SARS-CoV-2 Mutation

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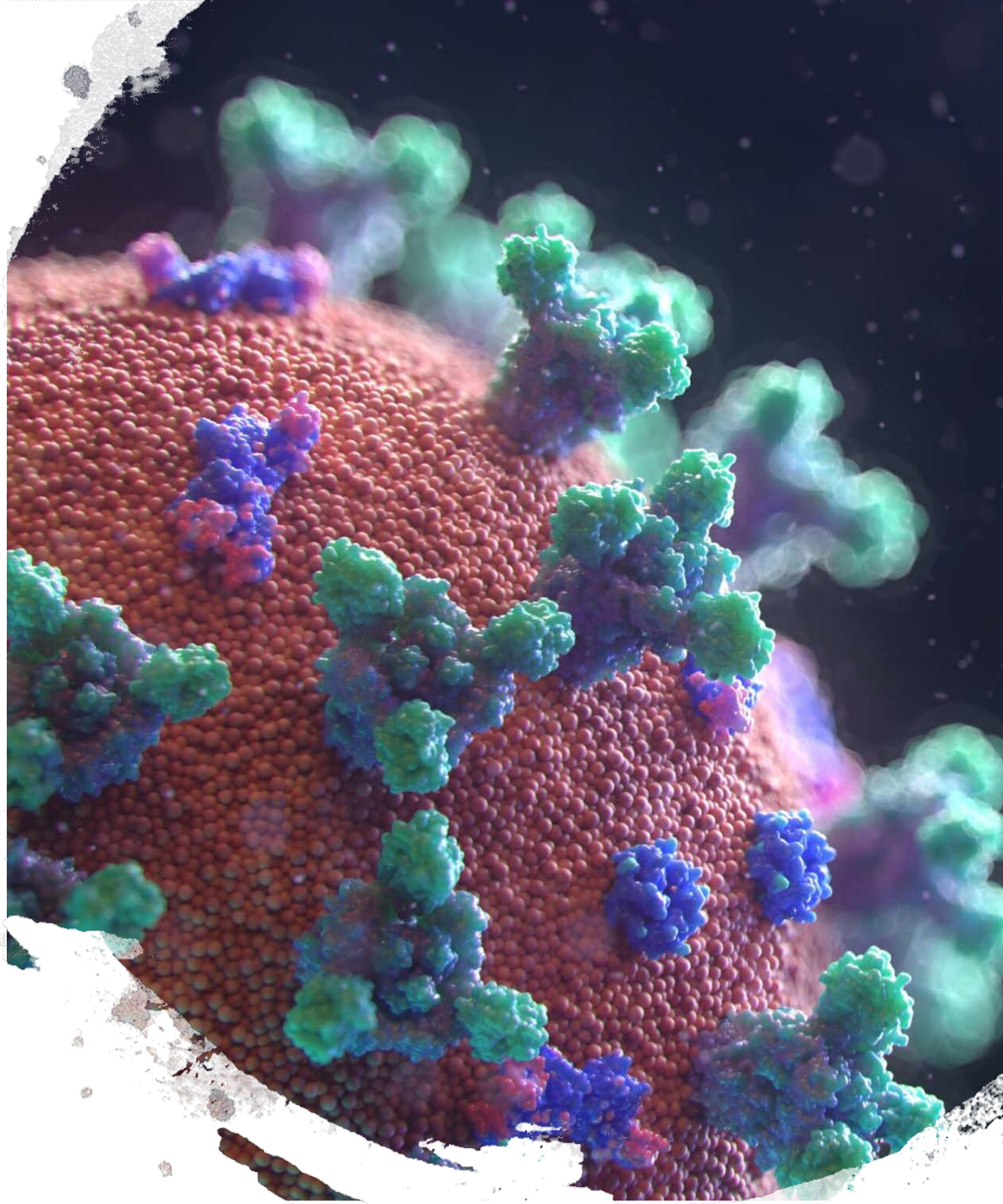
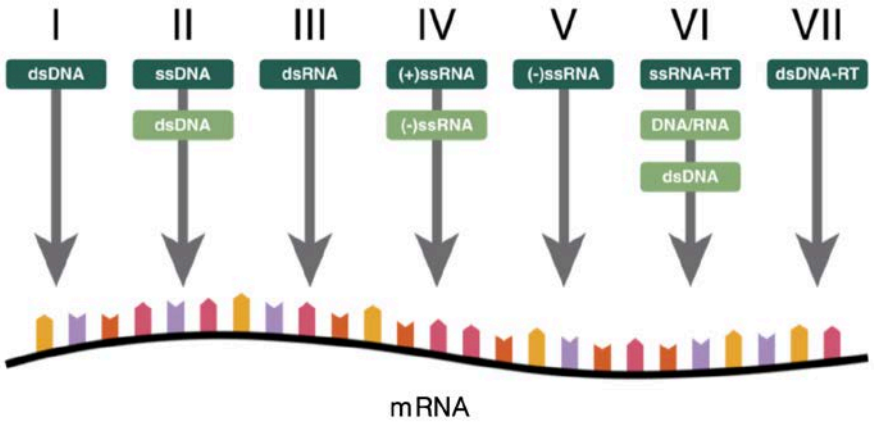


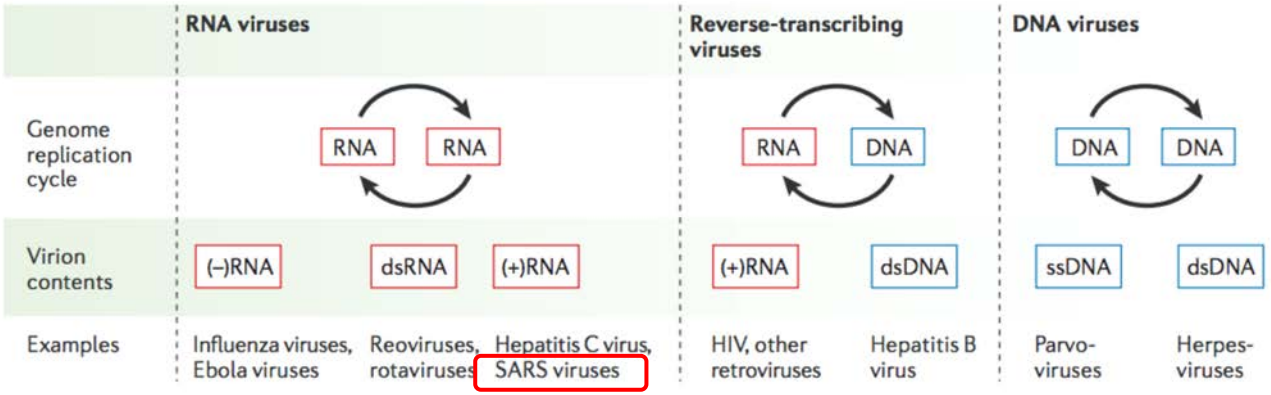
Presentation Outline

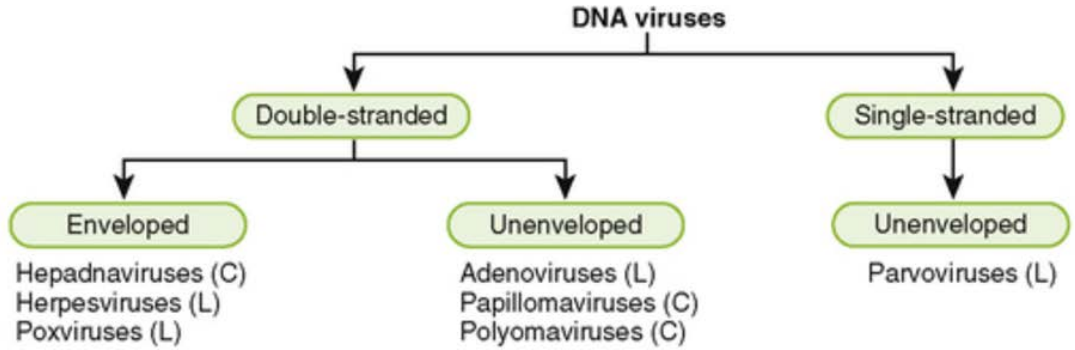
1. **Classifying Viruses**
2. **Understanding SARS-CoV-2 genome**
3. **Defining Mutation (Type, Rate and Fate of Mutation)**
4. **Major Clades/Types SARS-CoV-2 (PANGOLIN, GISAID, and NEXTSTRAIN classification system)**
5. **The Impact of Mutation (Notable Mutation, Variant of Concern, Preliminary Findings of COVID-19 Vaccine against Variants)**
6. **Genomic Surveillance (Importance, Prioritization, and Sample size estimation)**
7. **Genomic Sequencing Effort for SARS-CoV-2 in Indonesia**

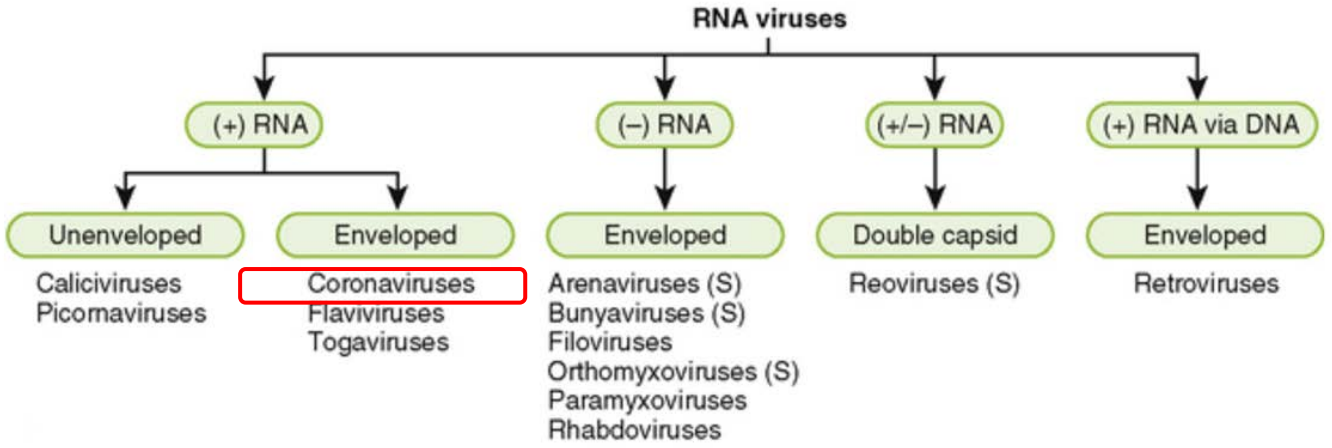
1. Classifying Viruses

- In 1971, **David Baltimore** created a classification system for viruses into 7 groups depending on nucleic acid (DNA/RNA), strandedness (single/double), sense (+/-), and whether it use reverse transcription to make mRNA.
- Baltimore classification of virus:









Baltimore D. Expression of animal virus genomes. Bacteriol Rev. 1971 Sep; 35(3):235-41
https://oncohemakey.com/viral-structure-classification-and-replication/

2. Understanding SARS-CoV-2 Genome

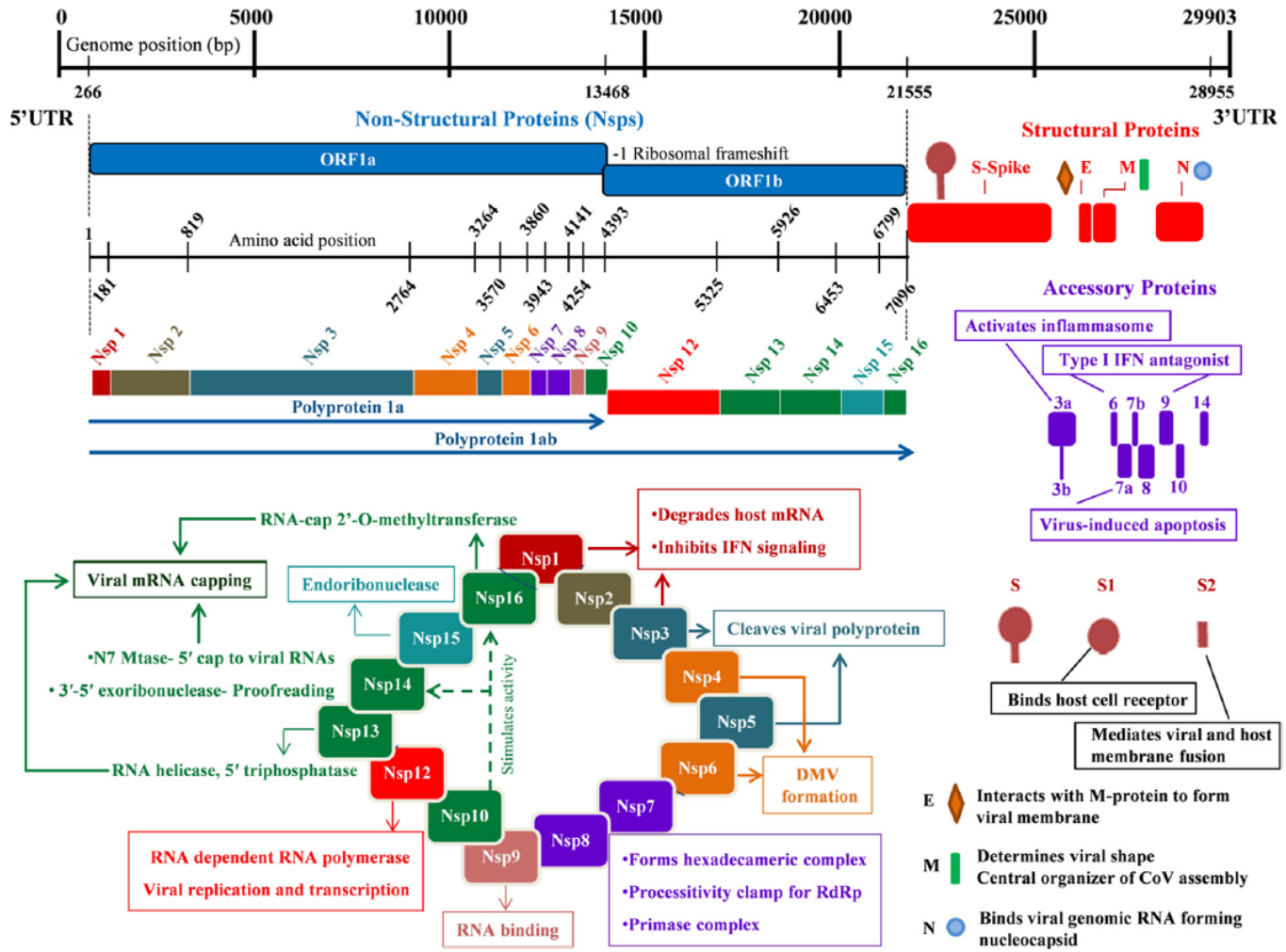


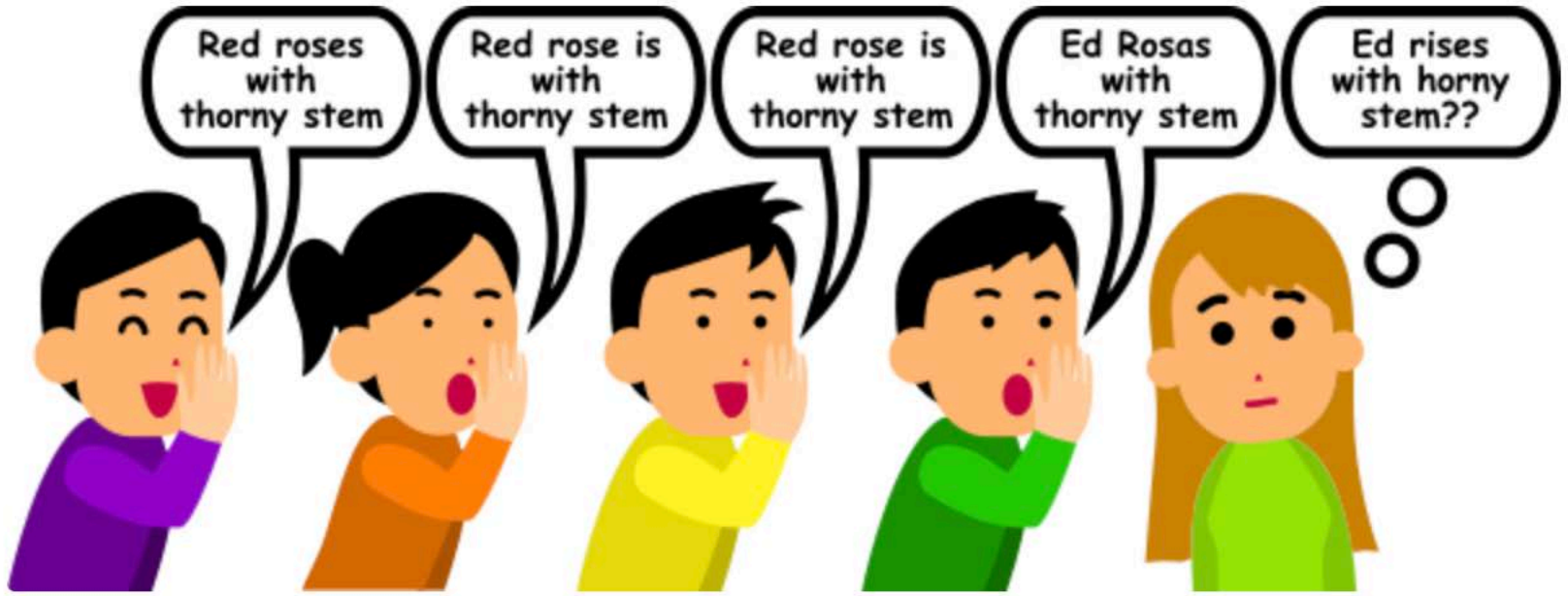
Fig. 1. Genome organization of the SARS-CoV-2. The viral genome encodes 16 Non-structural proteins (Nsps) required for replication/transcription along with the structural proteins required for the assembly of new virions. The proteins are marked below the genome with their respective coding regions. A short description of the functions of different proteins is also shown.

- ✓ Betacoronavirus from the Coronaviridae family
- ✓ Positive-sense, single-stranded RNA (+ssRNA) enclosed in nucleocapsid (N) protein
- ✓ The viral genome (RNA) is ~ **30,000** nucleotides
- ✓ With almost 30kbp size, Coronavirus has the largest genomes of the known RNA viruses
- ✓ Has 14 open reading frames (ORFs) encoding 29 proteins (ppa1 protein, ppa1ab protein, 15 non-structural proteins, 4 structural proteins, and 8 accessory proteins)

Arya R, Kumari S, Pandey B, Mistry H, Bihani SC, Das A, Prashar V, Gupta GD, Panicker L, Kumar M. Structural insights into SARS-CoV-2 proteins. Journal of molecular biology. 2021 Jan 22;433(2):166725.

3. Defining Mutation

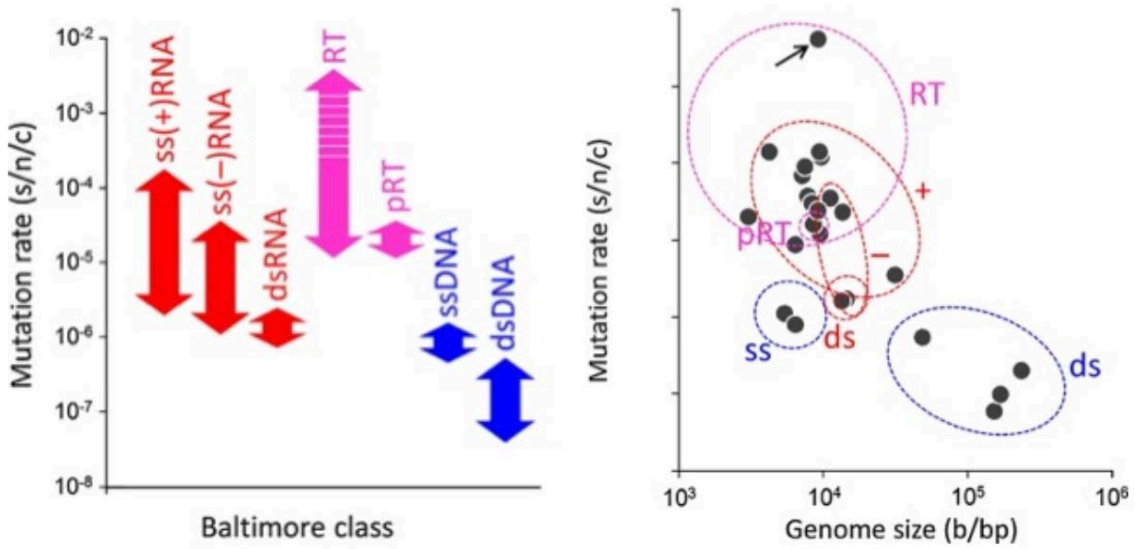
Why mutation occurs?



Have you ever play a telephone game?

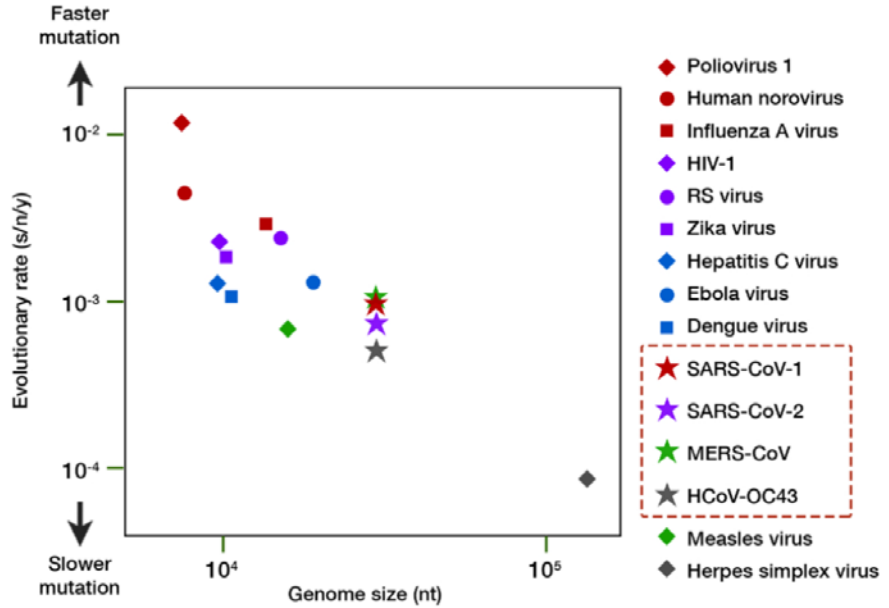
As long as the ”message” (virus) jump from one person to another without “editor” (proofread) correcting it, thus you can imagine the “distortion” (mutation).

- As replication occurred, mutation is not unexpected
- Viruses (especially RNA viruses) have a very fast pace of mutation due to high-error rate of genomic replication → lack of proofreading activity
- If mutation rate is high, the fittest genome will survive.



Rule of Thumb Mutation Rate:

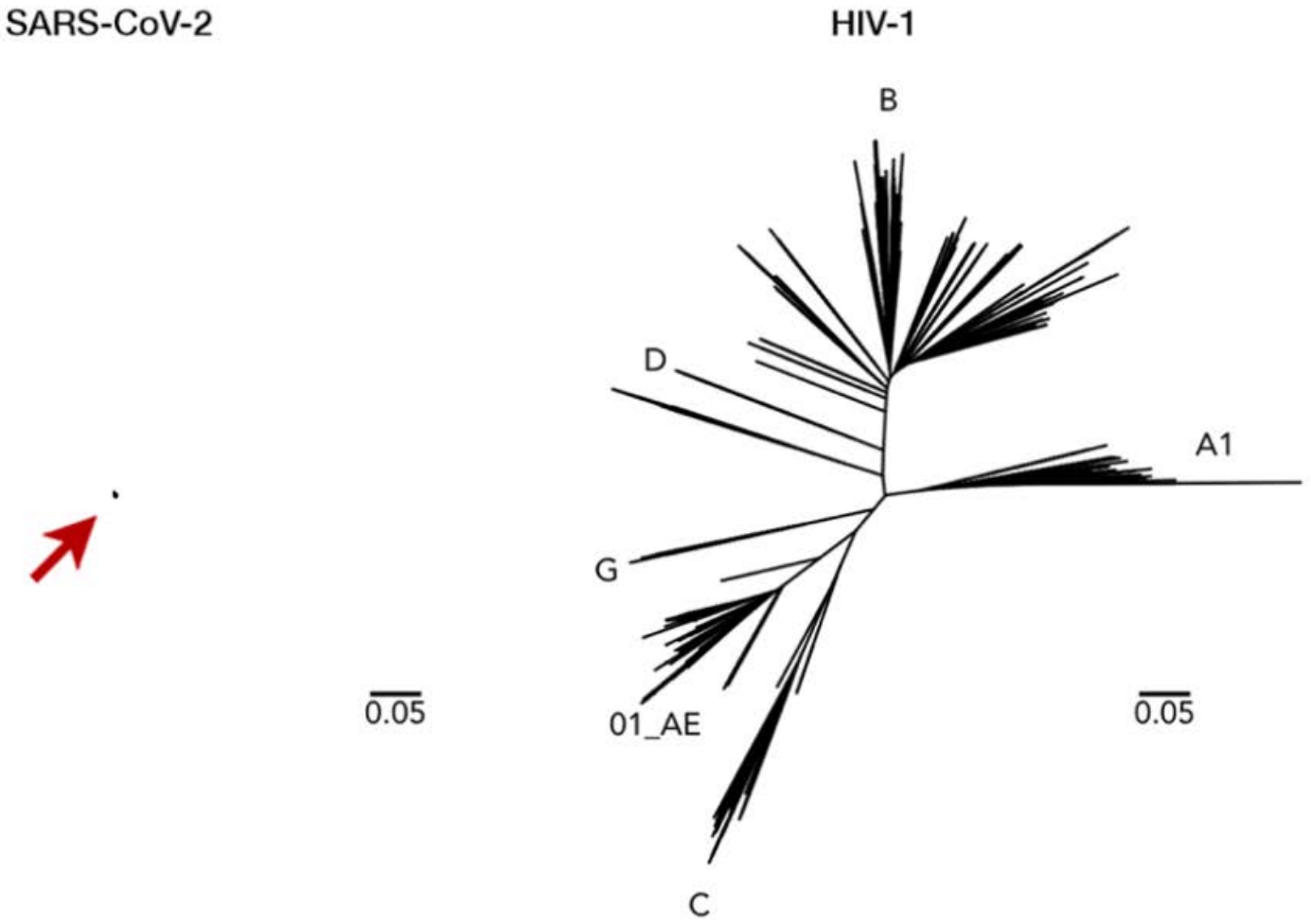
1. RNA Virus > DNA Virus
2. Single-strand > double strand
3. Smaller genome > Larger genome
4. Retrovirus (RT) > non-RT



1. The mutation rate of SARS-CoV-2 is estimated to be 8 × 10^-4 subs per site per year.
2. This translates to an 8 in 10,000 chance of mutation for every nucleotide (A-to-U, C-to-G, and so on).
3. Approximately 25 nucleotide changes can be expected throughout its genome after one year of full circulation (about 2 nucleotides/month) → a guideline for reinfection definitive cases
4. For comparison, seasonal flu virus has at least 50 changes a year with only half the genome size
5. Coronaviruses change more slowly than most other RNA viruses, probably because of a 'proofreading' enzyme (exonuclease activity / ExoN on nsp14) that corrects potentially fatal copying mistakes

Sanjuán, R., Domingo-Calap, P. Mechanisms of viral mutation. Cell. Mol. Life Sci. 73, 4433–4448 (2016). https://doi.org/10.1007/s00018-016-2299-6
https://covid-19chronicles.cseas.kyoto-u.ac.jp/post-041-html/
https://nextstrain.org/ncov/global

Genetic diversity of SARS-CoV-2 → "still a baby"



Comparison of genetic diversity between SARS-CoV-2 (left, arrow for clarity) and HIV-1 (right).

Longer lines mean amore distant relationship between two strains, i.e. a more mutated genome.

This juxtaposition with the same scaling illustrates how miniscule the diversity of SARS-CoV-2 has been so far.

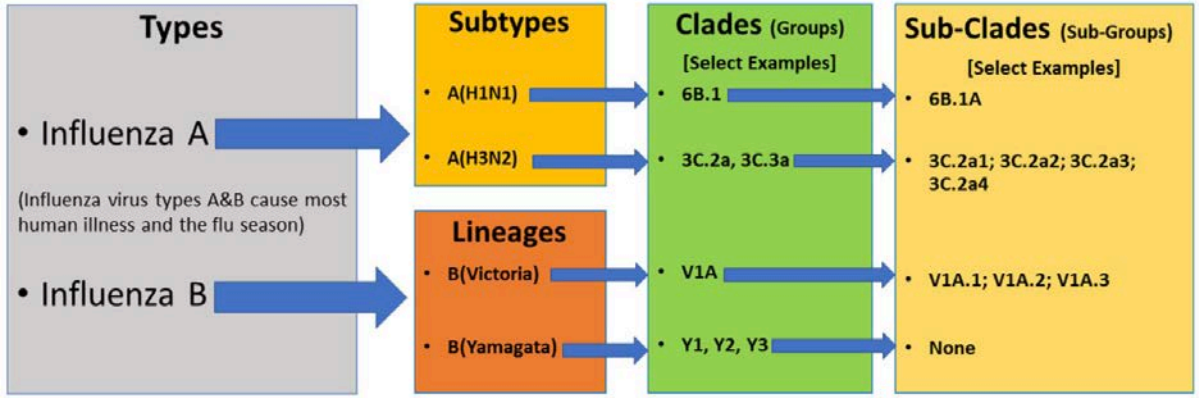
While a vaccine with enormous breadth is necessary to cover all variations of HIV-1, a single vaccine is likely to cover all of SARS-CoV-2.

https://covid-19chronicles.cseas.kyoto-u.ac.jp/post-041-html/?fbclid=IwAR0tfUKLW1kAystKB-CMUZjV6grzIc9tAxzunDf9R8_U4WkkqXMy8qx6o4c

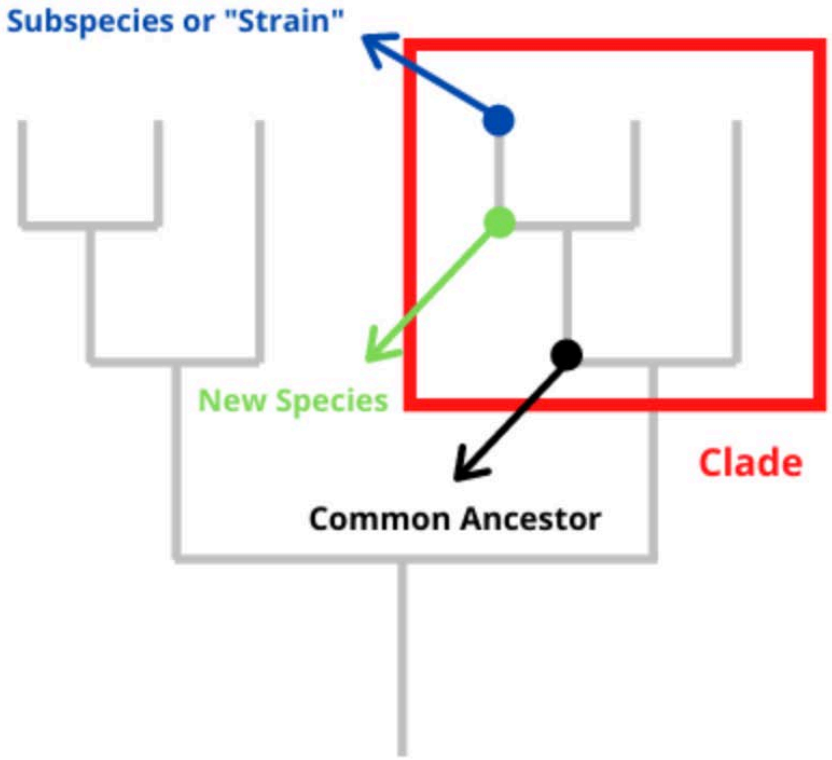
4. Major Clades/Types SARS-CoV-2

Before we jump into mutation/variants of SARS-CoV-2, lets meet Clades

In case of Influenza virus:



In case of SARS-CoV-2, why not call it a strain or a new species? Generally this is because the variation isn't meaningful enough for it to be a new species



Clades can be defined in various ways to show how species and/subspecies relate to one another. The example above shows an ancestral species that splits into two new species which have their own subspecies or "strain".

Mutation vs. Strain vs. Variant

- Although the terms mutation, variant, and strain are often used interchangeably in describing the epidemiology of SARS-CoV-2, the distinctions are important.
- Mutation refers to the actual change in sequence: D614G is an aspartic acid-to-glycine substitution at position 614 of the spike glycoprotein.
- Genomes that differ in sequence are often called variants. This term is somewhat less precise because 2 variants can differ by 1 mutation or many.
- Strictly speaking, a variant is a strain when it has a demonstrably different phenotype (eg, a difference in antigenicity, transmissibility, or virulence).

Lauring AS, Hodcroft EB. Genetic Variants of SARS-CoV-2—What Do They Mean?. JAMA. 2021.

SARS-CoV-2 Nomenclature

No nomenclature for evolutionary lineages of SARS-CoV-2 is universally accepted, but as of January 2021, the World Health Organization is working on "standard nomenclature for SARS-CoV-2 variants that does not reference a geographical location

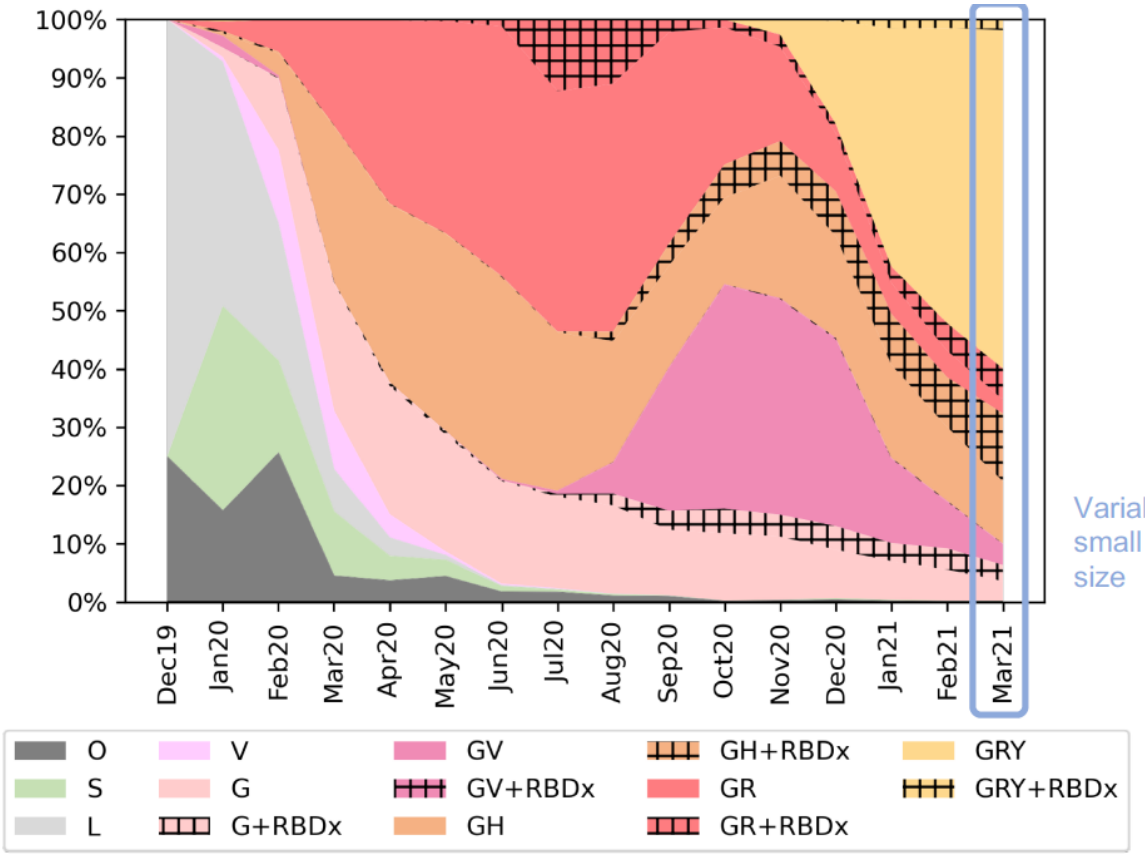
Current Nomenclature System:

- GISAID Clades
- Nextstrain Clades
- Phylogenetic Assignment of Named Global Outbreak Lineage (PANGOLIN)

Notably, the names “variant,” “strain” and “lineage” are all interchangeable terms used by the press and scientific community at this time to describe SARS-CoV-2.

GISAID Clades

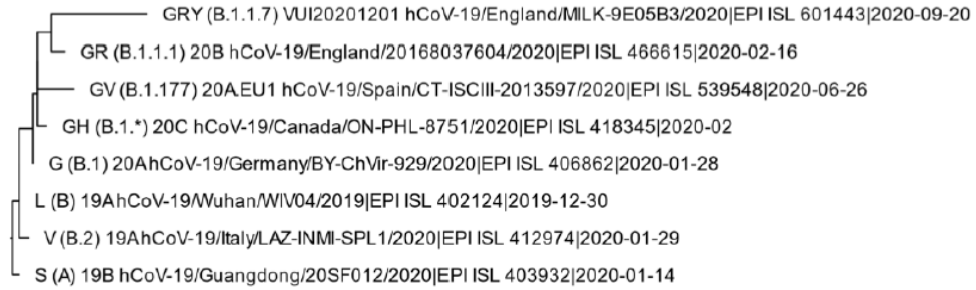
- As of April 2021, GISAID had identified eight global clades (S, O / L, V, G, GH, GR, GV and GRY)



Variable, small sample size

Given the widespread appearance and large numbers of B.1.1.7 genomes globally surpassing numbers of other existing clades, we have elevated the B.1.1.7 lineage (GR/501Y.V1) to its own clade GRY for simple reporting purposes, descending from clade GR with addition of spike markers including N501Y.

Clade references and Pango lineages



Larger Clades in GISAID were named in context of marker variants relative to WIV04-reference:

S	: C8782T, T28144C, NS8-L84S
L	: C241,C3037,A23403,C8782,G11083,G25563,G26144,T28144,G28882 (WIV04-reference)
V	: G11083T,G26144T NSP6-L37F + NS3-G251V
G	: C241T,C3037T,A23403G S-D614G
GH	: C241T,C3037T,A23403G,G25563T S-D614G + NS3-Q57H
GR	: C241T,C3037T,A23403G,G28882A S-D614G + N-G204R
GV	: C241T,C3037T,A23403G, C22227T S-D614G + S-A222V
GRY	: C241T,C3037T,A23403G,G28882A S-D614G includes S-H69del, S-V70del, S-Y144del, S-N501Y + N-G204R

RBDx: *Relevant changes near receptor and antibody binding sites (relative to clade reference)*

We gratefully acknowledge the Authors from Originating and Submitting laboratories of sequence data on which the analysis is based.

GISAID

by BII/GIS, A*STAR Singapore

https://www.gisaid.org/

Nextstrain Clades

- In 2017, Hadfield et al. announced Nextstrain, intended "for real-time tracking of pathogen evolution".
- Nextstrain has later been used for tracking SARS-CoV-2, identifying **12 major clades** (19A, 19B, and 20A–20J):
  - 19A and 19B emerged in Wuhan and have been dominating the early outbreak
  - 20A emerged from 19A out of dominated the European outbreak in March and has since spread globally
  - 20B and 20C are large genetically distinct subclades 20A emerged in early 2020
  - 20D to 20J have emerged over the summer of 2020 and include three "variants of concern" (VOC) with signature mutations S:N501Y.

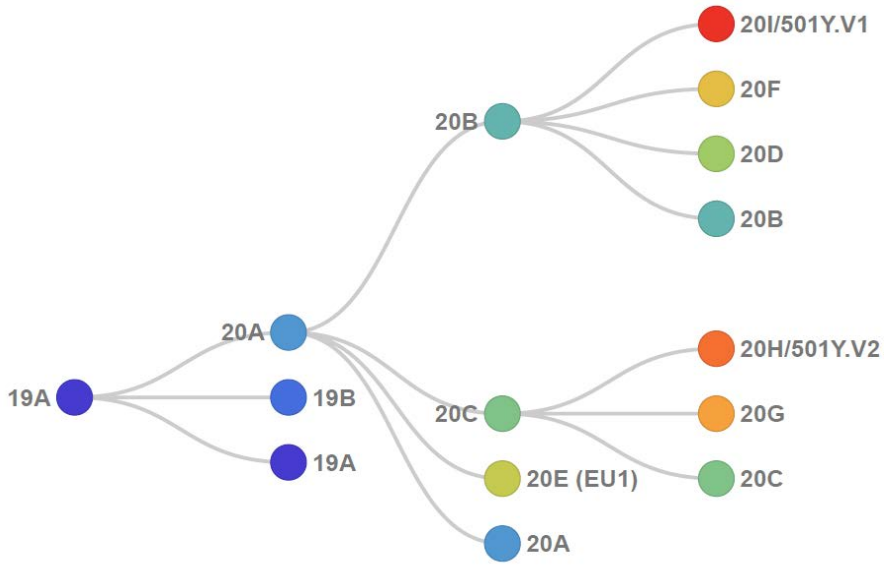
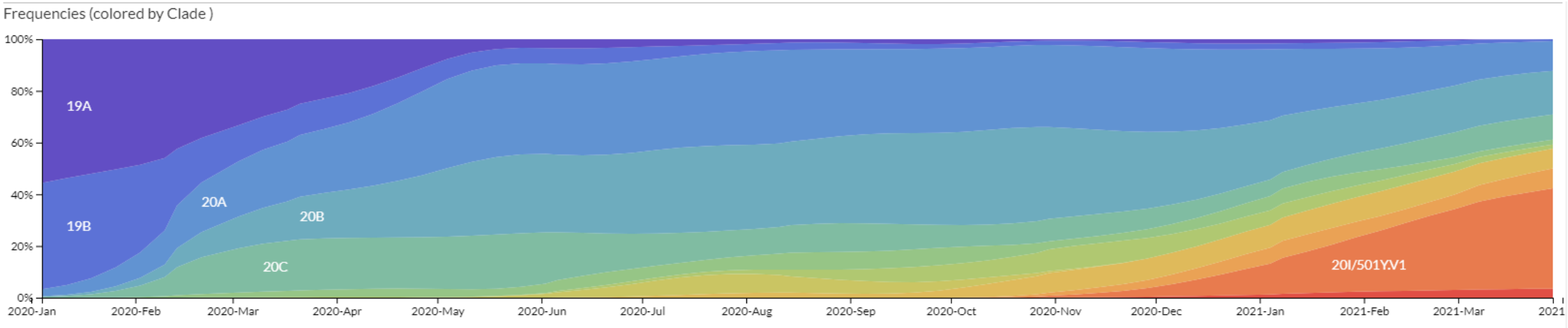
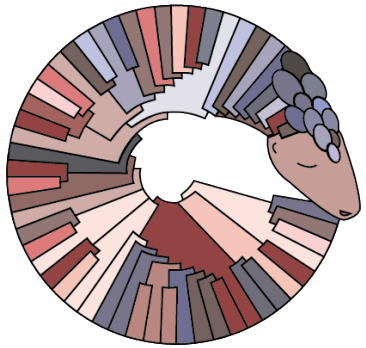


Fig.1. Illustration of phylogenetic relationship of clades, as defined by Nextstrain

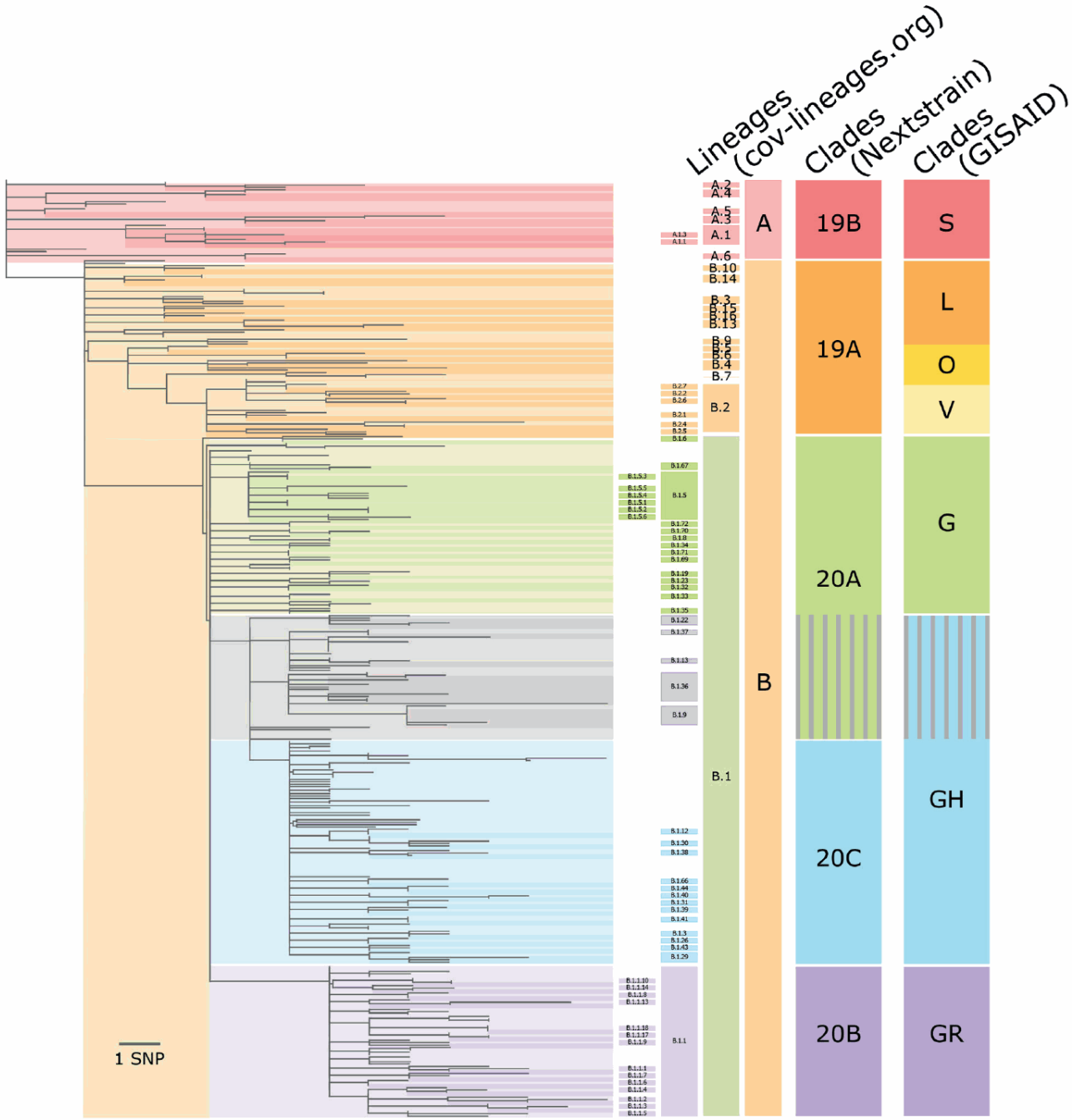


https://clades.nextstrain.org/



PANGOLIN Lineage

- In 2020, Rambaut et al. of the Phylogenetic Assignment of Named Global Outbreak LINeages (PANGOLIN) software team proposed that lineages be used for subtypes of SARS-CoV-2
- As of April 2021, **six major lineages** (A, B, B.1, B.1.1, and B.1.177 and B.1.1.7) had been identified



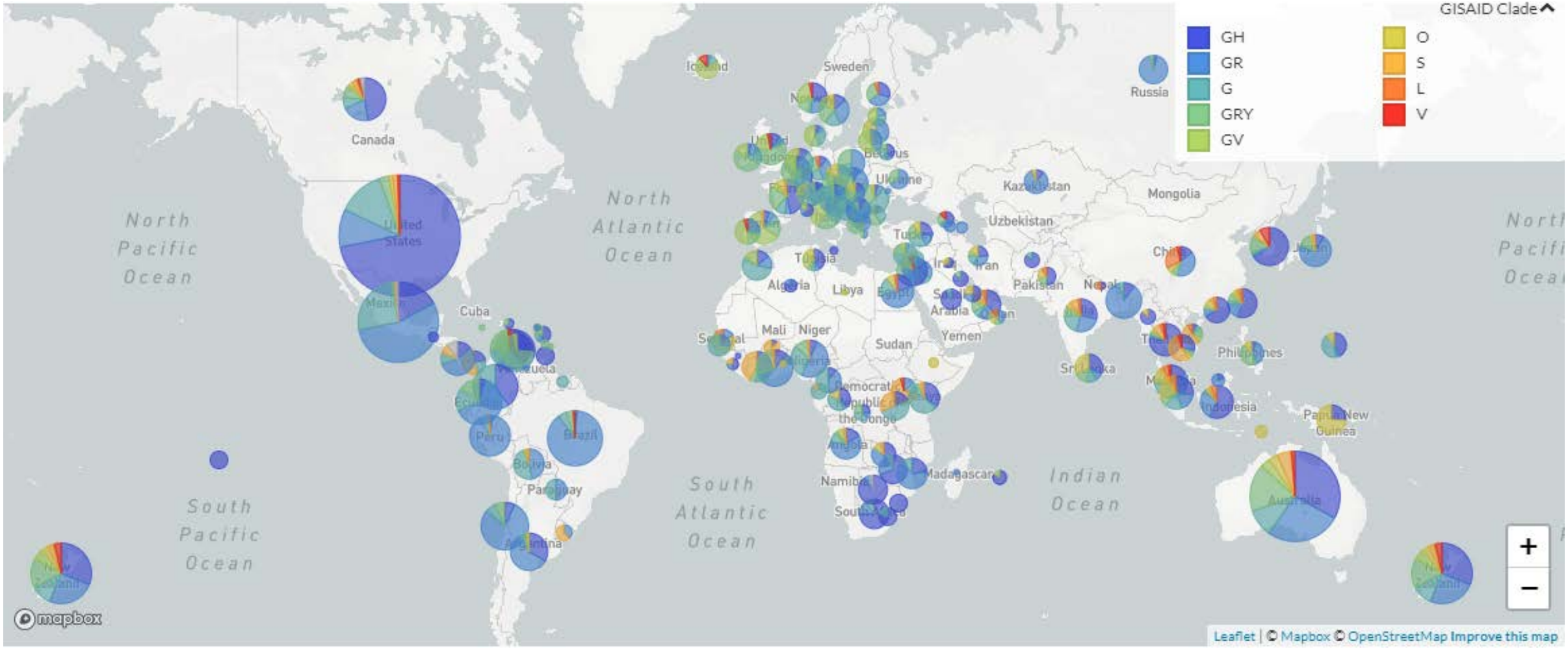
https://cov-lineages.org/pangolin.html

SARS-CoV-2 corresponding nomenclatures

Pangolin Lineages	Notes to Pangolin Lineages	Nextstrain Clades	GISAID clades	Notable variants or mutations
A.1–A.6		19B	S	Base of this lineage also lies in China, with many global exports at the early outbreaks, two distinct **SNPs C8782T and T28144C** define this lineage
B.3–B.7, B.9, B.10, B.13–B.16		19A	L	**Emerged in Wuhan** and have been dominating the early outbreak, **C8782 and T28144** define this lineage
			O	B.4 is probably the primary Iranian outbreak
B.2			V	G11083T,G26144T NSP6-L37F + **NS3-G251V**, comprises Italian outbreak
B.1	B.1.5, B.1.6, B.1.8, other B.1.n that overlap GISAID clade G	20A	**G**	Lineage B.1 in the PANGOLIN system, basal pandemic lineage bearing **S-D614G** that's globally distributed
	B.1.9, B.1.13, B.1.22, B.1.36, B.1.37		GH	20A (partial) and GH (partial)
	B.1.3, B.1.12, B.1.26, other B.1.n that overlap GISAID clade GH	20C		derived from 20A bearing **ORF3a-Q57H** and ORF1a 265I, also globally distributed
		20G		derived from 20C bearing ORF1b 1653D, ORF3a 172V, N 67S and N 199L, concentrated in the United States
		20H		Derived from 20C bearing S 80A, S 215G, **S-E484K, S-N501Y, S-K417N**, and S 701V. Includes **20H/501Y.V2** a.k.a. **South Africa Variant (B.1.351)**
	B.1.1	20B	GR	Derived from 20A bearing N 203K, **N-G204R** and ORF14 50N, globally distributed
		20D		Derived from 20B bearing ORF1a 1246I and ORF1a 3278S, concentrated in South America, southern Europe and South Africa (**Brazil Lineage or B.1.1.28**)
		20F		Derived from 20B bearing ORF1a 300F and **S S-477N**, concentrated in Australia
		20I		Derived from 20B bearing **S- N501Y**, S-A570D, **S-P681H, S-del 69-70**. ORF8 Q27*. Includes Variant of Concern (VOC) 202012/01 or **20I/501Y.V1** or lineage **B.1.1.7** or **UK variant**
		20J		Is a branch of the B.1.1.28 lineage (**B.1.1.28.1**), contains three mutations in the S-RBD: **K417T, E484K, and N501Y**. a.k.a. **20J/501Y.V3** or **P.1 lineage or Brazil variant**
	B.1.177	20E (EU1)	GV	Derived from 20A bearing N 220V, ORF10 30L, ORF14 67F and **S-A222V**, concentrated in Europe

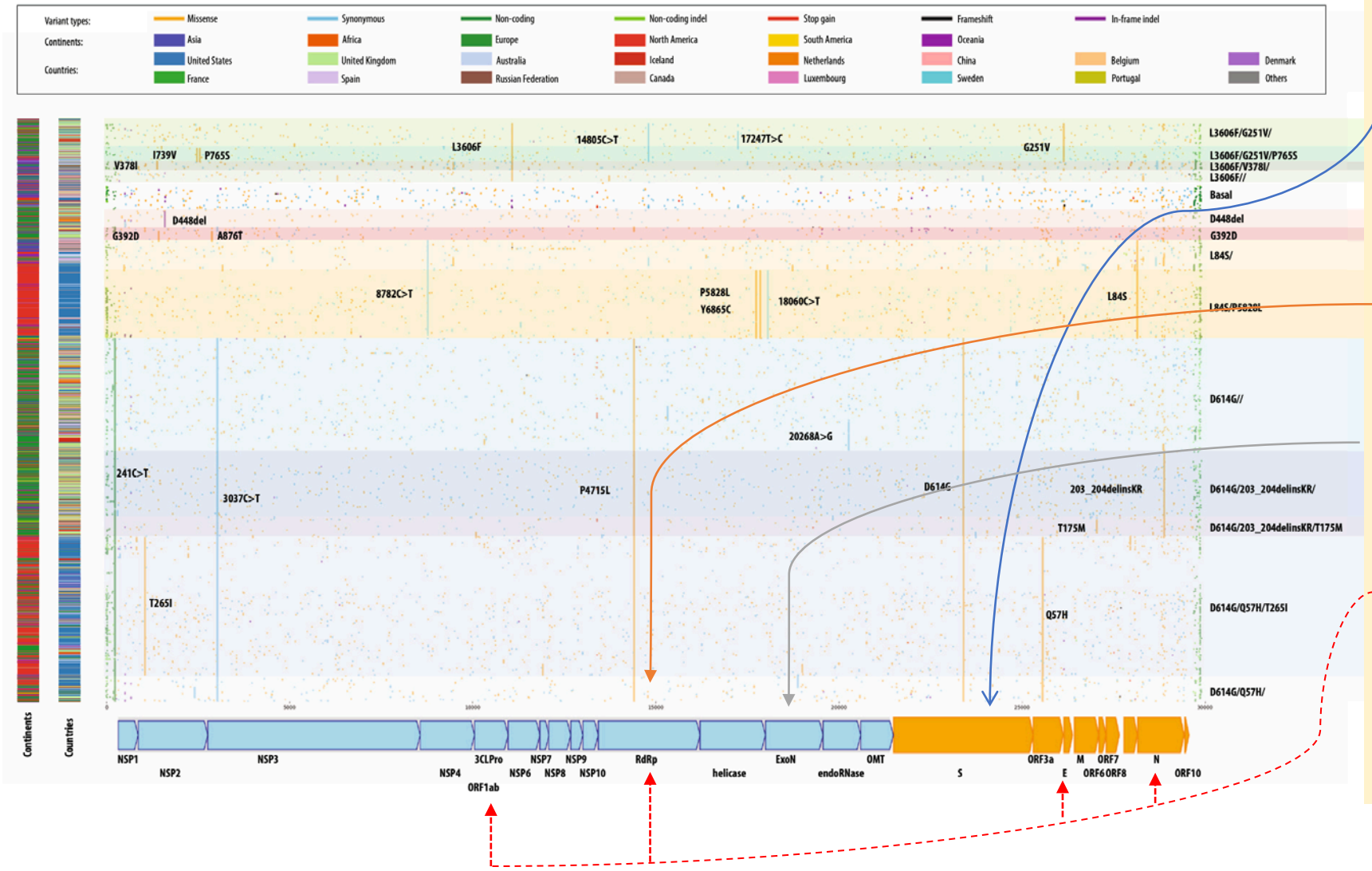
Source: GISAID, Nextstrain, PANGOLIN, ECDC, CDC, Virological, genome-euro.ucsc

Geographic distribution of the genetic variants of SARS-CoV-2



Source: GISAID. Available at: https://bit.ly/3qA9nXI Accessed on March 30. 2021

5. The Impact of Mutation

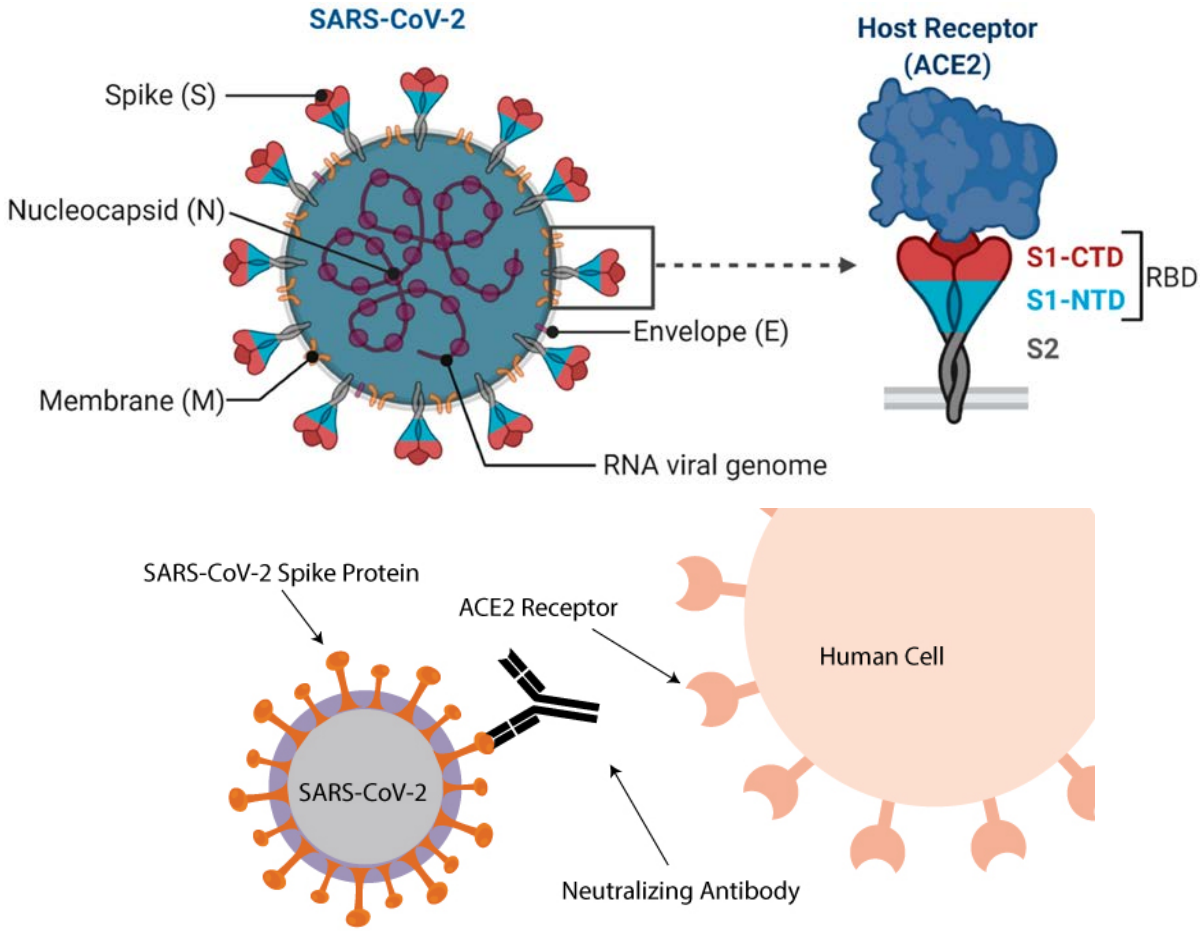


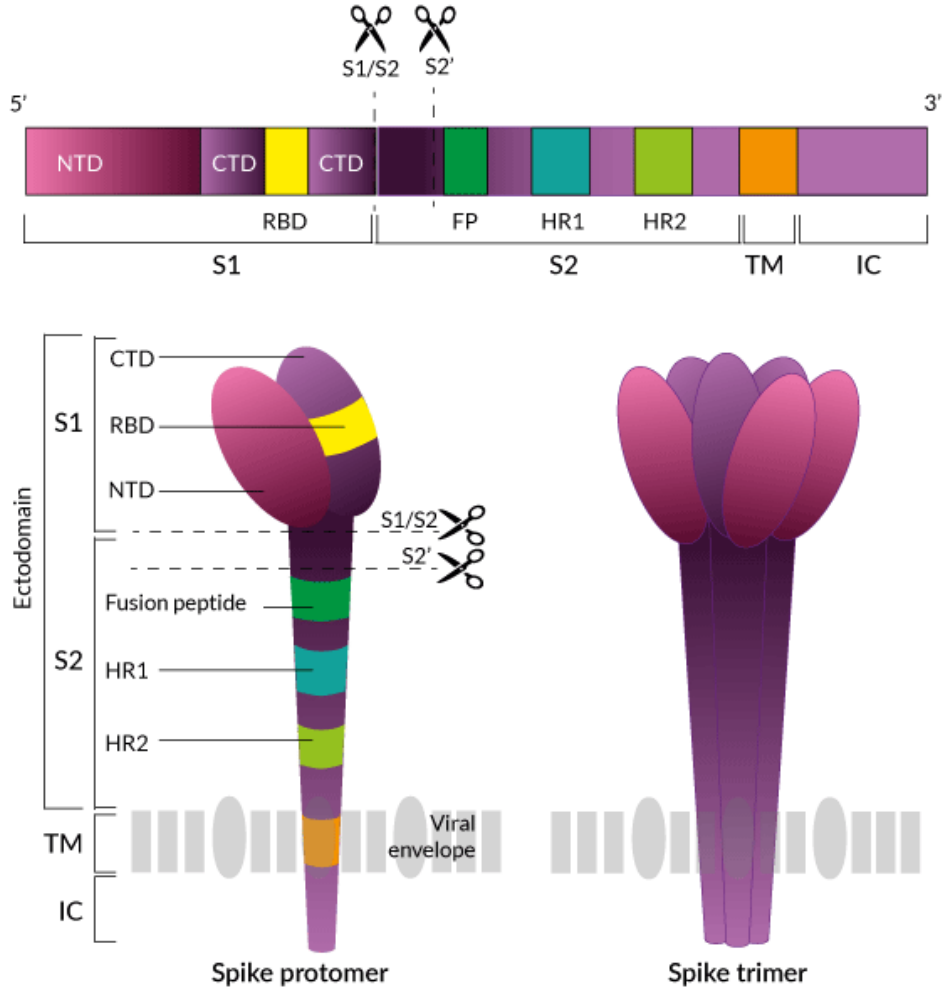
Potential Hot-spot Areas:

1. Mutation on the spike/RBD → impact on viral infectivity and antigenicity → increase infectiousness / vaccine escape?
2. Mutation on RdRP → target of currently used / potential drugs → drug resistance?
3. Mutation on "Proofreading" encoding gene → uncontrolled mutation?
4. Mutation on gene targeted by current PCR assay primer → false negative detection?
5. Other mutation sites → related with mild disease?

Spike Mutation

Site of binding with host-receptor and neutralizing Antibody → hot-spot site for mutation!





https://www.invivogen.com/sars2-spike
Jain S, Batra H, Yadav P, Chand S. COVID-19 Vaccines Currently under Preclinical and Clinical Studies, and Associated Antiviral Immune Response. Vaccines. 2020 Dec;8(4):649.
https://www.epigentek.com/catalog/sars-cov-targeted-assay-kits-lp-41.html

Notable Mutations	Spike Region	Potential effects	Variant Containing Mutation
D614G	S1 subunit - SD2 domain	Enhance infectivity by increasing RBD “up” state and enhances S1/S2 junction proteolysis	Predominant circulating worldwide
N501Y	S1 subunit - RBM	Enhance binding with ACE2 through new forms of hydrogen bond and open conformation of RBD.	B.1.1.7, B.1.135, P.1
E484K	S1 subunit - RBM	• Broadly Immune escape the neutralization by antibodies and human convalescent sera. • Enhance the binding with ACE2.	B.1.135, P.1, P.2, B.1.526, B.1.525, and some B.1.1.7 (not all) India: E484Q with L425R
ΔH69/V70	S1 subunit - NTD	• May increase infectivity and alter Ab recognition. • Cause S-gene target failure (SGTF)	B.1.1.7, B.1.525, cluster 5 ‘mink variant’, some 20A/S:N439K
K417N/T	S1 subunit - RBD	• Immune escape the neutralization by several monoclonal antibodies (included LY-CoV016 and others). • Decrease binding with ACE2.	K417N : B.1.315 K417N/T : P.1
L425R	S1 subunit - RBM	• Immune escape the neutralization by monoclonal antibodies and human convalescent sera. • Enhance the infectivity.	B.1.427, B.1.429 India: L425R with E484Q
P681H	S2 subunit	Potentially affect the furin processes and fusion, which may be important for immune recognition	B.1.1.7
Y453F	S1 subunit - RBM	Immune escape the neutralization by several monoclonal antibodies (REGN10933) and human convalescent sera.	cluster 5 ‘mink variant’
S477N	S1 subunit - RBM	• Broadly resistance to monoclonal antibodies, whereas sensitive to human convalescent sera. • Enhance the binding with ACE2.	Some B.1.526, 20F (Australia)
N439K	S1 subunit - RBM	• Immune escape the neutralization by monoclonal antibodies (REGN10987 and others) and human convalescent sera. • Enhance the binding with ACE2 through new forms a salt bridge with ACE2.	Reported to circulate in Indonesia and Europe (Scotland)
Q677H	S1 subunit - SD2 domain	Still unclear	B.1.525, 20G ("20C-US") clade
A222V	S1 subunit - NTD	No evidence that it spreads faster or affects severity	B.1.177 or 20A.EU1 (GISAID clade GV) “holiday summer travel variant”

Source: https://www.nytimes.com/interactive/2021/health/coronavirus-variant-tracker.html , https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html , https://doi.org/10.3390/ijms22063060 , https://covariants.org/ , https://www.bbc.com/news/world-asia-india-56517495

Mutation on other region than Spike gene

Mutation of the "proofread"

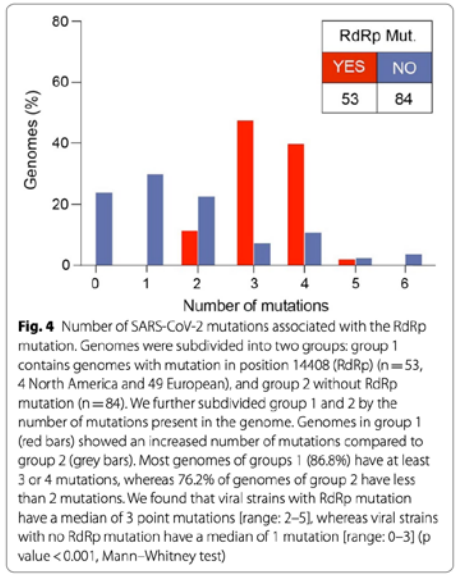


Fig. 4 Number of SARS-CoV-2 mutations associated with the RdRp mutation. Genomes were subdivided into two groups: group 1 contains genomes with mutation in position 14408 (RdRp) (n=53, 4 North America and 49 European), and group 2 without RdRp mutation (n=84). We further subdivided group 1 and 2 by the number of mutations present in the genome. Genomes in group 1 (red bars) showed an increased number of mutations compared to group 2 (grey bars). Most genomes of groups 1 (86.8%) have at least 3 or 4 mutations, whereas 76.2% of genomes of group 2 have less than 2 mutations. We found that viral strains with RdRp mutation have a median of 3 point mutations [range: 2–5], whereas viral strains with no RdRp mutation have a median of 1 mutation [range: 0–3] (p value < 0.001, Mann–Whitney test)

The RdRp mutation, located at position 14408, which is present in European viral genomes starting from February 20th, 2020, is associated with a higher number of point mutations compared to viral genomes from Asia. Given that RdRp works in complex machinery that includes proofreading activities (in cooperation with other viral cofactors, like ExoN, nsp7, and nsp8), it is tempting to speculate that this mutation has contributed in impairing its proofreading capability.

Pachetti M, Marini B, Benedetti F, Giudici F, Mauro E, Storici P, Masciovecchio C, Angeletti S, Ciccozzi M, Gallo RC, Zella D. Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. Journal of Translational Medicine. 2020 Dec;18:1-9.

382-nucleotide deletion (Δ382) in ORF8 region

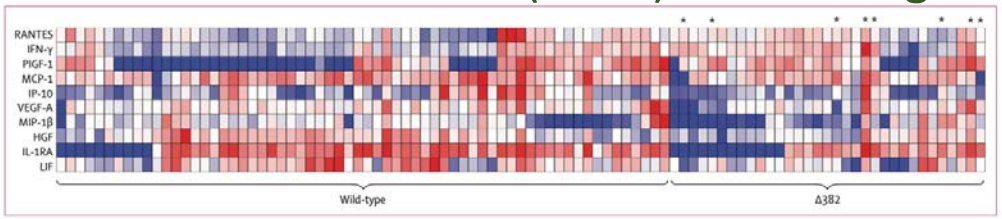
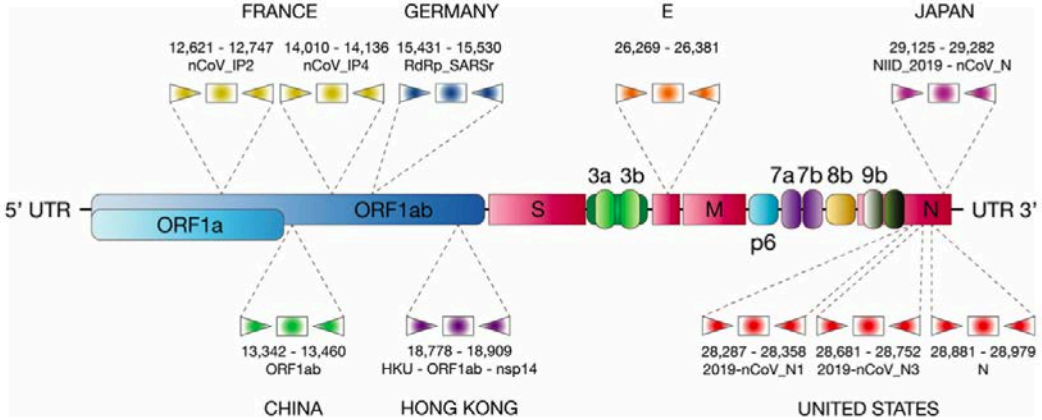


Figure 2: Concentrations of 45 immune mediators quantified using a 45-plex microbead-based immunoassay
Heatmap of immune mediator levels in plasma samples of patients infected with either wild-type (n=64), Δ382 variant (n=25), or mixed wild-type and Δ382 variant severe acute respiratory syndrome coronavirus 2 (n=8; indicated by asterisks in figure) during the first collection timepoint upon hospital admission (median 8 days from symptom onset). Each colour represents the relative concentration of a particular analyte (blue=low concentration; red=high concentration). Δ382=382-nucleotide deletion.

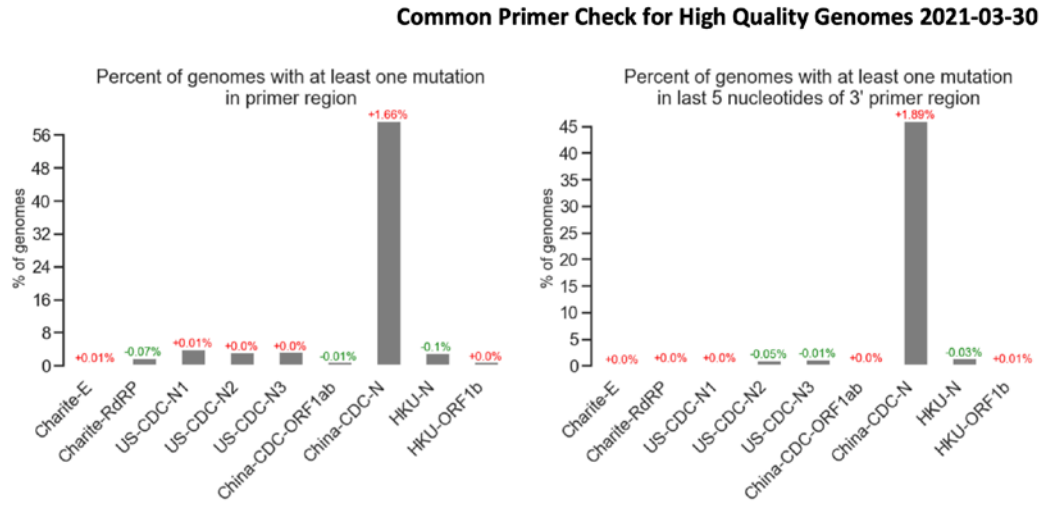
Δ382 variant was first detected in cluster cases Singapore, and was associated with a less severe infection and lower concentrations of proinflammatory cytokines associated with severe COVID-19. Might be less effective at establishing infection in a new host because of the loss of the immune evasion functions of ORF8 → possible target for therapeutic intervention ?

Young BE, Fong SW, Chan YH, Mak TM, Ang LW, Anderson DE, Lee CY, Amrun SN, Lee B, Goh YS, Su YC. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. The Lancet. 2020 Aug 29;396(10251):603-11.

Mutation of the Target Genes by PCR



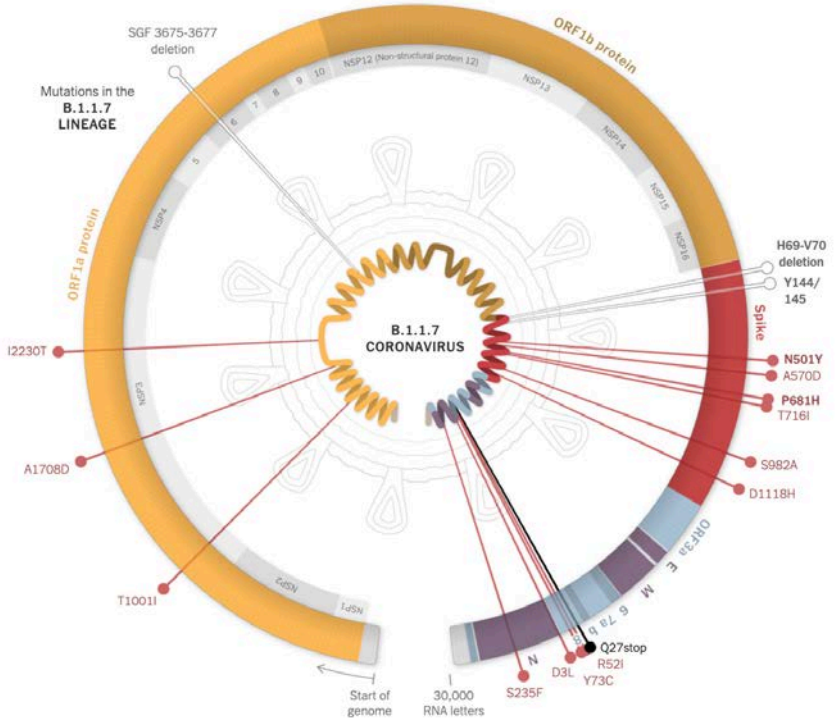
Most of the mismatches observed in primers of SARS-CoV-2 diagnostic assays were not near the 3' end and may be tolerated. Mismatches at the 3' end are known for their deleterious effect on PCR amplification. **May cause false-negative PCR?**

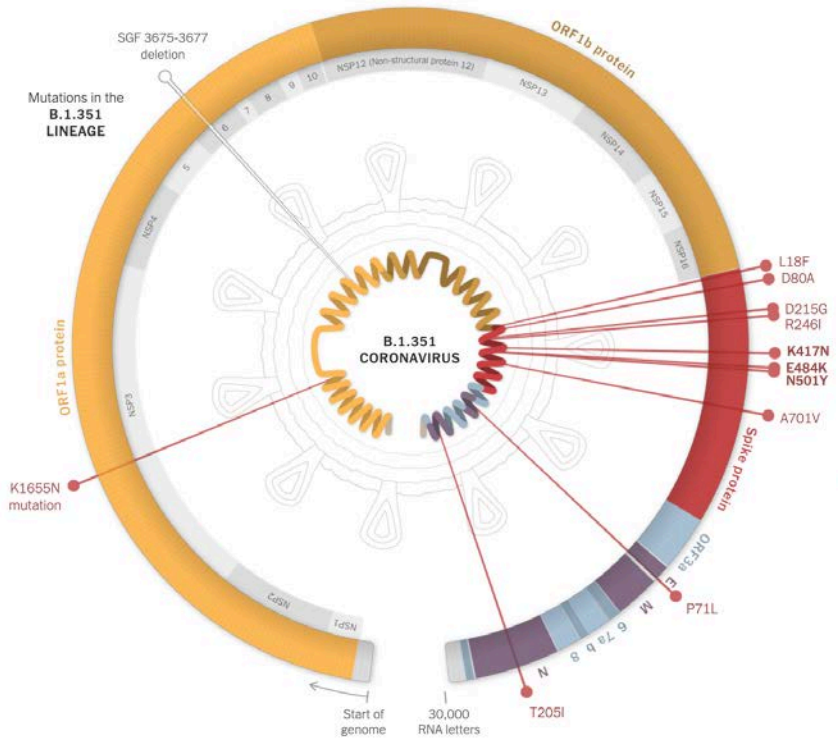


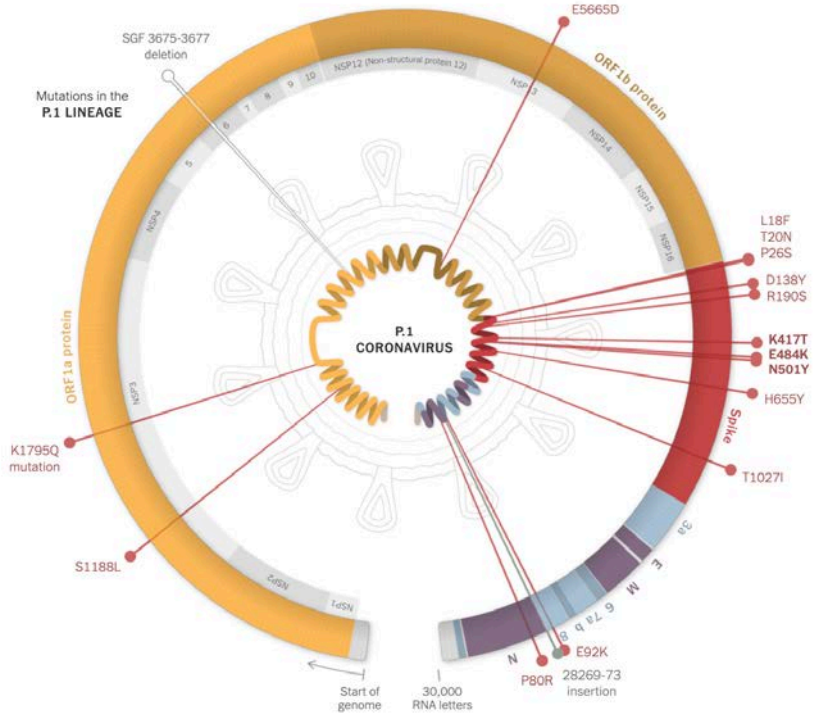
Khan KA, Cheung P. Presence of mismatches between diagnostic PCR assays and coronavirus SARS-CoV-2 genome. Royal Society Open Science. 2020 Jun 10;7(6):200636 and GISAID Database

Mutations of SARS-CoV-2:
current variants of concern

Mutations of SARS-CoV-2 that cause COVID-19 have been observed globally. While some mutations may not have a significant impact, others may provide the virus with a selective advantage, such as increased transmissibility







https://www.nytimes.com/interactive/2021/health/coronavirus-variant-tracker.html
https://www.astrazeneca.com/what-science-can-do/topics/disease-understanding/the-natural-evolution-of-sars-cov-2.html

WHO Working Definition (25 February 2021)

- **Working Definition of "SARS-CoV-2 Variant of Interest":**
  - A SARS-CoV-2 isolate is a variant of interest (VOI) if it is phenotypically changed compared to a reference isolate or has a genome with mutations that lead to amino acid changes associated with established or suspected phenotypic implications[1] ;

  AND
  - has been identified to cause community transmission[2] /multiple COVID-19 cases/clusters, or has been detected in multiple countries;

  OR
  - is otherwise assessed to be a VOI by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group.

- **Working Definition of "SARS-CoV-2 Variant of Concern" :**
  - A VOI (as defined above) is a variant of concern (VOC) if, through a comparative assessment, it has been demonstrated to be associated with
    - Increase in transmissibility or detrimental change in COVID-19 epidemiology;
    - Increase in virulence or change in clinical disease presentation; or
    - Decrease in effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics.

  OR
  - assessed to be a VOC by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group.

[1]Phenotypic changes include changes in the epidemiology, antigenicity, or virulence or changes that have or potentially have a negative impact on available diagnostics, vaccines, therapeutics or public health and social measures. WHO will provide guidance on amino acid changes with established or suspected phenotypic implications, and may be informed by a database on key amino acid changes, or as reported in the scientific literature.
[2]See WHO public health surveillance for COVID-19: interim guidance for definitions

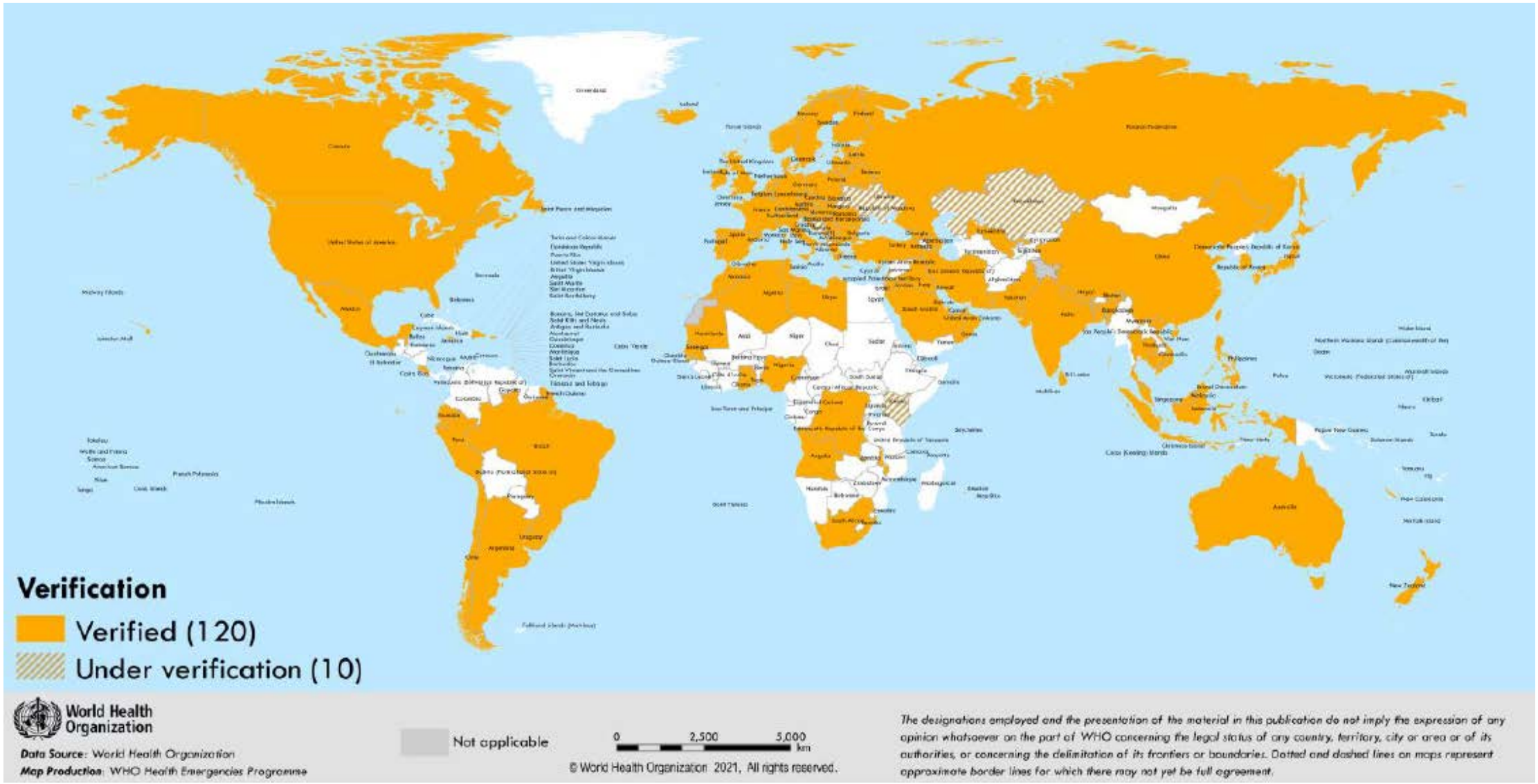
https://www.who.int/publications/m/item/covid-19-weekly-epidemiological-update

VOC Summary (as updated 31 March, 2021)

Pangolin Lineage	B.1.1.7	B.1.351	P.1
Nextstrain Clade	20I/501Y.V1	20H/501.V2	20J/501Y.V3
GISAID Clade	GRY	GH	GR
Alternate names	VOC 202012/01	VOC 202012/02	-
Key Spike Mutation	Δ69/70, Δ144Y, N501Y, A570D, D614G, P681H Some: E484K, S494P	K417N, E484K, N501Y, D614G, L242/A243/L244 deletion	K417N/T, E484K, N501Y, D614G
Key Mutation in common	S106/G107/F108 deletion in Non-Structural Protein 6 (NSP6)		
First Detected	United Kingdom	South Africa	Brazil
Transmission	~50-70% increased transmission	~50% increased transmission	Increased, % unresolved
Lethality	Likely increased severity based on hospitalizations and case fatality rates (~60%)	?	?
Immune evasion	Minimal impact on neutralization by EUA monoclonal antibody therapeutics, convalescent and post-vaccination sera	Moderate impact on neutralization by EUA monoclonal antibody therapeutics, convalescent and post-vaccination sera	Moderate impact on neutralization by EUA monoclonal antibody therapeutics. Reduced neutralization by convalescent and post-vaccination sera
Countries reported	130	80	45

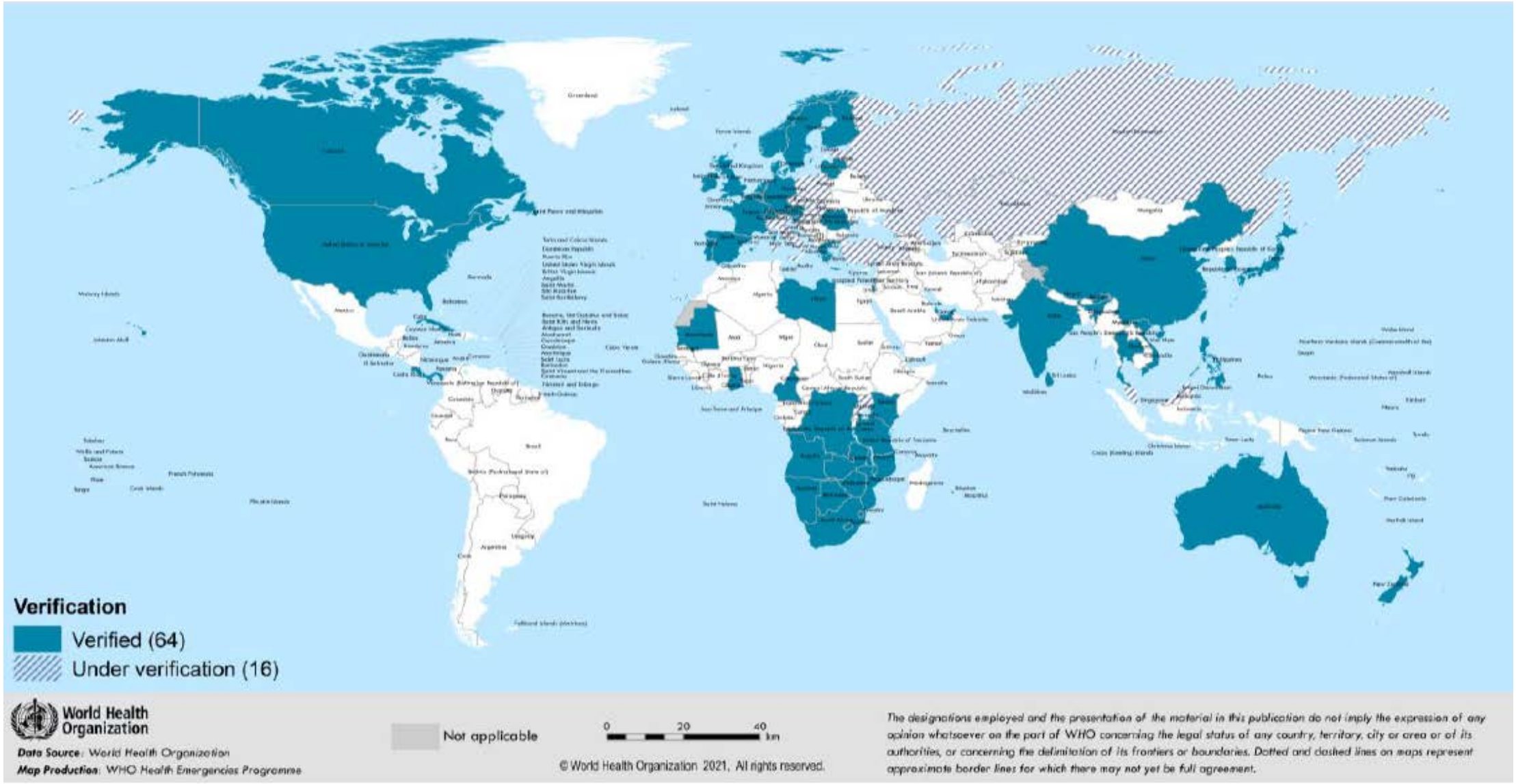
https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---31-march-2021
https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html

Countries, territories and areas reporting SARS-CoV-2 VOC 202012/01 as of 30 March 2021



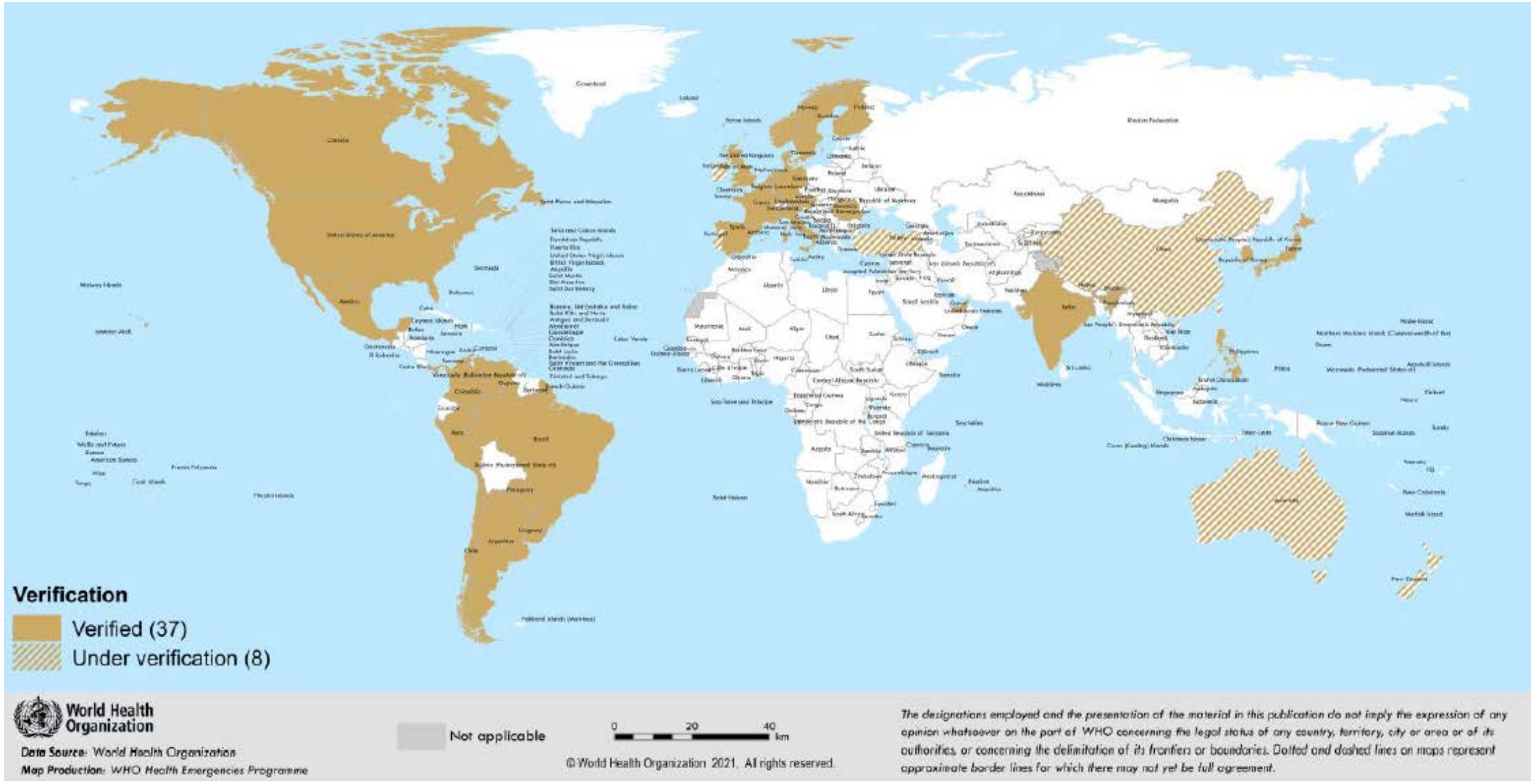
https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---31-march-2021

Countries, territories and areas reporting SARS-CoV-2 variant 501Y.V2 as of 30 March 2021



https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---31-march-2021

Countries, territories and areas reporting SARS-CoV-2 variant P.1 as of 30 March 2021



https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---31-march-2021

Overview of variants of interest (VOIs), as of 30 March 2021

Nextstrain clade	20C	20C/S.452R	20B/S.484K	Not yet assigned	20C	20C
PANGO lineage	B.1.525	B.1.427/B.1.429	B.1.1.28.2, alias P.2	B.1.1.28.3 alias P.3	B.1.526 (with E484K or S477N)	B.1 descendant with 9 mutations
GISAID clade	G/484K.V3	GH/452R.V1	GR	Not yet assigned	GH	GH
Alternate names		CAL.20C/L452R		PHL-B.1.1.28		
First detected by	United Kingdom and Nigeria	United States of America	Brazil	Philippines and Japan	United States of America	France
First appearance	December 2020	June 2020	April 2020	February 2021	November 2020	January 2021
Key spike mutations	H69-V70 deletion; Y144 deletion; Q52R; E484K; Q677H; D614G; and F888L	L452R; W152C; S13I; and D614G	L18F; T20N; P26S; F157L; E484K; D614G; S929I; and V1176F	141-143 deletion; E484K; N501Y; and P681H	L5F; T95I; D253G; D614G; A701V; and E484K or S477N	G142 deletion; D66H; Y144V; D215G; V483A; D614G; H655Y; G669S; Q949R; and N1187D

https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---31-march-2021

Pfizer Vaccine

- They investigated SARS-CoV-2-S pseudoviruses bearing either the Wuhan reference strain or the B.1.1.7 lineage spike protein with sera of 16 participants in a previously reported trial with the mRNA-based COVID-19 vaccine BNT162b2.
- The immune sera had slightly reduced but overall largely preserved neutralizing titers against the B.1.1.7 lineage pseudovirus. These data indicate that the B.1.1.7 lineage will not escape BNT162b2-mediated protection

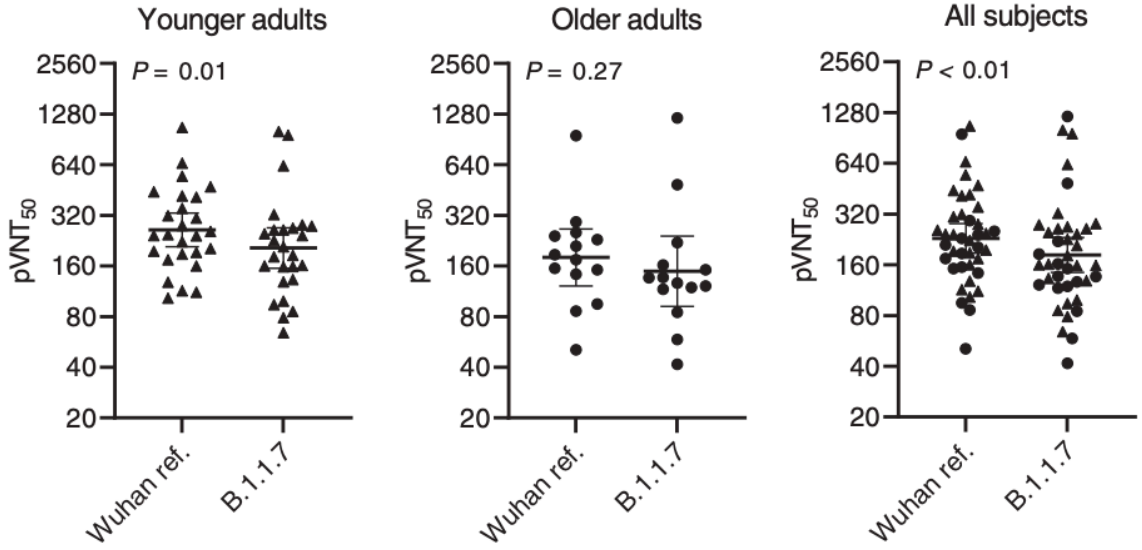
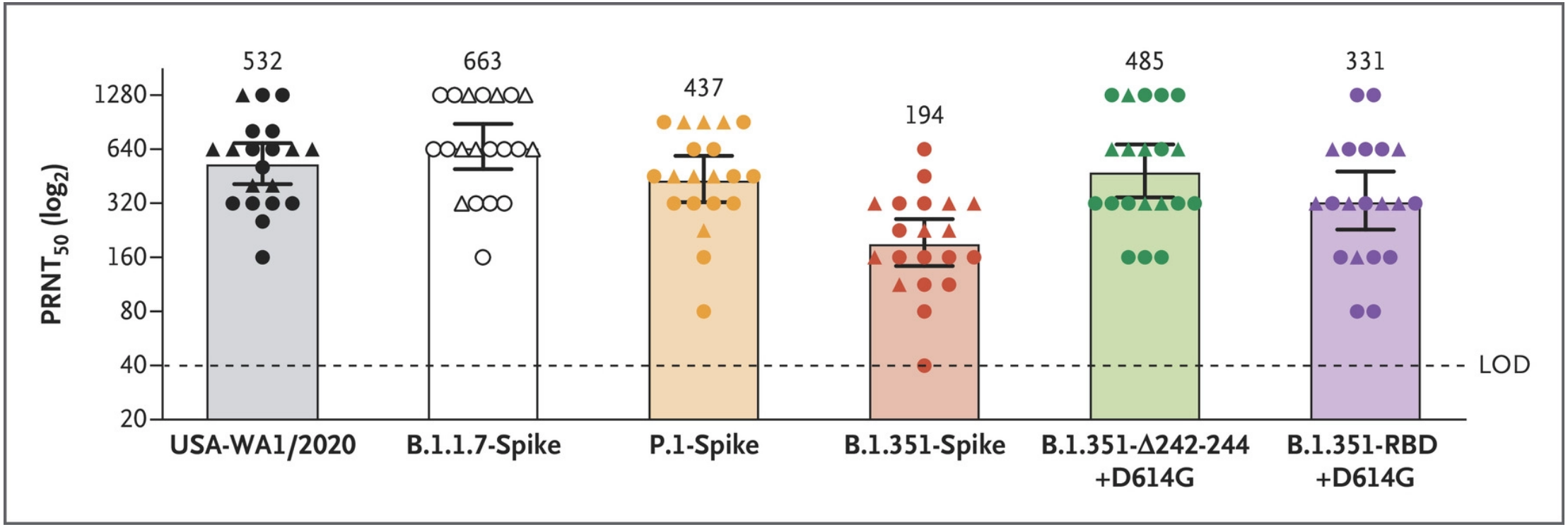


Fig. 1. 50% pseudovirus neutralization titers ($pVNT_{50}$) of 40 sera from BNT162b2 vaccine recipients against VSV-SARS-CoV-2-S pseudovirus bearing the Wuhan reference strain or lineage B.1.1.7 spike protein. Sera from *n* = 26 younger adults (aged 23 to 55 years; indicated by triangles) and *n* = 14 older adults (aged 57 to 73 years; indicated by circles) drawn at either day 29 or day 43 (7 or 21 days after vaccine dose two) were tested. Statistical significance of the difference between the neutralization of the VSV-SARS-CoV-2-S pseudovirus bearing the Wuhan or lineage B.1.1.7 spike protein was calculated by a Wilcoxon matched-pairs signed rank test. Two-tailed *P* values are reported. GMTs and 95% CIs are indicated.

Muik A, Wallisch AK, Sänger B, Swanson KA, Mühl J, Chen W, Cai H, Maurus D, Sarkar R, Türeci Ö, Dormitzer PR. Neutralization of SARS-CoV-2 lineage B. 1.1. 7 pseudovirus by BNT162b2 vaccine–elicited human sera. Science. 2021 Mar 12;371(6534):1152-3.

Special Report of Vaccine Efficacy Against Variant

In this study, they performed 50% plaque reduction neutralization testing ($PRNT_{50}$) using **20 serum samples** that had been obtained from **15 participants** in the pivotal trial[1,2] 2 or 4 weeks after the administration of the second dose of 30 µg of BNT162b2 (which occurred 3 weeks after the first immunization)



As compared with neutralization of USA-WA1/2020, **neutralization of B.1.1.7-spike and P.1-spike viruses was roughly equivalent**, and neutralization of **B.1.351-spike virus was robust but lower.** This fndings also suggest that mutations that result in amino acid substitutions K417N, E484K, and N501Y in the receptor-binding site have a greater effect on neutralization than the 242–244 deletion affecting the N-terminal domain of the spike protein.

Liu Y, Liu J, Xia H, Zhang X, Fontes-Garfias CR, Swanson KA, Cai H, Sarkar R, Chen W, Cutler M, Cooper D. Neutralizing activity of BNT162b2-elicited serum. New England Journal of Medicine. 2021 Mar 8.

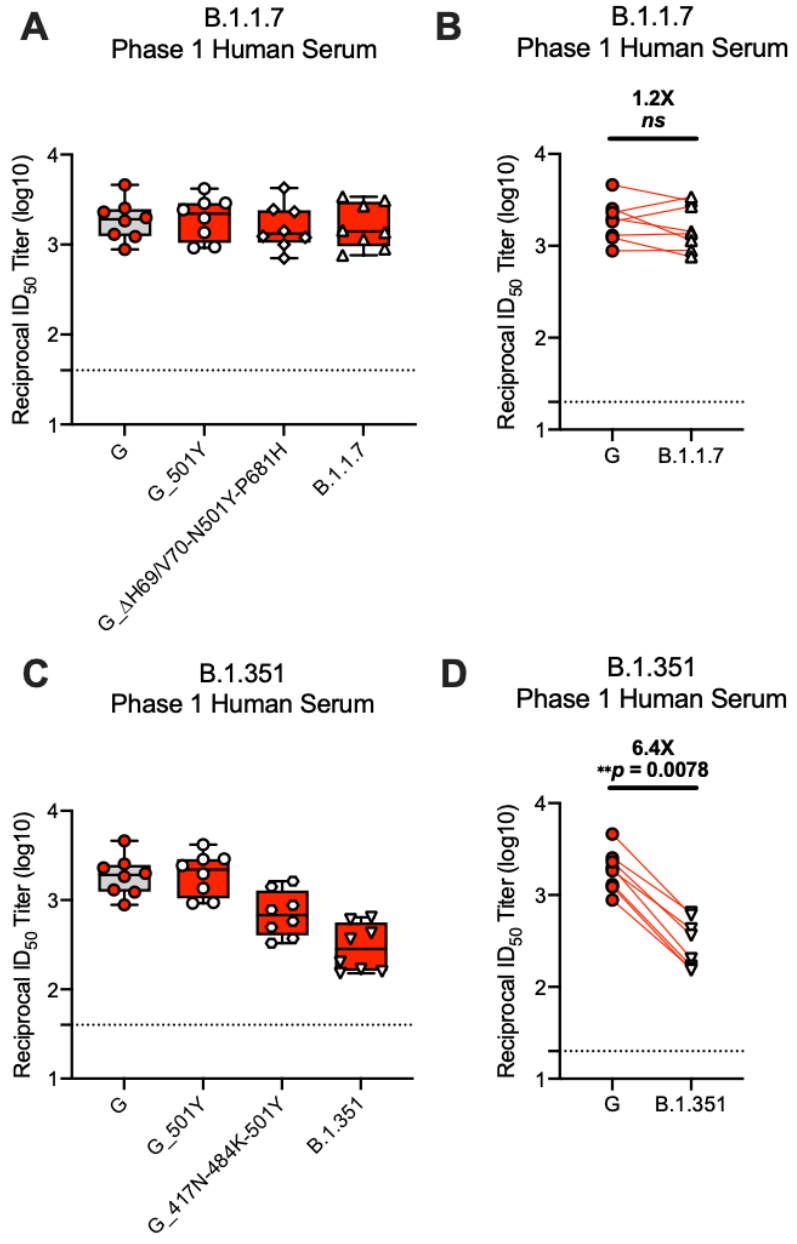
Special Report of Vaccine Efficacy Against Variant

Moderna Vaccine

The in vitro study assessed the ability of mRNA-1273 to elicit potently neutralizing antibodies against the new SARS-CoV-2 variants, using sera from eight Phase 1 clinical trial participants (aged 18-55 years) who received two 100 µg doses of mRNA-1273, and separately using sera from non-human primates (NHPs) immunized with two doses of 30 µg or 100 µg of mRNA-1273.

For the B.1.1.7 variant, neutralizing antibody titers remained high and were generally consistent with neutralizing titers relative to prior variants. No significant impact on neutralization was observed from either the full set of mutations found in the B.1.1.7 variant or from specific key mutations of concern. Although these mutations have been reported to lessen neutralization from convalescent sera and to increase infectivity, sera from the Phase 1 participants and NHPs immunized with mRNA-1273 were able to neutralize the B.1.1.7 variant to the same level as prior variants.

For the B.1.351 variant, vaccination with the Moderna COVID-19 Vaccine produces neutralizing antibody titers that remain above the neutralizing titers that were shown to protect NHPs against wildtype viral challenge. While the Company expects these levels of neutralizing antibodies to be protective, pseudovirus neutralizing antibody titers were approximately 6-fold lower relative to prior variants. These lower titers may suggest a potential risk of earlier waning of immunity to the new B.1.351 strains.



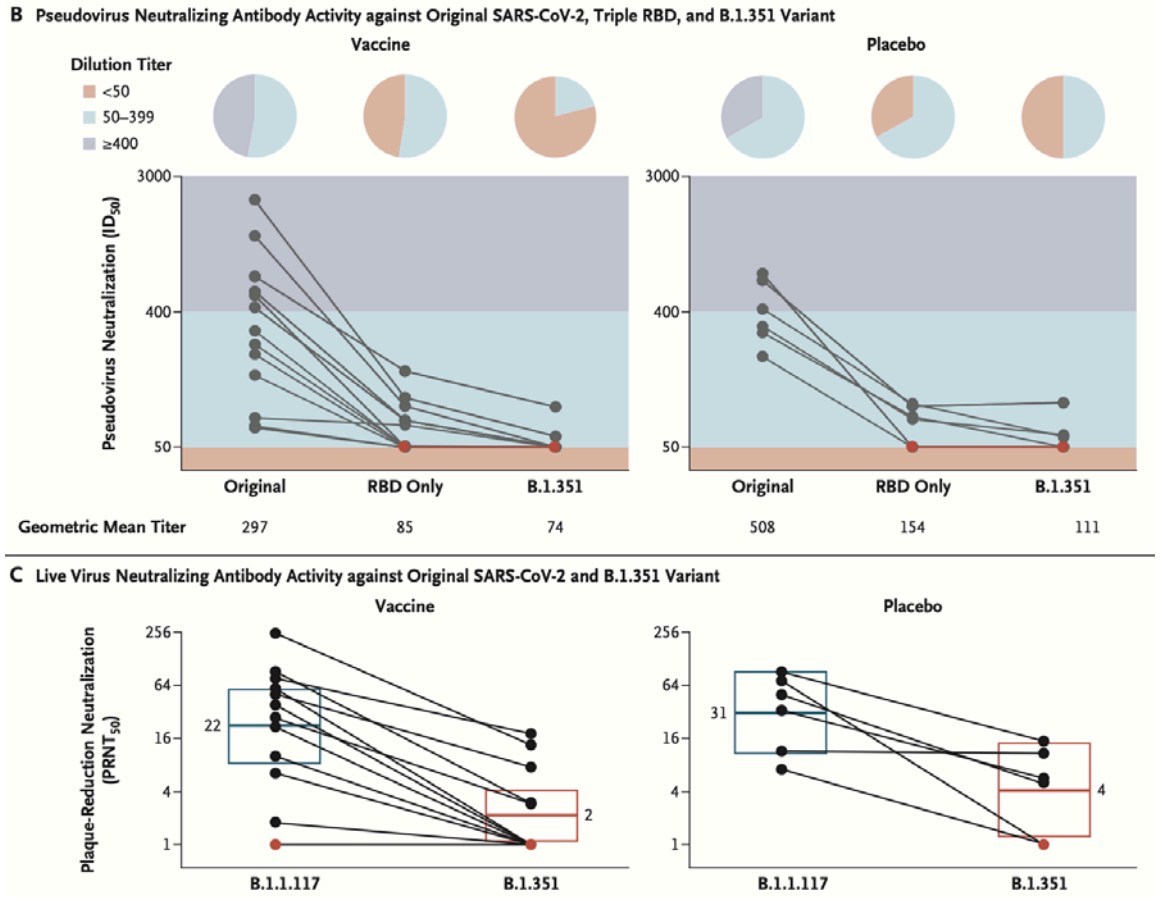
The mutations present in the B.1.1.7 variant, either the full panel of S mutations or key mutations in the RBD region, had minimal effect on neutralization of mRNA-1273 Phase1 participant sera (Figure A-B). In contrast, a significant decrease in neutralizing titers was measured against both the full set of S mutations and the partial list of RBD mutations in the B.1.351 variant. In the VSV assay, using Phase 1 one-week post-boost sera samples, they detected a 2.7- and 6.4-fold reduction in neutralizing titers against the partial or full panel of mutations, respectively

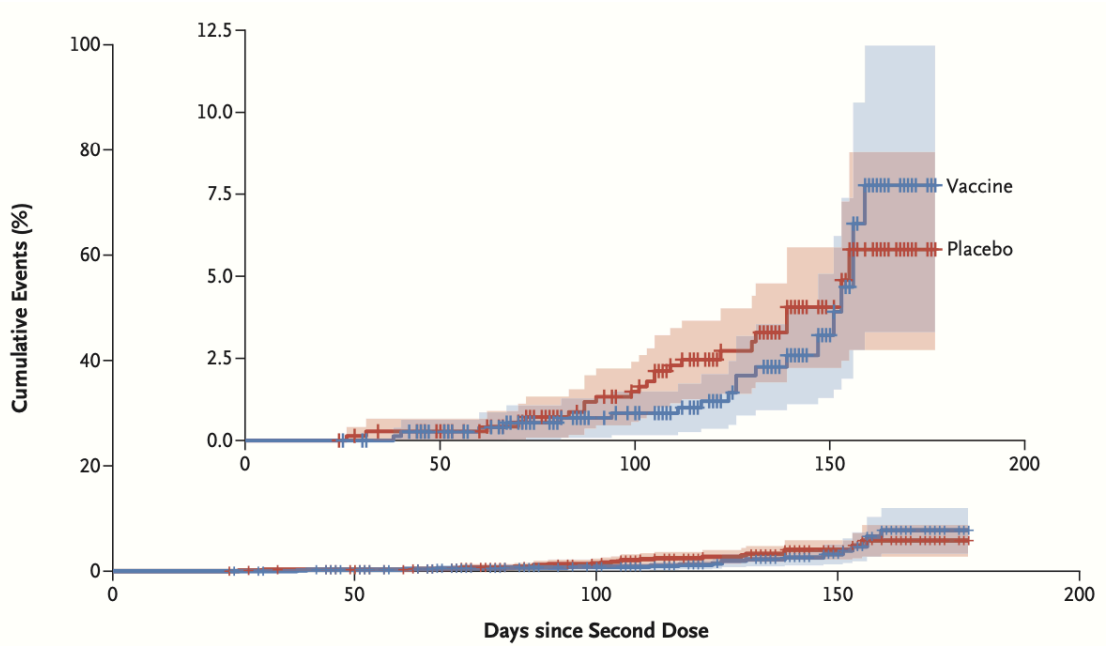
Wu K, Werner AP, Moliva JI, et al. mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. BioRxiv. Preprint posted online January 25, 2021. doi:10.1101/2021.01.25.427948

Special Report of Vaccine Efficacy Against Variant

AstraZeneca Vaccine

AstraZeneca





No. at Risk					
Vaccine	750	738	674	137	0
Placebo	717	707	632	124	0

Cumulative No. of Events					
Vaccine	0	2	6	14	19
Placebo	0	2	10	21	23

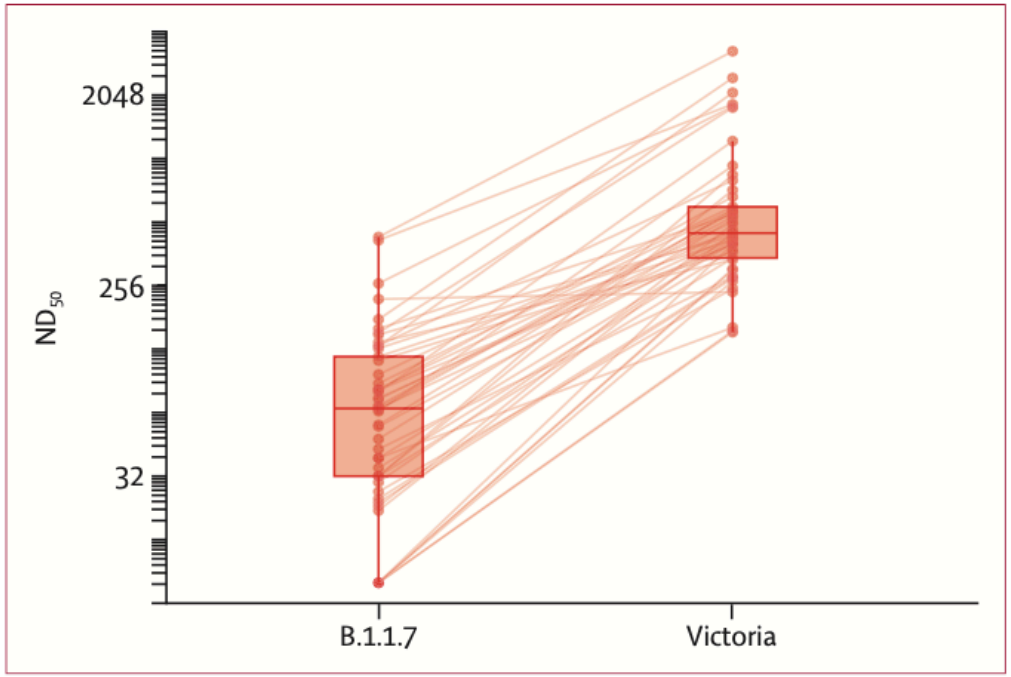
Figure 3. Kaplan–Meyer Plot of ChAdOx1 nCoV-19 Vaccine Efficacy against Symptomatic Covid-19 Illness of Mild or Moderate Severity after Two Doses, as Compared with Placebo.

The shading represents 95% confidence intervals. The tick marks indicate data censored at the time of one of the following events: a Covid-19 infection that did not meet the trial criteria for symptomatic Covid-19 illness, withdrawal from the trial, or death. The inset shows the same data on an expanded y axis.

A two-dose regimen of the ChAdOx1 nCoV-19 vaccine did not show protection against mild-to-moderate Covid-19 due to the B.1.351 variant.

Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L, Padayachee SD, Dheda K, Barnabas SL, Bhorat QE, Briner C. Efficacy of the ChAdOx1 nCoV-19 covid-19 vaccine against the B. 1.351 variant. New England Journal of Medicine. 2021 Mar 16.

Special Report of Vaccine Efficacy Against Variant



AstraZeneca

Figure 6: **Live-virus microneutralisation antibody titres of sera against B.1.1.7 and a canonical non-B.1.1.7 strain (Victoria)**

The geometric mean titre is 58 (95% CI 44–77) for B.1.1.7 and 517 (424–631) for Victoria. The geometric mean ratio (Victoria *vs* B.1.1.7) is 8·9 (95% CI 7·2–11·0). The midlines of the boxes show medians and the outer bounds of the boxes show IQRs. Error bars show the most extreme point within 1·5 × IQR above or below the 75th or 25th percentile. Lines connect samples from the same participant collected at the same trial timepoint (n=49). ND_{50}=titre at which 50% virus neutralisation is achieved.

ChAdOx1 nCoV-19 showed reduced neutralisation activity against the B.1.1.7 variant compared with a non-B.1.1.7 variant in vitro, but the vaccine showed efficacy against the B.1.1.7 variant of SARS-CoV-2

	Cases*	ChAdOx1 nCoV-19 vaccine (n=4244)	Control vaccine (n=4290)	ChAdOx1 nCoV-19 vaccine efficacy (95% CI)
Primary symptomatic COVID-19				
B.1.1.7	52 (19%)	12	40	70·4% (43·6 to 84·5)
Other variants	95 (35%)	15	80	81·5% (67·9 to 89·4)
No sequence result†	30 (11%)	5	25	80·2% (48·3 to 92·4)
Not sequenced‡	92 (34%)	27	65	59·1% (36·0 to 73·9)
Total cases	269	59	210	72·3% (63·1 to 79·3)
Asymptomatic or unknown infection				
B.1.1.7	19 (9%)	8	11	28·9% (–77·1 to 71·4)
Other variants	34 (16%)	8	26	69·7% (33·0 to 86·3)
No sequence result†	64 (31%)	36	28	–27·0% (–108·1 to 22·5)
Not sequenced‡	92 (44%)	45	47	5·6% (–42·3 to 37·3)
Total cases	209	97	112	14·6% (–12·1 to 34·9)
Any NAAT positive infection§				
B.1.1.7	75 (14%)	21	54	61·7% (36·7 to 76·9)
Other variants	144 (28%)	27	117	77·3% (65·4 to 85·0)
No sequence result†	101 (19%)	44	57	23·7% (–13·0 to 48·5)
Not sequenced‡	200 (38%)	81	119	32·9% (11·0 to 49·5)
Total cases	520	173	347	50·9% (41·0 to 59·0)

Data include SD/SD and LD/SD seronegative efficacy cohorts only. NAAT=nucleic acid amplification test. SD=standard dose. LD=low dose. *Data in this column are n (%) or n. †No viable sequence obtained or unprocessed due to cycle threshold >30. ‡Sample did not enter sequencing pipeline, was destroyed, or sequencing results are yet to be obtained. §Includes primary symptomatic cases, non-primary symptomatic cases (those with other symptoms such as nausea or diarrhoea; not shown separately), asymptomatic cases, and cases for which symptoms were unknown.

Table: **Vaccine efficacy against B.1.1.7 and non-B.1.1.7 variants**

Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. Lancet. Mar 30, 2021. https://doi.org/10.1016/S0140-6736(21)00628-0

Special Report of Vaccine Efficacy Against Variant

Sinovac Vaccine

RACE FOR A CURE FEBRUARY 18, 2021 / 12:52 AM / UPDATED A MONTH AGO

Sinovac vaccine works on UK, South African variants - Brazil institute

REUTERS

SERRANA, Brazil (Reuters) - The COVID-19 vaccine developed by China's Sinovac Biotech is effective against the UK and South African variants, the vaccine's Brazilian partner said on Wednesday, citing test results in Chinese trials.

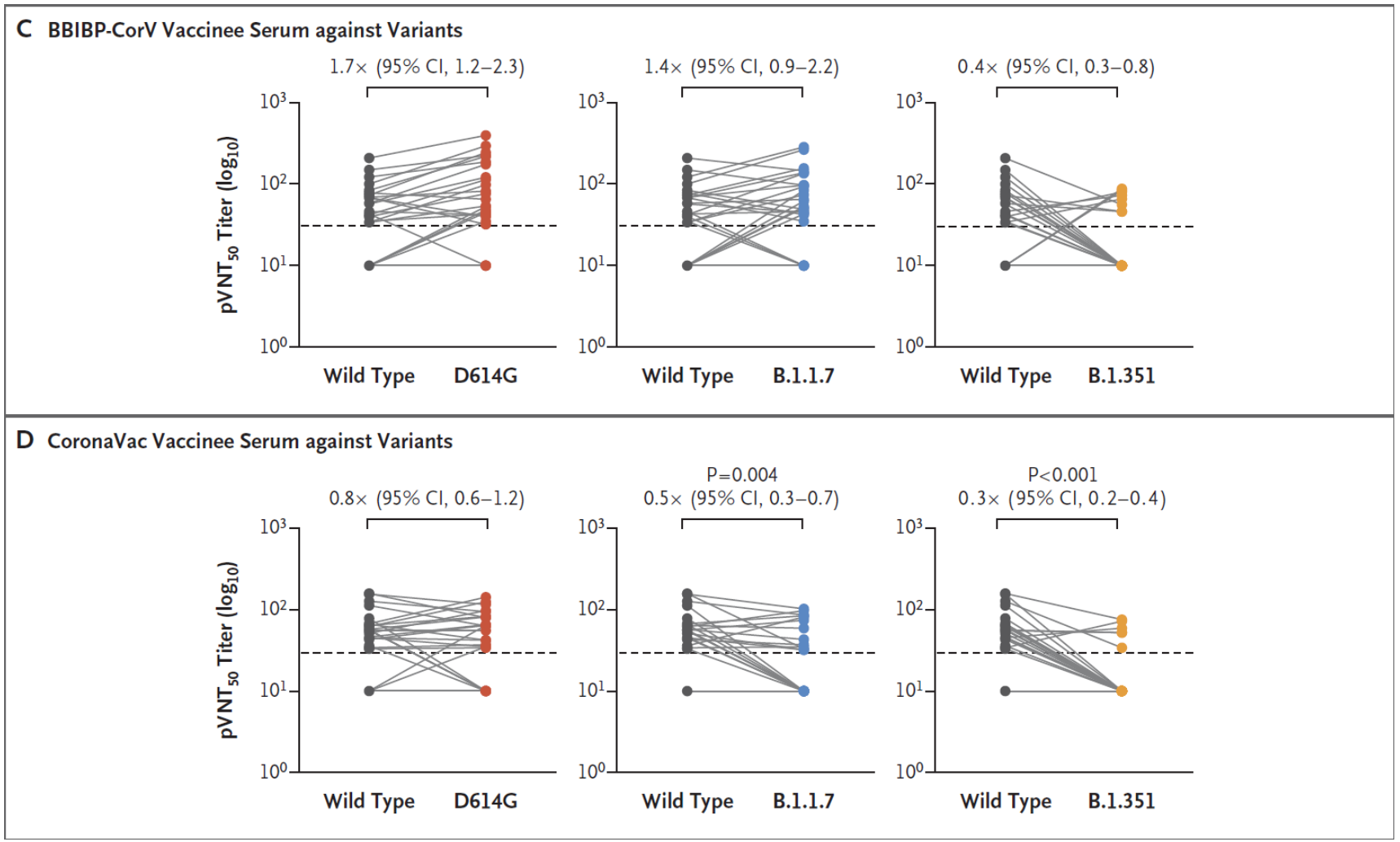
"We have tested this vaccine in China against the English and the South African variants, with good results," said Dimas Covas, head of the Butantan biomedical center in Sao Paulo which lead domestic trials of the Chinese vaccine and is supplying doses to Brazil's Health Ministry.

Covas did not give any more details on exactly how effective the vaccine proved against these strains.

https://www.reuters.com/article/us-health-coronavirus-brazil/sinovac-vaccine-works-on-uk-south-african-variants-brazil-institute-idUSKBN2AH2H0

Special Report of Vaccine Efficacy Against Variant

Sinovac (CoronaVac) and Sinopharm (BBIBP-CorV) Vaccine



Analysis of serum samples after vaccination with BBIBP-CorV or CoronaVac vaccines in China showed neutralizing-antibody titers against the B.1.1.7 variant that were similar to those against the "wild-type" (Wuhan) isolate but were lower against the B.1.351 variant.

- For the BBIBP-CorV vaccinee serum samples, some showed complete or partial loss of neutralization against B.1.351.
- For the CoronaVac vaccinee serum samples, a marked decrease in the GMTs in the serum neutralization of B.1.1.7 (by a factor of 0.5; 95% CI, 0.3 to 0.7) and B.1.351 (by a factor of 0.3; 95% CI, 0.2 to 0.4) was observed

Wang G-L, Wang Z-Y, Duan L-J, Meng Q-C, Jiang M-D, Cao J, et al. Susceptibility of Circulating SARS-CoV-2 Variants to Neutralization. N Engl J Med [Internet]. 2021 Apr 6; Available from: https://doi.org/10.1056/NEJMc2103022

Novavax Vaccine

- The SARS-CoV-2 vaccine produced by the US biotechnology company Novavax is 96.4 % effective against the original variant of SARS-CoV-2 (UK trial) but also provides protection against the newer variants B.1.1.7 (86.3%) in UK phase 3 trial and B.1.351 (48.6%) in South Africa Phase 2b trial. Novavax shows 100% protection against severe disease

NOVAVAX
Creating Tomorrow's Vaccines Today

https://ir.novavax.com/news-releases/news-release-details/novavax-confirms-high-levels-efficacy-against-original-and-0

Johnson & Johnson Vaccine

- This Vaccine Candidates 66% Effective Overall at Preventing Moderate to Severe COVID-19, 28 Days after Vaccination
- The vaccine's efficacy rate dropped from 74.4% in the United States to 52% in South Africa, where 94.5% of viral sequences were from B.1.351 lineage

Johnson & Johnson

Oliver SE, Gargano JW, Scobie H, Wallace M, Hadler SC, Leung J, Blain AE, McClung N, Campos-Outcalt D, Morgan RL, Mbaeyi S. The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Janssen COVID-19 Vaccine—United States, February 2021. Morbidity and Mortality Weekly Report. 2021 Mar 5;70(9):329.

SARS-CoV-2 Variants vs. Vaccines (summary)

Table 1. Summary Results on SARS-CoV-2 Vaccine Trial Efficacy and Viral Neutralization of the B.1.1.7, P.1, and 501Y.V2 Variants, as Compared with Preexisting Variants.*

Vaccine (Company)	Preexisting Variants			Neutralization by Pseudovirion or Live Viral Plaque Assay			Efficacy in Settings with 501Y.V2 Variant
	Sample Size	Efficacy in Preventing Clinical Covid-19	Efficacy in Preventing Severe Covid-19	B.1.1.7 Variant	P.1 Variant	501Y.V2 Variant	
	no.	*% (no. of events with vaccine vs. placebo)*					%
Ad26.COV2.S (Johnson & Johnson)	43,783	66 (NA)	85 (NA)	NA	NA	NA	57†, 85‡
BNT162b2 (Pfizer)	34,922	95 (8 vs. 162)	90 (1 vs. 9)	Decrease by 2×	Decrease by 6.7×	Decrease by ≤6.5×	NA
mRNA-1273 (Moderna)	28,207	94 (11 vs. 185)	100 (0 vs. 30)	Decrease by 1.8×	Decrease by 4.5×	Decrease by ≤8.6×	NA
Sputnik V (Gamaleya)	19,866	92 (16 vs. 62)	100 (0 vs. 20)	NA	NA	NA	NA
AZD1222 (AstraZeneca)	17,177	67 (84 vs. 248)	100 (0 vs. 3)	NA	NA	Decrease by ≤86× to complete immune escape	22§
NVX-CoV2373 (Novavax)	15,000	89 (6 vs. 56)	100 (0 vs. 1)	Decrease by 1.8×	NA	NA	49§
CoronaVac (Sinovac)¶							
Brazil	12,396	51 (NA)	100 (NA)	NA	NA	NA	NA
Turkey	7,371	91 (3 vs. 26)	NA	NA	NA	NA	NA
BBIBP-CorV (Sinopharm)	NA	79 (NA)	NA	NA	NA	Decrease by 1.6×	NA

* Data were available up to March 18, 2021. The definitions of mild, moderate, and severe coronavirus disease 2019 (Covid-19) vary across the vaccine trials. A list of references associated with these vaccines is provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org. NA denotes not available, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.
† Shown is the efficacy of the vaccine, as compared with placebo, against moderate-to-severe Covid-19.
‡ Shown is efficacy of the vaccine, as compared with placebo, against severe Covid-19 and hospitalization.
§ Shown is efficacy of the vaccine, as compared with placebo, against symptomatic Covid-19.
¶ Data are shown separately for the trial sites in Brazil and Turkey.

Abdool Karim SS, de Oliveira T. New SARS-CoV-2 Variants—Clinical, Public Health, and Vaccine Implications. New England Journal of Medicine. 2021 Mar 24.

6. Genomic Surveillance

Main objectives

- Early detection and characterization of emerging variant viruses to define if they are of particular concern;
- Assessing the impact of genetic and antigenic variant viruses for the pandemic and monitoring them over time to guide public health action.

Specific objectives

- Investigating virus transmission dynamics and introductions of novel genetic variants;
- Modelling the antigenic properties of the virus to assess the risk of vaccine escape and selecting viruses for vaccine composition;
- Assessing the impact of mutations on the performance of molecular diagnostic, antigen characterization and serological methods;
- Investigating the relationship between clades/lineages and epidemiological data such as transmissibility and disease severity or risk groups;
- Understanding the impact of response measures on the virus population;
- Assessing relatedness of viral strains within epidemiological clusters and supporting contact tracing and other public health interventions;
- Assessing and confirming reinfections; and Assessing the impact of mutations on the performance of antiviral drugs;
- Monitoring emerging lineages within wild/domestic/farmed animal populations that may impact human health;
- Prompting further basic research investigation to confirm the relevance of observed mutations in the pathogenesis of the disease (e.g., infectivity, receptors binding);
- Assessing the potential incidence of vaccine-derived virus infections and transmissions should live SARS-CoV-2 vaccines become available.

https://www.ecdc.europa.eu/en/publications-data/sequencing-sars-cov-2

Prioritization of Genomic Surveillance

- From individuals vaccinated for SARS-CoV-2 but who later become infected with SARS-CoV-2 despite exhibiting an appropriate immune response to the vaccine;
- In risk settings, such as where there is close human–animal interaction with a large number of animals that are susceptible to SARS-CoV-2 infection, or where there are immunocompromised patients with prolonged shedding, especially when receiving antibody therapy against SARS-CoV-2;
- When there is an unexpected increase or change in SARS-CoV-2 transmissibility and/or virulence;
- When there is suspicion of a change in the performance of diagnostic (antibody, antigen, molecular assays) methods or therapies; and
- During cluster investigations when sequencing can support understanding of transmission events and/or evaluate the efficacy of infection control procedures.

WHO. SARS-CoV-2 genomic sequencing for public health goals: Interim guidance, 8 January 2021. Geneva: WHO; 2021. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-genomic_sequencing-2021.1

7. Genomic Sequencing Effort for SARS-CoV-2 in Indonesia

Genome Sequences per Cases (by Country)

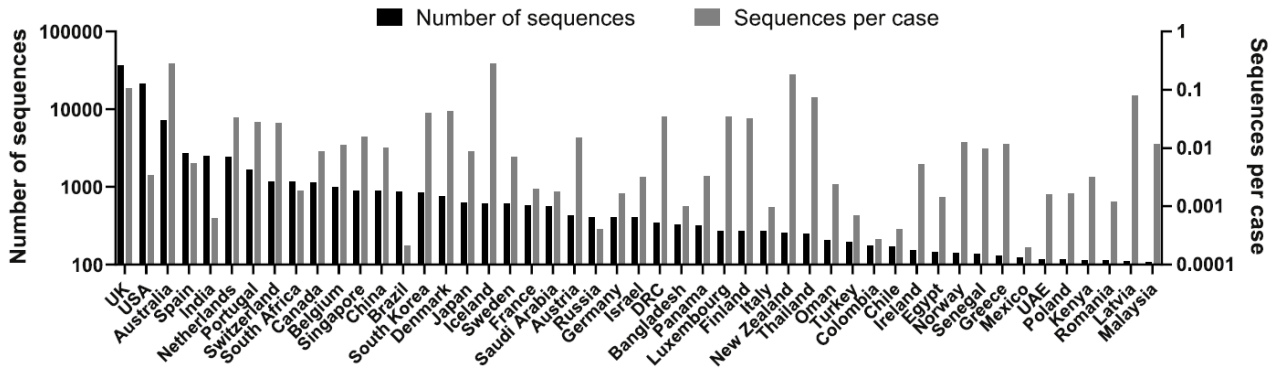


Figure 1. Number of genomic sequences of SARS-CoV-2.
Countries in which more than 100 genomic sequences had been published as of September 2020 are listed in order of the number of sequences. The number of SARS-CoV-2 genomic sequences per reported COVID-19 case in each country is also shown. UK, the United Kingdom; USA, the United States of America; DRC, the Democratic Republic of the Congo; UAE, the United Arab Emirates.

RANK	COUNTRY	REPORTED CASES	SAMPLES SEQUENCED	PERCENTAGE OF CASES SEQUENCED
1	Australia	28,238	16,537	58.6%
2	New Zealand	2,128	1,034	48.6
3	Taiwan	776	137	17.7
4	Denmark	144,047	16,790	11.7
5	Iceland	5,683	601	10.6
6	Gambia	3,791	360	9.5
7	Vietnam	1,421	113	8.0
8	Britain	2,116,609	157,439	7.4
9	Thailand	5,762	343	6.0
10	Japan	207,001	9,599	4.6
43	**United States**	**18,229,260**	**51,212**	**0.3**

Note: Timor-Leste has sequenced 58% of its 33 reported cases.

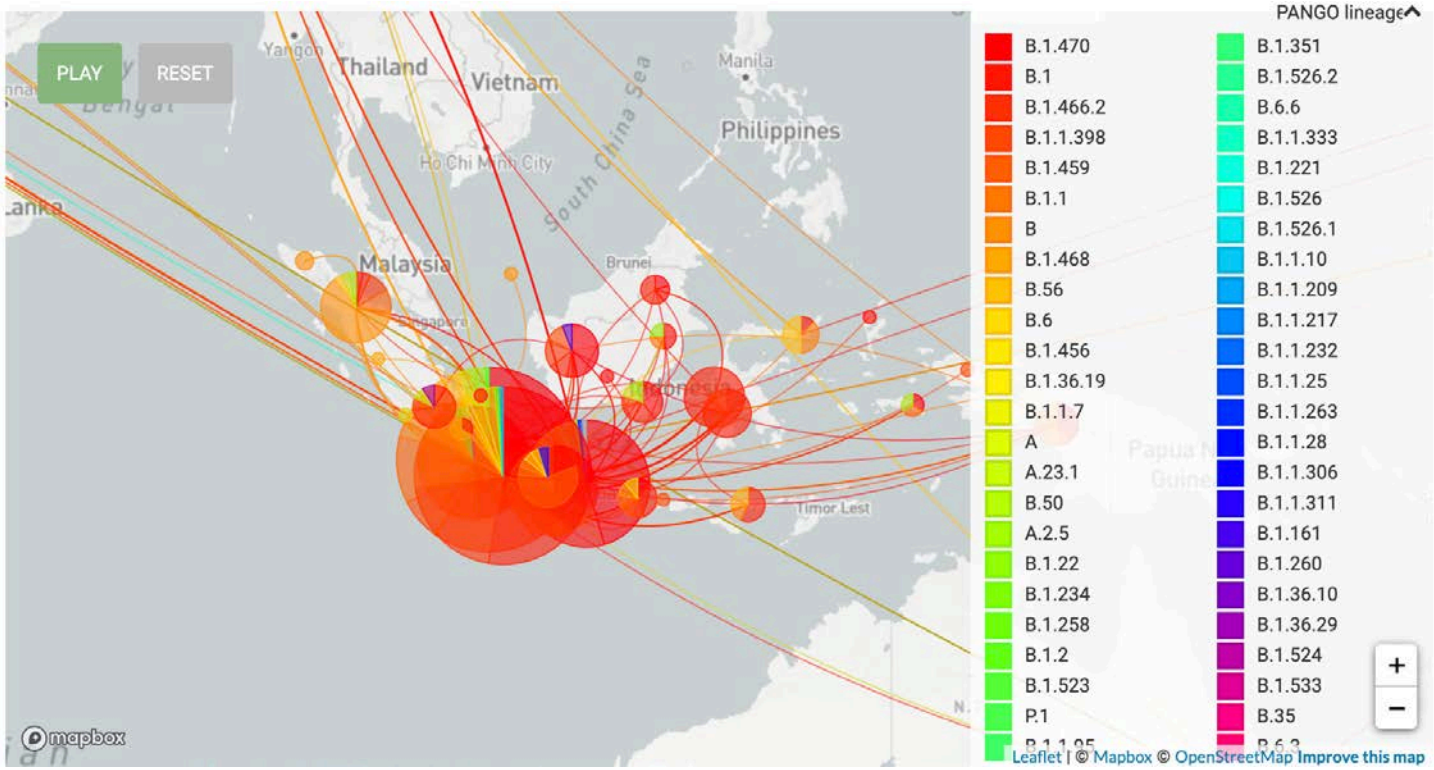
Sources: GISAID Initiative, COVID-19 Genomics UK Consortium, Johns Hopkins University, Post reporting

HARRY STEVENS/THE WASHINGTON POST

As of April 4th, 2021, Total 957 SARS-CoV-2 complete sequences (per 1,534,255 total cases) had been submitted to GISAID → **% genome / case = 0.065%** → **very low** compared with other countries

Furuse Y. Genomic sequencing effort for SARS-CoV-2 by country during the pandemic. International Journal of Infectious Diseases. 2021 Feb 1;103:305-7
https://www.washingtonpost.com/world/2020/12/23/us-leads-world-coronavirus-cases-ranks-43rd-sequencing-check-variants/ .

Phylodynamics of pandemic coronavirus in Indonesia



Updated by the COVID-19 Research and Innovation Consortium (Ristek/BRIN) and enabled by data from GSAID 896 genomes collected between March 2020 and April 2021, last updated 2021-04-01

https://www.gisaid.org/phylodynamics/indonesia/

As of April 4th, 2021,
Total **957** SARS-CoV-2 complete sequences had been submitted to GISAID

GISAID Clades:
GH Clade: 609 viruses
GR Clade: 176 viruses
L/O Clade: 94 viruses
G Clade: 17 viruses
GRY Clade: 10 viruses
S Clade: 5 viruses

Variants:
B.1.1.7 lineage: 10 viruses
B.1.351 lineage: 0 virus
P.1 lineage: 0 virus

Mutations:
Spike D614G: 824 viruses
Spike N439K: 184 viruses
Spike N501Y: 11 viruses
Spike E484K: 1 virus
Spike E484Q: 1 virus

Conclusion

- ❑Mutation is a nature of virus, although SARS-CoV-2 seems to have a sluggish mutation rate compared with other RNA virus
- ❑Mutation can have no effect (mostly), or produce worrisome or even beneficial effect towards clinical course
- ❑Recent studies had reported some mutation may help virus to evade from antibodies, that **may reduce vaccine effectiveness**
- ❑Given the SARS-CoV-2 genome's evolving nature, scientists and drug or vaccine developers should continue to be vigilant for the emergence of new variants or sub-strains of the virus → **Genome surveillance**
- ❑To stop virus mutation → Stop virus transmission! (5M and 3T)


Thank
You