SARS-CoV-2 evolution in a patient with secondary B-cell immunodeficiency: A clinical case

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ABSTRACT

The article highlights the course of long-term SARS-CoV-2 infection in a patient with a secondary immunodeficiency developed with B-cell-depleting therapy of the underlying disease. Analysis of the intrapatient virus evolution revealed an inpatient S/SGA mutation that alters the 72GNTGKR78 motif of the S-protein, with a possible role in binding to alternative cellular receptors. Therapy with a ready-made COVID-19-globulin preparation (native human immunoglobulin G (IgG) derived from the plasma of convalescent COVID-19-patients) resulted in rapid improvement of the patient's condition, fast, and stable elimination of the virus, and passive immunization of the patient for at least 30 days. The results suggest the use of products containing neutralizing antibodies opens new prospects for treatment algorithms for patients with persistent coronavirus infection, as well as for passive immunization schemes for patients with a presumably reduced specific response to vaccination.

Introduction

Recent studies on the course of SARS-CoV-2 have shown that repeated positive polymerase chain reaction (PCR) tests in convalescent patients with coronavirus disease (COVID-19) are very common. The proportion of repeat positive tests in discharged patients with COVID-19 ranged from 2.4% to 69.2% and persisted from 1 to 38 days after discharge, depending on population size, patient age, and sample type. At the moment, several potential reasons for repeated positive SARS-CoV-2 test results in convalescent patients with COVID-19 are considered false-positive tests, viral reactivation, and reinfection. Cases of repeated positive PCR tests in convalescent, PCR-negative patients raise several important questions in the treatment and diagnosis of SARS-CoV-2 infection. First, whether a re-infection with SARS-CoV-2 is still possible in convalescent patients, and why SARS-CoV-2 RNA might be re-detected in some symptomatically recovered COVID-19 patients. Second, whether a symptomatically recovered PCR-positive patient is still contagious. And third, how the patients with COVID-19 with repeated positive tests for SARS-CoV-2 should be managed. The main causes of recurrent infection in some patients include primary or secondary immunodeficiencies with impaired formation of specific antibodies.

The use of convalescent plasma has been reported as a possible option for antiviral therapy in patients with primary and secondary B-cell deficiencies, which require long-term inpatient treatment with minimal improvement on maintenance therapy. The present article describes an intrapatient SARS-CoV-2 evolution during long-term SARS-CoV-2 infection in a patient with a secondary B-cell immunodeficiency.

Materials and methods

To detect the virus by PCR testing we used the RealBest RNA SARS-CoV-2 test system, manufactured by Vector-Best, Russia. The reagent kit is to detect SARS-CoV-2 coronavirus RNA in real-time PCR. The reaction mixture includes all necessary components as well as reverse transcriptase and Taq polymerase. Analytical sensitivity of the method is 25 copies per PCR sample, which corresponds to 1 x 103 copies/ml of the original sample. The swab for the study was taken from the nasal mucosa and the posterior pharyngeal wall, and then the sample was sent to the laboratory, where the presence or absence of the SARS-CoV-2 coronavirus (RNA) genetic material was determined by polymerase chain reaction (PCR). The response format in PCR testing was qualitative: positive/negative.
The levels of IgM and IgG antibodies to the SARS-CoV-2 coronavirus in the blood serum were detected by the chemiluminescent immunoassay, the Mindray test system, China, with venous blood used as biomaterial, and the result presented in numerical terms. Reference values: Negative IgM test for SARS-CoV-2 < 2 IU/mL; Negative IgG test for SARS-CoV-2 < 10 IU/mL.

A COVID globulin (Nacimbio) is a native human IgG derived from the plasma of COVID-19 convalescent patients. Each immunoglobulin batch is made of a pool of donor plasma containing antibodies against SARS-CoV-2, individually tested for the absence of hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus, antibodies to human immunodeficiency virus HIV-1 and HIV-2 and antigen p24 HIV-1, as well as antibodies to the causative agent of syphilis (Treponema pallidum). Only plasma with negative test results is used in production. In the manufacture of the medicine, molecules of IgG are not subject to change due to chemical or enzymatic effects. Antibody activity is fully preserved. The drug is administered once by intravenous drip without dilution at a dose of 2–4 ml/kg of body weight.

The 32 slice spiral CT scanner was used at center.

Genotyping of the virus isolated from the patient was performed to look for possible mutations. Vero E6 cells were used for isolation and primary passaging. The sample was added to freshly seeded Vero E6 cells per T25 flask in Dulbecco’s modified Eagle’s medium (DMEM) with 2% heat-inactivated fetal bovine serum (FBS) supplemented with penicillin, streptomycin, and gentamicin, the total cytopathic effect was determined after 3 days, and the medium was collected for further virus multiplication and analysis. Total ribonucleic acid (RNA) was isolated using ExtractRNA Reagent (Eurogen, Moscow, Russia) according to the manufacturer’s instructions. Viral RNA was fragmented and reverse transcribed using random hexameric primers using the RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific), followed by dsDNA synthesis using the NEBNext Ultra II (NEB) non-directional second strand RNA synthesis module. A desoxyribonucleic acid (DNA) library was created using the NEBNext Fast DNA Library Prep Set for Ion Torrent (NEB) and sequenced using an Ion 530 chip, IonChef instrument, and IonTorrent SSXL sequencer (ThermoFisher Scientific, Waltham, MA, USA). The quality of the raw reads was controlled using vsearch v2.14.2, mapped to the Wuhan-Hu 1 reference sequence (GenBank accession NC_045512.2) using BWA v0.7.17. Consensus sequences were obtained using FreeBayes v1.3.2, bcftools v1.9 and bedtools v2.29.2. The isolated virus belongs to genetic line B.1.1.397 (Pangolin nomenclature).

To distinguish changes in the viral genome resulting from intrapatient evolution, we placed a virus sample obtained from a patient on the SARS-CoV2 phylogenetic tree using the online version of the UShER tool. One of the sister samples (Russia/ SPE-RII-MH1516S/2020[EPI_ISL_596287] 202,020-09-11) was identical to the parent node (branch length equal to zero), and we used its sequence as the ancestor.

To test the possible effect of mutations on antigen presentation, we used best percentile rank (BR, Best Rank_EL) and PHBR (Patient Harmonic-mean Best Rank) metrics as, pipeline described in Refs. 5,10 For each altered site, we considered all peptides 8–14 amino acids long for human leukocyte antigen (HLA) I and 12–18 amino acids long for HLA II that overlapped this site in the ancestral and derived states. The binding affinity of all peptides to the patient’s HLA alleles was calculated in percentile ranks (Rank_EL score), reflecting percentile rank of studying peptide among the set of random natural peptides, using the NetMHCpan and NetMHCIIpan tools.11 Next, the BR was defined as the rank of the most affine peptide, as an estimate of the site presentation in the ancestral and derived states at a particular allele. Comparison of BR between states shows the effect of the mutation on antigen presentation at a particular HLA allele. Decrease of BR score due to mutation corresponds to strengthening of binding affinity, and increase to weakening, respectively.

To assess the overall effect of a mutation on presentation at all HLA alleles of the same class, we used PHBR, which is the average of the harmonic best ranks between all HLA alleles of the same class:

$$PHBR = \frac{6}{\sum_{i=1}^{n} \frac{1}{BR_i}}$$

To test whether these sites are parts of immunogenic peptides that have been shown to provoke a T-cell response in patients with identical HLA alleles, the peptides were tested in the Immune Epitope Database (IEDB).12

**Results**

**Clinical case presentation**

In May 2021, patient C., 48 years old, was seen by the immunologist due to the spectrum of complaints: shortness of breath, decreased tolerance to minimal physical activity, dry cough, elevated body temperature to 37.5°C in the evening and at night, profuse sweating, and chronic weakness, weight loss from 94 to 72 kg in 6 months. COVID-19 was proven by a positive PCR test result. The patient was managed according to the current version of the clinical protocol for the treatment of patients with the new coronavirus infection COVID-19. The medical history analysis revealed a series of SARS-CoV-2 RNA re-detection occurred within the 6-month period from the previous COVID-19 episodes, and a sample of the virus was isolated for further assays.

Of note, in June 2018, the patient was diagnosed with mantle cell lymphoma (Ann-Arbor grade IV) with peripheral, intra-abdominal lymph nodes, spleen, stomach, and bone marrow involvement. Between 11 August 2018 and 2 November 2018, the patient received intensive immunotherapy under the R-BAC#2/R-Hd-Ara-C#2 program and underwent splenectomy. From 5 December 2018 to 10 December 2018, high-dose conditioning with CEAM was performed and followed by hematopoietic stem cell autotransplantation (auto-HSCT) on 12 December 2018. Complete remission of the lymphoma was achieved. From March 2019 to October 2020, the patient was on Rituximab maintenance therapy. The last injection of 700 mg of Rituximab was given on 26 October 2020.

On 7 November 2020, the patient first noted an increase in body temperature to febrile values, with the maximum of 39°C. On 7 November 2020 and further on PCR test results for
SARS-CoV-2 RNA were positive (Table 1). The patient received outpatient treatment with Umiferovir and Azithromycin. A chest computer tomography (CT) performed on 22 November 2020, revealed signs of bilateral polysegmental pneumonia, most likely of viral etiology (CT class 1, see Supplementary Table S1). Between 7 November 2020 and 7 May 2021, the patient continuously complained of shortness of breath, severe weakness, episodes of febrile temperature, with the maximum of 39°C. In this period, the results of PCR testing for SARS-CoV-2 were five times positive, and the titer of IgM and IgG to SARS-CoV-2 remained below the reference values (Table 1). The patient underwent multiple chest CT scans, which showed a progressive increase in lung tissue involvement (see Supplementary Table S1). Twice in this period, the patient was hospitalized for inpatient treatment, where he received antibiotic therapy, antifungal therapy, physiotherapy and did breathing exercises. On 7 May 2021, a chest CT scan showed residual changes in both lungs after viral pneumonia with the presence of fresh infiltrative changes as bilateral polysegmental pneumonia, most likely of viral origin. Compared to the CT scan of 2 April 2021, there was a negative trend, namely the appearance of fresh lesion foci. In May 2021, the patient continued replacement therapy with normal human IVIG (50 mg/mL, 2.5 g protein once every 3 days, 2 transfusions) as prescribed by the hematologist. The hematologist referred the patient to an allergist-immunologist for a consultation to decide whether replacement therapy with native human immunoglobulin against COVID-19 is possible. Tests of the patient’s immunological status were not performed, and only on the basis of the patient’s complaints, history, and clinical examination the diagnosis of secondary immunodeficiency, general variant immunodeficiency was established. PCR confirmed the presence of COVID-19, and hospital-acquired bilateral pneumonia was also confirmed.

On 20 May 2021, the patient underwent day care replacement therapy with 150 mL of COVID globulin (Nacimbio). On the same day, the patient was discharged in satisfactory condition, with recommendations for control PCR test for SARS-CoV-2 RNA, blood tests for IgM and IgG antibody levels to SARS-CoV-2, and a control chest CT scan 3 weeks after treatment. As a result of the therapy one day later, the patient noted a significant improvement in his general condition, normalization of body temperature, reduction of dyspnea, absence of sweating. On the fourth day after treatment, a PCR test result was negative. Seven days after immunofluorescence analysis, specific IgG to SARS-CoV-2 at 33.21 U/mL was observed in serum. On examination performed 21 days after transfusion of human COVID-19 globulin a PCR test for SARS-CoV-2 RNA was also negative, while the serum IgG level to SARS-CoV-2 was 17.29 U/mL. There was also positive dynamics on chest CT, namely complete resolution of inflammatory process and partial resolution of areas of gross consolidation. The patient noted improvement of his general condition, increased tolerance to physical activity, including absence of dyspnea on heavy physical activity, absence of sweating, and normalization of body temperature. Currently, dynamic monitoring of the patient continues, including determination of the level of antibodies to SARS-CoV-2 and genotyping of the viral isolates for possible mutations.

Viral evolution

The sample of SARS-CoV-2 isolated from the patient on 19 May 2021 (hereinafter “derived state”) differed from the nearest ancestor (hereinafter “ancestral state”) by 5 single-nucleotide changes (C4901T, C16338T, C19269T, G21786C, C25609A). Two of them (G21786C and C25609A) were nonsynonymous and resulted in S:G75A and ORF3a:L73I amino acid changes, respectively. Among them, S:G75A is of particular interest because G75 is part of the 72GTNGTKR78 motif of the SARS-CoV-2 protein. This insertion, which is absent in SARS-CoV, presumably plays a role in binding to alternative cellular receptors.13

To estimate the possibility of the site to be presented on HLA allele of the patient as a part of viral peptide, we used BR metric (Best Rank_EL, see Materials and methods10), which reflects the binding score of the peptide with the best binding affinity among all viral peptides, covering the altered site. Our analysis revealed that both sites with amino-acid modifying mutations are parts of peptides that can be presented on a patient’s HLA allele (BR < 2 for HLA class I and BR < 10 for HLA class II, Table 2).

However, the observed mutations do not significantly reduce or even slightly improve the prediction of the presentation of the altered sites, which is manifested by a decrease in BR scores in the derived state in comparison with the ancestral one in most alleles. A comparison of PHBR scores showing the overall level of site presentation at all HLA alleles of the same class demonstrates the same effect (Table 3). There was no data on the immunogenicity of these sites on the patient’s HLA alleles for T-cells in IEDB.

Taken together, these results indicate that escape from antigenic presentation was not the driving force behind the observed viral evolution. However, this analysis provides no information on the immunogenicity of the mutated sites for the T-cell clones. Thus, a scenario that the amino acid change interferes with recognition by an existing patient T-cell clone cannot be ruled out.

Discussion

Patients with primary and secondary immunodeficiencies are at risk of prolonged non-elimination of the virus, which can lead to an undulating course of the disease, the so-called true long COVID, or due to some hypothesis serve as a reservoir for further virus mutation, which can have negative consequences both for this patient cohort and for the public health system as a whole.14 spreading. Our clinical case demonstrates a successful treatment protocol and short-term safety, consistent with data published by other authors.15–17 And also demonstrates no change in virus type during the period of carrier, despite the described cases of rapid viral evolution in immunosuppressed patients with persistent SARS-CoV-2 infection.18 Limitations of this case report include the lack of evaluation of the specific T-cell response to SARS-CoV-2, as well as prior therapy with antiviral drugs, broad spectrum antibiotics, and replacement therapy with normal human IVIG prior to the introduction of COVID globulin. This cohort of patients does not form a specific immune response after illness and cannot form specific protective immunity after vaccination. Currently little is known about the value of the T-cell specific immunity assessment, which can be a prioritized unmet need in this particular cohort, bringing more information about the cross-
**Table 1.** Dynamics of PCR results and serum levels of IgM and IgG to SARS-CoV-2.

| Date       | PCR for SARS-CoV-2 RNA* | IgM to SARS-CoV-2, IU/mL** | IgG to SARS-CoV-2, IU/mL*** | Additional Information |
|------------|--------------------------|-----------------------------|-----------------------------|------------------------|
| Oct 23, 2020 | Negative                 | 0.13                        | 0.00                        | The last injection of 700 mg of Rituximab Oct 26, 2020 |
| Nov 7, 2020  | Positive                  | Not performed               | Not performed               | Febrile body temperature; Outpatient treatment: Umifirov + Azithromycin |
| Nov 17, 2020 | Negative                 | Not performed               | Not performed               | Bilateral polysegmental pneumonia – CT class 1 |
| Dec 23, 2020 | Positive                  | 0.11                        | 0.14                        | Bilateral polysegmental pneumonia – CT class 2 |
| Jan 3, 2021  | Negative                  | 0.11                        | 0.19                        | Worsening of clinical symptoms, hospitalization to January 12 |
| Jan 23, 2021 | Negative                  | Not performed               | Not performed               | Re-hospitalization, change of therapy to February 5 |
| Mar 15, 2021 | Positive                  | Not performed               | Not performed               | Bilateral polysegmental pneumonia – CT class 2. Outpatient treatment: Aprepitant +Methylprednisolone+ Levofloxacin, human intravenous immunoglobulin (IVIG) replacement therapy (50 mg/mL, 2.5 g protein once every 3 days, 3 transfusions) |
| Mar 31, 2021 | Negative                  | Not performed               | Not performed               | Therapy with 150 mL of COVID globulin (Nacimbo), May 2021 |
| Apr 7, 2021  | Positive                  | 0.09                        | 0.0                         | Negative trend on CT scan. Outpatient treatment: Triazaverine 250 mg (1 tablet 3 times daily) + Acetylcysteine (600 mg daily) |
| Apr 18, 2021 | Positive                  | Not performed               | Not performed               | Improvement of clinical manifestations |
| May 24, 2021 | Negative                  | Not performed               | Not performed               | Increased tolerance to physical activity. Positive dynamics on chest CT |
| May 27, 2021 | Not performed             | 0.12                        | 33.21                       | |
| Jun 11, 2021 | Negative                  | 0.1                         | 17.29                       | |

*Analytical sensitivity of the method: 25 copies per PCR sample, corresponding to 1 × 10⁷ copies/ml of the original sample. The response format for PCR testing was qualitative: positive/negative.

**Negative IgM test for SARS-CoV-2 < 2 IU/mL.

***Negative IgG test for SARS-CoV-2 < 10 IU/mL; CT- chest computed tomography with a 32-slice CT scanner.

**Table 2.** BR scores of mutated sites in ancestral and derived states for HLA alleles of the patient.

| Mutation | Class of HLA | Allele | BR in ancestral state | BR in derived state |
|----------|---------------|--------|-----------------------|---------------------|
| S:G75A  | HLA I         | HLA-A *25:01 | 1.28                  | 0.85                |
| S:G75A  | HLA I         | HLA-A *32:01 | No binding            | No binding          |
| S:G75A  | HLA I         | HLA-B *40:02 | No binding            | No binding          |
| S:G75A  | HLA I         | HLA-B *44:02 | No binding            | No binding          |
| S:G75A  | HLA I         | HLA-C *02:02 | No binding            | No binding          |
| S:G75A  | HLA I         | HLA-C *07:04 | No binding            | No binding          |
| ORF3a: L37I | HLA I    | HLA-A *25:01 | 0.35                  | 0.18                |
| ORF3a: L37I | HLA I    | HLA-A *32:01 | 0.09                  | 0.05                |
| ORF3a: L37I | HLA I    | HLA-B *40:02 | No binding            | No binding          |
| ORF3a: L37I | HLA I    | HLA-B *44:02 | 1.18                  | 1.02                |
| ORF3a: L37I | HLA I    | HLA-C *02:02 | 0.24                  | 0.13                |
| ORF3a: L37I | HLA I    | HLA-C *07:04 | 0.50                  | 0.55                |
| S:G75A  | HLA II        | HLA-DRB1 *11:01 | 2.63                  | 2.88                |
| S:G75A  | HLA II        | HLA-DRB1 *02:02 | 4.00                  | 2.37                |
| S:G75A  | HLA II        | HLA-DQB1 *03:01 | 5.76                  | 2.40                |
| ORF3a: L37I | HLA II   | HLA-DRB1 *11:01 | 9.02                  | 7.66                |
| ORF3a: L37I | HLA II   | HLA-DRB1 *02:02 | 2.70                  | 2.24                |
| ORF3a: L37I | HLA II   | HLA-DQB1 *03:01 | 2.38                  | 1.11                |
| ORF3a: L37I | HLA II   | HLA-DQB1 *03:01 | 2.38                  | 1.11                |

BR – Best Rank. EL score. Ancestral – the nearest known SARS-CoV-2 ancestor. Derived – SARS-CoV-2 isolated from the patient.

**Table 3.** PHBR scores of mutated sites in ancestral and derived states for two classes of HLA alleles.

| Mutation | PHBR before mutations | PHBR after mutation | Class of HLA |
|----------|------------------------|---------------------|---------------|
| S:G75A  | 3.53                   | 2.68                | HLA I         |
| ORF3a: L37I | 0.27               | 0.17                | HLA I         |
| S:G75A  | 3.73                   | 2.52                | HLA II        |
| ORF3a: L37I | 3.33               | 2.03                | HLA II        |

PHBR – Patient Harmonic-mean Best Rank. Ancestral – the nearest known SARS-CoV-2 ancestor. Derived – SARS-CoV-2 isolated from the patient.

**Conclusions**

The article presents a clinical case of a long-term course of COVID-19 due to chronic hematological disease in the inactive phase and development of a secondary immunodeficiency state due to massive prior B-cell-depletion immunosuppressive therapy. There was no formation of a protective antibody titer to the virus, which presumably resulted in an inability to eliminate the virus, and a gradual increase in the area of lung tissue damage. Laboratory markers of hyperimmune response as well as clinical signs of respiratory failure accompanied the viral lung involvement. The infectious process lasted a total of 6 months and was stopped by etiotropic therapy with plasma-derived COVID-19 globulin. The therapy resulted in rapid and effective elimination of the virus and passive immunization for at least 30 days.

**Note**

[a] https://mosgorzdrav.ru/professional/covid-19.

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References

1. Han Z, Battaglia F, Terlecky SR. Discharged COVID-19 patients testing positive again for SARS-CoV-2 RNA: a minireview of published studies from China. J Med Virol. 2021;93(1):262–274. doi:10.1002/jmv.26250.

2. Dao TL, Hoang VT, Gautret P. Recurrence of SARS-CoV-2 viral RNA in recovered COVID-19 patients: a narrative review. Eur J Clin Microbiol Infect Dis. 2021;40(1):13–25. doi:10.1007/s10096-020-04088-x.

3. Ikegami S, Benirschke R, Flanagan T, Tanna N, Klein T, Elue R, Deboz P, Mallek J, Wright G, Guariglia P, Kang J. Persistence of SARS-CoV-2 nasopharyngeal swab PCR positivity in COVID-19 convalescent plasma donors. J Med Virol. 2020;92(11):2263–2267. doi:10.1002/jmv.26056.

4. Kang H, Wang Y, Tong Z, Liu X. Retest positive for SARS-CoV-2 RNA of “recovered” patients with COVID-19: persistence, sampling issues, or re-infection? J Med Virol. 2020;92(11):2263–2265. doi:10.1002/jmv.26114.

5. Staniech O, Alekseeva E, Sergeeva M, Fadeev A, Komissarova K, Ivanova A, Simakova T, Vasiliev K, Shurygina AP, Stukova M, et al. SARS-CoV-2 escape from cytotoxic T cells during long-term COVID-19. Researchsquare. Version 1, Posted28 Jul, 2021. doi:10.21203/rs.3.rs-750741/v1.

6. Avouac J, Drumez E, Seror R, Georgin-Lavialle S, El Mahou S, Pertuiset E, Pham T, Marotte H, Servetaz A, et al. COVID-19 outcomes in patients with inflammatory rheumatic and musculoskeletal diseases treated with rituximab: a cohort study. Lancet Rheumatol. 2021;3(6):e419–e426. doi:(21)00059-X.1,0.0.0X10.1016/S2665-9913(21)00059-X.

7. Jin H, Reed JC, Liu ST, Ho HE, Lopes JP, Ramsey NB, Waqar O, Rahman F, Aberg JA, Bouvier NM, et al. Three patients with X-linked agammaglobulinemia hospitalized for COVID-19 improved with convalescent plasma. J Allergy Clin Immunol Pract. 2020;8(5):3594–3596.e3. doi:10.1016/j.jaip.2020.08.059.

8. Hueso T, Poudourex C, Pérez H, Beaumont AL, Raillon LA, Ader F, Chatenoud L, Eshagh D, Szwebel TA, Martinot M, Camou F. Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19. Blood. 2020;136(20):2290–2295. doi:10.1182/blood.2020008423.

9. Turakchia Y, Thornlow B, Hinrichs AS, De Maio N, Gozashi L, Lanfer R, Haussler D, Corbett-Detig R. Ultrafast sample placement on existing tRees (USHER) enables real-time phylogenetics for the SARS-CoV-2 pandemic. Nat Genet. 2021;53(6):809–816. doi:10.1038/s41588-021-00862-7.

10. Marty R, Kaabinejadian S, Rossell D, Slikker MJ, van de Haar J, Engin HB, de Prisco N, Iderer T, Hildebrand WH, Font-Burgada J, et al. MHC-I genotype restricts the oncogenic mutational landscape. Cell. 2017;171(6):1272–1283.e15. doi:10.1016/j.cell.2017.09.050.

11. Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M. NetMhcpan-4.1 and NetMhcipan-4.0: improved predictions of MHC antigen presentation by current motif deconvolution and integration of MS MHC eluted ligand data. Nucleic Acids Res. 2020;48(W1):W449–W454. doi:10.1093/nar/gkaa379.

12. Vita R, Mahajan S, Overton JA, Dhanka SK, Martini S, Cantrell JR, Wheeler DK, Sette A, Peters B. The immune epitope database (IEDB): 2018 update. Nucleic Acids Res. 2019;47(D1):D339–D343. doi:10.1093/nar/gky1006.

13. Behloul N, Baha S, Shi R, Meng J. Role of the G1NTGKR motif in the N-terminal receptor–binding domain of the SARS-CoV-2 spike protein. Virus Res. 2020;286:198058. doi:10.1016/j.virusres.2020.198058.

14. Corey L, Beyrer C, Cohen MS, Michael NL, Bedford T, Rolland M. SARS-CoV-2 variants in patients with immunosuppression. N Engl J Med. 2021;385(6):562–566. PMID: 34347959; PMCID: PMC8494465. doi:10.1056/NEJMsb2104756.

15. Nyström K, Hjorth M, Fust R, Nilsdotter-Augustinsson Å, Larsson M, Niward K, Nyström S. Specific T-cell responses for guiding treatment with convalescent plasma in severe COVID-19 and humoral immunodeficiency: a case report. BMC Infect Dis. 2022;22(1):362. PMID: 35410137; PMCID: PMC8996199. doi:10.1186/s12879-022-07323-4.

16. Hueso T, Poudourex C, Pérez H, Beaumont AL, Raillon LA, Ader F, Chatenoud L, Eshagh D, Szwebel TA, Martinot M, et al. Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19. Blood. 2020;136(20):2290–2295. PMID: 32959052; PMCID: PMC7702482. doi:10.1182/blood.2020008423.

17. London J, Boutboul D, Lacombe K, Pirene F, Heym B, Zeller V, Baudet A, Ouedraogo A, Bérezné A. Severe COVID-19 in patients with B cell lymphoplasia and response to convalescent plasma therapy. J Clin Immunol. 2021;41(2):356–361. Epub 2020 Nov 20. PMID: 32321949; PMCID: PMC7678568. doi:10.1007/s10875-020-00094-5.

18. Choi B, Choudhary MC, Regan J, Sparks JA, Pader BR, Qiu X, Solomon IH, Kuo HH, Boucai J, Bowman K, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med. 2020;383(23):2291–2293. Epub 2020 Nov 11. PMID: 33176080; PMCID: PMC7673033. doi:10.1056/NEJMc2031364.