Unlocking the mystery

What we need to know about SARS-CoV-2 variants

When Public Health England (PHE) announced on 14 December 2020 that it had identified a new mutated variant of the SARS-CoV-2 virus in South East England with potentially greater infectivity than the original wild type, a new and worrying phase in the history of the pandemic began. The announcement was greeted with widespread alarm, not to mention some dramatic newspaper headlines: several countries temporarily closed their borders with the UK in an effort to stop the spread of the variant, while *The New York Times* among others described the UK as "plague island."

The UK authorities were quick to point out that the variant had been identified first in the UK as a result of the country's advanced viral genome monitoring and sequencing capability, and that it was probably already present in other parts of the world. This proved to be the case, with other countries soon confirming its presence.

The mutant, known as VUI 202012/01 (variant under investigation, year 2020, month 12, variant 01) or B.1.1.7, was first identified in the UK in September 2020, at which time it accounted for approximately 25% of new diagnoses of Covid-19. By the time of PHE's announcement in mid-December, this had increased to almost two-thirds of new cases in London. Despite its apparent increase in transmissibility, the variant was not initially thought to be linked to any change in disease severity, although this supposition is still not definite.

"There is ample evidence that new variants can lead to reinfection."

The B.1.1.7 variant is defined by mutations that result in a number of changes to viral proteins, including 14 amino acid substitutions and three deletions. One of these substitutions, known as N501Y (where an asparagine residue is substituted by tyrosine at position 501 in the spike protein), results in a change in one of the six key contact residues in the receptor binding domain (RBD) of the protein, resulting in increased binding affinity to angiotensin-converting enzyme 2, (ACE2, the receptor used by the virus to gain access to the cell). It is this change that is believed to underlie the increased transmissibility of the virus.

News of the new variant came as little surprise to the scientific community, as it is common for viruses to mutate as they spread through host populations (as, for example, with seasonal influenza). The more widely a virus is distributed in the host population, the more likely it is that one or more mutations will emerge. This latter characteristic underlines the importance of controlling outbreaks of novel viral diseases, for example through vaccination, as quickly as possible.

There are a number of different types of mutation, but essentially, they all arise when mistakes occur as the nucleic acid (DNA or, in this case, RNA) in the viral genome is read and copied. Substitution and deletion mutations, where one nucleotide is erroneously substituted for another, or not copied at all, have already been mentioned. Other types of mutation arise when an extra nucleotide is randomly inserted into the viral RNA or DNA, or a particular section of the genome is copied in the wrong direction or more than once. Another type of mutation, known as recombination, where sections of the genome from two different viruses become joined together, is thought to have been involved in the emergence of SARS-CoV-2 in the first place.

Many mutations are of little consequence while others confer some specific advantage on the virus (indeed, unless a mutation imparts an advantage that increases the virus's survivability it is likely to disappear via normal evolutionary pressures). Compared with some other viruses like HIV or influenza virus, SARS-CoV-2 appears to mutate relatively slowly, although the number of individual mutations found in B.1.1.7 was surprisingly high.

B.1.1.7 was not the first variant of SARS-CoV-2 to be discovered, however. According to the World Health Organization, a variant with a D614G substitution (aspartic acid residue replaced by glycine) in the gene encoding the spike protein was detected in late January or early February 2020, and went on to replace the initial SARS-CoV-2 strain identified in China as the dominant form of the virus circulating globally. In August and September 2020, a variant with a previously unreported combination of mutations was linked to an outbreak of SARS-CoV-2 infections among farmed mink in Denmark that subsequently spread to humans.

South Africa, Brazil

Within days of the PHE announcement, the authorities in South Africa revealed that they had detected a new variant of SARS-CoV-2 which was spreading rapidly in three provinces of that country. The variant was named B.1.351, and like the UK variant it displayed the N501Y mutation. Early data suggested that the variant was associated with a higher viral load, a characteristic that could contribute to greater transmissibility. Since then the B.1.351 variant has been detected in at least 20 countries outside South Africa. As of the end of January there was no evidence to suggest that it has any impact on disease severity.

At the beginning of 2021 another new variant with high transmissibility was detected in Japan in four travellers who had arrived from Brazil. The variant, named P.1, has 17 unique amino acid changes, three deletions, four synonymous mutations and one insertion (a synonymous

mutation is a change in the nucleic acid sequence that codes for a particular protein, but which does not change the encoded amino acid). The changes in P.1 include three mutations in the spike protein RBD. One of these is the N501Y mutation seen in the UK and South African variants. According to the US Centers for Disease Control and Prevention (CDC), there is some evidence that some of the mutations in the P.1 variant may affect its transmissibility and antigenic profile. P.1 has subsequently become the dominant form of the virus in at least some parts of Brazil, and has been detected outside Brazil, notably in the US.

A second variant of SARS-CoV-2, P.2, has also been detected in Brazil, in the state of Rio de Janeiro. Unlike P.1 it has not so far been designated as a variant of concern. Other variants have been detected in Nigeria and in parts of the US.

The emergence of SARS-CoV-2 variants raises a number of questions. Concern about whether the new variants are more easily transmitted than the wild type virus, as with the B.1.1.7 variant, and whether they cause more serious disease, has already been mentioned. Other questions include:

- What is the potential impact of new variants on the frequency of reinfection?
- What is their potential impact on vaccine match and effectiveness?
- What are the implications for the treatment of Covid-19 disease, particularly with monoclonal antibodies such as Regeneron's antibody cocktail, REGEN-COV (casirivimab/imdevimab)?
- Are current diagnostic tests adequate to detect the new variants?

There is ample evidence that new variants can lead to reinfection. In the city of Manaus in Brazil, for example, it is estimated that as many as 75% of the population had already been infected with the original SARS-CoV-2 when the P.1 variant emerged at the end of 2020, causing a dramatic rise in the infection rate. This strongly suggests reinfections were occurring. In January 2021, physicians in the UK reported a confirmed case of reinfection with the second episode due to the B.1.1.7 variant. The initial infection had occurred in the first wave of the pandemic and resulted in mild illness, but eight months later reinfection with the B.1.1.7 variant was confirmed and caused a critical illness.

Vaccine effectiveness maintained

As far as the effectiveness of vaccines is concerned, all current products target the viral spike protein, which is where the known variants have mutations. The total length of the SARS-CoV-2 spike protein is 1273 amino acids, so theoretically at least a large number of mutations would be necessary for the virus completely to evade existing vaccines. There are limits to how much a virus can mutate without losing some of its transmissibility or virulence.

Manufacturers of Covid-19 vaccines have been quick to publish data attempting to show that their products are effective against variants. A preliminary report published on 7 January stated that sera from individuals who had received the Pfizer Inc/BioNTech SE vaccine had equivalent neutralising titres to both the wild type virus and variants with the N501Y mutation. Moderna Inc issued a statement on 25 January that sera from individuals who had received the Moderna vaccine had demonstrated neutralising activity against SARS-CoV-2 variants from the UK and South Africa. Although activity against the B.1.1.7 variant was similar to that against prior variants, a six-fold reduction in neutralising titres was observed with the B.1.351 variant. The company said however that the titres remained above levels expected to be protective.

At the end of January Novavax Inc released data to show that NVX-CoV2373, its protein-based Covid-19 vaccine candidate, had an efficacy of 89.3% in a Phase 3 clinical trial in the UK during a period when B.1.1.7 was emerging and circulating widely. But in a much smaller, Phase 2b trial in South Africa, efficacy was only 60% for the prevention of mild, moderate and severe COVID-19 disease in HIV-negative individuals (when HIV-positive individuals were included in the study, efficacy was 49%). On the same day, Johnson & Johnson announced that interim analysis of the Phase 3 ENSEMBLE trial of the Janssen Pharmaceutica NV Covid-19 vaccine showed it to be 66% effective overall in preventing moderate to severe disease 28 days after vaccination. However, the level of protection varied from 72% in the US arm of the trial to 66% in Latin America and 57% in South Africa.

In early February, *The Lancet* published a preprint of a paper by the Oxford Vaccine Group and others which showed that the efficacy of the Oxford/AstraZeneca vaccine against the B.1.1.7 variant was similar to that against the original virus, and that it resulted in a reduction in the duration of shedding and viral load, with implications for the transmission of disease. However, on 7 February Oxford University announced that a preprint analysis of a small (2000 patients, average age 31 years) Phase 1/2 trial of the vaccine in South Africa had found that viral neutralisation sera induced by the vaccine against the B.1.351 South African variant were substantially reduced when compared with the original strain of the coronavirus. The trial did not assess protection against moderate-to-severe disease, hospitalisation or death.

Therapeutics retain potency

There is so far little information about how the effectiveness of various antiviral therapies might be affected by new variants. Gilead Sciences Inc, whose product Veklury (remdesivir) has been approved or authorised for temporary use as a Covid-19 treatment in approximately 50 countries worldwide, issued a statement on 21 January stating that an internal company analysis had concluded that it is highly likely that the B.1.1.7 and B.1.351 variants will remain fully susceptible to remdesivir. Sequence analysis of the new variants did not detect any amino acid changes in the viral RNA-dependant RNA polymerase gene that would impact the efficacy of remdesivir.

At the end of January, Regeneron Pharmaceuticals Inc announced that studies carried out by the company and by researchers at Columbia University had independently confirmed that REGEN-COV successfully neutralises both the B.1.1.7 (UK) and B.1.351 (South Africa) variants *in vitro*. Of the two components of REGEN-COV, the company

Table: Main SARS-C0V-2 variants of concern

Variant	Where/when first detected	Countries reporting cases	Main mutations	Increased transmissibility?	Other concerns (unconfirmed)
B.1.1.7	UK - September 2020	70	• 69/70 deletion • 144Y deletion • N501Y • A570D • D614G • P681H	Yes	More serious disease
B.1.351	South Africa - October 2020	>30	• K417N • E484K • N501Y • D614G	Probably	Reduced vaccine effectiveness
P.1	Japan/Brazil - January 2021	>4	• E484K • K417N/T • N501Y • D614G	Probably	Reduced vaccine effectiveness

Source: US Centers for Disease Control and Prevention; European Centre for Disease Prevention and Control

Note: PHE reported at the beginning of February that the E484K mutation, which causes conformational changes in the spike protein and thus makes it less easily recognised by the immune system, had also been found in some B.1.1.7 isolates.

noted that casirivimab's potency against B.1.351 was reduced, but that it was still comparable to the potency that other single antibodies in development have against the original virus.

Regeneron added that it expected REGEN-COV to retain its potency against the P.1 variant (Brazil), and that it is conducting additional preclinical research in order to confirm this.

There is also little information available about the performance of diagnostic tests in the presence of new variants of SARS-CoV-2. PCR assays use multiple targets so the impact of any particular mutation is likely to be small. FIND, the Foundation for Innovative New Diagnostics, has reported that the B.1.1.7 variant has minimal impact on molecular diagnostics such as PCR or on antigen-based tests (including rapid lateral flow devices).

Similarly, the impact of the South African variant on molecular diagnostics is minimal, and although there are no data yet on the impact on antigen-based testing, no major performance deficits are anticipated. As yet there are no data on the impact of mutations on the performance of serological antibody tests.

At the beginning of February, Novacyt Group, an international clinical diagnostics company that specialises in cancer and infectious diseases, announced the launch of a portfolio of PCR assays for the genotyping of SARS-CoV-2 variants. According to the company, the SNPsig assays enable the identification of the non-variant virus and the B.1.1.7, B.1.351 and P.1 variants, as well as any variant carrying the N501Y mutation.

Global response

In response to the emergence of new variants of SARS-CoV-2, the World Health Organization has recommended increased monitoring and is currently working with countries to improve virus surveillance systems. A Virus Evolution Working Group was set up in June 2020 to boost mechanisms for identifying and prioritising potentially relevant mutations, to study their impact on viral characteristics, and to evaluate possible mitigation strategies to reduce their negative impact. For example, where possible, systematic sequencing of SARS-CoV-2 isolates should be increased, and sequence data shared internationally, WHO says. In addition, it advises that genetic sequencing should be considered as part of investigations of unusual transmission events.

Should existing measures prove inadequate to control new variants of SARS-CoV-2, all is not lost. Manufacturers of the current generation of vaccines are already understood to be working on how to improve their products to be ready for new coronavirus variants. The mRNA technology used in current vaccines is extremely flexible, meaning that the configuration of vaccines could be adjusted very easily, perhaps even easier than the annual production of seasonal influenza vaccines.

Professor Andrew Pollard, director of the Oxford Vaccine Group and lead investigator in the team that developed the Oxford/AstraZeneca vaccine, has said that such vaccines could be available later this year if needed.

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