

OMICRON'S SPIKE PROTEIN MUTATIONS AND VACCINE EFFICACY: UNDERSTANDING THE IMPACT AND FUTURE STRATEGIES IN COMBATING SARS-CoV-2 VARIANTS: REVIEW

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ABSTRACT

Omicron Spike protein has a notably high number of mutations. These mutations are related to the unique characteristics of the SARS-CoV-2 Omicron Virus. The symptoms of Covid-19 reported up till now are fever, cough, sore throat, Shortness of breath or chest pain, headache, nausea or fatigue, stuffy or runny nose, sore eyes, loss of smell or taste, muscle pain, sneezing, reduced appetite and abdominal pain. In some studies Omicron shows considerably but not a complete escape from Pfizer r BNT162b2 Vaccine in which they engineered lungs cell line. Pfizer-BioNTech booster post data was compared there was a drop of vaccine efficacy was seen from 96.5% against delta to 80.1% against omicron Pfizer-BioNTech booster dose is going to be very effective to alleviate the future effects of omicron virus in different countries. However in near future Omicron variant specific vaccines are much needed in current situation the risk of new variant can be reduced by vaccinating a large number of people to limit the space of virus. If efficacy of vaccine is partial, it still protects people from getting sick. The vaccine works by boosting immune system on sensing any SARS-CoV-2 virus. It may be said that the future of SARS-CoV-2 is still in hands of human. By vaccinating more people, changes in virus that cause a new variant to emerge can be stop. According to Rambaut, virus has multiple directions in which it can go.

Key-words: SARS-CoV-2 Omicron Virus, Vaccination, Prevention from disease, Cell line, organs.

INTRODUCTION

Virus Definition

The SARS-CoV-2 Omicron Virus was first reported in South Africa. Its First case was reported on 24th of November in 2021. World Health organization named this virus as a “variant of concern” (VOC) because of its rapid spread rate within 2 days. Omicron Spike protein has a notably high number of mutations. These mutations are related to the unique characteristics of the SARS-CoV-2 Omicron Virus (Wei *et al.*, 2021b). This virus variant shows exceptionally high number of mutations, this is the cause of its high spread rate find out in all preliminary tests. These finding clearly shows that the SARS-CoV-2 Omicron Virus has growth advantage over the other variant. (*Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern*, 2021).

The rise of SARS-CoV-2 Omicron Virus

In early 2021 SARS-CoV-2 spread was high. In November 2020 scientist spotted a variant called B.1.1.7. This variant was named as Alpha which contains large number of mutations in spike proteins. This Virus was first observed in Southeast London with 50% faster rate than previous virus variant. At the same time another mutation-laden variant called B.1.351 now known as Beta was found (Mallapaty, 2021). This Virus was responsible to the second wave of infection. After some time another variant Gamma variant was found in Amazonas state in Brazil.

Origin state of Corona virus Variant	Virus Variant name
Alpha B.1.1.7 Variant	Southeast London
Beta B.1.351 Variant	South Africa
Gamma B.1.1.28 Variant	Amazonas state in Brazil
Delta B.1.617.2variant	India's Maharashtra state
Omicron B.1.351Variant	South Africa's Gauteng province

All of these variants have mutation within the Spike regions with interacts with host-cell ACE2 receptors. It was most likely known that descendant of Alpha carry many bunch of mutations which evades immune response giving rise to new form of Corona Viruses (Mallapaty, 2021).

Symptoms:

The symptoms of Covid-19 reported up till now are fever, cough, sore throat, Shortness of breath or chest pain, headache, nausea or fatigue, stuffy or runny nose, sore eyes, loss of smell or taste, muscle pain, sneezing, reduced appetite and abdominal pain (Rafael, 2021). Omicron cases reported up till now show approximately similar symptoms with mild severity. Comparison of both Covid-19 and Omicron symptoms is enlisted in the following Table1.

Table 1. Comparison of Covid-19 and Omicron symptoms.

Symptoms	Omicron	Covid-19
Fever	Sometimes.	Common.
Cough.	Sometimes.	Common.
Shortness of breath or chest pain.	Rare.	Common.
Headache.	Common.	Common.
Nausea or fatigue.	Common.	Common.
Stuffy or runny nose.	Common.	Common.
Sore throat.		Common.
Loss of smell or taste.	Rare.	Common.
Muscle pain.	Common.	Common.
Sneezing.	Common.	Rare.
Reduced appetite.	Rare.	Common.
Abdominal pain.	Rare.	Common.

Milder Symptoms than other variants:

As reported by early studies, Omicron-infected patients and animals showed relatively mild symptoms. Omicron variant only slightly activated the NF-kB pathway is the main reason for the onset of mild symptoms (Maurya *et al.*, 2023). Several mutations have been observed in spike (S) protein of omicron. These mutations result in alteration of conformation and may also affects its ability to interact with neutralizing antibodies, receptors and some other interactors (Gobeil *et al.*, 2021). The researchers used pseudovirus-based vesicular stomatitis virus (VSV) and cell-cell fusion systems as well as NF-kB reporter systems to detect the impact of S mutations (Cai *et al.*, 2021). Even though the T716I mutation was present, the bands of Omicron cleaved were weak, suggesting that other mutations around the Furin cleavage site are responsible for the S protein proteolytic cleavage. A flow cytometry experiment demonstrated the soluble receptor angiotensin converting enzyme 2 (sACE2) binding affinity to S protein expressed in 293T. In turn, this cleavage may modify the conformation of the S trimer and result in the instability of virions. Various levels of ACE2 and transmembrane protease serine 2 (TMPRSS2) have been detected to be infective in cell lines harboring this variant. Omicron variant had a weaker infectivity. There are also several known membrane-bound serine proteinases (TMPRSS2/11D/11F/13) that may play a significant role in facilitating all variants infection, excluding Omicron. Besides the cathepsin-mediated entry from endosomal vesicles, SARS-CoV-2 is also capable of entering from cytoplasmic membranes via TMPRSS-like proteases (Li *et al.*, 2022). According to recent studies Omicron followed opposite path as compare to other strains of Covid-19. SARS-CoV-2 virus is different from SARS-CoV-1 because of a different cleavage site called Furin. Deleted Furin site let most of SARS-CoV-2 virus enter though E64d-sensitive pathway, similar to SARS-CoV-1 virus. A mutation in Omicron S (K, R, H) leads to the accumulation of amino groups (-NH3+), which increases protonation and renders it

susceptible to hydrolysis by endosomal enzymes such as cathepsin B/L. Compared to other variants of Covid-19, Omicron S protein is more likely to enter through the endocytic pathway.

The fusion of cells caused by S protein and ACE2 may cause excessive inflammation by inducing pyroptosis. Omicron S protein might alter its pro-inflammatory activity as a result of its fusogenicity change. Omicron variants do not substantially activate the NF- κ B pathway, confirming early reports that infected humans and animals show relative mild symptoms. Also, TMPRSS2, which activates NF- κ B pathway, is also involved in syncytial formation, implying that syncytial formation is responsible for the activation of pro-inflammatory pathways.

Host immunity:

The challenge of detecting SARS-CoV2 immune correlates is explained by various factors. It is a member of a family of viruses that is known to cause infections and recurrences by default. It is also true that different individuals respond differently to the same virus (Sette and Crotty, 2021). The following example illustrates this point. Approximately 95 percent of Indian adult males are 5'5" in height, with a range of 5'5" and 6'5". Consequently, a height parameter of 5'5 represents the average height for almost all members of the population (Plotkin, 2010). The range of antibodies produced against the SARS-CoV2 virus is so varied, however, that it is impossible to create an average level, i.e., one above which patients are protected and one below which they remain susceptible. Third, there are two mechanisms of immunity toward this virus. The first of these is the ability to prevent the virus from infecting the individual, which is related to antibodies against the virus, while the other is protection from diseases and organ damage caused by the infection, which is unrelated (McMahan *et al.*, 2021; Sette and Crotty, 2021).

Host Evasion:

In Omicron strain, there are several mutations in the spike (S) protein, which may alter its conformation and interactions with its receptor, neutralizing antibodies, or other interactions.

Impact of S mutations:

Recent researches relied upon the use of vesicular stomatitis virus-based pseudoviruses, cell-cell fusions, and nuclear factor kappaB (NF- κ B) reporter systems to detect S mutations. These researches revealed that initially, the expression of variant S proteins was comparable, but the cleaved bonds (S2) differed. There was evident weakness in the bands cleaved by Omicron and Alpha in spite of the P681H mutation, indicating that other mutations existed near Furin's sites of cleavage. With flow cytometry, the researchers found that soluble receptor angiotensin-converting enzyme 2 (sACE2) could bind to 293T S protein. The S trimer conformation can be destabilized and virions become unstable by cleavage.

Infectivity in Cell lines:

Several research studies have demonstrated that these variants infect cell lines with varying levels of ACE2 and transmembrane protease serine 2 (TMPRSS2). The infectivity of Delta, Lambda, and Mu pseudoviruses was similar to, or higher than, that of D614G. In contrast, Omicron variant was less infectious than D614G when infected with either of the Caco2-ACE2/TMPRSS2 or the Calu3 cells, but more infectious when infected with Caco2, Huh7, or Vero-E6 cells (Jamil *et al.*, 2022; Meng *et al.*, 2022). There are also several known membrane-bound serine proteinases (TMPRSS2/11D/11F/13) that may play a significant role in facilitating all variants of infection, apart from Omicron. SARS-CoV-2 also enters endosomes via cathepsin, in addition to the TMPRSS like proteases at cytoplasmic membrane. Pre-treating 293T/Caco2- ACE2-TMPRSS2 cells with inhibitors (Camostat and E64d) was used to analyze the selection of the entry path. While Delta pseudovirus entered more frequently through the Camostat-sensitive path than Omicron, the reverse was true for D614G. In SARS-CoV-2 the deletion of the Furin site resulted in the virus entering via a path sensitive to E64d, making it similar to SARS-CoV-1. In addition, as with Delta, addition of a basic amino acid (K, R, H) to Omicron S protein results in the formation of more amino groups (-NH₃⁺) and sensitivity to hydrolytic enzymes like cathepsin B/L. Contrary to Delta and other variants, Omicron S prefers endocytic entry (Peacock *et al.*, 2022).

Fusogenicity:

The Delta variant has strong fusogenicity and pathogenicity due to the P681R mutation (Saito *et al.*, 2021). The split GFP/renilla luciferase (Rluc8) reporter system was used to quantitatively characterize the fusogenicity of cells fusional mediated by S and ACE2. Mu and Omicron variants had weaker fusogenicity than WT or D614G despite their P681H mutation, while Delta, Lambda, and P681H/R were the most powerful. Interestingly, Alpha S protein showed similar Furin-related cleavage with Omicron, but also showed the same fusogenicity with WT and D614G. These findings suggest undefined mutations (e.g., N679K, N856K, Q954H, N969K, and L981F) on the cleavage

sites of S1/S2, S2', and the heptad repeat 1 (HR1) in Omicron S protein facilitate. A comparison of Omicron's fusion rate with SARS-CoV-2 variants also showed that it is similar to that of SARS-CoV-1. Another T7 polymerase-mediated cell–cell fusion reporter system also demonstrated this pattern.

Inflammatory Responses:

Researchers speculated that the fusogenicity change could alter Omicron S protein's pro-inflammatory effects based on the recent report that cell-cell fusion mediated by S protein and ACE2 could exacerbate excessive inflammatory responses (Ma *et al.*, 2021). As measured by reporter assay, S proteins of Delta, Lambda, and Mu variants significantly activated NF- κ B pathway in comparison to WT and D614G. NF-KB pathway activation by Omicron variant was only modest, which fits with early reports of relatively mild symptoms in both humans and animals infected with Omicron (Maslo *et al.*, 2021; McMehan *et al.*, 2022). Also, TMPRSS2, which activates NF-KB pathway, is also involved in syncytial formation, implying that syncytial formation is responsible for the activation of pro-inflammatory pathways.

Tendency of Immune escape:

Omicron S has undergone heavy mutations; therefore, the tendency of immune evasion from existing vaccination protection is worrying. Monoclonal neutralizing antibodies (mNAb) to variants Mu and Omicron are also compromised. The first difference is that Omicron prefers cathepsin-dependent (E64d-sensitive) entry pathways, unlike Delta and other variants (Camostat-sensitive). It is possible that these researches may explain the variation of TMPRSS-like proteases in host tissues and cells and suggest that cathepsin and TMPRSS-like inhibitors might be useful for treating all SARS-CoV-2 variants. Furthermore, Omicron and Mu are significantly less fusogenicity than other variants in spite of the P681H mutation. Third, Omicron S proteins have a tempered pro-inflammatory effect consistent with fusogenicity. In addition, mu and omicron variants have the greatest ability to escape immunity to vaccination and mNAb because of heavy mutations. Omicron possesses all of these characteristics and is therefore unique compared to Delta and other variants, which provide the ability to spread broadly among fully vaccinated individuals, and change the clinical symptoms and tropisms. Omicron and future variants of Coronavirus may be best combated with a combination of multiple treatment modalities, as well as variant-specific and pan- β -coronavirus antibodies and vaccines (He *et al.*, 2021). Mutations in S proteins of lambda, mu, and omicron variants alter infectivity, toxicology, and immunology, severely jeopardizing current therapies and prophylaxis. This highlights the need for strict epidemic prevention policies.

Sex and Gender Bias in COVID-19 Case Reports:

A study published in *Frontiers in Global Women's Health* found that case reports of patients with COVID-19 were biased in the same way as previous studies, including an over representation of males. Nicole Woitowich, PhD, research assistant professor of Medical Social Sciences, and senior author of the study, says that recognizing biases caused by sex and gender is an important step towards changing them. Woitowich says education and research should both strive for gender- and sex-balanced information. Nearly 500 clinical case reports of patients with COVID-19 were studied by Woitowich and her colleagues: 45 percent had menstrual dysfunction, 30 percent had females, and only 25 percent of the reports had males and females (Woitowich *et al.*, 2020). Men were more likely to be the senior authors of case reports with male patients compared to women. Similarly, female authors included more patients of both sexes in their works. Woitowich said gender imbalances are not a new phenomenon in biomedical science, but COVID-19's novelty means researchers should take extra time to investigate any gender differences in illness, a search that is hampered by reports of gender bias. "Following a new paradigm of assessing how conscious and unconscious bias may affect decision-making and clinical care, the biomedical community has made progress, Woitowich said. Nevertheless, this study suggests that much more work needs to be done and it may fall to journal editors to make sure such reports are balanced and representative (Woitowich *et al.*, 2020).

Ascertainment bias:

A major problem of the ongoing pandemic is that perception often leads to bias. The vast majority of Omicron patients, for example, might appear critically ill to someone who only attends to patients in the intensive care unit. If they only see outpatients, they might think the majority of Omicron patients have mild symptoms. Incoming passengers are screened by airport staff who may conclude that most do not have symptoms. This is a phenomenon known as ascertainment bias, and it is caused by not taking a representative sample.

Spread of omicron Virus

SARS-CoV-2 Omicron variant (B.1.1.529) is spreading in almost more than 100 countries. However this is more likely that in many countries this virus is present but it is not currently been reported. (Del-Rio *et al.*, 2021). After the initial identification from South Africa this virus has been detected in 46 US states where there was already Delta Virus was at its peak. In US on December 20, 2021, 70,000 infected with SARS-CoV-2 were hospitalized with 1300 death per day. Further within 3 weeks omicron become becomes dominant over delta Virus (Del-Rio *et al.*, 2021).

Vaccination effectiveness against

Many highly effective vaccines are developed for this virus targeting SARS-CoV-2 spike protein. All these vaccines greatly helped to reduce the infection rate and deaths. However this vaccination efficacy is put at the risk because of the high mutations in spike proteins of SARS-CoV-2 strain. It is more likely that SARS-CoV-2 Omicron variant will show more resistant towards currently used vaccines and therapeutic mAbs.

Van Blargan *et al.* (2022) found in their study many highly neutralizing antibodies shows inhibitory activity towards SARS-CoV-2 Omicron in their advance clinical development. Monotherapy and combination therapy was evaluated in patients that have pervious history SARS-CoV-2 isolates. Amino Acid changes in omicron B.1.1.529 variant shows a negative effect on neutralizing antibodies .AstraZeneca shows a moderate reduction of antibody neutralization (Van Blargan *et al.*, 2022). Other Vaccines like Regeneron, Eli Lilly, Celltrion were not quit effective and show almost complete loss of neutralizing activity. However these results need to be validated in vivo.

Pfizer Vaccine Effectiveness on Omicron Variant

In some studies Omicron shows considerably but not a complete escape from Pfizer r BNT162b2 Vaccine (Cele *et al.*, 2021) in their study in which they engineered lungs cell line. These cell lines were engineered to over express hACE2 receptor. This cell line was generated to check both to isolate omicron and for its neutralization ability. In this study they compare the neutralization capacity of study participants which were vaccinated with Pfizer and those who were not vaccinated.19 plasma samples were tested.6 samples were from those patients who have np previous history of SARS-CoV-2 infection and remaining samples were previously infected and vaccinated one. They found out that samples having previous history of infection and vaccination show higher neutralization capacity. But this capacity was lower relative to neutralize the ancestor viruses i.e delta or alpha virus.

Effect of booster vaccine on Omicron Variant

Vaccines are very essential to mitigate this severe COVID -19 diseases (Hogan *et al.*, 2021).However all of this vaccines loss their efficacy whenever new variant of virus appears. (Hogan *et al.*, 2021).shows in their report that NAT (neutralizing antibody titres) is less to 4.5 fold for omicron hen compared to Omicron variant. However, when 60 days Pfizer-BioNTech booster post data was compared there was a drop of vaccine efficacy was seen from 96.5% against delta to 80.1% against omicron. (Hogan *et al.*,2021).This data clearly suggests that Pfizer-BioNTech booster dose is going to be very effective to alleviate the future effects of omicron virus in different countries. However in near future Omicron variant specific vaccines are much needed in current situation.

Evolution of Covid-19 and Omicron and their relationship

Positive sense, single-stranded RNA is present in members of Coronaviridae (CoV) family. SARS CoV disease emerges from bats (Baghchechi *et al.*, 2020) like MERS but not cause any disease in host (Wertheim *et al.*, 2013). There are four genera of corona virus on the basis of genetic characteristics i.e. *Alphacoronavirus* (*alpha-CoV*), *Betacoronavirus* (*beta-CoV*), *Gammacoronavirus* (*gamma-CoV*), and *Deltacoronavirus* (*delta-CoV*) (Forni *et al.*, 2017). The coronavirus which cause recent pandemic belongs to *Betacoronavirus*, which is mainly present in animals then it gain ability to transmit to animals Sharma and Lal, 2017). They adapt themselves and cross animal-human species barrier and cause severe damage (Chowell *et al.*, 2014). Since 2019, SARS-CoV-2 have been mutating and these mutations have effect on ability of virus to infect cells. According to a study, genomic sequence of SARS-CoV-2 resembles with SARS-like bat coronaviruses RaTG13 with a percentage of 96.2% collected from Yunnan, with 79% to the SARS-CoV and 51.8% of MERS-CoV (To *et al.*, 2013).

Omicron is another variant of SARS-CoV-2 firstly reported from South Africa. It has more ability to transmit than delta variant (Del-Rio *et al.*, 2021). Large number of mutations are present in open reading frame of Omicron virus (Zhou *et al.*, 2020) which has characteristics of infection. There are many hypothesis regarding the evolution of Omicron. First one is that, Omicron could have ambiguously outspread and take its course in a population with inadequate viral observation. Secondly, evolution of Omicron could also be in incurable Covid-19 patient because it

provides a suitable environment. Thirdly, Evolution took place in non-human host and then transmit to human. (Callaway, 2021). Second hypothesis is most accepted and popular one (Wei *et al.*, 2021).

The Omicron variant possess a new monophyletic clade whose properties are distant from other SARS-CoV-2 variants. Omicron variant is closely related to alpha. On the Basis of percentage of sequence identity, the closest variants of SARS-Cov-2 were in the following order: Omicron, Alpha, Gamma, Delta, Beta, Mu, and then the SARS-CoV-2 (Fig.1).

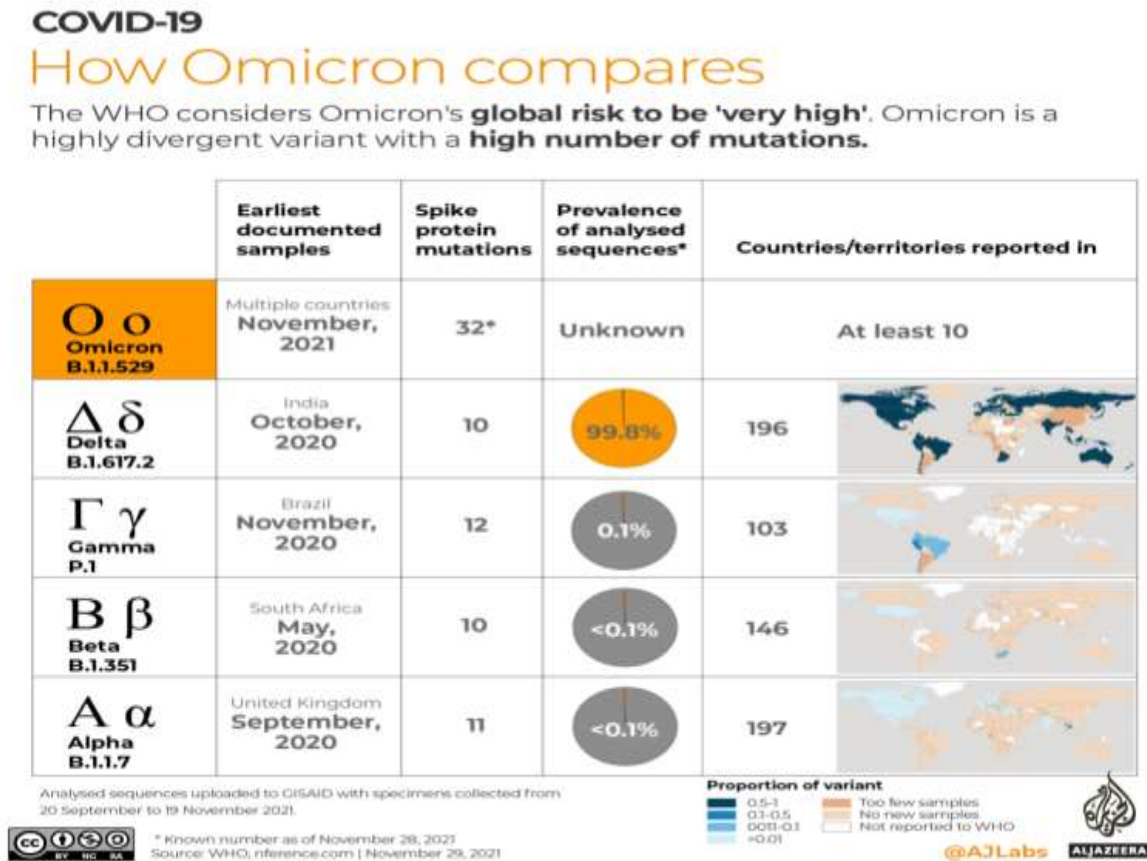


Fig. 1. Future virus spread and more SAR virus evolution and suggestion to combat or boost virus development (WHO | Coronavirus pandemic News | Al Jazeera).

According to a study, in future virus may be able to take two turns either converted to more transmissible form by quickly replicating or spread through sneezing, coughing or by controlling host immune response. According to genome sequencing of virus (performed in earlier days of pandemic), rate of mutation in SARS CoV virus is less than that of HIV and influenza (Callaway, 2021). There will be two possibilities i.e. either during omicron variant people get natural immunity so that virus will be suppress to low levels or it may also happen that virus get some mutation, rise up some new variant that widen the pandemic era. We cannot say with surety what will happen in future but virus will not go in coming generations.

According to Karen Mossman (Mossman, 2021), a professor of pathology and molecular medicine at McMaster University in Ontario, it is expected that new variants will come out but it is not necessary that every variant cause same damage. According to Mossman, the new variant will cause less severity of disease but will be more transmissible. SARS-CoV-2 can resist against many mutations while keeping its ability to infect human cells. According to Dr. Jonathan Abraham (Abraham, 2022), an assistant professor of microbiology at Harvard Medical School, flexibility of spike protein shows that there will be a new variant of SARS-CoV-2 in future (Marshall, 2022).

According to Chandran (Chandran, 2021), the risk of new variant can be reduced by vaccinating a large number of people to limit the space of virus. If efficacy of vaccine is partial, it still protects people from getting sick. Vaccine works by boosting immune system on sensing any SARS-CoV-2 virus. It may be said that the future of

SARS-CoV-2 is still in hands of human. By vaccinating more people, changes in virus that cause a new variant to emerge, can be stopped.

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