INTERNAL MEDICINE

□ CASE REPORT □

The Amelioration of Myelofibrosis with Thrombocytopenia by a JAK1/2 Inhibitor, Ruxolitinib, in a Post-polycythemia Vera Myelofibrosis Patient with a *JAK2* Exon 12 Mutation

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Abstract

Less than 5% of patients with polycythemia vera (PV) show *JAK2* exon 12 mutations. Although PV patients with *JAK2* exon 12 mutations are known to develop post-PV myelofibrosis (MF) as well as PV with *JAK2*V617F, the role of JAK inhibitors in post-PV MF patients with *JAK2* exon 12 mutations remains unknown. We describe how treatment with a JAK1/2 inhibitor, ruxolitinib, led to the rapid amelioration of marrow fibrosis, erythrocytosis and thrombocytopenia in a 77-year-old man with post-PV MF who carried a *JAK2* exon 12 mutation (*JAK2*H538QK539L). This case suggests that ruxolitinib is a treatment option for post-PV MF in patients with thrombocytopenia or *JAK2* exon 12 mutations.

Key words: myelofibrosis, polycythemia vera, thrombocytopenia, ruxolitinib, *JAK2* exon 12 mutation

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Introduction

Myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), are characterized by the proliferation of mature blood cells and extramedullary hematopoiesis (1). In addition to PMF, secondary myelofibrosis (MF) occasionally arises from PV (post-PV MF) and ET. Mutations in *JAK2* (*Janus Kinase 2*), *MPL* (*MPL proto-oncogene, thrombopoietin receptor*), and *CALR* (*calreticulin*) activate the JAK-STAT (signal transducer and activator of transcription) signaling pathway, which drives proliferative hematopoiesis in the MPNs (2). *JAK2*V617F is found in more than 90% of PV patients and approximately half of ET and PMF patients (3-6), whereas *JAK2* exon 12 mutations are rarely - but almost exclusively - detected in PV (7, 8). MPNs also show mutations in epigenetic modifiers including *DNMT3A* (*DNA Methyltransferase 3a*), *TET2* (*Tet Methylcytosine Dioxygenase 2*), *ASXL1* (*Additional Sex Combs Like 1*), and *EZH2* (*Enhancer of Zeste Homolog 2*) (9). These epigenetic-related mutations are detected in MF more frequently than other types of MPNs and may contribute to the disease progression and shorter survival of MF patients (10-12).

PMF is well known to have a poor prognosis (12, 13). Recently, patients with PV and ET have also been reported to have a reduced life expectancy (14, 15). The disease-related complications of PV that may reduce survival rates include thrombosis, hemorrhagic events, and evolution to acute leukemia or post-PV MF (16). The cumulative incidence of MF evolution at 15 years after the diagnosis of PV has been reported to approximately 6-14%, and median survival term of patients with post-PV MF is less than 6 years. Recently, Passamonti et al. (17) demonstrated that the rates of MF evolution and survival in PV patients with *JAK2* exon 12 mutations were similar to those with *JAK2*V617F; however, these two mutational subtypes show distinct clini-

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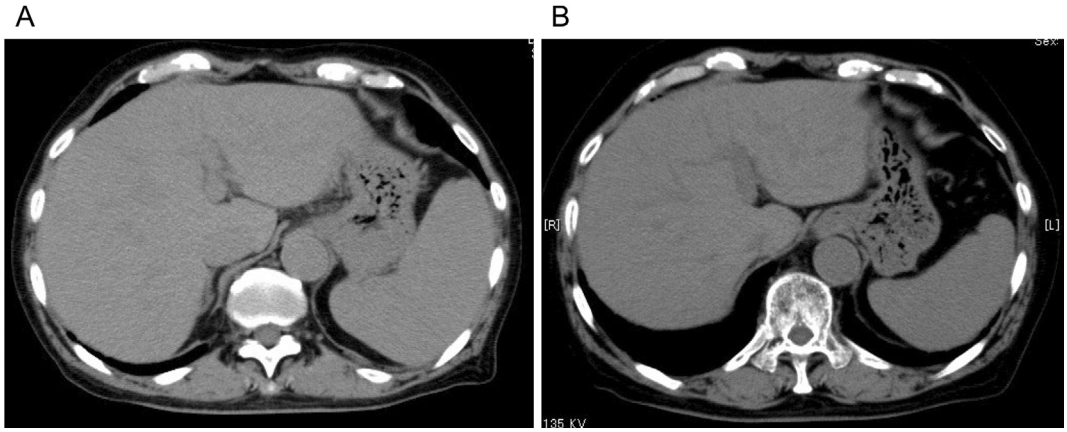

Figure 1. Computed tomography (CT). CT at diagnosis (A) and at 8 months after the initiation of treatment with ruxolitinib (B).

cal phenotypes at the clinical onset (7, 8). PV patients with *JAK2*V617F show leukocytosis and thrombocytosis as well as erythrocytosis, while PV patients carrying *JAK2* exon 12 mutations mainly show isolated erythrocytosis. Thus, PV patients with *JAK2* exon 12 mutations or *JAK2*V617F should receive meticulous care in terms of disease-related complications, including post-PV MF.

Ruxolitinib is an oral JAK1/2 inhibitor that targets the JAK-STAT signaling pathway. The Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment (COMFORT)-I and COMFORT-II studies first demonstrated the rapid and durable reduction of splenomegaly and disease-related symptoms in patients with intermediate-2 or high-risk MF in comparison to patients who received placebo and the best available therapy (18, 19). Ruxolitinib was also superior to the best available therapy in controlling the hematocrit levels in PV patients who could not tolerate or who showed an insufficient response to hydroxyurea (20). Despite being an unintended endpoint, the further analysis of the COMFORT data suggested that ruxolitinib improved the overall survival (21, 22) and reduced the allele burden of *JAK2*V617F (23) in MF. However, reports showing the histological improvement of bone marrow (BM) fibrosis following ruxiolitinib treatment are extremely rare (24-26). Thus far, the role of ruxolitinib in PV or post-PV MF in patients with *JAK2* exon 12 mutations is almost unknown.

We herein describe a case in which ruxolitinib treatment led to a reduction of BM fibrosis with improvements in thrombocytopenia and erythrocytosis in a patient with post-PV MF who carried a *JAK2* exon 12 mutation.

Case Report

A 77-year-old Japanese man was referred to us because of erythrocytosis and thrombocytopenia with fatigue, weight loss (3 kg over 6 months), and splenomegaly (Fig. 1A). Laboratory tests showed peripheral erythrocytosis with 6.75 $\times 10^{12}$/L erythrocytes, 18.8 g/dL hemoglobin, and 56.8% hematocrit; thrombocytopenia with 81×10^{9}/L platelets; elevated serum LDH at 347 U/L [reference interval (RI) ≤226]; and decreased plasma erythropoietin with 1.4 mIU/mL (RI: 4.2-23.7). Although the patient's leukocyte count was normal (4.9×10^{9}/L), metamyelocytes were present in the peripheral blood; myeloblasts and erythroblasts were not detected. A BM biopsy demonstrated hypercellularity with trilineage growth and reticulin fibrosis (Fig. 2A). No chromosomal abnormalities were found in the BM cells. Mutational assays (27-29) did not detect *JAK2*V617F, *MPL*W515K/L, or *CALR* exon 9 mutations in the peripheral leukocytes. However, the patient was diagnosed with post-PV MF based on the detection of endogenous erythroid colony (EEC) formation and a known *JAK2* exon 12 mutation [*JAK2*H538QK539L (8)] (Fig. 3), along with erythrocytosis, a decreased erythropoietin level (1.4 mIU/mL; RI, 4.2-23.7), and BM hypercellularity. The previous data that were available also showed that he first developed erythrocytosis and thrombocytopenia at 2 years before his diagnosis (Fig. 4). Ruxolitinib (5 mg, twice daily) was administered for the treatment of post-PV MF; this provided rapid histological (Fig. 2B) and hematological (Fig. 4) responses with an increase in platelets and a reduction of erythrocytosis. Moreover, the patient's splenomegaly (Fig. 1B) improved, and his body weight recovered to baseline. These responses are ongoing at 16 months since the initiation of treatment.

Histopathological findings

Before treatment, hypercellularity and MF-2 grade reticulin fibrosis (30) was seen throughout the BM (Fig. 2A). A BM biopsy performed at 10 months after the initiation of treatment showed a reduction in the cellularity and MF-0 to MF-1 grade fibrosis in approximately 70% of the BM, whereas 30% remained hypercellular with MF-2 grade fibrosis (Fig. 2B).

Target sequencing

The target sequencing of the Human Myeloid Neoplasms Panel (Qiagen, Hilden, Germany; Catalog No. NGHS-003X) was performed using a next-generation sequencer (MiSeq;

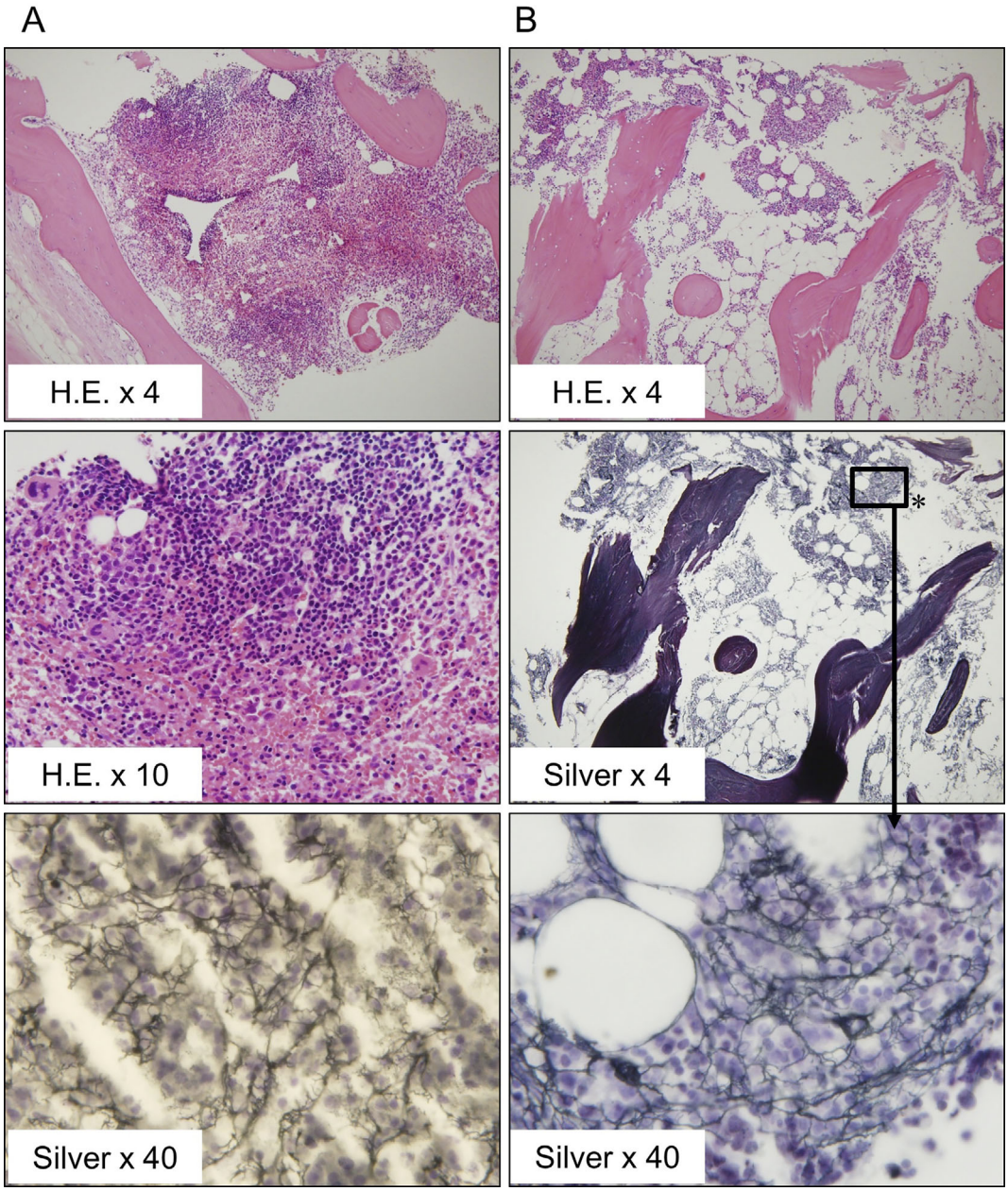

Figure 2. **The histopathological findings of the bone marrow specimen. (A) Diffuse trilineage proliferation with MF-2 fibrosis was present in whole marrow upon the diagnosis of post-PV MF. (B) Hypercellularity with fibrosis was significantly reduced and still partly present (*) at 8 months after starting the initiation of ruxolitinib treatment. Hematoxylin and Eosin staining and Gomori's silver impregnation (silver) are shown.**

Illumina, San Diego, CA, USA). This identified a known somatic mutation, *DNMT3A*R882C (31), in addition to *JAK2*H538QK539L in the peripheral leukocytes. While the allele burden of *JAK2*H538QK539L showed a slight reduction (56.3% to 48.5%) at 10 months after the initiation of treatment, that of *DNMT3A*R882C barely changed (30.5% to 29.9%).

Discussion

In the present case, treatment with ruxolitinib resulted in a major improvement of thrombocytopenia and erythrocytosis and a reduction of fibrosis in a patient with post-PV MF associated with *JAK2* exon 12 mutation. In a phase 2 clinical trial for thrombocytopenic MF with a platelet count of 50-100×10^9/L, 7 of 50 patients showed increased platelet counts ≥15×10^9/L (in comparison to baseline) at week 24 (32). Younger age, a recent diagnosis, a low-risk classification in the dynamic international prognostic scoring system, primary disease (PMF), and low neutrophil count were associated with platelet count increases; the report did not mention the *JAK2* mutational status. The characteristics of our patient might have been different because the low neutrophil count was the only comparable variable. Recently,

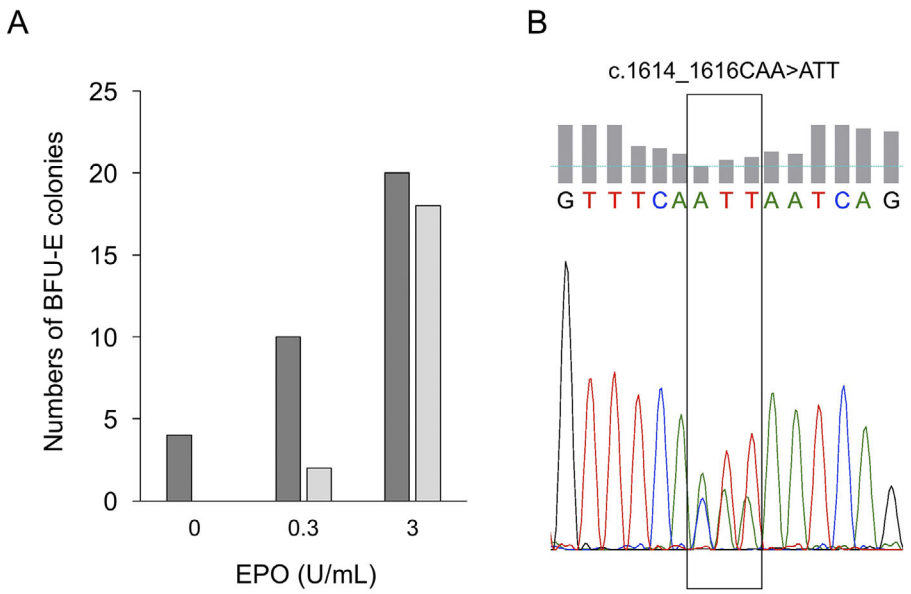

Figure 3. **Evidence of underlying PV. (A) Burst-forming unit erythroid (BFU-E) colonies grown from 1×10^5 peripheral blood mononuclear cells (PBMNCs) with the indicated concentrations of erythropoietin (EPO) were counted using an inverted microscope. Some BFU-E colonies were seen in this case (dark gray) in the absence (0 U/mL) or presence of low-concentration EPO (0.3 U/mL). In contrast, in the PBMNCs of 2 healthy controls, BFU-E colonies barely grew at 0 and 0.3 U/mL EPO (light gray). The mean colony numbers of 2 plates are shown. (B) The Sanger sequence indicated substitutions of nucleotides that corresponded to *JAK2*H538QK539L.**

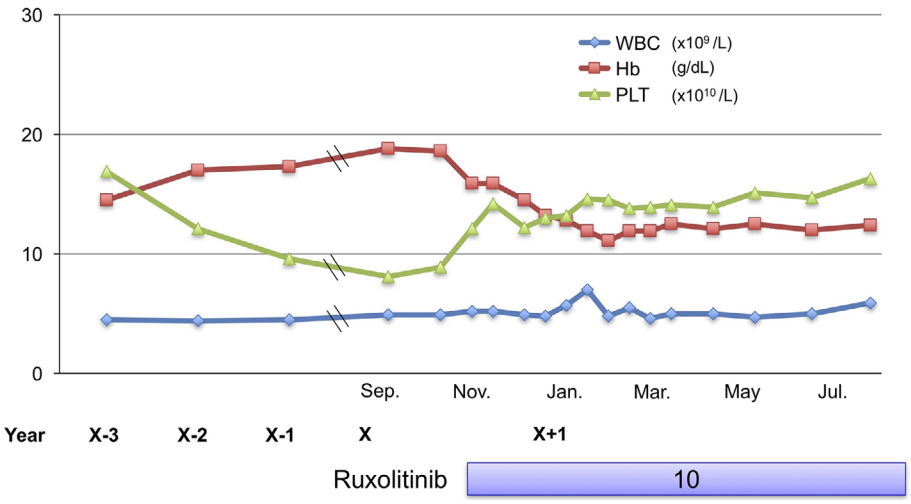

Figure 4. **The clinical course. At 3 years before the diagnosis of post-PV MF (year X-3), the patient's peripheral blood cell count was normal. This patient had already developed erythrocytosis and thrombocytopenia at 2 years before the diagnosis (year X-2). In year X, after starting ruxolitinib treatment, his hemoglobin level (Hb) and platelet count (PLT) rapidly normalized; this effect has persisted for 16 months since the initiation of treatment. The patient's white blood cell count (WBC) has been stable.**

platelet increases have also been reported in two patients with thrombocytopenic post-PV MF with *JAK2*V617F (33). These studies and our present case indicate that ruxolitinib is a treatment option for thrombopenic post-PV MF regardless of the *JAK2* mutation type.

The mechanisms by which ruxolitinib increases the platelet count in patients with thrombocytopenic MF remain unclear; however, the reduction in splenomegaly, the improvement in the BM microenvironment through decreased inflammatory cytokine production and the preferential suppression of the neoplastic clones have been suggested as possible causes (33). In our present patient, we observed a reduction in the size of the spleen (Fig. 1), which is a major effect of ruxolitinib in many cases (18, 19). A partial, but

significant amelioration of fibrosis was also observed (Fig. 2), which is a rare effect of ruxolitinib (24-26). The recovery of producible thrombopoiesis thanks to the amelioration of fibrosis possibly contributed to the increase in his platelet count. In the present case, it is unclear whether ruxolitinib improved the BM microenvironment or eliminated a neoplastic clone in our case. However, the environmental improvement is likely to be more important than the elimination of a neoplastic clone, because his disease-related symptoms, which were probably due to inflammatory cytokines (34), disappeared with ruxolitinib. In contrast, only a slight reduction was seen in the allele burden of the mutant *JAK2* exon 12. However, the long-term follow-up of COMFORT-I recently revealed major molecular responses determined by the allele burden of *JAK2*V617F in some MF patients (23). Thus, the gradual reduction in the allele burden of the mutant *JAK2* exon 12 may have also be important for a durable effect of ruxolitinib in the future care of our patient. At this point, the mutant *DNMT3A* remains at a very stable allele burden relative to the mutant *JAK2* exon 12. This is probably consistent with a finding that *DNMT3A*, *ASXL1*, and *EZH2* mutations were correlated with poor responses to ruxolitinib in MF (35). In the present case, the changes in the allele burdens of the mutants suggest that ruxolitinib can slightly decrease the numbers of clones that carry a *JAK2* exon 12 mutation alone, but not clones that carry both *JAK2* exon 12 and *DNMT3A* mutations or *DNMT3A* mutations alone. Our patient presented with thrombocytopenia when he first showed erythrocytosis. In addition, MF-2 fibrosis was found at only two years after the development of erythrocytosis; however, a cohort study indicated that MF occurred at least 20 years after the onset of PV in most patients with *JAK2* exon 12 mutations (17). Thus, it is difficult to exclude PMF in our present patient; however, we are of the opinion that it represents a case of post-PV MF because EEC formation and *JAK2* exon 12 mutations are usually exclusive to PV.

It has been reported that older age, leukocytosis, splenomegaly, thrombocytosis, a masked-PV phenotype (PV characteristics with lower hemoglobin levels than criteria targets), a high *JAK2*V617F allele burden, and chromosome 12 abnormalities are associated with the evolution of MF in PV (16, 36). Among these factors, only old age and splenomegaly fit our case, and the cause of his MF evolution remains unknown. *HMGA2* is located on chromosome 12; thus, since our previous study (29) and the studies of other authors (37-39) have reported that *HMGA2* is highly expressed in the hematopoietic cells from the vast majority of MF patients, we measured the *HMGA2* mRNA level in his granulocytes. The expression of *HMGA2* mRNA in his granulocytes at the time of the diagnosis was 3-fold higher than that in healthy controls, as well as the MF patients in our previous study (29). However, it is unclear whether *HMGA2* contributes directly to the evolution of MF, because the overexpression of *HMGA2* causes MPN-like hematopoiesis without MF in mouse models (40, 41).

To our knowledge, this is the first reported case in which post-PV MF was ameliorated by ruxolitinib leading to a resolution of thrombocytopenia in a patient with a *JAK2* exon 12 mutation. The mechanisms underlying the improvement of both MF and thrombocytopenia should be studied further; however, we should at least consider the use of ruxolitinib in the treatment of thrombocytopenic MF, including post-PV MF in patients with *JAK2* exon 12 mutations.

The authors state that they have no Conflict of Interest (COI).

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