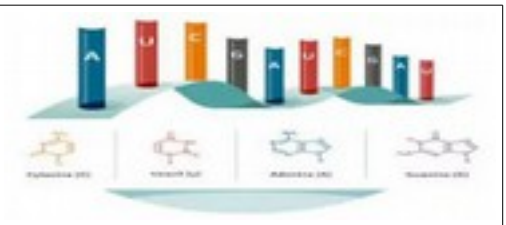
SARS-CoV-2 : THE VARIANTS TRIAL

« British », « South African » or « Brazilian », who are they, and what are their capabilities?

Authors: Menahem Bregegere, Ph.D. Molecular Biology and Genetics **Contributors**: Irene Fermont MD, MSc, Immuno-hematology and Pharmacovigilance, Ayalah Livneh, MD, MSc, Laboratory Medicine	

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SARS-CoV-2 general profile

The SARS-CoV-2 genome is an RNA strand of 29,903 nucleotides. Its first half codes for a long polypeptide, which is cleaved into 15 "non-structural" proteins, involved in virus multiplication. Its terminal half codes for 4 structural proteins: S ("spike"), E ("envelope"), M ("membrane") and N ("nucleocapsid"), and 8 accessory proteins.
In total, the SARS-CoV-2 genome contains 14 open reading frames, which direct **the synthesis of 29 proteins** (1). It is one of the largest known RNA viruses.

Variants mutations are simply replication errors of the RNA genome. RNA viruses are considered genetically unstable: their average mutation rate is 100 times higher than that of DNA viruses (**2**). They usually lack an error correction mechanism, which facilitates genetic variation. This evolutionary flexibility can be advantageous for small viruses, but it becomes a drawback as the size of the genome increases, as well as the probability of mutations with negative effects.

SARS-CoV-2 avoids this problem by producing an exoribonuclease (ExoN), specifically assigned to correct its replication errors (**3**). Therefore its mutation rate is low for an RNA virus, and **less variants occur than in influenza and AIDS for example.**
Phylogenetic studies show that its 29 proteins mutate at different speeds: 27 show little or no genetic variability, while the **S and N proteins**, which are the targets of immune or therapeutic molecules, **are much more variable** (**4**).

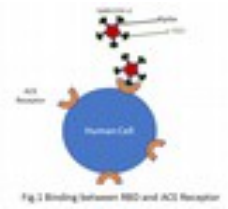
The evolution of **SARS-CoV-2** since its appearance in 2019 has been traced through **systematic sequencing of viral genomes**, collected from all parts of the world. Lineages have been defined, which constitute the branches of SARS-CoV-2 phylogenetic tree (**5**).
Three lineages are currently dominant: the "British variant" (B.1.1.7), the "South African variant" (B.1.351), and of the "Brazilian variant" (P.1). We will briefly present these three variants, then provide an overview of variants multiplication, virus variability limits, and of our means to defeat it.

The "British variant", B.1.1.7

The British variant **B.1.1.7** appeared in September 2020. Compared to the **Wuhan** strain of January 2020, it has 17 mutations. Three deletions and seven substitutions of aminoacids are located in the **S** protein, which serves as a “key ” to enter the cell (**6**). Two of them, N501Y and P681H, are located in the **S** binding domain (RBD), which binds to the ACE2 membrane receptor.

ACE2 is the “lock” that allows the virus to open the cell membrane and pass through it. These two mutations facilitate the interaction between the "key" and the "lock", and are probably responsible for the increase in virulence (7).
Several studies have shown that **B.1.1.7** increases COVID-19 severity and mortality (**8**-**9**).

This lineage has already supplanted the “ancestral” lineage of **Wuhan** in several European countries, America and China. In Israel, it is responsible for 90% of cases since January 2021. Fortunately, the vaccines available today (Pfizer, Moderna, AstraZeneca, Johnson & Johnson and Novavax) retain an effective rate of neutralization against this lineage (**10**).



The "South African" variant, B.1.351

B.1.351 spread to South Africa in October 2020, carrying five new mutations in protein S, including three amino acid substitutions in the RBD: K417N, E484K and N501Y. Then were added three substitutions and one deletion in the N-terminal domain. **As for B.1.1.7, these mutations seem to be responsible for the 50% hypertransmissibility** of B.1.351, and hence for its selective advantage (11).

In vitro,
they increase the resistance to monoclonal antibodies (12), and to sera of patients exposed to the original Wuhan strain (13-fold less neutralization).
Sera from vaccinated subjects also have a reduced capacity to neutralize **B.1.351**.

- Using the antisera elicited by the **Pfizer vaccine, the neutralizing ability is 7.6 times weaker against B.1.351** compared with the Wuhan strain.
- Using the antisera elicited by the **AstraZeneca vaccine, the neutralizing ability is 9 times weaker.** Since the AstraZeneca vaccine is already 3.6 times less potent than the Pfizer, its residual efficacy on B.1.351 is very low (13).

Epidemiologic data
Concurrently, available epidemiological data show that the protection provided by the

- Novavax vaccine, which is 95.6% against the Wuhan strain, falls to 85.6% against B.1.1.7, and to 60% in South Africa, where B.1.351 represents 92.6% of infections.
- **The Janssen single-dose vaccine** (Johnson & Johnson), which protects **72% against moderate to severe disease, protects only 57% in South Africa.**
- Finally, **the AstraZeneca vaccine protects only 10.6% against mild to moderate symptoms in cases of infection with B.1.351 (13).**

Outside of South Africa, the B.1.351 lineage is now widely represented in North America, Europe and Israel.

The "Brazilian" variant, P.1

Another variant, named **P.1**, was characterized in Brazil in December 2020. This variant carries 17 amino acid substitutions and three deletions. Three substitutions in the RBD (K417N, E848K and N501Y), and one deletion in the orf1b gene (del11288-11296), are common with the South African variant **B.1.351**. They appeared independently, **suggestive of a convergent evolution** (14). **N501Y increases transmissibility, and E484K hampers neutralization by antibodies elicited by the Wuhan strain.** Both variants have established themselves in natural selection against their competitors, and have spread to areas largely infected with the Wuhan strain, **suggesting that they at least partially escape the immunity acquired through a primary infection with (15).**

In vitro,
P.1 and B.1.351 RBDs are very similar, but when vaccine-elicited immunity to the S protein is analyzed in pseudoviruses (16), pseudo-P.1 turns out to be less resistant to neutralization than pseudo-B.1.351.

- Using antisera elicited by the **Pfizer vaccine, neutralization of pseudo-P.1 is reduced 6.7 times** in comparison, neutralization of pseudo-B.1.351 is reduced 41 times),
- Using antisera elicited by the **Moderna vaccine, it is reduced 4.5 times.** (in comparison, neutralization of pseudo-B.1.351 is reduced 21 times)

This difference between P.1 and B.1.351 suggests that more mutations, that lay outside the RBD, also impact neutralization (6,17-18). These conclusions have recently been confirmed by another study, performed with SARS-CoV-2 viruses rather than pseudoviruses (19).

Finally, P.1 sensitivity to neutralization by sera from vaccinated subjects is of the same order as that of the British variant B.1.1.7, and markedly greater than that of the South African variant B.1.351.

New emerging lineages and their dangers

The virus continues to evolve around the world, with particular dynamism in regions with a high viral load, where its circulation is most active. Emerging variants generally carry S protein mutations which **increase their transmissibility,** giving them a selective advantage. This is the case of the **N501Y mutation, present in the three major variants** and in many others, and of the **L452R mutation,** which is found in the **Californian variant B.1.427 / B.1.429** (**20C**) (**20**).

Other mutations **attenuate the neutralizing ability of antibodies**, such as **E484K** which is present in the **South African** and **Brazilian** variants, and appears independently in the **New York lines** B.1.526 (21) and B.1.1.220 (22), **Arizonian** B.1.243.1 (23), and **African A.VOI.V2** (**24**), which indicates a convergent evolution.

Other lines follow **different evolutionary strategies**, such as the **Ugandan** line A.23.1, which presents an original set of mutations (**25**).

A recently reported Indian variant, B.1.617, combines the mutations L452R of B.1.427/B.1.429 and E484Q of B.1.351 and P.1 (43).

The appearance of variants naturally gives rise to fears that the evolution of the virus may allow it to escape natural and vaccine-elicited immunity, and indeed mutations reduce the neutralizing ability of monoclonal antibodies and of antisera. Epidemiological data in Brazil and South Africa point in the same direction, showing that the second waves of epidemics in these countries, which coincided with

the appearance of variants, developed by reinfection of subjects naturally immunized against the original strain (26). **It is therefore necessary to prepare for a possible immune escape of new variants.**

In conclusion :

It is possible to conclude on an optimistic note, noting the strengths of our defense against variants:

1) **In vitro** neutralization assays of variants by antisera from cured or vaccinated subjects shows that sera neutralization ability is greater after vaccination than after natural infection, including variants neutralization (10, 17, 27-30).

2) The production of neutralizing antibodies (humoral response) is the first adaptive response of the immune system against the virus. Antibodies block virus particles, prevent them from infecting cells, and get them to macrophages, which digest them. But **removing the virus from the body is a task of cellular immunity**, and especially cytotoxic CD8 + T lymphocytes, which detect infected cells that are sources of the virus, and kill them (31). **Cellular immunity might be sufficient to clear the virus even in the absence of neutralizing antibodies (32)**. It is therefore important that RNA vaccines elicit not only a powerful humoral response, but also a substantial cellular response (27).

The antigenic determinants recognized by CD8+ T lymphocytes are different from those recognized by antibodies. They are short peptide fragments taken from the entire protein sequence, and accommodated on the cell membrane by class I histocompatibility antigens (33-35). They can be located in buried and less variable regions of the protein, which are conserved in most variants. Cellular immunity therefore has a much larger targets than humoral immunity, and can combat variants that would escape neutralization by humoral immunity (36).

3) **Messenger RNA technology greatly facilitates the adaptation of vaccines to the evolution of pathogens.** To adapt a vaccine to new variants, you have just to replace a sequence by another in the RNA of the **S** protein, a genetic engineering operation that can be carried out within a day. This prompted the CEO of BioNTech to say that a new vaccine could be ready in 6 weeks (37). In addition, different strategies can be considered today to produce a vaccine with a broad specificity. For example, one could insert the sequence of the variant B.1.351, which elicits an effective humoral response against all variants tested (38, 39).

4) **An alternative to conventional vaccination** consists in inducing specific cellular immunity. For this purpose, one has to determine the immunodominant epitopes of viral proteins recognized by T cells, and to prepare a vaccine with these peptides. **The vast majority of immunodominant S protein epitopes, and therefore T cell response, have been shown to be unaffected by mutations in current variants** (40, 41). Indeed, 33 epitopes restricted by the human HLA-A2 class I antigen were used to prepare “peptide cocktail” vaccines, which elicited strong specific responses in wild-type or HLA-A2/DR1 transgenic mice (42).

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