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BRIEF REPORT

Severe Acute Respiratory Syndrome Coronavirus 2 Evolution and Escape From Combination Monoclonal Antibody Treatment in a Person With HIV

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) escape from combination monoclonal antibody treatment is rarely reported. We describe an immunocompromised individual with human immunodeficiency virus and persistent SARS-CoV-2 infection in whom substantial SARS-CoV-2 evolution occurred, including the emergence of 2 mutations associated with escape from the monoclonal antibody cocktail received.

Keywords. SARS-CoV-2; immunocompromised; HIV; monoclonal antibody; virus evolution.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mutations that confer neutralizing antibody escape have emerged during persistent infections in immunocompromised individuals, often associated with exogenous antibody treatment [1, 2]. Currently, few longitudinal studies have investigated persistent SARS-CoV-2 infection in immunocompromised people with human immunodeficiency virus (PWH). Two case reports of persistently SARS-CoV-2–infected PWH described the emergence of spike mutations that have also been found in variants of concern and are associated with immune escape [3]. However, the implications of selective pressure from monoclonal antibody treatment in PWH has yet to be explored. In addition, while escape mutations following single-agent monoclonal antibody have been well described [4–6], less is known about combination monoclonal antibody cocktail treatments. Because these use antibodies that bind to different spike domains, they are expected to select for escape mutations less frequently [4]. Here, we investigated SARS-CoV-2 evolution in an immunocompromised PWH treated with combination monoclonal antibody therapy.

CASE REPORT

A 44-year-old Hispanic man with diagnosis of SARS-CoV-2 infection 10 months prior to presentation was admitted to the hospital in the fall of 2021 with significant unintentional weight loss, chills, intermittent fevers, night sweats, and fatigue over the previous 6-month period, along with a subacute worsening of productive cough and dyspnea on exertion for several weeks. Upon presentation, the patient was hypotensive, tachycardic, and tachypneic. Pulse oximetry was >90% on room air. Laboratory testing revealed pancytopenia, high serum ferritin, and elevated liver enzymes (Supplementary Table 1). Bilateral patchy ground glass opacities were observed via lung computed tomography (Supplementary Figure 1), and abdominal imaging uncovered hepatosplenomegaly and diffuse mesenteric lymphadenopathy.

A human immunodeficiency virus (HIV) fourth-generation antibody/antigen test was positive, and the patient had an HIV viral load of >1 million copies/mL and a CD4 count of 14 (4%) cells/μL. This HIV diagnosis was new. On hospital day 0, a nasopharyngeal swab was positive for SARS-CoV-2 with a cycle threshold (Ct) value of 25.7. He had completed the Pfizer-BioNTech coronavirus disease 2019 vaccine 2-dose series, 3 weeks prior to this presentation. As his respiratory symptoms had been ongoing for a few weeks, remdesivir was not administered. Likewise, he did not meet criteria for dexamethasone, as he was not hypoxemic. The patient was treated with casirivimab/imdevimab (REGEN-COV) monoclonal antibodies on day 2. Following treatment, he regained respiratory stability but remained febrile. As his respiratory symptoms had improved, bronchoscopy and workup for additional respiratory viruses were not performed. Serum cryptococcal antigen, urine histoplasma antigen, and urine *Legionella* antigen were all negative. He tested positive for *Mycobacterium avium* complex (MAC) in sputum and blood, consistent with disseminated MAC (DMAC) infection. He was started on combination antiretroviral therapy (ART) for HIV with tenofovir disoproxil fumarate, emtricitabine, and dolutegravir. He was also started on azithromycin/rifabutin/ethambutol for DMAC, and trimethoprim/sulfamethoxazole for pneumocystis pneumonia prophylaxis. He was discharged home with close outpatient

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follow-up. A day after discharge, results were received of a positive *Pneumocystis jirovecii* DNA test from induced sputum. The clinical team contacted the patient, who remained without cough or shortness of breath; given his severe immunocompromise, he was started on a 21-day treatment course of trimethoprim/sulfamethoxazole.

A month later, the patient returned with hypotension, tachycardia, and fever. He was admitted to the hospital with concern for sepsis versus DMAC–immune reconstitution inflammatory syndrome versus hemophagocytic syndrome. Another nasopharyngeal SARS-CoV-2 swab was positive with a Ct value of 15.2; this was on day 31 since initial presentation. The patient left the hospital but returned <2 weeks later due to persistent fevers, tachycardia, and hypotension. Upon his return, a third nasopharyngeal swab revealed a SARS-CoV-2 Ct value of 14.9 (day 44). His respiratory symptoms had diminished, but he had persistent fever. He underwent an excisional inguinal lymph node biopsy, and pathology was negative for malignancy. Lymph node tissue culture was positive for MAC. The patient was continued on ART and DMAC treatment. After being afebrile for several days, he was discharged home. Unfortunately, the patient did not return to his clinic appointment 2 days after discharge and was unable to be contacted by the clinical team. A family member reported that he ceased his medication regimen due to swallowing difficulties, then stopped eating and drinking and died approximately 2 months after his last discharge.

METHODS

To investigate SARS-CoV-2 evolution over the course of the patient's infection, we generated full-length viral genome sequences from residual nasopharyngeal samples obtained on days 0, 31, and 44. We used 2 library construction approaches and a rigorous method for analyzing intrasample single-nucleotide variants (iSNVs), as described in the Supplementary Material and Supplementary Figure 2. This study was approved by the Emory University and Grady Health System Institutional Review Boards.

RESULTS

We successfully sequenced complete SARS-CoV-2 genomes with high depth at days 0, 31, and 44 (Supplementary Table 2). All sequences belonged to Delta lineage AY.119 and clustered together on a phylogenetic tree (Supplementary Figure 3). We observed substantial within-patient SARS-CoV-2 evolution: Across the 3 time points, there were a total of 78 iSNVs (Figure 1*A*) and 4 deletions (Supplementary Data File). We observed variability across the genome, with the largest number of mutations in the spike gene, whereas the 5′ end of the genome was relatively conserved. Eight consensus-level mutations arose over the course of infection, the majority of which (7/8) were nonsynonymous (Table 1). There were 12 other mutations whose frequency changed by 10% or more (Figure 1*B*). We identified 2 potential haplotypes based on alleles with similar frequencies over the course of infection. The first potential haplotype was comprised of 3 mutations (T17442C_P1325P in helicase, G18181T_D44Y in exoribonuclease, and G22289Y_A243S in spike) that were not present on day 0, were at low frequency (4%–9%) on day 31, and were at high frequency (61%–71%) on day 44 (Figure 1*B*). The second potential haplotype was comprised of 7 mutations (Nsp 2 C811_Y2Y, Nsp 3 A3779G_N354D, Nsp 3 C3924T_P402L, Nsp 3 C8344T_D1875D, spike C21621A_T20N, Orf3A C25635_C81C, and N C29167T_Y298Y) that were all at low frequency on day 0 (0%–3%), peaked on day 31 (23%–46%), and declined again on day 44 (0%–25%) (Figure 1*B*). Linkage between alleles was unable to be directly confirmed based on our short-read sequencing. Most (5/6) of the synonymous iSNVs whose frequency changed over the course of infection were found in 1 of these potential haplotypes along with 1 or more nonsynonymous iSNVs, compatible with linkage (Figure 1*B*).

Notably, we observed 2 amino acid mutations in spike that would be expected to confer escape from casirivimab (REGN10933) or imdevimab (REGN10987), and both arose between day 0 (before the patient received casirivimab/imdevimab) and day 31. Specifically, based on an experimental study that mapped escape mutations to therapeutic antibodies using scanning mutagenesis [7], spike V445A confers a high degree of escape from imdevimab (Supplementary Figure 4*A*) and spike Y453F confers a moderate to high degree of escape from casirivimab (Supplementary Figure 4*B*). Additionally, spike G476S, which was fixed in all 3 time points, occurred at a position where other amino acid substitutions have been associated with escape from casirivimab [7]; the relevance of this mutation in our patient is unknown.

DISCUSSION

Our results suggest selection for SARS-CoV-2 escape by a combination monoclonal antibody cocktail; importantly this was due to the emergence of 2 spike mutations, 1 of which was previously demonstrated to confer escape from casirivimab, and the other from imdevimab. Both rose to consensus-level SNPs between day 0 and day 31 (after administration of casirivimab/imdevimab at day 2), and both SNPs remained predominant at day 44. While casirivimab/imdevimab is not currently in use due to its ineffectiveness against Omicron variants, our study highlights the possibility of multiple resistance mutations occurring in an individual treated with combination monoclonal antibody therapy, supporting the need for further research into broader antibody cocktails or targets. Our results also emphasize that PWH with advanced immunosuppression are at risk for prolonged SARS-CoV-2 infection and underscore the importance of HIV diagnosis and treatment amid the SARS-CoV-2 pandemic.

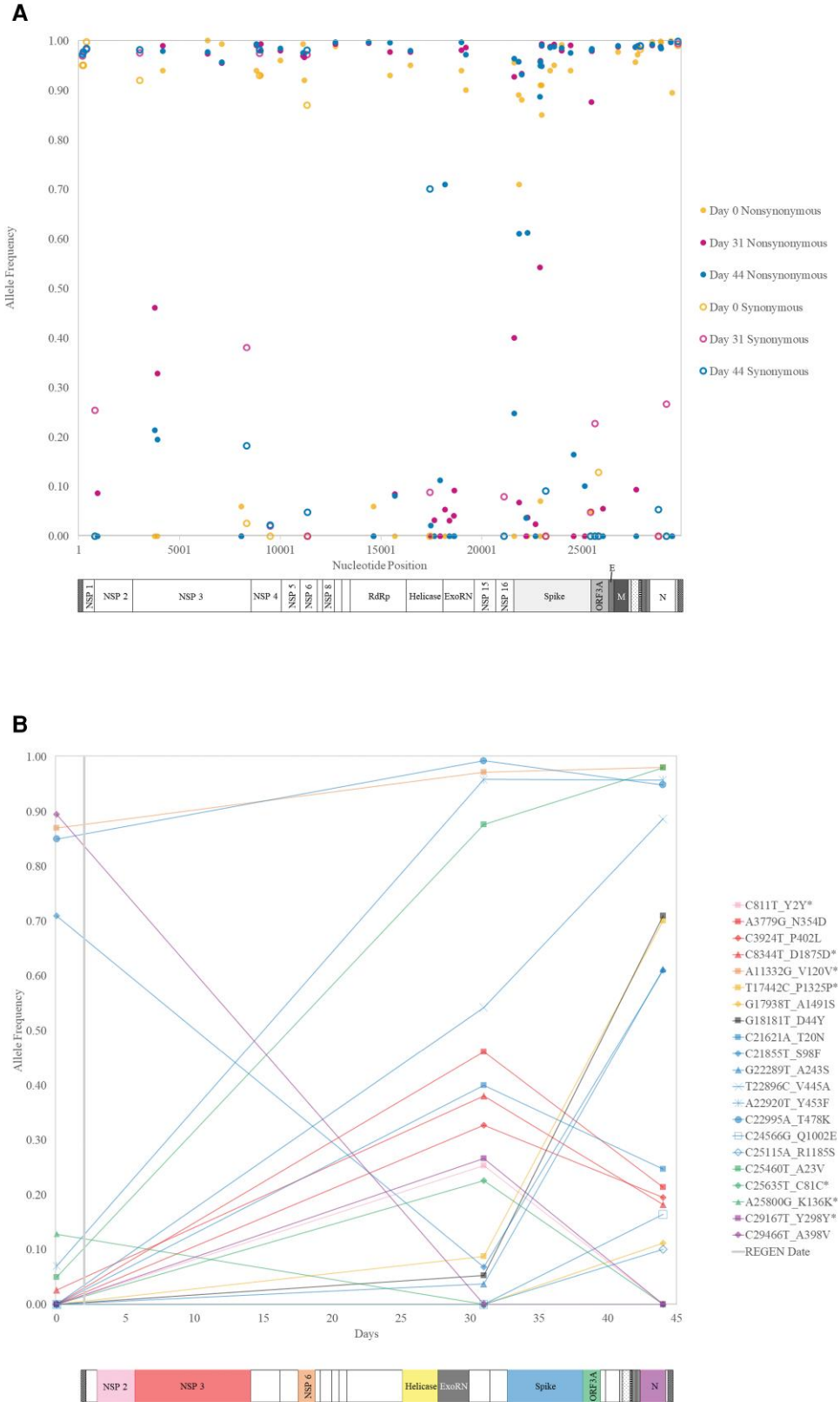

Figure 1. iSNV allele frequency in relation to nucleotide position and time, compared to reference sequence Wuhan-Hu-1 (NC_045512). (*A*) iSNV allele frequency (y-axis) is plotted against nucleotide position (x-axis). Open or closed circles represent synonymous or nonsynonymous mutations, respectively. The SARS-CoV-2 genome map below the x-axis highlights genes of interest. (*B*) iSNV allele frequency (y-axis) is plotted against time (x-axis). Only iSNVs that differed in allele frequency by more than 10% between any two time points are included. Asterisks mark synonymous changes. Vertical line indicates treatment with casirivimab/imdevimab.

Table 1. Consensus-Level Severe Acute Respiratory Syndrome Coronavirus 2 Mutations That Arose Over the Course of Infection

Nucleotide Change	Amino Acid Change	Gene	Allele Frequency at Day 0	Allele Frequency at Day 31	Allele Frequency at Day 44
T17442C	Synonymous	Helicase	0%	9%	70%
G18181T	D44Y	Exoribonuclease	0%	5%	71%
C21855T	S98F	S	71%	7%	61%
G22289T	A243S	S	0%	4%	61%
T22896C	**V445A**	**S**	**0%**	**54%**	**89%**
A22920T	**Y453F**	**S**	**7%**	**96%**	**96%**
C25460T	A23V	orf3a	5%	88%	98%
C29466T	A398V	N	90%	0%	0%

Mutations are reported relative to reference sequence Wuhan-Hu-1, and fixed mutations present at all 3 time points are not included. Four of the 8 mutations occurred in the spike gene, and most (7/8) were nonsynonymous. Mutations V445A and Y453F (in bold) occurred in regions known to confer escape from 1 of the monoclonal antibodies in casirivimab/imdevimab; both mutations rose to high frequency after administration of casirivimab/imdevimab on day 2.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. D. K., H. S., and A. P. analyzed the data and drafted and edited the manuscript. C. F. K., S. M. R., C. J. G., Y. F. W., B. C. Z., and V. D. C. obtained the samples and clinical data and drafted and edited the manuscript. P. N., J. J. W., and D. K. performed laboratory work and edited the manuscript. R. M. L. and A. B. analyzed the data and edited the manuscript.

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Patient consent. Not applicable as the patient is deceased.

Data availability. All sequence data (cleaned of human reads) are available from the National Center for Biotechnology Information under BioProject PRJNA634356. The BioSample accession numbers for the samples are SAMN30730674, SAMN30730675, and SAMN30730676. The unaligned reads are available under Sequence Read Archive accession numbers SRR23022001, SRR23022002, SRR23022003, SRR21492037, SRR 21492034, SRR21492033, SRR21492036, SRR21492032, SRR21492031, SRR21492035, SRR21492030, and SRR21492029. SARS-CoV-2 consensus sequences are available in GISAID (accession numbers EPI_ISL_ 7950344, EPI_ISL_7950343, and EPI_ISL_7950345) and GenBank (accession numbers OP422635, OP439735, and OP422634).

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Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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