BRIEF REPORT

Vaccine Breakthrough Infections with SARS-CoV-2 Variants

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SUMMARY

Emerging variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are of clinical concern. In a cohort of 417 persons who had received the second dose of BNT162b2 (Pfizer–BioNTech) or mRNA-1273 (Moderna) vaccine at least 2 weeks previously, we identified 2 women with vaccine breakthrough infection. Despite evidence of vaccine efficacy in both women, symptoms of coronavirus disease 2019 developed, and they tested positive for SARS-CoV-2 by polymerase-chain-reaction testing. Viral sequencing revealed variants of likely clinical importance, including E484K in 1 woman and three mutations (T95I, del142–144, and D614G) in both. These observations indicate a potential risk of illness after successful vaccination and subsequent infection with variant virus, and they provide support for continued efforts to prevent and diagnose infection and to characterize variants in vaccinated persons. (Funded by the National Institutes of Health and others.)

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections caused more than 83 million known cases of coronavirus disease 2019 (Covid-19) by the end of 2020, but tremendous progress has been made with the authorization and deployment of vaccines and antibody therapies. These strategies are directed at the viral spike protein, but the emergence of viral variants, particularly in the *S* gene, threatens their continued efficacy.

These concerns have provided an impetus to increase testing and sequencing of viral DNA in infected persons in order to understand the transmissibility, virulence, and ability of variants to evade current vaccines. New York City has seen a troubling increase in viral variants. Most of these variants, which accounted for more than 72% of new cases as of March 30, 2021, were the B.1.1.7 variant first identified in the United Kingdom (in 26.2% of the cases) and the B.1.526 variant first identified in New York City (in 42.9%).[1] Two areas of concern relate to the ability of variants to evade vaccine-induced immunity and cause asymptomatic infection (and thereby promote viral spread) or illness. Both consequences are important, both need to be considered independently, and both are largely unknown.

We describe two fully vaccinated persons in whom subsequent breakthrough infections with SARS-CoV-2 variants harboring a number of substitutions of interest developed. Despite evidence that the first dose of vaccine led to a strong antibody response to spike protein in Patient 1, saliva-based polymerase-chain-reaction

(PCR) screening at Rockefeller University identified infection 19 days after the second dose (the booster vaccination). Testing was also positive for infection in Patient 2, who had completed vaccination 36 days previously. Together, these observations provide support for current strategies to monitor multiple variables proactively. These strategies include viral testing of symptomatic and asymptomatic persons, sequencing of viral RNA, and monitoring of neutralizing antibody titers, particularly in vaccinated persons who subsequently become infected.

METHODS

SPECIMEN COLLECTION AND PROCESSING

Beginning in the fall of 2020, all employees and students at the Rockefeller University campus (approximately 1400 persons) were tested at least weekly with a saliva-based PCR test developed in the Darnell Clinical Laboratory Improvement Amendments–Clinical Laboratory Evaluation Program laboratory (approval number, PFI-9216) and approved for clinical use by a New York State emergency use authorization. Protocols for the collection of saliva samples for clinical SARS-CoV-2 testing were reviewed by the institutional review board at Rockefeller University and were deemed not to be research involving human subjects. Institutional review board–approved written informed consent for the analysis of antibody titers was obtained from Patient 1, and the study was conducted in accordance with International Council for Harmonisation Good Clinical Practice guidelines.

In accordance with New York State regulations regarding eligibility, 417 employees who had received a second dose of either the BNT162b2 (Pfizer–BioNTech) or mRNA-1273 (Moderna) vaccine at least 2 weeks previously were tested between January 21 and March 17, 2021, and weekly testing continued thereafter. The demographic characteristics of these 417 persons and of 1491 unvaccinated persons tested in parallel at Rockefeller University during the same period are shown in Table S1 of the Supplementary Appendix, available with the full text of this article at NEJM.org.

The employees and students were instructed to provide a saliva sample in a medicine cup and transfer 300 μl into a vial containing 300 μl of Darnell Rockefeller University Laboratory (DRUL) buffer (5 M of guanidine thiocyanate, 0.5% sarkosyl, and 300 mM of sodium acetate [pH 5.5]).[2] Samples were processed on the Thermo KingFisher Apex system for rapid RNA purification, and complementary DNA (cDNA) was amplified with the use of TaqPath 1-Step RT-qPCR (reverse-transcriptase quantitative PCR) Master Mix (Thermo Fisher Scientific) and multiplexed primers and probes that were validated under a Food and Drug Administration emergency use authorization (Table S2) with the 7500 Fast Dx Real-Time PCR detection system (Applied Biosystems). Samples were considered to be interpretable if the housekeeping control (RNase P) cycle threshold (Ct) was less than 40, and viral RNA was considered to be detected with both viral primers and probes (N1 and N2, detecting two regions of the nucleocapsid [*N*] gene of SARS-CoV-2) at a Ct of less than 40.

VIRAL LOAD CALCULATION

We calculated the viral load per milliliter of saliva using chemically inactivated SARS-CoV-2 (ZeptoMetrix) spiked into saliva at various dilutions. Extractions and RT-PCR were performed as described previously to determine the corresponding Ct values for each dilution (Fig. S1).

TARGETED SEQUENCING

Reverse transcription of RNA samples was performed with the iScript mix (Bio-Rad) according to the manufacturer's instructions. PCR amplification of cDNA was performed with the use of two primer sets (primer set 1: forward primer 1 [CCAGATGATTTTACAGGCTGC] and reverse primer 1 [CTACTGATGTCTTGGTCATAGAC]; primer set 2: forward primer 2 [CTTGTTTTATTGCCACTAGTC] and reverse primer 1). PCR products were then extracted from gel and sent to Genewiz for Sanger sequencing.

NEUTRALIZATION ASSAY

Neutralization assays with pseudotyped replication defective human immunodeficiency virus type 1 modified with SARS-CoV-2 spike protein were performed as previously described.[3] Mean serum neutralizing antibody titers (50% neutralization testing [NT_{50}]) were calculated as an average of three independent experiments, each performed with the use of technical duplicates, and statistical significance was determined with the two-tailed Mann–Whitney test.

WHOLE VIRAL RNA GENOME SEQUENCING

Total RNA was extracted as described above, and a meta-transcriptomic library was constructed for paired-end (150-bp reads) sequencing with an Illumina MiSeq platform. Libraries were prepared with the SureSelect XT HS2 DNA System (Agilent Technologies) and Community Design Pan Human Coronavirus Panel (Agilent Technologies) according to the manufacturer's instructions. FASTQ files (a text-based format for storing both a biologic sequence and its corresponding quality scores) were trimmed with Agilent Genomics NextGen Toolkit (AGeNT) software (version 2.0.5) and used for downstream analysis. The SARS-CoV-2 genome was assembled with MEGAHIT with default parameters, and the longest sequence (30,005 nucleotides) was analyzed with Nextclade software (https://clades.nextstrain.org/) in order to assign the clade and call mutations. Detected mutations were confirmed by aligning RNA sequencing reads on the reference genome sequence of SARS-CoV-2 (GenBank number, NC_045512) with the Burrows–Wheeler Aligner (BWA-MEM).

PATIENT HISTORIES

Patient 1 was a healthy 51-year-old woman with no risk factors for severe Covid-19 who received the first dose of mRNA-1273 vaccine on January 21, 2021, and the second dose on February 19. She had adhered strictly to routine precautions. Ten hours after she received the second vaccine dose, flulike muscle aches developed. These symptoms resolved the following day. On March 10 (19 days after she received the second vaccine dose), a sore throat, congestion, and headache developed, and she tested positive for SARS-CoV-2 RNA at Rockefeller University later that day. On March 11, she lost her sense of smell. Her symptoms gradually resolved over a 1-week period.

Patient 2 was a healthy 65-year-old woman with no risk factors for severe Covid-19 who received the first dose of BNT162b2 vaccine on January 19 and the second dose on February 9. Pain that developed in the inoculated arm lasted for 2 days. On March 3, her unvaccinated partner tested positive for SARS-CoV-2, and on March 16, fatigue, sinus congestion, and a headache developed in Patient 2. On March 17, she felt worse and tested positive for SARS-CoV-2 RNA, 36 days after completing vaccination. Her symptoms plateaued and began to resolve on March 20.

Table 1. Clinically Relevant Mutations in the *S* Gene and Corresponding Results in Patients 1 and 2.*

Mutation	Patient 1	Patient 2
69/70 deletion	Not detected	Not detected
D80A	Not detected	Not detected
T95I	Detected	Detected
D138Y	Not detected	Not detected
del142–145	Detected (del144)	Detected (G142V, del144)
R190T	Not detected	Detected
D215G	Not detected	Not detected
F220I	Not detected	Detected
R237K	Not detected	Detected
Δ242–244	Not detected	Not detected
R246T	Not detected	Detected
D253G	Not detected	Not detected
K417T	Not detected	Undetermined
L452R	Not detected	Undetermined
S477N	Not detected	P/H†
E484K	Detected	Undetermined
N501Y	Not detected	Undetermined
A570D	Detected	Not detected
D614G	Detected	Detected
H655Y	Not detected	Not detected
P681H	Detected	P/H†
A701V	Not detected	Undetermined
D796H	Detected	Undetermined

* These mutations are defined by the Centers for Disease Control and Prevention.[6]

† P/H indicates partial heterozygosity (sequences from both the variant strain and the original strain first identified in Wuhan, China, were detected).

RESULTS

Serial PCR tests of saliva samples were performed before and during the course of the illness in both patients (Table S3). At the time of diagnosis, PCR testing showed a Ct value that corresponded to a viral load of approximately 195,000 copies per milliliter of saliva in Patient 1 and approximately 400 copies per milliliter in Patient 2 (Fig. S1). Given the unusual clinical histories of these two patients, RNA obtained from the saliva samples was sequenced after reverse transcription and targeted PCR amplification of the SARS-CoV-2 *S* gene. This test revealed a number of differences between the sequences and the original sequence first identified

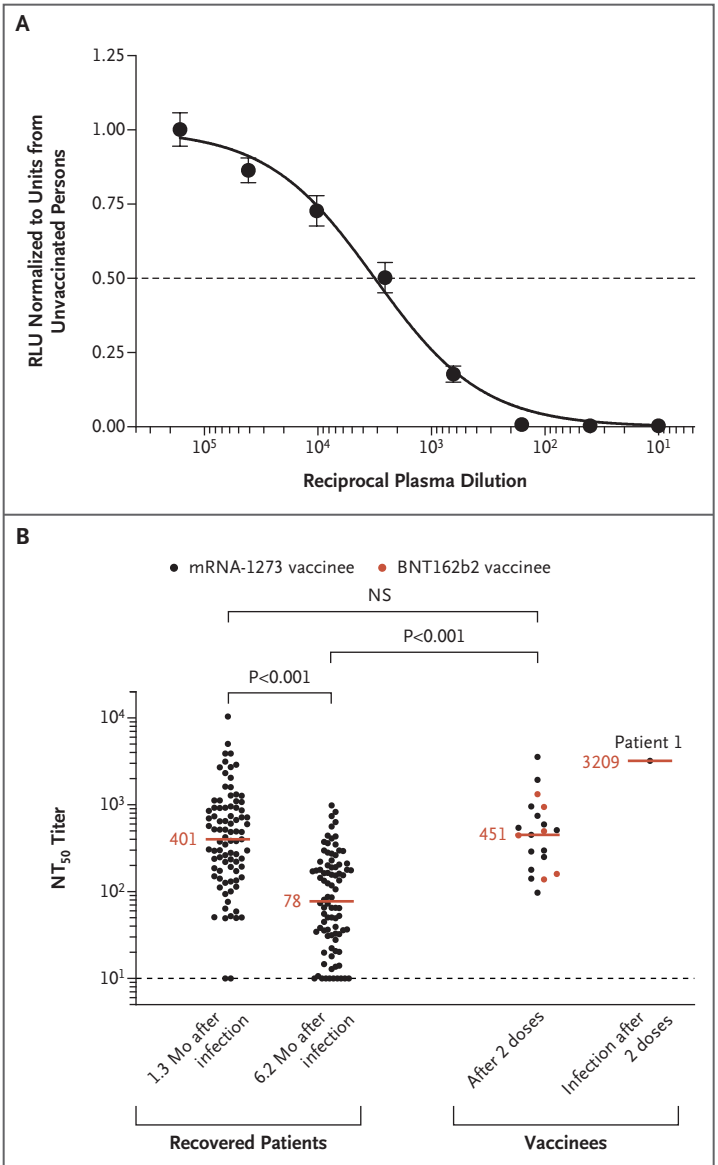

Figure 1. Evidence of Vaccine Efficacy in Patient 1.

Panel A shows the results of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pseudotype neutralization assay[7] in Patient 1. Relative luminescence units (RLUs) normalized to units from unvaccinated persons are shown. The I bars indicate 95% confidence intervals. Panel B shows the neutralizing antibody titers in serum obtained from recovered patients 1.3 months[8] and 6.2 months[9] after infection, from persons who had received both doses of either the mRNA-1273 or BNT162b2 vaccine,[4] and from Patient 1 (after vaccination and infection). The 50% neutralization testing (NT_{50}) titer among recovered patients at 1.3 months after infection did not differ significantly from that among persons who received both doses of either mRNA-1273 or BNT162b2 vaccine. The red bars indicate geometric means; high levels of neutralizing activity (mean [±SD] NT_{50}, 3209±760) in Patient 1 are shown. The mean serum neutralizing antibody titers are from three independent experiments, each performed in duplicate. NS denotes not significant.

in Wuhan, China, including several variants that have been suggested to be of potential clinical concern. In Patient 1, these mutations included E484K (which confers resistance to a commonly elicited class of neutralizing antibodies[4,5]) and D614G, and in Patient 2, these mutations included D614G and S477N (Table 1).

A serum sample obtained 4 days after the onset of symptoms in Patient 1 was analyzed for neutralizing antibodies to SARS-CoV-2 in a pseudotype neutralization assay.[3] These results revealed extremely high titers of neutralizing antibodies (Fig. 1). These data, together with the clinical history, provide strong evidence that this patient probably had neutralizing antibodies elicited by vaccination.

To explore the variants in Patient 1 in more detail, we used excess RNA available from extracted saliva to undertake whole viral genome sequencing. This analysis confirmed the sequence changes found in the *S* gene by targeted amplification and suggested that the infection resulted from a SARS-CoV-2 variant that is related to but distinct from the known variants of concern (the B.1.1.7 variant first identified in the United Kingdom and the B.1.526 variant first identified in New York City) (Figs. 2 and 3).

We therefore further tested the serum sample obtained from Patient 1 to measure its effectiveness against the wild-type virus, the E484K mutant, and the B.1.526 variant, and we found that the serum was equally effective against each (Fig. 4). These data suggest that the antibody response in Patient 1 recognized these variants but was nonetheless insufficient to prevent a breakthrough infection.

DISCUSSION

Clinical symptoms of Covid-19 developed 19 days after Patient 1 received the second dose of vaccine and 36 days after Patient 2 received the second dose. Both patients had histories consistent with a clinical response to vaccine boost. In Patient 1, documented high titers of neutralizing antibodies were present shortly after the development of symptoms. Although a baseline antibody test before illness and after vaccination would have been ideal, it remains possible that she became infected before the booster shot took full effect. Considering the clinical history,

time course, and neutralizing antibody titers measured, we conclude that it is very likely that both patients had effective immune responses to the vaccines. Although these patients presented with clinically mild symptoms, it will be very important to ascertain whether severe symptoms can or cannot develop in others despite vaccination as variants continue to evolve.[10] Taken together, our observations support the conclusion that we have characterized bona fide examples of vaccine breakthrough manifesting as clinical symptoms. Moreover, data from Patient 1 indicate that infection with variant virus can be sustained with a high viral load despite high levels of neutralizing antibodies to variants.

Examination of the SARS-CoV-2 sequences revealed that both patients were infected with variant viruses. Rapid identification of sequence variants by targeted PCR amplification showed that neither sequence precisely fit any known clade. Some of the substitutions in Patient 1 (T95I, del144, E484K, A570D, D614G, P681H, and

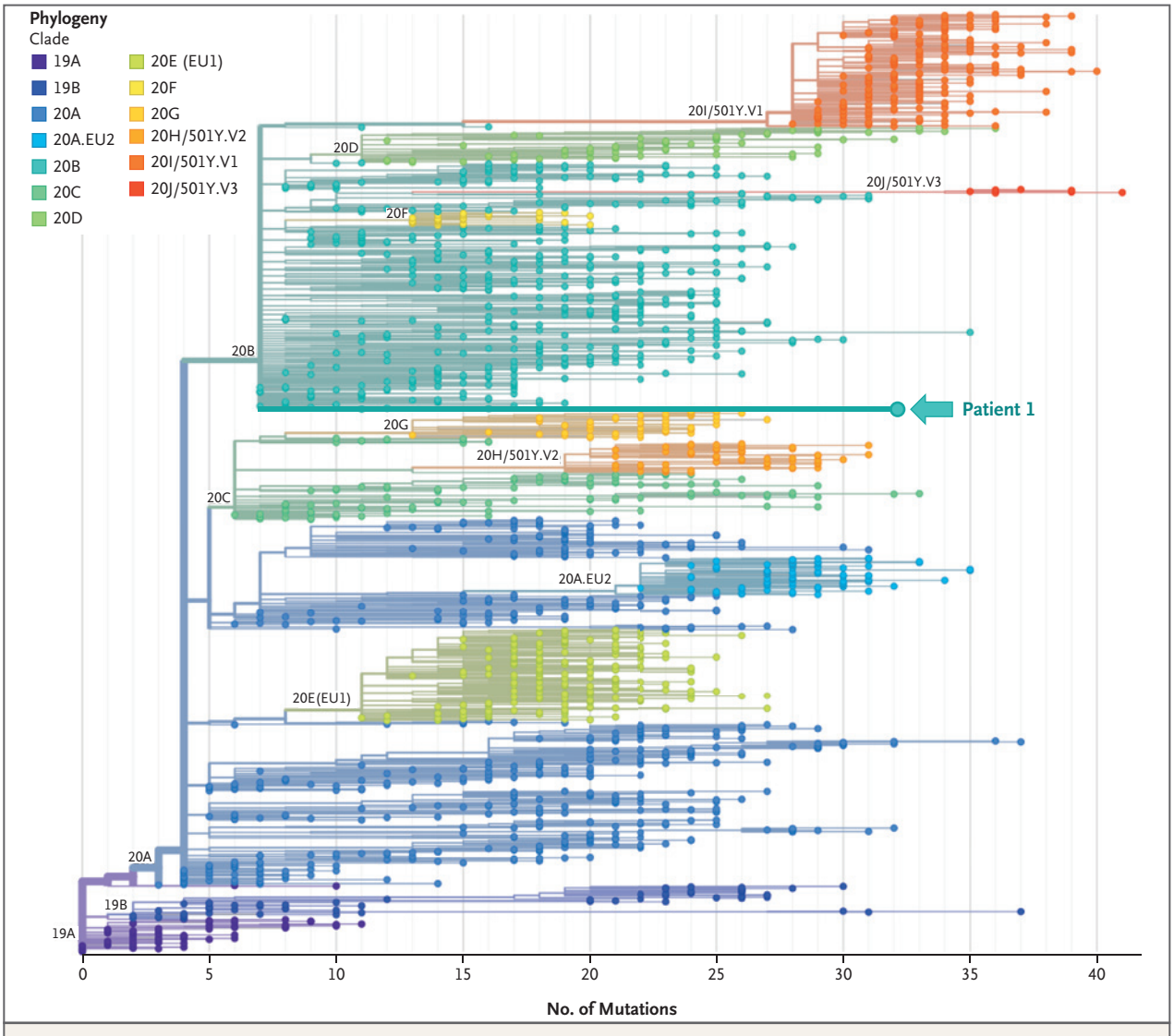

Figure 2. Phylogenetic Tree.

In the clade analysis for Patient 1, the closest match was between clades 20B and 20C. The SARS-CoV-2 variant first identified in the United Kingdom (B.1.1.7) is clade B, and the variant first identified in New York City (B.1.526) is clade 20C.

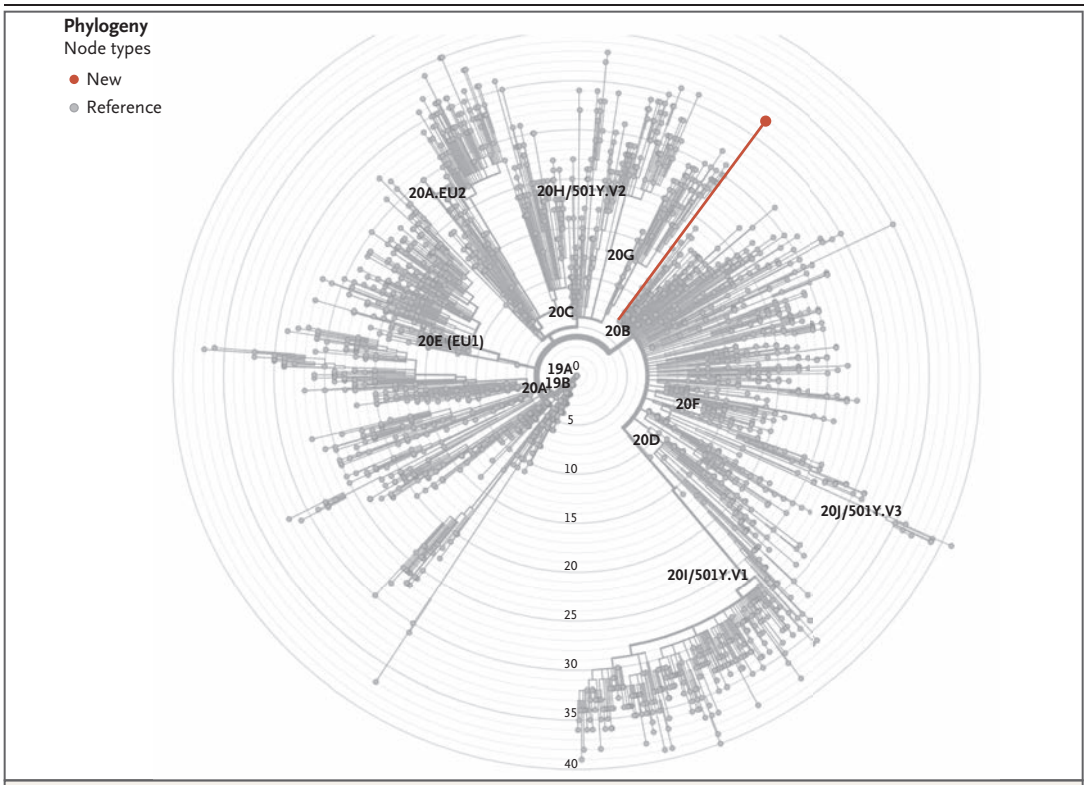

Figure 3. Phylogenetic Comparisons of SARS-CoV-2 Variants.
The red line indicates Patient 1.

D796H) were shared with B.1.526 (T95I, E484K, and D614G[6]), and three substitutions were shared with Patient 2 (in whom the variants T95I, G142V and del144, F220I, R190T, R237K, R246T, and D614G were detected). Whole viral genome sequencing revealed several additional substitutions, including D796H, present in a guanine–cytosine–rich region not identified by targeted PCR. These substitutions may decrease sensitivity to convalescent serum[11] and may have some unique noncoding changes as compared with the clades first identified in Wuhan, the United Kingdom, and New York City.

Although more detailed analysis of whole-genome sequencing from Patient 1 was undertaken, we could not conclude that the variant in this patient was a Pango lineage because it was only present in a single person.[12] Its closest links on the phylogenetic tree were the variant first identified in the United Kingdom (B.1.1.7) and the variant first identified in New York City (B.1.526), but with considerable differences (Figs. 2 and 3). It will be of interest to determine whether this may have resulted from a recombination event between B.1.1.7 and B.1.526, as has been recently reported for recombination between the B.1.1.7 lineage and the "wild-type" lineage first identified in Wuhan.[13] Alternatively, shared substitutions may be the result of convergent evolution.

These observations in no way undermine the importance of the urgent efforts being taken at the federal and state levels to vaccinate the U.S. population. They also lend support to efforts to advance a new vaccine booster (as well as a pancoronavirus vaccine) to provide increased protection against variants. In January 2021, Moderna announced clinical efforts to target a new variant of SARS-CoV-2 that was first identified in South Africa and includes three mutations

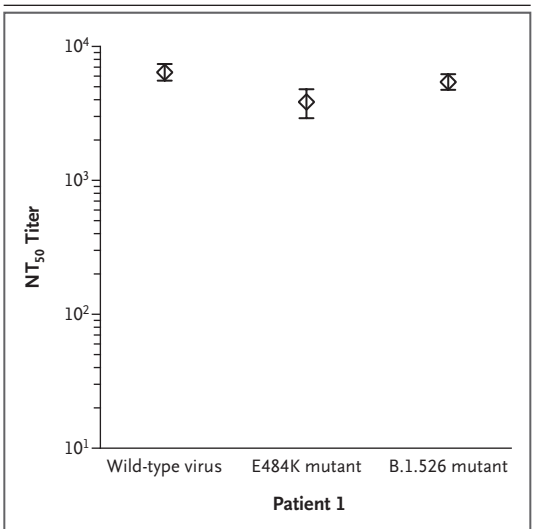

Figure 4. Neutralization Assays for Antibodies to the Wuhan-Hu-1 Isolate, the E484K Variant, and the B.1.526 Variant.

Pseudotype neutralization assays for the wild-type (Wuhan-Hu-1 isolate) virus, the E484K mutant, and the B.1.526 variant first identified in New York City (including the substitutions L5F, T95I, D253G, E484K, D614G, and A701V) were performed with a serum sample obtained from Patient 1. Shown are means and standard deviations (I bars) of three independent experiments. The E484K substitution alone or together with other substitutions in the B.1.526 variant were not significantly more resistant to neutralization by the serum from Patient 1 than the wild-type virus.

(E484K, N501Y, and K417N) in the angiotensin-converting–enzyme 2 receptor-binding domain. These efforts are of critical value because recent studies have shown that immunizations are proving to be less potent against the variant first identified in South Africa (B.1.351), which might have acquired a partial resistance to neutralizing antibodies generated by natural infections or vaccinations.[14,15] At the same time, our observations underscore the importance of the ongoing race between immunization and the natural selection of potential viral escape mutants. During this critical period, our data support the need to maintain layers of mitigation strategies, including serial testing of asymptomatic persons, open publication and analysis of vaccination and infection databases (such as those accruing data in New York City), and rapid sequencing of SARS-CoV-2 RNA obtained from a variety of high-risk persons.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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