Published in final edited form as:

Expert Opin Pharmacother. 2014 July ; 15(10): 1465–1473. doi:10.1517/14656566.2014.923404.

Efficacy of Ruxolitinib for Myelofibrosis

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Abstract

Introduction—The discovery of the activating JAK2V617F mutation in patients with myelofibrosis (MF) led to the development of JAK2 inhibitors. The first such inhibitor to enter clinical trials was ruxolitinib. This review summarizes pre-clinical and clinical data of ruxolitinib in MF.

Areas covered—A literature search through Medline employing the terms “ruxolitinib”, “INCB018424” and “myelofibrosis” was undertaken. The results from phase I/II studies in patients with MF showed that ruxolitinib led to durable improvements in splenomegaly, and symptoms associated with MF. Two phase III trials have compared ruxolitinib against placebo and best available therapy and in both studies ruxolitinib demonstrated superior rates of spleen control and symptom improvement, and additional analysis demonstrated a survival benefit with ruxolitinib treatment. The main toxicities seen with ruxolitinib are cytopenias, which are managed with dose adjustments. Recent reports documented sporadic cases of immunosuppression-related infections. Ruxolitinib is the first drug ever approved for the therapy of patients with MF.

Expert Opinion—Understanding the factors that predict the rate and duration of response to ruxolitinib would improve our ability to manage patients treated with this medication. Clinical trials combining ruxolitinib with novel compounds that are also active in MF will further improve therapy for this disease.

Keywords

Myelofibrosis; Ruxolitinib; JAK2 V617F

1 Introduction

Myelofibrosis (MF) is a Philadelphia-negative (Ph-negative) myeloproliferative neoplasm (MPN) characterized by bone marrow fibrosis, abnormalities in peripheral blood cell counts, extramedullary hematopoiesis (particularly in the spleen) and an increased risk of

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Conflicts of interest: FPSS has received research funding from Novartis; SV has received research support from Incyte

Declaration of interest:

The authors have received research funding from Novartis and Incyte

transformation to acute myeloid leukemia (AML)¹. It is an uncommon disease, with an incidence estimated at 1.46 cases/100,000 persons per year, and median age at diagnosis is 67 years². MF can be primary MF (PMF) or secondary to other Ph-negative MPNs, such as polycythemia vera (post-PV MF) and essential thrombocythemia (post-ET MF)¹. Patients with MF usually refer a myriad of symptoms, including extreme fatigue, fever, weight loss, night sweats, pruritus, abdominal and bone pain and have very poor quality of life^{3,4}.

MF is a disease that has few treatment options. For patients who develop massive splenomegaly, treatment options included hydroxyurea, other chemotherapeutic agents (e.g. cladribine), splenic radiation and splenectomy⁵. For patients who have transfusion dependent anemia, drugs such as erythropoietin, androgens (e.g. danazol), and immunomodulatory compounds (e.g. lenalidomide) can be used⁶. No curative drug option exists, and median survival of newly-diagnosed patients is in the range of 5–6 years⁷. Allogeneic stem cell transplantation is a potential curative treatment approach; however it is applicable only in a minority of patients due to risks associated with procedure⁸.

The scenario for MF started to change with the report in 2005 of activating mutation in the JAK2 tyrosine kinase (TK) in 60% of patients with MF and ET, and 90% of patients with PV^{9–12}. The JAK2 TK is part of the JAK family of TKs¹³. JAK2 plays an essential role in hematopoiesis, as evidenced by knock-out mice studies^{13–15}. The V617F mutation leads to increased activity of the JAK2 TK, and consequently causes increased proliferation and resistance to apoptosis in the transformed hematopoietic cell¹⁰. Subsequently, mutations in other genes that also lead to increased JAK2 TK activity, and general dysregulation of JAK-signal transducer and activator of transcription (STAT) intracellular signaling pathway were described in patients with JAK2V617F-negative MF and ET (e.g. *MPL*, *CALR*)^{16,17}. These findings demonstrate that JAK-STAT plays an essential role in the pathogenesis of MF and other classic Ph-negative MPNs.

The discovery of JAK2V617F spurred the development of JAK2 TK inhibitors (TKIs). The first such inhibitor to be developed and tested in clinical trials was ruxolitinib (formerly known as INCB028424; Jakafi®, Incyte, Wilmington, DE; Jakavi®, Novartis AG, Basel, Switzerland [outside the US]). Ruxolitinib is an orally available, potent JAK1 and JAK2 inhibitor¹⁸. Initial clinical trials demonstrated that ruxolitinib could lead to dramatic reductions in spleen size and symptom burdens in patients with MF¹⁸. Subsequently, the results of two phase III trials confirmed the efficacy of ruxolitinib for patients with MF, and it was the first drug to be approved for this indication by the FDA in November, 2011^{19,20}. This article will review the current data on the efficacy of ruxolitinib for patients with MF.

2 Pre-clinical studies

2.1 Chemistry

Ruxolitinib phosphate is a compound of the class of pyrrolopyrimidines that has activity as tyrosine kinase inhibitor. The chemical name of ruxolitinib is (*R*)-3-(4-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl)-3-cyclopentylpropanenitrile phosphate.²¹ The molecular weight of ruxolitinib phosphate is 404.36 g/mole and its molecular formula is C₁₇H₂₁N₆O₄P. Ruxolitinib phosphate is a white to off-white to light pink powder; it is soluble in aqueous

buffers across a pH of 1–8.²¹ Tablets of ruxolitinib phosphate²¹ should be stored at room temperatures of 20°C to 25°C (68°F to 77°F). The synthesis of ruxolitinib has been summarized before.²²

2.2 Pharmacodynamics

The pre-clinical characterization of ruxolitinib was published in 2010 by Quintás-Cardama et al²³. The authors demonstrated that ruxolitinib inhibited both JAK1 and JAK2 TKs with a half-maximal inhibitory concentration of 3.3 and 2.8 nM, respectively, while maintaining modest activity against TYK2 and minimal activity against JAK3²³. Whole blood stimulated with cytokines (Interleukin-6 [IL-6] and thrombopoietin [TPO]) confirmed the efficacy of ruxolitinib in decreasing phosphorylation of STAT3, a substrate of both JAK1 and JAK2 after stimulation with the aforementioned cytokines.

The activity of ruxolitinib against cells harboring JAK2V617F was demonstrated against both Ba/F3 cells transformed by JAK2V617F and erythropoietin receptor (Ba/F3-EPOR-JAK2V617F cells) and HEL cells (human erythroleukemia cell line carrying the JAK2V617F mutation)²³. Treatment of both cell lines with ruxolitinib reduced cell proliferation (Ba/F3 IC₅₀=126 nM; HEL IC₅₀=186 nM) and blocked phosphorylation of both STAT3 and STAT5²³. In Ba/F3-EPOR-JAK2V617F cells, treatment with ruxolitinib led to an increase in the number of apoptotic cells²³.

Ruxolitinib led to a dose-dependent decrease in proliferation of erythroid progenitors from patients with PV compared to healthy controls (IC₅₀ 223 nM vs. 407 nM)²³. Further evaluation demonstrated that ruxolitinib also inhibited endogenous EPO-independent endogenous erythroid colony formation from PV samples (IC₅₀=67 nM). These results suggested that ruxolitinib inhibited hematopoietic cell proliferation, with greater activity against JAK2-mutated cells versus normal cells²³.

In a mouse model of MPN, Ba/F3-EPOR-JAK2V617F cells were injected into the tail vein of Balb/c mice, and mice were randomized to treatment with vehicle or ruxolitinib²³. Vehicle-treated mice developed massive splenomegaly and became moribund in 2–3 weeks. In contrast, most mice treated with ruxolitinib survived (90% survival at 22 days, versus 10% in the control group)²³. At 15 days post-inoculation, vehicle-treated mice displayed splenomegaly (mean splenic weight 471 mg), while the spleen of mice treated with ruxolitinib were more similar to normal controls (mean weight 110 mg). Treatment with ruxolitinib also led to a marked reduction in the plasma levels of IL-6 and tumor necrosis factor alpha (TNF-α) compared to vehicle-treated mice, similarly to what has been observed in humans²³.

2.3 Pharmacokinetics and metabolism

In healthy human subjects receiving a single dose of 25 mg of [¹⁴C]ruxolitinib in oral solution, results of pharmacokinetics analysis showed rapid absorption (mean time to peak concentrations 0.63h), with a mean peak concentration (*C*_{max}) of 1093 nM and AUC_{0-∞} of 3200 nM.h²⁴. Ruxolitinib concentration declined in a monophasic or biphasic fashion (*t*_{1/2} of 2.32h and 5.81h, respectively)²⁴. The excretion of ruxolitinib-derived radioactivity was rapid, with 96% of administered radioactivity being recovered within 24 hours (74% in urine

and 22% in feces)²⁴. Metabolic profiling in urine and feces demonstrated hydroxyl and oxo metabolites as well as glucuronide conjugates of ruxolitinib, with the parent compound accounting for <1% of the excreted dose, suggesting that after an oral dose >95% of ruxolitinib is absorbed²⁴. There were no major differences in concentrations of the parent compound or metabolites after single or multiple dosing, suggesting a lack of drug accumulation. In another pharmacokinetic study, healthy human subjects received either single, ascending doses of ruxolitinib (5 to 200 mg) or multiple ascending doses both once and twice daily²⁵. Results of this study showed a good oral bioavailability of ruxolitinib and dose-proportional systemic exposures²⁵. A high-fat meal reduced C_{max} by 24%, but had no effect on ruxolitinib AUC. The maximum tolerated doses (MTD) in healthy volunteers were determined to be 25 mg twice daily and 100 mg once daily²⁵.

3 Clinical studies

3.1 Phase I/II Study

Ruxolitinib was first evaluated in a phase I/II study (NCT00509899) that recruited 153 patients with MF¹⁸. Doses of ruxolitinib ranged from 10–50 mg twice daily to 25–200 mg once daily. The MTD was determined to be 25 mg twice daily, and the dose limiting toxicity (DLT) was thrombocytopenia¹⁸. However, an individualized dose schedule whereupon patients started with 15 mg twice daily and dose was escalated monthly in case there was no response and no significant toxicity proved to be the best strategy. Ruxolitinib led to significant improvements in spleen size and constitutional symptoms. By the International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) response criteria, a clinical improvement (CI) in splenomegaly (50% in spleen reduction by palpation) was seen in 44% of patients across all dose levels¹⁸. Interestingly, responses were observed in both JAK2V6171F-positive (51%) and –negative (45%) patients, further evidence that deregulated JAK signaling is important to the pathogenesis of both mutated and wild type JAK2¹⁸. There were significant improvements in the symptomatic burden of patients, including reductions in pruritus, night sweats, weight loss, fatigue, bone and abdominal pain, and patients had an improvement in their exercise ability¹⁸. A decrease in pro-inflammatory cytokines mirrored the improvement in systemic symptoms¹⁸.

Long term follow-up data for the cohort of patients participating in the aforementioned phase I/II trial who were treated at the M.D. Anderson Cancer Center has been published²⁶. After a median follow-up of 2 years, the median overall survival (OS) of 107 patients with MF who received ruxolitinib was 69%²⁶. A CI in splenomegaly by the IWG-MRT was reached at some point in 61 patients (57%)²⁶. Responses were durable, as median duration of response was estimated around 3.2 years²⁶. MF-related symptoms were assessed by serial completion of the Myelofibrosis Symptom Assessment Form (MFSAF). The total symptom score (TSS; composite score of night sweats, itching, abdominal pain/discomfort, bone pain) decreased a median of 60% during 2 years of therapy. Overall, 58 (54%) patients were still being treated with ruxolitinib at the time of report. The 2-years OS for these patients was 92% (International Prognostic Scoring System [IPSS] Intermediate-2) and 88% (IPSS High)²⁶. A total of 49 patients (46%) discontinued therapy with ruxolitinib. The 2-years OS for these patients was 32% (IPSS Intermediate-2) and 21% (IPSS high 21%). Most frequent

reasons for therapy discontinuation were death (12%) and progressive disease (11%). Patients who had a 50% reduction in spleen size had an improved survival compared to patients who had lesser degrees of spleen reduction (HR=0.223; 95% CI 0.09–0.51; p=0.0001)²⁶.

The survival of the 107 patients treated with ruxolitinib at M.D. Anderson Cancer Center was compared to the outcomes of 310 historical matched controls that would have fulfilled enrollment criteria for the phase I/II trial²⁶. Therapy with ruxolitinib significantly improved survival compared to the historical control group (HR=0.58; 95% CI 0.39–0.85; p=0.005)²⁶. The benefit was more evident in patients in the high IPSS risk group (HR=0.50; 95% CI 0.31–0.81; p=0.006). The OS at 2 years for high-risk IPSS was 83% in the ruxolitinib-treated patients versus 58% in the historical control group.²⁶ The Mayo Clinic also published a report on the long-term outcome of 51 patients with MF treated with ruxolitinib in that center during the phase I/II study²⁷. Contrary to the study based on the larger M.D. Anderson cohort, no improvement in OS was detected when their outcome was compared to that of 410 historical control patients with MF (all IPSS risk categories) treated at the same center (unadjusted p=0.43; p=0.58 after adjusting for the Dynamic International Prognostic Scoring System-Plus Score [DIPSS-Plus])²⁷. This difference in results obtained may have stemmed from the very high treatment discontinuation rate in the Mayo cohort (1 year-51%; 3 years-89%)²⁷, which were higher than the discontinuation rates at M.D. Anderson Cancer Center (1 year- 24%; 3 years-46%)²⁶. The most common reasons for discontinuation in the Mayo cohort were patient withdrawal of consent (29.4%), physician decision (23.5%) and disease progression (19.6%)²⁷. Additionally, the mean dose of ruxolitinib over time was much lower in the Mayo cohort compared to the M.D. Anderson cohort²⁶.

Initial reports from the phase I/II study did not show any appreciable reduction in JAK2V617F allele burden or decrease in BM fibrosis¹⁸. However, longer term follow-up has demonstrated that this may not always be true. A recent report compared the reduction in the grade of BM fibrosis among patients receiving BAT (best available therapy; historical control) and ruxolitinib²⁸. After 2 years (74% [ruxolitinib] vs. 60% [BAT]; odds ratio [OR] 2.62), 4 years (79% vs. 46%; OR 9.40) and 5 years (76% vs. 32%; OR 15.39) of therapy, more patients in the ruxolitinib-treated group had an improvement or stabilization in the grade of BM fibrosis compared to patients in the BAT group. Conversely, at 4 years (25% vs. 76%) and 5 years (26% vs. 75%), more patients in the BAT group had worsening of BM fibrosis. There was a strong correlation between reduction in splenomegaly and BM fibrosis reduction at 4 years in patients receiving ruxolitinib. One case report also described a patient with post-PV MF who participated in the COMFORT-II clinical trial (see below) and had complete resolution of BM fibrosis after 168 weeks of therapy with ruxolitinib²⁹. This patient also had an improvement in hematopoiesis and a dramatic reduction in JAK2V671F allele burden. Both studies suggest that in selected cases, long-term therapy with a JAK2 inhibitor may reverse BM fibrosis.

3.2 Phase III Studies

There were two phase III clinical trials conducted with ruxolitinib in patients with MF. In the phase III COMFORT-I clinical trial (that was conducted in North America; NCT00952289),

a total of 309 patients with intermediate-2 and high-risk IPSS MF were randomized (1:1) between ruxolitinib (15–20 mg twice daily, depending on the platelet count) and placebo¹⁹. Spleen response rate (RR) at 24 weeks was the primary endpoint, and was defined as a 35% reduction in spleen volume by magnetic resonance imaging (MRI), that corresponded to a decrease 50% of spleen size by palpation. After a median follow-up of 32 weeks, the RR was 41.9% in ruxolitinib-treated patients vs. 0.7% in placebo-treated patients ($p<0.0001$)¹⁹. Patients receiving ruxolitinib had a mean reduction in spleen volume of 31.6% at 24 weeks, compared to a mean increase of 8.1% in spleen volume at 24 weeks in placebo-treated patients¹⁹. There was a 50% reduction in the MFSAF-TSS in 46% of ruxolitinib-treated patients versus 5.3% of placebo-treated patients ($p<0.0001$)¹⁹. Most patients who were treated with ruxolitinib had an improvement in symptoms, whereas most patients treated with placebo had worsening of symptoms. No subgroup of patients (subtype of MF [primary vs. secondary], age, JAK2 mutational status, spleen size, hemoglobin, IPSS risk score) was identified that had a differential response to ruxolitinib¹⁹. After a median follow-up of 51 weeks, therapy with ruxolitinib led to an improvement in overall survival of patients with Intermediate-2/High risk MF (HR 0.5; $p=0.04$ versus placebo)¹⁹.

In the 3-years update of the COMFORT-I trial, after a median follow-up of 2.86 years, 77 of 155 patients randomized to ruxolitinib were still receiving therapy and 111 of 154 patients randomized to placebo were crossed-over to ruxolitinib³⁰. The mean spleen volume reduction after 144 weeks was 34.1%³⁰. Among all patients randomized to ruxolitinib, 59% achieved a 35% reduction in spleen volume at any point during study; the probability of maintaining this response after 132 weeks was 53%³⁰. Patients who crossed over from placebo to ruxolitinib also had significant improvements in spleen volume (mean 30.0% reduction). Improvements in symptoms and quality of life were maintained in the majority of patients after 2 years of therapy³¹. After 3 years, a survival benefit with ruxolitinib was still present (HR=0.69, 95% CI 0.46–1.03, $p=0.067$), despite the fact that by 80 weeks all patients in the placebo group had discontinued or crossed over to ruxolitinib³⁰.

In the companion trial COMFORT-II (conducted in Europe; NCT00934544), 219 patients with Intermediate-2/High risk MF were randomized (2:1) among ruxolitinib and best available therapy (BAT)²⁰. The primary endpoint was the spleen RR at 48 weeks as assessed by a MRI. The most commonly used agent in the BAT arm was hydroxyurea (47%). At 48 weeks, the majority of patients in the ruxolitinib arm had a reduction in spleen volume, and 28% achieved a 35% reduction in spleen volume²⁰. Median time to response was 12.3 weeks. In the BAT arm, 0% of patients achieved a spleen volume response ($p<0.0001$)²⁰. Mean relative change in spleen volume at 48 weeks was –30.1% (ruxolitinib) and +7.3% (BAT; $p<0.001$)²⁰. Ruxolitinib also led to improvements in patient-reported quality of life measurements (assessed by the EORTC QLQ-C30 and FACT-Lym subscales) compared to patients receiving BAT, who consistently worsened during study period. Correlative studies showed that levels of proinflammatory cytokines (IL-6, TNF- α , C-reactive protein) decreased in patients receiving ruxolitinib and there was an increase in erythropoietin and leptin²⁰.

A 3-years update of COMFORT-II was published recently³². After a median follow-up of 151 weeks, 97% of patients receiving ruxolitinib had some degree of spleen reduction, and

51% achieved a response (35% reduction) in spleen volume at some point³². The probability of maintaining this response at 144 weeks was 50%. Among patients with MF who were treated with ruxolitinib and were positive for the JAK2V617F mutation, a 20% reduction in JAK2V617F allele burden at 48 weeks was seen in 22% of patients³². This was associated with a higher spleen volume RR (73% vs. 19%; odds ratio [OR] 4.6). The probability of survival at 144 weeks was 81% in the ruxolitinib arm and 61% in the BAT arm (HR=0.48; p=0.009), consistent with a survival benefit for ruxolitinib, similarly to the results seen with COMFORT-I³².

One study reported on a pooled survival analysis of both COMFORT studies, and the outcomes of 301 patients with MF who received therapy with ruxolitinib were compared to 227 patients who received control therapy³³. Results of this analysis demonstrated that therapy with ruxolitinib reduced the risk of death (HR =0.65; 95% CI 0.46–0.90)³³. In another post-hoc analysis, the survival of MF patients who were treated with ruxolitinib in the COMFORT-II study was compared to the survival of a matched cohort of patients in the dynamic IPSS (DIPSS) database³⁴. Survival was measured from the date of diagnosis, not from the date of start of ruxolitinib therapy. Results from this study demonstrated that patients who received ruxolitinib at some point during the course of their disease had improved survival (median OS 5 years vs. 3.5 years; HR 0.61; 95% CI 0.41–0.91; p=0.0148)³⁴. Another report analyzed the impact of high-risk mutations (i.e. mutations in *ASXL1*, *EZH2*, *IDH1/2*, *SRSF2*) on RR to ruxolitinib in patients treated in the COMFORT-II trial³⁵. There was no difference in the RR in patients in the high-risk molecular group, and there was a trend for a reduced risk of death in patients with high risk mutations treated with ruxolitinib compared to BAT (HR=0.57; 95% CI 0.30–1.80)³⁵.

Recently, two papers reported on novel calreticulin gene (*CALR*) mutations that occur in patients with Ph-negative MF and ET who are negative for both *JAK2* and *MPL* mutations^{16,36}. These mutations are found in 67% of *JAK2/MPL*-negative ET patients and up to 88% of *JAK2/MPL*-negative MF patients³⁶. *CALR* mutations are always frameshift insertions and deletions that occur in exon 9, and the most common mutations are a 52-bp deletion (c.1092_1043del; type 1 mutation) and a 5-bp insertion (c.1154_1155insTTGTC).³⁶ Mutated calreticulin appears to induce STAT5 activation through an unknown mechanism³⁶, and immunohistochemistry directed against mutated calreticulin have revealed that the protein is mostly expressed in megakaryocytes, with a faint staining in erythroid and granulocytic cells³⁷. Patients with *CALR* mutations have higher platelet counts, decreased thrombotic risk and improved survival compared to patients harboring either *JAK* or *MPL* mutations and triple-negative (*JAK/MPL/CALR*-negative) patients^{36,38}. One paper reported recently on two patients with *CALR*-mutated MF who were treated with the JAK2 inhibitor fedratinib³⁹. Both patients had a clinical response, as evidenced by a decrease in spleen size³⁹. This report suggests that ruxolitinib and other JAK2 inhibitors may be effective in patients harboring *CALR* mutation, and may explain the observed fact that the JAK2V617F mutational status did not predict response to ruxolitinib in the aforementioned clinical trials. However, there is still need for a formal evaluation of the clinical response to ruxolitinib in patients with *CALR*-mutated MF.

The results of clinical trials with ruxolitinib are summarized in table 1.

3.4 Safety and tolerability

The most common side effects observed with ruxolitinib are dose-dependent anemia and thrombocytopenia (table 2). Data from the COMFORT-I trial demonstrate that ruxolitinib leads to grade 3–4 anemia in 45.2% of patients compared to 19.2% of placebo-treated patients (34% increase) and causes grade 3–4 thrombocytopenia in 12.9% of patients compared to 1.3% in patients treated with placebo (11.6% increase) when these rates of hematological side effects were assessed at any time during the study (initial follow-up period of 51 weeks)¹⁹. Grade 3–4 neutropenia is uncommon (7.1% in COMFORT-I). Anemia and thrombocytopenia are more common in the early phases of therapy, and tend to improve with time¹⁹. The most common pattern is for patients to present with a fall of 1.5 g/dL in hemoglobin during the first 8–12 weeks of therapy, followed by an increase to a new steady state with 24 weeks of therapy¹⁹. In COMFORT-I, the majority of novel cases of grade 3–4 anemia/thrombocytopenia occurred within the first 6 months of therapy, and after 12 months the incidence of new cases was lower (i.e. 12% [anemia] and 2.8% [thrombocytopenia])³⁰. Despite the relatively high incidence of cytopenias in the first 12 months of therapy, few patients (only one in each phase III trial) had to discontinue therapy due to cytopenias. Ruxolitinib dose adjustments and transient red blood cell transfusions can be implemented in case of drug-induced cytopenias. Data from COMFORT-I suggests that patients who were dose-reduced to 10 mg twice daily after nadir hemoglobin had faster recovery to pre-treatment levels, and had similar benefits regarding spleen volume reduction, symptom control and survival^{30,31}.

Non-hematological side effects are usually grade 1–2 in severity, and rarely lead to treatment discontinuation (8% and 11% in both phase III trials, discontinuation rate similar to that seen in control arms)^{19,20}. Most common non-hematological side effects compared to placebo were included ecchymosis (18.7% vs. 9.3%), headache (14.8% vs. 5.3%) and dizziness (14.8% vs. 6.6%)¹⁹.

Recently, there were several cases reports of systemic, opportunistic infections in patients being treated with ruxolitinib, including hepatitis B virus reactivation⁴⁰, herpes simplex reactivation⁴¹, *Cryptococcus neoformans* pneumonia⁴², toxoplasmosis retinitis⁴³, tuberculosis⁴⁴ and progressive multifocal leukoencephalopathy (the latter being only a single case)⁴⁵. There is some in vitro evidence to suggest that ruxolitinib impairs dendritic cell functions, which may result in impaired CD4+ and CD8+ T-lymphocyte activation⁴⁶. On the other hand, one study reported on a single patient with MF who had leukopenia and recurrent infections and had an improvement in white blood cell count and reduced risk of infections after receiving therapy with low-dose ruxolitinib⁴⁷. While there is no conclusive data on the immunosuppressive potential of ruxolitinib, the physician should be mindful of possible atypical infectious complications in patients being treated with this drug⁴⁸.

After discontinuing therapy, patients experience a rapid relapse of symptoms after a median of 7 days post drug discontinuation¹⁹. One study reported on the development of a cytokine rebound “syndrome”, similar to an inflammatory shock state, after ruxolitinib abrupt discontinuation⁴⁹. In the COMFORT-I trial, however, there was no increased rate of severe adverse events after treatment discontinuation in patients receiving ruxolitinib versus patients receiving placebo (6.1% vs. 5.6%, respectively)¹⁹. Despite this, the prescribing

information for ruxolitinib in the United States recommends dose tapering (5 mg BID per week) to decrease the risk of withdrawal return of symptoms.

4 Regulatory Affairs

Ruxolitinib was discovered and developed within Incyte. A new drug application (NDA) was submitted to the U.S. Food and Drug Administration (FDA) in June 8, 2011. The drug was approved by the FDA for treatment of patients with intermediate and high-risk MF in November 16, 2011. Ruxolitinib was also approved in the European Union in August 29, 2012 for therapy of disease-related splenomegaly or symptoms in adults with PMF, post-PV MF and post-ET MF.

5 Conclusion

The approval of ruxolitinib represented a milestone in the history of therapy for MF, since it is the first drug that was ever approved for the treatment of this disease. Ruxolitinib is an effective drug for treating splenomegaly and disease-related symptoms in patients with MF. Its effects on splenomegaly are durable, and it improves patients' quality of life and functioning (patients walk more and gain weight). There is now strong data from both pivotal phase III trials demonstrating that therapy with ruxolitinib improves survival of patients with MF. Furthermore, the drug is active in all subsets of MF, including patients who do not harbor the JAK2V617F mutation. The main toxicity of ruxolitinib is drug-related cytopenias, which can be treated with dose adjustments and rarely lead to treatment interruption.

6 Expert Opinion

While it is clear that ruxolitinib can improve splenomegaly, MF-associated signs and symptoms, as well as survival, there is more that needs to be learned. There are several areas of research that we believe of importance to further consolidate the role, and improve the use of ruxolitinib as therapy for patients with MF.

It is important to determine biomarkers of response to ruxolitinib to best select patients for this therapy. It is already known that the JAK2V617F mutational status does not predict for response to ruxolitinib, as JAK2V617F negative patients have the same response as those with mutation. The concomitant presence of additional epigenetic and chromatin-modifying mutations, such as has been reported recently for therapy with pegylated interferon- α 2a in PV and ET⁵⁰, should be explored. It is also clear that some patients present with significant responses to ruxolitinib, manifested by major decreases in spleen size, JAK2V617F allele burden and BM fibrosis reduction. If we could unravel the molecular mechanisms behind those patients with outstanding response this could help us to better understand the mechanism of action of ruxolitinib in MF and possibly identify biomarkers of response and duration of response.

Additionally, the use of ruxolitinib should be explored in patients with lower-risk disease. Patients with low risk MF have relatively preserved blood cell count, no significant constitutional symptoms, and very rarely symptomatic splenomegaly. The use of ruxolitinib

in these patients, possibly at lower doses to decrease the incidence of drug-related cytopenias, would mark a shift from treating patients with established advanced, symptomatic disease, to treating patients to prevent the development of signs and symptoms.

Allogeneic stem cell transplantation is the only known curative treatment for patients with MF. Improvements in conditioning regimen-related toxicities and HLA typing have made transplantation a possible therapeutic option for a greater percentage of patients than in the past.⁸ While clinical trials with ruxolitinib have focused solely on patients deemed ineligible for transplantation, the use of ruxolitinib as a pre-transplant therapy in transplant eligible patients should be explored. Ruxolitinib therapy may decrease spleen size, which may possibly improve engraftment time and graft function after transplant. Patients may improve performance status and gain weight, and be in better physical condition for the procedure. Preliminary experience of such use of ruxolitinib have already been reported.^{51,52} A question that is important is whether responding patients should be transplanted at the time of optimal response or when they start to lose response to the drug. Well conducted clinical trials will help us better define the role of ruxolitinib in transplant-eligible patients with MF.

While ruxolitinib represents an important advance for therapy of MF, it is clear that it is not a curative option, and over time patients will lose response to the drug. Loss of response may present as worsening splenomegaly, worsening symptoms, and/or worsening cytopenias, after prolonged stable response to therapy. Little is known about this process. There is *in vitro* data suggesting that resistance to ruxolitinib is related to stabilization and increase of JAK2 protein levels followed by heterodimerization with JAK1 and TYK2 that leads to JAK2 transactivation and resumption of signaling⁵³. Stopping exposure of cells to ruxolitinib leads to a regain of sensitivity. While these results are intriguing, this strategy still needs to be confirmed in the clinical setting, and will be explored in an upcoming clinical trial (ReTreatment trial; NCT02091752)⁵³. Combinatorial trials evaluating ruxolitinib with additional agents known to be active in this disease, or shown active in preclinical models, are of utmost importance. It is known that inhibition of Heat Shock Protein 90 (Hsp90) leads to JAK2 degradation, and compounds such as Hsp90 Inhibitors⁵⁴ and histone deacetylase 6 inhibitors⁵⁵ have pre-clinical activity in animal models of JAK2-mediated MPN. Clinical trials of these compounds in combination with ruxolitinib are already underway (PRIME trial; NCT01693601), and hopefully will add other therapeutic options for the treatment of patients with MF.

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Drug Summary Box

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Drug name: Ruxolitinib

Phase: III

Indication: Intermediate and High risk Myelofibrosis

Mechanism of action: Tyrosine kinase inhibitor, inhibits JAK1 and JAK2 signaling

Route of administration: oral

Chemical structure: $C_{17}H_{18}N_6$

Pivotal trial(s): COMFORT-I and COMFORT-II

Table 1

Summary of clinical studies conducted with ruxolitinib

Study	Number of patients	Phase	Therapy	Spleen Response Rate [*]	Other response endpoints
Verstovsek et al. ¹⁸	153	I/II	Ruxolitinib (all patients)	44%	Reduction in pruritus, night sweats, weight loss, fatigue, bone pain, abdominal pain; improvement in exercise ability
Verstovsek et al. ¹⁹ (COMFORT-I)	309	III	Ruxolitinib (155 patients) vs. placebo (154 patients)	41.9% (ruxolitinib) vs. 0.7% (placebo) ^{**}	Symptom improvement: 46% (ruxolitinib) vs. 5.3% (placebo) ^{**} Overall Survival: HR 0.50 (vs. placebo) [‡]
Harrison et al. ²⁰ (COMFORT-II)	219	III	Ruxolitinib (146 patients) vs. best available therapy (73 patients)	28% (ruxolitinib) vs. 0% (best available therapy) ^{**}	Symptom and quality of life improvement with ruxolitinib Overall survival: HR 0.48 (vs. best available therapy) [‡]

^{*} -spleen response was defined as 50% reduction in spleen size by palpation in the phase I/II trial; spleen response was defined as 35% reduction in spleen volume by magnetic resonance imaging at 24 weeks (COMFORT-I) and 48 weeks (COMFORT-II).

^{**} - p<0.0001.

[‡] - p=0.04.

[‡] - p=0.009

Table 2

Incidence of anemia and thrombocytopenia in clinical studies with ruxolitinib

Study	Therapy	Anemia Grade 1–2	Anemia Grade 3–4	Thrombocytopenia Grade 1–2	Thrombocytopenia Grade 3–4
Verstovsek et al. ¹⁸	Ruxolitinib	NR	23%	NR	20%
Verstovsek et al. ¹⁹ (COMFORT-I)	Ruxolitinib	51%	45%	57%	13%
Verstovsek et al. ¹⁹ (COMFORT-I)	Placebo	68%	19%	29%	1.5%
Harrison et al. ²⁰ (COMFORT-II)	Ruxolitinib	54%	42%	60%	8%
Harrison et al. ²⁰ (COMFORT-II)	Best available therapy	63%	31%	22%	7%

NR means not reported;