Acknowledgments
We thank Debasis Jana (Arignar Anna Zoological Park, Chennai, India) for providing the clinical information and for sending samples for SARS-CoV-2 molecular investigations for the lions. We also thank V.P. Singh for providing infrastructure facilities to carry out this study, Atul Kumar Pateriya for assistance with quantitative reverse transcription PCR, and the originating and submitting laboratories for sharing SARS-CoV-2 genomic sequence data via GISAID. This study was funded by the service project of ICAR-National Institute of High Security Animal Diseases (ICAR-NIHSAD), Bhopal, India, and ICAR-National Agricultural Science Fund (grant no. NASF/ABA-8027/2020-21).

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References
1. World Organisation for Animal Health. SARS-COV-2 in animals—situation report 1 [cited 2021 Jun 16]. https://www.oie.int/app/uploads/2021/06/sars-cov-2-situation-report-1.pdf
2. Frisk AL, König M, Moritz A, Baumgärtner W. Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. J Clin Microbiol. 1999;37:3634–43. https://doi.org/10.1128/JCM.37.11.3634-3643.1999
3. Elbe S, Buckland-Merrett G. Data, disease and diplomacy: GISAID’s innovative contribution to global health. Glob Chall. 2017;1:33–46. https://doi.org/10.1002/gch2.1018
4. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1. https://doi.org/10.1093/bioinformatics/btp461
5. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. https://doi.org/10.1093/molbev/mst010
6. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3. https://doi.org/10.1093/bioinformatics/btu033
7. McAloose D, Laverack M, Wang L, Killian ML, Caserta LC, Yuan F, et al. From People to Panthera: natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. MBio. 2020;11:e02220–20. https://doi.org/10.1128/mBio.02220-20

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SARS-CoV-2 Variants in Immunocompromised Patient Given Antibody Monotherapy

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DOI: https://doi.org/10.3201/eid2710.211509

A 72-year-old immunocompromised man infected with severe acute respiratory syndrome coronavirus 2 received bamlanivimab monotherapy. Viral evolution was monitored in nasopharyngeal and blood samples by melting curve analysis of single-nucleotide polymorphisms and whole-genome sequencing. Rapid emergence of spike receptor binding domain mutations was found, associated with a compartmentalization of viral populations.
the intensive care unit on day 13. A high dose of corticosteroids was given on days 21–26. This treatment resulted in an improvement of his respiratory condition, but the patient remained dependent on supplemental oxygen (6 L/min). The patient was discharged from the intensive care unit and returned

| Disease course, day† | Clinical manifestations | Treatment/action | Clinical samples‡ | RT-PCR results (mean C<sub>t</sub> value) | VirusNIPI Kit results | NGS clade |
|----------------------|------------------------|------------------|-------------------|------------------------------------------|-----------------------|-----------|
| ‒20                  | First dose mRNA vaccine§ |                  | NP                | Positive (20)                            | E484, N501Y           | 20I/501Y.V1 |
| ‒17                  | Venetoclax, rituximab   |                  |                  |                                          |                       |           |
| ‒3                   | Cough, fever, diarrhea, asthenia |                  | NP                | Positive (20)                            | E484, N501Y           | 20I/501Y.V1 |
| 0                    | Bamlanivimab (700 mg)   |                  | NP                | Positive (20)                            | E484, N501Y           | 20I/501Y.V1 |
| 3                    | Hospitalized at infectious diseases department |                  | Blood Serum (30.7) | Positive (20)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 6                    | Convalvescent-phase plasma |      | NP                | Positive (21)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 7                    | High-flow nasal oxygen |                | Blood Serum (23.2) | Negative                                |                       |           |
| 10                   | Improved in respiratory condition |          | NP                | Positive (17)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 11                   | High-dose corticotherapy protocol |        | NP                | Positive (19)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 33                   | Transferred to infectious disease department |        | Blood Serum (30.8) | Positive (17)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 39                   | Improvement in respiratory condition |        | NP                | Positive (20)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 45                   | High-dose corticotherapy protocol |        | NP                | Positive (21)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 47                   | Treatment with remdesivir (10 d) |        | NP                | Positive (19)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 52                   | Hospitalization for follow-up care |        | NP                | Positive (20)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |
| 54                   | Hospitalization for follow-up care |        | NP                | Positive (20)                            | E484Q, N501Y          | 20I/501Y.V1 + E484Q |

*Blank cells indicate that clinical status was stable on that day, and no treatment was given. COVID-19, coronavirus disease; C<sub>t</sub>, cycle threshold; D, day; ICU, intensive care unit; NA, not available; NP, nasopharyngeal swab specimen; NGS, next-generation sequencing; RT-PCR, reverse transcription PCR.  †Day 0 indicates first day of follow-up care at hospital.  ‡Serologic results given by using the Wantai antibody test (index of positivity = 1).  §Vaccine BNT162b2 (Pfizer/BioNTech. https://www.pfizer.com).  ¶Test was performed in an external laboratory (no sample was available for further analysis).
to the infectious disease department on day 33, but still had a high viral load in nasopharyngeal swab specimens ($C_t \geq 20$ on day 45).

Because of this persistent viral replication, the patient was given remdesivir on day 47 and this treatment was continued for 10 days (200 mg for 1 day, followed by 100 mg/d for 9 days). SARS-CoV-2 carriage in a nasopharyngeal swab specimen decreased during treatment, and the patient was discharged from the infectious disease department and transferred to a rehabilitation center. The nasopharyngeal swab specimen viral load became negative on day 61.

To monitor viral evolution, we performed a multiplex RT-PCR based on melting curve analyses with VirSNIP Kits (TIB Molbiol, https://www.tib-molbiol.de) to evaluate the presence of the S: E484K and S: N501Y mutations in SARS-CoV-2 variants. Three days after mAb treatment (day 3), RT-PCR results suggested the presence of S: N501Y and an absence of S: E484K on an nasopharyngeal swab specimen. On day 6, the S: N501Y mutation was still present but was also found associated with an undetermined mutation at position 484 (melting temperatures different from those of wild-type E and the mutated strain K). On day 11, we detected the S: N501Y mutation in a blood sample but found no mutation at position 484. No nasopharyngeal swab specimen or blood sample from before mAb administration was available for analysis and comparison.

We performed whole-genome sequencing on 12 clinical samples by using amplicon-based technology on the Ion Torrent Platform (ThermoFisher, https://www.thermofisher.com) according to the protocol of and plug-ins used by Sjaarda et al. (1). We confirmed results of this analysis by using the minimap2 program (2). This analysis detected clade 20I/501Y.V1, Alpha variant (Pangolin: B.1.1.7), on day 3 in nasopharyngeal swab specimens. Three days later (day 6), a novel mutation (G23012C, S: E484Q) appeared in nasopharyngeal swab specimens at frequency of 82%, which rapidly reached >99% (S: E484Q) 10 days after mAb treatment (Table; Figure). Eleven days after the mAb infusion, we detected an additional nucleotide mutation A23040G (S: Q493R) in only a blood sample at a frequency of 64%. This rate reached 76% at day 17 without any detection in nasopharyngeal swab specimens.

Clinical trials of monotherapy treatment for SARS-CoV-2 infection have shown that subsequent dynamic shifts in the viral population appear to be frequent (3,4). An in vitro model showed that E484 and Q493 are 2 amino acid mutations of the spike protein that are known to be critical for bamlanivimab binding (5,6). The S: E484Q mutation is a hotspot of escape and could reduce susceptibility to bamlanivimab by >1,000-fold (6) and S: Q493R by >6,666-fold (7). Use of bitherapy with bamlanivimab and etesevimab decreases the risk for emergence of drug-resistant variants (5,8). However, an escape mutation after use of this drug combination was recently described (7).

Our analysis identified signs of compartmentalized viral populations on the basis of sequences
recovered in blood and nasopharyngeal swab samples (notably on day 17). Such a phenomenon has been reported in clinical trials (9,10). Further analysis is needed to distinguish genetic changes that occur in the primary viral population from apparent changes to clarify whether such escape mutants are enough to spread and persist in humans and how SARS-CoV-2 displays compartmentalized replication. Genomic surveillance for SARS-CoV-2 variants is encouraged for COVID-19 patients given mAbs as monotherapy or biotherapy.

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Dr. Truffot is a physician in the Department of Virology, University Hospital of Grenoble, Grenoble, France. Her research interests include novel sequencing technologies for genome diagnosis and follow-up of major pathogens, including SARS-CoV-2, and quantification of monoclonal antibodies by high-performance liquid chromatography/mass spectrometry.

References
1. Sjaarda CP, Rustom N, Evans GA, Huang D, Perez-Patregion S, Hudson ML, et al. Phylogenomics reveals viral sources, transmission, and potential superinfection in early-stage COVID-19 patients in Ontario, Canada. Sci Rep. 2021;11:3697. https://doi.org/10.1038/s41598-021-83355-1
2. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018;34:3094–100. https://doi.org/10.1093/bioinformatics/bty191
3. Gottlieb RL, Nirula A, Chen P, Boscia J, Heller B, Morris J, et al. Effect of bamlanivimab as monotherapy or in combination with etesevimab on viral load in patients with mild to moderate COVID-19: a randomized clinical trial. JAMA. 2021;325:632–44. https://doi.org/10.1001/jama.2021.0202
4. Lohr B, Niemann D, Verheyen J. Bamlanivimab treatment leads to rapid selection of immune escape variant carrying E484K mutation in a B.1.1.7 infected and immunocompromised patient. Clin Infect Dis. 2021 May 1 [Epub ahead of print]. https://doi.org/10.1093/cid/ciab392
5. Baum A, Fulton BO, Wloga E, Copin R, Pascal KE, Russo V, et al. Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. Science. 2020;369:1014–8. https://doi.org/10.1126/science.abc0831
6. Starr TN, Greaeaey AJ, Dingens AS, Bloom JD. Complete map of SARS-CoV-2 RBD mutations that escape the monoclonal antibody LY-CoV555 and its cocktail with LY-CoV016. Cell Rep Med. 2021;2:100255. https://doi.org/10.1016/j.xcrm.2021.100255
7. Focosi D, Novazzi F, Genoni A, Dentali F, Dalla Gasperina D, Baj A, et al. Emergence of SARS-CoV-2 spike protein escape mutation Q493R after treatment for COVID-19. Emerg Infect Dis. 2021;27:2728–31. https://doi.org/10.3201/eid2710.211538
8. Copin R, Baum A, Wloga E, Pascal KE, Giordano S, Fulton BO, et al. The monoclonal antibody combination REGEN-COV protects against SARS-CoV-2 mutual escape in preclinical and human studies. Cell. 2021;184:3949–3961.e11. https://doi.org/10.1016/j.cell.2021.06.002
9. Jary A, Leducq V, Malet I, Marot S, Klement-Frutos E, Teysou E, et al. Evolution of viral quasispecies during SARS-CoV-2 infection. Clin Microbiol Infect. 2020;26:1560.e1–4. https://doi.org/10.1016/j.cmi.2020.07.032
10. Rueca M, Bartolini B, Gruber CE, Piralla A, Baldanti F, Giombini E, et al. Compartmentalized replication of SARS-CoV-2 in upper vs. lower respiratory tract assessed by whole genome quasispecies analysis. Microorganisms. 2020;8:1302. https://doi.org/10.3390/microorganisms8091302

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Emergence of SARS-COV-2 Spike Protein Escape Mutation Q493R after Treatment for COVID-19

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DOI: https://doi.org/10.3201/eid2710.211538

We report in vivo selection of a severe acute respiratory syndrome coronavirus 2 spike mutation (Q493R) conferring simultaneous resistance to bamlanivimab and etesivimab. This mutation was isolated from a patient who had coronavirus disease and was treated with these drugs.

Variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) usually result from random mutations in humans or other hosts, but accelerated evolution can also occur under selective pressure from therapeutic interventions using