Emerging RNA-Dependent RNA Polymerase Mutation in a Remdesivir-Treated B-cell Immunodeficient Patient With Protracted Coronavirus Disease 2019

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Remdesivir failure with SARS-CoV-2 RNA-dependent RNA-polymerase mutation in a B-cell immunodeficient patient with protracted Covid-19

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Abstract

SARS-CoV-2 is a new pandemic virus for which Remdesivir is the only antiviral available. We report the occurrence of a mutation in the RdRP (D484Y) following failure of remdesivir in a 76-year-old woman with a post-rituximab B-cell immunodeficiency and persistent SARS-CoV-2 viremia. Cure was reached after supplementation with convalescent plasma.

Key words: SARS-CoV-2, Remdesivir, SARS-CoV-2 RNA-dependent RNA-polymerase mutation, COVID-19, CoVID-19 convalescent plasma.

Background

Coronavirus disease 2019 (COVID-19) has recently emerged as a new pandemic due to the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). Alongside the well-described acute pulmonary presentation of Covid-19, a more chronic evolution of the disease is being reported in patients with B-cell immunodeficiency, probably associated with the inability to produce anti-SARS-CoV-2 antibodies[1]. Remdesivir is an RNA-dependent RNA-polymerase (RdRP) inhibitor of SARS-CoV-2, the first antiviral to be given in the USA [2] and Europe [3], with an impact on the hospital duration stay but not on mortality [4]. SARS-CoV-2 mutation, especially in RNA-dependent RNA polymerase (RdRP), may affect remdesivir susceptibility [5]. More recently, anti-SARS-CoV-2 antibody supplementation by COVID-19 convalescent plasma (CP) has emerged as a promising therapy but the characterization of the patients most likely to benefit from CP is critical [6].

We report the case of a 76-year-old woman with B-cell immunodeficiency who presented with severe protracted COVID-19 and persistent SARS-CoV-2 viremia, in whom remdesivir treatment failure was associated with the emergence of a point mutation in the RdRP and cure was achieved only after CP therapy.

Methods

SARS-CoV-2 RT-PCR and antibodies testing

In house real-time reverse transcriptase PCR (RT-PCR) tests for SARS-CoV-2 nucleic acid were performed using nasopharyngeal swab, bronchoalveolar lavage (BAL) and plasma samples. Primer and probe sequences targeted two regions on the RdRP gene and were specific to SARS-CoV-2. Assay sensitivity was around 10 copies/reaction (Institut Pasteur, Paris, France).

Sera were tested for anti-SARS-CoV-2 antibodies using the Elecsys Anti-SARS-CoV-2 electrochemiluminescence immunoassay for detection of IgA and IgG against the SARS-CoV-2 nucleocapsid antigen (Roche Cobas 6000®). This immunoassay was approved by the FDA regarding its excellent analytical performances (sensitivity 99.5% and specificity 99.8%). The positive cut-off value of optical density index ratio was 1.10.

RdRP sequencing

Screening for mutation was performed after reverse transcriptase with Transcriptase inverse SuperScript™ III (ThermoFisher), 4 nested PCR were performed to amplify the entire RdRP gene of 2795 nucleotides (nt), or 932 amino-acids (aa), in length (Supplementary data Table 2). Sanger sequencing was performed with BigDye Terminator chemistry (Thermo Fisher Scientific®) and the analysis of the reaction products on an ABI sequencer.

Sequences were analyzed on Geneious 11.1.4 as following: after cleaning base with low quality at 5' and 3' ends (error probability limit set at 0.05), the four fragments were mapped on the annotated SARS-CoV-2 reference sequence NC_045512 and consensus sequences were generated for each sample. Multiple alignments of nt and aa consensus sequences were performed with Mafft v7.388 implemented in Geneious software. Finally, the differences between sequences were identified visually.

CP collection and preparation

CP collection was approved by the ethical committee (Comité de Protection des Personnes d'Ile-de-France) on March 26th, 2020. The plasma was obtained as previously described [7].

RESULTS

A 76-year-old woman arrived at the emergency room on the 11th of April 2020 presenting with a cough, fever, and dyspnea that had persisted for 4 days. COVID-19 was diagnosed via SARS-CoV-2 positive RT-PCR on a nasopharyngeal swab.

Her medical history included recent chronic lymphocytic leukemia diagnosed in 2019. She was successfully treated by 4 doses of Rituximab / Bendamustine, last cure in October 2019. While the patient was considered in complete remission, she exhibited secondary B-immunodeficiency requiring immunoglobulin substitution stopped in February 2020.

Upon admission for COVID-19, laboratory tests exhibited a low Ig level (1.2 g/l) for which intravenous Ig were administered and Lymphocytes (Lc) immunophenotyping revealed 70 Lc/mm^3 with 88% CD3+ T Lc, <1%CD19 B Lc and 11% CD16+CD56+ NK Lc. The patient was treated by cefotaxime without improvement as she consequently required increased oxygen need with fever and increased inflammation (Supplementary data Figure 1).

Broad spectrum antibiotherapy was administered and hydoxychloroquin was initiated for 9 days (29th April-7th May) with corticosteroids without improvement. On May 27, the patient still suffered from fever and hypoxemia, with an inflammatory syndrome (CRP at 137 mg/L) and a measurable SARS-CoV-2 viremia (3.88$\log_{10}$ copies/ml) while testing negative for anti SARS-CoV-2 antibodies (Roche Cobas 6000®). Remdesivir was introduced for 5 days (compassionate use GS-US-540-5821, 200mg at day 1 followed by 100 mg per day). The patient experienced an early clinical and biological improvement with a normalization of oxygen saturation in room air and a decreased CRP level to 38mg/L. However, SARS-CoV-2 viremia remained detectable (3.94$\log_{10}$ copies/ml, 9th June). Five days following treatment discontinuation, the patient experienced a new clinical degradation with fever and required intra nasal oxygenation (9th June). The patient was then treated with CP (4 X 200 ml from the 12th to 15th June 2020) and experienced a rapid improvement of her clinical, biological and radiological parameters, without detection of SARS-CoV-2 in blood on day 7 and 28 after CP transfusion (22th June) (Supplementary data Table 1, Figure 1**)**. The patient was discharged on the 22th of June and six weeks after CP transfusion, no SARS-CoV-2 RNA was detected in blood and in rhinopharynx.

Virology and RdRP mutation

Screening for mutations in the RdRP gene was performed on 2 expectorations (24th April and 15th May and 1 BAL from 27th April) and 1 blood sample (from 27th May) collected before remdesivir treatment and 2 blood samples collected after remdesivir treatment (on 9th and 12th June). Sequencing was successful for all samples except the last one collected on 12th June. The three respiratory samples collected before treatment as well as the blood sample collected the day of remdesivir initiation harbored strictly the same amino-acid sequences. In the blood sample collected after treatment (9th June), a substitution of one nucleotide in position 1449 (G>T) in the RdRP gene led to a non-synonymous mutation changing an acid aspartic to tyrosine in position 484 (Figure 1).

DISCUSSION

We demonstrate the occurrence of a mutation in RdRP polymerase following failure of remdesivir treatment in a 76-year-old woman with B-cell immunodeficiency and persistent SARS-CoV-2 viremia. RNA viruses are characterized by a high mutation rate and the occurrence of such point mutations (STPs) has already been described mainly in the different viral proteins of SARS-CoV-2 and reported as a risk for the emergence of resistance to treatment [5]. We report the first case of occurrence of a STP in RdRP *in vivo* following remdesivir treatment with identification of a single point mutation in RdRP gene not present before treatment. Although phenotypic assay was not performed, the non-synonymous mutation D484Y was only evidenced in the sample collected after treatment. Furthermore, it occurred in the finger subdomain (extended from aa 395 to 581)[8] which is one of the 3 subdomains constituting the catalytic domain of the RdRP. Lastly, D484Y is proximate to other mutations already described in SARS-CoV (F480L) and other coronaviruses (F476L) to induce resistance to remdesivir in vitro [9-11].

Remdesivir treatment is to date the only COVID-19 antiviral treatment with a reported efficacy and thus, identification of putative resistance mutations is of utmost importance. It is unclear whether the emergence of this mutation was favored by the profound B-cell depletion but our case illustrates however, the likelihood of appearance of SARS-CoV-2 resistant strains under treatment pressure and the need of new antiviral treatments aiming at different targets alongside the viral cycle, such as elbasvir or EIDD-1931 [10, 12].

We used a 5 day treatment duration in our patient, which was considered not inferior to a 10 day course for patients not requiring mechanical ventilation. However, the specific case of immunodeficiencies was not addressed in this trial, a subpopulation for which a longer treatment duration could be considered. The patient did respond clinically to a 5-day treatment course with receding of fever, dyspnea and lifting of oxygen need. However, the persistence of an inflammatory syndrome at the end of treatment as well as a persistent viremia associated with the occurrence of the D484Y mutation in RdRP may be predictive of treatment failure even with a 10-day course of remdesivir.

The other originality of this case is the effectiveness of CP transfusion. By allowing for the provision of anti-SARS-CoV2 antibodies to patients unable to mount a humoral response because of B-cell immunodeficiency, CP treatment is emerging as a safe and efficient treatment in these cases characterized by an ongoing chronic viremia and a protracted Covid-19, as illustrated by a series of 17 cases that we recently reported [7].

In conclusion, mutations such as D484Y in the RdRP of SARS-CoV-2 may emerge *in vivo* during a 5-day course of remdesivir treatment and lead to treatment failure. Patients with a B-cell immunodeficiency experiencing a protracted form COVID-19 represent a population subgroup at risk of viral failure because of prolonged SARS-CoV-2 viremia. In this situation, SARS-CoV-2 convalescent plasma may represent an efficient therapeutic option.

Competing interests

KL, AGM and VC have received grants and honoraria from ViiV Healthcare, Gilead and MSD outside of COVID-19 and present work. KL reports expert meeting fees and travel grants from Gilead, Janssen, MSD, and Abbvie, outside the submitted work.

The other authors declare no competing interests.

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Figure 1: RNA-dependent RNA-polymerase amino-acid sequences of SARS-CoV-2 before and after remdesivir treatment. The 3 respiratory samples were collected before treatment (Expecto_042420, BAL_042720 and Expecto_051520), one blood sample was collected the day of remdesivir initiation (Blood_052720) and the other blood sample was collected nine days after the end of remdesivir treatment (Blood_060920). Red cross shows the non-synonymous mutation identified only in sample collected after treatment (D484Y). Black cross show the amino-acid where mutation to remdesevir in vitro has already been described in SARS-CoV (F480L) and other coronaviruses (F476L).

Figure 1