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

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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

neutralizing antibodies (*1*). Bamlanivimab has been recently withdrawn as a monotherapy because of treatment failure against E484K SARS-CoV-2 virus variants. Emergency use remains authorized for the bamlanivimab/etesevimab cocktail, targeting overlapping epitopes (*2*), for which no completely resistant variant has been reported to date. This cocktail has been effective in reducing hospitalizations when administered early after infection (*3*).

Given increasing reports of accelerated intrahost evolution of drug-resistant SARS-CoV-2 clades after neutralizing antibody–based treatments (*4*,*5*), we began screening patients who failed to show virus-negative results for nasopharyngeal swab (NPS) specimens after they were given bamlanivimab/etesevimab. We report an in vivo case of a spike protein escape mutation conferring combined resistance to bamlanivimab and etesevimab.

A 73-year-old man had cholangiocarcinoma diagnosed during February 2021. While he was waiting for chemotherapy, sepsis developed, and he was admitted to Varese Hospital (Varese, Italy) on April 12 and given a steroid and antimicrobial drugs. At admission, reverse transcription PCR (RT-PCR) for SARS CoV-2 in an NPS specimen showed a negative result, but the same test showed a positive result on April 24.

Given that he had recovered from sepsis, the patient was moved to the coronavirus disease unit of the hospital on April 25. He satisfied 1 of the frail-patient categories for emergency use of spike protein monoclonal antibodies approved by the Italian Drug Agency. The patient was also seronegative for S1/S2 IgG against spike protein (Diasorin, https://www.diasorin.com).

On April 26, the patient received a single intravenous infusion of bamlanivimab (700 mg) and etesevimab (400 mg) at the hospital. RT-PCR performed on an NPS specimen collected before the infusion was positive for SARS-CoV-2 and showed a cycle threshold (C_t) of 12 (Alinity Analyzer; Abbott Laboratories, https://www abbott.com).

Follow-up analysis of NPS specimens showed positive results on Apr 28 (C_t 15) and May 3 (C_t 24). Chest computed tomography on April 30 showed progression to interstitial pneumonia, and the patient was given noninvasive ventilation. No additional bamlanivimab/etesevimab infusion was performed, and the patient died on May 14.

According to national guidelines for breakthrough infections, we sequenced SARS-CoV-2–positive samples. We performed a SARS CoV-2 RT-PCR on NPS specimens by using the Alinity Platform (Abbott Laboratories), and measured S1/S2 IgG by using a chemiluminescent immunoassay (Diasorin). We used the Sanger method to sequence the spike gene as reported (*6*), analyzed sequences by using NextStrain (https://nextstrain.org), and deposited sequences in GenBank.

Spike gene sequencing of the NPS specimen obtained on April 24 clade B.1.1.7 (Alpha; NextStrain clade 20I/501Y.V1; GenBank accession no. MZ157261), which was 94% prevalent in Italy at that time. However, the May 3 specimen showed a secondary A1478G peak in the spike protein gene, corresponding to the spike Q493R mutation, which became predominant by May 8 (C_t 18; GenBank accession no. MZ157275) (Figure).

E484, F490, Q493, and S494 are the 4 aa residues within the spike protein receptor-binding motif that are known to be critical for bamlanivimab binding. Q493 is also among the many more receptor-binding motif residues crucial for interactions with etesivimab. Q493R/K (which can be selected in vitro by bamlanivimab [*7*,*8*]) is to date the only mutation that causes resistance to bamlanivimab and etesivimab. This residue also causes resistance to other class 3 monoclonal antibodies (*8*) (i.e., those that do not overlap with the angiotensin-converting enzyme 2 binding site and have accessibility to the receptor-binding domain epitope in the up and down conformations).

In pseudoviral neutralization assays, Q493R reduces susceptibility to bamlanivimab by >6,666-fold, to etesevimab by 232-fold, and to the combination of both drugs by >100-fold (*2*). In a flow cytometry competitive assay, Q493R reduces the 50% inhibitory concentration >100-fold for bamlanivimab and 42-fold for etesivimab (*7*). Q493R has a frequency of 0.006% in the GISAID database (https://www.gisaid.org; 85 of 1,424,998 deposited sequences as of May 8, 2021; https://covid19dashboard.regeneron.com/?tab=Mutation_Details&subTab=Spike), making the occurrence of co-infection with a Q493R-positive strain extremely unlikely in our patient.

It remains unclear how such risk extends to different spike protein monoclonal antibody cocktails targeting nonoverlapping epitopes. Although different mutations can similarly cause immune escape by the nonoverlapping REGN-CoV-2 (imdevimab plus casirivimab) cocktail, hamster models and clinical trials showed no increased emergence of variants (R. Copin et al., Regeneron Pharamaceuticals Inc., pers. comm., 2021 Jun 22). Nevertheless, Choi et al. reported a patient having detectable SARS-CoV-2 for 154 days, and accelerated viral evolution in the spike protein after being given remdesivir and REGN-CoV-2 (*4*).

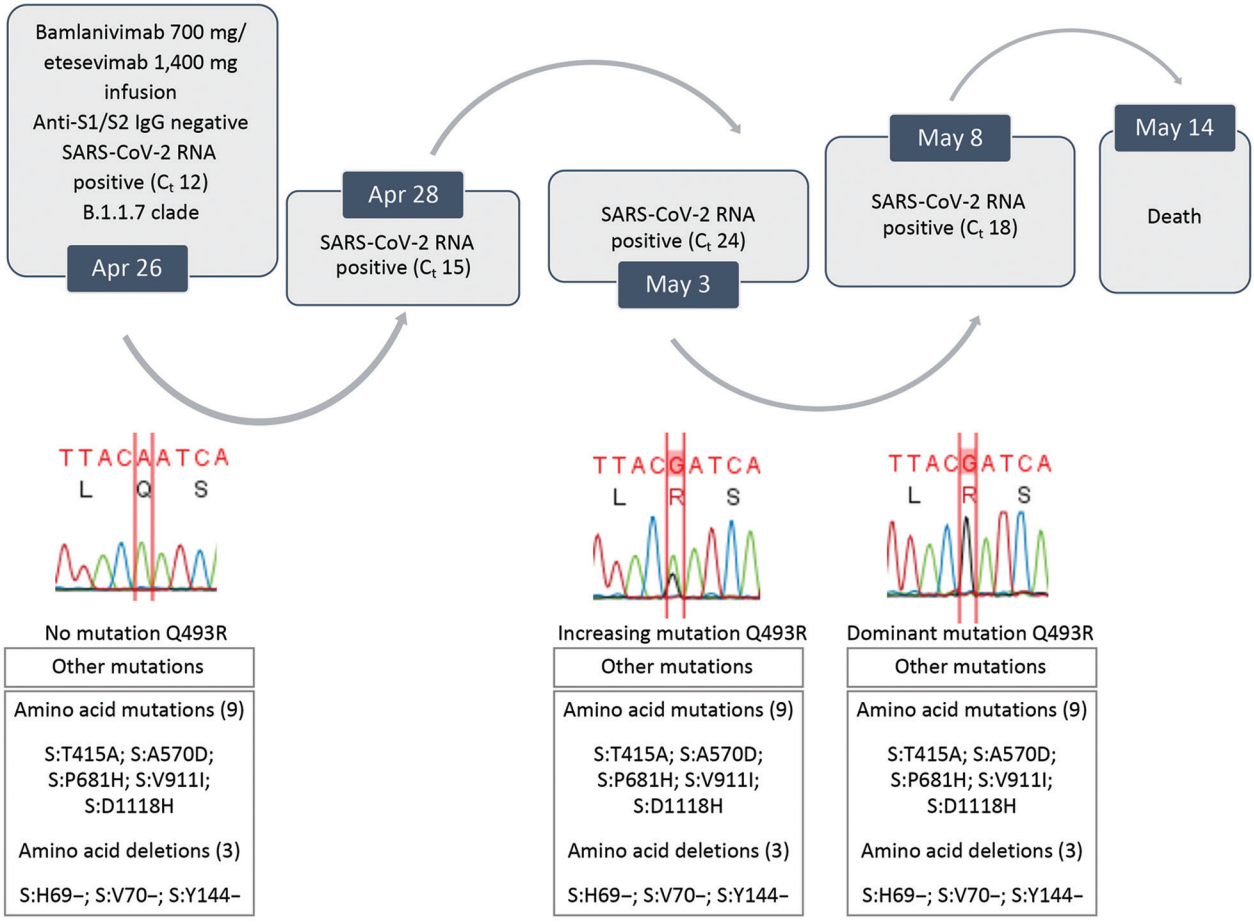

Figure. Evolution of SARS-CoV-2 variants in a patient who had coronavirus disease who was given bamlanivimab and etesivimab, Italy. C_t, cycle threshold; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

The nonoverlapping AZD7442 (COV2–2130 and COV2–2196) cocktail also seems resistant to rapid escape (J. Dong et al., Huazhong University of Science and Technology, pers. comm., 2021 Jun 21), but again, such in vitro or animal models could miss rare in vivo events.

In conclusion, SARS-CoV-2 mutations conferring resistance to bamlanivimab and etesevimab can arise in vivo after specific selective pressure; Q493 mutations increase binding affinity to the angiotensin-converting enzyme 2. Additional studies are needed to clarify whether such escape mutations can spread and persist in humans. Genomic surveillance for SARS-CoV-2 variants is encouraged for coronavirus disease patients who do not respond well to treatment with spike protein monoclonal antibodies.

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Indoor and Outdoor Rodent Hosts of *Orientia tsutsugamushi*, Shandong Province, China

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During December 2012–July 2016, we tested small indoor and outdoor mammals in Qingdao, China, for *Orientia tsutsugamushi* infection. We found that outdoor *Apodemus agrarius* mice, *Cricetulus barabensis* hamsters, and *Niviventer confucianus* rats, as well as indoor *Mus musculus* mice, tested positive for *O. tsutsugamushi* by PCR.

Scrub typhus is an emerging infectious disease caused by *Orientia tsutsugamushi* (*1*), which is transmitted through the bites of infected chiggers, the larvae of trombiculid mites of the genus *Leptotrombidium*. Scrub typhus has been documented in southern China for thousands of years (*2*) and emerged in northern China during the 1990s (*3*). Several studies have investigated the animal hosts of *O. tsutsugamushi* (*4*,*5*), but the major hosts and seasonality of *O. tsutsugamushi* in northern China remain unclear. We collected small animals in Qingdao, a city in eastern China, to investigate the hosts and seasonality of *O. tsutsugamushi*.

During December 2012–July 2016, we used indoor and outdoor mousetraps to capture 162 small mammals (154 rodents and 8 shrews) in 2 villages in Huangdao District, Qingdao (119°30′–121°00′E, 35°35′–37°09′N) (Figure). All animal samples were obtained in accordance with the Implementation Regulations of the People's Republic of China on the Protection of Terrestrial Wild Animals (http://www.gov.cn/zhengce/2020-12/25/content_5574749.htm). The collection of rodents for microbiological studies was approved by the Ethics Committee of Prevention Medicine of Shandong University (Jinan, China; approval no. 20150501).

We classified samples by morphologic characteristics. We captured all 7 *Cricetulus barabensis* hamsters, 18 *Tscherskia triton* hamsters, and 8 *Niviventer confucianus* rats in the fields, as well as 98.6% (69/70) of *Apodemus agrarius* mice. We captured most *Rattus norvegicus* rats (22/24; 92%) and *Mus musculus* mice (18/27; 67%) in indoor settings, as well as 25% (2/8) of *Crocidura lasiura* shrews.

We extracted and screened DNA from rodent spleens for *O. tsutsugamushi* by nested PCR selective for the 56-kDa type-specific antigen gene with outer primers (5′- TCAAGCTTATTGCTAGTGCAATGTCTGC-3′ and 5′-AGGGATCCCTGCTGCTGTGCTTGCTGCG-3′) and inner primers (5′-GATCAAGCTTCCTCAGCCTACTATAATGCC-3′ and 5′-CTAGGGATCCCGACAGATGCACTATTAGGC-3′) (*6*,*7*). Overall, 4.5% of 154 rodents but none of the 8 shrews were positive for *O. tsutsugamushi*.

All infected rodents were captured during autumn (i.e., September–November); among rodents captured in autumn, the infection rate was 8.1% (7/86; $p>0.05$ by 1-sided Fisher exact test). None of the 68 rodents captured during spring, summer, and winter tested positive for *O. tsutsugamushi* (Table). The absence of *O. tsutsugamushi* infection among rodents collected during spring, summer, and winter indicated that these rodents were not reservoirs but temporary amplifying hosts for *O. tsutsugamushi*. The presence of *O. tsutsugamushi* among rodents during autumn months is consistent with the seasonality of