Emergence of the E484K mutation in SARS-CoV-2-infected immunocompromised patients treated with bamlanivimab in Germany

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A B S T R A C T

Background: Monoclonal antibodies (mAb) have been introduced as a promising new therapeutic approach against SARS-CoV-2. At present, there is little experience regarding their clinical effects in patient populations underrepresented in clinical trials, e.g. immunocompromised patients. Additionally, it is not well known to what extent SARS-CoV-2 treatment with monoclonal antibodies could trigger the selection of immune escape viral variants.

Methods: After identifying immunocompromised patients with viral rebound under treatment with bamlanivimab, we characterized the SARS-CoV-2-isolates by whole genome sequencing. Viral load measurements and sequence analysis were performed consecutively before and after bamlanivimab administration.

Findings: After initial decrease of viral load, viral clearance was not achieved in five of six immunocompromised patients treated with bamlanivimab. Instead, viral replication increased again over the course of the following one to two weeks. In these five patients, the E484K substitution known to confer immune escape was detected at the time of viral rebound but not before bamlanivimab treatment.

Interpretation: Treatment of SARS-CoV-2 with bamlanivimab in immunocompromised patients results in the rapid development of immune escape variants in a significant proportion of cases. Given that the E484K mutation can hamper natural immunity, the effectiveness of vaccination as well as antibody-based therapies, these findings may have important implications not only for individual treatment decisions but may also pose a risk to general prevention and treatment strategies.

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1. Introduction

Monoclonal antibodies (mAb), such as bamlanivimab (LY-CoV555), represent a promising new treatment option for early SARS-CoV-2 infection. They have the potential to prevent complications of severe COVID-19 disease for the individual patient and therefore might help to reduce the burden on health systems [1,2]. Compared to previous approaches of using convalescent plasma, monoclonal antibodies have several advantages, for example, their

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that antiviral resistance to neuraminidase inhibitors readily develops mutations under therapy pressure. For in achieving rapid viral clearance, thereby favoring the selection of viral nocompromised COVID-19-patients may be at increased risk of not mutations [3]. In SARS-CoV-2 infection, several viral variants of con- have also become clear, such as immune escape by selection of viral such as HIV therapy, potential drawbacks of monoclonal antibodies transmission of infectious agents. However, in other disease contexts, such as influenza, it has been shown that antiviral resistance to neuraminidase inhibitors readily develops in immunocompromised individuals with persistent viral shedding [6]. It has been already reported that viral persistence and evolution

2. Methods

2.1. Design and setting

All patients were treated with bamlanivimab for SARS-CoV-2 infection at an academic tertiary referral center in Germany because of their increased individual risk for progression to severe COVID-19. Viral load was measured consecutively before and after bamlanivi- mab administration. After identifying immunocompromised patients with viral rebound under treatment with bamlanivimab, we charac- terized the available SARS-CoV-2-isolates with cycle threshold < 30 by whole genome sequencing.

2.2. Isolation of viral genomic material and SARS-CoV-2 quantification

Respiratory samples from nasopharyngeal swabs were used for total nucleic acid extraction using the EZ1 Virus Mini Kit v2.0 on an EZ1 Advanced XL (Qiagen, Germany) according to the manufacturer’s instructions. SARS-CoV-2 was detected as previously described [8] by the cobas® SARS-CoV-2 test on the cobas®6800 system (Roche), or by the SARS-CoV-2 test on the NeuMoDx™ platform (Qiagen) with a plasmid-standard for quantification [9].

2.3. SARS-CoV-2 whole genome sequencing

Viral RNA was reversely transcribed to single-strand cDNA using random hexamers and SuperScript reverse transcriptase (Thermo- Fisher) [10]. Viral cDNA was PCR-amplified using the Artic network SARS-CoV-2 protocol with V3 primers [11,12], employing an extended annealing/extension time of 10 min. Prior to library prep- aration, for each sample, Artic PCR pools 1 and 2 were combined (500 ng DNA per pool). Sequencing was carried out on the Oxford Nanopore MinION device, utilizing MIN106 flow cells and the SQL- LSK109 ligation sequencing kit. Barcoding was carried out with the native Barcoding Expansion 96 Kit (EXP-NBD196).

Data analysis and generation of consensus sequences were carried out as previously described [13]. Briefly, after base-calling with Guppy v3.4.5+ fl/b1bb, the Artic pipeline1 with default settings was applied to each sequencing run, analyzing each sample independently with Nano- polish and Medaka [14]. Generated VCF files and consensus FASTA sequences were manually curated by a) carrying out a comparison between the Nanopolish- and Medaka-based VCF files and b) visual inspection with IGV [15], checking for i) false-positive calls; ii) polymor- phic positions with more than one plausible allele; iii) false-negative calls.

2.4. Role of the funding source

The funders had no role in study design, data collection, data anal- ysis, interpretation or writing of the report.

3. Results

After bamlanivimab became available for clinical use in Germany as the first monoclonal antibody directed against SARS-CoV-2, our clinic treated six patients in whom we feared a severe course in the setting of SARS-CoV-2 with known severe humoral and/or cellular immunodeficiency. The main clinical characteristics of the six patients are presented in Table 1.
and died on day 20 due to multi-organ failure. Unfortunately, the patient could not be stabilized before the treatment with bamlanivimab (Fig. 1A). Interestingly, at day 8, SARS-CoV-2 RNA levels remained high for about one week and then further increased up to 2.9 × 10^9 copies/mL with simultaneous detection of the E484K substitution. Due to persistently high viral replication levels, the patient was treated with the antiviral drug remdesivir and two units of CP, resulting in a decrease of SARS-CoV-2 RNA by three log levels over the following 10 days. At day 32 the patient could be discharged from hospital with two consecutive negative SARS-CoV-2 qPCR tests.

Patient 3 (Fig. 1C) was a caucasian male in his early sixties with relapsed follicular lymphoma, who had persistent positive SARS-CoV-2 qPCR approximately 2 months after the first positive SARS-CoV-2 RT-qPCR in November 2020 (day 1). In order to ensure viral clearance before a scheduled and urgently indicated high-dose chemotherapy and autologous hematopoietic stem cell transplantation (HSCT), 2 units of CP were administered on day 57 and one unit on day 59. After nasopharyngeal swabs repeatedly tested negative for SARS-CoV-2, high-dose chemotherapy with obinutuzumab, thiopeta, cytarabine, etoposide, cyclophosphamide, azathioprine, and prednisolone was switched to dexamethasone 6 mg qd. Viral load dropped to 4.62 × 10^5 copies/mL on day 8. However, his clinical condition further deteriorated and by day 12, the viral load had increased again by four log levels (2.27 × 10^9 copies/mL). This prompted us to perform whole genome sequence analysis, which revealed the presence of the E484K immune escape mutation in strain B.1. Of note, this substitution was not present before the treatment with bamlanivimab (Fig. 1A). Interestingly, at day 15 we observed continuous evolution to E484Q, reverting back to E484K on day 16 after administration of three units of convalescent plasma (CP). Unfortunately, the patient could not be stabilized and died on day 20 due to multi-organ failure.

Patient 4 (Fig. 1D) was a caucasian male in his late sixties with relapsed follicular lymphoma who had persistent positive SARS-CoV-2 qPCR with 22 log improvements at the time of bamlanivimab administration. The patient reported only mild symptoms (fatigue), and bamlanivimab 700 mg was administered intravenously on day 2. On day 4, his respiratory situation deteriorated with the need for oxygen supplementation, thus prednisolone was switched to dexamethasone 6 mg qd. Viral load dropped to 4.62 × 10^5 copies/mL on day 8. In parallel, clinical and laboratory parameters improved slowly and could be discharged from the hospital at day 40 with two negative consecutive SARS-CoV-2 qPCR tests.
Fig. 1. Selection of E484K in SARS-CoV-2 infected patients with severe immunosuppression. 

(A) Patient 1 with ANCA-associated vasculitis and pronounced immunosuppression; (B) Patient 2 with severe HIV-associated immunosuppression; (C) Patient 3 with follicular lymphoma and severe immunosuppression due to high-dose chemotherapy; (D) Patient 4 with severe immunosuppression due to heart transplantation; (E) Patient 5 with severe immunosuppression due to chronic lymphocytic leukemia; (F) Patient 6 with severe immunosuppression due to kidney transplantation. Bamla, bamlanivimab; CP, convalescent plasma; RDV, remdesivir; REGN, REGN-ECOV2, casirivimab/imdevimab; CTx, chemotherapy; HTx, heart transplantation; PRDL, prednisolone; DXM, dexamethasone; MMF, mycophenolate mofetil; TAC, tacrolimus; CsA, cyclosporin A; AZA, azathioprine; CCL, chronic lymphocytic leukemia; E484K, substitution in the receptor-binding domain (RBD) associated with immune escape. Time points are color coded according their sequence. White, not determined; black, 484E; red, 484K; blue, 484Q (for details see suppl. Table 1).
SARS-CoV-2 RT-qPCR (3.12 × 10^7 copies/mL, strain B1.258, harbouring E484E) 44 days after the first positive SARS-CoV-2 RT-qPCR in December 2020 before scheduled initiation of CLL treatment with ibritinib. In view of the current risk constellation and the previous illnesses, bamlanivimab 700 mg was applied on day 45, which resulted in only a transient decrease in SARS-CoV-2 viral load. A subsequent increase of the viral load to 4.82 × 10^6 copies/mL prompted us to perform whole-genome sequencing on day 52 (day 8 after bamlanivimab administration), which identified the E484K mutant. An off-label-use therapy with remdesivir was then initiated for a total of 10 days. In addition, cumulatively 3 units of CP were administered (Fig 1E). In the further course a high SARS-CoV-2 viral load persisted (>2.26 × 10^6 copies/mL, still harbouring E484K) so that we decided to administer imdevimab/casirivimab, which was well tolerated by the patient. Viral load subsequently decreased and became negative on day 91. The patient could be discharged from hospital in moderately reduced clinical condition.

Patient 6 (Fig. 1F) was a caucasian female in her mid-sixties with allogeneic cadaveric kidney transplantation performed about 2 months before hospital admission for SARS-CoV2-infection. Her immunosuppressive therapy consisted of 3 mg tacrolimus bid, mycophenolate mofetil 1 g bid ( paused after admission) and prednisolone 20 mg qd. At admission, her SARS-CoV-2 viral load was 9.36 × 10^6 (strain B1.1.60, harbouring E484E). Considering the increased risk for a severe course of COVID-19, we decided to administer 2 units of CP on the day of admission. Because viral load did not decrease over the next 2 weeks, we additionally administered 700 mg of bamlanivimab. Viral load subsequently dropped and became negative on day 26. Without further complications, the patient could be discharged from hospital in good clinical condition.

4. Discussion

Of six severely immunosuppressed SARS-CoV-2-infected patients treated with bamlanivimab, we observed viral immune escape in five patients. The E484K mutation selected here in common SARS-CoV-2 variants is also present in different VOCs associated with immune evasion, including the variant B1.351 initially described in South Africa (WHO label Beta) and the variants P1 (WHO label Gamma) and P2 (WHO label Zeta) detected in Brazil [16-18] which are currently rare in Germany. To investigate the local baseline prevalence of the E484K mutation, all SARS-CoV-2 whole genome sequences available from the Düsseldorf region at that time were screened. Remarkably, only 3 out of the 1270 available sequences presented the E484K mutation and were characterized as B1.351 isolates. This observation and the fact that all our patients harbouring variants with the E484E mutation at baseline support our hypothesis that the E484K mutation was indeed newly selected under the specific immune pressure of bamlanivimab in five of six patients with impaired humoral and cellular immunity. It should also be mentioned that the recently described variant B1.617.1 (first documented in India, WHO label Kappa) harbours the E484Q mutation [19], which was also selected in patient 1.

While it was reported in vitro that SARS-CoV-2 variants harbouring the mutations E484K or E484Q are resistant against neutralization by the monoclonal antibody bamlanivimab [16,18], our clinical observation that these mutations newly emerged under bamlanivimab therapy and potentially impaired clinical outcomes of patients could have important implications not only for the clinical management of individual patients but also concerning epidemiological measures for pandemic control. Especially when used in immunocompromised patients in the outpatient setting, there would be a risk of transmission of viruses with immune escape mutations, which may become highly relevant when such mutations are selected in VOCs associated with increased viral transmission such as the variant B.1.1.7. Indeed, there are already different reports that describe the E484K substitution in the context of B.1.1.7. Due to the complexity of the immunosuppressed patients treated with bamlanivimab, we treated them exclusively as inpatients in single rooms in a dedicated COVID-19 isolation ward with staff trained and highly experienced in PSA, and there was no evidence of transmission of these emerging viral strains harbouring E484K in this setting. After the potential threat was recognized upon receipt of sequencing results, all patients were followed for an extended period of time and only discharged after persistently low and eventually negative SARS-CoV-2 PCR results.

Cautious management and strict adherence to infection prevention and control practices appear to be highly advisable in the context of mAb treatment of SARS-CoV-2-positive immunosuppressed individuals.

Gottlieb and colleagues reported an emergence of escape mutants (E484K; E484Q; E490S and S494P) in 28/297 (9.4%) patients who received bamlanivimab monotherapy and even in 7/145 (4.8%) of patients receiving placebo in the phase 2/3 BLAZE-1 trial [2]. The fact that we observed the occurrence of E484K in a much higher percentage (5/6; 83.3%) when treating severely immunosuppressed patients suggests a significantly higher risk of viral escape in this setting. While the exact reason for the observed emergence of immune escape mutants predominantly in immunocompromised patients is unclear, it is likely that the persistent impairment of humoral and cellular immune control in these patients results in prolonged intervals of viral replication. In addition, with mAb targeting specific epitopes of SARS-CoV-2, escaping antibody neutralization by mutation is easier in the context of a single mAb compared to e.g. polyclonal immune sera and natural immunity. The combination of prolonged intervals of viral replication under narrowly focused selection pressure may explain the rapid viral immune escape observed in our patients.

The differential therapeutic response to bamlanivimab in terms of viral load with at least initial decrease in patients 1, 4, 5, and 6, but on the other hand, unchanged or increasing SARS-CoV-2 viral load after bamlanivimab administration in patients 2 and 3 could be explained by a combination of several factors. First, therapy occurred at different time points within the natural history of SARS-CoV-2 infection, with typically an initial rapid increase in viral load, subsequent stabilization at a high level, and subsequent clearance by the onset of the adaptive immune response. However, this would certainly not explain the course of patient 3, who had been SARS-CoV-2 positive for an extended period of time. Similarly, the development of the immune escape mutation E484K during the course of the disease cannot conclusively explain the lack of timely response to mAb therapy in patients 2 and 3.

We therefore hypothesize that in the context of the severe immunosuppression of these bamlanivimab-treated patients, their own immune function, which changed significantly in some of the patients during the course of the disease, contributed quite substantially to these viral load courses. Patient 2 initially had a very severe cellular immunodeficiency with CD4+ cells of 0/µL, which improved only gradually after initiation of antiretroviral therapy, whereas in patient 3 cellular immunity was transiently profoundly impaired by high-dose chemotherapy with consecutive aplasia. In our view, it should therefore be discussed to what extent a certain degree of cellular immune function may be essential for a successful therapeutic response after administration of monoclonal antibodies.

CP and casirivimab/imdevimab (REGN—COV2) appeared to remain at least partially effective from a clinical perspective when used in our patients with viral rebound even in the presence of E484K (Fig. 1). However, due to the different disease courses, the additional use of remdesivir in two cases and the small number of patients, further data are needed regarding the efficacy in this specific clinical setting.

It is also noteworthy that the only severely immunosuppressed patient without viral rebound (patient 6) had previously been given CP
and thus had not actually received mAb monotherapy. Combinations of two or more mAbs or polyclonal antisera may therefore increase the genetic barrier sufficiently to largely prevent escape of the immune system as known from other viral infections [20]. Furthermore, in the context of variants of concern that already harbour immune escape mutations, it should be kept in mind that functional monotherapy may also be present when a combination of two mAbs is used. However, this needs to be thoroughly evaluated, especially when treating severely immunocompromised patients infected with SARS-CoV-2. The U.S. Food and Drug Administration has recently withdrawn the Emergency Use Authorization for bamlanivimab in consideration of the increasing prevalence of immune escape mutants in the USA. The European Medicines Agency (EMA) issued a recommendation on treatment with bamlanivimab and etesevimab in early March 2021. The EMA concludes that this mAb combination can be used to treat confirmed COVID-19 in patients who do not require supplemental oxygen and who are at high risk for progression of COVID-19 to a severe disease course. The agency also examined the use of bamlanivimab alone, which was available as monotherapy in Germany from the end of January 2021, and concluded that it could be also considered as a treatment option despite uncertainties about the benefits of monotherapy.

Until further data will be available, our results suggest that caution is warranted in the use of monoclonal antibodies in immunocompromised patients infected with SARS-CoV-2.

Contributors

BJ, NL, TF, AW, JT, TL were responsible for conceptualization and supervised the study. BJ, TF, VK, TL, ML, NF, DS, TB, DK, RH, NL, AW, JT contributed to investigation and data curation. BJ, NL, AW, OA, AD, JT conducted the formal analysis. BJ, TF, NL, AW, JT were responsible for methodology, data validation and visualization. BJ, NL, TF, VK, AW, JT, TL contributed to the original draft. All authors critically revised the manuscript and approved the final version of the manuscript.

Data availability statement

Raw data were generated at University Hospital Düsseldorf. Derived or additional data supporting the findings of this study are available from the corresponding author [BJ] upon reasonable request.

Declaration of interests

BJ received honoraria for presentations from Gilead (Remdesivir) as well as Falk, Janssen-Cilag, MSD, BMS, Abbvie, Viiv, Gilead, Boehringer, Fresenius Medical Care (outside the submitted work) and served on advisory boards for Viiv, BMS, Gilead, Theratechnologies (outside the submitted work). VK received lecture fees from Abbvie, Falk, Albireo, Gilead (outside the submitted work). TF was PI for a Gilead clinical trial (Remdesivir) and served on Gilead advisory boards. All other authors declare no competing interests regarding this work.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.lanepe.2021.100164.

References

[1] Palfetta AM, Kim C, Gordon SM, Kim A. Monoclonal antibodies for treating COVID-19. Cleve Clin J Med 2021.
[2] Gottlieb RL, Nirula A, Chen P, 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(7):632–44.
[3] Schoofs T, Klein F, Braunshweig M, et al. HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. Science 2016;352(6288):997–1001.
[4] Kemp SA, Collier DA, Darie RP, et al. SARS-CoV-2 evolution during treatment of chronic infection. Nature 2021.
[5] Collier DA, De Marco A, Ferreira I, et al. SARS-CoV-2 B.1.1.7 sensitivity to mRNA vaccine-elicited, convalescent and monoclonal antibodies. medRxiv 2021.
[6] Kossyvakis A, Menti’s AA, Tryfonopoulou K, et al. Antiviral susceptibility profile of influenza A viruses; keep an eye on immunocompromised patients under prolonged treatment. Eur J Clin Microbiol Infect Dis 2017;36(2):361–71.
[7] Choi B, Choudhary MC, Regan J, et al. Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. N Engl J Med 2020;383(23):2291–3.
[8] Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25(3).
[9] Lübbe N, Seifert T, Scherger S, et al. Extraction-free SARS-CoV-2 detection by rapid RT-qPCR universal for all primary respiratory materials. J Clin Virol 2020;130:104579.
[10] Walker A, Houwaart T, Wienemann T, et al. Genetic structure of SARS-CoV-2 reflects clonal superspreading and multiple independent introduction events. North-Rhine Westphalia, Germany, February and March 2020. Euro Surveill 2020;25(22).
[11] Quick J. ARTIC amplicon sequencing protocol for MinION for nCoV-2019. 2020.
[12] Quick J, Grabbaugh ND, Pullan ST, et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat Protoc 2017;12(6):1261–76.
[13] Walker A, Houwaart T, Finzer P, et al. Characterization of SARS-CoV-2 genetic structure and infection clusters in a large German city based on integrated genomic surveillance, outbreak analysis, and contact tracing. 2021.
[14] Loman NJ, Quick J, Simpson JT. A complete bacterial genome assembled de novo using only nanopore sequencing data. Nat Methods 2015;12(8):733–5.
[15] Robinson JT, Thuroldsdottir H, Winkler W, et al. Integrative genomics viewer. Nat Biotechnol 2011;29(1):313–8.
[16] 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. bioRxiv 2021.
[17] Whimoto CR, Ayres F, Hermanus T, et al. SARS-CoV-2 S0Y1.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med 2021.
[18] Widera M, Wilhelm A, Hehl S, et al. Bamlanivimab does not neutralize two SARS-CoV-2 variants carrying E484K in vitro. 2021.
[19] Cherven S, Potdar V, Jadhav S, et al. Convergent evolution of SARS-CoV-2 spike mutations, L452R, E484Q and P681R, in the second wave of COVID-19 in Maharashtra, India. bioRxiv 2021;2021.04.22.440352.
[20] Margolis DM, Koup RA, Ferrari G. HIV antibodies for treatment of HIV infection. Immunol Rev 2017;275(1):313–23.