Use of the JAK1/JAK2 inhibitor ruxolitinib in the treatment of patients with myelofibrosis

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Myelofibrosis (MF), including primary MF and MF secondary to polycythemia vera or essential thrombocytopenia, is a chronic, clinically heterogeneous hematologic malignancy characterized by inefficient hematopoiesis, bone marrow fibrosis, and shortened survival. Typical clinical manifestations include progressive splenomegaly, debilitating symptoms, and anemia. MF is associated with dysregulation of Janus kinase (JAK)-signal transducer and activator of transcription (JAK/STAT) pathway affecting hematopoiesis and inflammation. Ruxolitinib, an oral JAK1/JAK2 inhibitor, was approved for the treatment of patients with intermediate or high-risk MF based on the results of 2 phase 3 studies [Controlled MyeloFibrosis Study with Oral JAK Inhibitor Treatment [COMFORT]-I and COMFORT-II]. In these trials, ruxolitinib treatment was associated with reductions in spleen size and symptom burden, and improvements in quality of life. The most common adverse events were dose-dependent cytopenias, which were managed by dose modifications, treatment interruptions, and red blood cell transfusions (for anemia). Ruxolitinib was effective regardless of MF type, risk status, or JAK2V617F mutation status, and across various other MF subpopulations. Two-year follow-up data from the COMFORT trials also demonstrate that ruxolitinib has durable efficacy and may be associated with a survival advantage relative to placebo and best available therapy. Preliminary data from ongoing studies support possible dosing strategies for patients with low platelet counts.

Myelofibrosis (MF) is a Philadelphia chromosome-negative myeloproliferative neoplasm (MPN) that affects primarily older patients and is characterized by progressive bone marrow fibrosis, inefficient hematopoiesis, and shortened survival. MF may occur as a primary disorder (PMF) or secondary to polycythemia vera (PV) or essential thrombocythemia (ET).1 The clinical and laboratory features of primary, post-PV, and post-ET MF are often indistinguishable, with shared cytogenetic abnormalities and an increased risk of transformation to secondary acute myeloid leukemia (AML).1 Typical clinical manifestations of MF include splenomegaly, anemia, and debilitating symptoms.1 Most patients die of complications or disease progression without leukemic transformation.2

Ruxolitinib, an oral Janus kinase 1 (JAK) 1 and JAK2 inhibitor, is the first and only therapy approved to date for patients with intermediate- or high-risk MF,3 based on the results of 2 randomized controlled phase 3 trials.4,5 Here, we review currently available evidence of the clinical benefits of ruxolitinib in patients with MF, including recent data on the use of ruxolitinib in various patient subpopulations.

Clinical phenotype and natural history of MF

MF typically is associated with leukoerythroblastosis and, as the disease progresses, with increasingly ineffective hematopoiesis and consequent extramedullary hematopoiesis, which may lead to potentially massive and painful organ enlargement.1,6 Splenomegaly, a common manifestation of MF, is often symptomatic (pain under the left ribs, early satiety, and/or abdominal discomfort), and may result in severe complica-
tions such as portal hypertension and splenic infarcts. As a result of inefficient hematopoiesis, progressive anemia may affect one-third or more of patients with MF, and often leads to transfusion dependence.

High circulating levels of inflammatory cytokines in patients with MF are believed to contribute to debilitating symptoms, such as fever, night sweats, fatigue, bone and joint pain, and pruritus. These symptoms may greatly impair a patient’s quality of life (QoL) and prognosis. Progression of MF may ultimately result in bone marrow failure, secondary AML, or potentially life-threatening complications such as thrombosis and hemorrhage.

Life expectancy in patients with MF varies depending on the presence of risk factors, including age (> 65 years), hemoglobin level (< 10 g/dL), leukocyte count (> 25 × 10⁹/L), circulating blasts (≥ 1%), presence of constitutional symptoms, platelet count (< 100 × 10⁹/L), transfusion dependence, and unfavorable karyotype. Estimated median survival times range from 11-15 years for low-risk patients to around 2 years for high-risk patients.

Pathogenesis of MPNs and MF

Two major features of MF place aberrant JAK-signal transducer and activator of transcription (STAT) signaling at the core of disease pathobiology: clonal expansion of hematopoietic progenitors associated with dysregulated JAK2-STAT signaling and a pro-inflammatory state likely involving aberrant JAK1-STAT and JAK2-STAT signaling (Figure 1).

Dysregulated JAK2-STAT signaling in hematopoietic stem cells is the result of somatic mutations in JAK2 or regulators of JAK2-STAT signaling, including epigenetic modifiers. JAK2 is a cytoplasmic tyrosine kinase required for normal hematopoiesis, and it is activated through association with type I cytokine receptors, such as the receptors for erythropoietin, thrombopoietin, or granulocyte colony-stimulating factor. JAK2 mutations involved in the pathogenesis of MPNs result in constitutive activation of JAK2, leading to increased proliferation and resistance to apoptosis of mutant cells. JAK2V617F, the most common mutation in MPNs, affects > 95% of patients with PV and approximately 50%-60% of patients with ET or PMF. JAK2V617F is a point mutation in the regulatory (pseudo kinase or JH2) domain of JAK2 that leads to constitutive JAK2 kinase activity (residing in the catalytic JH1 domain) in the absence of upstream cognate receptor stimulation. However, the role of JAK2V617F in MPNs is unlike that of the disease-determining Philadelphia chromosome in chronic myelogenous leukemia (CML). Whereas the BCR-ABL genetic fusion resulting from the Philadelphia chromosome is sufficient to cause chronic phase CML, JAK2V617F is not the disease-initiating event in MPNs, and it is neither necessary nor specific for MPN or MF phenotype. Other mutations leading directly or indirectly to dysregulated JAK2-STAT signaling have been identified in patients with MPNs, and variable combinations of genetic and epigenetic factors likely account for the considerable heterogeneity of MF phenotypes.

The relationship between mutant allele burden and clinical phenotype is indirect, complex, and incompletely understood. A higher JAK2V617F mutant allele burden has been associated with a higher rate of pruritus and fibrotic transformation in patients with PV. Patients with post-PV MF appear to have the highest allele burden compared with ET, PV, and PMF. However, allele burden or even the presence of JAK2V617F has not been reliably associated with prognosis or transformation to AML. Furthermore, whereas signaling patterns in patients with ET, PV, or MF were correlated with clinical phenotype, JAK2 genotype and mutant allele burden were not.

In addition to its role in aberrant myeloproliferation, dysregulated JAK-STAT signaling, likely involving both JAK1 and JAK2, has a broader role in the pathophysiology of MF by facilitating aberrant production of cytokines, including pro-inflammatory and pro-fibrotic cytokines. Increased cytokine levels have been associated with marked splenomegaly, transfusion dependency, thrombocytopenia, and shortened survival. The interconnectedness of JAK1 and JAK2 in their respective signaling cascades, and their involvement in a globally dysregulated, malicious network of cytokine overproduction driving multiple disease manifestations, provide a rationale for the concurrent inhibition of JAK1 and JAK2 in MF and MPNs. This concept is further supported by evidence suggesting that cytokine-mediated cross-talk between neoplastic and stromal cells may protect JAK2 mutant neoplastic cells against pure JAK2 inhibitors.

Clinical effects of ruxolitinib

Ruxolitinib, which was recently approved in the United States for the treatment of patients with intermediate- or high-risk MF, is a potent JAK1/JAK2 inhibitor with similar in vitro activity against isolated kinase domains of JAK1 (concentration at half-maximal inhibition [IC₅₀] = 3.3 nM) and JAK2 (IC₅₀ = 2.8 nM). The clinical efficacy and safety of ruxolitinib were evaluated in a phase 1/2 dose-finding study and 2 randomized controlled phase 3 trials: the Controlled Myelofibrosis Study with Oral JAK1/JAK2 Inhibitor Treatment (COMFORT)-I against placebo and
COMFORT-II against what then was considered best available therapy.5

Reduction in splenomegaly and symptom burden
In a phase 1/2 trial of ruxolitinib in 153 patients with MF, 44% of those with an enlarged spleen at baseline experienced a ≥ 50% reduction in palpable spleen length within the first 3 months of therapy. In addition, the majority of patients experienced improvement of MF-related symptoms.9

In the placebo-controlled COMFORT-I study of 309 patients with intermediate-2 or high-risk MF, 41.9% of patients receiving ruxolitinib (n = 155) compared with 0.7% in the placebo group (n = 154) achieved the primary endpoint of a ≥ 35% reduction in spleen volume at week 24 as assessed by abdominal imaging (P < .001). Most patients treated with ruxolitinib had some reduction in spleen volume (Figure 2A).4 Longer-term data for the ruxolitinib group, with 100 patients still on therapy after a median follow-up of 102 weeks, showed that a reduction in spleen size was maintained: mean changes from baseline in spleen volume ranged from −31.6% at weeks 24 and 48 to −34.9% at week 96.23

At week 24, ruxolitinib also was associated with a 46.1% mean reduction in Total Symptom Score (TSS, assessed using the modified Myelofibrosis Symptom Assessment Form [MFSAF] version 2.0) compared with a 41.8% mean increase in TSS in the placebo group (P < .001). Most patients treated with ruxolitinib had improvement in TSS (Figure 2B).4 These symptom improvements, which included abdominal symptoms (abdominal discomfort, pain under the left ribs, and early satiety), non-abdominal symptoms (night sweats, itching, and bone/muscle pain), and inactivity, were accompanied by rapid improvements in QoL measures, including the European Organization for Research and Treatment of Cancers (EORTC) QoL Questionnaire—Core 30 (QLQ-C30) subscales for global health status/QoL and physical, role, social, and emotional functioning, and by substantial reversal of signs of cachexia (with documented weight gain).4,24 Clinically meaningful improvements in EORTC QLQ-C30 global health status/QoL were maintained with longer-term therapy.23 Even patients who achieved <10% reduction in spleen volume with ruxolitinib therapy experienced signifi-

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**FIGURE 1** Dysregulation of JAK-STAT signaling in MF. A, Normal JAK-STAT signaling is mediated by circulating levels of cytokines and growth factors, including EPO, TPO, and G-CSF, which are essential for normal hematopoiesis. B, Overactive JAK-STAT signaling in MF is associated with malignant growth of hematopoietic clones and a pro-inflammatory state caused by overproduction of circulating cytokines. The JAK2V617F mutation, which is present in 50% to 60% of patients with primary or post-ET MF, may cause constitutive JAK2 activation in the absence of ligand-receptor binding. In addition, overactive JAK-STAT signaling may result from a number of other genetic and epigenetic abnormalities. Abbreviations: EPO, erythropoietin; ET, essential thrombocythemia; G-CSF, granulocyte colony-stimulating factor; JAK, Janus kinase; MF, myelofibrosis; STAT, signal transducer and activator of transcription; TPO, thrombopoietin.
cant improvements in TSS and perceived change in their condition compared with patients who received placebo. \(^{24}\) Post hoc analyses showed that ruxolitinib was effective in reducing spleen size and symptom burden across subgroups defined by age, MF type, \(JAK2\) \(^{V617F}\) mutation status, or various other baseline characteristics (Figure 3).\(^{25}\)

In COMFORT-II, which compared the effects of ruxolitinib versus best available therapy, 28% of patients in the ruxolitinib group achieved the primary endpoint of \(\geq 35\%\) reduction in spleen volume at week 48 compared with no patients in the best available therapy group \((P < .001)\).\(^{5}\) The probability of maintaining a \(\geq 35\%\) reduction in spleen volume was 75% \((95\% \text{ CI}, 61\%-84\%)\) at week 48 and 58% \((95\% \text{ CI}, 35\%-76\%)\) at week 84.\(^{26}\) Patients receiving ruxolitinib had a 56% mean decrease from baseline to week 48 in palpable spleen length, compared with a 4% mean increase with best available therapy. Ruxolitinib therapy also improved MF-associated symptoms (including fatigue, insomnia, appetite loss, and dyspnea) and QoL measures compared with best available therapy (Figure 4).\(^{5}\)

In both COMFORT trials, ruxolitinib was associated with reductions in plasma levels of the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor–alpha and the inflammation marker C-reactive protein (CRP), whereas placebo and best available therapy were associated with no change or increases in corresponding plasma levels.\(^{4,5}\) These findings confirm previous observations from the phase 1/2 study\(^{9}\) and are consistent with the notion that symptom relief and reversal of cachexia-associated weight loss may be associated with the effect of ruxolitinib on MF-associated systemic inflammation.

**Overall survival**

A survival analysis of COMFORT-I at a median follow-up of 51 weeks suggested a survival advantage for patients randomized to ruxolitinib versus placebo \((\text{hazard ratio [HR]}, 0.50; 95\% \text{ CI}, 0.25-0.98; P = .04)\).\(^{4}\) At a median follow-up of 102 weeks, patients randomized to ruxolitinib continued to show a survival advantage over those randomized to placebo \((\text{HR}, 0.58; 95\% \text{ CI}, 0.36-0.95; P = .028)\), although all patients randomized to placebo had by then discontinued or crossed over to ruxolitinib therapy (which was allowed based on prespecified criteria of disease progression).\(^{23}\) In COMFORT-II, ruxolitinib was not associated with a survival advantage relative to best available therapy based on an analysis at a median follow-up of 61 weeks \((\text{HR}, 1.01; 95\% \text{ CI}, 0.32-3.24)\).\(^{5}\) However, recently presented 2-year follow-up data suggest that there may also be a survival advantage for ruxolitinib compared with best available therapy \((\text{median follow-up of 112 weeks; HR, 0.51; 95\% CI, 0.27-0.99; P = .041})\).\(^{26}\)

**Effect of \(JAK2\) \(^{V617}\) status and allele burden on response to ruxolitinib treatment**

COMFORT-I subgroup analyses demonstrated that the efficacy of ruxolitinib in reducing spleen size and symptom burden was independent of \(JAK2\) \(^{V617F}\) mutation status or MF subtype (Figures 3A and 3B). Among \(JAK2\) \(^{V617F}\)-positive patients, those treated with ruxolitinib achieved a mean reduction in spleen volume of 34.6%, whereas those in the placebo group experienced a mean increase of 8.1%.\(^{4}\) The corresponding changes in \(JAK2\) \(^{V617F}\)-negative patients were a 23.8% reduction with ruxolitinib and an 8.4% increase with placebo. Similar to spleen volume, TSS decreased (improved) with ruxolitinib in both \(JAK2\) \(^{V617F}\)-positive and \(JAK2\) \(^{V617F}\)-negative patients \((\text{mean change of } -52.6\% \text{ and } -28.1\%, \text{ respectively})\), but increased with placebo \((\text{mean change of 42.8\% and 37.2\%, respectively})\).\(^{4}\) Overall, these findings clearly
FIGURE 3  A, Percentage of change in spleen volume and B, Total Symptom Score from baseline to week 24 by COMFORT-I patient subgroup. Dashed lines represent the mean percentage of change from baseline for the overall treatment group.

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; Int-2, Intermediate-2; IPSS, International Prognostic Scoring System; MF, myelofibrosis; PET, post-essential thrombocythemia; PMF, primary myelofibrosis; PPV, post-polycythemia vera; SEM, standard error of the mean.

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indicate that the presence of the JAK2V617F mutation is not a prerequisite for ruxolitinib efficacy (or worsening of MF signs and symptoms with placebo). Consistent with these findings, ruxolitinib-mediated reductions in spleen size and MFSAF TSS among patients with PMF or post-ET MF were quantitatively similar to those among patients with post-PV MF, even though PV is an almost exclusively JAK2V617F-driven MPN (Figures 3A and 3B). Similar findings were observed in COMFORT-II.5

Ruxolitinib had only a modest effect on JAK2V617F allele burden. In COMFORT-I,4 there were mean reductions of 10.9% and 21.5% at weeks 24 and 48, respectively, and a median reduction of 7.0% at week 48 in COMFORT-II.27 In contrast, patients in the placebo group of COMFORT-I experienced a mean increase in JAK2V617F allele burden of 6.3% at week 48, and the median change in the allele burden with best available therapy in COMFORT-II was 0%.4,27 These data show that while ruxolitinib affects mutant clonal proliferation, it is not specific for mutant forms of JAK2. However, given the complex involvement of both JAK1 and JAK2 in multiple pathogenic pathways independent of JAK2V617F clonal proliferation, as well as the fact that disease clinical phenotype in MF is driven primarily by secondary non-clonal reactive effects,28 such as a global pro-inflammatory state, insufficient hematopoiesis, and bone marrow fibrosis, it can be concluded that reduction in allele burden is not required for patients to experience treatment benefit with ruxolitinib. It remains to be seen whether this statement holds true for other JAK inhibitors.

Safety and tolerability
The results of COMFORT-I and COMFORT-II suggest that ruxolitinib is generally well tolerated.4,5 Non-hematologic adverse events related to therapy were generally of low grade,4,5 and occurred at a rate similar to placebo.5 However, consistent with the mechanism of action of ruxolitinib, the most common adverse events were dose-dependent anemia and thrombocytopenia. In COMFORT-I, 45.2% of patients in the ruxolitinib group versus 19.2% in the placebo group experienced grade 3 or 4 anemia at the time of the primary analysis; the proportions of patients experiencing grade 3 or 4 thrombocytopenia were 12.9% for ruxolitinib and 1.3% for placebo.5 In COMFORT-II, 42% of patients treated with ruxolitinib and 31% of those who received best available therapy had grade ≥ 3 anemia, and 8% and 7% of patients in the ruxolitinib and best available therapy groups, respectively, had grade ≥ 3 thrombocytopenia.5

During 2 years of follow-up in both trials no additional safety concerns or unexpected long-term toxicities were identified.23,26 Overall, rates of grade 3 and 4 anemia reported for the ruxolitinib arm of COMFORT-I at a median follow-up of 102 weeks were 37.4% and 14.8%, respectively, and rates of grade 3 and 4 thrombocytopenia were 11.0% and 5.2%, respectively.23 Few patients experienced leukemic transformation. Over the entire 2-year follow-up period in COMFORT-I, 2 patients randomized to ruxolitinib and 2 patients randomized to placebo (after crossover to ruxolitinib) acquired secondary AML.4,23 In COMFORT-II, 2 cases of leukemic transformation in patients randomized to best available therapy were reported in the primary analysis.5

Management of cytopenias and dose adjustments
Starting doses of ruxolitinib in both COMFORT trials depended on the platelet count at baseline (ie, patients received 15 and 20 mg twice daily [BID] if counts were 100 to 200 × 10^9/L and >200 × 10^9/L, respectively).4,5 In both trials, anemia and thrombocytopenia generally were managed successfully by dose modifications, treat-
ment interruptions, and red blood cell (RBC) transfusions (for anemia), rarely leading to treatment discontinuation. Among patients randomized to ruxolitinib (in either trial), only 1 patient discontinued treatment because of anemia (in COMFORT-I) and only 2 patients (1 in each trial) discontinued treatment because of thrombocytopenia at the time of the primary analysis.4,5 In COMFORT-I, rates of grade 3 or 4 anemia in the ruxolitinib group peaked around weeks 8 to 12 and subsequently decreased to placebo values.4 RBC transfusions followed a similar pattern, gradually approaching baseline levels and remaining stable throughout the remainder of the 2-year follow-up.4,23 Similarly, platelet counts during ruxolitinib therapy stabilized after initial decreases during the first 12 weeks.4

After dose adjustments (most of which occurred during the first 8 to 12 weeks of treatment), the median final titrated doses (calculated from the average doses received from weeks 21 to 24) in the 2 platelet groups in COMFORT-I were 10 and 20 mg BID, respectively.29 Doses of 10 mg BID and higher were associated with treatment benefit.24,29 For example, final titrated doses of 10, 15, 20, and 25 mg BID were associated with a 71.1%, 59.6%, 67.7%, and 66.2% reduction in TSS, respectively.24

**Ruxolitinib therapy in patients with low platelet counts**

Patients with MF and platelet counts < 100 x 10^9/L were not eligible for participation in the COMFORT trials4,5 because of the dose-dependent risk of further reductions in platelet count with ruxolitinib. Two early-phase clinical studies are currently evaluating ruxolitinib dosing strategies for patients with platelet counts of 50 to 100 x 10^9/L. Interim results of a phase 2 trial in patients receiving a starting dose of 5 mg BID showed that most of those treated for 24 weeks reached final doses of 10 mg BID or higher, with no treatment discontinuations attributable to thrombocytopenia or bleeding events, and with efficacy data showing clinically meaningful reductions in spleen volume and symptoms.30 In an ongoing ruxolitinib dose-finding study (EXPAND), preliminary results showed no dose-limiting toxicities with ≤ 10 mg BID in patients with platelet counts of 75 to 99 x 10^9/L and 5 mg BID in patients with counts of 50 to 74 x 10^9/L.31

**Future directions**

A number of JAK inhibitors are currently in clinical development for the treatment of MF, including but not limited to fedratinib (SAR302503), pacritinib (SB1518), and momelotinib (CYT387).32 Fedratinib, a selective JAK2 inhibitor currently in phase 3 (ClinicalTrials.gov identifier, NCT01437787), was associated with dose-dependent spleen size reductions, symptom improvements, and cytopenias in a randomized dose-ranging phase 2 study in patients with intermediate-2 or high-risk MF.33 The JAK2 inhibitor pacritinib, which has been suggested to induce minimal myelosuppression based on the results of a small phase 2 study,34 is now being further studied in a randomized phase 3 trial (ClinicalTrials.gov identifier, NCT01773187). Results from an early phase trial suggest that momelotinib may elicit transfusion independence in a moderate proportion of patients,35 but this will need to be confirmed in randomized controlled trials. Additional treatment approaches for MF currently under investigation include combination therapy of ruxolitinib with anti-anemia and anti-fibrotic agents.32

**Conclusion**

MF is a debilitating, highly heterogeneous, progressive malignancy, in which clinicopathologic manifestations are driven not only by a state of excessive myeloproliferation, but also by general dysregulation of cytokine signaling, including overproduction of pro-inflammatory cytokines. Although JAK2V617F is present in about 60% of patients with MF, it is neither specific for a MF subtype nor predictive of clinical phenotype. The efficacy of ruxolitinib in patients with MF is mediated by the inhibition of dysregulated JAK-STAT signaling, resulting in significant and durable reductions in spleen size as well as marked improvements of MF-related symptoms and QoL measures. In addition, recent data suggest that ruxolitinib is associated with a survival benefit over placebo or best available therapy. These treatment benefits appear to apply across various patient populations, including patients with and without the JAK2V617F mutation. Thus, instead of being viewed primarily as a therapy that targets mutant kinase activity in hematopoietic stem cells, ruxolitinib should be regarded as a targeted pathway inhibitor that interferes with multiple pathophysiologic processes in this chronic disease.

Although ruxolitinib is generally well-tolerated, treatment-related onset or exacerbation of pre-existing cytopenias may require dose modifications and/or temporary treatment interruptions or RBC transfusions (for anemia). Such treatment adjustments may help to preserve overall efficacy of therapy in individual patients and prevent permanent treatment discontinuation. In patients with low platelet counts (≤ 100 x 10^9/L), a low starting dose (5 mg BID), followed by up-titration may be a preferable strategy for maximizing treatment benefit.

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