Article

Pharmacokinetics Profile and Genetics of Double Antiviral Therapy with Remdesivir and Nirmatrelvir/Ritonavir for Prolonged COVID-19 in Patients Treated with Rituximab: A Real-Life Study and Literature Review

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Abstract: Introduction: Patients with hematologic malignancies are more likely to develop severe and prolonged SARS-CoV-2 infection, often showing viral persistence despite the use of authorized antivirals. Herein, we report the cases of four patients who received rituximab for different conditions and developed persistent COVID-19 treated with an extended course of dual antivirals, Nirmatrelvir/Ritonavir and Remdesivir. Moreover, we describe the pharmacokinetics and pharmacogenetics (PK/PG) characteristics of Nirmatrelvir/Ritonavir and Remdesivir treatment in two of these patients. Methods: Plasma specimens for evaluation of trough concentrations (C_{trough}) were collected 10 min before the daily dose administration, in addition to 3 h (C_{max}), 4 h (C4h), 6 h (C6h) and 1 h (C_{max}) after the administration of Nirmatrelvir/Ritonavir and Remdesivir, respectively. The following gene single-nucleotide polymorphisms (SNPs) were investigated: *ABCB1* 3435 (rs1045642) C > T, *ABCB1* 1236 (rs1128503) C > T, PXR 63396 (rs2472667) T > C, *CYP2D6* (rs1135840) G > C, and *CYP3A4**1B (rs2740574) G > A. Results: Double antiviral treatment was successful in terms of symptoms resolution, whereas three out of four patients achieved microbiological eradication. Based on our results, concentrations of Nirmatrelvir ranging from 50 to 5000 ng/mL were effective, whereas a higher concentration (range 1068–3377 ng/mL), compared to that previously reported in patients with similar weight and BMI, was evidenced for Ritonavir. Considering the genetic variant analysis, *ABCB1* 3435 CT and 1236 CT genotypes were found in patient 1; and *ABCB1* 3435 CC and 1236 CC in patient 2. In conclusion, this real-life study supports the usefulness of TDM and genetics in immunocompromised patients with persistent SARS-CoV-2 infection, a challenging setting for clinicians in which personalized medicine may improve outcome.

Keywords: pharmacokinetics; pharmacogenetics; therapeutic drug monitoring; COVID-19; remdesivir; nirmatrelvir/ritonavir; rituximab; immunocompromised host; hematological patients

1. Introduction

Patients with hematologic malignancies are more likely to develop severe and prolonged SARS-CoV-2 infection, often showing viral persistence despite the use of authorized antivirals [1,2]. The effectiveness of Remdesivir and Nirmatrelvir/Ritonavir has been demonstrated in vitro and in randomized controlled trials for the treatment of early symptomatic COVID-19 in order to avoid the risk of progression to severe disease [3,4]; however, unfortunately, these therapies are not approved for patients with prolonged and late COVID-19, and the off-label use of direct antivirals and their combination has been reported in few cases as a possible and effective approach in immunocompromised populations [5–8].

COVID-19 can persist for up to 100 days in immunocompromised patients, with a prolonged viral shedding that is likely to be attributable to both humoral and cellular immune deficiency [9]. Innate and adaptive immune responses synergistically cooperate to limit SARS-CoV-2 infection. In particular, CD8+ T cells take part in acute reactions, while B and CD4+ T cells play a pivotal role in viral clearance and prevention of SARS-CoV-2 recurrence. In fact, patients receiving B-cell targeting treatments, such as rituximab, are exposed to a decrease in humoral immunity that limits the clearance of SARS-CoV-2, even after a full vaccination cycle [2,10].

Remdesivir acts against viral RNA polymerase, whereas Nirmatrelvir is a direct antiviral agent that targets the SARS-CoV-2 3-chymotrypsin-like cysteine protease enzyme, thus determining a strong inhibition of Mpro activity and virus replication across a broad spectrum of coronaviruses. Furthermore, Nirmatrelvir is a substrate of the efflux pump P-glycoprotein (P-gp, encoded by *ABCB1* gene) and Cyp3A4, whereas Ritonavir, besides being an inhibitor of *CYP3A4* and *CYP2D6*, also represents their substrate [11–13]. Moreover, pregnane-X-receptor (PXR) transcription factor can regulate the expression of *ABCB1*, *CYP2D6* and *CYP3A4* genes [14].

Herein, we report the cases of four patients who received Rituximab for different clinical conditions and presented persistent COVID-19 infection treated with an extended course of dual antivirals, Nirmatrelvir/Ritonavir and Remdesivir; we also report the pharmacokinetics and genetic analysis (PK/PG) of Nirmatrelvir/Ritonavir and Remdesivir treatment in two of these patients. Moreover, a literature revision regarding therapeutic approaches to prolonged COVID-19 infection in immune-compromised patients is presented.

2. Materials and Methods

Patients were admitted to the City of Health and Sciences, Molinette Teaching Hospital in Turin, Italy, from November 2022 to February 2023. Patients were treated for ten days with Nirmatrelvir/Ritonavir 300/100 mg q12h if estimated glomerular filtration rate (EGFR) > 60 mL/min or Nirmatrelvir/Ritonavir 150/100 mg q12h if EGFR > 30–60 mL/min and Remdesivir 200 mg loading dose, followed by 100 mg as recommended by Italian Regulatory Agency (https://www.aifa.gov.it/documents/20142/1123276/Remdesivir_update03_06.06.2022.pdf; https://www.aifa.gov.it/documents/20142/1616529/PAXLOVID_PT_04.04.2023.pdf, accessed on 20 December 2023). The off-label 10-day combination of Remdesivir and Nirmatrelvir/Ritonavir was authorized from the Internal Ethical Committee as of 24 January 2020, protocol number 0008195; a signed informed consent form was collected for each patient.

2.1. Molecular Assays

Nucleic acid amplification tests (NAATs) are the methods of choice for SARS-CoV-2 diagnostic testing. In particular, real-time RT-PCR is the most widely used test for viral RNA detection of SARS-CoV-2. Prior to nucleic acid amplification, specimens are purified by using defined extraction protocols. Generally, SARS-CoV-2 nucleic acids from whole blood or plasma samples are obtained by extraction performed with many automated instruments. More complex matrices, such as biopsies and feces, require specific pretreatments, including incubation with lysis buffer and proteinase K at 56 °C in order to

obtain the total disintegration of samples. Viral RNA amplification is performed by using commercial qualitative kits that contain fluorescent probes designed to detect the specific *SARS-CoV-2 E, N, ORF8* and *RdRp* genes.

In addition to conventional molecular assays, RT-PCR rapid tests are widely used for identification of RNA SARS-CoV-2, as they provide rapid results in about 45 min with minimal operator intervention. Among these, the Xpert Xpress SARS-CoV-2 assay, developed for the Cepheid GeneXpert platform, contains specific primers and probes for qualitative detection of unique sequences in the nucleocapsid (N), envelope (E), and RNA-dependent RNA polymerase (*RdRP*) genes of the SARS-CoV-2 genome in upper respiratory specimens, including nasopharyngeal, anterior nasal, mid-turbinate nasal, and oropharyngeal swab, as well as nasal wash/aspirate specimens. The Xpert Xpress CoV-2 plus test was used in the present study.

2.2. Next-Generation Sequencing (NGS) of SARS-CoV-2 Genome

With the development of high-throughput next-generation sequencing (NGS) approaches allowing for the analysis of SARS-CoV-2 whole genome, identification of COVID-19 pandemic origins and variants, delineation of transmission events and the monitoring of viral populations' evolution over time can be demonstrated. Viral RNA extraction and metagenomic analysis are performed with a whole-genome shotgun sequencing approach using different platforms (Illumina, Ion Torrent) that provide automated library and template preparation.

Drug quantification by LC/MS-MS: Plasma samples for therapeutic drug monitoring of Nirmatrelvir/Ritonavir and Remdesivir (detected as GS-441524, the predominant metabolite of remdesivir circulating in the serum) were collected on day 6 of treatment after steady-state achievement. Plasma samples to determine trough concentrations (C_{trough}) were collected 10 min before the daily dose administration. The other considered timings for Nirmatrelvir/Ritonavir were 3 h (C_{max}), 4 h (C_{4h}), and 6 h (C_{6h}) after administration of Nirmatrelvir/Ritonavir, whereas for GS-441524, 1 h (C_{max}) after one-hour infusion. Plasma samples were isolated after whole blood centrifugation at $1400 \times g$ for 10 min at 4 °C and then stored at -80 °C until the analysis. Plasma samples were shipped on dry ice in a time span of 30 min to the Laboratory of Clinical Pharmacology and Pharmacogenetics, Amedeo di Savoia Hospital in Turin, Italy.

The quantification was performed on total Nirmatrelvir/Ritonavir (free and bounded drug fraction to plasma proteins) through a validated ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) assay, using a method developed in the Laboratory of Clinical Pharmacology and Pharmacogenetics, Amedeo di Savoia Hospital in Turin, under validation. The analytical process consisted in a fast protein precipitation of a mixture of 50 µL of plasma and 50 µL of internal standard solution with 4000 µL of freezing precipitating solution; samples were then kept 10 min at room temperature in a sonicator bath before being ultra-centrifuged at $10,000 \times g$ 10 min. All of the supernatant was transferred into a vacuum concentrator. All of the dried samples were then resuspended with 250 µL of diluting solution and, finally, 1.50 µL was injected into the column and analyzed with the Perkin Elmer LX-50® UHPLC system coupled with a Triple Quadrupole QSight 220® (Perkin Elmer, Milan, Italy).

Genotyping of SNPs: Patients' DNA was extracted from whole blood samples with a semi-automated instrument (MagnaPure Compact, Roche, Monza, Italy). This kit contains materials allowing for DNA purification starting from 200 µL of blood or plasma. Genotypes were assessed with allelic discrimination (Taqman, Thermofisher: Waltham, MA, USA) through a real-time polymerase chain reaction allelic discrimination system (LightCycler 96, Roche, Monza, Italy). The following gene single nucleotide polymorphisms (SNPs) were investigated: *ABCB1 3435 (rs1045642) C > T, ABCB1 1236 (rs1128503) C > T, PXR 63396 (rs2472667) T > C, CYP2D6 (rs1135840) G > C,* and *CYP3A4*1B (rs2740574) G > A.*

3. Results

Case Descriptions

The main clinical and demographic characteristics of patients are summarized in Table 1. In two out of four patients, pharmacokinetic (Figure 1) and genetic analysis was performed.
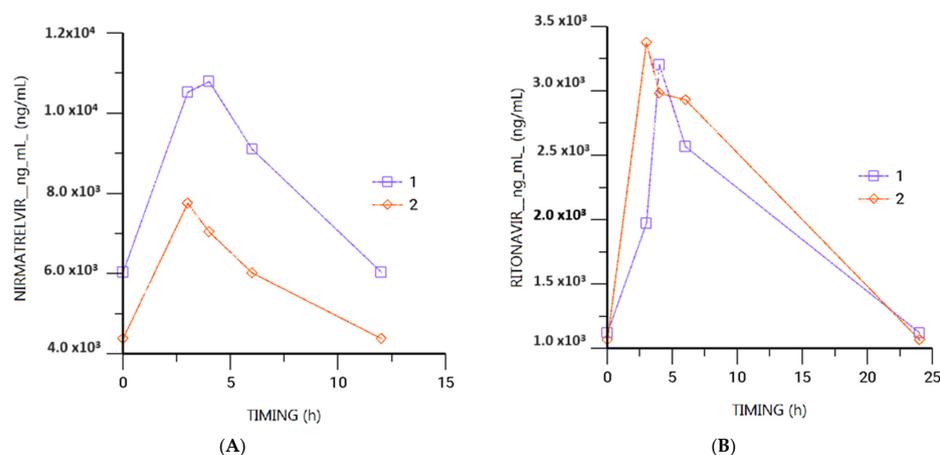

Figure 1. Nirmatrelvir (**A**) and Ritonavir (**B**) plasma area under the curve (AUC) levels available for patients 1 and 2 (2 out of 4 patients underwent PK/PG analysis). Patient 1 concentrations are described by purple line, patients 2 concentrations are described by orange line.

Patient 1. A 3-dose SARS-CoV2 mRNA-vaccinated 65-year-old man (weight 70 kg, BMI 23.2, EGFR 60 mL/min) with glomerulonephritis was hospitalized in November 2022 for exacerbation of nephrotic syndrome and was treated with Rituximab, Cyclosporine and Prednisone until December 2022. By the beginning of December, he was hospitalized for progressively worsening dyspnea and tested positive for SARS-CoV-2 infection. High-resolution computed tomography (HRCT) chest scans revealed bilateral posterior basal areas of consolidation, and he required O_2 supplementation up to HFNC. Intravenous steroid therapy was incremented with daily Dexamethasone 12 mg with partial beneficial effect, but after tapering of steroid, worsening dyspnea and recurrent fever reappeared. A new course of Dexamethasone 6 mg was started; however, following the appearance of multifocal areas of ground opacities, a bronchoalveolar lavage (BAL) was collected and tested positive for *P. jirovecii* DNA. A 21-day course of Trimethoprim/Sulfamethoxazole was administered. Due to the lack of clinical improvement and the persistence of fever, asthenia and oxygen demand, an extensive microbiological investigation was performed and evidenced only SARS-CoV-2 positivity with a low cycle threshold at the early-February 2023 timepoint (Table 2). The patient was treated with combination therapy with Remdesivir and Nirmatrelvir/Ritonavir for 10 days. Nevertheless, positive molecular assay results persisted with a low cycle threshold at the mid-February 2023 timepoint (Table 2). The patient was treated with off-label combination therapy with Remdesivir and Nirmatrelvir/Ritonavir for 10 days. The $C_{through}$ of Nirmatrelvir was 4386 ng/mL, C_{3h} 7759 ng/mL, C_{4h} 7045 ng/mL, and C_{6h} 6022 ng/mL. The $C_{through}$ of Ritonavir was 1068 ng/mL, C_{3h} 3377 ng/mL, C_{4h} 2983 ng/mL, and C_{6h} 2933 ng/mL. The $C_{through}$ of Remdesivir was not detectable, whereas C_{max} was 356 ng/mL. The $C_{through}$ of GS-441524 was 41 ng/mL, whereas C_{max} was 61 ng/mL. Concomitantly administered drugs were levotiroxine 0.125 microg, alprazolam 0.25 mg, entecavir 0.5 mg, furosemide 50 mg, allopurinol 150 mg, canrenone 50 mg, and ganciclovir 200 mg. For suspected organizing pneumonia, the patient also received Methylprednisolone 250 mg, followed by slow steroid tapering. Concerning genetic analysis, the following genotypes were described: *ABCB1* 3435 CC, *ABCB1* 1236 CC, *PXR63396* CC and *CYP2D6* CG and *CYP3A4*1B* AA.

Table 1. Clinical characteristics of patients treated for prolonged COVID-19.

Patient	Sex/Age	COVID-19 Vaccine	Hematological Malignancy/Chemotherapy	Total Days of Hospital Stay	Time from Last Anti-CD20 to COVID-19	Treatment of the First COVID-19	Duration of COVID-19 till Admission	Symptoms	Radiology	Serology Title	Treatment of the Prolonged COVID-19	Clinical and Virological Outcome
Pt 1	M/65	mRNA 3 doses	MCD/Rituximab ++ Cyclosporine and Prednisone	71	6 days	Prednisone 50 mg, Dexamethasone 12 mg	2 months	Dyspnea, fever, asthenia	COVID-19 related pneumonia with ground-glass opacities and development of organizing pneumonia	negative	Remdesivir + Nirmatrelvir/Ritonavir 10 days + Prednisone 50 mg tapering	Afebrile and improvement of dyspnea and asthenia Tested negative for SARS-CoV-2
Pt 2	F/81	mRNA 5 doses	DLBCL/Rituximab and Chlorambucil	34	2 days	Dexamethasone 6 mg, then 12 mg, then 6 mg	45 days	Dyspnea, fever, asthenia, cough	COVID-19 related pneumonia with ground-glass opacities	NA	Remdesivir + Nirmatrelvir/Ritonavir 10 days + Methyl-prednisolone 250 mg tapering	Asymptomatic at discharge
Pt 3	M/63	mRNA 3 doses	CLL/FCR	61	4 years	Remdesivir 5 days + prednisone 1 mg/kg/die	161 days	Dyspnea, fatigue, fever	COVID-19 related pneumonia with ground-glass opacities	negative	Remdesivir + Nirmatrelvir/Ritonavir 10 days + Methyl-prednisolone 80 mg tapering	Asymptomatic at discharge Tested negative for SARS-CoV-2
Pt 4	M/66	mRNA 3 doses	Follicular lymphoma/R-CHOP	51	5 months	Remdesivir 5 days + Dexamethasone	51 days	Fever, dyspnea, asthenia, dry cough	COVID-19 related pneumonia and pulmonary embolism	1360 BAU/mL	Remdesivir + Nirmatrelvir/Ritonavir 10 days	Afebrile and complete weaning of oxygen supplementation Tested negative for SARS-CoV-2

Abbreviation: M: male; F: female; R-CHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone; CLL: Chronic lymphatic leukemia; FCR: fludarabina, ciclofosfamide and rituximab; DLBCL: diffuse large B cell lymphoma. BAU: binding antibody unit.

Table 2. Cycle threshold according to envelope (E), nucleocapsid (N) and RNA-dependent RNA polymerase (*RdRp*) in Nirmatrelvir/Ritonavir and Remdesivir treated patients.

	Date	Envelope (E)	Nucleocapsid (N)	RNA-Dependent RNA Polymerase (*RdRp*)
Patient 1	31 January 2023	19.8	22.8	22.1
	2 February 2023	19.5	22.9	21.6
	12 February 2023	29.6	33.3	32.1
Patient 2	1 January 2023	19.8	23.0	21.7
	9 January 2023	20.3	22.9	22.7
	31 January 2023	22.2	25.0	24.6
Patient 3	10 January 2023	19.0	21.6	21.3
	19 January 2023	16.8	19.5	19.2
	26 January 2023	27.9	31.5	30.4
	31 January 2023	30.9	34.8	33.2
	7 February 2023	37.9	41.9	0.0
Patient 4	31 January 2023	23.6	26.6	25.9

By the end of antiviral therapy, he obtained respiratory weaning from oxygen supplementation and clinical resolution of fever and asthenia. SARS-CoV-2 nasopharyngeal swab tested negative in late February 2023. Nonetheless, despite virological negativization, the patient's condition worsened again, determining the need for restarting non-invasive ventilation due to a recurrence of *P. jirovecii* and concomitant bacterial septic shock that eventually led to death.

Patient 2. A 5-dose SARS-CoV-2 mRNA-vaccinated 81-year-old woman (weight 48 kg, BMI 19.3, EGFR 50 mL/min), with renal B-cell lymphoma treated with Rituximab at 4 doses/week since July 2022 and stabilization therapy with Chlorambucil, was hospitalized for fever and cough in late December 2022 and tested positive for SARS-CoV-2. Chest HRCT revealed blurred bibasal ground-glass infiltrates, requiring treatment with steroid therapy and prednisone 50 mg. Due to the patient's worsening clinical condition and the enlargement of HRCT chest infiltrates, intravenous (iv) steroid therapy with Dexamethasone 6 mg was started. Since clinical resolution of symptoms was achieved and steroid therapy tapered, she was discharged with a persistent positive SARS-CoV-2 swab. However, she came back to the Emergency Department in mid-February 2023 for remittent and worsening dyspnea, cough and chest pain. At this point, chest CT scan excluded pulmonary thromboembolic events and revealed further increase in ground-glass opacities and areas of parenchymal consolidation with pleural effusion; SARS-CoV-2 nasopharyngeal swab repeatedly tested positive. Antibiotic therapy with empiric Ceftriaxone 2 gr q24h iv, dexamethasone 12 mg iv and O_2 supplementation with nasal cannula were started. Double antiviral therapy was started with Remdesivir and Nirmatrelvir/Ritonavir 150/100 mg q12h for 10 days. The $C_{through}$ of Nirmatrelvir was 6042 ng/mL, C_{3h} 10,527 ng/mL, C_{4h} 10,794 ng/mL, and C_{6h} 9104 ng/mL. The $C_{through}$ of Ritonavir was 1123 ng/mL, C_{3h} 1973 ng/mL, C_{4h} 3205 ng/mL, and C_{6h} 2569 ng/mL. The $C_{through}$ of Remdesivir was not detectable, whereas the C_{max} was 1377 ng/mL. The $C_{through}$ of GS-441524 was 299 ng/mL, whereas the C_{max} was 393 ng/mL. Concomitant drugs were enoxaparin 4000 UI, amlodipine 2.5 mg, and alizapride 50 mg. Rapid resolution of clinical symptoms and normalization of lab tests were observed on the third day of treatment, and a complete weaning from supplementary O_2 was obtained as well as tapering of Prednisone. SARS-CoV-2 nasopharyngeal swab was still positive by the time of discharge (Table 2). Concerning genetic analysis, the following genotypes were described: *ABCB1* 3435 CT, *ABCB1* 1236 CT, *PXR63396* CT, *CYP2D6* CG and *CYP3A4**1B AA.

Patient 3. A 3-dose SARS-CoV-2 mRNA-vaccinated 63-year-old male patient with chronic lymphatic leukemia (CLL), previously treated with fludarabine, cyclophosphamide and rituximab, underwent a chest HRCT scan in September 2022 because of episodes of remittent fever, revealing a ground-glass pneumonia. A nasal antigenic test was negative for SARS-CoV-2, whereas the bronchoalveolar lavage (BAL) fluid tested positive. A five-day course of Remdesivir was started with rapid clinical response, but the patient experienced a relapse of fever. HRCT scan showed no improvement. The patient started oral Prednisone, but as soon as the dose was tapered, fever reappeared, alongside dyspnea with minimum effort and desaturation. The patient was readmitted at the end of December 2022, requiring low flows of oxygen, and tested positive for SARS-CoV-2 on molecular swab. Treatment with 1 mg/kg/day of Prednisone was started, resulting in the resolution of fever. Nevertheless, following 24 days of hospitalization, the results of an IgG titer against SARS-CoV-2 was negative, and viral persistence of SARS-CoV-2 was noted. Furthermore, a widening of the ground-glass areas with consolidative opacities was detected by a CT scan of chest alongside increasing oxygen requirement up to the combination of continuous positive-airway pressure (CPAP) sessions and high-flow nasal cannula (HFNC). Hence, an off-label 10-day combination of Remdesivir and Nirmatrelvir/Ritonavir 150/100 mg q12h was started, and intravenous Methylprednisolone 80 mg was added. A rapid resolution of fever and progressive lowering of inflammation markers were observed. Supplementary oxygen was subsequently reduced, until complete weaning. Results of molecular SARS-CoV-2 tests showed a much-increased cycle threshold (Table 2), and an HRCT showed a clear improvement of bilateral lung opacities.

Patient 4. A 66-year-old man (weight 73 kg, BMI 24.4, EGFR 82 mL/min) who received a 3-dose SARS-CoV-2 mRNA vaccination was admitted to the emergency department at the beginning of February 2023 due to fever, asthenia, and dry cough. A month before admission, the patient was hospitalized at our center for severe COVID-19 related pneumonia and pulmonary embolism treated with a 5-day regimen of Remdesivir, supplementary O_2, anticoagulant, and dexamethasone. After a rapid worsening of respiratory failure, the patient's condition significantly improved during the following days. He was finally discharged at the end of January with molecular assay still testing positive for SARS-CoV-2. Patient history evidenced follicular lymphoma with mediastinal, abdominal, and hepatic involvement, diagnosed in late 2021 and treated with 6-month Rituximab-based chemotherapy with cyclophosphamide, vincristine, and prednisone administered monthly in complete remission. Given the recent clinical history, a nasopharyngeal swab for SARS-CoV-2 was repeated and turned out positive. A general physical examination showed mild respiratory failure and breathlessness; oxygen supplementation was started via nasal cannula. A chest CT revealed a new onset of parenchymal thickening in the right upper lobe. Chronic treatment with prednisone 10 mg/day was continued, and broad-spectrum antibiotic treatment with ceftobiprole and azithromycin was started. Serological anti-SARS-CoV-2 levels were sustained (IgG antibodies 1360 BAU/mL; reference < 33.8 BAU/mL). On day 7, due to worsening symptoms, including high fever, sweating, and persistent dry cough with negative cultures and serum galactomannan antigen, he was administered combination therapy with Remdesivir and Nirmatrelvir/Ritonavir 300/100 mg q12h for 10 days. Treatment also included pantoprazole 20 mg, metoprolol 100 mg, and lorazepam 1 mg. No adverse effect was observed during treatment. From day 9, the patient remained afebrile, with clinical improvement and subsequent reduction and finally complete weaning of oxygen demand. On day 21, nasopharyngeal swab for SARS-CoV-2 RT-PCR was negative.

4. Discussion

Herein, we presented the cases of four patients previously treated with anti-CD20 monoclonal antibodies (mAbs), who developed prolonged COVID-19 infection and were treated with off-label antiviral combination therapy with Nirmatrelvir/Ritonavir and Remdesivir. In our case series, prolonged treatment with Remdesivir and Nirmatrelvir/Ritonavir allowed the patients to achieve a clinical and virological improvement with no reported

adverse effect. An increase in the cycle threshold of SARS-CoV-2 (likely suggesting a reduction in viral load) was achieved on molecular nasal swab in three patients out of four by the end of the 10-day treatment. We reviewed the available literature on prolonged COVID-19 management in immunocompromised patients in Table 3. The association of two different antiviral pharmacodynamic mechanisms has been considered useful in order to eradicate SARS-CoV-2 infections in immunocompromised patients [5,11]. In the cases reported by Trottier and Snell [6,11], a 20-day and 10-day course of Remdesivir and Nirmatrelvir/Ritonavir, respectively, resulted in the clinical improvement and negativization of nasopharyngeal swab in patients with prolonged SARS-CoV-2 positivity. Even a prolonged course or retreatment with Remdesivir alone up to 30 days has been shown effective in clinical improvement of symptoms in the works by Rüfenacht et al. [5] and Martinez et al. [15], despite no proof of virological negativization in the former. In addition to the work of Ford and colleagues [7], the largest published case series by Mikulska et al. [16], including 22 patients, showed a higher rate of virological and clinical response in immunocompromised patients with prolonged/relapsed COVID-19 after combination therapy with two antivirals (mainly Remdesivir and Nirmatrelvir/Ritonavir), including mAbs.

The second aim of the study was to determinate the pharmacokinetics of nirmatrelvir/ritonavir and investigate whether polymorphisms in the genes involved in their metabolism could affect the interindividual pharmacokinetic variability of nirmatrelvir/ritonavir and ritonavir concentrations in patients with COVID-19. Results showed that concentrations of Nirmatrelvir appeared comparable to those reported by Liu et al. [17], ranging from 50 to 5000 ng/mL, whereas Ritonavir measurements showed higher concentrations (range 1068–3377 ng/mL) compared to the 10–1000 ng/mL range previously reported in patients with similar weight and BMI. Considering the genetic variant analyses, in patient 1, genotypes *ABCB1* 3435 CT and 1236 CT were found. P-glycoprotein (P-gp) is one of 49 members in the superfamily of human adenosine triphosphate ATP-binding cassette transporters (ABC), responsible for cellular homeostasis and drug efflux (https://www.pharmgkb.org/vip/PA166170352; accessed on 20 December 2023). In the literature, several data points described the sequence variations for *ABCB1*; however, there is no clear consensus regarding the contribution of *ABCB1* variation to disease risk. Instead, the literature reported the role of *ABCB1* genetic polymorphisms in different phenotypes, such as P-gp expression, function, drug response, and disease susceptibility, with little consensus (https://www.pharmgkb.org/vip/PA166170352; accessed on 20 December 2023). Some of the most common SNPs in the protein coding region are 3435 and 1236 (https://www.pharmgkb.org/vip/PA166170352; accessed on 20 December 2023): they have been studied in the context of pharmacokinetics and disease, obtaining controversial results (https://www.pharmgkb.org/vip/PA166170352; accessed on 20 December 2023). In our study, patient 1 presented *ABCB1* 3435 CT and 1236 CT genotypes: these are associated with an intermediate activity of drug extrusion. In addition, the *PXR* 63396 CT genotype is associated with an intermediate modulation of *ABCB1* gene expression: overall, this can contribute to the slightly increased values of nirmatrelvir. In particular, PXR is encoded by the *NR1I2* gene and is a member of the nuclear receptor superfamily of transcription factors modulating the induction of many genes.

CYP2D6 is one of the key pharmacogenes involved in the implementation of pharmacogenomics all around the world. It is one of the most polymorphic genes and has an impact on the metabolism of up to 25% of the drugs commonly used in routine therapeutic management. Concerning the *CYP2D6* 840 SNP, the CC genotype is related to a faster elimination rate. Patient 1 presented the CG genotype: consequently, he could have had a possible slowdown in metabolism, potentially affecting drugs concentrations (particularly for ritonavir) and viral clearance. In addition, it should be noted that *CYP2D6* is highly polymorphic; thus, other genetic variants could have an impact on metabolism (further studies with an increased number of patients have to be performed). Furthermore, basically, conflicting data on *CYP2B6* genetic variants are present in the literature [18,19] (differences could be highlighted in other populations, for example in the African population).

Table 3. Literature review of the studies describing off-label treatment for prolonged COVID-19 in immunocompromised patients.

References	Sex and Median Age of Patient	COVID-19 Vaccine	Immunocompromising Condition/Chemotherapy	Median Length of Hospital Stay (days)	Time from Last Anti-CD20 to COVID-19	Treatment of the First and Prolonged COVID-19	Duration of COVID-19 after Admission	Symptoms	Radiology	Serum Title	Cycle Threshold	Treatment of the Prolonged COVID-19	Clinical and Virological Outcome
Snell et al. [6]	M/60	4 doses	Follicular Lymphoma/ R-CHOP	117	5 years	Prednisone 60 mg, Remdesivir 10 days Remdesivir 5 days + Methylprednisone 500 mg + Sotrovimab 500 mg Sotrovimab 1000 mg + Paxlovid + Baricitinib + Methylprednisolone 1000 mg	4 months	Dyspnea, fever, cough	COVID-19 related pneumonia with ground-glass opacities + pulmonary embolism	negative	NA	Remdesivir 10 days + Nirma-trelvir/Ritonavir 10 days	Shortness of breath—afebrile, no cough, improvement of exercise tolerance Negative SARS-CoV-2 swab
Rüfenacht et al. [5]	4 M/ 7 F/ 66	NA	6 Lymphoma 1 Optic neuritis 1 Multiple sclerosis 1 CLL 1 Limbic encephalitis	42	NA	NA	NA	Fever, dyspnea, respiratory failure	NA	negative	24.6 at the beginning 29.7 after Remdesivir course	8 Patients: Remdesivir 5 days 2 Patients: Remdesivir 10 days 1 Patient: Remdesivir 17 days	Clinical improvement in 8 of 11 patients Not available SARS-CoV-2 swab
Ford et al. [7]	M/40	NA	B-cell ALL/Rituximab + hyperCVAD	150	1 month	Sotrovimab Remdesivir 10 days + Dexamethasone Remdesivir iv + Prednisone 0.5/kg	5 months	Dyspnea, cough	COVID-19 related pneumonia with ground-glass opacities ground and interstitial disease	negative	NA	Nirmatrelvir/Ritonavir 20 days + Remdesivir 10 days	Afebrile Improvement of dyspnea and asthenia Negative SARS-CoV-2 swab
Ueda et al. [9]	F/63	NA	Follicular Lymphoma/GB	30	NA	Prednisolone 60 mg	122 days	Fever, cough, dyspnea, rhinorrhea	COVID-19 related pneumonia and development of organizing pneumonia	negative	23 at the beginning 33.9 and 34.3 after one month of disease	Remdesivir 5 days + Dexamethasone tapering	Asymptomatic at discharge Not available SARS-CoV-2 swab
Trottier et al. [12]	M/64	3 doses	CCL/ Venetoclax + Obinutuzumab	75	1 month	Nirmatralvir/ritonavir 5 days Dexamethasone 6 mg + Remdesivir 10 days Prednisone 60 mg + Bebtelovimab 80 mg	5 months	Dyspnea, cough, recurrent fever, body aches	COVID-19 related pneumonia with ground-glass opacities and development of organizing pneumonia	negative	22 serum 25 BAL	Nirmatrelvir/Ritonavir 20 days + Remdesivir 20 days	Afebrile Resolving of dyspnea and asthenia Negative SARS-CoV-2 swab
Martinez et al. [15]	M/44	NA	Granulomatosis with polyangiitis	142 days	4 years	Remdesivir 5 days + Dexamethasone 6 mg Plasma IVIG	108 days	Fever, chills, exertional dyspnea, and diarrhea	COVID-19 related pneumonia	negative	>50	Remdesivir 30 days	Clinical recovery Negative SARS-CoV-2 swab
Thorton et al. [20]	M/49	No	Follicular lymphoma	107 days	1 month	Dexamethasone 6 mg Remdesivir 5 days + Dexamethasone 6 mg	189 days	Fever, cough, asthenia, dyspnea	Remittent ground-glass opacities with bilateral interstitial pneumonia evolving in organizing pneumonia	negative to the nucleocapsid antibody/positive to the spike antibody	>50	Bamlanivimab 700 mg + IVIG 75 mg + Remdesivir 10 days	Afebrile, clinical recovery, improvement in exercise tolerance Negative SARS-CoV-2 swab
Mikulska et al. [16]	13 M/ 9 F/70	3 doses	Hematological malignancies and renal transplant/Anti CD20 in previous 12 months	NA	<12 months	1 Bam-lanivimab/Casirivimab, 3 Sotrovimab 5 Molnupiravir, 3 nirmatrelvir/ritonavir 5 days, 1 Remdesivir 5 days	42 days	Oxygen requirement in 8 patients	NA	8 patients positive	NA	Remdesivir and nirmatrelvir/ritonavir or molnupiravir (of which 18 with mAbs)	Alive, asymptomatic and negative SARS-CoV-2 swab at last follow-up 82%
Colombo et al. [21]	F/67	No	Follicular Lymphoma/R-CHOP	70	1 month	Remdesivir + Corticosteroids Methylprednisolone 1.5 mg/Kg	90 days	Fever, cough, dyspnea	COVID-19 related pneumonia	negative	NA	Corticosteroids + IVIG + Hyperimmune plasma	Afebrile, no respiratory distress Not available SARS-CoV-2 swab

Table 3. *Cont.*

References	Sex and Median Age of Patient	COVID-19 Vaccine	Immunocompromising Condi-tion/Chemotherapy	Median Length of Hospital Stay (days)	Time from Last Anti-CD20 to COVID-19	Treatment of the First and Prolonged COVID-19	Duration of COVID-19 after Admission	Symptoms	Radiology	Serum Title	Cycle Threshold	Treatment of the Prolonged COVID-19	Clinical and Virological Outcome
Wada et al. [22]	M/51 M/74	Yes	2 Follicular lymphoma	14 and 19 days	NA	Remdesivir	NA	NA	NA	negative	18.6 and 19.8 at the beginning	Pt 1: Remdesivir 10 days Pt 2: Nirmatrelvir/Ritonavir for the other	Clinical improvement Not available SARS-CoV-2 swab
Rabscall et al. [23]	M/35	NA	Myasthenia gravis in Thymoma with pleural metastasis	NA	4 months	Remdesivir 5 days + Dexamethasone 6 mg	35 days	Remittent fever, cough, dyspnea	Ground-glass opacities and consolidation consistent with COVID-19	negative to the nucleocapsid antibody/positive to the spike antibody	NA	Casirivimab–Imdevimab	Afebrile, clinical recovery Negative SARS-CoV-2 swab
Breeden et al. [24]	F/79 M/72 M/72 F/60	3 doses 2 doses 2 doses 4 doses	Follicular lym-phoma/Lenalidomide, rituximab, zandelisib, and epcoritamab Large B-cell lymphoma/chimeric antigen receptor T-cell therapy Mantle cell lymphoma/venetoclax, lenalidomide, and rituximab Multiple myeloma and HSCT, then B-cell ALL	180 NA NA NA	2 years 1 year 2 years	Prior tixagevimab/cilgavimab, then molnupinavir and remdesivir Prior tixagevimab/cilgavimab, then nirmatrelvir/ritonavir 5 days Prior tixagevimab/cilgavimab Prior tixagevimab/cilgavimab, then nirmatrelvir/ritonavir 5 days	42 days 30 days 60 days 60 days	cough, shortness of breath, and fatigue fever and cough, then hypoxia shortness of breath and night sweats recurrent fatigue and fevers	Ground-glass opacities and multilobar consolidation Multifocal ground-glass opacities NA Bilateral airspace opacities	NA NA NA	20–25 27.5 28.2 NA	Nirmatrelvir/ritonavir 21 days Remdesivir 10 days followed by nirmatrelvir/ritonavir 20 days Remdesivir 10 days, then nirmatrelvir/ritonavir 15 days Remdesivir 7 days and bebtelovimab once, then nirmatrelvir/ritonavir 20 days	Symptoms resolved Negative SARS-CoV-2 swab

Abbreviations: CLL: chronic lymphocytic leukemia; NA: not applicable; R-CHOP: rituximab-cyclophosphamide-hydroxydaunorubicin-Oncovin-prednisone; ALL: acute lymphocytic leukemia; IVIG: immunoglobulin IV.

Finally, the *CYP3A4* AA genotype is associated with a normal function activity in metabolism, but its impact in our population seems to be weak, since most patients belong to the AA genotype.

Concerning patient 2, the *ABCB1* 3435 CC and 1236 CC genotypes were reported: they seem to be associated with an increased efflux function, and *PXR63396* influences *ABCB1* gene expression. *CYP2D6* CG is related to an intermediate speed of metabolism, and *CYP3A4**1B AA with a normal function allele. Consequently, on this subject, genetics seems to have no impact on drug exposure. It is important to highlight that these data refer to a single patient; therefore, more defined conclusions could be obtained in a larger cohort of patients.

In conclusion, despite the low number of the reported case series, this real-life study supports the usefulness of TDM and genetics in immunocompromised patients with persistent SARS-CoV-2 infection, a challenging setting for clinicians in which personalized medicine may improve outcomes. To the best of our knowledge, no other study reporting this kind of analysis has been published; therefore, further studies are warranted because other genetic and non-genetic factors could contribute to these AUC levels.

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