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CASE REPORT

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SARS-CoV-2 genomic evolution during a severe and long-lasting omicron infection under antiviral therapy

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Abstract

Background Prolonged SARS-CoV-2 infection observed in immunocompromised individuals even in the presence of antiviral treatment provides opportunities for viruses to evolve in immune escape and drug-resistant variants.

Case presentation A 72-year-old male with IgG4-related disease was admitted to the Emergency Department of a city Hospital in Milan and then transferred to Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in December 2023, due to respiratory distress due to SARS-CoV-2 infection diagnosed in November 2023. After 117 days since the onset of the infection, and two cycles of sotrovimab/remdesivir combined therapy, the clinical improvement allowed the hospital discharge, notwithstanding the persistent SARS-CoV-2 positivity. Fifteen days later, the patient was re-admitted to the hospital due to worsening clinical conditions. After a third cycle of sotrovimab/remdesivir combined therapy prolonged with nirmatrelvir/ritonavir, nasopharyngeal load dropped and clinical conditions improved, ending with a successful discharge. SARS-CoV-2 whole genome sequences, obtained at six time-points of infection, showed an FL.1.5.1 recombinant form infection and a genetic distance of median (IQR) 0.00052 (0.00041–0.00066) similar to the genetic distance observed among the 43 contemporaneous FL.1.5.1 recombinant forms ($p=0.098$). De novo SNPs were observed at all time points, with a peak ($n=70$) at day 133 of infection, corresponding to the time of the second hospitalization. Six non-synonymous mutations (three in the RdRp and three in the spike protein, four of them known to be associated with drug resistance) appeared transiently, after the third and fourth course of sotrovimab 500 mg/remdesivir combination. Five de novo SNPs, three of them in the spike protein, were fixed over the long-lasting infection. The spike N856K, associated with reduced fusogenicity and infectivity in Omicron BA.1, was completely replaced by constitutive N at day 136.

Conclusions This clinical case confirms the intra-host evolution dynamics of SARS-CoV-2 in an immunocompromised, prolonged-infected individual, involving positions associated with drug resistance and fusogenic traits of SARS-CoV-2. These results underscore the importance of the early detection of SARS-CoV-2

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infection in immunocompromised individuals, and its rapid containment using highly effective treatment, to limit serious complications and the risk of new and potentially concerning viral variants emergence.

Keywords SARS-CoV-2 evolution, Long term infection, Immunocompromised host, Antiviral agents, Antiviral drug resistance

Background

As widely described since the first phases of the pandemic, immunocompromised individuals are at higher risk of developing prolonged SARS-CoV-2 infection with respect to the general population [1–6]. This fragile population cannot efficiently respond to vaccination and is impaired in producing neutralizing antibodies [7]. The inability to efficiently clear the virus poses immunocompromised individuals at risk of serious complications and life-threatening COVID-19 syndrome. Moreover, persistent SARS-CoV-2 replication in the presence of impaired immune responses provides opportunities for viruses to evolve immune escape and possibly other phenotypes [8–10]. SARS-CoV-2 Omicron lineage was first reported in November 2021 in South Africa and quickly spread, immediately becoming the dominant lineage worldwide, and differentiating in different sublineages and recombinant forms [11]. As well as alpha-variant, this variant of concern probably evolved in persistently infected, immunocompromised hosts [12].

Here we describe a case of Omicron FL.1.5.1 long-lasting evolution in a patient affected by IgG4-related disease on Rituximab therapy every six months and experiencing 149 days of persistent SARS-CoV-2 positivity accompanied by critical COVID-19 syndrome. SARS-CoV-2 whole genome sequencing was performed at six-time points, revealing an intra-host viral evolution.

Case presentation

A 72-year-old Caucasian male with IgG4-related disease, involving the pituitary, pulmonary, and lymph nodes, on rituximab therapy every six months (last administered in September 2023) was admitted to the Emergency department of a city Hospital in Milan on December 2023 with fever and dyspnoea caused by SARS-CoV-2 infection (diagnosed 25 days earlier by a rapid antigen swab, Day 25) (Fig. 1). The patient had received the last mRNA anti-SARS-CoV-2 vaccine dose (Pfizer Biontech) one year before. A chest computed tomography (CT) scan revealed interstitial pneumonia, necessitating continuous positive airway pressure (cPAP). Due to the deterioration of clinical condition, the patient was transferred to the Intensive Care Unit (ICU) of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan on Day 37. Here, the respiratory support was escalated to non-invasive ventilation (NIV) and extracorporeal membrane oxygenation (ECMO). SARS-CoV-2 positivity was confirmed by an antigen positive swab. The patient received remdesivir and sotrovimab 500 mg on Day 38. Additionally, following pulmonary and immunological evaluations, the patient underwent high-dose steroid bolus therapy (methylprednisolone 10 mg/kg/day for three days, followed by 1 mg/kg/day for other three days) for an accelerated phase of pulmonary interstitial disease and received IgVena infusion for replacement therapy (Day 40).

After 10 days of ICU stay, the patient was transferred to the Infectious Diseases Department, where a tapering regimen of steroid therapy from methylprednisolone 80 mg to prednisone 50 mg (ongoing at discharge) and reduction of respiratory support to 3 l/min via nasal cannula continued (Day 47). The patient continued to test positive at the nasopharyngeal swab by rapid antigenic test. The hospitalization was complicated by systemic CMV reactivation, successfully treated with ganciclovir for 7 weeks, and Enterococcus faecalis urinary sepsis, treated with ampicillin for 14 days (Day 55). The persistent positivity to SARS-CoV-2 was confirmed by RT-PCR assay, revealing a mean Ct value of 18.9, and a positive result of SARS-CoV-2 culture isolation (Day 79; Fig. 1). Due to persistent SARS-CoV-2 positivity, cixagevimab/cilgavimab 300/300 mg IM was administered (Day 80) and clinical parameters temporarily improved.

At Day 96, 71 days after the first episode, the patient experienced a second episode of severe COVID-19 when fever reappeared accompanied by an increase in C-reactive protein (CRP) (peak 16 mg/dL), worsening radiological findings, and respiratory function. Microbiological investigations on blood and urine were negative, and an initial course of empiric antibiotic therapy with piperacillin/tazobactam did not yield improvement. A peak of nasopharyngeal SARS-CoV-2 load was observed at Day 106 with a mean Ct value of 16.9. Therefore, two days later, a second course of antiviral therapy with remdesivir and sotrovimab 500 mg was carried out. This resulted in clinical improvement, with fever resolution and CRP reduction (4 mg/dL at discharge), even if SARS-CoV-2 load remained high (mean Ct 19 at Day 111). At Day 117, the patient was transferred to a Rehabilitation Institute to continue respiratory physiotherapy.

After 133 days since the onset of the first infection and 16 days after the last hospital discharge, the patient was re-admitted to the hospital with fever and respiratory distress (Fig. 1). This third episode of severe COVID-19 was accompanied by the continuous SARS-CoV-2 positivity of nasopharyngeal swab, with a mean Ct value of

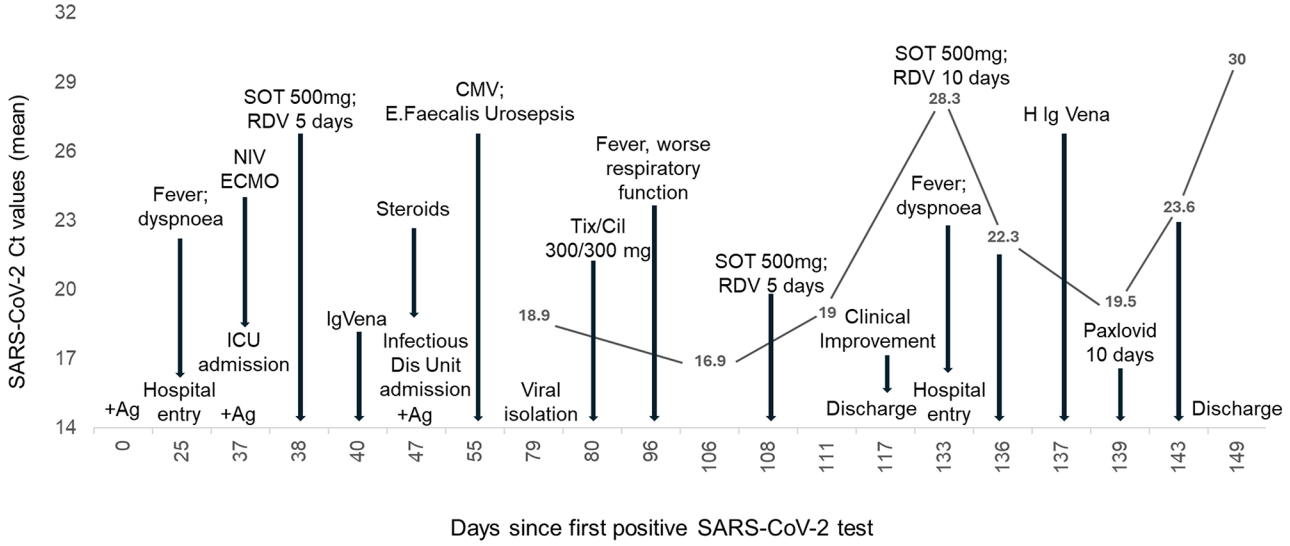

Fig. 1 Clinical case history. Timeline of the case. SARS-CoV-2 load expressed as mean Ct values obtained by real time PCR over time. Black arrows indicate clinical history while gray arrows indicate antiviral treatment cycles. CT: cycle threshold values; +Ag: Positive rapid antigenic test; NIV: non-invasive ventilation; ECMO: extracorporeal membrane oxygenation; SOT: Sotrovimab; TIX/CIL: tixagevimab/cilgavimab; 300/300 mg; RDV: remdesivir; H IgVena: intravenous normal human immunoglobulin

28 (Day 133). Antiviral treatment based on sotrovimab infusion and remdesivir for initial 5 days was started. Nasopharyngeal swabs were repeated Day 136 and Day 139, resulting in a progressive increase of viral load (mean Ct: 22 and 19.5, Fig. 1). Due to SARS-CoV-2 persistent positivity, infusion of intravenous normal human immunoglobulin solution 400 mg/kg in a single administration was carried out at Day 137 and remdesivir treatment continued until Day 143. This treatment approach was combined with nirmatrelvir/ritonavir, administered at Day 139 for 10 days. RT-PCR of nasopharyngeal swabs were repeated at days 4, and 10 of nirmatrelvir/ritonavir treatment, resulting in a progressive sharp drop in viral load (mean Ct: 23.6 and negative, Fig. 1).

After 149 days since the first onset of symptoms, the patient had the nasopharyngeal SARS-CoV-2 RT-PCR test negative, and clinical conditions improved. Thus, the patient was successfully discharged.

SARS-CoV-2 whole genome sequences were obtained at six time-points since the first SARS-CoV-2 positive test (T0_Day79; T1_Day106; T2_Day111; T3_Day133; T4_Day136; T5_Day143) (Fig. 2 and Supplementary Figs. 1 and 2). All sequences belonged to Omicron FL.1.5.1 recombinant form. The six strains clustered together when placed with 43 genetically similar background isolates (bootstrap = 100%, Fig. 2), consistent with prolonged infection caused by a single strain. The genetic distance among the six strains was a median (IQR) of 0.00052 (0.00041–0.00066) base substitution per site, similar to the genetic distance observed among the 43 contemporaneous FL.1.5.1 recombinant forms (0.00063 [0.00051–0.00075], $P = 0.098$). This confirms the ability of mutation accumulation of prolonged infections [13].

To account for the single nucleotide polymorphisms (SNP) detected, any SNP present in all the six samples analyzed was considered constitutive, while any SNP present de novo in at least one sequence was considered non-constitutive. When occurring in the gene coding regions, SNPs were defined synonymous or nonsynonymous, depending on the maintenance or alteration of amino acid in the corresponding codon.

Among the constitutive SNPs, notable mutations included the non-synonymous G671S in RdRp, which was associated with a < 2.5-fold reduction in remdesivir susceptibility [14] and had an intrapatient prevalence of over 60% in all six strains, and the spike S371F, associated with a 25-fold reduction in sotrovimab susceptibility [14], which was detected with 100% intrapatient prevalence in all six strains (Fig. 3).

Regarding non-constitutive SNPs, 17 were detected at T0_Day79 (median intrapatient prevalence: 7.2% [IQR: 6.3–9.2]; non-synonymous: 13). SNPs increased to 34 and 35 at T1_Day106 and T2_Day111 respectively (intrapatient prevalence: 16.3% [86.9–44.7] and 15.1% [7.6–22.7]; non-synonymous: 27 and 23, respectively). The number of SNPs reached a peak at T3_Day133 ($n = 70$; intrapatient prevalence: 10.4% [6.5–17.2]; non-synonymous: 52), at the time of the second hospitalization, and decreased to 33 and 19 respectively at T4_Day136 and T5_Day143 (intrapatient prevalence: 17.7% [8.3–27.9] and 49.2% [37.8–97.8]; non-synonymous: 26 and 12, respectively) (Supplementary Fig. 2).

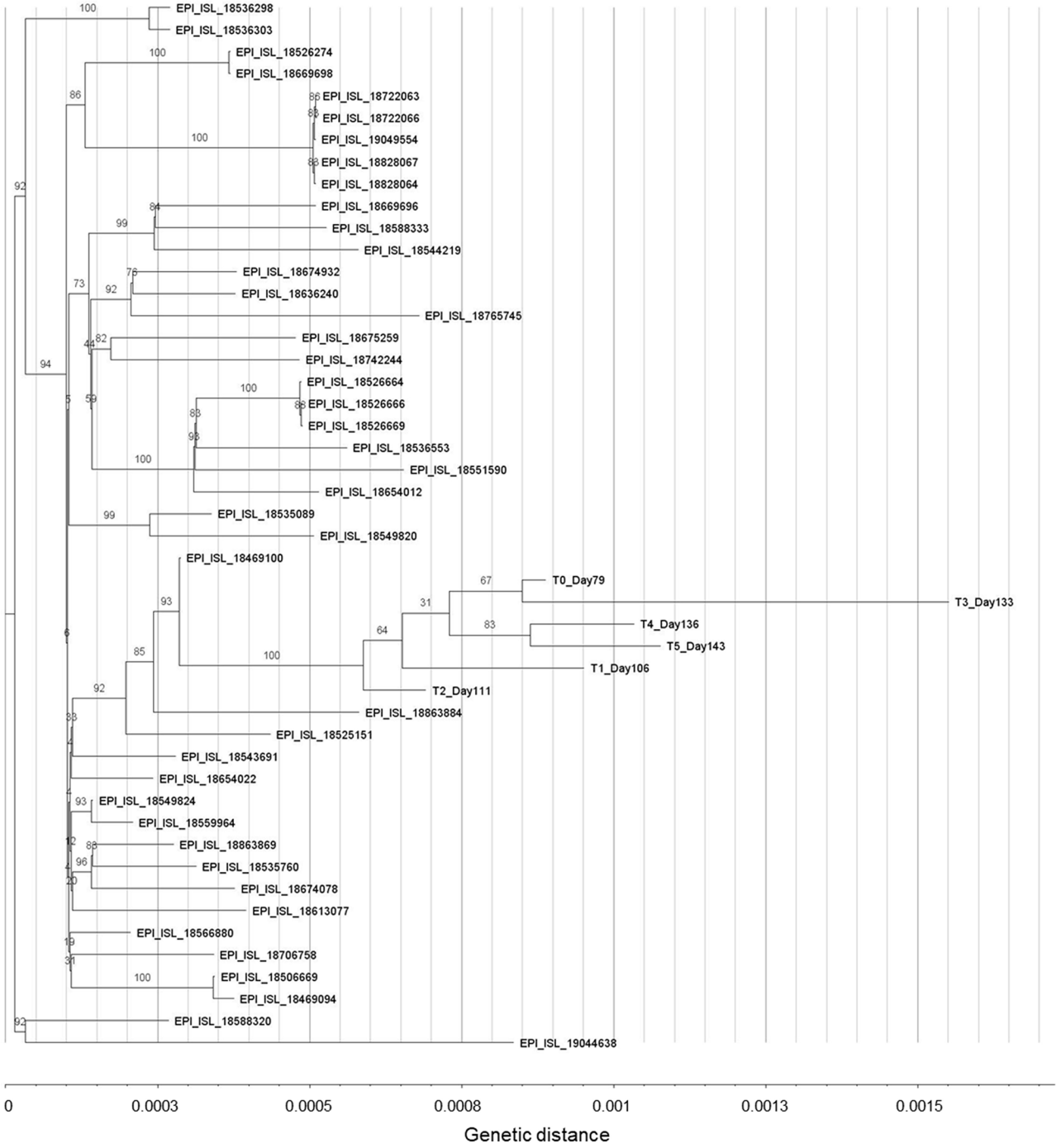

Fig. 2 Phylogenetic tree. Estimated maximum likelihood (ML) phylogeny of SARS-CoV-2 genomes from the six samples of the same patient and additional 43 FL.1.5.1 contemporaneous sequences. All SNPs having a minimum supporting read frequency of 40% and a depth ≥ 100 reads were included in the whole genome SARS-CoV-2 sequences. The ML phylogeny was estimated with IqTree using the best-fit model of nucleotide substitution TIM + F + I with 1000 replicates fast bootstrapping. T0_Day79 since the first SARS-CoV-2 positive test; T1_Day106; T2_Day111; T3_Day133; T4_Day136; T5_Day143

Among non-synonymous non-constitutive SNPs, six mutations appeared transiently (Fig. 3). The RdRp Q573K emerged on T1_Day106 (intrapatient prevalence: 20.0%), after 25 days of the third course of sotrovimab 500 mg/remdesivir combination, and persisted since T3_Day133 (intrapatient prevalence: 18.0%). The spike L542R and K356T, associated with reduced susceptibility to cilgavimab and sotrovimab, respectively, (intrapatient prevalence: 80.2% 16.4%), appeared transiently at T3_Day133, 52 days after the cixagevimab/cilgavimab administration and after 25 days of the third course of sotrovimab 500 mg/remdesivir combination. The RdRp V637L and

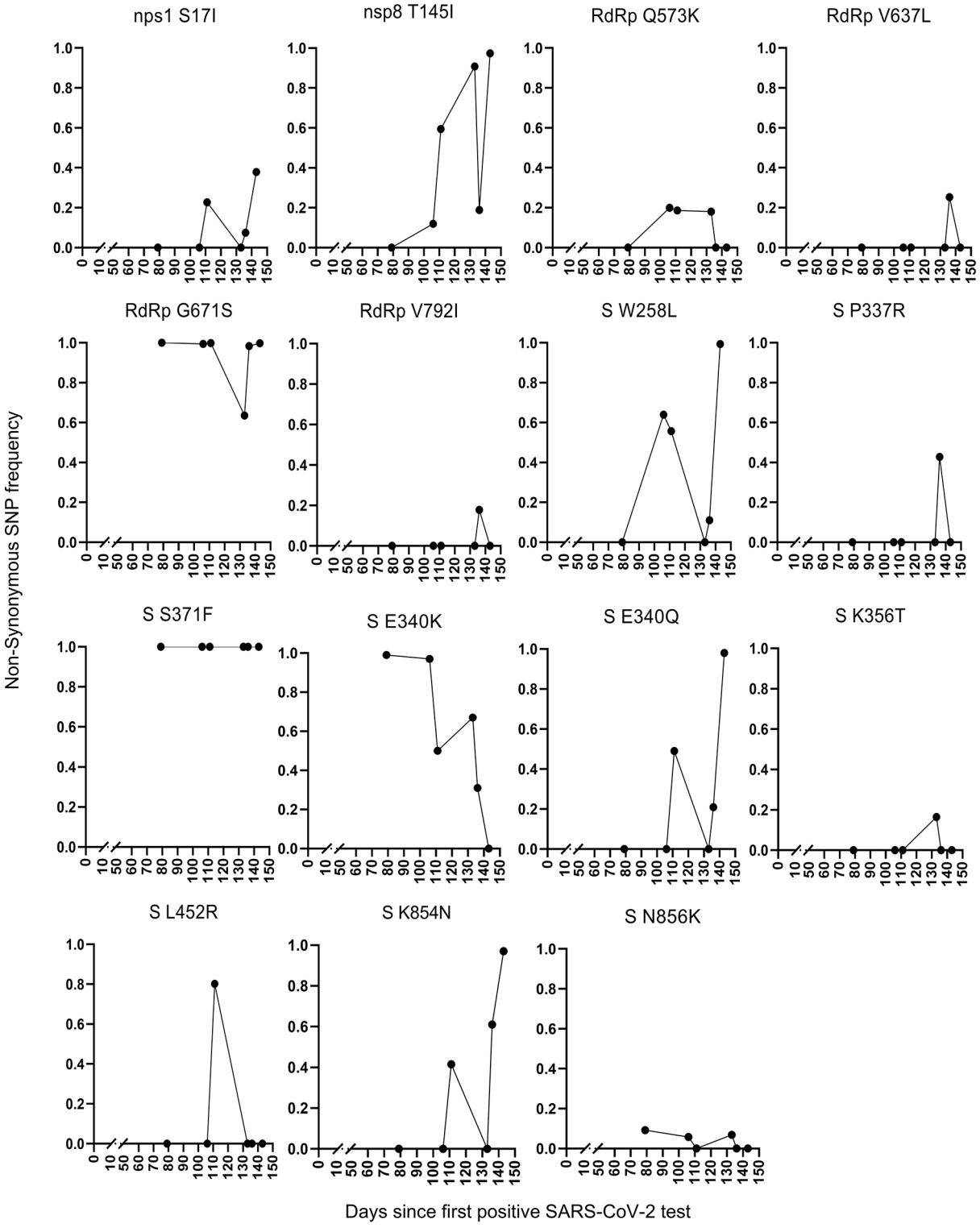

Fig. 3 Dynamics of drug resistance and non-synonymous de novo selected or disappearing SNPs during SARS-CoV-2 infection. Temporal frequencies of 15 SNPs identified in the SARS-CoV-2 genome. Time points with SARS-CoV-2 sequence available: Day79 since the first SARS-CoV-2 positive test; Day106; Day111; Day133; Day136; Day143

V792I and the spike P337R (intrapatient prevalence: 25.2%, 17.8% and 42.8%, respectively), associated with >100-fold reduced sotrovimab susceptibility, emerged at T4_Day136, after 28 days of the third course and 3 days of the fourth course of sotrovimab 500 mg/remdesivir combination.

Five non-synonymous non-constitutive SNPs were fixed over the long-lasting infection (Fig. 3). The nsp8 T145I and the spike W258L were first detected at T1_Day106 (intrapatient prevalence: 12.0% and 64.0%, respectively), and reached 97.4% and 99.4% at T5_Day143. The nsp1 S17I, and the spike E340Q and K854N were first detected at T2_Day111 (intrapatient prevalence: 22.0%, 49.0% and 41.6%, respectively), and reached 37.8%, 97.8%, 97.1%, respectively, at T5_Day143.

Two non-synonymous SNPs progressively decreased. The spike E340K, associated with >25-fold reduction in sotrovimab susceptibility [14] and constitutively present in Omicron sublineages, progressively decreased from 99% at T0_Day79 to 31% at T4_Day136. The spike N856K, associated with reduced fusogenicity and infectivity in Omicron BA.1 [15] and found with an intrapatient prevalence <10% at T0_Day79, T1_Day106, and T3_Day133 was instead replaced by constitutive N at T4.

For details regarding material and methods and sequencing statistics refer to Supplementary Material.

Discussion and conclusion

Here we described three episodes of severe COVID-19 in an immune-suppressed patient with a 149-day-long infection sustained by Omicron FL.1.5.1 recombinant form. Recurring symptomatic SARS-CoV-2 infection have been documented in immunocompromised individuals, particularly, patients with hematologic malignancy with B-cell depletion after anti-cluster of differentiation (CD) 20 antibodies or chimeric antigen receptor T-cell therapy [16].

The characterization of SARS-CoV-2 whole genome sequences at the available six-time points suggests a considerable within-host evolutionary rate, much higher than the contemporaneous SARS-CoV-2 inter-host variation [13]. At 79 days after the infection, the virus was still viable, as suggested by the positivity of viral culture, a time much higher than those reported by literature in the general population infected by Omicron variants and in immunocompromised persons [17–18].

The patient was also treated with three cycles of sotrovimab/remdesivir combined therapy. In this regard, three RdRp mutations with an intrapatient prevalence never above 30% appeared transiently during the courses of these treatments. Among them, the V792I, localized in the Domain II of the enzyme, was already described to enhance the I779-mediated hydrophobic interaction with remdesivir-triphosphate, preventing entry of the drug into the active site [19]. After the third course of sotrovimab and remdesivir combination, sotrovimab resistance mutations K356T and P337R appeared transiently with an intrapatient prevalence of 16.4% and 42.8%, respectively. Even if not fixed, the emergence of these mutations is generally consistent with previous findings of mAb resistance [6, 20].

SARS-CoV-2 Omicron variants are also known to harbor spike protein mutations responsible for their attenuated fusogenic phenotype [15, 21], like mutations at amino acid 547 (T547K), 655 (H655Y), 856 (N856K), and 969 (N969K). In this clinical case, one of these mutations, the spike N856K, localized in the S2 domain, was found with a low intrapatient prevalence at Day 79, Day 106, and Day 133, and disappeared at Day 136 and Day 143. The spike K854N, localized two amino acids before the N856K and rarely reported in delta variants [22], appeared after 111 days of the diagnosis, increased its intrapatient prevalence over time, and was finally fixed between Day 136 and 143. Based on the increased viral load and severe manifestations occurring at Day 136 and 143, we cannot exclude that these modifications in the S2 domain increased the S1/S2 cleavage, restored the viral fusogenicity, and possibly impacted the clinical course.

Among the other fixed non-constitutive mutations, the spike W258L and E340Q were localized in the N-terminal domain (NTD) and Receptor-Binding-Domain (RBD), respectively. While the E340Q is known to be a sotrovimab-resistant mutation [14], the exact functional role of W258L remains object of study, even if this mutation has been already found [23], and has been localized in the NTD, known to potentially influence tissue tropism and pathogenicity, and to potentially confer evasion from neutralizing antibodies due to its variability [24]. Finally, the fixed nsp8 T145I mutation has been recently described for its role in stabilizing the viral replication protein complex [25].

Overall, these findings are in line with those already described in SARS-CoV-2 intra and inter-host evolution [26–28]. The evolution of SARS-CoV-2 variants is shaped by a complex process in which variants can occasionally be fixed or lost during a long-term infection. These mechanisms have been described mainly in persistent SARS-CoV-2 infection in immunosuppressed individuals [29]. Many nonsynonymous substitutions generated in the host during long-term infection (positive selection) tend to be unfixed as SNPs in the population (negative selection). Thus, many mutations selected and fixed occasionally in long-term infections are not persistent over time, and only the few that offer some selective advantages are likely to persist and be transmitted.

Also, in this clinical case, the variant dynamics showed fluctuations in certain SNPs, with some experiencing a drop in prevalence, even falling below the detection

threshold, only to return to high frequencies several days later. This occurred primarily on Day 133 of infection, which coincided with the third episode of severe COVID-19, marked by ongoing SARS-CoV-2 positivity in the nasopharyngeal swab with a mean Ct value of 28. On Day 133, the prevalence of some mutations decreased (nsp1 S17I, nsp8 T145I, RdRp G671S, Spike W258L, E340Q, K854N), while others emerged at low frequencies (RdRp V637L and V792I, Spike K356T). The sharp fluctuations in SARS-CoV-2 were previously described by Fario et al., J Vir 2024 [30], who suggested that these fluctuations could be linked to a bottleneck effect, potentially driven by the spatial structuring of within-host viral genetic diversity. Alternatively, such fluctuations might be due to sampling artifacts from poor-quality viral population samples. While the latter is unlikely, given the 100x depth coverage thresholds used, we cannot entirely rule out this possibility because of the correlation between high Ct values and noise in SNP detection [30].

As a limitation of our study, the SARS-CoV-2 genome at the baseline of infection or before the first sotrovimab/remdesivir treatment is unavailable. This prevents us from tracing the virus's evolutionary trajectories from the early stages of infection. This limitation may also account for the higher genetic distance characterizing the first SARS-CoV-2 sequence available (corresponding to Day 79 of infection) with respect to the background sequences (Fig. 2).

The viability of the virus was tested at a single time point, after 79 days of infection, limiting the possibility of speculating about the shedding of newly emerged and drug-resistant SARS-CoV-2 variants. No information regarding potential SARS-CoV-2 evolution was available after the nirmatrelvir/ritonavir treatment, due to the rapid decrease of viral load observed. However, the rapid elimination of the virus after this last therapy course suggests a null or negligible viral evolution.

Regarding the treatment approach used in this clinical case, sotrovimab/remdesivir combined therapy was administered at three different time-points to limit viral replication at different stages, offering a broader antiviral activity. Nirmatrelvir/ritonavir was administrated during the third episode of severe COVID-19, successfully reducing viral replication. This drug combination has shown the ability to reduce the severity of COVID-19 in high-risk patients when administered within the first 5 days of symptom onset, even if some real-world evidence suggests a role of this drug in reducing replication and disease course also in individuals with more than 5-days long infection [31–32].

Overall, this clinical case confirms the intra-host evolution dynamics of SARS-CoV-2 in an immunocompromised, prolonged-infected individual, involving positions associated with drug resistance or fusogenic phenotype of SARS-CoV-2. Once again, these results underscore the importance of the early detection of SARS-CoV-2 infection in immunocompromised individuals, and its rapid containment thanks to highly effective treatment, to limit serious complications and the risk of emergence of new and potentially concerning viral variants.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10740-w.

Supplementary Material 1

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Author contributions

MB and SUR performed data analysis, data interpretation, and writing; LA and AL performed clinical evaluation and help in data interpretation and writing; BZP and AP helped in data processing; SUR and AP processed samples; AG and AB critically revised the manuscript; AM and CA conceived the clinical case. All authors revised and approved the manuscript.

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Data availability

The SARS-CoV-2 sequences characterizing this episode are openly available on the SRA portal under the accession numbers SAMN42723708-SAMN42723713 (BioProject: PRJNA1138626) ad the following link: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1138626. This published article includes all the other data analyzed during this study [and its supplementary information files].

Declarations

Ethics approval and consent to participate

The study protocol has been approved by the Territorial Ethical Committee Lombardy 3 (5164_Case.Report_11.09.2024_P). This study was conducted following the principles of the 1964 Declaration of Helsinki. The data used in this study were anonymized before use.

Consent for publication

A written informed consent was obtained by the patient.

Competing interests

The authors declare no competing interests.

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References

1. Baang JH, Smith C, Mirabelli C, Valesano AL, Manthei DM, Bachman MA, Wobus CE, Adams M, Washer L, Martin ET, Lauring AS. Prolonged severe acute respiratory syndrome coronavirus 2 replication in an immunocompromised patient. J Infect Dis. 2021;223(1):23–7. https://doi.org/10.1093/infdis/jiaa666.
2. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, Solomon IH, Kuo HH, Boucau J, Bowman K, Adhikari UD, Winkler ML, Mueller AA, Hsu TY, Desjardins M, Baden LR, Chan BT, Walker BD, Lichterfeld M, Brigl M, Kwon DS, Kanjilal S, Richardson ET, Jonsson AH, Alter G, Barczak AK, Hanage WP, Yu XG, Gaiha GD, Seaman MS, Cernadas M, Li JZ. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med. 2020;383(23):2291–3. https://doi.org/10.1056/NEJMc2031364.
3. Chaguza C, Hahn AM, Petrone ME, Zhou S, Ferguson D, Breban MI, Pham K, Peña-Hernández MA, Castaldi C, Hill V, Yale, Schulz W, Swanstrom RI, Roberts SC, Grubaugh ND. Accelerated SARS-CoV-2 intrahost evolution leading to distinct genotypes during chronic infection. Cell Rep Med. 2023;4(2):100943. https://doi.org/10.1016/j.xcrm.2023.100943.
4. Wilkinson SAJ, Richter A, Casey A, Osman H, Mirza JD, Stockton J, Quick J, Ratcliffe L, Sparks N, Cumley N, Poplawski R, Nicholls SN, Kele B, Harris K, Peacock TP, Loman NJ. Recurrent SARS-CoV-2 mutations in immunodeficient patients. Virus Evol. 2022;8(2):veac050. https://doi.org/10.1093/ve/veac050.
5. Raglow Z, Surie D, Chappell JD, Zhu Y, Martin ET, Kwon JH, Frosch AE, Mohamed A, Gilbert J, Bendall EE, Bahr A, Halasa N, Talbot HK, Grijalva CG, Baughman A, Womack KN, Johnson C, Swan SA, Koumans E, McMorrow ML, Harcourt JL, Atherton LJ, Burroughs A, Thornburg NJ, Self WH, Lauring AS. Investigating respiratory viruses in the acutely ill (IVY) network. SARS-CoV-2 shedding and evolution in patients who were immunocompromised during the Omicron period: a multicentre, prospective analysis. Lancet Microbe. 2024;5(3):e235–46. https://doi.org/10.1016/S2666-5247(23)00336-1.
6. Marques AD, Graham-Wooten J, Fitzgerald AS, Sobel Leonard A, Cook EJ, Everett JK, Rodino KG, Moncla LH, Kelly BJ, Collman RG, Bushman FD. SARS-CoV-2 evolution during prolonged infection in immunocompromised patients. mBio. 2024;15(3):e0011024. https://doi.org/10.1128/mbio.00110-24.
7. Hogan JI, Duerr R, Dimartino D, Marier C, Hochman SE, Mehta S, Wang G, Heguy A. Remdesivir resistance in transplant recipients with persistent coronavirus disease 2019. Clin Infect Dis. 2023;76(2):342–5. https://doi.org/10.1093/cid/ciac769.
8. Machkovech HM, Hahn AM, Garonzik Wang J, Grubaugh ND, Halfmann PJ, Johnson MC, Lemieux JE, O'Connor DH, Piantadosi A, Wei W, Friedrich TC. Persistent SARS-CoV-2 infection: significance and implications. Lancet Infect Dis 2024 Feb 7:S1473-3099(23)00815-0. doi: https://doi.org/10.1016/S1473-3099(23)00815-0.
9. Ghafari M, Liu Q, Dhillon A, Katzourakis A, Weissman DB. Investigating the evolutionary origins of the first three SARS-CoV-2 variants of concern. Front Virol. 2022;2:942555.
10. Tegally H, Moir M, Everatt J, Giovanetti M, Scheepers C, Wilkinson E, Subramoney K, Makatini Z, Moyo S, Amoako DG, Baxter C, Althaus CL, Anyaneji UJ, Kekana D, Viana R, Giandhari J, Lessells RJ, Maponga T, Maruapula D, Choga W, Matshaba M, Mbulawa MB, Msomi N, NGS -SA consortium;, Naidoo Y, Pillay S, Sanko TJ, San JE, Scott L, Singh L, Magini NA, Smith-Lawrence P, Stevens W, Dor G, Tshiabuila D, Wolter N, Preiser W, Treurnicht FK, Venter M, Chiloane G, McIntyre C, O'Toole A, Ruis C, Peacock TP, Roemer C, Kosakovsky Pond SL, Williamson C, Pybus OG, Bhiman JN, Glass A, Martin DP, Jackson B, Rambaut A, Laguda-Akingba O, Gaseitsiwe S, von Gottberg A, de Oliveira T. Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. Nat Med. 2022;28(9):1785–1790. https://doi.org/10.1038/s41591-022-01911-2
11. Dhama K, Nainu F, Frediansyah A, Yatoo MI, Mohapatra RK, Chakraborty S, Zhou H, Islam MR, Mamada SS, Kusuma HI, Rabaan AA, Alhumaid S, Mutair AA, Iqhrammullah M, Al-Tawfiq JA, Mohaini MA, Alsalman AJ, Tuli HS, Chakraborty C, Harapan H. Global emerging Omicron variant of SARS-CoV-2: impacts, challenges and strategies. J Infect Public Health. 2023;16(1):4–14. https://doi.org/10.1016/j.jiph.2022.11.024.
12. Markov PV, Ghafari M, Beer M, et al. The evolution of SARS-CoV-2. Nat Rev Microbiol. 2023;21:361–79.
13. Chaguza C, Hahn AM, Petrone ME, et al. Accelerated SARS-CoV-2 intrahost evolution leading to distinct genotypes during chronic infection. Cell Rep Med. 2023;4:100943.
14. https://covdb.stanford.edu/drms, last access 05/02/2025.
15. Sun C, Wang H, Yang J, et al. Mutation N856K in Spike reduces fusogenicity and infectivity of Omicron BA.1. Sig Transduct Target Ther. 2023;8:75. https://doi.org/10.1038/s41392-022-01281-8.
16. Aydillo T, Gonzalez-Reiche AS, Aslam S, van de Guchte A, Khan Z, Obla A, Dutta J, van Bakel H, Aberg J, García-Sastre A, Shah G, Hohl T, Papanicolaou G, Perales MA, Sepkowitz K, Babady NE, Kamboj M. Shedding of viable SARS-CoV-2 after immunosuppressive therapy for Cancer. N Engl J Med. 2020;383(26):2586–8. https://doi.org/10.1056/NEJMc2031670.
17. Wu Y, Guo Z, Yuan J, Cao G, Wang Y, Gao P, Liu J, Liu M. Duration of viable virus shedding and polymerase chain reaction positivity of the SARS-CoV-2 Omicron variant in the upper respiratory tract: a systematic review and meta-analysis. Int J Infect Dis. 2023;129:228–35. https://doi.org/10.1016/j.ijid.2023.02.011.
18. Kang SW, Kim JW, Kim JY, Lim SY, Jang CY, Chang E, Yang JS, Kim KC, Jang HC, Kim D, Shin Y, Lee JY, Kim SH. Characteristics and risk factors of prolonged viable virus shedding in immunocompromised patients with COVID-19: a prospective cohort study. J Infect. 2023;86(4):412–4. https://doi.org/10.1016/j.jinf.2023.01.024.
19. Igari H, Sakao S, Ishige T, et al. Dynamic diversity of SARS-CoV-2 genetic mutations in a lung transplantation patient with persistent COVID-19. Nat Commun. 2024;15:3604. https://doi.org/10.1038/s41467-024-47941-x.
20. Palomino-Cabrera R, Tejerina F, Molero-Salinas A, Ferris M, Veintimilla C, Catalán P, Rodríguez Macias G, Alonso R, Muñoz P, García de Viedma D, Pérez-Lago L. Gregorio Marañón Microbiology-ID COVID 19 study group. Frequent emergence of resistance mutations following complex Intra-Host genomic dynamics in SARS-CoV-2 patients receiving Sotrovimab. Antimicrob Agents Chemother. 2023;67(7):e0026623. https://doi.org/10.1128/aac.00266-23.
21. Park SB, Khan M, Chiliveri SC, et al. SARS-CoV-2 Omicron variants harbor Spike protein mutations responsible for their attenuated fusogenic phenotype. Commun Biol. 2023;6:556. https://doi.org/10.1038/s42003-023-04923-x.
22. Baj A, Novazzi F, Drago Ferrante F, Genoni A, Tettamanzi E, Catanoso G, Dalla Gasperina D, Dentali F, Focosi D, Maggi F. Spike protein evolution in the SARS-CoV-2 Delta variant of concern: a case series from Northern Lombardy. Emerg Microbes Infect. 2021;10(1):2010–5. https://doi.org/10.1080/22221751.2021.
23. Paul D, Pyne N, Paul S. Mutation profile of SARS-CoV-2 Spike protein and identification of potential multiple epitopes within Spike protein for vaccine development against SARS-CoV-2. Virus Disease. 2021;32(4):703–26. https://doi.org/10.1007/s13337-021-00747-7.
24. Ciccozzi M, Pascarella S. Two sides of the same coin: the N-terminal and the receptor binding domains of SARS-CoV-2 Spike. Future Virol. 2023 Feb. https://doi.org/10.2217/fvl-2022-0181.
25. Subong BJJ, Ozawa T. Bio-Chemoinformatics-Driven analysis of nsp7 and nsp8 mutations and their effects on viral replication protein complex stability. Curr Issues Mol Biol. 2024;46(3):2598–619. https://doi.org/10.3390/cimb46030165.
26. Lythgoe KA, Hall M, Ferretti L, de Cesare M, MacIntyre-Cockett G, Trebes A, Andersson M, Otecko N, Wise EL, Moore N, Lynch J, Kidd S, Cortes N, Mori M, Williams R, Vernet G, Justice A, Green A, Nicholls SM, Ansari MA, Abeler-Dörner L, Moore CE, Peto TEA, Eyre DW, Shaw R, Simmonds P, Buck D, Todd JA, Oxford Virus Sequencing Analysis Group (OVSG), Connor TR, Ashraf S, da Silva Filipe A, Shepherd J, Thomson EC, COVID-19 Genomics UK (COG-UK) Consortium, Bonsall D, Fraser C, Golubchik T. SARS-CoV-2 within-host diversity and transmission. Science. 2021;372(6539):eabg0821. https://doi.org/10.1126/science.abg0821.
27. Li J, Du P, Yang L, Zhang J, Song C, Chen D, Song Y, Ding N, Hua M, Han K, Song R, Xie W, Chen Z, Wang X, Liu J, Xu Y, Gao G, Wang Q, Pu L, Di L, Li J, Yue J, Han J, Zhao X, Yan Y, Yu F, Wu AR, Zhang F, Gao YQ, Huang Y, Wang J, Zeng H, Chen C. Two-step fitness selection for intra-host variations in SARS-CoV-2. Cell Rep. 2022;38(2):110205. https://doi.org/10.1016/j.celrep.2021.110205.
28. Hou M, Shi J, Gong Z, Wen H, Lan Y, Deng X, Fan Q, Li J, Jiang M, Tang X, Wu CI, Li F, Ruan Y. Intra- vs. Interhost evolution of SARS-CoV-2 driven by uncorrelated Selection-The evolution thwarted. Mol Biol Evol. 2023;40(9):msad204. https://doi.org/10.1093/molbev/msad204.
29. Kemp SA, Collier DA, Datir RP, Ferreira I, Gayed S, Jahun A, Hosmillo M, Rees-Spear C, Mlcochova P, Lumb IU, et al. SARS-CoV-2 evolution during treatment of chronic infection. Nature. 2021;592:277–82.
30. Farjo M, Koelle K, Martin MA, Gibson LL, Walden KKO, Rendon G, Fields CJ, Alnaji FG, Gallagher N, Luo CH, Mostafa HH, Manabe YC, Pekosz A, Smith

RL, McManus DD, Brooke CB. Within-host evolutionary dynamics and tissue compartmentalization during acute SARS-CoV-2 infection. J Virol. 2024;98(1):e0161823. https://doi.org/10.1128/jvi.01618-23.
31. Choi MH, Wan EYF, Wong ICK, Chan EWY, Chu WM, Tam AR, Yuen KY, Hung IFN. Comparative effectiveness of combination therapy with nirmatrelvir-ritonavir and Remdesivir versus monotherapy with Remdesivir or nirmatrelvir-ritonavir in patients hospitalised with COVID-19: a target trial emulation study. Lancet Infect Dis. 2024;24(11):1213–24. https://doi.org/10.1016/S1473-3099(24)00353-0.
32. Qiu Y, Wen H, Wang H, Sun W, Li G, Li S, Wang Y, Zhai J, Zhan Y, Su Y, Long Z, Li Z, Ye F. Real-world effectiveness and safety of nirmatrelvir-ritonavir (Paxlovid)-treated for COVID-19 patients with onset of more than 5 days: a retrospective cohort study. Front Pharmacol. 2024;15:1401658. https://doi.org/10.3389/fphar.2024.1401658.

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