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Case report

Antiviral combination treatment of SARS-CoV-2 after repeated treatment failures of remdesivir monotherapy: A case report

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ABSTRACT

Immunocompromised patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can have a longer duration of viral shedding and persistence of symptoms. The optimal treatment strategy for these patients remains to be established. This case describes a male in his late sixties with follicular lymphoma and persistent symptoms of infection with SARS-CoV-2 variant BA.2 who was treated with remdesivir five times over a period of six months. The clinical effect of remdesivir treatment decreased over time, and further viral sequencing revealed the emergence of mutations across the SARS-CoV-2 genome. Due to the lack of other treatment options, the patient was treated with a combination of remdesivir and molnupiravir for 10 days, and epcoritamab was discontinued, which led to the cessation of symptoms. This case illustrates the risk of a diminished effect of remdesivir with prolonged use and the need for treatment guidelines for immunocompromised patients with persistent COVID-19.

Introduction

Immunocompromised patients infected with SARS-CoV-2 can experience a prolonged duration of viral shedding and persistence of symptoms due to coronavirus disease 2019 (COVID-19) [1].

Clinical guidelines recommend early antiviral treatment with nirmatrelvir/ritonavir as the first choice and molnupiravir and remdesivir as the second and third choices, respectively, for the treatment of nonhospitalized, high-risk patients [2]. Due to the emergence of new SARS-CoV-2 variants, the neutralizing effect of monoclonal antibodies has been reduced, and the number of available treatment options is therefore limited [3].

Protracted disease course due to COVID-19 has been a particular concern in patients with hematologic malignancy. A study of 368 patients with lymphoid malignancy showed that 13.6 % of the patients were PCR positive for SARS-CoV-2 beyond 30 days after the primary COVID-19 diagnosis [4]. The optimal management of immunocompromised patients with persistent symptoms and viral shedding still needs to be established [5]. Case reports have described the successful use of several courses of remdesivir in immunocompromised patients, and repeated remdesivir treatments have therefore been used as a treatment strategy for patients with prolonged viral shedding [6,7]. Despite the widespread use of remdesivir, a very low rate of resistance has been observed in COVID-19 patients treated with remdesivir. Only a few published case reports have demonstrated remdesivir resistance. A study by Focosi et al. demonstrated that real-world remdesivir resistance is remarkably rare and associated with poor viral fitness due to the instability of the virus over time [8].

In this case report, we describe how the clinical effect of remdesivir diminished after several courses of remdesivir treatment and the emergence of several mutations in the SARS-CoV-2 genome. In addition, we describe how combination therapy with remdesivir and

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molnupiravir cleared the infection with SARS-CoV-2 after six months of viral shedding in a patient with lymphoid malignancy.

Case presentation

A male in his late sixties was originally diagnosed with stage II follicular lymphoma (FL) in 2010. After a period of watchful waiting, three lines of immunochemotherapy from 2016 to 2019 followed with short periods of remission. Progressive disease with CD20-positive FL was diagnosed in an iliac lymph node and in the bone marrow in May 2020. A phase 1 trial of CD3×CD20 bispecific antibody (epicoritamab) was offered, and the patient started treatment in late September 2020. Complete metabolic remission was observed at the first interim PET-CT scan in late October 2020. Symptoms of lymphoma abated, and complete remission was ongoing during 2021. As part of the clinical routine, the patient was tested for SARS-CoV-2 13 times during 2021, all of which were negative. In May 2022, upper airway symptoms and fever developed. He tested positive for the SARS-CoV-2 variant Omicron BA.2. After undergoing a three-day remdesivir treatment, all symptoms remitted. Approximately three weeks later, he experienced fever above 38 degrees and cough. He was admitted to the hematologic department in mid-June, where SARS-CoV-2 antigen levels were measured above 1000 ng/L. This time, the patient was treated with remdesivir for nine days and administered one dose of tixagevimab/cilgavimab. By the end of treatment, he had a temperature below 37 degrees, SARS-CoV-2 antigen was measured at 0.88 ng/L, and he was without any symptoms.

At this point, the patient was undergoing treatment with epicoritamab for lymphoma.

Epicoritamab was administered 11 days after the initial remdesivir treatment and then again six days after the second treatment. Treatment for his lymphoma was paused thereafter.

Approximately three weeks after the second remdesivir treatment, the patient presented again with a fever above 38°, cough, and fatigue. After experiencing three weeks of continuous fever, a five-day remdesivir treatment was administered. Fever was absent after one day.

He experienced his third relapse with low-grade fever, cough and fatigue just a few days after the last remdesivir dose. The patient was again treated with remdesivir, but this time the treatment was extended to 10 days. The fever abated after two days of treatment, and the cough was absent at the end of the treatment. SARS-CoV-2 antigen was 3,2 ng/L after 7 days of the treatment.

Only five days after the last remdesivir dose, the patient again experienced a low-grade fever, cough, and headache. This time, the patient was treated with remdesivir for 20 days, and the fever resolved after 14 days. SARS-CoV-2 antigen was 37 ng/L on the first day after remdesivir administration, and at 8.3 ng/L by end of treatment. Three days after the termination of this extended remdesivir treatment, the patient's fever returned.

After the fifth treatment with remdesivir and persistent symptoms related to SARS-CoV-2 infection, an examination for viable virus was performed. Furthermore whole-genome sequencing (WGS) was analyzed from samples at day 60, 144 and 148 in the disease course. These analyses revealed viable virus based on cytopathic effects, RT—PCR detected viral replication, and WGS revealed multiple mutations appearing across the consensus genome sequence over time and up to at least seven in the final sample inclusive one mutation (SQ1180K) in the spike region (Fig. 1).

At this time, the only marketed SARS-CoV-2 antivirals in Denmark were remdesivir and molnupiravir. The patient was therefore treated with a 10-day combination of remdesivir and molnupiravir. SARS-CoV-2 antigen were 293 ng/L before treatment and < 0.1 ng/L after 7 days of treatment, and 4 days after end of treatment. During this treatment, the patient experienced immediate improvement in symptoms, and after six months of persistent COVID-19 symptoms, the assessment was that he had finally cleared the SARS-CoV-2 infection. Episodes of fever and all antiviral treatments are shown in Fig. 2.

Throughout the entire disease course, the patient received several treatments with antibiotics without effect, and blood and sputum cultures showed no presence of microbes. The patient was reverse transcription polymerase chain reaction (RT—PCR) positive for SARS-CoV-2 with subtype Omicron BA.2 at all relapses. During relapses, SARS-CoV-2 antigen was monitored in blood, with decreasing values during treatment and elevated values at relapses. Throughout the course, the remdesivir treatment was paused at three different times during weekends.

The patient did not experience any fever for 61 days, but in December 2022, he debuted with low-grade fever and a sore throat. An oropharyngeal swab revealed that he was again infected with SARS-CoV-2, but this time the variant was Omicron XBC.1. This time, he was treated with nirmatrelvir/ritonavir, which in the meantime had been marketed in Denmark, and he was without symptoms after five days of treatment.

The patient has not received treatment for his lymphoma since June 2022, and in April 2023, a positron emission tomography and computed tomography (PET-CT) scan showed complete remission, and the patient therefore exited the protocol.

Materials and methods

SARS-CoV-2 RNA was detected using the Cobas® 6800 (Roche Diagnostics) with the Cobas® SARS-CoV-2 Qualitative kit. For detection of viable virus, an oropharyngeal swab sample in Universal Transport Medium™ (Copan) was cultured on the same day of sampling. Briefly, the sample was inoculated onto confluent VERO E6 cells (ATCC CRL-1586) and allowed to infect the cells for a one-hour infection period, followed by the addition of 5 mL of cell culture media. After 6 days of culture, cells were inspected for cytopathic effects, and cell media aliquots collected immediately after infection and after 6 days were

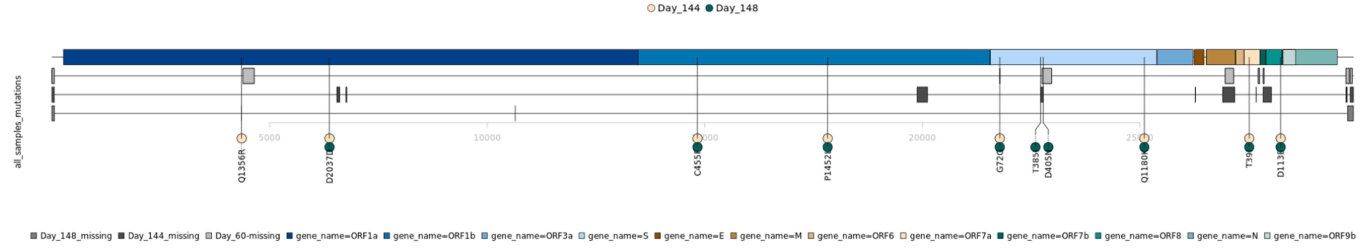

Fig. 1. Mutations over time. Lollipop plot of new amino acid mutations accrued over the course of treatment along the SARS-CoV-2 genome and their locations in genes compared with the first analyzed sequence at day 60 in the disease course. Created in R using Gviz and trackViewer. Top bar is the different regions of the SARS-CoV-2 genome, where the light blue region labeled "gene_name = S" in the legend symbolizes the region coding for spike. As missing data might hide a mutation, gaps in the assemblies have been visualized as well, displayed as lines below the genome overview with gray boxes marking the gaps. At the bottom are the colored 'bubbles' indicating whether a particular mutation is present in the given sequence. In the samples from day 144 there are three non-synonymous mutations, two in ORF1b (C455F, P1452L) and one in Spike (Q1180K).

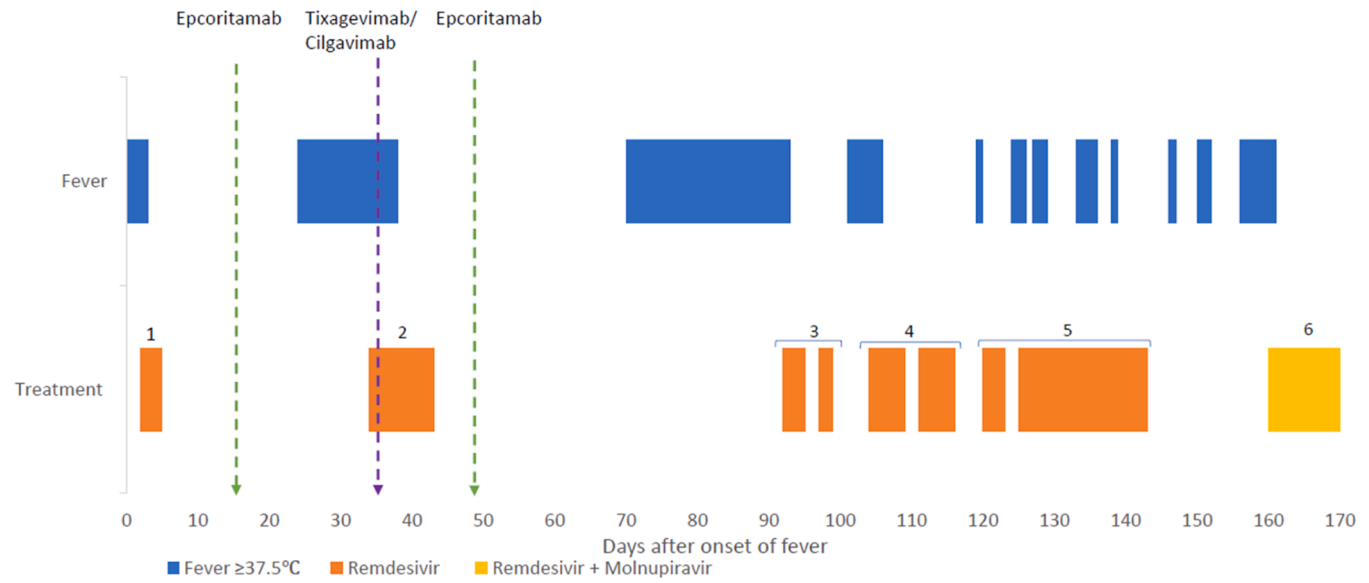

Fig. 2. Disease course. Disease course showing days with fever ≥ 37.5 °C, remdesivir courses, single-dose epcoritamab, single-dose tixagevimab/cilgavimab and combined remdesivir and molnupiravir course.

analyzed by RT—PCR to assess viral replication. Replicative SARS-CoV-2 replication was confirmed by a drop in 7 Ct values [1].

Consensus sequences for all samples were obtained with the medaka workflow of the ARTIC pipeline; lineages were subsequently assigned with Pangolin 4.1.2 [9] in usher mode, and mutations were identified using Nextclade v 2.6.0 (dataset version 2022-10-04) [10]. Mutations were visualized in R using the gviz [11] and TrackViewer [12] libraries.

Discussion

This case report describes the diminished clinical effect of remdesivir and the emergence of mutations in the SARS-CoV-2 genome in an immunosuppressed patient with follicular lymphoma treated repeatedly with remdesivir over six months due to recurrent fever and subjective symptoms of SARS-CoV-2 infection. The infection was cleared after combination therapy with remdesivir and molnupiravir.

Remdesivir is an inhibitor of the RNA-dependent RNA polymerase of SARS-CoV-2. Mutations, particularly in the RNA-dependent RNA polymerase, have the potential to affect the effectiveness of remdesivir by altering its susceptibility to the drug [13].

In this case report, SARS-CoV-2 samples taken from the patient developed seven certain mutations over the course of the treatment. Of these, ORF1b:C455F is notable, as it is close to ORF1b:P314L and ORF1b:F471L/S/C, which have been associated with remdesivir resistance in in vitro studies [8].

Interruption and the repeated use of remdesivir may have contributed to the development of resistance in this case.

Combination therapy is a state-of-the art treatment for chronic viral infections such as human immunodeficiency virus and chronic hepatitis C virus to reduce the emergence of drug resistance variants and increase the antiviral effect. Combination therapy is not commonly used to treat acute viral respiratory infections such as influenza and SARS-CoV-2. The use of combination therapy in patients with influenza has been tested in the FLAGSTONE study, and although it resulted in shortened viral shedding, it did not result in superior clinical improvement [14].

There are no randomized studies investigating antiviral combination treatment for SARS-CoV-2, and knowledge has been limited to small retrospective studies, case reports, and animal studies [15]. These cases has primarily described successful use of combination therapy with remdesivir and nirmatrelvir/ritonavir with or without monoclonal antibodies in immunocompromised patients with prolonged SARS-CoV-2 infections [5,16]. The combination of molnupiravir with remdesivir has been described in two immunocompromised patients with divergent results [5]. A case report describes successful treatment outcome of triple therapy with the addition of molnupiravir to remdesivir and nirmatrelvir/ritonavir after an immunocompromised patient experienced treatment failure after treatment with remdesivir, nirmatrelvir/ritonavir and tixagevimab/cilgavimab [17]. In vitro studies have described a synergistic effect of molnupiravir combination treatments with boosted antiviral activity against SARS-CoV-2 [18], and the combination treatment of remdesivir and molnupiravir showed a markedly potent antiviral effect in SARS-CoV-2-infected hamsters [19]. However, a recently published study showed lower risk of mortality for patients treated with monotherapy nirmatrelvir-ritonavir compared to combination therapy with remdesivir and nirmatrelvir-ritonavir in primarily immunocompetent hospitalized COVID-19 patients [20].

The strength of this case report is the investigation of viable virus, which emphasizes that this patient's symptoms were due to infection with SARS-CoV-2. Furthermore, by including sequencing of the viral genome, several emergences of mutations in the SARS-CoV-2 genome were revealed during the disease course. As a limitation, it would have been preferable to examine viable virus throughout the entire disease course and follow the viral dynamics with quantification of viable virus.

Prolonged viral shedding of SARS-CoV-2 remains a challenge for immunocompromised patients, and this case report illustrates the need for guidelines for the repeated use of antivirals and the role of antiviral combination therapy in this patient population.

CRediT authorship contribution statement

Rune Micha Pedersen: Methodology. **Kat Steinke:** Methodology. **Thomas E. Andersen:** Methodology. **Line L. Bang:** Methodology. **Anders Jensen:** Methodology. **Lone W. Madsen:** Writing – original draft, Supervision, Conceptualization. **Michael R. Clausen:** Conceptualization. **Anne Cathrine Bay:** Writing – original draft. **Thomas V. Sydenham:** Methodology. **Birgit Thorup Røge:** Conceptualization.

Author statement

All co-authors have read and approved the revised manuscript.

Consent

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Ethical approval

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Abbreviations

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; RT−PCR: Reverse transcription polymerase chain reaction; PET-CT: Positron emission tomography and computed tomography; WGS: whole-genome sequencing.

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Competing of interest

On behalf of all authors, the corresponding author states that there are no competing interests.

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Availability of data and materials

The dataset generated and analyzed for this cohort study is not publicly available due to the Danish Data Protection Law in accordance with approval by the Danish Data Protection Agency (j.nr. 20/16202).

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