Genetic surveillance for COVID-19 in the light of the novel SARS-CoV-2 variants

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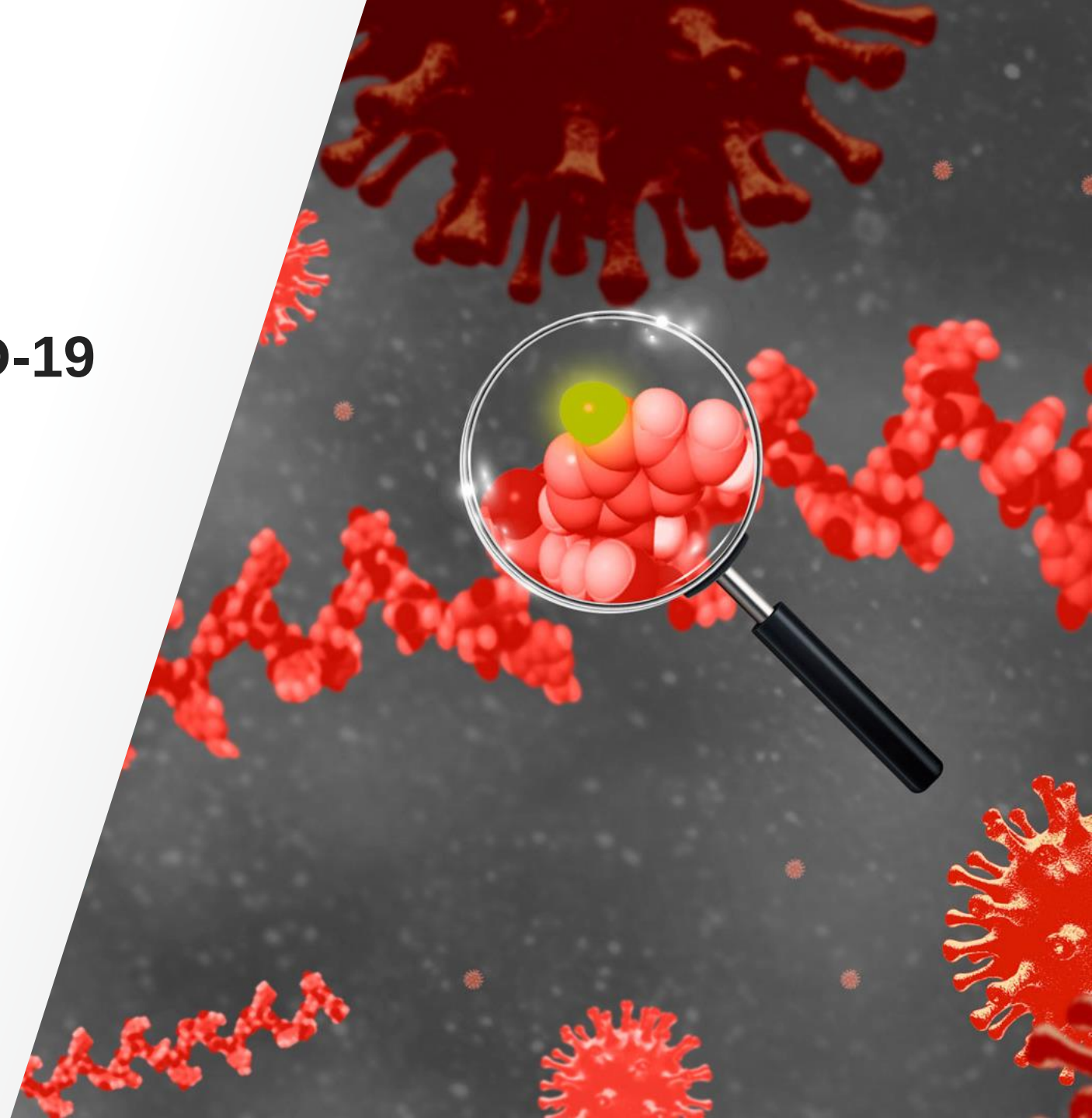
June 16th 2021



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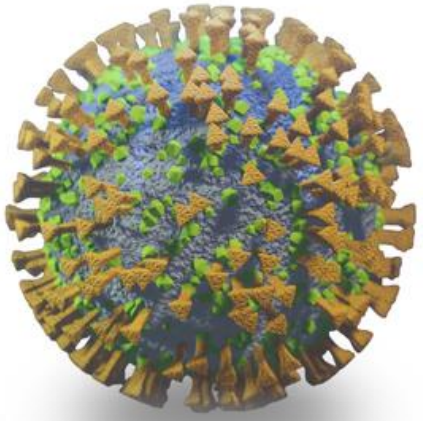
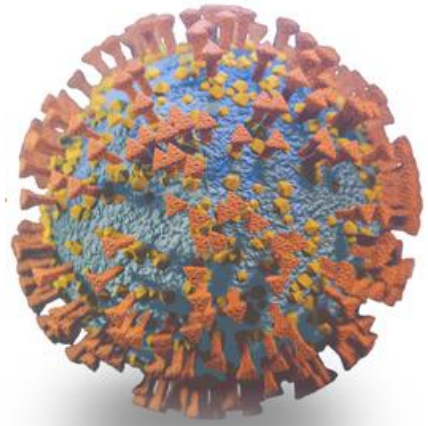
SARS-CoV-2 Viral Mutations

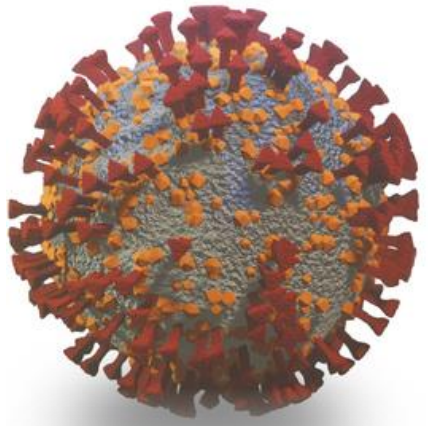
Viruses mutate. RNA viruses, like SARS-CoV-2, mutate at high rates in response to selective pressures



Continued uncontrolled transmission of SARS-CoV-2 in many parts of the world is creating conditions for significant virus evolution



SARS-CoV-2 has been mutating at a rate of about one to two mutations per month*



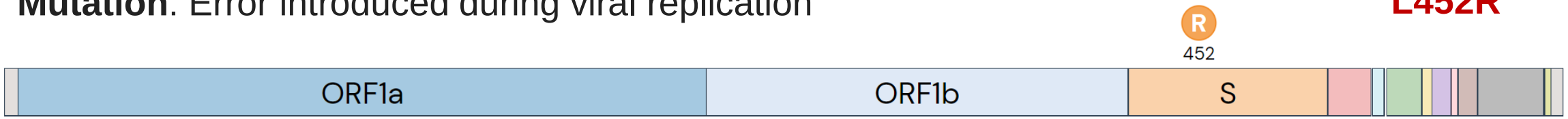
Some recently identified variants, however, have acquired mutations much more rapidly than scientists expected

*Nature https://www.nature.com/articles/d41586-020-02544-6

Mutation vs Variant vs Lineage

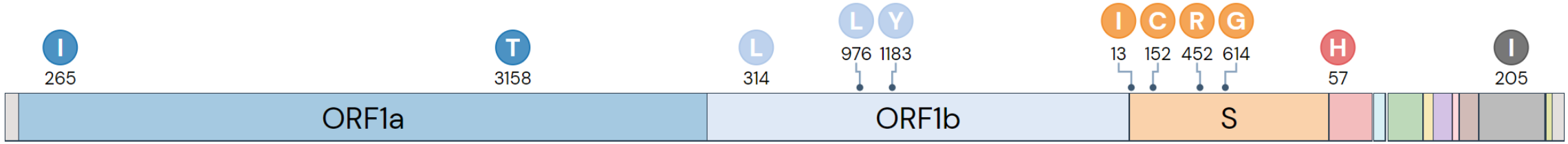
- **Mutation**: Error introduced during viral replication

L452R

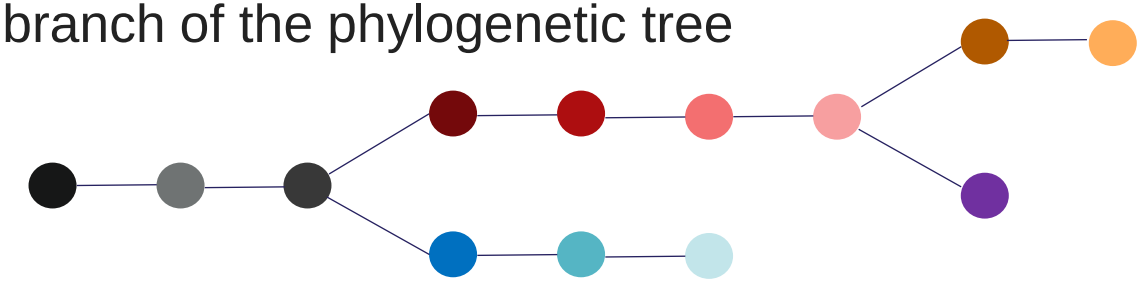


- **Variant**: Genome that contains a particular set of mutations

B.1.427



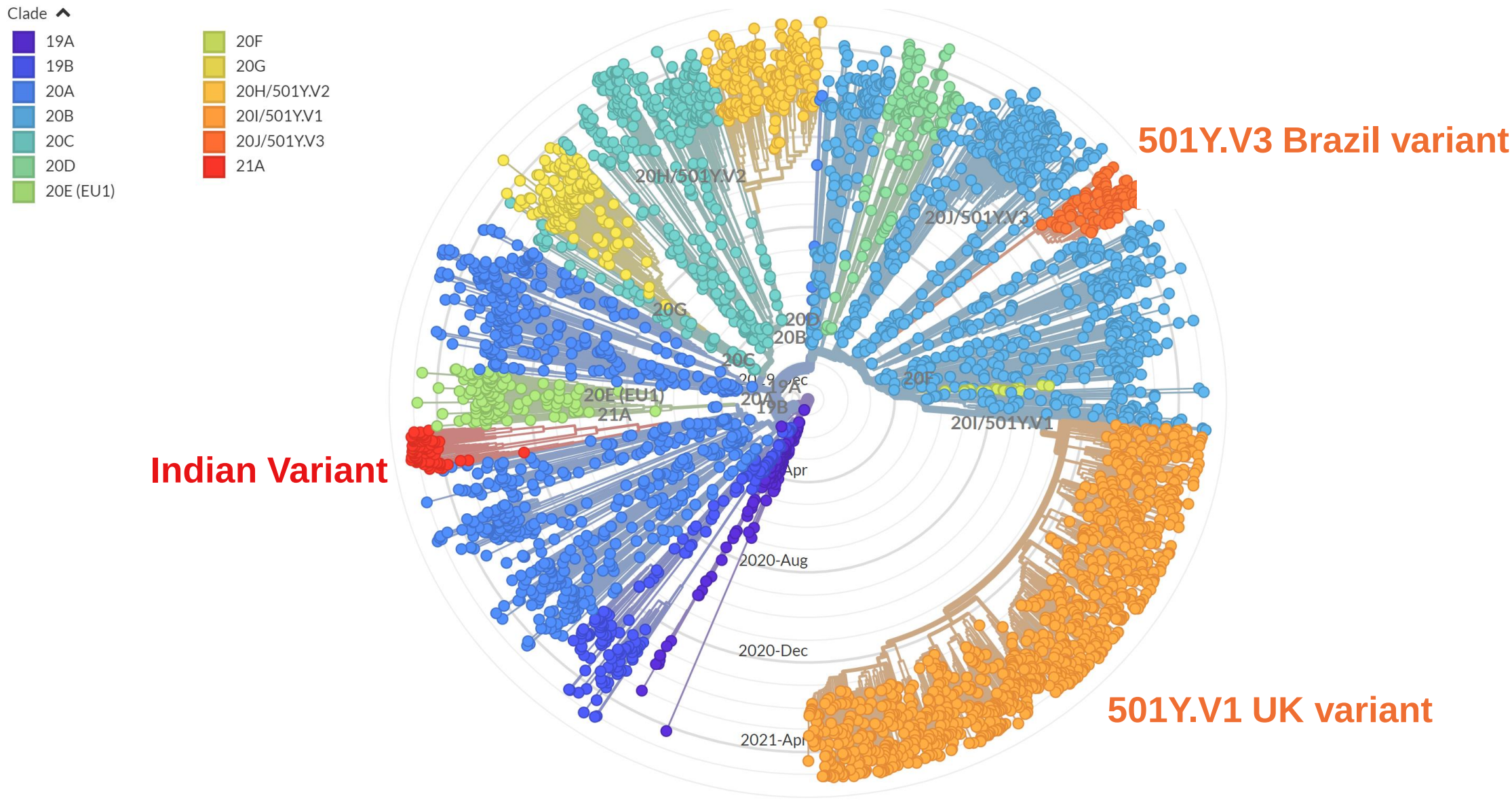
- **Lineage:** All descendants of a branch of the phylogenetic tree



https://outbreak.info/situation-reports/caveats

https://outbreak.info/situation-reports#Lineage_Mutation

Phylogenetic Tree of SARS-CoV-2



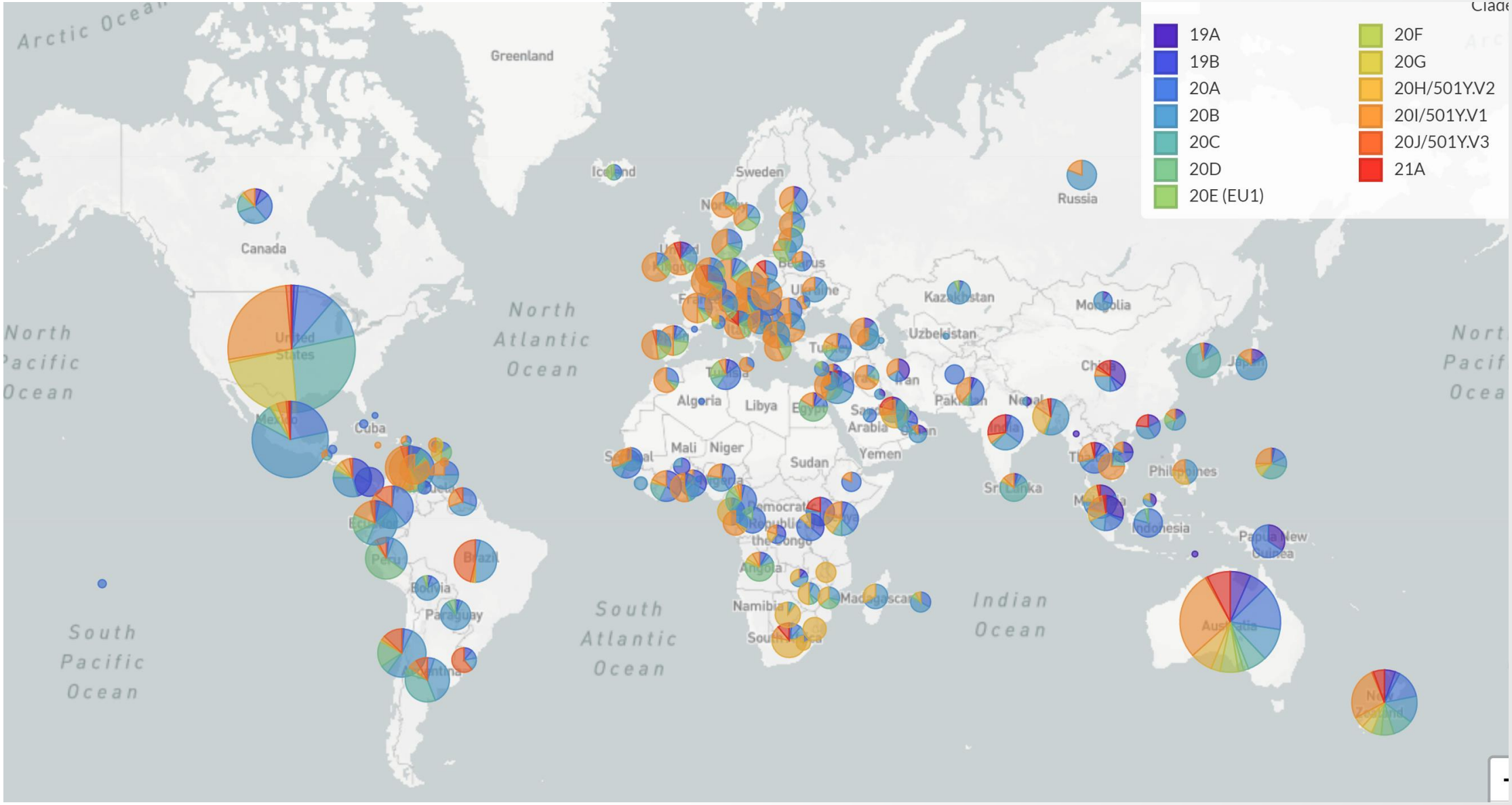
501Y.V3 Brazil variant

Indian Variant

501Y.V1 UK variant

 https://nextstrain.org/ncov/global

Worldwide Distribution of SARS-CoV-2 Variants

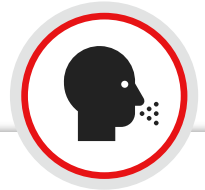


https://nextstrain.org/ncov/global

Potential Implications of New SARS-CoV-2 Variants

Variant Impact

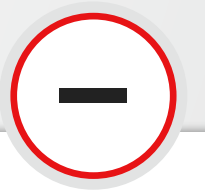
Speed of human to human transmission & increased infectivity



Change disease severity

Susceptibility to therapeutic agents (i.e., monoclonal antibodies, drugs)

Evade vaccine-induced and/or natural immunity



Impact detection by diagnostic tests

Variant of Interest (VOI)

Variant of Concern (VOC)

Variant of High Consequence (VHC)

US CDC https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html

https://asm.org/Articles/2021/February/SARS-CoV-2-Variants-vs-Vaccines

Variants of Concern (VOC) Circulating in the US

ThermoFisher SCIENTIFIC

B.1.1.7 (501Y.V1) – UK variant	P.1 (501Y.V3) – Brazil variant	B.1.351 (501Y.V2) – SA variant	B.1.427 / B.1.429 – California variants
• Δ69_70 • Δ144 • N501Y • A570D • D614G • P681H • T716I • S982A • D1118H	• L18F • T20N • P26S • D138Y • R190S • K417T • E484K • N501Y • D614G • H655Y • T1027I	• D80A • D215G • Δ241_242 • K417N • E484K • N501Y • D614G • A701V	• S13I • W152C • L452R • D614G

Spike protein mutations are often shared among different SARS-CoV-2 variants.

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html

B.1.1.7 or 501Y.V1 (UK) Variant

B.1.1.7 or 501Y.V1 Variant

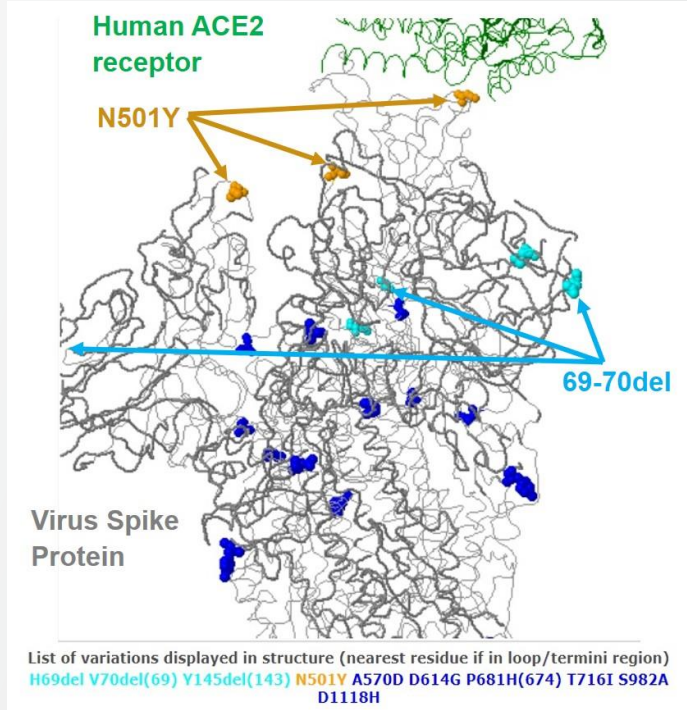
Emerged in the UK in Sept 2020, reported widely in Dec 2020, now reported in 60+ countries*

- 17 mutations (8 of which in the S gene) acquired more quickly than expected

- Concerns
  - Increased transmission rates may stress at-capacity health care systems
  - Questions about how mutations affect sensitivity claims of diagnostic tests

- Includes del69_70 S gene mutation
- Effect on ACE2 binding and virus replication resulting in estimated 70% more transmissibility**
- Recent studies indicate possibility of increased risk of death in patients infected with B.1.1.7***
- Highly likely that COVID-19 vaccines remain effective against this variant****

The UK Variant †



*As of 20 January 2021: https://www.forbes.com/sites/roberthart/2021/01/20/infectious-uk-covid-19-variant-detected-in-60-countries-who-says/?sh=3a10ab5b562d

**European Centre for Disease Prevention and Control 20 Dec 2020 Threat Assessment Brief

***https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/955239/NERVTAG_paper_on_variant_of_concern__VOC__B.1.1.7.pdf

****https://www.npr.org/sections/coronavirus-live-updates/2020/12/22/949150817/biontech-ceo-says-highly-likely-vaccine-is-effective-against-u-k-virus-variant

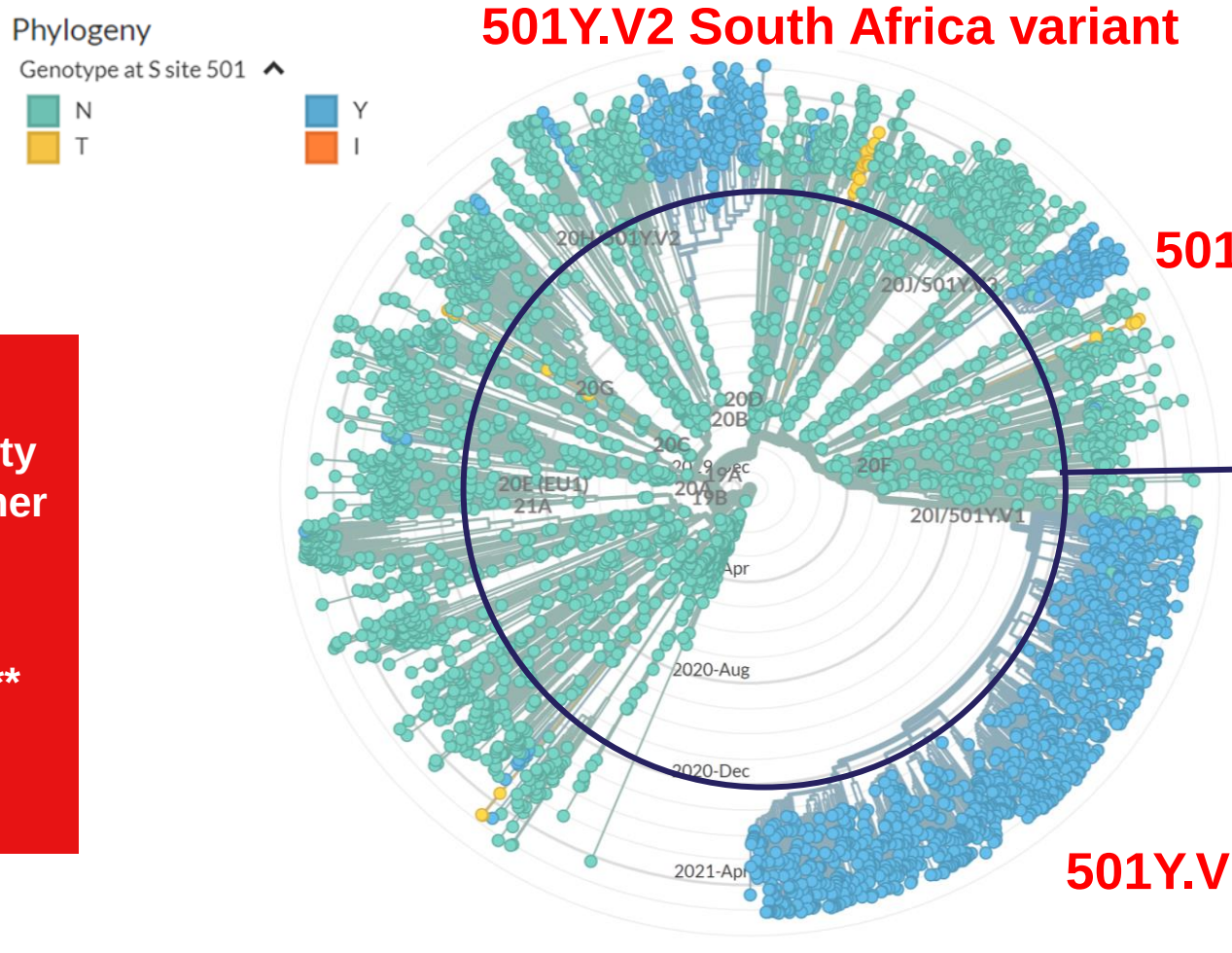
† https://www.gisaid.org/references/gisaid-in-the-news/uk-reports-new-variant-termed-vui-20201201/

https://outbreak.info/situation-reports#Lineage_Mutation

N501Y Increases SARS-CoV-2 Transmissibility

Spike protein mutations are often shared among different SARS-CoV-2 variants.

- **Key contact residue***
- **Increases binding affinity to ACE2 receptor = higher infectivity***
- **Increases resistance to neutralizing antibodies****
- **Diminishes antibody production*****

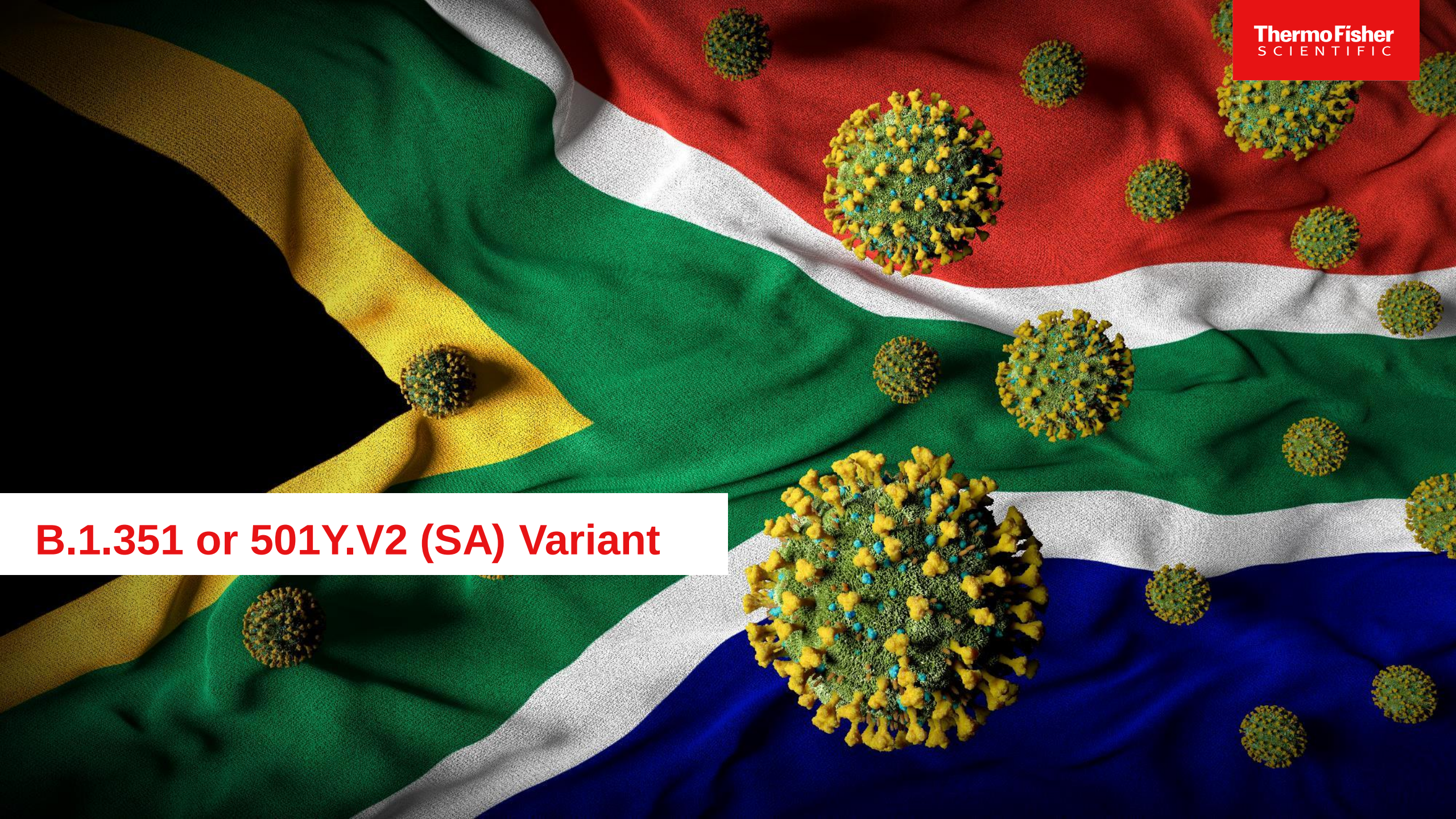


https://nextstrain.org/ncov/global

*Luan B et al. (2021) Enhanced binding of the N501Y-mutated SARS-CoV-2 spike protein to the human ACE2 receptor: insights from molecular dynamics simulations. FEBS Lett doi: https://doi.org/10.1002/1873-3468.14076

**Deshpande A et al. (2021) Epitope classification and RBD binding properties of neutralizing antibodies against SARS-CoV-2 variants of concern. bioRxiv 2021.04.13.439681; doi: https://doi.org/10.1101/2021.04.13.439681

***Castro A et al. (2021) Potential global impact of the N501Y mutation on MHC-II presentation and immune escape. bioRxiv 2021.02.02.429431; doi: https://doi.org/10.1101/2021.02.02.429431

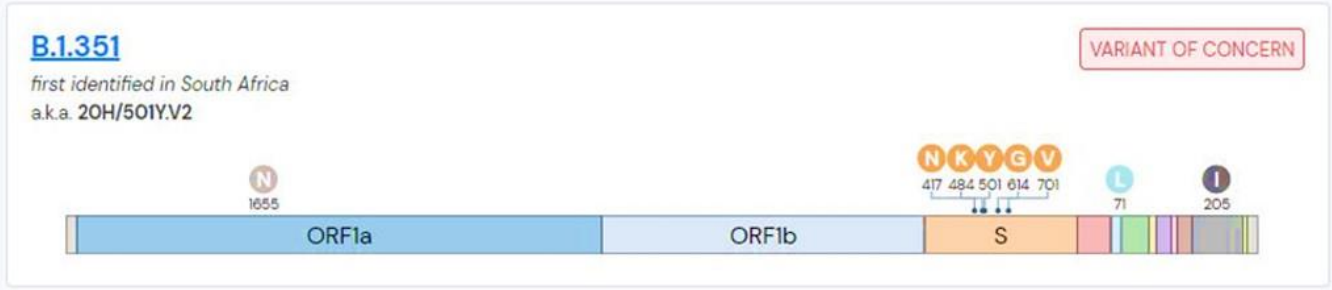


B.1.351 or 501Y.V2 (SA) Variant

B.1.351 or 501Y.V2 Variant

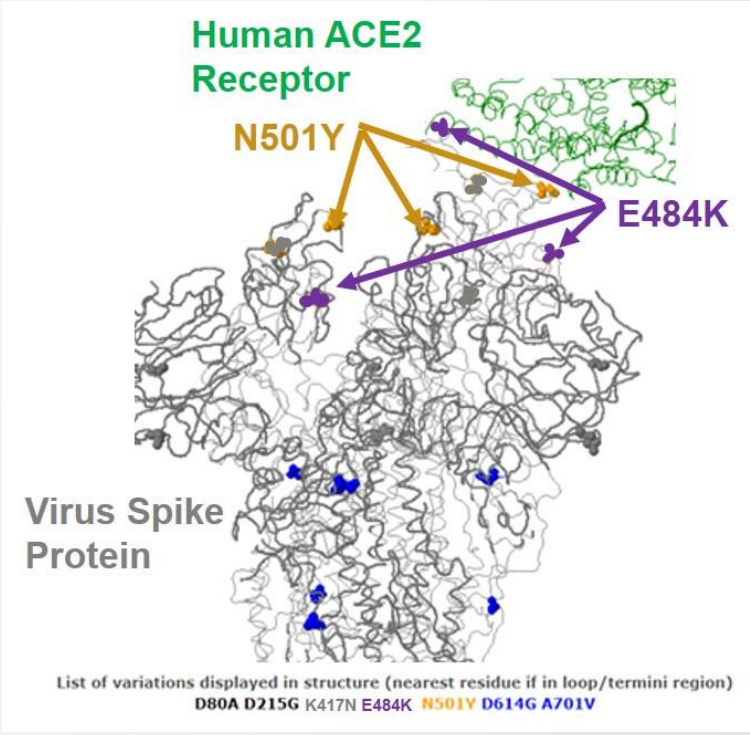
First reported in South Africa in December 2020. Now 30+ countries*

- Reportedly more contagious, but no evidence of increased disease severity**
- Shares N501Y mutation with B.1.1.7 (UK variant), but emerged independently and is phylogenetically different



- Concerns:
  - Linked to higher viral load and increased transmission, which could stress at-capacity health care systems
  - May be associated with poor response to antibody-based therapies***
  - Worries about current vaccine effectiveness due to multiple mutations (N501Y, E484K, K417N) in receptor binding domain (RBD)**

The South Africa Variant †

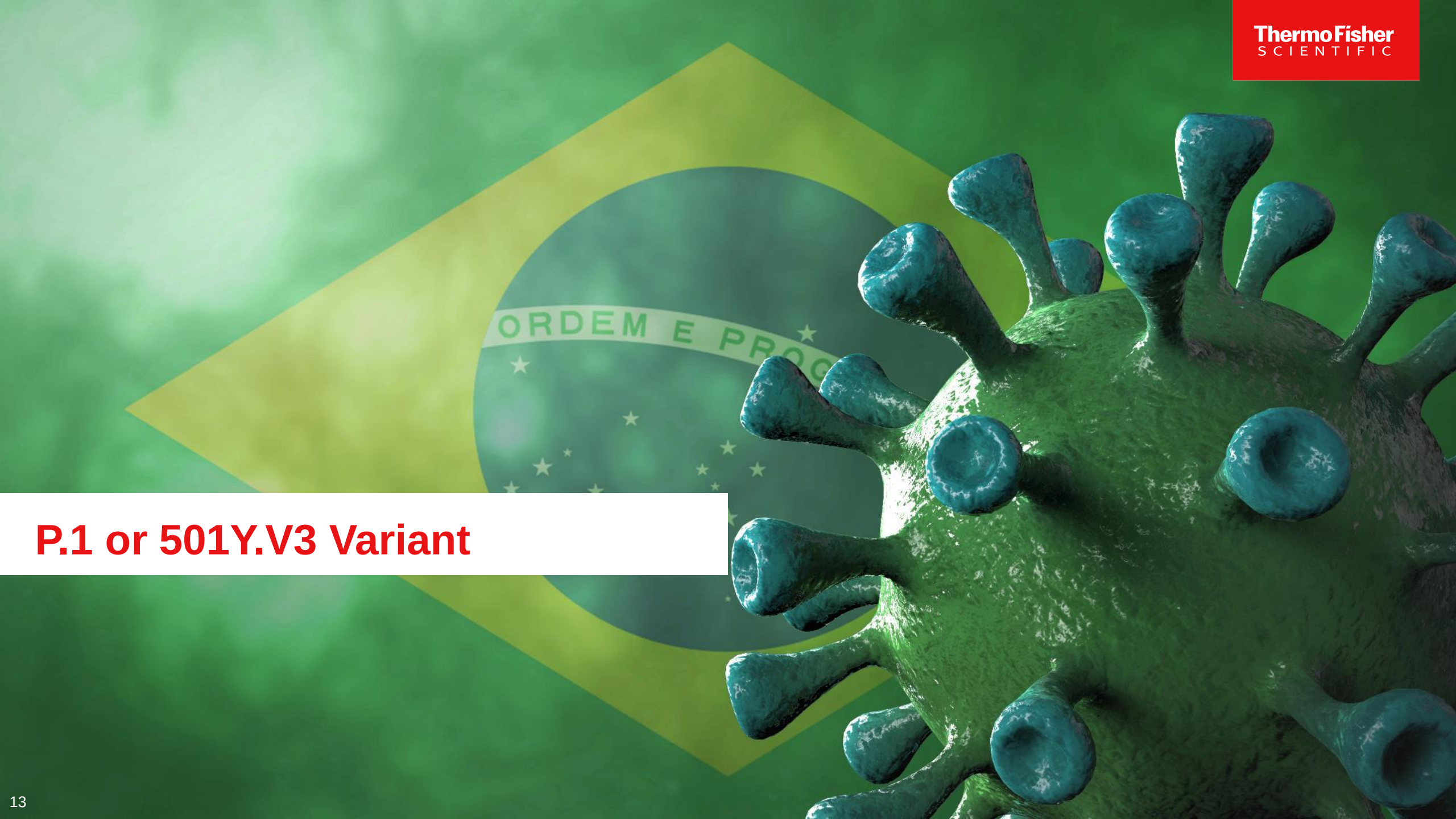


*As of 29 Jan 2021: https://apnews.com/article/coronavirus-variant-south-africa-us-2bc397370cdd44afe916ddd6edbaf870

** WHO: https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/

***https://www.cnbc.com/2021/01/05/south-africa-covid-variant-appears-to-obviate-antibody-drugs-dr-scott-gottlieb-says.html

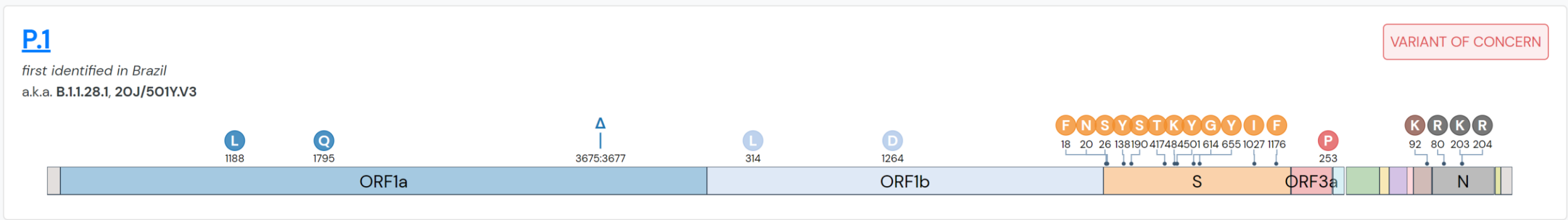
† https://www.gisaid.org/references/gisaid-in-the-news/novel-variant-combination-in-spike-receptor-binding-site/



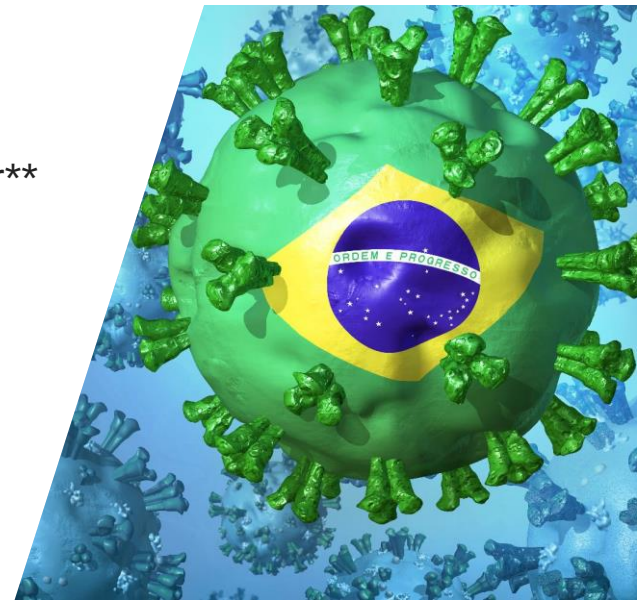
P.1 or 501Y.V3 Variant

P.1 or 501Y.V3 Variant

First detected in Brazil in December 2020. Now in at least 37 countries*



- Contains several spike protein mutations, including: N501Y, K417T, E484K
- Shares N501Y mutation with B.1.1.7 (UK variant), but emerged independently and is phylogenetically different
- P.1 concerns:
  - Potential increased transmissibility due to higher affinity of mutant S protein for ACE2 receptor**
  - Propensity for SARS-CoV-2 re-infection of individuals with previous non-P.1 infection**
  - Poor response to antibody-based therapies***



*New York Times Variant Tracker - accessed 18 Feb 2021: https://www.nytimes.com/interactive/2021/health/coronavirus-variant-tracker.html

**Faria N. R. et al., (2021) Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil; Science Doi: 10.1126/science.abh2644

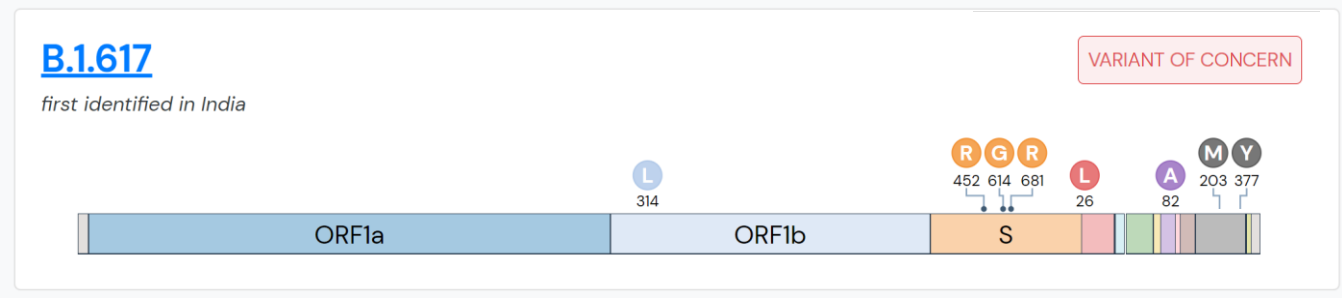
***Dejnirattisai W, el al, (2021) Antibody evasion by the P.1 strain of SARS-CoV-2ö Cell Doi:10.1016/j.cell.2021.03.055

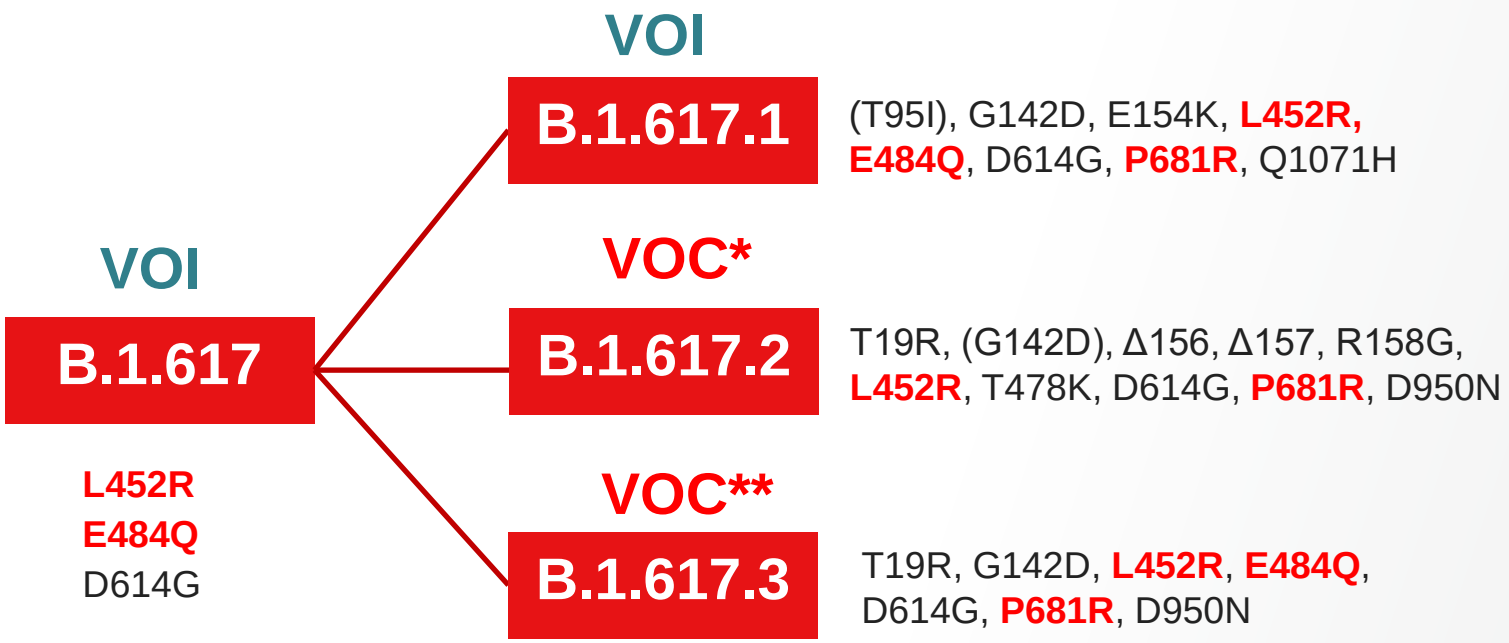
https://outbreak.info/situation-reports#Lineage_Mutation

B.1.617 (India) Variants

B.1.617 lineage

- Appeared in October 2020 and now the most prevalent lineage in India
- 49 countries now reporting B.1.617 lineage
- “Double mutant” (L452R and E484Q) & “Triple mutant” due to addition of P681R





Concerns:

- Increased transmissibility[1]
- Limited evidence of effect on disease severity[1]
- Potentially reduced effectiveness of monoclonal antibody treatments[2,3]
- Potential reduction in neutralization by post-vaccination sera[4,5,6]

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html
[1]https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---11-may-2021
[2] https://www.fda.gov/media/145802/download
[3] https://www.fda.gov/media/145611/download
[4] Yadav PD, Sapkal GN, Abraham P, et al. BioRxiv 2021. DOI: https://doi.org/10.1101/2021.04.23.441101
[5] Greaney AJ, Loes AN, Crawford KHD, et al. Cell 2021. DOI: https://doi.org/10.1016/j.chom.2021.02.003
[6] Deng X, Garcia-Knight MA, Khalid MM, et al. MedRxiv 2021. doi: https://doi.org/10.1101/2021.03.07.21252647
* https://www.reuters.com/world/uk/public-health-england-says-coronavirus-variant-b16172-is-variant-concern-2021-05-07/
** https://www.cnbc.com/2021/05/10/who-classifies-triple-mutant-covid-variant-from-india-as-global-health-risk-.html

E484K/Q– Enhance Neutralizing Antibodies Escape

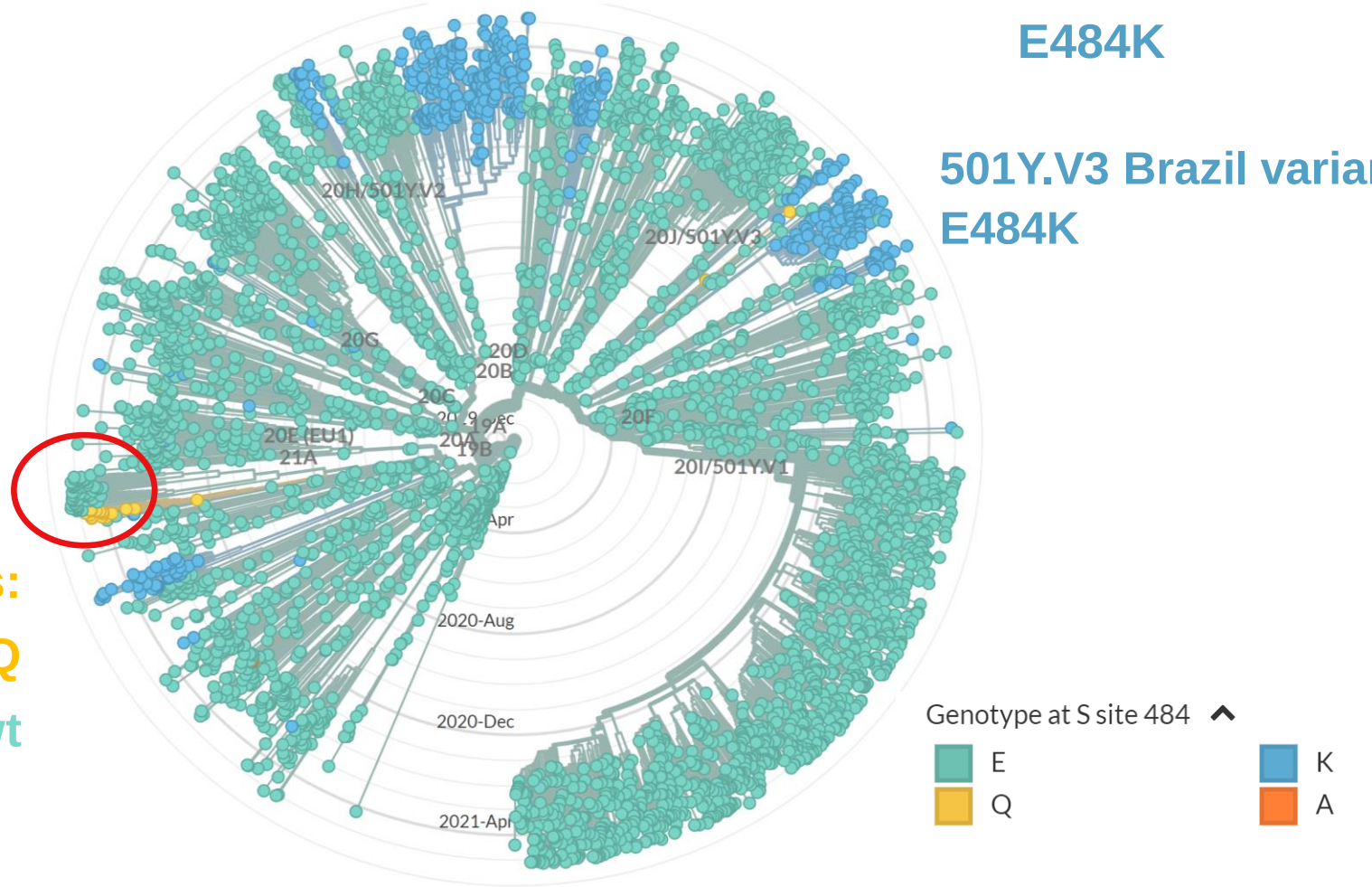
- **Contributes to resistance to neutralizing antibodies[1,2,3,4]**
- **Decreases sensitivity to convalescent and vaccine-induced immune sera[1,2,3,4]**
- **Increases affinity for the human ACE2 receptor***

501Y.V2 South Africa variant

E484K

501Y.V3 Brazil variant

E484K



Indian variants:

B1.617, B.1.617.1, B.1.617.3 E484Q

B.1.617.2 E484wt

https://nextstrain.org/ncov/global

[1]Chen RE et al. (2021) Nat Med 27, 717-726 https://doi.org/10.1038/s41591-021-01294-w

*Nelson et al. (2021) bioRxiv 2021.01.13.426558 https://doi.org/10.1101/2021.01.13.426558

[2]Jangra SA et al. (2021) Lancet Microbe; https://doi.org/10.1016/S2666-5247(21)00068-9

[3]Garcia-Beltran et al., (2021) Cell 184, 2372–2383 https://doi.org/10.1016/j.cell.2021.03.013

[4]Tada, et al. (2021) bioRxiv 2021.05.14.444076 https://doi.org/10.1101/2021.05.14.444076

How Can We Detect and Discriminate the Emerging Variants?

Global Surveillance of SARS-CoV-2 Variants

Public health partners

- Monitor viral genome
- Detect & Inform on emerging mutations
- Determine impact on detection, spread and vaccine / therapy effectivity

Clinical

Isolate

Isolation of SARS-CoV-2 RNA

How: automated isolation using magnetic beads

Detect

Detection of SARS-CoV-2

How: multiplex real-time RT-PCR

Research

Identify

Genetic Surveillance

How: Sequence the full viral genome or specific sections / genes of interest

Confirm

Mutation Verification

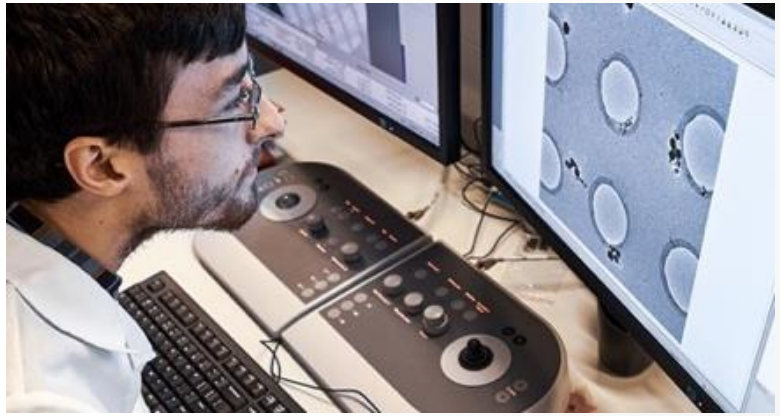
How: interrogate specific sections or areas of the viral genome by sequencing or genotyping

RT-PCR Applications for Surveillance

Detection of SARS-CoV-2

- Target amplification failure in a multiplex RT-PCR diagnostic test can be caused by mutations in emerging variants
- If the assay provides sufficient redundancy, it can be used as a screening tool

Multiplex RT-PCR



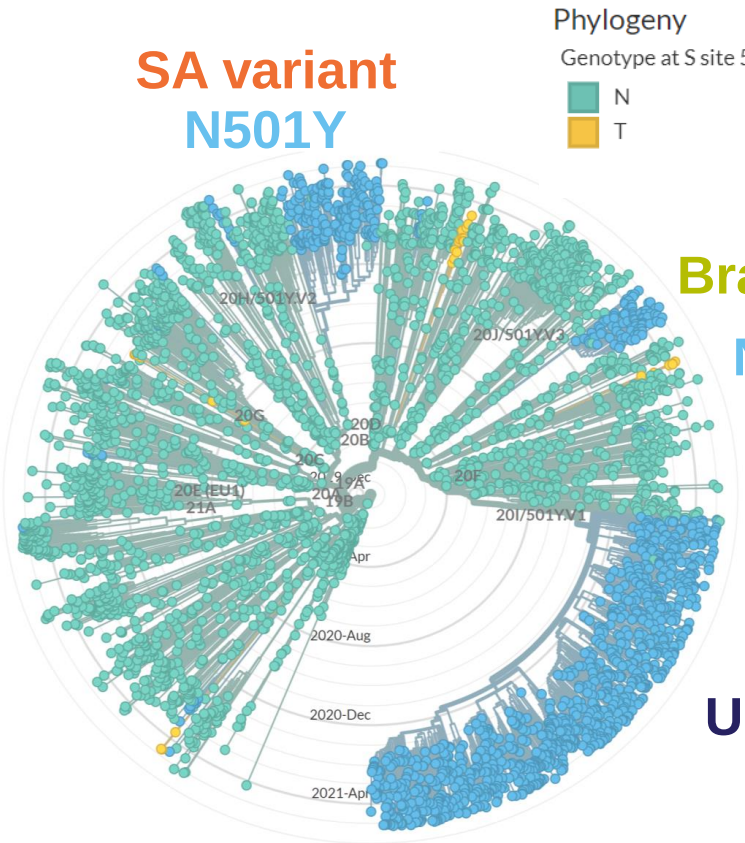
- Multiplexed RT-PCR solutions identify simultaneously the presence of several mutations in the viral genome
- They can be applied to detect only known variants of concern

Mutation Panel

- Individual genotyping assays detecting mutations shared or specific for each variant of concern can also be used
- Provides flexibility and allows customization to the particular epidemiological situation
- Novel assays can be easily added for emerging variants

How to Discriminate the Variants Using a Mutation Panel?

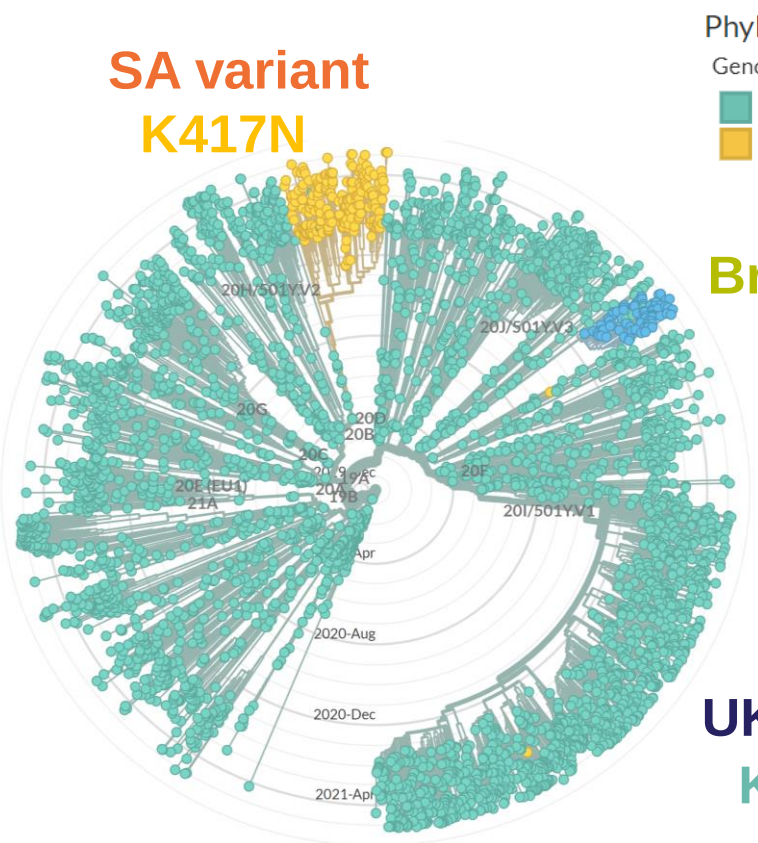
SA variant
N501Y



Brazil variant
N501Y

UK variant
N501Y

SA variant
K417N



Brazil variant
K417T

UK variant
K417 wt

N501Y + K417 wt = UK variant

N501Y + K417T = Brazil variant

N501Y + K417N = South Africa variant

If we test for N501Y, K417N and K417T we can discriminate the 3 VOC

https://nextstrain.org/ncov/global

Sequencing as a Surveillance Tool

NGS Assay

Surveillance of the full viral genome to discover and identify new and emerging variants and mutations.

SARS-CoV-2 Whole Genome Sequencing

- One assay surveys the complete genome
  - >99% genome coverage (~30 kb)
  - Covers all potential serotypes
- Use with biological research samples
- Viral loads as low as 20 copies
- Confirm individual amplicons with Sanger sequencing

Sanger Sequencing

Gold standard to sequence short stretches of the viral genome, detect mutations and confirm NGS results.

Surveillance Sanger Sequencing Protocols

- Cost effective sequencing of targeted genes or emerging mutations
- Sanger sequencing protocols and primer sequence sets available for surveillance
  - full S gene sequencing
  - targeted to specific variants

Multiple Research Solutions for Surveillance and Verification

	Verification		Surveillance
	RT-PCR	**Sanger sequencing by capillary electrophoresis**	**Next-generation sequencing (whole viral genome)**
Use Case	Mutation Verification of known mutations	Rapid Verification of genes / areas of interest with known mutations, strains or lineages	Surveillance of the full viral genome to discover and identify new and emerging variants and mutations
Lab profile	Use the same PCR instrument for both detection and mutation Verification	Cost effective and able to see full sequence of mutations	Manual prep resources and skills Experienced with NGS
Number of targets	1-20	1-20	>20 - 10000
Solution Complexity	*	**	***
Surveillance Research Solutions	Primer / Probe sets mutations found in the main circulating variants	Primer sets for specific variants or full S-gene sequencing	Full viral genome
Sample Prep	Manual or Automated	Manual or Automated	Manual or Automated
Sensitivity	**	**	***
Time to Result	hours	hours	days

Thank you

The line has been unmuted for questions.