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COVID-19: Challenges of Viral Variants

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Abstract

The COVID-19 pandemic has been accompanied by SARS-CoV-2 evolution and emergence of viral variants that have far exceeded initial expectations. Five major variants of concern (Alpha, Beta, Gamma, Delta, and Omicron) have emerged, each having both unique and overlapping amino acid substitutions that have affected transmissibility, disease severity, and susceptibility to natural or vaccine-induced immune responses and monoclonal antibodies. Several of the more recent variants appear to have evolved properties of immune evasion, particularly in cases of prolonged infection. Tracking of existing variants and surveillance for new variants are critical for an effective pandemic response.

INTRODUCTION

Viruses evolve as a result of genetic errors (mutations) made during replication—including recombination between viral genomes—and selective forces in the environment that favor one variant over the other. Although each class of viruses (RNA and DNA) has unique life cycle characteristics that contribute to their evolution, mammalian viruses tend to evolve toward an optimal balance of replication, transmission efficiency, and immune evasion that leads to persistence within the susceptible host population. RNA viruses are well known for their plasticity, due in part to low-fidelity RNA-dependent RNA polymerases and lack of genomic repair mechanisms, such as proofreading and mismatch repair (1, 2). Since its emergence in late 2019, SARS-CoV-2 has shown remarkable plasticity in evolutionary adaptation, initially in the setting of unchecked worldwide spread and subsequently within a complex landscape of selective forces including natural and vaccine-induced immune response and monoclonal antibodies (mAbs). Herein, we review (*a*) the chronology of SARS-CoV-2 evolution as of this writing (May 2022), with a focus on variants of concern (VOCs) designated by the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO); (*b*) within-host evolution of SARS-CoV-2; (*c*) viral immune escape; and (*d*) the impact of variants on COVID-19 prevention and treatment strategies.

SARS-COV-2 VARIATION

When SARS-CoV-2 first emerged, many predicted that its evolutionary rate would be at the lower end of the spectrum of RNA viruses, largely because the unique proofreading function of the coronavirus-encoded replicase complex results in less frequent mutations than other well-known RNA viruses (3, 4). However, the evolutions of SARS-CoV-2 have outpaced initial expectations. One likely reason is the sheer speed and geographic breadth of infection. To date, there have been approximately 500 million reported infections worldwide and many billions of cycles of viral replication, with each cycle capable of generating consequential amino acid substitutions. Additionally, the species jump from bats to humans, which is supported by available phylogenetic evidence, provided substantial selective pressure for viral evolution (5). A virus that is able to overcome such a species barrier has innate plasticity. Finally, viral recombinants can arise when two different viral variants infect the same host and replicate simultaneously in the same cell. This dual infection can lead to template switching of the viral polymerase from one viral genome to the other, generating a hybrid or recombinant viral genome containing portions of the two original genomes. Such recombinants can be incorporated into progeny virions and can infect and replicate in new cells if the recombinant is viable, which is not always the case. Viable recombination can result in a marked shift in virulence, transmissibility, and immune evasion and has been proposed as the evolutionary process leading to emergence of SARS-CoV-2 in humans (6–9). RNA viruses, including coronaviruses, are well known to recombine regularly (7), and recombination is most likely during periods of high transmission when more than one variant is circulating (10). Given the rapid and wide spread of SARS-CoV-2 variants, it is not surprising that viral recombinants have been detected (10, 11), although they have not become dominant frequently. Ongoing surveillance and analysis to identify successful recombinants are critical since they can be highly consequential.

EARLY VARIATION OF SARS-COV-2

In the absence of vaccine or infection-elicited immunity early in the pandemic, optimal transmission was the main driver of viral evolution. The first significant evolutionary change was the D614G substitution in the Spike glycoprotein identified in early 2020, which quickly became dominant globally (12) and is now established in all B-derived lineages. [B lineages are derived

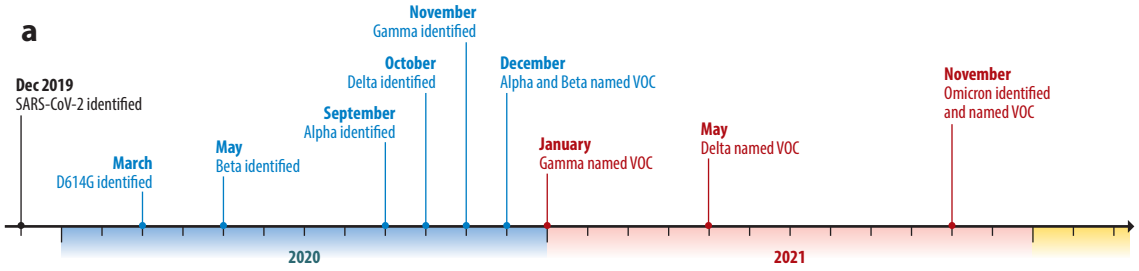
from the ancestral strain Wu-Hu-1, sampled December 26, 2019, in Wuhan, China (GenBank accession no. MN908947). All VOCs fall within this lineage.] Work by Korber et al. and others showed that the D614G substitution may provide a transmission advantage by increasing the ability to infect ACE2-expressing target cells, resulting in higher amounts of infectious virus in the host and thus greater likelihood of transmission (12–14). Animal studies have since confirmed that D614G leads to higher viral levels in the upper airways and increased transmissibility (15, 16).

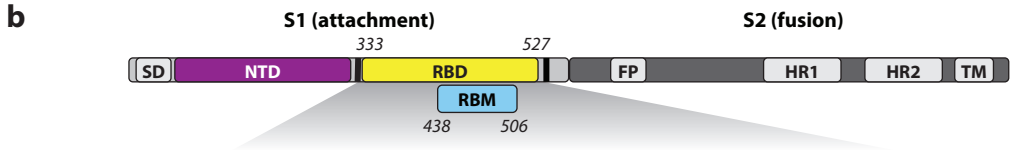
SARS-COV-2 VARIANTS OF CONCERN

Since 2020, the world has gained partial population-level immunity from natural infection and vaccination that has added an extra barrier for the virus to overcome to maintain replication fitness and persistence in human populations. This shift coincided with the emergence and recognition of VOCs, each with greater transmissibility than ancestral SARS-CoV-2 and varying abilities to cause more severe disease and to evade host immunity. As it became clear that SARS-CoV-2 would continue to meaningfully evolve, the US CDC and WHO, among other international public health agencies, began identifying and tracking variants with potential for serious public health consequences. According to the CDC, a variant with evidence of (*a*) impact on diagnosis, treatment, or prevention of disease; (*b*) increased transmissibility; and/or (*c*) increased disease severity is likely to be classified as a VOC if its prevalence is expected to increase substantially (18). It is important to note, however, the challenges in determining the relative transmissibility of variants. Transmissibility can be difficult to quantify because it involves a complex interplay between properties of viral infectivity, host population susceptibility to infection, and host population behavior (19). Many variants have been described (20), but we have focused this review on those designated by WHO and CDC as VOCs as of May 2022. The temporal emergence of VOCs is illustrated in **Figure 1*a***.

ALPHA VARIANT OF CONCERN

The Alpha, or B.1.1.7, VOC was identified in the United Kingdom in September 2020, was classified as a VOC in December 2020 (**Figure 1*a***), and was noted to contain an unusually large number of mutations (21, 22). It contains 13 non-synonymous mutations and 4 deletions (**Figure 1*b***), a surprising jump in evolution given the lack of apparent evolutionary change in SARS-CoV-2 since the discovery of D614G. Six substitutions and 2 deletions were found in Spike, leading some to hypothesize that Alpha evolved in a single immunocompromised host with prolonged infection (23). Alpha was eventually determined to be ∼50% more transmissible than D614G (**Figure 1*c***), and it quickly spread to replace D614G as the dominant variant worldwide (24). Alpha showed an increase in disease severity as measured by undesirable outcomes (need for critical care or death) (25), but it did not show evidence of significant immune evasion (antibody resistance) from natural infection or vaccine-elicited immunity (26, 27). Important genetic changes include the 69/70 deletion, which has been found independently and repeatedly and has been proposed to aid in evasion of N-terminal domain (NTD)-specific antibodies that play an important role in immunity to SARS-CoV-2 (28); it has also been proposed as compensatory for RBD mutations that also mediate immune escape (26). The N501Y substitution in the RBD of Alpha has also arisen repeatedly and has been shown to enhance viral fitness and transmissibility by increasing affinity to the ACE2 host receptor (29, 30). Importantly, genetic variation in the receptor binding domain (RBD) of Spike is highly consequential since it is an immunodominant region (17), it is critical for ACE2 receptor binding, and most vaccines that are currently in use induce production of Spike as the immunogen, which contains the RBD.





	RBD																		
Alpha																	N501Y		
Beta								K417N						E484K			N501Y		
Gamma								K417N						E484K			N501Y		
Delta											L452R		T478K						
Omicron BA.1	G339D	S371L	S373P	S375F				K417N	N440K	G446S		S477N	T478K	E484A	Q493R	Q496S	Q498R	N501Y	Y505H
Omicron BA.2	G339D	S371F	S373P	S375F	T376A	D405N	R408S	K417N	N440K			S477N	T478K	E484A	Q493R		Q498R	N501Y	Y505H

c

	Transmission efficiency	Pathogenicity	mAb Resistance*	Protection from previous infection	Vaccine efficacy
Alpha	↑	↑	None	No change	No change
Beta	↑	↑	Bamlanivimab-Etesevimab	↓	↓
Gamma	↑	↑	Bamlanivimab-Etesevimab	↓	↓
Delta	↑	↑	Bamlanivimab	↓	↓
Omicron BA.1	↑	↓	Bamlanivimab-Etesevimab, Casirivimab-Imdevimab	↓	↓
Omicron BA.2	↑	↓	Bamlanivimab-Etesevimab, Casirivimab-Imdevimab, Sotrovimab	↓	↓

*Resulting in modification or rescinding of EUA.

(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Emergence of SARS-CoV-2 variants of concern (VOCs) and their defining substitutions. (*a*) Timeline of SARS-CoV-2 VOCs. Date of first identification indicates earliest documented samples. Panel adapted from Timeline (6 Segments, Horizontal, Black and White) 4 by BioRender.com (2022), retrieved from **https://app.biorender.com/biorender-templates**. (*b*) Defining substitutions present in the receptor binding domain (RBD) of the Spike glycoprotein of variants as compared to the ancestral Wu-Hu-1 strain (GenBank accession no. MN908947). Substitutions within the RBD are colored yellow, and substitutions within the receptor binding motif (RBM) of the RBD are colored blue. (*c*) Characteristics of SARS-CoV-2 VOCs as compared to the ancestral Wu-Hu-1 strain.

BETA VARIANT OF CONCERN

Beta (B.1.351) was identified in South Africa in May 2020 and declared a VOC by the CDC alongside Alpha in December 2020. Beta quickly ignited a large surge of infection in South Africa. This raised concerns worldwide, since preliminary analyses of the Spike substitutions suggested extensive immune evasion as evidenced by reductions in neutralizing antibody titers of sera from people with prior infection with variants other than Beta (31, 32). The Beta variant was shown to cause more severe disease and higher transmissibility than ancestral SARS-CoV-2 (33, 34), although it did not result in the magnitude of global spread that was initially feared, for reasons that are still unclear. Some have suggested that Beta's advantage in South Africa was related to the immune status of the South African population at the time of its emergence. The country had just experienced a large wave of infections leading to high population-based immunity. In other global communities without such immunity, immune evasion properties of a VOC might not have been as favored. This thesis would also suggest that immune pressure contributed to the selection and emergence of Beta. The immune resistance of Beta has been ascribed to a trio of RBD substitutions: K417N, E484K, and N501Y (35, 36). The E484K and K417N substitutions have been shown to reduce antibody neutralization (36, 37), whereas N501Y increases binding to ACE2 to enhance transmissibility, as noted above.

GAMMA VARIANT OF CONCERN

Gamma (P.1) was detected in November 2020 in Brazil and was classified as a VOC in January 2021. Similar to Beta, Gamma ignited a significant surge locally without leading to substantial worldwide spread. Analyses suggested that it was more transmissible and more likely to result in death than other variants circulating at the time (38). Notably, Gamma contains substitutions in the same trio of amino acids in the RBD as Beta (K417T rather than K417N, E484K, and N501Y, noted above), which enable humoral immune evasion and enhance infectivity. These traits were further confirmed by a high SARS-CoV-2 reinfection rate in the region of Manaus, Brazil (39, 40). The emergence of the triple mutation in the RBD (K417N/T, E484K, and N501Y) in at least two different VOCs seemed to suggest convergent evolution (38, 40, 41), leading some to postulate restricted plasticity of the SARS-CoV-2 Spike glycoprotein (42). This hypothesis has been proved to be incorrect by the emergence and worldwide spread of highly divergent VOCs.

DELTA VARIANT OF CONCERN

Delta (B.1.617.2) was the first variant after Alpha to cause rapid global spread, displacing Alpha as the dominant variant in most places. The earliest samples containing Delta were found in India in October 2020, and it was declared a VOC in May 2021. Of note, it was during this period that vaccines were being rolled out globally. Data on disease severity of Delta compared to Alpha are conflicting. Some reports suggested that Delta may cause more severe illness than Alpha based on increased hospitalization rates (43, 44), but an analysis by the CDC showed that Delta did not cause more severe disease than Alpha as measured by intensive care unit admission,

mechanical ventilation, or death. The higher rate of hospitalization was attributed to more infections in demographics with low vaccine coverage (45, 46). Delta's increased transmissibility was evident almost immediately based simply on its rapid global spread, and many studies have unequivocally confirmed that at the time of its emergence Delta was the most transmissible VOC to date (47–50). An early study attributed greater transmissibility to higher levels of virus in the respiratory tract based on cycle threshold (Ct) values obtained by qRT-PCR (quantitative reverse transcription polymerase chain reaction) assays (48). Laboratory studies of Delta viral isolates showed accelerated growth kinetics, higher levels of released virions, and higher levels of cleaved Spike compared to Alpha (51), providing an explanation for higher virus levels. Analysis of the RBD and other Spike substitutions in Delta, coupled with a higher rate of breakthrough infections in vaccinated individuals, led to the hypothesis that Delta was evading host humoral immune responses (51–54), a hypothesis further strengthened by the results of lab experiments using blood from previously infected and vaccinated people to neutralize authentic or pseudotyped virus (51, 55).The two substitutions present in the Delta Spike RBD, L452R and T478K, did not increase ACE2 binding (52); however, the L452R substitution has been shown to confer approximately fivefold reduced susceptibility to polyclonal antibodies in plasma from vaccinated individuals (56). In addition, several NTD substitutions that lie within antigenic supersites (G142D, E156G, and del 157/158) reduce antibody binding of NTD-specific mAbs by at least tenfold (52).

OMICRON VARIANT OF CONCERN

Omicron (B.1.1.529) is the most recently identified VOC as of May 2022. The earliest Omicron samples were identified in November 2021, and it was officially classified a VOC at the end of that month. Omicron contains an impressive number of mutations in Spike (>30), including a collection of Spike substitutions that were previously identified and characterized in other circulating variants and are known to aid in immune evasion and increase infectivity (**Figure 2**). In addition, it contains deletions and substitutions in the NTD antigenic supersite, including the 69/70 deletion seen in multiple previously circulating variants, most notably Alpha. Omicron also

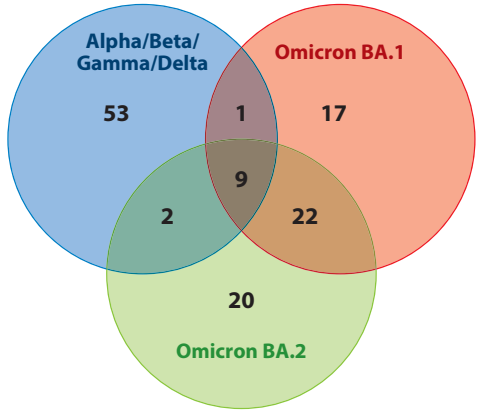


Figure 2

Venn diagram of all substitutions present in variants of concern compared to the ancestral SARS-CoV-2 strain, Wu-Hu-1 (GenBank accession no. MN908947). All substitutions in Alpha, Beta, Gamma, and Delta combined are in blue, Omicron BA.1 in red, and Omicron BA.2 in green. Venn diagram and associated table generated using **https://bioinformatics.psb.ugent.be/webtools/Venn/**.

contains the infectivity-enhancing N501Y substitution in conjunction with E484A (rather than K) and K417N, which is the same trio of changes that reduced antibody sensitivity in the Beta and Gamma VOCs. Collectively, the substitutions concentrated around the regions involved in viral fusion and host cell receptor engagement are likely to enhance viral infectivity (57), whereas the high number of substitutions in the NTD and RBD regions likely contribute to humoral immune evasion. The latter has been confirmed in multiple studies showing reduced neutralization by sera from vaccinated individuals or those previously infected with other variants (58–67). The enhanced transmissibility of Omicron is likely due to a combination of higher virus levels in the upper respiratory tract and humoral immune evasion (66, 68, 69). Although more transmissible than previously circulating VOCs, Omicron causes less severe disease both in animal models and by epidemiological assessments (70–74), possibly due to a lower level of viral replication in the lower respiratory tract (68, 70–72). It should be noted that despite the lower proportion of deaths caused by Omicron, the absolute number of those deaths remains substantial because of the sheer volume of COVID-19 cases caused by Omicron (75).

Three sublineages of Omicron emerged at around the same time, BA.1, BA.2, and BA.3. Although BA.1 had been the dominant lineage globally, at the time of this writing BA.2 has begun to overtake BA.1 in multiple regions of the world including Asia, Europe, and the United States. By contrast, BA.3 has not spread widely. An early study indicated that BA.2 may be more transmissible than BA.1 (76), although pre-existing immunity from prior infection (including BA.1 infection) and vaccination does seem to protect against infection or severe disease from BA.2 (77). The rapid replacement of BA.1 by BA.2 in some locations argues that it has a selective advantage, likely from higher transmissibility because the sensitivity of BA.1 and BA.2 to immune sera appears to be similar. Additionally, the Omicron sublineages BA.2.12.1, BA.2.13, BA.4, and BA.5 may exhibit higher transmissibility than BA.2, with stronger neutralization evasion against the plasma of individuals who had received three-dose vaccines and, most strikingly, of vaccinated BA.1 convalescents (78).

The origin of Omicron has been widely discussed because it contains a remarkable number of previously unobserved substitutions (**Figure 2**) and because phylogenetic analyses suggest it was not derived from prior circulating VOCs (57). The three main theories addressing its emergence as a highly mutated variant are (*a*) undetected circulation in an isolated human population, accumulating mutations over time; (*b*) evolution in a single prolonged chronic infection; and (*c*) re-emergence from an animal reservoir (57). Studies are ongoing to determine which of these theories is correct.

EMERGENCE OF VARIANTS OF CONCERN IN IMMUNOCOMPROMISED HOSTS

It has been hypothesized that VOCs emerged in immunodeficient hosts who were unable to control viral replication, thereby facilitating intrahost viral evolution, which then spread to other individuals (79). Although this hypothesis has been difficult to prove, multiple case reports and case series have demonstrated that certain immunocompromised individuals are at risk for protracted viral replication and intrahost evolution of multiply-mutated variants (**Table 1**). For instance, in a patient who developed COVID-19 following administration of chimeric antigen T cell receptor modified therapy, six different SARS-CoV-2 sequence variants were identified over the span of approximately 2.5 months (80). The Spike sequences of the initial infecting strain matched the GH clade (containing D614G), but additional mutations, such as R190K and G1124D, developed within 2 weeks of infection. Over time, multiple additional mutations developed, such as a Y144 deletion and a D215G substitution, which were later identified in the Alpha and Beta variants, respectively. More recently, researchers in South Africa characterized intrahost evolution of SARS-CoV-2 over 6 months in an individual with advanced HIV infection (81). The patient's

Table 1 Summary of reports of prolonged SARS-CoV-2 infection with intrahost viral evolution

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
98	CLL (anti-CD20 monoclonal antibody + cyclophosphamide, doxorubicin, prednisone)	Pneumonia, death	Remdesivir (×2), CP (×2)	Culture positive on day 119	NR	Antibody negative or low-positive	Multiple SARS-CoV-2 variants present simultaneously; mutations appeared prior to antiviral therapy
99	CLL	Asymptomatic, survival	CP (×2)	Culture positive on day 70	NR	Antibody negative except after CP	Several SNPs within the ORF1ab, Spike, M, and ORF8 coding sequence; deletions in Spike coding region and NTD (141–144, 139–145)
100	Antiphospholipid syndrome (steroids, cyclophosphamide, rituximab)	Pneumonia, death (day 154)	Steroids, remdesivir (×3), casirivimab-imdevimab	Culture positive on day 143	Detected for >150 days	Low levels of B cells, robust T cell responses to SARS-CoV-2, delayed rise in SARS-CoV-2 IgG, waxing/waning IgM, low levels of neutralizing antibodies	Deletions at residues 141–144 of the Spike NTD
101	HCT ($n = 18$), CAR-T cell therapy ($n = 2$)	55% with severe disease; 20% mortality at 30 days	Remdesivir ($n = 2$), CP ($n = 8$), tocilizumab ($n = 5$), steroids ($n = 9$)	Samples from 3 patients with HCT/CAR-T within <6 months grew SARS-CoV-2 >20 days after infection (days 25, 26, and 61)	NR	7 seropositive patients out of 15 tested	Multiple longitudinal mutations in Spike and other domains
80	Multiple myeloma, CAR-T cell therapy	Pneumonia, death (day 74)	Remdesivir, CP (×2), steroids	Culture positive on day 72	Detected for >70 days	0% CD19+ B cells; antibody negative (marginal effect of CP); attenuated SARS-CoV-2–specific T cell responses	Emergence of 6 sequence variants with mutations in Spike (including NTD and RBD regions). The following mutations were identified: Y144 deletion, H146 deletion, D215G, N501T, L241-Y248

(*Continued*)

Table 1 (*Continued*)

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
102	Diffuse large B cell lymphoma, chemotherapy rituximab	Pneumonia, death (day 101)	Remdesivir (×3), CP (×3), steroids (×2), tocilizumab (×1)	Virus could not be cultured from stored respiratory samples, but clinical course and continued viral evolution suggest ongoing replication up to day 101	NR	Diminished T cell responses; antibodies only detected after CP	Various mutations in Spike, RdRp, and other regions (e.g., N501Y); deletions emerged after CP
103	AIDS (CD4 = 0/mm^3; HIV VL 275,000 copies/mL)	Pneumonia	NR	Culture positive on day 95	NR	Antibody negative	Sequencing of SARS-CoV-2 on days 19, 35, 53, and 75 showed no evidence of evolution; transient appearance of a C23718T mutation (Spike) on day 53
	Heart transplant (belatacept, mycophenolic acid, prednisone)	Pneumonia	NR	Culture positive on day 103	NR	Antibody negative	Various mutations in ORF1a, ORF1b, Spike (D614G), and ORF3a (virus sequenced on days 80, 91, and 103); minimal evolution
	Rheumatoid arthritis, rituximab	Pneumonia	Steroids	Culture positive on day 84	NR	Antibody negative up to day 121; 0% CD19+ B cells	Various mutations in Spike (D614G, L8W), ORF1a, ORF1b, E, and M; mention of possible superinfection
104	Non-Hodgkin's lymphoma, chemotherapy, rituximab, bendamustine, cytarabine (0% CD19+ B cells)	Recurrent pneumonia and respiratory failure, with >9 months of active SARS-CoV-2 infection before death	Steroids, tocilizumab, CP, remdesivir	Positive culture on day 268	Detected for >260 days	Antibody negative; 0% CD19+ B cells	Various mutations in Spike (D614G, W1214C, I1221K, G1223C, H69Y, H69P, V70G, S982A),ORF1a, ORF1b, ORF3a, and N

(*Continued*)

Table 1 (*Continued*)

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
105	20 patients (10 SOT, 5 hematologic malignancy)	Various	Various	2 patients with positive viral cell cultures (CLL receiving venetoclax-obinutumzab, day 23; marginal zone lymphoma with bentamustine and rituximab, day 22)	NR	NR	D614G in the CLL patient; D614G, S98F, S813I in the lymphoma patient
106	Non-Hodgkin's lymphoma, rituximab	Fever, pneumonia	Steroids, remdesivir	Positive culture on day 10	NR	Antibody negative >270 days	Multiple
107	Kidney transplant, rituximab for ITP	Recurrent episodes of fever and pneumonia	None	Positive culture on ~day 90	NR	NR	G142D; 143–145 deletion in NTD of Spike
108	Follicular lymphoma, rituximab	Recurrent episodes of fever and pneumonia >200 days	Steroids, remdesivir (×3), bamlanivimab	Culture positive on day 90, negative on day 107, positive on day 118	NR	Anti-N negative; anti-Spike detected	D614G
81	AIDS (CD4 = 6 cells/μL	Hypoxia at initial presentation, otherwise mild symptoms	Dexamethasone	Culture positive on days 6, 20, 34, 71, 106, and 190 (negative on days 204, 216)	NR	Anti-RBD IgG negative to borderline through day 106; positive as of day 190	Intrahost evolution of SARS-CoV-2 over span of 6 months, with emergence of mutations found in VOCs; mutations were clustered in the NTD and RBD, including K417T, F490S, and N501Y, and others

(*Continued*)

Table 1 (*Continued*)

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
109	Diffuse large B cell lymphoma, CAR-T cell therapy	Recurrent pnemumonia for ~1 year, with varying severity	CP (×2), remdesivir, steroids	NR, but PCR positive for ~1 year, with emergence of novel mutations	NR	Anti-Spike and -N IgG negative	Multiple mutations and deletions in Spike and other parts of genome, suggesting coexistence of several variants; WGS indicated persistent infection, not reinfection
110	AIDS (CD4 = 2 cell/mm^3)	Pneumonia followed by asymptomatic infection	Dexamethasone, CP	NR, but PCR positive for 71 days, with emergence of novel mutations	NR	Anti-Spike and -N IgG negative on day 47, low-positive after CP; T cell responses to SARS-CoV-2 antigens negative; no Spike-specific B cells	Emergence of the following mutations on day 71 versus day 12: ORF1a (V3718A), N (P13L and R185C), ORF9b (P10S), and Spike (T724D and the Y144 deletion)
111	Kidney transplant, non-Hodgkin's lymphoma, chemotherapy, rituximab	Waxing and waning pneumonia, intermittently positive and negative PCRs	Steroids, remdesivir	Culture positive on days 64 and 86	NR	Anti-Spike and -N IgG negative; robust T cell responses to SARS-CoV-2-specific peptide pools	WGS indicated persistent infection, with emergence of several SNPs during course of illness (location of SNPs in genome not described)
112	B cell ALL, HCT, blinatumomab, inotuzumab	Waxing and waning course between mild infection and pneumonia	Steroids, remdesivir	Culture positive on day 78, negative on days 91 and 97	NR	Anti-S1 IgG negative (day 87)	Multiple mutations in ORF1ab, Spike, and N; on day 97 emergence of Y144 deletion and S494P

(*Continued*)

Table 1 (*Continued*)

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
113	CLL (venetoclax, rituximab)	Recurrent pneumonia followed by asymptomatic infection	Steroids, CP, remdesivir	NR, but emergence of multiple variants throughout disease course (44 days of illness)	NR	NR	Multiple mutations in Spike
	Hodgkin's lymphoma (chemotherapy)	Pneumonia, recurrent fever, followed by asymptomatic infection	Steroids, bamlanivimab	NR, but emergence of multiple variants throughout disease course (~90 days of illness)	NR	NR	Multiple mutations in Spike
114	Kidney transplant (tacrolimus, mycophenolate, prednisone)	Asymptomatic infection (ground glass opacities on chest CT)	Steroids, remdesivir	Culture positive through day 105 but not beyond	NR	Anti-N IgG detected on day 12; anti-S1 IgG detected on day 140	Multiple mutations in Spike associated with escape from antibody neutralization
115	Lung transplant, CVID, kidney transplant, myelodysplasia	NR	Sotrovimab	Culture positive for up to 24 days	NR	NR	Treatment-emergent mutations in the E340K/A/V locus (and to a lesser extent P337L/T) conferring resistance to sotrovimab emerged over span of 6–13 days
116	ALL, HCT, rituximab	Recurrent pneumonia	CP, remdesivir, casirivimab-imdevimab (×2)	NR, but >370 days of viral replication and clinical syndrome compatible with COVID-19; PCRs became negative by day 410, after second dose of casirivimab-imdevimab	Detectable for >200 days	NR	Intrahost evolution over >200 days, emergence of N501Y mutation and other mutations in Spike

(*Continued*)

Table 1 (*Continued*)

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
117	Non-Hodgkin's lymphoma, checkpoint inhibitor, rituximab, vinblastine	Asymptomatic initially, followed by fulminant pneumonia	Dexamethasone	Positive culture on day 33 (death on day 39)	NR	Lack of SARS-CoV-2–specific antibodies, B cells, and activated T helper cells	Genomic analysis suggested persistent infection but with minimal intrahost evolution [1 mutation in ORF1ab (P5371S) identified at day 26, and deletion in ORF1ab (del:11288:9) identified at day 33]
118	Kidney transplant, tacrolimus, mycophenolic acid, prednisone	Respiratory failure, with 94 days of illness	CP, tocilizumab	NR	NR	Increase in antibodies following CP therapy	Emergence of mixed viral populations with several Spike protein mutations (del 141–144 and del 243–244, E484K, Q493K, and Q493R) on day 21; predominance of del 141–144 E484K on day 27
119	Follicular lymphoma, rituximab	~200 days of recurrent pneumonia, death	Dexamethasone, remdesivir (×4), CP, tocilizumab	NR	NR	Antibody negative	Emergence of multiple SNPs within the Spike gene, ORF1ab, ORF7a, ORF8a
	Follicular lymphoma, rituximab	~120 days of recurrent pneumonia, recovery	Dexamethasone, remdesivir (×2), tocilizumab	Culture positive on days 24, 35, 47, 79, and 112	NR	Antibody negative	Coexistence of several viral variants with different mutations
	Follicular lymphoma, rituximab	~70 days of recurrent pneumonia, death	Dexamethasone, remdesivir (×2)	Culture positive on day 66	NR	Antibody negative	Coexistence of several viral variants with different mutations

(*Continued*)

Table 1 (*Continued*)

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
120	CLL, venetoclax, rituximab	~80 days of recurrent pneumonia	Bamlanivimab, remdesivir	NR, but evidence of intrahost evolution	Detectable for 33 days	NR	Compartmentalized viral populations with multiple mutations (e.g., E484, N501Y); mutations conferred reduced susceptibility to bamlanivimab
121	Angioimmunoblastic T cell lymphoma	Fever, pneumonia, survival	Bamlanivimab, CP	NR, but evidence of intrahost evolution	NR	NR	Emergence of several mutations by day 7, including E484Q, E484K, and E484A, conferring reduced susceptibility to bamlanivimab
	Mantle cell lymphoma	Fever, pneumonia, death	Bamlanivimab	NR, but evidence of intrahost evolution	Detectable on day 14	NR	Emergence of several mutations on day 7, including E484Q and E484K, conferring reduced susceptibility to bamlanivimab
122	Follicular lymphoma, obinutuzumab	Protracted pneumonia	No COVID-19–specific therapies	NR, but evidence of intrahost evolution	NR	Antibody negative	Coexistence of multiple viral variants
123	Diffuse large B cell lymphoma	Protracted respiratory insufficiency (~190 days)	Remdesivir, steroids	Viral culture attempted on sample from day 164, negative; WGS suggested persistent infection	NR	Antibody negative	Comparison of virus from day 1 and 164 revealed 3 deletions and 15 SNPs; mutations found in ORF1ab, Spike, ORF3a, E, M, ORF7a, N
	AIDS	Cough followed by prolonged asymptomatic infection	NR	NR, but evidence of intrahost evolution over 45 days	NR	Antibody negative until day 51	Emergence of NTD and RBD mutations over ~45 days associated with reduced antibody neutralization

(*Continued*)

Table 1 (*Continued*)

Reference	Immunosuppressive condition	Syndrome	Therapies given	Duration of persistent SARS-CoV-2 infection	Plasma RNAemia	Immune responses	Results of viral sequencing
124	CLL	Protracted and recurrent pneumonia	Remdesivir (×2), dexametha-sone, CP (×2)	Culture positive on days 129 and 197; still PCR positive on day 333	NR	Poor antibody response, unable to neutralize pseudovirus	Emergence of multiple viral variants, temporally associated with administration of CP
125	Follicular lymphoma, rituximab	Pneumonia; recovered, with relapsed symptomatic infection 4 months later	Remdesivir, CP during relapse	NR, but WGS suggested persistent and relapsed infection	NR	Antibody negative (low-positive IgG after CP)	Genomes of isolates at 0 and 4 months differed by 12 base-pair substitutions
126	B cell ALL, CAR-T cell therapy	Recurrent pneumonia	Remdesivir (×2), CP (×10)	Persistently culture positive through ~day 160 (PCR positive through day 250)	NR	Antibody negative; low-positive after CP	Increase in intrahost viral diversity over time; accumulation of multiple Spike gene mutations (e.g., **S**:Δ141–143, S:Δ145, and S:Δ141–144, a deletion-insertion at S:Δ211–212, and several nonsynonymous mutations including N440K, V483A, and E484Q)
	B cell ALL, chemotherapy	Recurrent pneumonia	Remdesivir	Culture positive through day 30; negative ~day 60; positive again ~day 140	NR	IgG negative for most of illness; low-positive IgG ~day 180	Increase in intrahost viral diversity over time; accumulation of multiple Spike gene mutations

Abbreviations: ALL, acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor-modified T; CLL, chronic lymphocytic leukemia; CP, convalescent plasma; CT, computed tomography; CVID, common variable immunodeficiency; HCT, hematopoietic cell transplant; ITP, idiopathic thrombocytopenia purpura; N, nucleocapsid; NR, not reported; NTD, N-terminal domain; ORF, open reading frame; PCR, polymerase chain reaction; RBD, receptor-binding domain; SNP, single nucleotide polymorphism; SOT, solid organ transplant; VL, viral load; WGS, whole-genome sequencing.

early viral sequence was similar to the ancestral sequence, but it evolved a multitude of mutations (primarily in the RBD and NTD regions of the Spike gene) found in VOCs. The variant showed evidence of sequential evolution of humoral immune escape, including variable degrees of humoral immune escape from the BNT162b2 vaccine, weak neutralization by self and convalescent plasma, and extensive escape of neutralization by antibodies elicited by the Delta variant.

IMPACT OF VARIANTS OF CONCERN ON VACCINE EFFICACY

Vaccines have demonstrated excellent efficacy against ancestral SARS-CoV-2 (82, 83). Although it is expected that the ability of vaccines to prevent infection will wane over time, the rapid emergence of SARS-CoV-2 VOCs has resulted in an unexpectedly accelerated reduction in vaccine neutralization ability, which appears most pronounced for Omicron (**Figure 1*c***) (58–65, 84–86). This reduction in neutralization is fortunately ameliorated with additional vaccine doses. A study of >200,000 patient encounters in the United States found that mRNA vaccine effectiveness against laboratory-confirmed COVID-19–associated emergency room visits after two vaccine doses was significantly lower during the Omicron wave compared to the Delta wave (64). In another study, receipt of three doses of an mRNA vaccine resulted in superior protection against symptomatic COVID-19 caused by either Omicron or Delta, although protection was still lower for Omicron than Delta (87). Similar findings have been observed after primary vaccination with ChAdOx1 nCoV-19 or BNT162b2, both of which were less effective at preventing symptomatic COVID-19 caused by Delta than Alpha (85) and provided limited protection against Omicron (59). Although protection against Omicron increased after a booster mRNA vaccine dose, it waned by 9 weeks after vaccination (59). Nonetheless, vaccination, particularly with the use of booster doses, continues to provide excellent protection against severe disease or death (58, 60, 61), with the exception of certain high-risk groups such as those with immunocompromising conditions (65, 86).

IMPACT OF VARIANTS OF CONCERN ON THERAPEUTIC OPTIONS

The emergence of VOCs has also impacted treatment and prophylaxis of COVID-19. Because many of the VOC-specific mutations are localized to the Spike gene, it is not surprising that the greatest effect has been observed for therapeutic mAbs, all of which target the RBD of the Spike protein. Due to the emergence of mAb-resistant VOCs, the US Food and Drug Administration has revoked authorization for several ineffective mAbs (**Figure 1*c***). For example, authorization for the use of bamlanivimab-etesevimab in the United States was revoked because of ineffective neutralization of the Gamma and Beta variants but reissued once Delta became dominant, as susceptibility to Delta was restored. Unfortunately, neither bamlanivimab-etesevimab nor casirivimab-imdevimab is capable of adequately neutralizing Omicron (88), resulting in revocation of their authorizations in the United States (89). Neutralization activity of sotrovimab was initially retained against all existing VOCs (88). Most recently, sotrovimab was shown to ineffectively neutralize BA.2 (90) and is no longer authorized for use in the United States (April 5, 2022) as BA.2 has dominated (91). One additional mAb, bebtelovimab, is active against BA.1 and BA.2 and has received emergency use authorization for treatment of mild–moderate COVID-19 based on limited phase II data (92).

The tixagevimab-cilgavimab combination, which has recently been authorized for use as preexposure prophylaxis among immunocompromised individuals (93), was shown to effectively neutralize the Beta, Gamma, and Delta variants, but neutralization of Omicron was several-fold lower (88). This reduction may be overcome by administering a double dose of tixagevimab-cilgavimab (93).

By contrast, resistance to small-molecule antivirals has not been observed. Despite the presence of mutations in RNA-dependent RNA polymerase and the main protease of SARS-CoV-2 in the Omicron variant, drugs targeting these enzymes (remdesivir, molnupiravir, and nirmatrelvir, respectively) continue to retain activity (88). Nevertheless, continued surveillance for emergence of resistance to small molecules is warranted.

PREDICTIONS FOR THE FUTURE

The pattern of evolution thus far for SARS-CoV-2 has not followed the ladder-like evolutionary pattern that well-known respiratory viruses such as seasonal coronaviruses and influenza viruses follow in response to immune selection (94, 95). This is not surprising given the absence of population immunity to SARS-CoV-2 when it first emerged. In a ladder-like evolutionary pattern, each new viral variant is derived from the previous variant, representing genetic drift in response to host immune pressure. Conversely, for SARS-CoV-2, divergent solutions to enhance transmission and evade humoral immune responses have been observed, particularly in the Omicron lineage. The most optimistic prediction for the future of SARS-CoV-2 evolution includes a shift toward a ladder-like evolutionary pattern, since significant human immunity has now been increased. This scenario would be unlikely to produce a variant with a major increase in virulence. However, as was the case with Omicron, emergence of a highly divergent variant originating from prolonged infection of an immunocompromised host or from an animal reservoir is not implausible. In this case, both increased virulence and immune evasion are possible.

Variants of SARS-CoV-2 continue to present a clear challenge for treatment, prevention, and diagnosis of COVID-19. A key unanswered question is whether prior VOCs will re-emerge with time, from the human population or an animal reservoir, or whether they are now extinct. In addition, methods to predict new mutations and combinations of mutations that could arise in variants may help in preparing for their emergence. For example, a recent report describes a new method to identify consequential mutations based on genomic and epidemiologic surveillance data; the method was able to retrospectively predict VOC-containing mutations (96), and functional assays have been used to probe the mutational landscape of the RBD to determine the capacity for escape from immune responses and therapies (30, 97). Although insightful, such methods may fall short of predicting variants that emerge in the future because of the complexity of host–virus interaction and the plasticity of virus biology. Additional studies to expand the prediction and detection of SARS-CoV-2 variants along with the prompt implementation of measures to contain their spread are critical for the future of global public health.

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