Sirolimus as a possible treatment in cleavage-resistant RIPK1-induced autoinflammatory syndrome

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Clinical Implications

We present the case of a girl with cleavage-resistant RIPK1-induced autoinflammatory (CRIA) syndrome treated with sirolimus with a significant reduction in the number of days with fever, the total dose of glucocorticoids used, and the inflammatory marker levels, together with a reduction in the double-negative T cells and control of phosphoinositide 3-kinases/mammalian target of rapamycin activity. We propose sirolimus as a valid therapeutic choice in patients with CRIA syndrome.

Cleavage-resistant RIPK1-induced autoinflammatory syndrome (CRIA syndrome) is a monogenic autoinflammatory disease (AID), caused by monoallelic variants in the *RIPK1* (receptor-interacting serine/threonine-protein kinase 1) gene, preventing the caspase-8-mediated RIPK1 cleavage. CRIA syndrome is characterized by early-onset recurrent fever, lymphadenopathy, splenomegaly, arthralgias, oral ulcers, and gastrointestinal manifestations, mainly abdominal pain. These episodes usually last 1 to 7 days and recur every 15 days to 1 month.[1] Inflammatory markers may be found high even during fever-free intervals.[2]

RIPK1 activation has a pivotal role in controlling TNF-mediated apoptosis and necroptosis, and the production of proinflammatory cytokines, such as IL-6.[2,3]

The loss of RIPK1 activity due to caspase-8 cleavage-resistant variants may provoke increased levels of inflammasome-related cytokines (IL-1 and IL-18) and cell death through both necroptosis and apoptosis, with an overproduction of IL-6, TNFs, and IFN-γ.[1] The cytokine production resembles that of other AIDs, such as NLR family pyrin domain containing 3 inflammasomopathies and tumor Necrosis factor Receptor Associated Periodic Syndrome, which respond to anti-IL-1 and/or anti-TNF drugs. Some patients with CRIA syndrome display only a partial clinical response to anti-IL-6 treatments (tocilizumab), whereas others fail to respond.[2] Moreover, treatment with either anakinra or etanercept was not able to fully suppress inflammation in all CRIA patients treated.[2]

Prednisone is usually effective in controlling the attacks but does not prevent recurrence of flares, and chronic administration may be associated with dreadful complications.

Another important effect of RIPK1 pathogenic variants is the unchecked activation of the extrinsic apoptotic pathway triggered by caspase-8 activation, leading to increased stimulation of the mTOR pathway with increased counts of both double-negative (DN) T cells (T-cell receptor α/β+CD4−CD8−) and naive B cells, resembling the immunological presentation of autoimmune lymphoproliferative syndrome among others.[2] Notably, increased mTOR activation can be modulated using targeted therapy such as sirolimus.[4]

Sirolimus efficacy and safety in controlling disease flares in patients with CRIA syndrome have not been assessed.

We herein report the clinical and laboratory response to sirolimus treatment in a patient with CRIA syndrome. To assess sirolimus efficacy we compared the number of days with fever, cumulative glucocorticoid dose (per kg/month), and C-reactive protein (CRP) levels before and after treatment was started.

The patient is currently an 18-year-old girl. Her symptoms began at 1 month of life with recurrent febrile episodes, lymphadenopathies, abdominal pain, and hepatosplenomegaly, occurring every 14 days, with a duration of 3 to 5 days. The flares were associated with increased levels of CRP and serum amyloid A (SAA); both the inflammatory markers always returned to normal levels between the attacks. The girl was regularly checked for early signs of amyloidosis (urine analysis with β2 microglobulin, liver function tests, liver ultrasound, and echocardiogram) and always turned out negative. Despite a normal number of total lymphocytes, T- and B-cell phenotyping revealed increased counts of both DN T cells (TCR+ CD4−CD8−/T lymphocyte 4.6%) and naïve B cells (IgD+ IgM+ CD27−/CD19+ lymphocyte 81.9%), while CD21lowCD38low B cells were lower than normal. Antinuclear antibodies, IgA anti-transglutaminase, and thyroid function tests were regularly checked and were always within normal limits. Immunoglobulin levels were appropriate for levels on multiple occasions, and there was regular antibody production to hepatitis B and tetanus vaccination.

While targeted Sanger sequencing and next-generation sequencing analysis over the years did not yield a definite genetic diagnosis, whole exome sequencing was performed at the age of 14 years, revealing a *de novo* heterozygous pathogenic variant c.970G>C (p.Asp324His) in *RIPK1*. Sanger sequencing confirmed the variant and the *de novo* origin, and the girl was diagnosed with CRIA syndrome.

During follow-up, several treatments were undertaken, besides glucocorticoids to manage the attacks, including anakinra, canakinumab, colchicine, azathioprine, and mycophenolate mofetil without a satisfactory response. In contrast, treatment with tocilizumab intravenously led to a reduction in the number of attacks and inflammatory markers. Unfortunately, during the second dose of tocilizumab, the patient experienced flushing, nausea, vomiting, and shortness of breath. Pulsossimetry and blood pressure were still in the normal range, the drug was immediately stopped, and hydrocortisone and antihistamines intravenously were administered with prompt resolution of symptoms. The introduction of sarilumab was not able to reduce disease flares (Figure 1). The chronic use of prednisone led to significant collateral effects (growth and pubertal delay).

Once the genetic diagnosis was achieved, and due to the impossibility of proceeding with tocilizumab treatment, sirolimus was administered as off-label use, after obtaining informed consent from the parents.

Sirolimus treatment was started at the dose of 1.7 mg/m^2 and then increased up to 2.5 mg/m^2, resulting in a satisfactory blood concentration of the drug (4-9.4 ng/mL). Clinical and laboratory

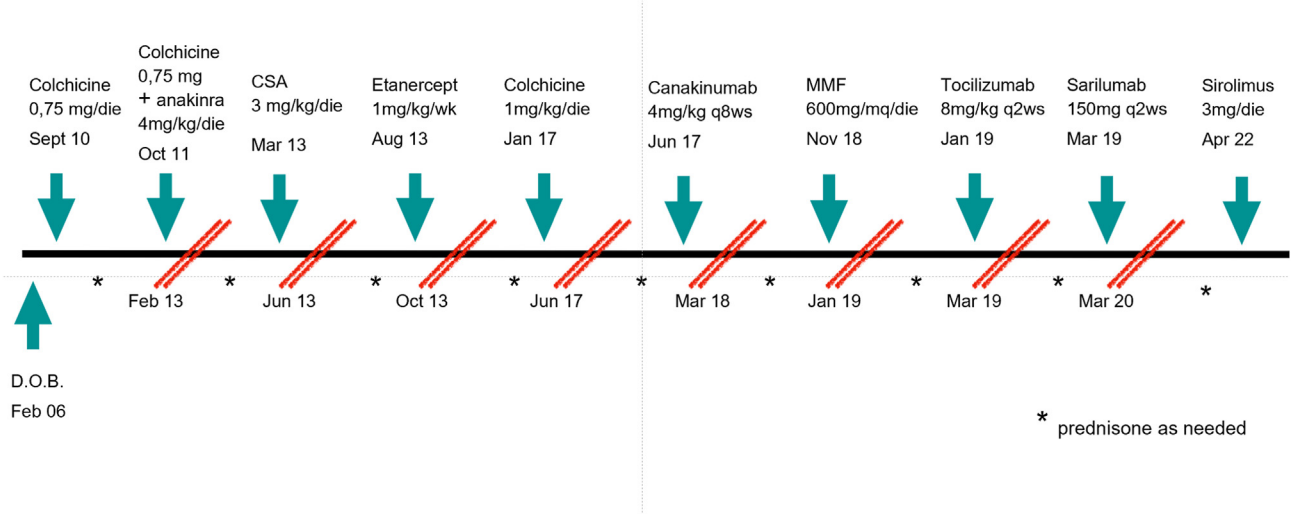

FIGURE 1. A timeline of the treatments used in the patient during time. *CSA*, Cyclosporin; *MMF*, micophenolate mofetil.

data were collected from 16 months before sirolimus treatment up to 24 months.

Clinical data analysis showed that before initiating sirolimus the patient experienced an average of 13.7 days per month with fever, lymphadenopathies, and abdominal pain, while the number of febrile episodes significantly decreased to an average of 3.2 days per month after the start of sirolimus ($P < .0001$) (Figure 2, *A* and *B*). Moreover, the fever episodes after introducing sirolimus were shorter, the body temperature was lower (between 38°C and 39°C), and the associated symptoms were perceived as milder by the girl and the parents with the absence of abdominal pain and lymphadenopathy. This substantial reduction highlights the effectiveness of sirolimus in alleviating the symptomatic burden of CRIA syndrome.

In addition, before the introduction of sirolimus, the patient required an average steroid dose of 1.225 mg/kg/month to manage symptoms, while after treatment, the average dose dropped dramatically to 0.119 mg/kg/month ($P < .0001$) (Figure 2, *C*). The average CRP level was 81 mg/L in the months preceding sirolimus treatment, indicative of persistent inflammation, and 14 mg/L ($P < .0001$) (Figure 2, *D*) after sirolimus introduction. The average SAA levels dropped from 307 mg/L (normal value <5 mg/L) in the 12 months before sirolimus to 114 mg/L in the 12 months after sirolimus ($P < .0001$). Also, hemoglobin levels improved from a mean value of 9 g/dL before treatment to a mean value of 12 g/dL after treatment.

As mentioned, the patient displayed a normal T-cell lymphocyte number, with an increased proportion of DN T cells. After 8 months of sirolimus treatment, DN T cells/CD3+ and DN T cells/T lymphocyte decreased from 4.6% to 3.1% and 2.8% to 2%, respectively. So far, we have not observed a parallel decrease in the number of naïve B lymphocytes. As our proposed rationale for sirolimus use was its inhibitory effect on mTOR, we measured *in vitro* phosphorylated S6 ribosomal protein expression in peripheral CD4+ and CD8+ T cells from the index patient (before and after treatment) and healthy control at steady state (gray) and on anti-CD3 stimulation (Figure E1, available in this article's Online Repository at www.jaci-inpractice.org) The pretreatment assay from the patient showed low levels of S6 phosphorylation, which were confirmed under sirolimus treatment. Because increased mTOR activity is a well-known phenomenon in CRIA syndrome, we believe that this was secondary to the chronic use of corticosteroids. Indeed, before sirolimus was started, the girl was on corticosteroids as needed, but experiencing 1 episode/week, so it was not possible to obtain a sample with adequate distance from the glucocorticoids. On the other hand, the post-treatment results suggest that controlling phosphoinositide 3-kinases/mammalian target of rapamycin activity may be a potential target for this disorder.

Besides experimental data, we believe that the clinical findings collectively suggest that sirolimus may be effective in controlling the clinical manifestations of CRIA syndrome, reducing both the frequency of symptomatic episodes and the need for steroids, while also reducing systemic inflammation as evidenced by CRP levels.

No side effects related to sirolimus occurred, other than recurrent oral ulcers from which the patient never suffered before starting the treatment. Notably, given the recurrent ulcers, we proposed to the patient to reduce the sirolimus dose, but she refused because she was more concerned about a disease relapse.

In conclusion, we provide preliminary evidence on the potential role of sirolimus as a therapeutic option for CRIA syndrome. We recognize that our level of evidence is low; still, sirolimus has a rationale for being effective in CRIA, and because the disease is extremely rare, a randomized trial would not be easily feasible. Therefore, we believe that our experience could be of great value for physicians treating patients with CRIA syndrome.

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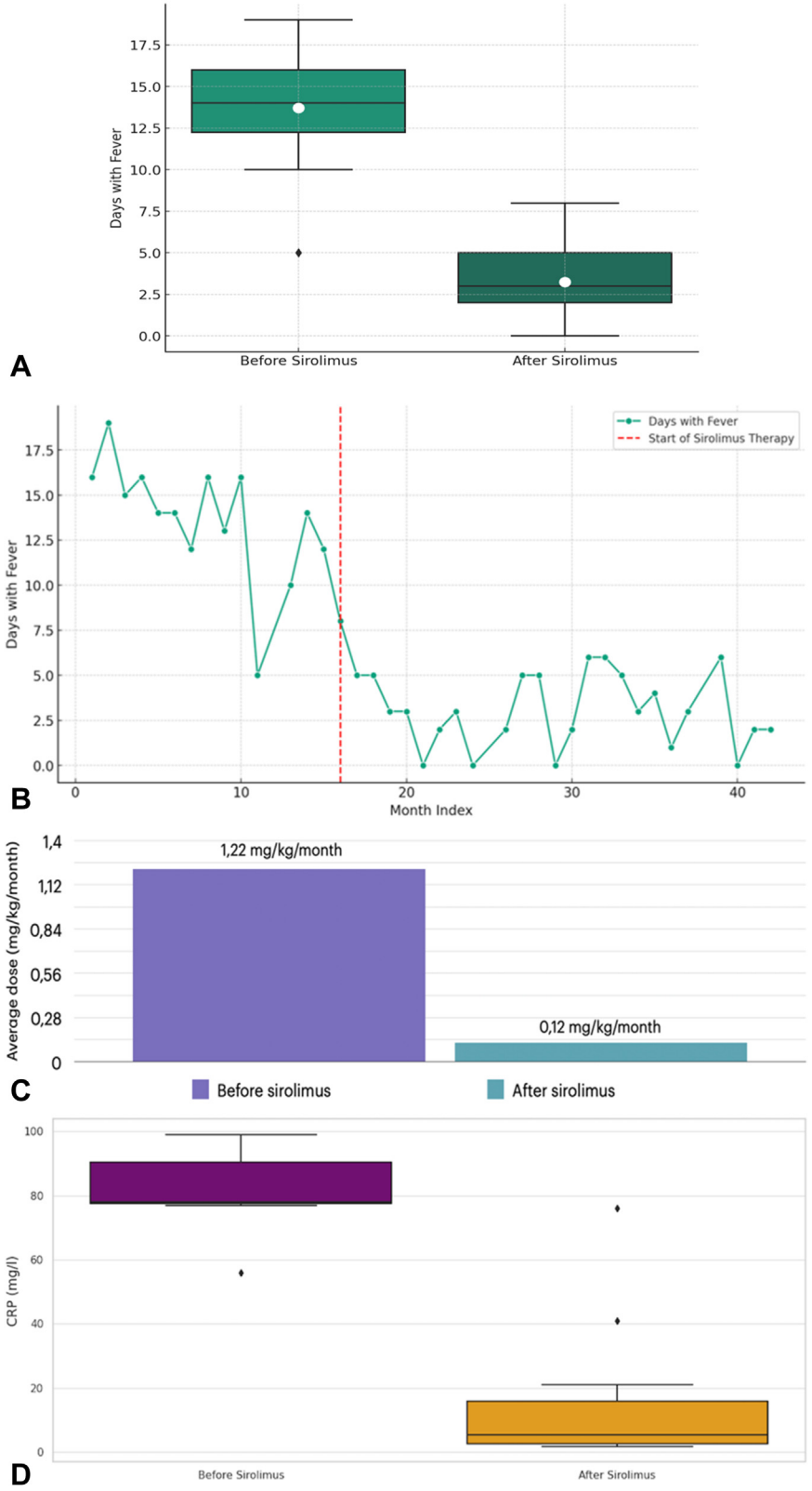

FIGURE 2. (**A**) Comparison of fever days before and after sirolimus treatment. (**B**) Trend of febrile days over time. (**C**) Comparison of average cumulative steroid dose during sirolimus therapy. (**D**) Changes in CRP levels during sirolimus therapy. *CRP,* C-reactive protein.

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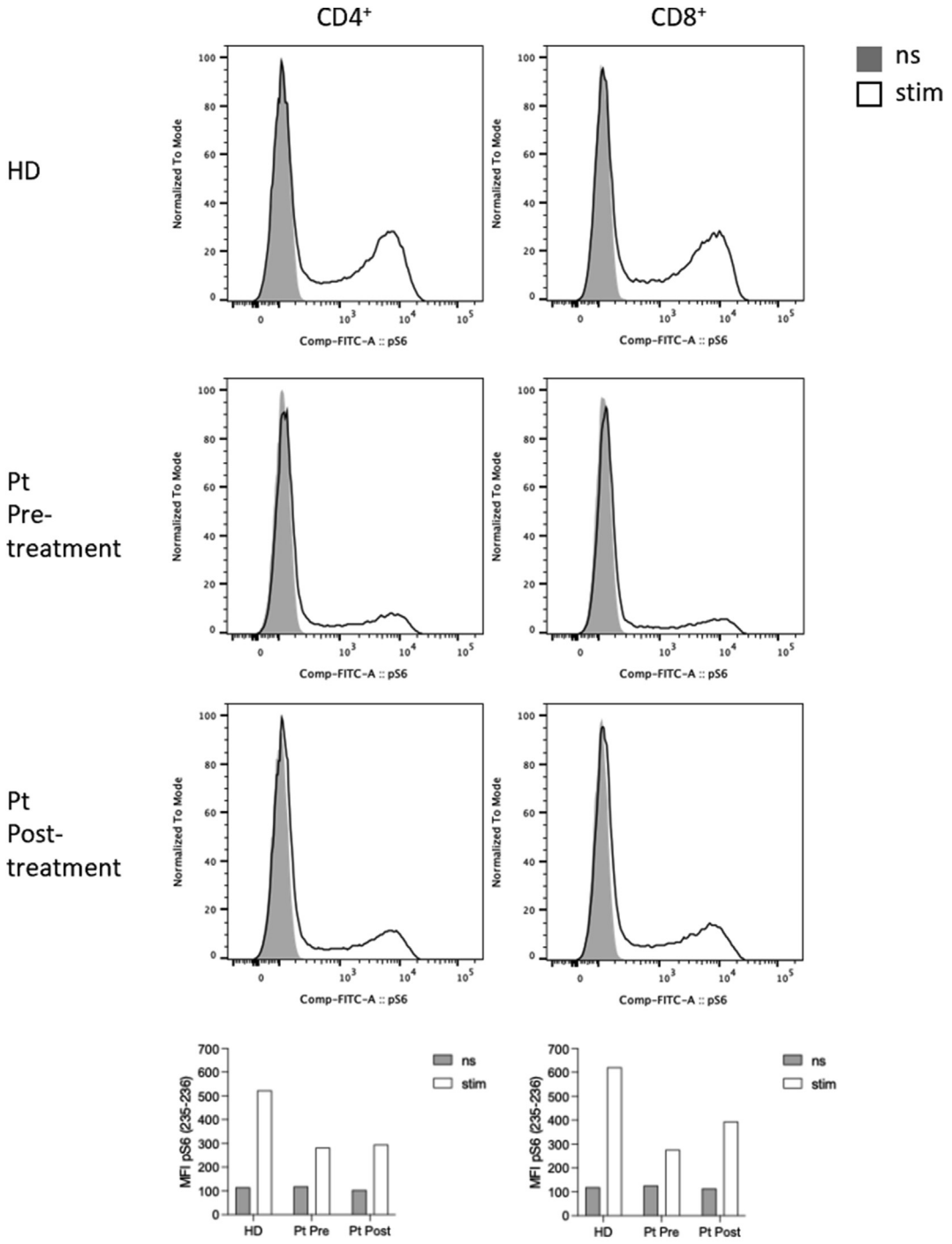

pS6 levels in peripheral CD4+ and CD8+ T cells from the index patient (pre and post-treatment) and healthy control at steady state (grey) and upon anti-CD3 stimulation (white). MFI values are summarized in the graphical bar below.

FIGURE E1. pS6 levels in peripheral CD4+ and CD8+ T cells from the index patient (before and after treatment) and healthy control at steady state (gray) and on anti-CD3 stimulation (white). MFI values are summarized in the graphical bar below. *FITC*, Fluorescein isothiocyanate; *MFI*, mean fluorescence intensity.