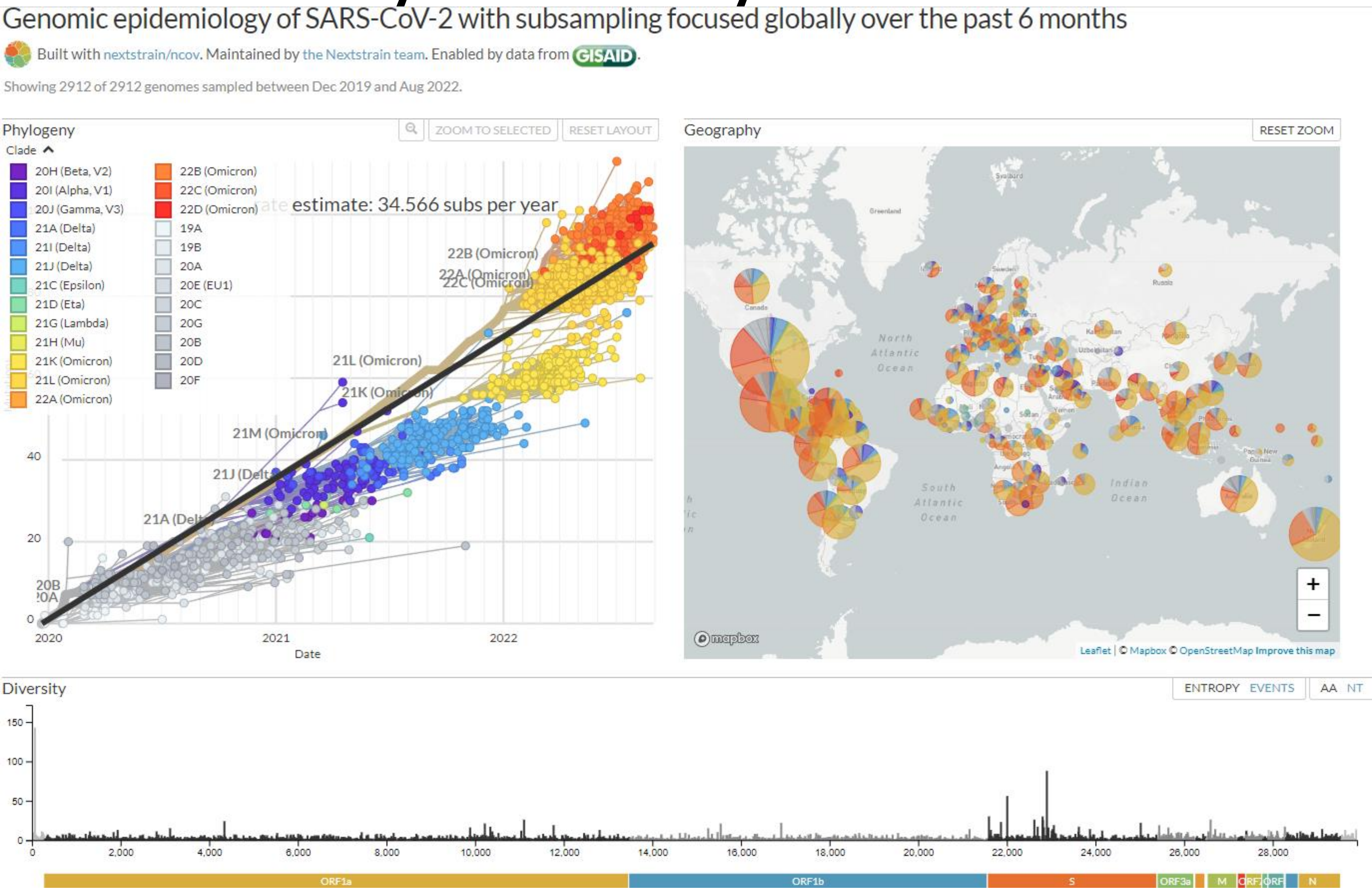
Escape calculator for SARS-CoV-2 RBD



https://jbloomlab.github.io/SARS2_RBD_Ab_escape_maps/escape-calc/
https://academic.oup.com/ve/article/8/1/veac021/6549895

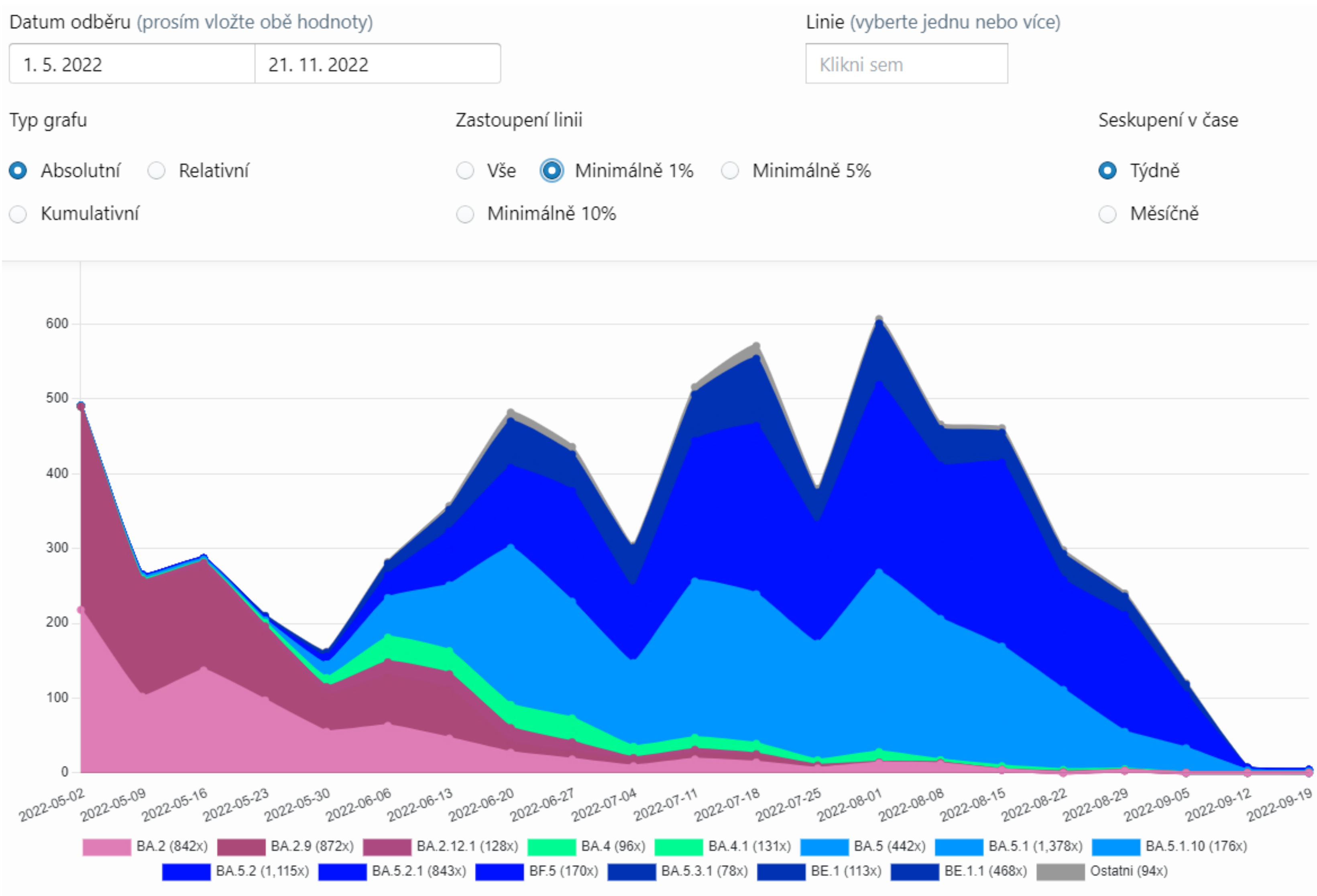
Genomic surveillance

Varianty - Genomy SARS-Cov-2



https://nextstrain.org/ncov/global?m=div

Varianty v ČR



https://virus.img.cas.cz/lineages

Therapeutics

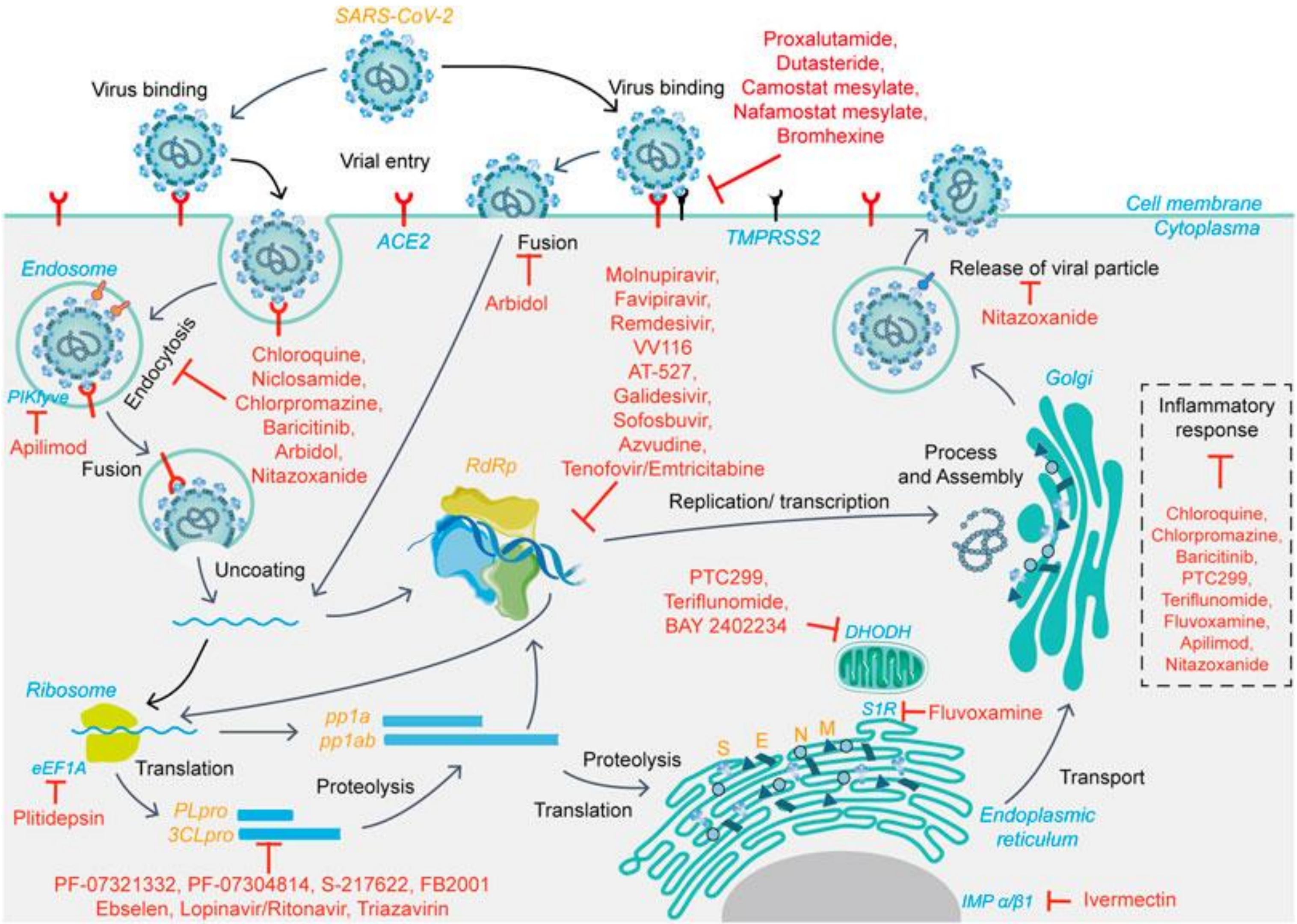
WHO guidelines (13.1.2023)

Non-severe w risk hospitalization	Severe and Critical
+ 3C-Protease inhibitor (nirmatrelvir-ritonavir)(Paxlovid©)	**+ Systemic corticosteroids (dexamethason)**
+ RdRp mutator (molnupiravir)	**+ IL-6 receptor blockers (tocilizumab)**
+ RdRp inhibitor (remdesivir)	**+ JAK inhibitor (baricitinib)**
- Systemic corticosteroids (dexamethason)	+ RdRp inhibitor (remdesivir)
- colchicine	- JAK inhibitors (ruxolitinib and tofacitinib)
- fluvoxamine	- RdRp inhibitor(remdesivir)w critical COVID-19
- convalescent plasma	- **convalescent plasma**

NOT recommended (regardless severity)
- Spike antibodies (sotrovimab and casirivimab-imdevimab) – due to Omicron
- Antimalaricum (hydroxychloroquine)
- Anti-HIV protease inhibitor (lopinavir-ritonavir)
- Antiparasitic (ivermectin)

https://app.magicapp.org/#/guideline/6914

Clinical trials



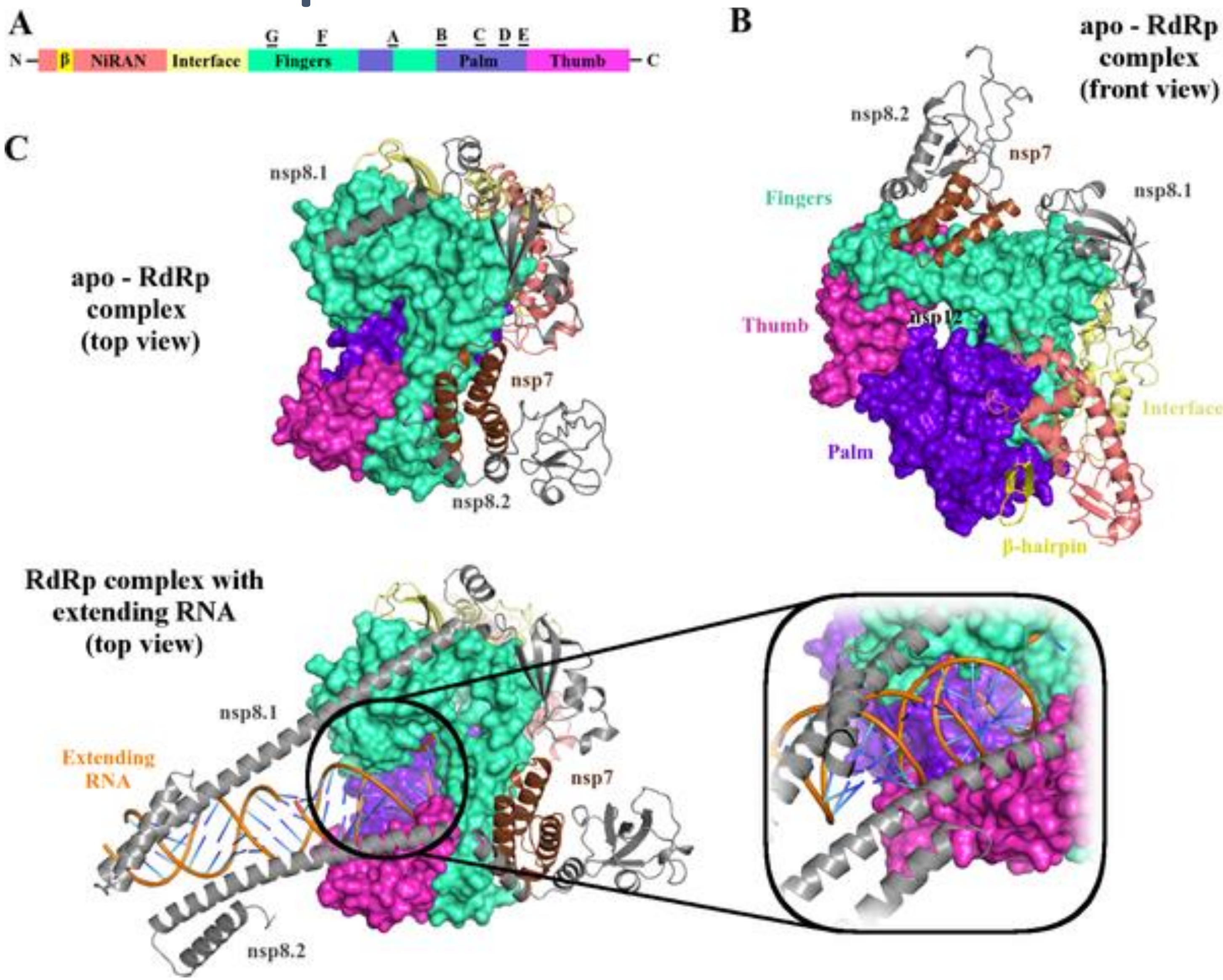
Zhao et al. Mechanism of Action of Small-Molecule Agents in Ongoing Clinical Trials for SARS-CoV-2: A Review
Front. Pharmacol., 25 February 2022 https://doi.org/10.3389/fphar.2022.840639

Drugs	No. of clinical trials registered[a]	Phase	Molecular target	Development strategy	Approval status (for COVID-19)
Remdesivir	77	4	RdRp	Repurposing	Approval by FDA
Favipiravir	46	4	RdRp	Repurposing	EUA in several countries
Molnupiravir	5	3	RdRp	Novel	Approval by MHRA; EUA by FDA
AT-527	3	3	RdRp	Novel	Non-approved
Galidesivir	1	1	RdRp	Repurposing	Non-approved
Sofosbuvir	8	4	RdRp	Repurposing	Non-approved
Azvudine	3	3	RdRp	Repurposing	Non-approved
Tenofovir/emtricitabine	5	3	RdRp	Repurposing	Non-approved
PF-07321332	8	3	3CLpro	Novel	EUA by FDA
PF-07304814	3	1	3CLpro	Novel	Non-approved
s-217622	—	2/3	3CLpro	Novel	Non-approved
FB2001	1	2/3	3CLpro	Novel	Non-approved
Ebselen	2	2	3CLpro	Repurposing	Non-approved
Lopinavir/ritonavir	24	4	3CLpro	Repurposing	Non-approved
Triazavirin	2	4	RNA synthesis/3CLpro	Repurposing	Non-approved
Chloroquine/hydroxychloroquine	46/276	4	Endosomal entry	Repurposing	EUA by FDA at earlier outbreak (chloroquine)
Umifenovir/arbidol	3	4	Endosomal entry	Repurposing	Non-approved
Niclosamide	11	3	Endosomal entry	Repurposing	Non-approved
Chlorpromazine	2	3	Endosomal entry	Repurposing	Non-approved
Baricitinib	20	4	Endosomal entry	Repurposing	EUA by FDA
Proxalutamide	5	3	Androgen receptor antagonist	Repurposing	Non-approved
Dutasteride	1	2	5-alpha-reductase inhibitor	Repurposing	Non-approved
Camostat mesylate	5	3	TMPRSS2 inhibitor	Repurposing	Non-approved
Nafamostat mesylate	2	2	TMPRSS2 inhibitor	Repurposing	Non-approved
PTC299	1	2	DHODH inhibitor	Repurposing	Non-approved
Teriflunomide	3	3	DHODH inhibitor	Repurposing	Non-approved
Nitazoxanide	23	4	Endosomal entry/Inflammatory response regulation	Repurposing	Non-approved
Fluvoxamine	1	3	Sigma-1 receptors agonist	Repurposing	Non-approved
Plitidepsin	3	3	eEF1A inhibitor	Repurposing	Non-approved
Ivermectin	69	4	IMPA/β1 inhibitor	Repurposing	Non-approved
Apilimod	1	2	PIKFYVE inhibitor	Repurposing	Non-approved

[a]*Registered on ClinicalTrials.gov.*

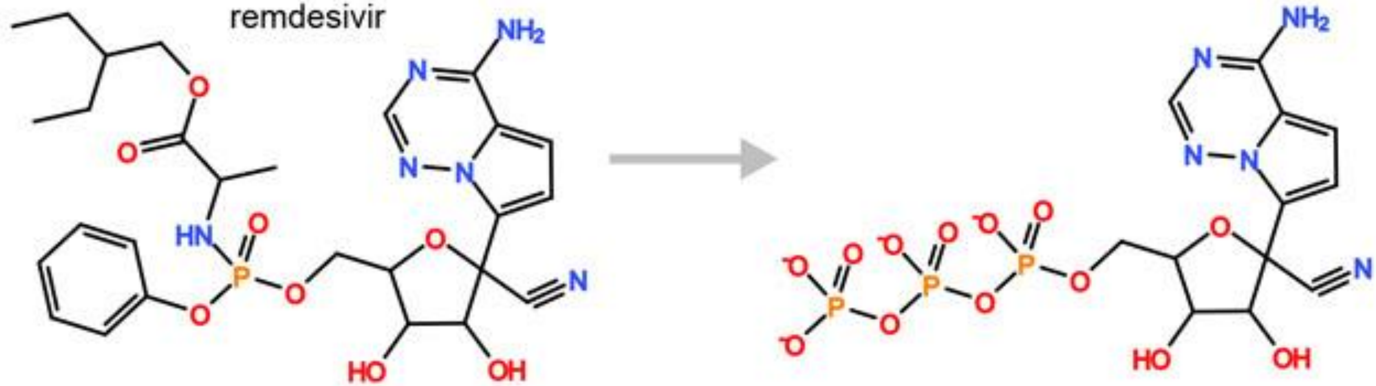
Zhao et al. Mechanism of Action of Small-Molecule Agents in Ongoing Clinical Trials for SARS-CoV-2: A Review
Front. Pharmacol., 25 February 2022 https://doi.org/10.3389/fphar.2022.840639

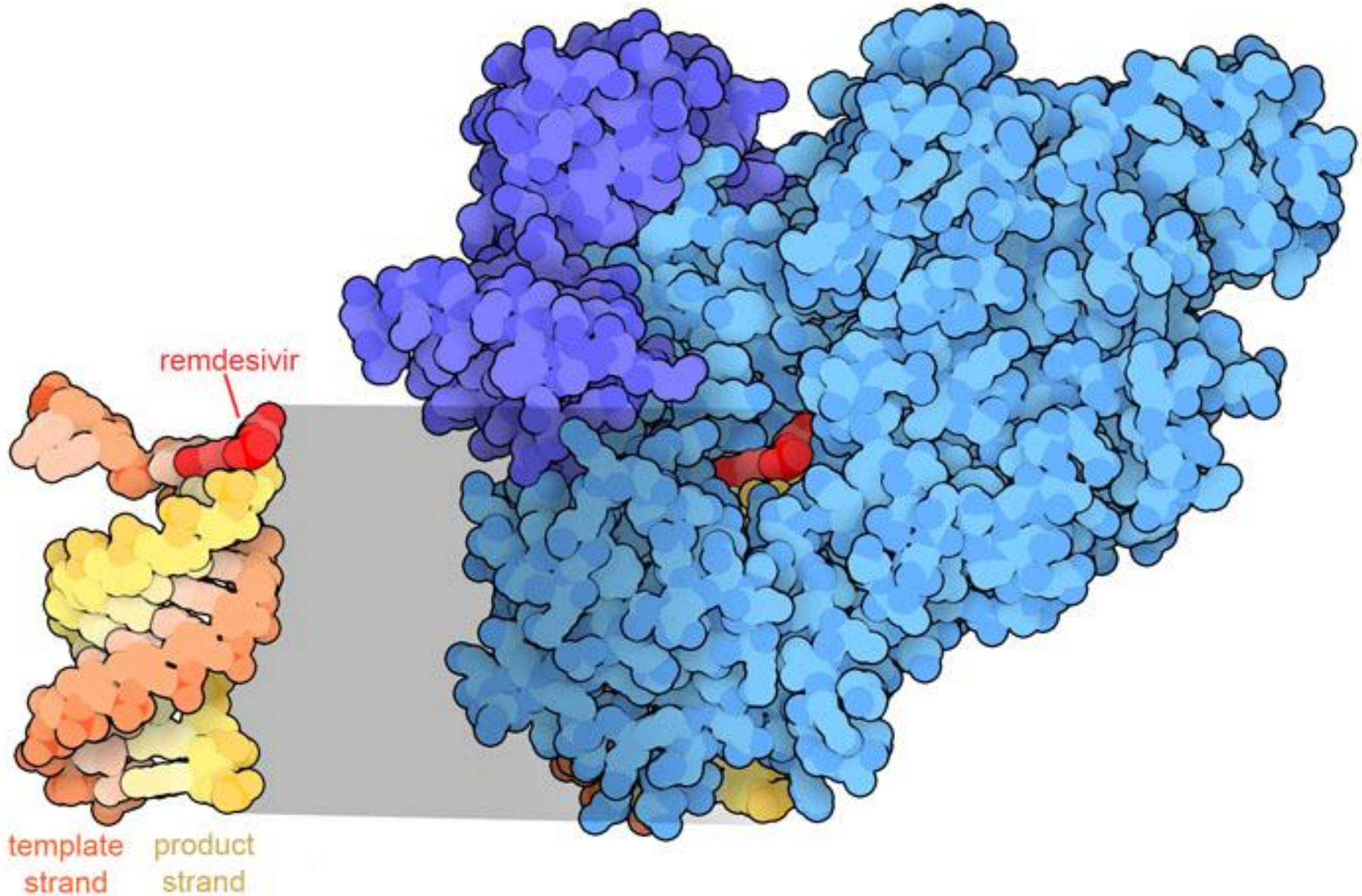
Cryo-EM RdRp of SARS-CoV-2.



Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, et al. (2020) COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. PLOS Pathogens 16(8): e1008762. https://doi.org/10.1371/journal.ppat.1008762
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008762

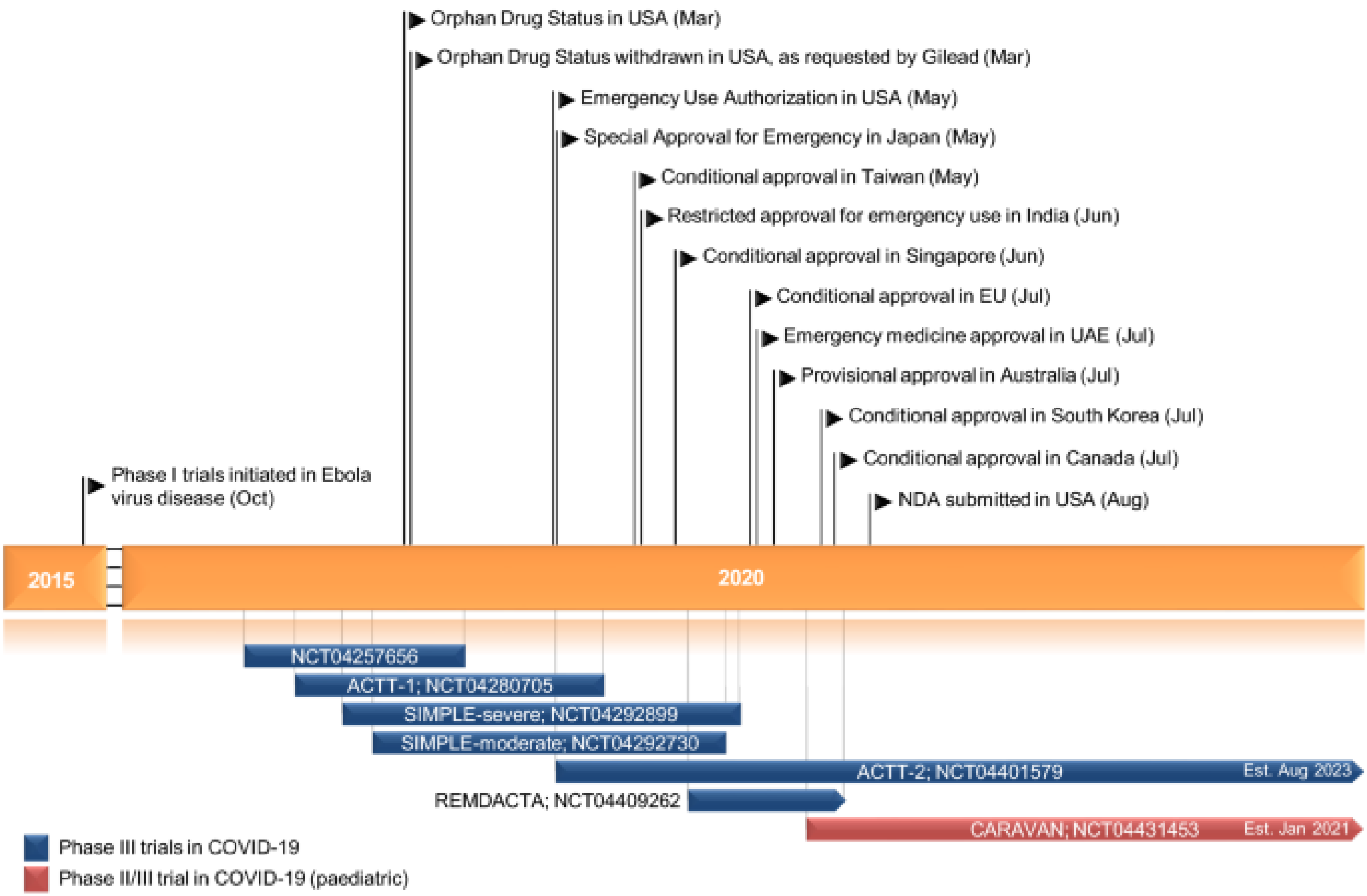
Remdesivir action





http://pdb101.rcsb.org/learn/resources-to-fight-the-covid-19-pandemic/the-search-for-drugs-to-fight-covid-19

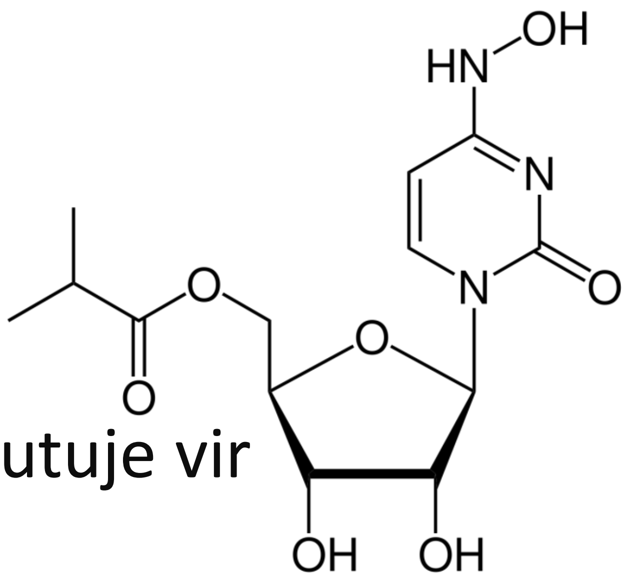
Remdesivir historie



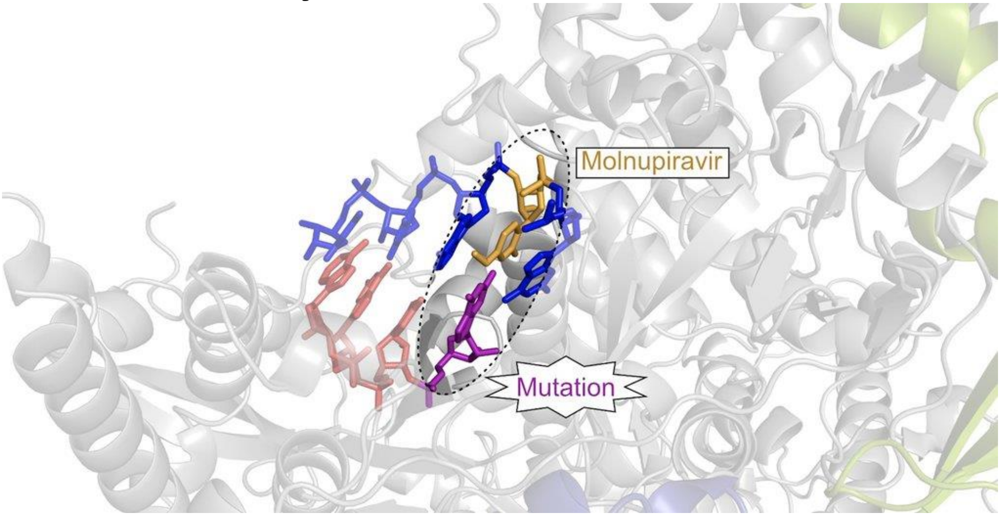
Remdesivir aktuálně

- WHO – uprava guidelines https://www.who.int/publications/i/item/therapeutics-and-covid-19-living-guideline
  - recommendation **against the use of remdesivir** in hospitalized patients with COVID-19, regardless of disease severity.
  - a conditional recommendation **for** remdesivir for non-severe COVID-19. (as of 13.1.2023)
- EMA – stále autorizovan od 20.7.2020
  - Čekají na kompletni výsledky WHO studie

Molnupiravir

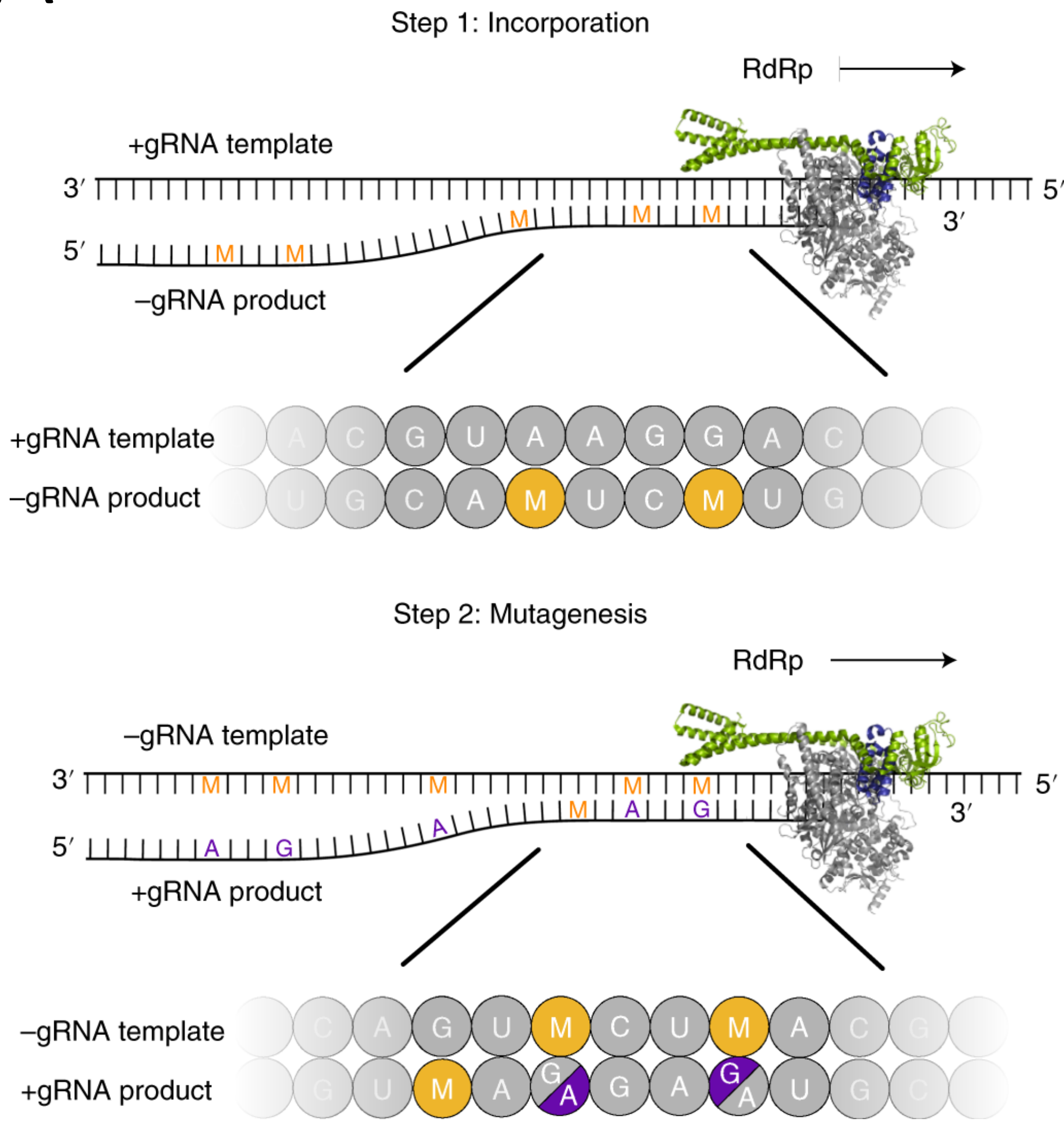


- Pilulka
- Blokuje replikaci SARS-CoV-2 – umutuje vir
- Žádné závažné vedlejší efekty na dobrovolnících
- prevence hospitalizace se závažnými formami a smrtí



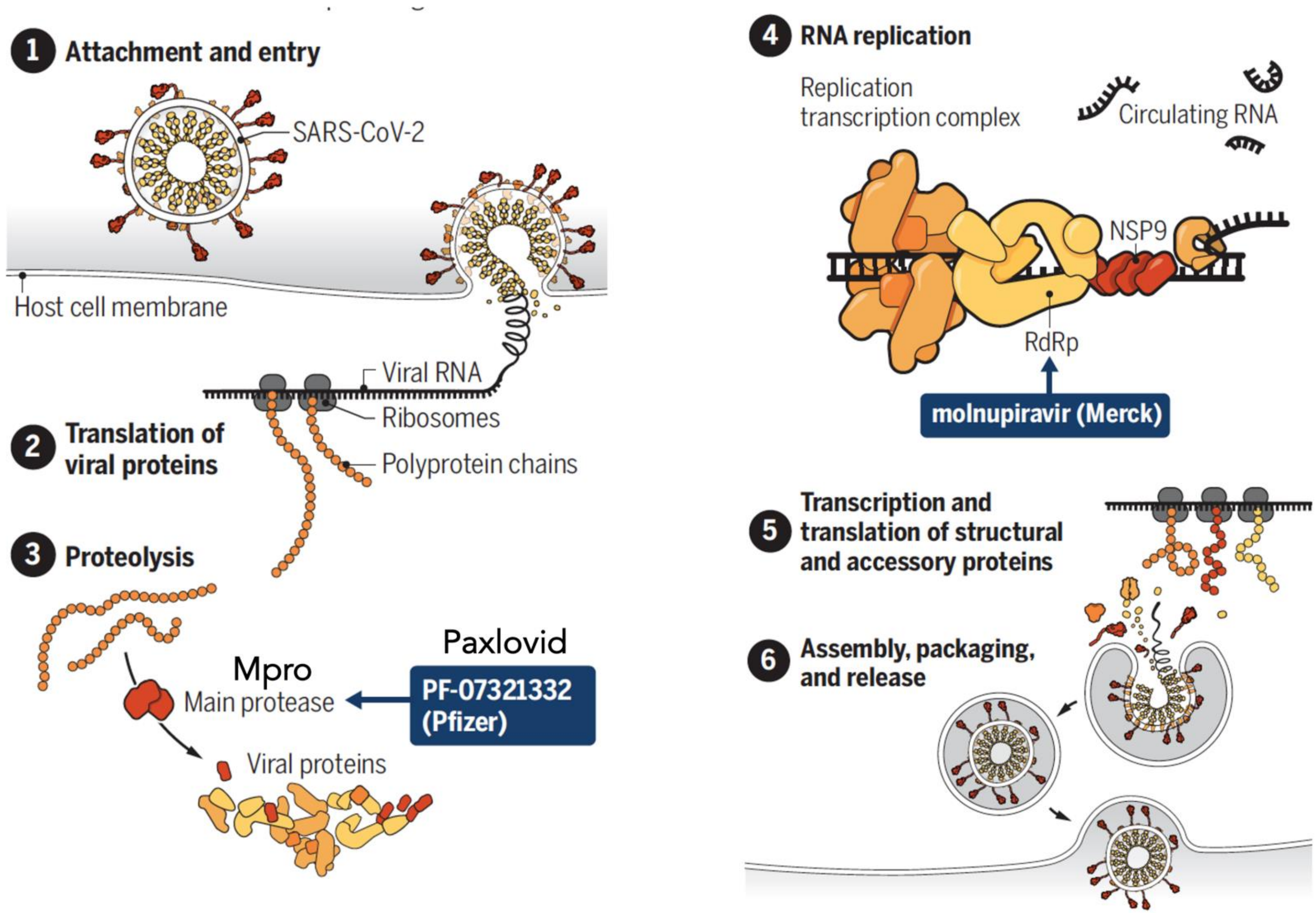
https://www.mpg.de/17362204/0812-bich-molnupiravir-152115-x

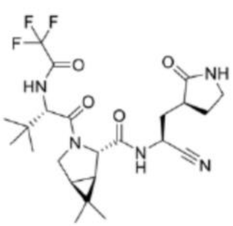
Molnupiravir MoA



Kabinger, F., Stiller, C., Schmitzová, J. *et al.* Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. *Nat Struct Mol Biol* **28**, 740–746 (2021). https://doi.org/10.1038/s41594-021-00651-0

Paxlovid vz Molnupiravir



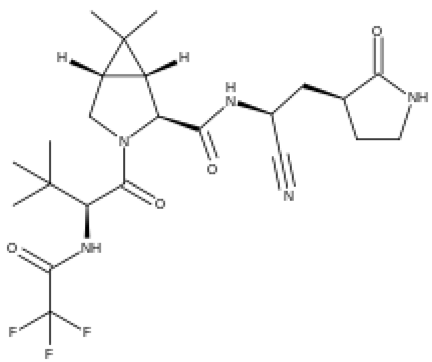


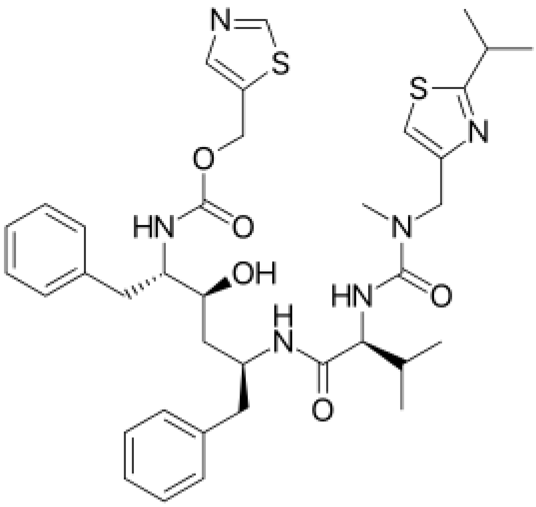
Chemical structure

https://erictopol.substack.com/p/why-paxlovid-is-a-just-in-time-breakthrough

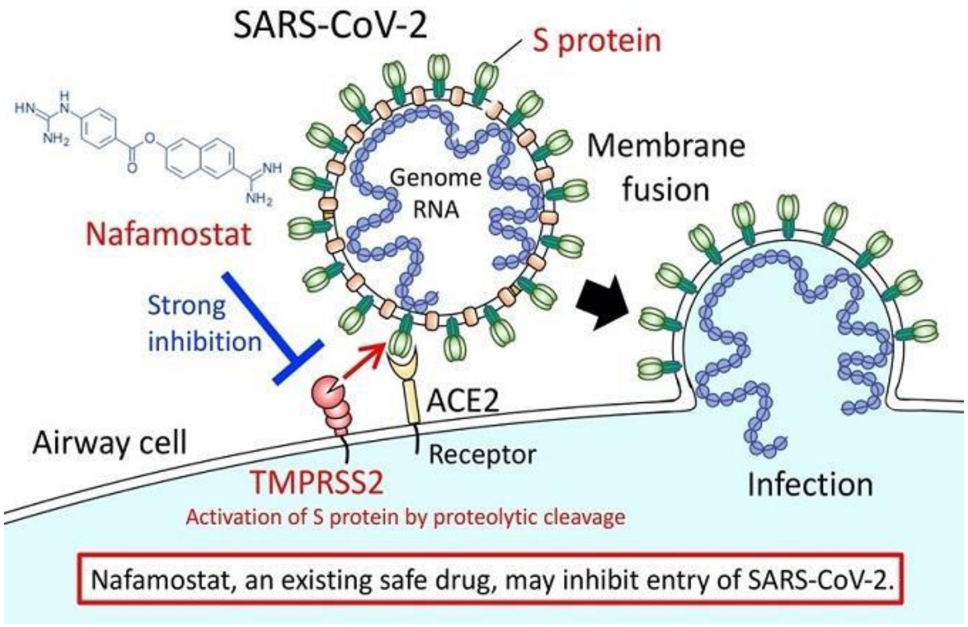
Paxlovid

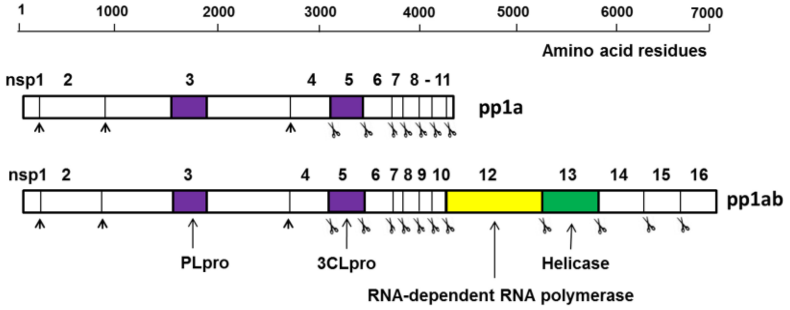
- **PF-07321332; ritonavir**
- orally active 3CL protease inhibitor
- November 2021, Pfizer positive phase II/III results, including 89% reduction in hospitalizations when given within three days after symptom onset





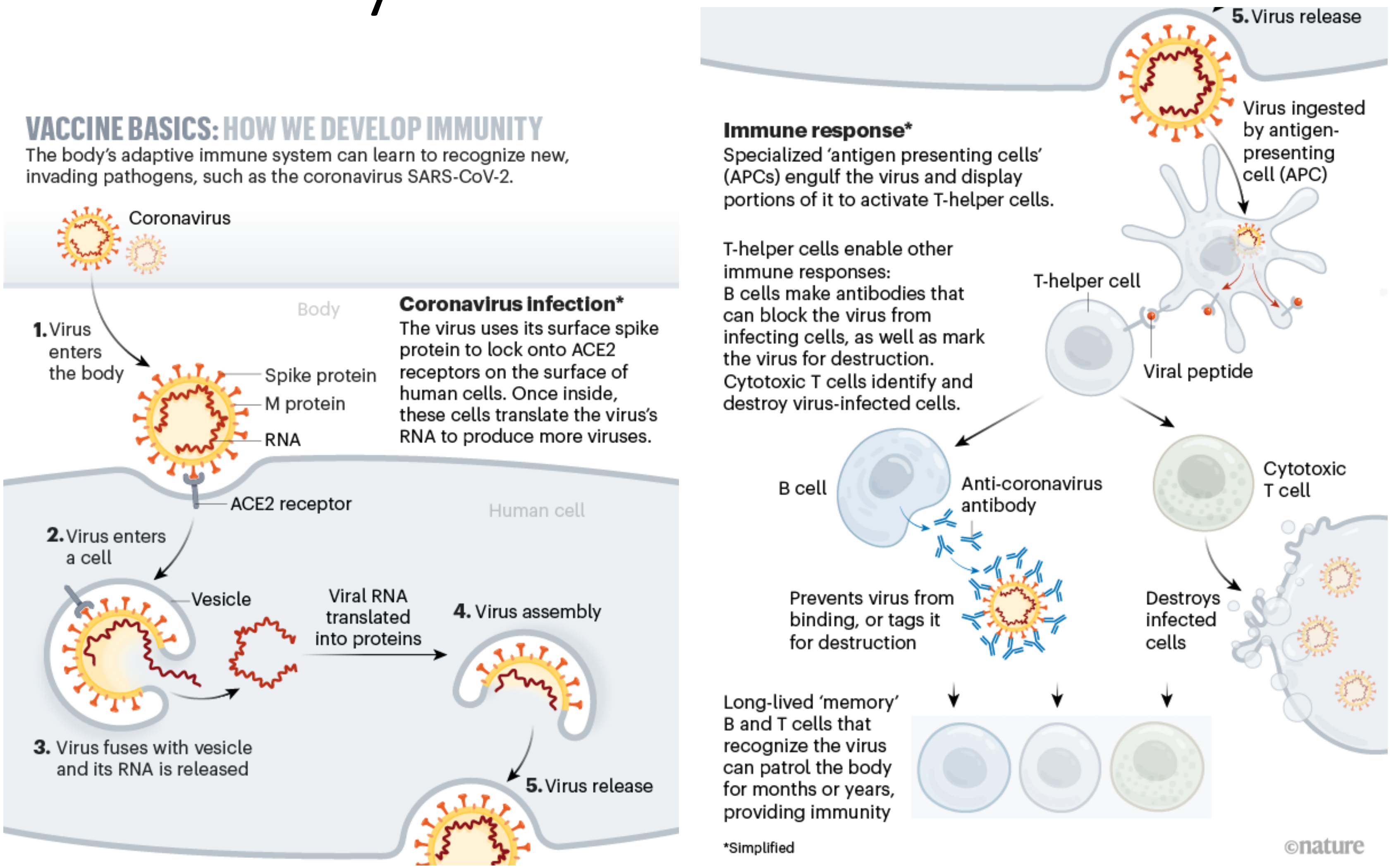
Protease Inhibitors – Spike and Maturation



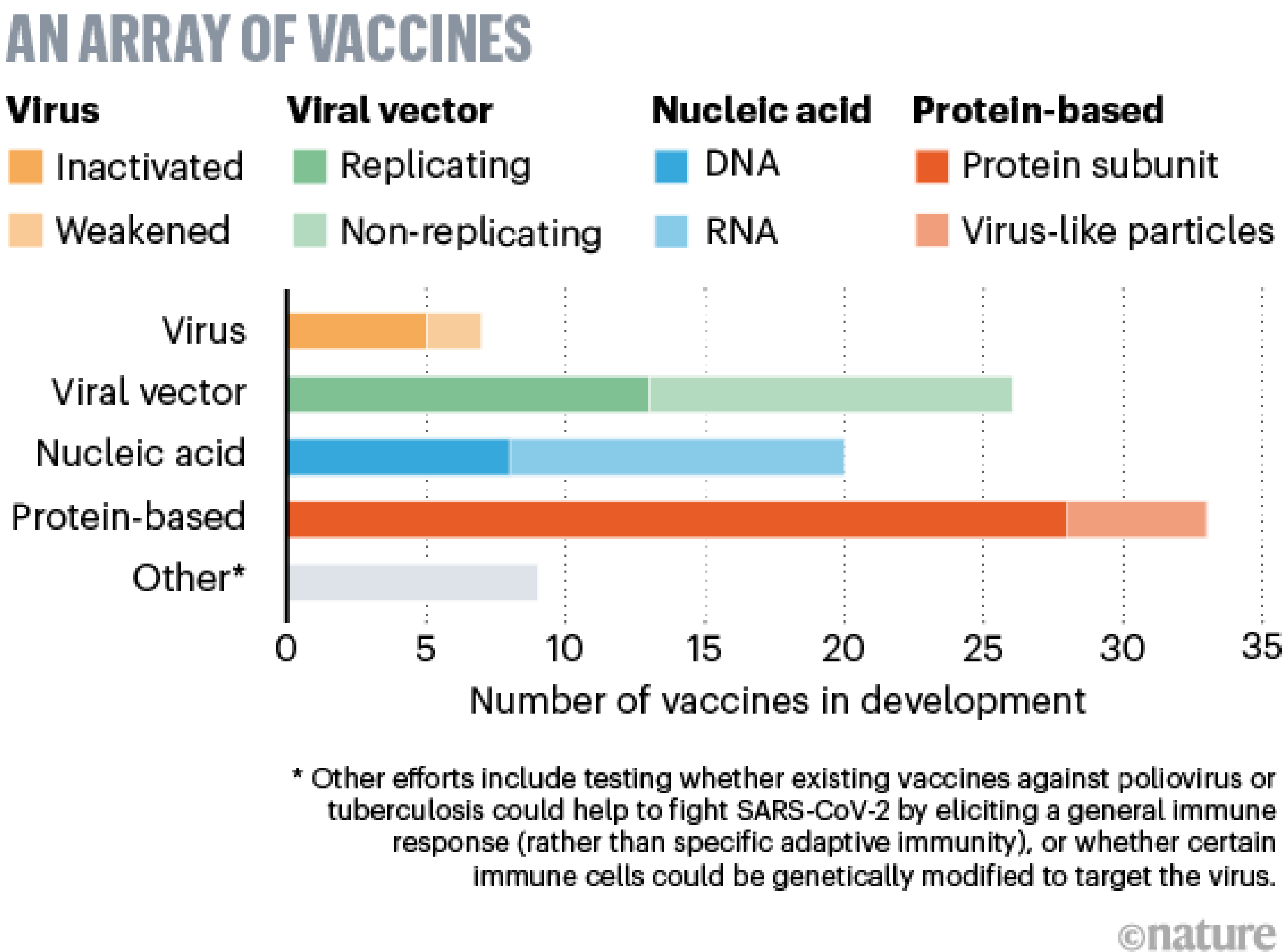


Ineffective – multiple access routes – some do not need TMPRSS2 involvement

Immunity



https://www.nature.com/articles/d41586-020-01221-y



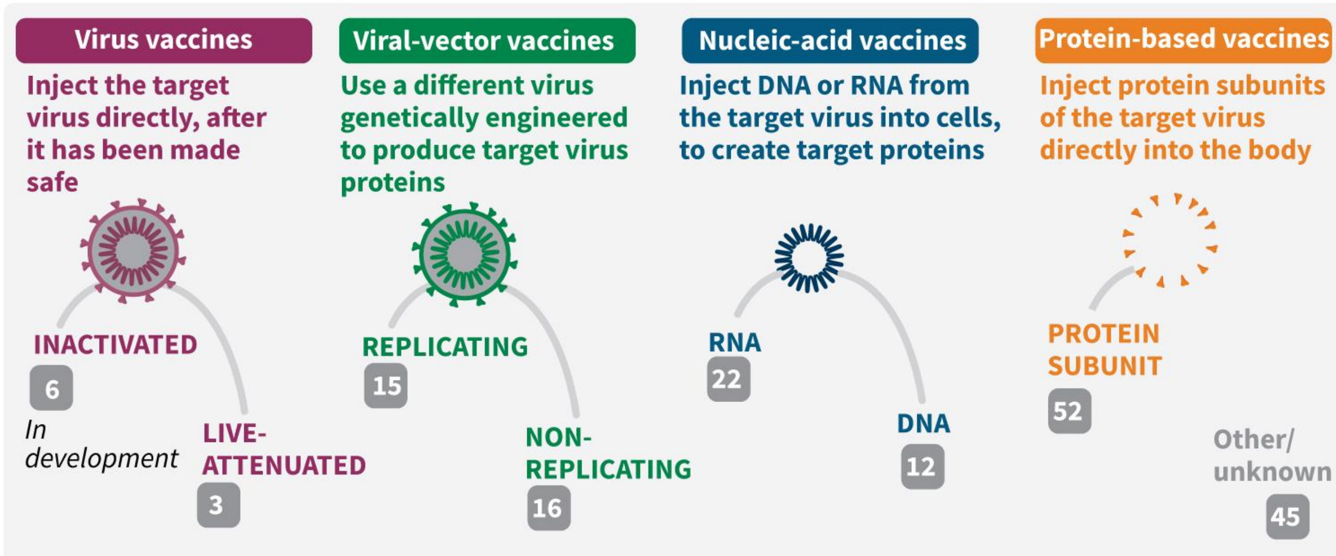
https://www.nature.com/articles/d41586-020-01221-y as of April 2020

Race for a COVID-19 vaccine

There are currently 171 vaccine candidates according to a tracker developed by the London School of Hygiene and Tropical Medicine

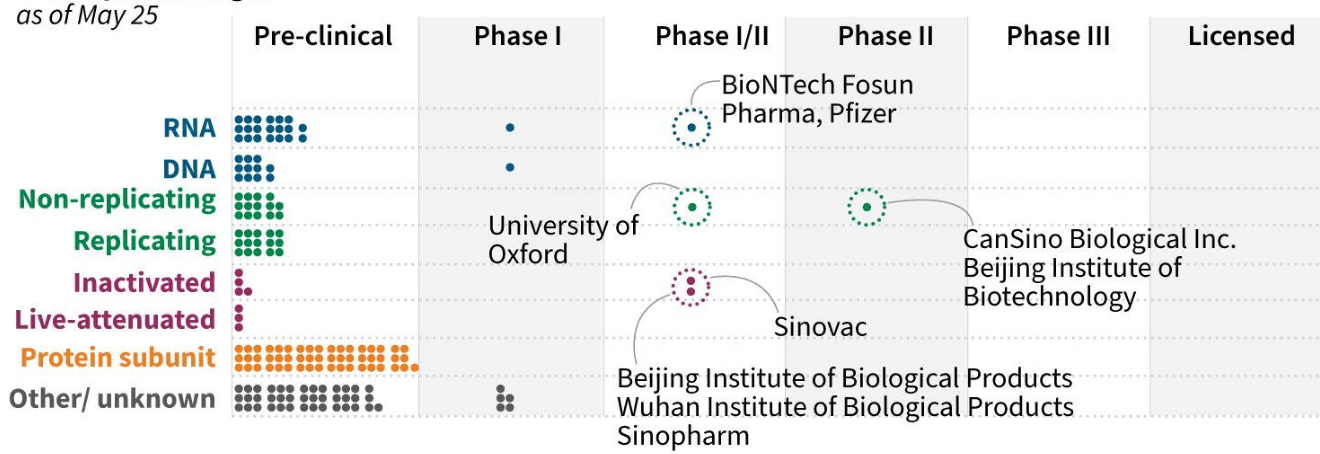
Four main approaches

All aimed at safely triggering the body's natural immune response to SARS-CoV-2

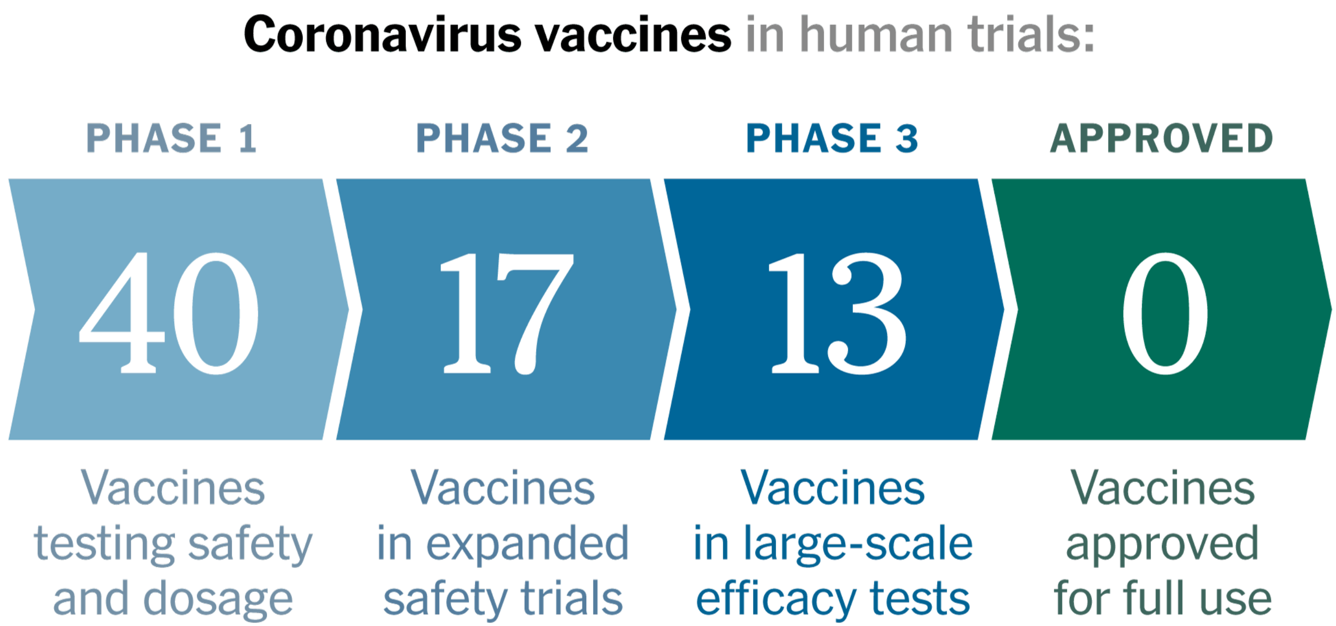


Development stages

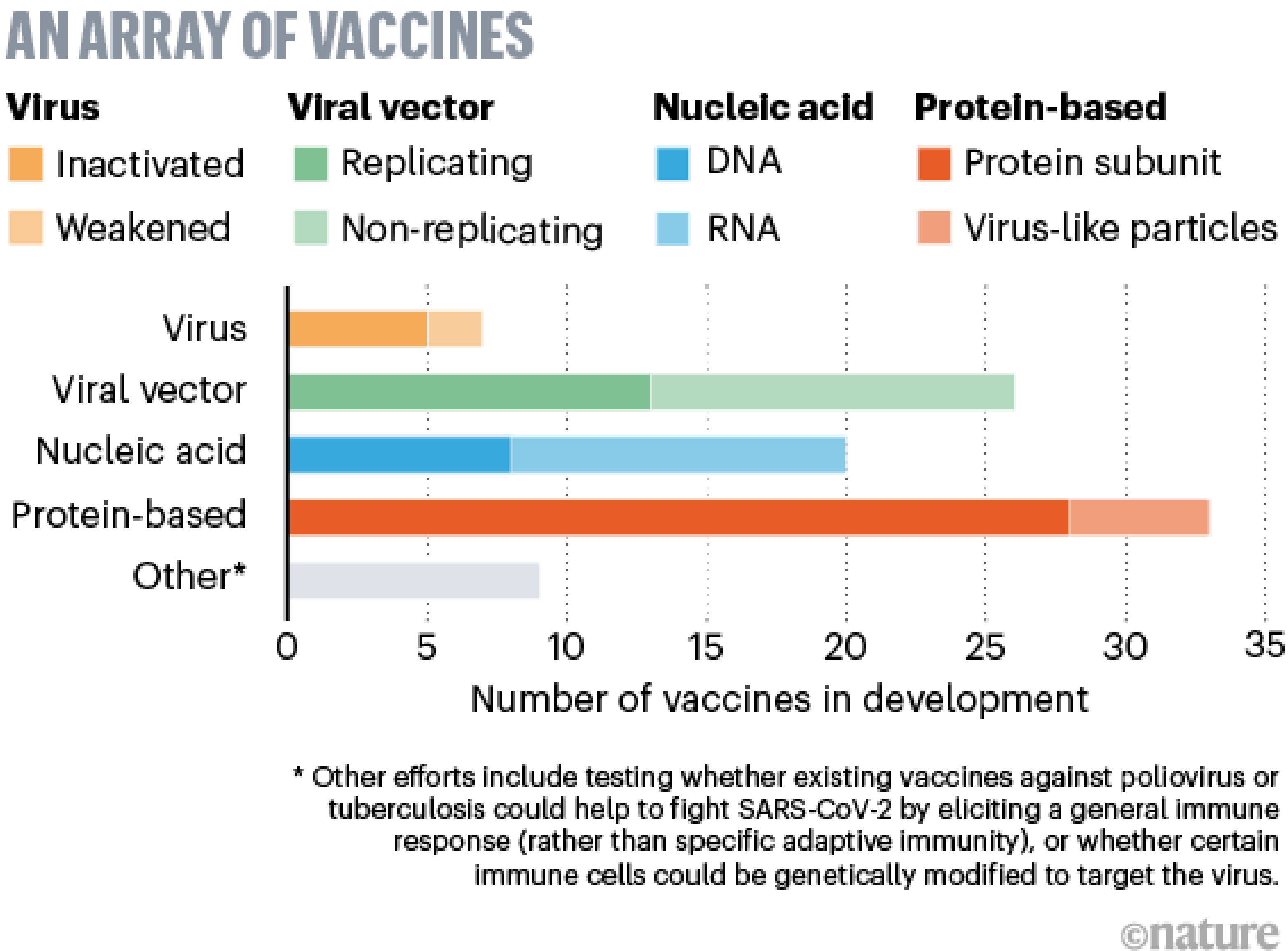
as of May 25



Source: Vaccine pipeline/Nature journal

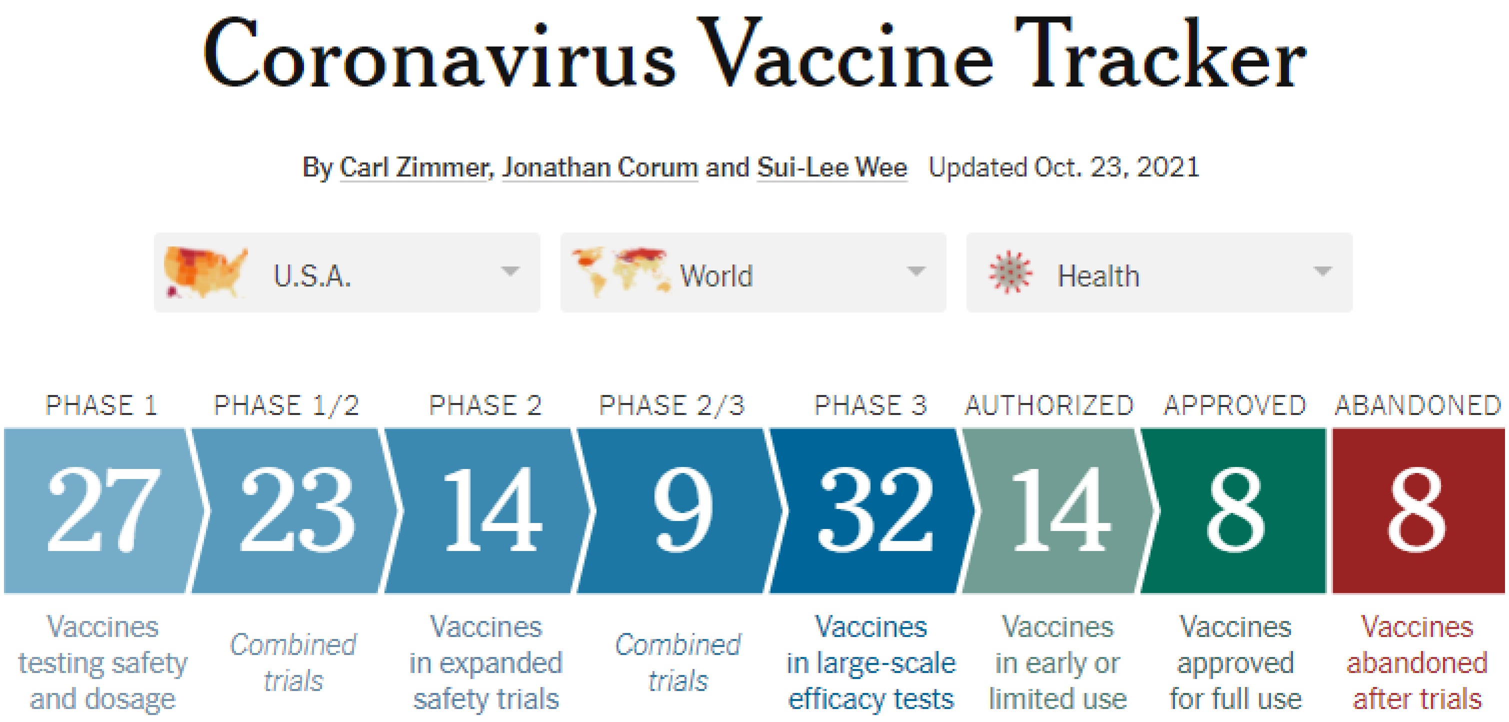


https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html



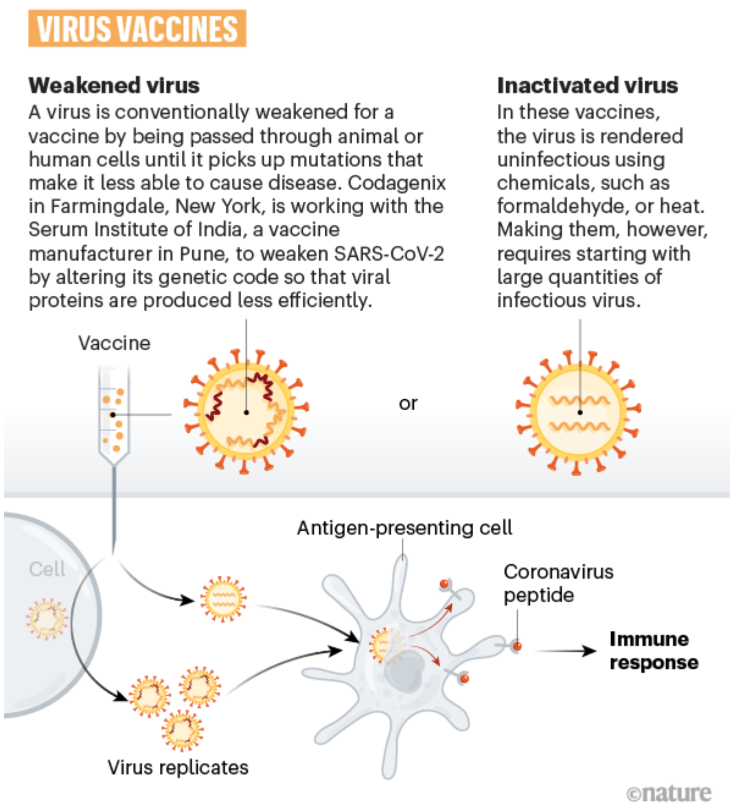
https://www.nature.com/articles/d41586-020-01221-y as of April 2020

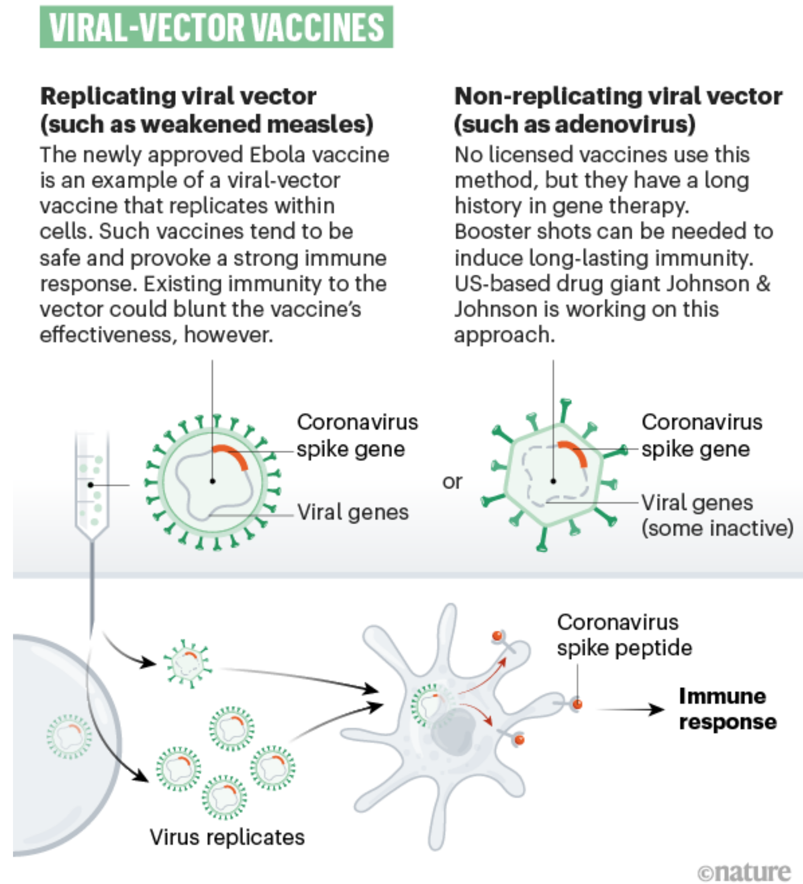
Vakcíny ve vývoji



https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html

Vakcíny I

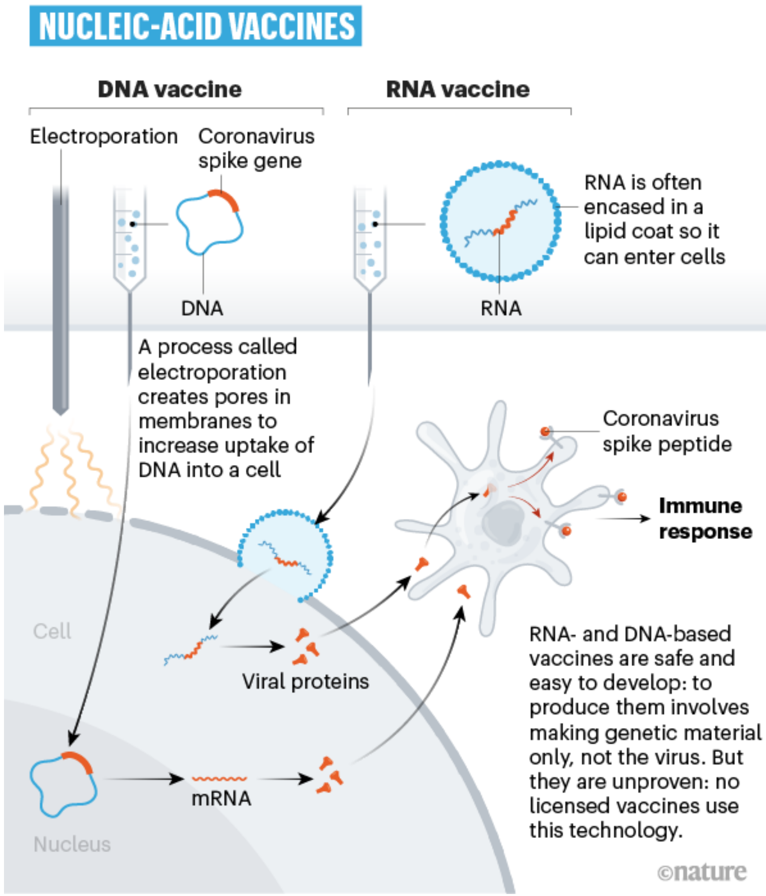


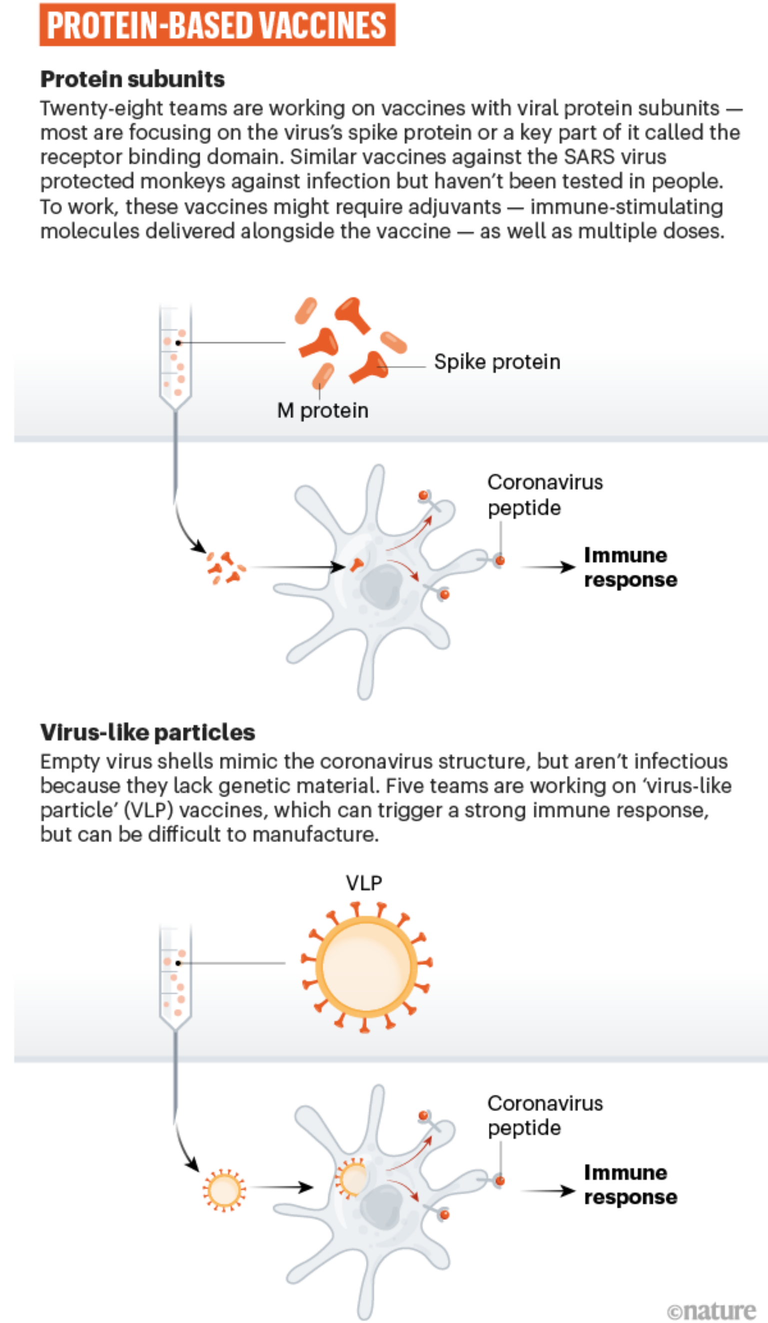


Sputnik V - two adenovirus vectors with Spike protein- rAd26-S + rAd5-S

https://www.nature.com/articles/d41586-020-01221-y

Vakcíny II

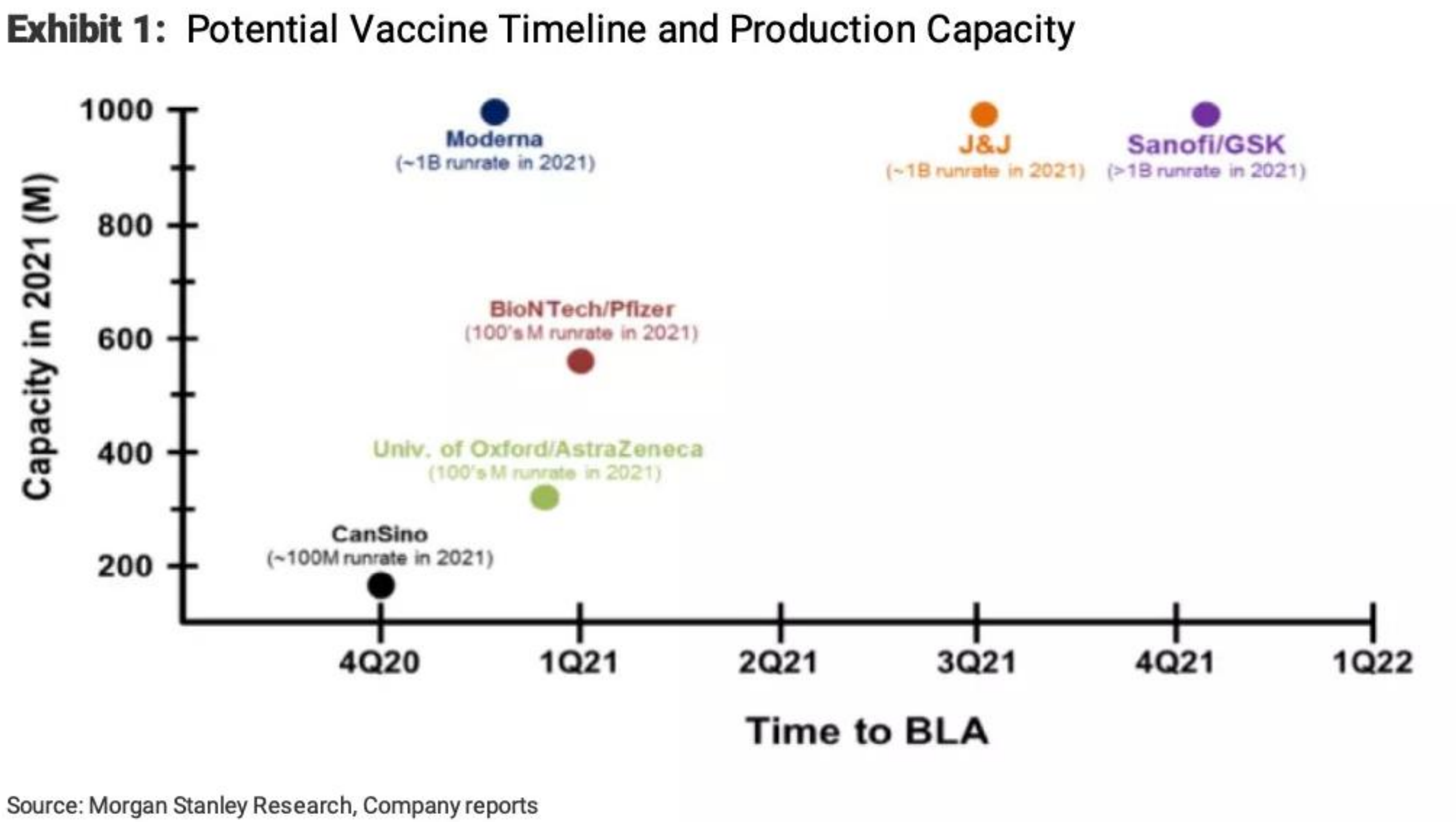




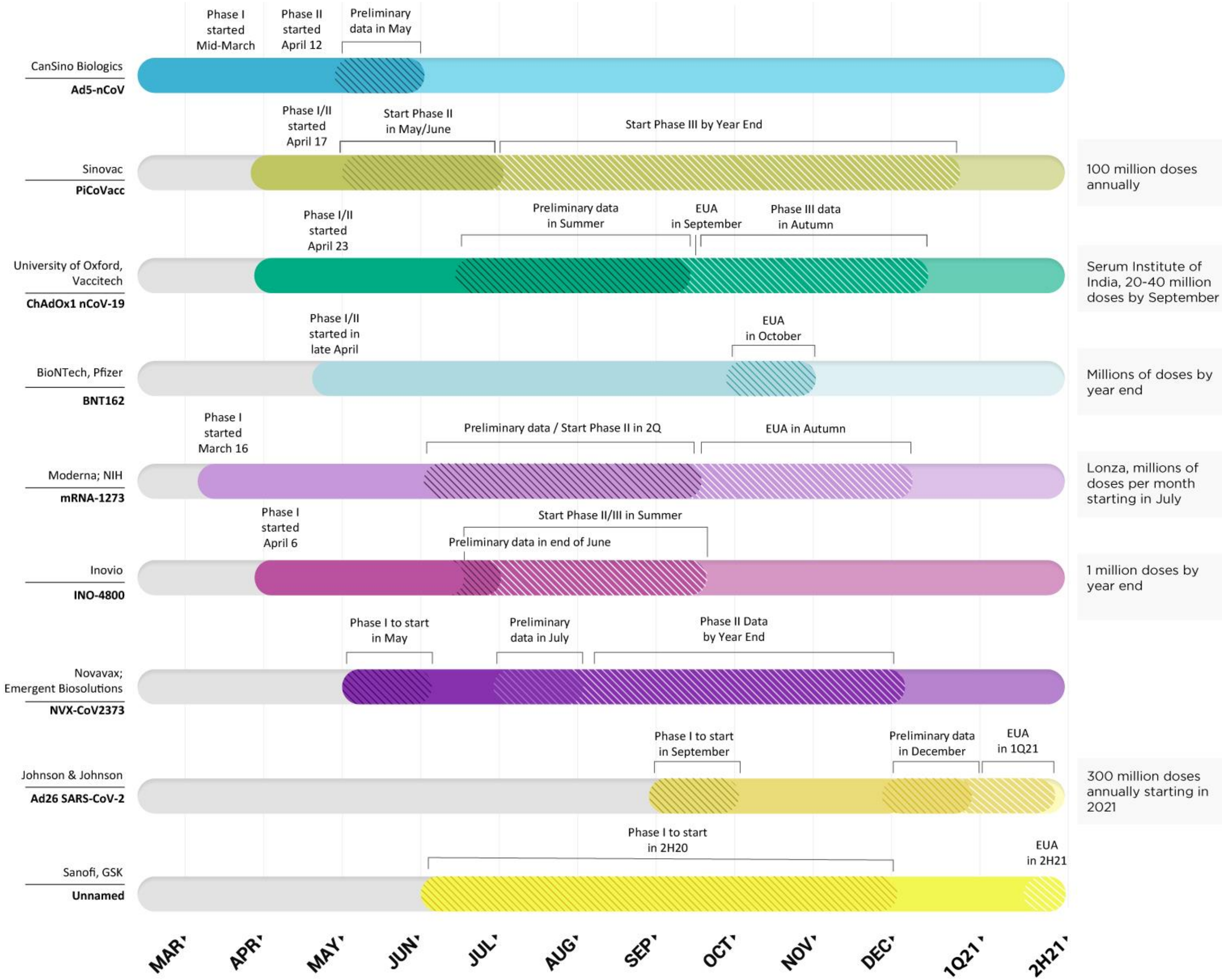
Pfizer-BioNtech – mRNA for Spike protein
Moderna - mRNA-1273 for Spike protein

https://www.nature.com/articles/d41586-020-01221-y

Who is winning the COVID vaccine race?



https://www.investopedia.com/who-is-winning-the-covid-19-vaccine-race-4845441



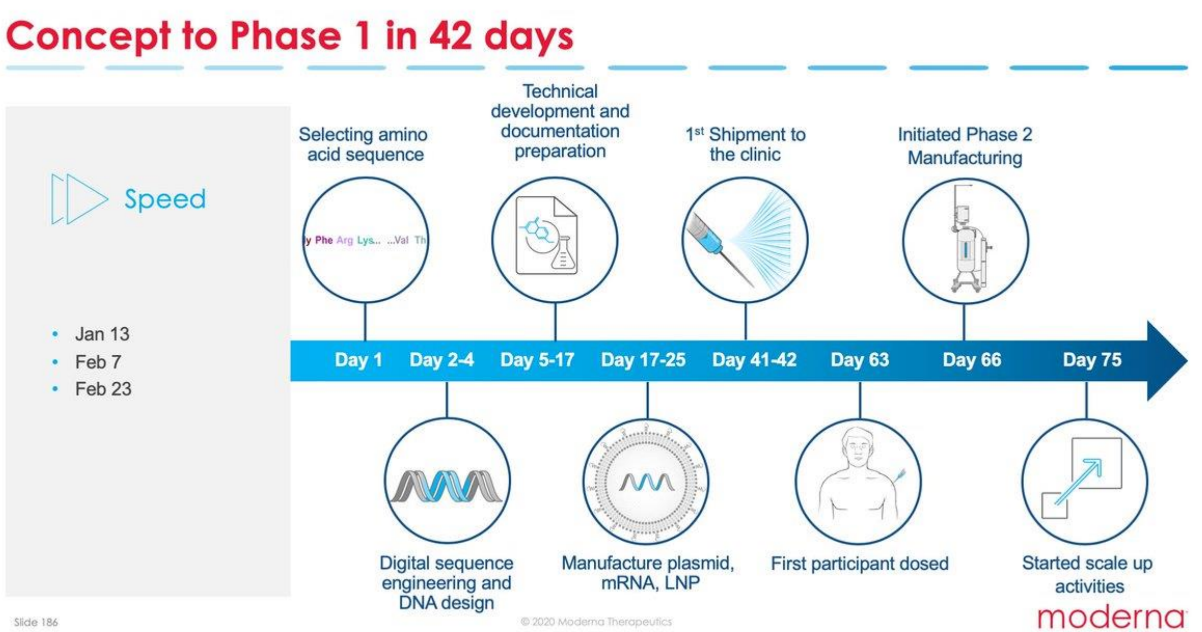
CanSino Biologics
Ad5-nCoV
Phase I started Mid-March
Phase II started April 12
Preliminary data in May
Sinovac
PiCoVacc
Phase I/II started April 17
Start Phase II in May/June
Start Phase III by Year End
100 million doses annually
University of Oxford, Vaccitech
ChAdOx1 nCoV-19
Phase I/II started April 23
Preliminary data in Summer
EUA in September
Phase III data in Autumn
Serum Institute of India, 20-40 million doses by September
BioNTech, Pfizer
BNT162
Phase I/II started in late April
EUA in October
Millions of doses by year end
Moderna; NIH
mRNA-1273
Phase I started March 16
Preliminary data / Start Phase II in 2Q
EUA in Autumn
Lonza, millions of doses per month starting in July
Inovio
INO-4800
Phase I started April 6
Start Phase II/III in Summer
Preliminary data in end of June
1 million doses by year end
Novavax; Emergent Biosolutions
NVX-CoV2373
Phase I to start in May
Preliminary data in July
Phase II Data by Year End
Johnson & Johnson
Ad26 SARS-CoV-2
Phase I to start in September
Preliminary data in December
EUA in 1Q21
300 million doses annually starting in 2021
Sanofi, GSK
Unnamed
Phase I to start in 2H20
EUA in 2H21
MAR'
APR'
MAY'
JUN'
JUL'
AUG'
SEP'
OCT'
NOV'
DEC'
1Q21'
2H21'

BIOCENTURY

On November 22, the *New York Times* published a fascinating account of the race to produce a coronavirus vaccine. The *Times* report included a number of interesting facts, but one really grabbed my attention: It turns out that the Moderna vaccine, which was just shown to be 95 percent effective, was actually developed by the company in just *two days* in January 2020.

That's right, they developed the vaccine in two days in January, but then needed to spend the following ten months performing tests in order to meet the FDA's standards for vaccine safety and efficacy.

Moderna/NIAID



https://twitter.com/moderna_tx/status/1250037483171131392

Moderna's COVID-19 Vaccine Timeline

- Jan 11: China shares genetic sequence of novel coronavirus
- Jan 13: Moderna finishes the sequence for its coronavirus vaccine
- Feb 7: First clinical batch of vaccine is complete
- Feb 24: Moderna ships vaccines to NIH for the Phase 1 clinical study
- Mar 4: FDA approves clinical trials
- Apr 27: Moderna asks FDA approval for Phase 2 trials
- May 12: FDA gives the vaccine fast track designation
- Oct 22: Moderna completes enrollment of Phase 3 trials
- Nov 16: Interim results of Phase 3 study show 94.5% efficacy
- Nov 30: Final data shows vaccine is 94.1% effective, Moderna requests U.S., Europe approval

https://globalnews.ca/news/7492076/moderna-coronavirus-vaccine-technology-how-it-works/

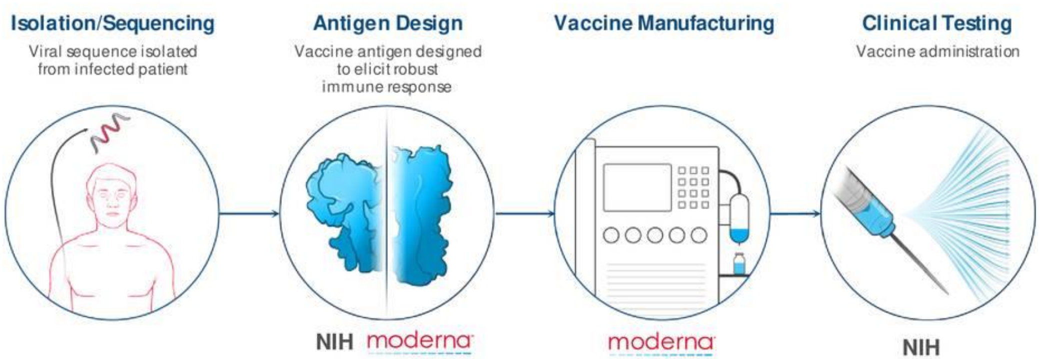
Moderna

mRNA vaccine (mRNA-1273) against SARS-CoV-2

- mRNA-1273 is an mRNA vaccine against SARS-CoV-2 encoding for a prefusion stabilized form of the Spike (S) protein of the novel coronavirus, which was selected by Moderna in collaboration with investigators at the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center (VRC), a part of the National Institutes of Health (NIH)

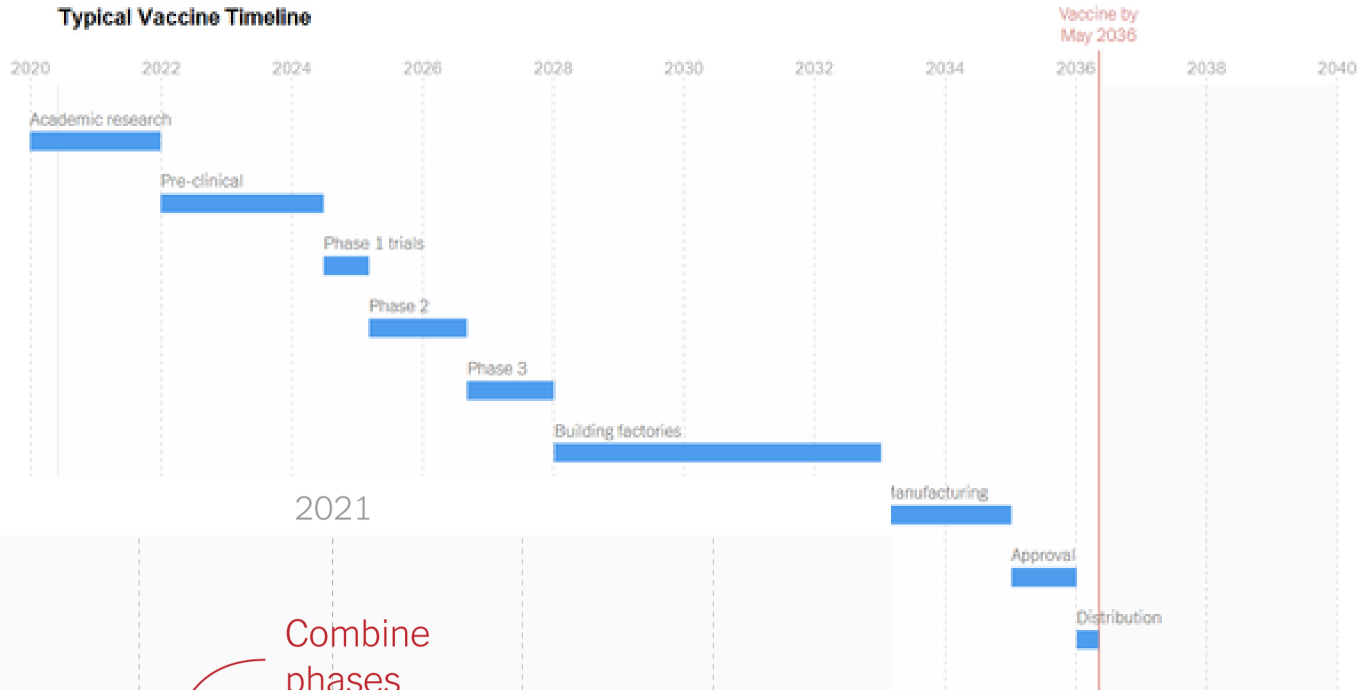
- First clinical batch for Phase 1, including fill and finishing of vials, was completed on February 7; Batch has been shipped to the NIAID for the Phase 1 study

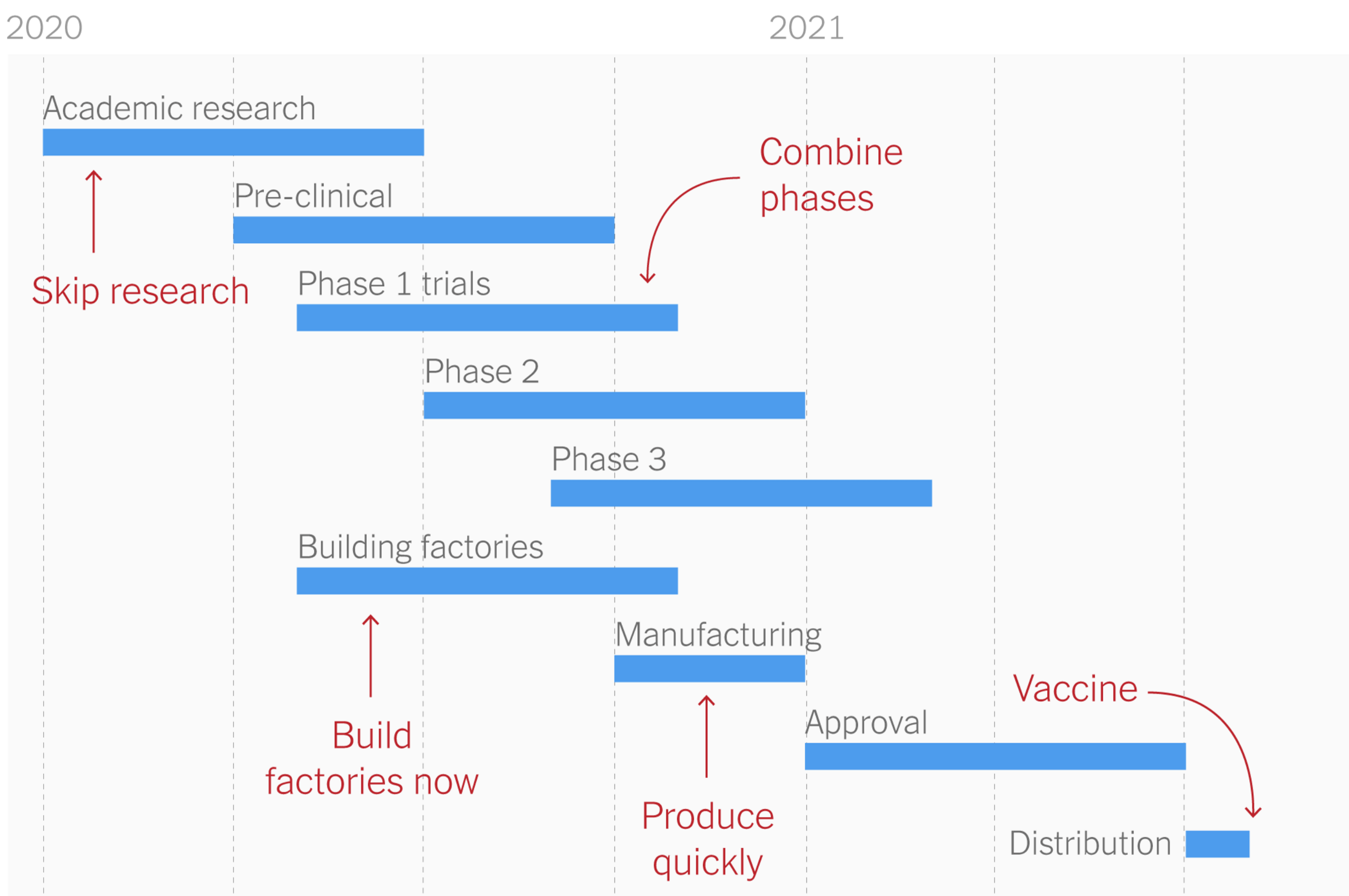
- NIAID will conduct the Phase 1 clinical study under their IND



moderna

Moderna vz typical





Plně povolené vakcíny – k 1.11.2021

Pfizer BIONTECH

VACCINE NAME: Comirnaty (also known as tozinameran or BNT162b2)

Bahrain, Brazil, Canada, NewZealand, SaudiArabia, Switzerland, UnitedStates.

moderna NIH National Institutes of Health Turning Discovery Into Health

VACCINE NAME: mRNA-1273 or Spikevax

Canada, Switzerland.

UNIVERSITY OF OXFORD AstraZeneca

VACCINE NAME: Vaxzevria (also known as AZD1222, or Covishield in India)

Brazil.

CanSinoBIO

VACCINE NAME: Convidecia (also known as Ad5-nCoV)

China

https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html

Plně povolené vakcíny – k 1.11.2021

ВЕКТОР

Turkmenistan

VACCINE NAME: EpiVacCorona, Aurora-CoV

SINOPHARM

Bahrain, China, United Arab Emirates.

VACCINE NAME: BBIBP-CorV

sinovac

China

VACCINE NAME: CoronaVac (formerly PiCoVacc)

SINOPHARM

武汉生物制品研究所有限责任公司

WUHAN INSTITUTE OF BIOLOGICAL PRODUCTS CO.,LTD.

China

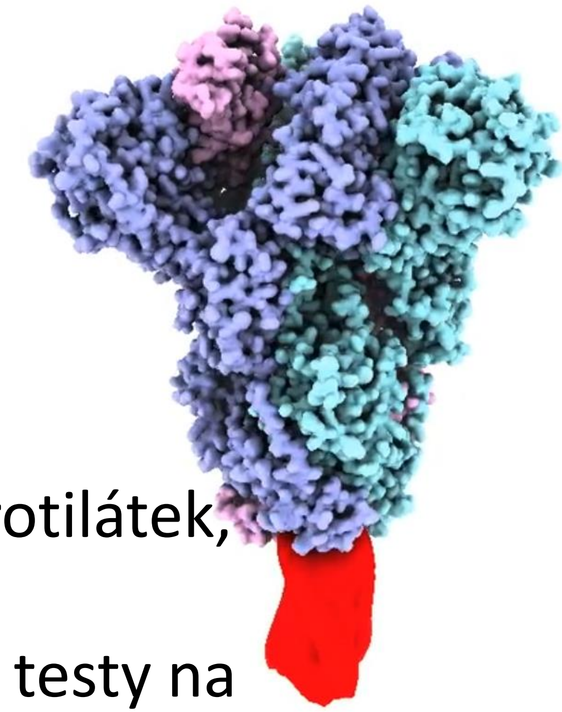
https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html

Zamítnuté vakcíny – zvláštní zmínka

ABANDONED

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

CSL



- Opuštěna 10.12.2020
- Na křečcích fungovala skvěle
- Fáze I – červenec 2020 – skvělé, hodně protilátek, žádné závažné vedlejší účinky
- Ale pak – dobrovolníci začali mít pozitivní testy na HIV, aniž by HIV virus měli
- Důvod: Aby udrželi S protein ve správném tvaru – drželi ho na místě pomocí „molecular clamp“ – ke kterému použili segment HIV proteinu – a ten chytaly protilátkové testy na HIV

https://www.nature.com/articles/d42473-020-00504-2?source=globalbiodefense

Závěr

- SARS-Cov-2 způsobuje chorobu COVID-19
- Hledání léku a jeho testování postupuje neskutečně rychle díky mezinárodní spolupráci a obrovskému nasazení vědců, firem a donátorů
- Zkouší se různé strategie léčby, ale většina bohužel nemá příliš silné výsledky
- Zatím nejnadějněji vypadají vakcíny