Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

SARS-CoV-2 evolution during persistent infection in a CAR-T recipient shows an escape to both sotrovimab and T-cell responses

Dear editor

Immunocompromised patients are at higher risk for severe COVID-19 due to higher baseline viral loads and more common persistent infections. We present the case of a 58 years-old male diagnosed with high-grade B-cell lymphoma in November 2019 and treated with 6 courses of R-CHOP and 4 courses of R-DHAP until November 2020. He remained seronegative at the Euroimmun anti-Spike (S) RBD IgG assay despite receiving 3 doses of BNT162b2 vaccine (last on December 2020). As shown in Fig. 1, on February 8, 2021, a nasopharyngeal swab (NPS) tested positive for SARS-CoV-2 clade G, and the patient recovered after mild symptoms. On April 2021 he received anti-CD19 autologous chimeric antigen receptor T-cell therapy (axicabtagene ciloleucel) achieving complete remission.

On January 29, 2022, he tested positive again for BA.1.1.16, and, on February 2, 2022, he received treatment with the anti-Spike monoclonal antibody sotrovimab. The NPS tested negative at PCR 3 days later, but the patient was admitted to the emergency room with bilateral pneumonia and his NPS turned positive on February 8. He was discharged from the hospital on February 24, but his NPS remained positive, and pneumonia progressed. He was thus readmitted on May 2 and finally discharged on August 16 after NPS negativization (at both PCR and antigen assays). Throughout this period and as shown in Supplementary Table 1, SARS-CoV-2 was detectable at high levels (Abbott Alinity® M SARS-CoV-2 assay cycle threshold 22–23, Lumipulse G® SARS-CoV-2 Ag assay 138–3473 pg/ml) and the viral genome was sequenced on June 15, July 15, and August 4 (Fig. 1). The consensus sequences were retrieved by annotating mutations with frequencies > 0.5.

During the second infection, we isolated the virus from NPS using Vero/TMPRSS2 cells, measured serum anti-S RBD and anti-N IgG levels using Abbott Architect® SARS-CoV-2 Ig assays and examined cell-mediated responses through ELISpot at different timepoints. Furthermore, we analyzed the class I and II HLA loci of the patient and used IEDB NetMHCIIPan 4.0 BA and IEDB NetMHCpan EL 4.1 to investigate changes in the presentation of a previously reported library of S epitopes to CD4+ and CD8+ lymphocytes, respectively (Supplementary Materials). As shown in Supplementary Table 1, serum anti-S RBD IgG increased over time. On May 5, the level was 2294 AU/mL, which increased to 13,896 AU/mL on June 8 and 15,033 AU/mL on

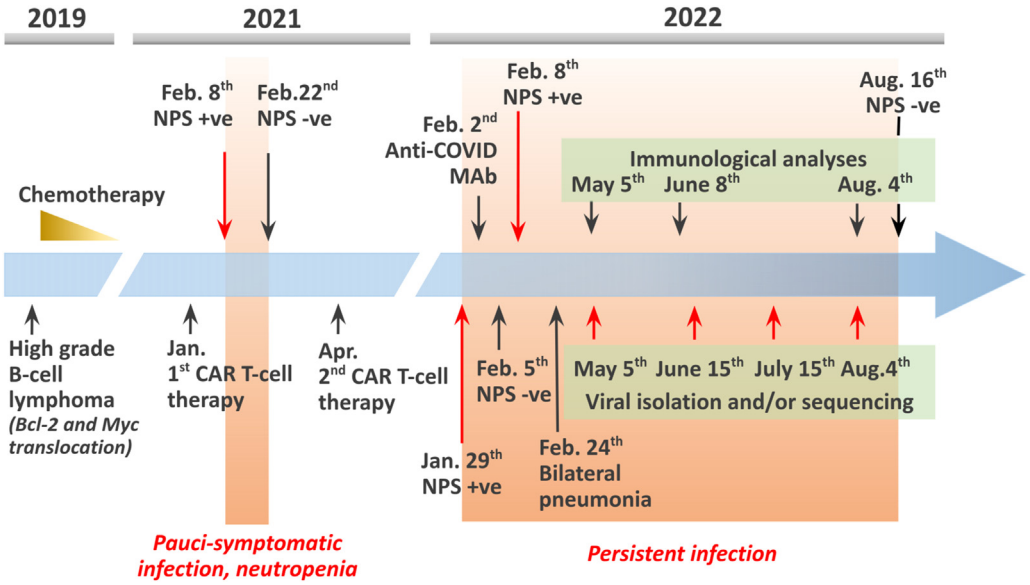

Fig. 1. Timeline outlining the key events in the study patient's SARS-CoV-2 infections. The red arrows indicate the times when PCR on nasopharyngeal swab (NPS) tested positives, either through antigenic or molecular assays, and when viral isolation and/or sequencing were performed. Additional details on the results of antigenic and molecular assays, whole genome sequencing, and immunological analyses are available in Supplementary Tables 1 and 2.

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August 4. Neutralizing antibodies had CPE_{90} titers 160–320 against SARS-CoV-2/Human/ITA/PAVIA10734/2020 (GISAID EPI_ISL_568,579 [1]). At all 3 time points, serum anti-N IgG levels remained negative (data not shown). ELISpot analysis detected S-specific spot forming units (SFU) ranging from 220 to 610 SFU/10^6 PBMCs, but no N-specific SFU. Viral isolation was successful on May 5 and June 8, but not on August 4. Whole viral genome sequencing revealed Spike mutations (L5F, Y145D, E340A, L582F, F855L, and L938F) after receiving sotrovimab, as shown in Supplementary Table 2 (GISAID accession EPI_ISL_16,849,243, EPI_ISL_16,849,243, EPI_ISL_16,849,245). Notably, S:E340A confers immune escape to sotrovimab [2–12]. We also observed an overall reduction in the number of S epitopes that could be presented by the patient's HLA alleles, as depicted in Supplementary Materials.

Our findings underscore the urgent need for tailoring treatment based on the patient's baseline sensitivity and closely monitoring the emergence of resistant variants to improve outcomes.

Authors contributions

Conceptualization: Paola Mazzetti, Pietro Giorgio Spezia, Giulia Freer, Michele Lai, Spartaco Sani, Mauro Pistello; *Methodology:* Paola Mazzetti, Pietro Giorgio Spezia, Anna Lisa Capria, Giulia Freer, Maria Sidoti, Alice Cara, Alfredo Rosellini, Susi Frateschi, Iacopo Franconi, Michele Curcio, Paola Quaranta, Giovanna Moscato, Maria Linda Vatteroni, Silvia Costarelli; *Investigation e formal analysis:* Paola Mazzetti, Pietro Giorgio Spezia, Anna Lisa Capria, Giulia Freer, Maria Sidoti, Alice Cara, Alfredo Rosellini, Giovanna Moscato, Maria Linda Vatteroni, Michele Curcio, Paola Quaranta, Silvia Costarelli, Daniele Focosi; *Resources:* Mauro Pistello; *Original draft preparation:* Paola Mazzetti, Pietro Giorgio Spezia, Giulia Freer, Maria Sidoti; *Writing – review and editing:* Pietro Giorgio Spezia, Giulia Freer, Maria Sidoti, Silvia Costarelli, Daniele Focosi, Mauro Pistello; *Supervision:* Daniele Focosi, Spartaco Sani, Mauro Pistello; *Submission:* Daniele Focosi. All authors have read and agreed to the last version of this manuscript.

Data availability statement

The data set is available on request.

Declaration of competing interest

The authors declare that there is no conflict of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2023.100149.

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