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BRIEF REPORT

Rapid Emergence of Potentially Transmissible Severe Acute Respiratory Syndrome Coronavirus 2 With Resistance to Combination Monoclonal Antibody Therapy

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Prolonged coronavirus disease 2019 may generate new viral variants. We report an immunocompromised patient treated with monoclonal antibodies who experienced rebound of viral RNA and emergence of an antibody-resistant (>1000-fold) variant containing 5 mutations in the spike gene. The mutant virus was isolated from respiratory secretions, suggesting the potential for secondary transmission.

Prolonged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in immunocompromised patients has been well documented [1]. It has been postulated that some variants of concern emerged in immunocompromised patients with prolonged infection [2] and that selection by monoclonal antibodies (mAbs) could accelerate the emergence of immune escape variants [3]. Here we provide clear evidence of rapid in vivo selection of multiply mutated SARS-CoV-2 following combination mAb treatment resulting in high-level resistance to the antibodies.

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CASE REPORT

A 55-year-old woman with refractory diffuse large B-cell lymphoma (DLBCL) who had received extensive chemotherapy and immunosuppression since 2018 was diagnosed with coronavirus disease 2019 (COVID-19) in December 2020 (day 0) (Figure 1*A*). Despite receiving bamlanivimab (Bam) on day 5, she developed additional symptoms with persistently positive clinical SARS-CoV-2 nasopharyngeal swab polymerase chain reaction (PCR) testing, eventually resulting in hospitalization on day 56. Chest computed tomography showed diffuse ground-glass opacities, consistent with COVID-19 pneumonia. She enrolled in an observational study and biobank of immunocompromised patients with COVID-19 at the University of Pittsburgh (STUDY21010051). Quantitation of SARS-CoV-2 RNA in saliva and plasma revealed high levels of viral RNA on day 58 (saliva, 2.52×10^9 copies/mL; plasma, 214 copies/mL), which increased 100-fold (23 680 copies/mL in plasma) by day 69 (Figure 1*A*).

She was rehospitalized on day 76 with cough and hypoxia and persistently positive SARS-CoV-2 PCR; chest imaging revealed worsening ground-glass opacities. She received remdesivir (days 76–84), dexamethasone (days 76–86), and casirivimab-imdevimab (C + I, day 76) (Figure 1*A*), which resulted in initial clinical improvement, and she was discharged home on day 88. SARS-CoV-2 viral RNA quantitation revealed a reduction in saliva and plasma viral RNA levels (Figure 1*B*). The patient was hospitalized again on day 126, and imaging revealed extensive progression of her malignancy. She developed bacterial pneumonia, shock, lactic acidosis, and gastrointestinal bleeding thought to be related to progression of her DLBCL. The family withdrew care, and the patient died on day 135.

METHODS

Patient Samples

Samples were collected after the patient enrolled in an observational study and biobank of immunocompromised patients with COVID-19 at the University of Pittsburgh (STUDY21010051), and institutional review board approval and patient consent were obtained. Samples were collected as follows: nasal swabs on day 0 (day of COVID-19 diagnosis) and days 58, 69, 76, 78, 83, 97, 100, 104, 111, 114, 118, 125, 128, and 133; plasma on days 58, 69, 76, 77, 78, 83, 87, 97, 100, 104, 111, 114, 118, 125, 128, and 133; saliva on days 58, 69, 83, 97, 100, 104, 111, 114, 118, 125, and 128; and sputum on day 135.

See the Supplementary Methods for experimental details.

RESULTS

Blood, nasal swab, and saliva samples were collected at various timepoints both before and after C + I treatment (Figure 1*A*).

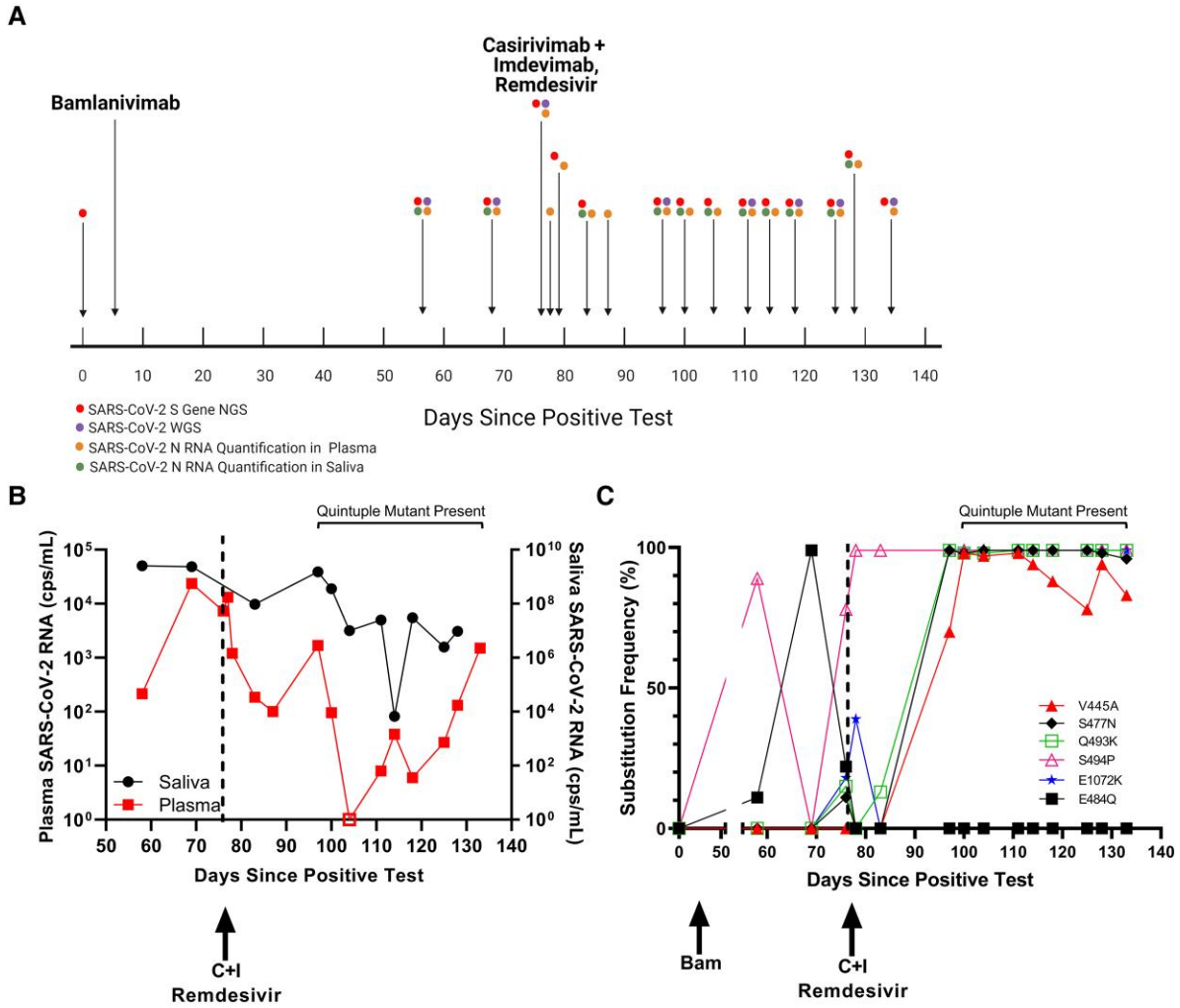
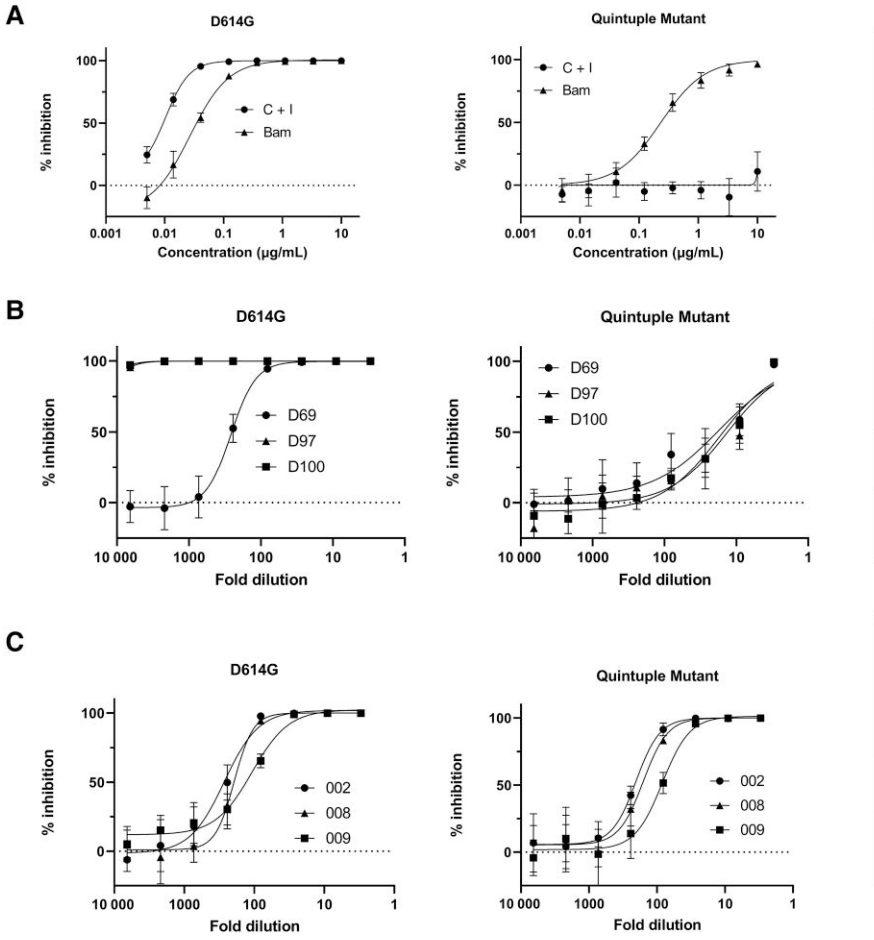


Figure 1. Dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA levels and spike substitutions. *A*, Timeline of relevant treatments, viral RNA quantification, and sequencing, in days since initial positive SARS-CoV-2 polymerase chain reaction test (created with BioRender.com). *B*, Viral RNA levels over time in saliva and plasma. Dashed vertical line indicates treatment with casirivimab + imdevimab and remdesivir. Samples with undetectable viral RNA are shown in open symbols and graphed as one-half the limit of detection. *C*, Substitution frequency in spike amino acid sequence over time determined by next-generation spike gene sequencing. Only substitutions that exceed 10% frequency at the given timepoint are shown. *D*, SARS-CoV-2 receptor-binding domain (RBD) of spike showing the RBD substitutions present in the quintuple mutant virus (left) and their overlap with monoclonal antibody footprints (center and right). RBD models are prepared using pymol, and antibody footprints are depicted based on published RBD/Antibody crystal structures (Protein data bank IDs: 6xdg for casirivimab + imdevimab; 7kmg for bamlanivimab). Abbreviations: Bam, bamlanivimab; C+I, casirivimab + imdevimab; cps, copies; N, nucleocapsid; NGS, next-generation sequencing; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WGS, whole genome sequencing.



	IC_{50} (µg/mL)	
	C+I	Bam
D614G	0.01	0.03
Quintuple Mutant	>10	0.20

	NT_{50} (reciprocal of dilution)		
	D69	D97	D100
D614G	251	>6561	>6561
Quintuple Mutant	17	14	17

	NT_{50} (reciprocal of dilution)		
	002*	008*	009*
D614G	268	193	108
Quintuple Mutant	203	159	83

Figure 2. Quintuple suite of substitutions in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike confer antibody resistance. Neutralization of SARS-CoV-2 pseudovirus containing D614G or quintuple mutant spike protein by monoclonal antibodies (*A*), autologous plasma from donor HMC-001 (D69, D97, and D100 indicate days 69, 97, and 100 since first positive clinical SARS-CoV-2 polymerase chain reaction test) (*B*), and sera from 3 vaccinated donors (donors 002, 008, and 009) (*C*). *Postvaccination sera from healthy volunteers. Error bars represent standard deviation. Abbreviations: Bam, bamlanivimab; C + I, casirivimab + imdevimab; IC_{50}, concentration leading to 50% inhibition; NT_{50}, titer leading to 50% neutralization.

In addition, a nasal swab sample was collected before Bam treatment, and a sputum sample was collected on day 135 (after C + I treatment). The longitudinal SARS-CoV-2 viral RNA levels in saliva and plasma showed incomplete viral clearance following treatment (Figure 1*B*), followed by viral rebound. High-depth next-generation sequencing (NGS) of the S gene of the pre-Bam nasal swab showed no mutations of interest, and whole genome sequencing (WGS) and high-depth S NGS of post-Bam, pre-C + I swabs showed emergence of 2 nonsynonymous mutations in the S gene: E484Q and S494P. WGS and S NGS of post-C + I swabs showed the emergence with viral rebound of a new viral population containing 5 nonsynonymous mutations in the S gene: V445A, S477N, Q493K, S494P, and E1072K (quintuple mutant, Figure 1*B* and 1*C* and Supplementary Figure 1). Of note, the E484Q and S494P substitutions emerged after Bam treatment as has been reported [4, 5] but subsequently decreased before C + I treatment began. The S494P substitution rapidly increased again following C + I treatment while E484Q remained undetectable, indicating repeated selection of S494P but not E484Q by C + I. These findings indicate that S494 can be selected by both Bam and C + I, which is consistent with the overlap of antibody footprints on the receptor-binding domain (RBD) (Figure 1*D*). The V445A and Q493K substitutions have emerged in an in vitro system of casivirimab or imdevimab selection, but escape mutations were not selected when C + I were added together [3, 6].

The rapid emergence and dominance of 5 substitutions after C + I treatment (V445A, S477N, Q493K, S494P, E1072K) temporally linked to rebound of viral RNA levels led us to postulate that this set of 5 substitutions conferred resistance to both mAbs (C + I). Indeed, SARS-CoV-2 pseudovirus bearing the quintuple mutant spike protein was completely resistant to neutralization by C + I up to 10 ug/mL (concentration leading to 50% inhibition [IC_{50}] >10 µg/mL, compared to IC_{50} 0.01 µg/mL

for pseudovirus bearing D614G spike), and nearly 10-fold less susceptible to neutralization by Bam than wild-type (D614G) spike-bearing pseudovirus (quintuple mutant $IC_{50} = 0.20$ μg/mL, D614G $IC_{50} = 0.03$ μg/mL; Figure 2*A*). Autologous patient plasma collected before C + I treatment (day 69; Figure 2*B*) neutralized D614G pseudovirus at titer leading to 50% neutralization (NT_{50}) of 1:251, and after C + I treatment (days 97 and 100) neutralized D614G pseudovirus completely at 1:6561 dilution (NT_{50} >6561, the largest dilution tested). This large increase in neutralization capability of the patient's plasma after C + I treatment is likely due to the presence of C + I, which is corroborated by high levels of S1 and RBD immunoglobulin G (IgG), but not Nucleocapsid (N) and S2 IgG, in the patient's sera (Supplementary Table 1). The same autologous patient plasma had a diminished ability to neutralize pseudovirus with the quintuple mutations at all 3 timepoints tested ($NT_{50} = 17$, 14, and 17 for days 69, 97, and 100, respectively; Figure 2*B*), a result concordant with the diminished ability of C + I and Bam to neutralize the quintuple mutant pseudovirus (Figure 2*A*). By contrast, sera from healthy individuals immunized with mRNA-1273 vaccines were tested for neutralization of both D614G and quintuple mutant pseudoviruses and showed no difference in neutralizing titer (Figure 2*C*), likely due to the polyclonal nature of vaccine-elicited antibody response.

Replicating SARS-CoV-2 was isolated from a sputum sample collected on day 135 after therapy with both Bam and C + I. The isolate had 5×10^8 copies/mL of SARS-CoV-2 viral RNA and an infectious virus titer of 1.5×10^4 plaque-forming units/mL. Spike-specific deep NGS of viral RNA in the sputum sample and in the infectious virus population isolated from sputum confirmed the presence of the quintuple mutations in both specimens at ≥90% frequency. This finding of infectious mutant virus in respiratory secretions demonstrates the potential for transmission and spread of new, mAb-resistant variants from immunocompromised patients with protracted SARS-CoV-2 infection to other susceptible persons.

DISCUSSION

While there are many reports of resistance-conferring mutations in response to mAb treatment [1, 7, 8], the current report highlights the potential public health risk of onward transmission. The rapid emergence of replication-competent virus with 5 mutations in the spike glycoprotein underscores the remarkable plasticity of SARS-CoV-2 that was not anticipated at the onset of the COVID-19 pandemic [1, 9]. Neutralization of the quintuple mutation in the current case by vaccine-induced antibodies in healthy individuals suggests that vaccination could prevent transmission. Nevertheless, we urge diligence in monitoring for clearance of infection in the immunocompromised population and strict adherence to infection prevention precautions to avoid onward transmission of resistant variants. Strategies to prevent emergence of viral resistance, such as combination of mAbs with antivirals targeting more structurally and functionally constrained components of SARS-CoV-2, should be explored [10].

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

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Patient consent. The patient's written informed consent was obtained. This study was reviewed and approved by the University of Pittsburgh Institutional Review Board (study ID: STUDY21010051).

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Advancing Translational Sciences (NCATS) or the National Institutes of Health (NIH).

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Potential conflicts of interest. J. W. M. is a consultant to AlloVir, Infectious Disease Connect, and Gilead Sciences; has received grant funding from Gilead Sciences to the University of Pittsburgh; receives compensation from Abound Bio (unrelated to the current work); and holds share options in Infectious Disease Connect and MingMed Biotechnology Co (unrelated to the current work). G. H. is a recipient of research grants from AlloVir, Karius, NIH, and AstraZeneca; reports consulting fees for serving on the advisory boards of Karius and AstraZeneca; and has received honoraria from MDOutlook. E. M. has served on advisory boards for Shionogi and AbbVie related to COVID-19 therapeutics and has received speaker honorarium from Shionogi related to COVID-19 therapeutics. U. M. P. reports consulting fees from Merck & Co. B. J. C. M. has received research funds from AstraZeneca. J. R. B. holds shares/share options in CarePoint Holdings. All other authors report no potential conflicts.

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