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Efficacy of the Janus kinase 1/2 inhibitor ruxolitinib in the treatment of vasculopathy associated with *TMEM173*-activating mutations in 3 children

To the Editor:

Gain-of-function mutations in *TMEM173* encoding stimulator of interferon genes (STING) underlie a novel type I interferonopathy,[1] termed SAVI (STING-associated vasculopathy with onset in infancy).[2,3] This disease is associated with high childhood morbidity and mortality. STING is a central component of DNA sensing that leads to the induction of type I interferons, which, in turn, drives the expression of IFN-stimulated genes (ISGs) through the engagement of a common receptor and subsequent activation of Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2).

We describe, for the first time, the use and efficacy of ruxolitinib, a selective oral JAK1/2 inhibitor, in 3 children with *TMEM173*-activating mutations over 6 to 18 months of follow-up. The patients, aged between 5 and 12 years, exhibited the phenotypic variability associated with *TMEM173*-activating mutations,[2-4] with lung disease and systemic inflammation being the major features in patient 1 (P1) and patient 3 (P3), while in patient 2 (P2) skin involvement was most prominent (Fig 1; see Supplemental Text and Table E1 in this article's Online Repository at www.jacionline.org). There was minimal response to a broad spectrum of immunosuppressive therapies including steroids, methotrexate, and anti-CD20 mAbs.

An increased expression of ISGs, a so-called type I IFN signature,[5] was observed in all 3 patients (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Increased levels of STAT1 phosphorylation were recorded in patients' T lymphocytes (P1, P2, and P3), T-cultured lymphoblasts (P1), and primary fibroblasts (P3) compared with controls (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org). Liu et al[2] demonstrated that, *in vitro*, 3 JAK1 inhibitors (ruxolitinib, tofacitinib, and baricitinib) were able to block the constitutive phosphorylation of signal transducer and activator of transcription 1 (STAT1) in lymphocytes from *TMEM173*-mutated patients, and we saw that exposure to ruxolitinib inhibited the constitutive phosphorylation of STAT1 and decreased the expression of IL-6 and 3 ISGs tested in T lymphoblasts from P1 (see Fig E2, *B* and *C*). Considering the severity of the phenotype and the poor response to conventional immunosuppressive therapies, we hypothesized that JAK1 inhibition would block IFN signaling in the context of activating mutations in *TMEM173*.

We observed a marked positive effect on all aspects of the phenotype in all 3 treated children. There was a general improvement in patient-reported well-being, a reduction in febrile episodes, an almost complete resolution of the associated cutaneous lesions, and a major improvement in pulmonary function (Fig 1 and Fig 2, *A* and *B*; also see Supplemental Text, Figs E3 and E4, and Tables E1-E4 in this article's Online Repository at www.jacionline.org). Concordant with these clinical observations, it was possible to taper, and then stop, steroid treatment in all 3 children. Ruxolitinib concentration was assessed during follow-up and showed peak levels consistent with published pharmacokinetic data (see Tables E5 and E6 in this article's Online Repository at www.jacionline.org).[6]

Ex vivo experimental data mirrored the favorable clinical effect that we observed. Treatment resulted in a trend to reduction of the IFN score in P1 and P3, whereas there was no significant change in the expression of ISGs in P2 (see Fig E1, *B*). Pretreatment transcriptomic analysis of whole blood showed differential expression of 119 genes as compared with healthy controls, including 35 upregulated ISGs ($P < .05$ and $Q < .25$; see Fig E5, *A* and *B*, in this article's Online Repository at www.jacionline.org). Among these 119 genes, the expression levels of 20 previously upregulated genes decreased significantly after treatment ($P < .05$; Fig E5, *C*). This list included genes associated with fever (*IRAK-2*, *IL18-RAP*, and *NFκB1*) and vasculopathy (*ICAM1* and *NOTCH1*).[2] In *ex vivo* flow cytometry assays, we collected blood from P1, P2, and P3 just before the morning drug intake (H0 equals 12 hours after last dose) and 4 hours after dosing (H4). At H0, when ruxolitinib concentration was minimally raised (see Table E6), STAT1 phosphorylation in T lymphocytes from patients was higher than in a healthy control. In contrast, at H4, T-lymphocyte STAT1 phosphorylation decreased in all patients (see Fig E6 in this article's Online Repository at www.jacionline.org). STAT1 phosphorylation dynamics were further explored by *ex vivo*

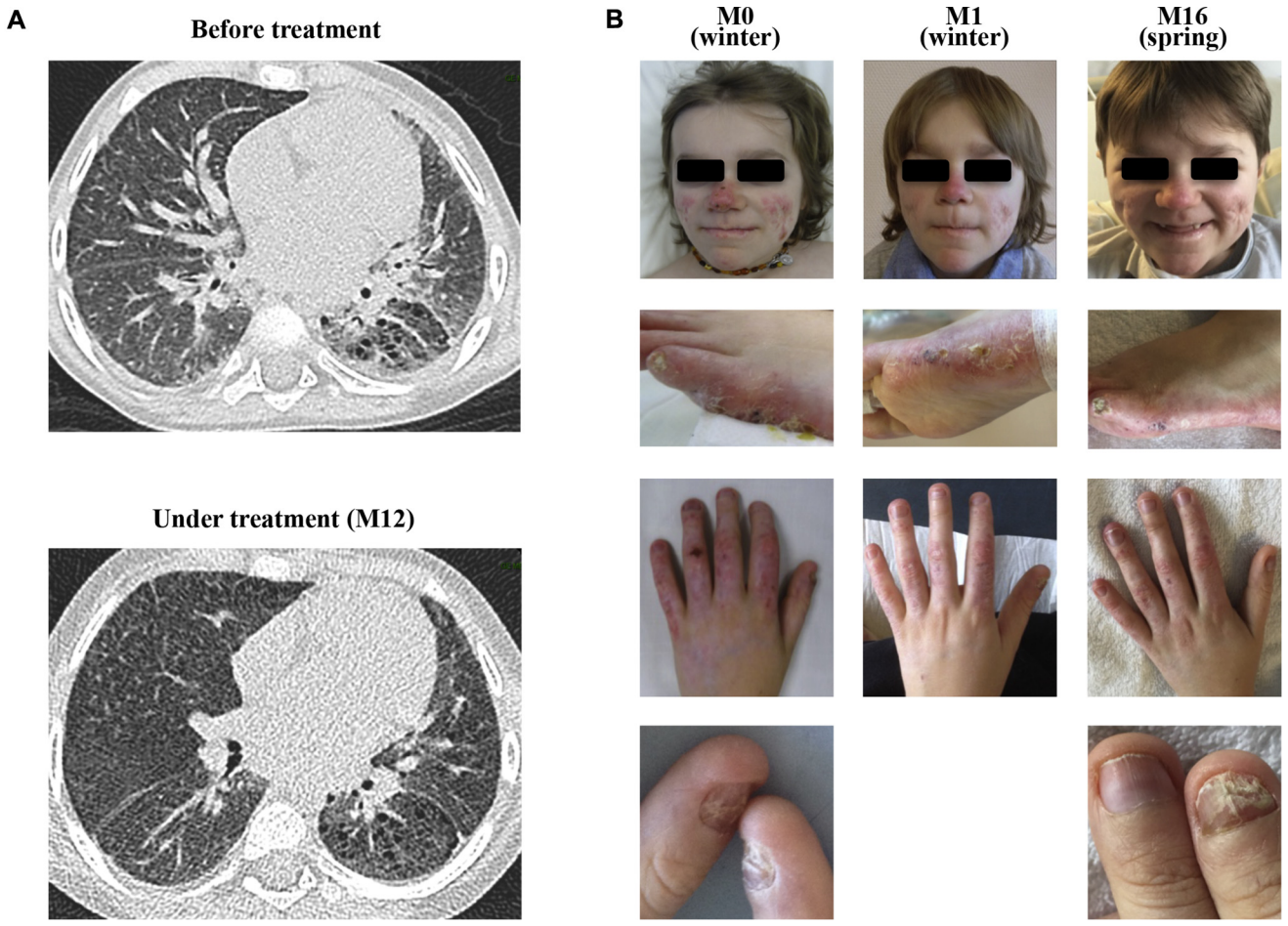

FIG 1. Clinical response of patients with *TMEM173* mutations to treatment with ruxolitinib. **A,** High-resolution chest computed tomography of P1 demonstrating improvement in ground-glass lesions and stabilization of fibrosis after 12 months (M12) of treatment with ruxolitinib. **B,** Cutaneous involvement observed in P2 before (M0) and after 1 (M1) and 16 (M16) months of treatment with ruxolitinib. Healing of the cheek and nose ulcerations was noticed within a month of the initiation of ruxolitinib. Ulcerations of the digits, erythematous scaling plaques of the feet, and nail dystrophy improved after 16 months of treatment. These improvements were not related to season (where such lesions are exacerbated by cold and thus are more prominent in the winter and spring months).

kinetic phosphorylation assays in P2. STAT1 phosphorylation in T lymphocytes and neutrophils from P2 began to decrease at H2, was at the lowest level at H4, increased again at H6, and was at its highest at H10 (Fig 2, *C*; see Fig E7, *A*, in this article's Online Repository at www.jacionline.org). Interestingly, in monocytes from P2, STAT1 phosphorylation showed a similar pattern, but was at its highest level at H8 (Fig 2, *C*). These dynamic changes were also observed in a further child with a *TMEM173*-activating mutation in whom ruxolitinib was recently initiated (see the Methods section and Fig E7, *B* and *C*, in this article's Online Repository at www.jacionline.org).

Ruxolitinib was well tolerated, particularly considering the hematological and infectious side effects described in the treatment of myelofibrosis.[7,8] Of importance, we observed no increased incidence of infection in any of the 3 treated children. In 1 patient with prominent stigmata of systemic inflammation (P1), an initial improvement was followed by a relapse when treatment was temporarily stopped (Fig 2, *A*, and see Fig E4, *A*, *C*, and *D*). The resolution of these features following reinstatement of the drug further indicates a causal relationship between ruxolitinib administration and the observed improvement. We note that a possible explanation for the intensity of the relapse could relate to a cytokine rebound effect, indicating the need for careful monitoring in the case of treatment interruption. Papillary edema secondary to intracranial hypertension was observed in 1 patient. It is unclear whether this, previously unreported, feature should be considered as a side effect of JAK inhibition. However, this observation indicates the need for careful surveillance fundoscopy in treated patients.

Despite marked clinical improvement, incomplete inhibition of type I IFN signaling likely accounts for the variable reduction in ISG expression and the modest fold changes in expression observed across a larger number of immune-related genes with ruxolitinib treatment. Such a possibility might explain the absence of an increased rate of infection in the treated patients, and also suggests the possibility for increased dosing according to clinical response. Modest and incomplete downregulation of ISG was recently described in splenic B cells of mice treated with tofacitinib, a JAK1/3 inhibitor, with differential signaling effects suggesting currently poorly understood facets of IFN regulation.[9]

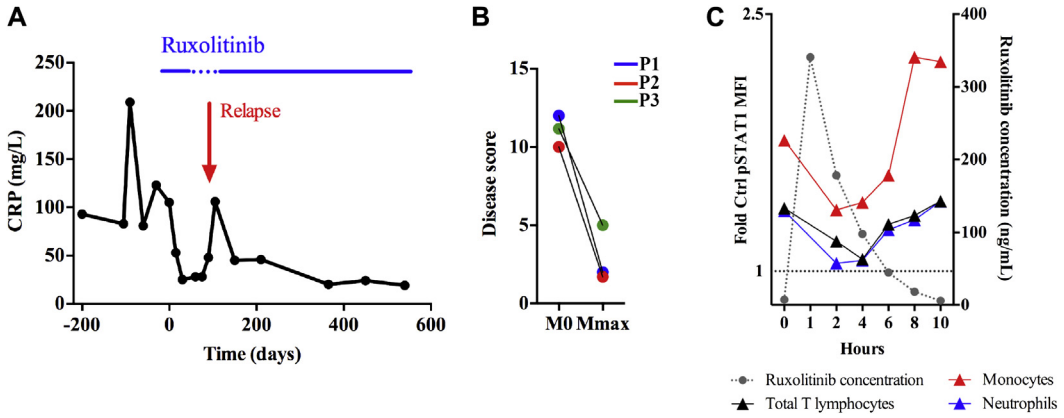

FIG 2. C-reactive protein (CRP) levels of P1, disease scores of patients under treatment, and *ex vivo* effect of ruxolitinib. **A,** CRP values (normal <2 mg/L, to convert to nmol/L, multiply by 9.524) of P1 are shown before and after treatment with ruxolitinib. Levels of CRP with treatment were found to be significantly decreased compared with levels without treatment (***$P < .001$, *t* test, and see Fig E4, *F*). The withdrawal of ruxolitinib, depicted by a dotted line, led to a clinical and biological relapse. **B,** Disease scores at screening (M0) and at maximal follow-up (Mmax) (see the Methods section). Disease scores were decreased with treatment in all 3 patients (see Tables E2, E3, and E4 and Fig E4, *C*). **C,** PBMCs were obtained from P2 before treatment (H0) and 2, 4, 6, 8, and 10 hours after treatment intake and ruxolitinib concentrations were measured simultaneously. As the treatment is taken twice daily, H0 is at 12 hours after the last dose and immediately before the next dose. Blood from the same healthy control was collected at each time point. STAT1 constitutive phosphorylation quantified in relative mean fluorescent intensity (MFI) in $CD3^+$ lymphocytes and neutrophils began to decrease at H2, was at the lowest level at H4, increased again from H6, and was highest at H10. STAT1 phosphorylation in monocytes showed a comparable pattern but was at maximum at H8. Similar results were observed in CD4+ and CD8+ lymphocytes (see Fig E7, *A*).

In this regard, our kinetic *ex vivo* experiments provide insights into the rapid dynamic changes in IFN signaling secondary to JAK1 blockade.

Overall, our findings suggest that JAK inhibition represents a highly promising and well-tolerated therapeutic approach to the multisystem sterile inflammation associated with *TMEM173*-activating mutations, warranting further long-term assessment. Specific inhibitors of JAK1 might be particularly attractive in this context. The potential for irreversible lung damage in STING-related disease indicates that early treatment should be considered to avoid progression to pulmonary failure. Considering the recognized phenotypic and pathophysiological overlap, treatment by JAK inhibition may also be relevant to other monogenic type I interferonopathies,[1] and the still wider spectrum of diseases associated with an activation of type I IFN such as subsets of systemic lupus erythematosus and dermatomyositis.[10]

For detailed methods, please see the Methods section in the Online Repository.

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