Emergence of SARS-CoV-2 Spike Escape Mutation Q493r After Bamlanivimab/Etesevimab Treatment for COVID-19

Daniele Focosi
North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy
Federica Novazzi
Laboratory of Microbiology, ASST Sette Laghi, Varese, Italy
Angelo Genoni
Department of Medicine and Surgery, University of Insubria, Varese, Italy
Francesco Dentali
Department of Medicine and Surgery, University of Insubria, Varese, Italy
Daniela Dalla gasperina
Department of Medicine and Surgery, University of Insubria, Varese, Italy
Andreina Baj (andreina.baj@uninsubria.it)
Department of Medicine and Surgery, University of Insubria, Varese, Italy https://orcid.org/0000-0003-0088-2712
Fabrizio Maggi
Department of Medicine and Surgery, University of Insubria, Varese, Italy

Case Report

Keywords: SARS-CoV-2, COVID-19, variant of interest, Spike, receptor-binding motif, Q493R

Posted Date: May 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-524959/v1

Version of Record: A version of this preprint was published at Emerging Infectious Diseases on July 27th, 2021. See the published version at https://doi.org/10.3201/eid2710.211538.

Abstract

SARS-CoV-2 variants are usually a consequence of random mutations in humans or other hosts, but accelerated evolution can also occur under selective pressure from therapeutic interventions with neutralizing antibodies1. Bamlanivimab has been recently withdrawn from the vendor as a monotherapy because of failure against E484K-positive SARS-CoV-2 variants, but emergency use authorization remains in place for the bamlanivimab/etesevimab cocktail2, for which no completely resistant variant has been reported to date. We report here the first *in vivo* case of a Spike escape mutation conferring combined resistance to both bamlanivimab and etesevimab.

Introduction

SARS-CoV-2 variants are usually a consequence of random mutations in humans or other hosts, but accelerated evolution can also occur under selective pressure from therapeutic interventions with neutralizing antibodies[1]. Bamlanivimab has been recently withdrawn from the vendor as a monotherapy because of failure against E484K-positive SARS-CoV-2 variants, but emergency use authorization remains in place for the bamlanivimab/etesevimab cocktail[2], for which no completely resistant variant has been reported to date. We report here the first *in vivo* case of a Spike escape mutation conferring combined resistance to both bamlanivimab and etesevimab.

Methods

A 73-years old male was diagnosed on February 2021 with cholangiocarcinoma: while waiting for chemotherapy, he developed sepsis and was admitted to Varese hospital on Apr 12 for steroid and antimicrobial treatment. Nasopharyngeal swab (NPS) for SARS CoV-2 RT-PCR was negative on admission, but positive on Apr 24. Given he had recovered from sepsis, on Apr 25 he was moved to the COVID unit of the hospital. Falling within one of the frail patient categories for emergency use of anti-Spike monoclonal antibodies approved by the Italian Drug Agency and being seronegative for anti-S1/S2 IgG (Diasorin, Italy), on Apr 26 the patient received an i.v. infusion of bamlanivimab 700 mg/etesevimab 1400 mg at the hospital. RT-PCR performed on the NPS collected before infusion was positive for SARS-CoV-2, with a cycle threshold (Ct) of 12 (Alinity, Abbott).

Follow-up NPS remained positive on Apr 28 (Ct 15) and May 3 (Ct 24); a chest CT scan on Apr 30 showed progression to interstitial pneumonia, and the patient was placed in noninvasive ventilation.

According to national guidelines, SARS-CoV-2-positive samples were sequenced. SARS CoV-2 RT-PCR on nasopharyngeal swab (NPS) was performed with Alinity platform (Abbott), and anti-S1/S2 IgG were measured using a chemiluminescent immunoassay (Diasorin). Sanger method was utilized to sequence the spike gene, then analyzed on NextStrain and deposited in GenBank.

Results

Spike gene sequencing on the Apr 24 NPS revealed a PANGOLIN clade B.1.1.7 (NextStrain clade 20I/501Y.V1, GenBank sequence MZ157261), which was 94% prevalent in Italy at that time: since May 3 we observed a double peak at A1478G of the *S gene*, corresponding to the Spike Q493R mutation, which became predominant as soon as May 8 (Ct 18; GenBank sequence MZ157275) (Fig. 1).

Discussion

E484, F490, Q493, and S494 are the 4 amino acid residues within the Spike receptor-binding motif (RBM) that are known to be critical for bamlanivimab binding. Q493 is also among the many more RBM residues crucial for interactions with etesivimab. Q493R/K (which can be selected *in vitro* by bamlanivimab[3], C121, or C144[4]) is to date the only mutation that causes resistance to both bamlanivimab and etesivimab. It also causes resistance to other class 3 monoclonal antibodies[5], i.e. the ones that do not overlap with ACE2 binding site and have accessibility to RBD epitope in “up”/”down” conformations. More in detail, in pseudoviral neutralization assays Q493R reduces susceptibility to bamlanivimab by > 6,666 folds, to etesevimab by 232 folds, and to the combination of both by > 100 folds[2]; accordingly, in a flow cytometry competitive assay, Q493R reduces IC_{50} of > 100 folds for bamlanivimab and 42 folds for etesivimab[3].

Q493R has a frequency of 0.006% in the GISAID database (85 out 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 from a Q493R-positive strain extremely unlikely in our patient.

In conclusion, we have shown here that mutations conferring resistance to both bamlanivimab and etesevimab can arise *in vivo*: Q493 mutations increase binding affinity to ACE2 [6], but further studies are needed to clarify whether such escape mutants are fit enough to spread and persist in humans. Genomic surveillance for SARS-CoV-2 variants is encouraged in COVID-19 patients showing refractoriness to anti-Spike monoclonal antibodies.

Declarations

We declare we have no conflict of interest related to this manuscript.

References

1. Chen L, Zody MC, Mediavilla JR, et al. Emergence of multiple SARS-CoV-2 antibody escape variants in an immunocompromised host undergoing convalescent plasma treatment. 2021:2021.2004.2008.21254791.
2. US Food and Drug Administration. Emergency Use Authorization (EUA) for Bamlanivimab 700 mg and Etesevimab 1400 mg IV Administered Together Center for Drug Evaluation and Research (CDER) Review 2021.

3. European Medicines Agency. Assessment report Eli Lilly and Company Limited use of bamlanivimab and etesevimab for the treatment of COVID-19. In:2021.
4. Weisblum Y, Schmidt F, Zhang F, et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *eLife.* 2020;28(9):e61312.
5. Starr TN, Greaney 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. *Cel Reports Medicine.* 2021;2(4):100255.
6. Ortega JT, Pujol FH, Jastrzebska B, Rangel HR. Mutations in the SARS-CoV-2 spike protein modulate the virus affinity to the human ACE2 receptor, an in silico analysis. *EXCLI journal.* 2021;20:585–600.

Figures

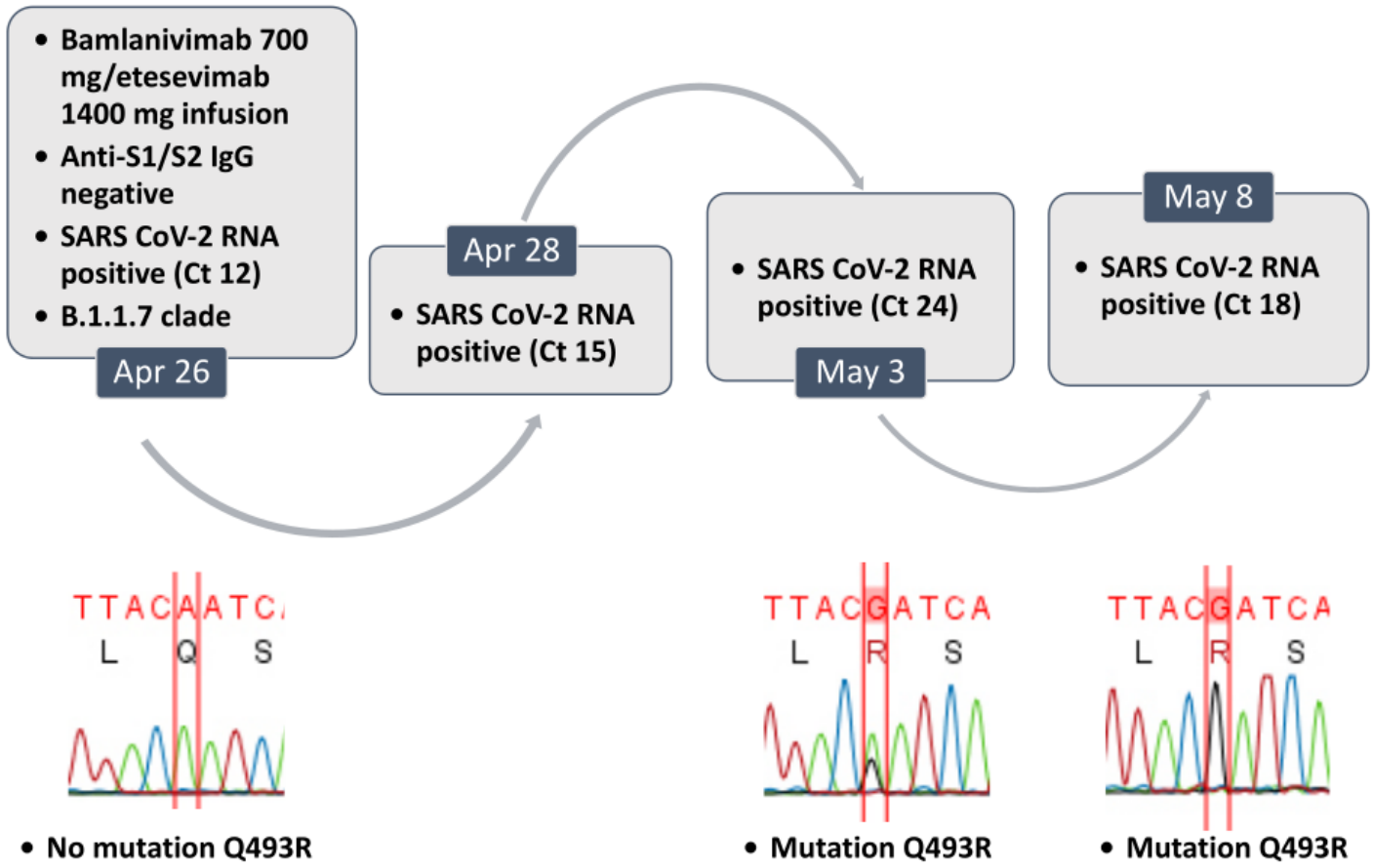

Figure 1

Spike gene sequencing on the Apr 24 NPS revealed a PANGOLIN clade B.1.1.7 (NextStrain clade 20I/501Y.V1, GenBank sequence MZ157261), which was 94% prevalent in Italy at that time: since May 3 we observed a double peak at A1478G of the S gene, corresponding to the Spike Q493R mutation, which became predominant as soon as May 8 (Ct 18; GenBank sequence MZ157275)