

# Myelofibrosis: Clinicopathologic Features, Prognosis, and Management

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**Abstract:** Myelofibrosis is one of the *BCR-ABL*-negative clonal disorders that collectively are known as myeloproliferative neoplasms (MPNs). It is caused by the proliferation of clonal hematopoietic stem cells, which over time leads to characteristic clinical features. The disease presentation is heterogeneous, however, with 30% of patients initially asymptomatic. This variation in clinical phenotype warrants careful risk stratification to guide appropriate management, and prognostic risk scores are continually being refined. Considerable advancements have been made in the understanding of MPN pathogenesis, in particular recognition of the driver mutations *JAK2* V617F, *CALR*, and *MPL*, which has led to the development of ruxolitinib, an inhibitor of Janus kinase 1 (JAK1) and JAK2 that has transformed therapy for myelofibrosis. Although ruxolitinib decreases symptoms and is associated with a survival advantage, it has no clear disease-altering activity, and allogeneic hematopoietic stem cell transplant remains the sole curative option for myelofibrosis. Ongoing studies are evaluating newer JAK inhibitors, combinations of ruxolitinib with other targeted drugs, and targeted therapies that do not inhibit JAK. This review provides further detail regarding the clinical features, pathogenesis, risk stratification, and current management of myelofibrosis, including older and newer targeted treatments.

## Introduction

The myeloproliferative neoplasms (MPNs) are a group of disorders of clonal myeloid proliferation. Myelofibrosis (MF), a neoplasm that is negative for the *BCR-ABL* translocation, originates in hematopoietic stem cells. The clonal proliferation of hematopoietic stem cells in the bone marrow leads to cytokine release, myeloid hyperproliferation, and bone marrow fibrosis. In some cases, osteosclerosis with subsequent extramedullary hematopoiesis develops, and in a proportion of patients, acute myeloid leukemia can occur. MF can present de novo as primary myelofibrosis (PMF) or can be secondary to an antecedent myeloproliferative neoplasm—namely, polycythemia vera (PV) or essential thrombocytosis (ET). The incidence is approximately 0.1 to 1 per 100,000 individuals per year,<sup>1</sup> with patients presenting at a median age of 64 years. The median survival was 5 years before 1995<sup>2</sup> and increased to 6.5 years between 1996 and 2007. Because

## Keywords

JAK inhibitors, myelofibrosis, ruxolitinib

this period preceded Janus kinase (JAK) inhibitor use,<sup>3</sup> most of the increase is attributed to improved supportive treatments and earlier diagnosis. Disease presentation is diverse. MF causes no symptoms in 30% of patients initially, whereas other patients have symptoms caused by cytopenias, leukocytosis, thrombocytosis, thrombosis, infections, aquagenic pruritus, splenomegaly, bone pain, and constitutional symptoms.

Allogeneic hematopoietic stem cell transplant (AHSCT) is the only curative treatment, but it cannot be applied in most cases. Until recently, the goal of treatments was to alleviate symptoms and improve quality of life. This has changed with the introduction of JAK inhibitors, which have eclipsed older agents. Here, we review the pathogenesis, clinical features, and prognostication of MF, and discuss current and future therapeutic strategies.

## Pathogenesis

Mutations in the *JAK2*, calreticulin (*CALR*), and myeloproliferative leukemia virus (*MPL*) genes have been identified to be driver mutations in the pathogenesis of MPNs, including MF. Of patients with MF, 45% to 68% have the *JAK2* V617F mutation<sup>4</sup> and 5% to 10% have an *MPL* mutation<sup>5,6</sup>; the most newly recognized mutation, *CALR*, occurs in 25% to 35% of patients.<sup>7</sup> All 3 mutations are absent in approximately 9% of patients, in which case the disease is denoted as “triple-negative.”<sup>8</sup>

Aberrant constitutive activation of the JAK signal transducer and activator of transcription (JAK-STAT) pathway is core to the pathogenesis of MPNs, regardless of which mutations are present or absent in so-called triple-negative disease.<sup>9</sup> This results in increased signaling via STAT, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and serine/threonine kinase (STK), and a downstream increase in gene transcription and expression. Additional somatic mutations are detected more often in MF than in other MPNs, implying a more complex disease ontogeny. These mutations often affect epigenetic regulation (eg, *EZH2*, *ASXL1*, and *TET2*) and manifest at a lower frequency (5%-25%)<sup>10</sup> than the driver mutations. Some are detected more commonly at time of leukemic transformation (eg, *IDH2*).<sup>11</sup>

An additional key feature of MF is elevation of proinflammatory cytokines. Transforming growth factor beta 1 (TGF- $\beta$ 1) is one such cytokine that, through mouse models, has been implicated in the development of bone marrow fibrosis in MF.<sup>12</sup> The malignant hematopoietic stem cells stimulate the production of multipotent stromal cells of osteoblastic lineage directly and also via cytokines, increasing fibrosis and trabecular thickening.<sup>13</sup>

## Diagnosis

Both clinical and laboratory findings are required to make the diagnosis of MF. Characteristic bone marrow morphology is essential, comprising fibrosis (graded on a World Health Organization [WHO] scale from 0 to 3), megakaryocytic proliferation, and megakaryocytic atypia.<sup>14</sup> The WHO updated its diagnostic criteria for MPN in 2016,<sup>15</sup> taking into consideration the importance of newly recognized molecular markers in diagnosis and prognostication. The presence of 1 of the 3 driver mutations is a major diagnostic criterion. In patients with triple-negative disease, the detection of one of the associated somatic mutations (eg, *EZH2*, *TET2*, *IDH1/2*, *ASXL1*, *SRSF2*, or *SF3B1*) suffices as the presence of a clonal marker for diagnostic purposes.<sup>15</sup> However, it is important to take into consideration the confounding effect of clonal hematopoiesis of indeterminate potential (CHIP).<sup>16</sup>

Standardized bone marrow morphology criteria are important to reduce interobserver variability. Concordance rates range between 76% and 88%.<sup>17</sup> The WHO update highlighted the need to recognize the distinct entity of prefibrotic primary myelofibrosis (pre-PMF) by bone marrow histology and clinical features. Pre-PMF can present similarly to ET, but distinguishing between these disorders is vital owing to their different prognostic ramifications—in particular, the decreased survival in those with prefibrotic MF. In a multicenter review of more than 1000 bone marrow samples<sup>18</sup> classified as ET, 16% were reclassified as pre-PMF according to the WHO 2008 criteria. The outcomes of patients whose samples were reclassified as pre-PMF were poorer than those of patients with ET; leukemic transformation occurred in 5.8% vs 0.7%, respectively, at 10 years, and progression to MF occurred in 11.7% vs 2.1%, respectively, at 10 years. These findings of poorer prognosis and survival in those with pre-PMF were reproduced by a group in Cologne and Vienna the same year.<sup>19</sup> The management of pre-PMF is unclear and is not discussed further in this review.

The natural course of MF after PV or ET may differ from that of PMF. Diagnostic criteria appear in the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) consensus report.<sup>20</sup>

## Prognostication

Given the heterogeneous clinical phenotype, it is essential to stratify patients to facilitate the process of choosing an appropriate therapy, particularly ascertaining transplant eligibility. The International Prognostic Scoring System (IPSS) was developed in response to this need.<sup>2</sup> Its utility is at the time of diagnosis with median survival impacted by the following 5 factors: age older than 65

**Table 1.** Prognostic Scoring Systems

Scoring System	IPSS	DIPSS	DIPSS Plus	MIPSS <sup>27,a</sup>	GPSS <sup>28,a</sup>
Factors (points)	<ul style="list-style-type: none"> <li>• Age &gt;65 y (1)</li> <li>• Hb &lt;100 g/L (1)</li> <li>• WCC &gt;25 × 10<sup>9</sup>/L (1)</li> <li>• PB blasts ≥1% (1)</li> <li>• C Sx (1)</li> </ul>	<ul style="list-style-type: none"> <li>• Age &gt;65 y (1)</li> <li>• Hb &lt;100 g/L (2)</li> <li>• WCC &gt;25 × 10<sup>9</sup>/L (1)</li> <li>• PB blasts ≥1% (1)</li> <li>• C Sx (1)</li> </ul>	<ul style="list-style-type: none"> <li>• Age &gt;65 y (1)</li> <li>• Hb &lt;100 g/L (1)</li> <li>• WCC &gt;25 × 10<sup>9</sup>/L (1)</li> <li>• PB blasts ≥1% (1)</li> <li>• C Sx (1)</li> <li>• Unfavorable karyotype<sup>b</sup> (1)</li> <li>• Transfusion dependency (1)</li> <li>• Plt &lt;100 × 10<sup>9</sup>/L (1)</li> </ul>	<ul style="list-style-type: none"> <li>• Age &gt;60 y (1.5)</li> <li>• Hb &lt;100 g/L (0.5)</li> <li>• Plt &lt;200 × 10<sup>9</sup>/L (1)</li> <li>• C Sx (0.5)</li> <li>• <i>JAK2</i> (0.5)</li> <li>• <i>MPL</i> (0.5)</li> <li>• <i>ASXL1/SRSF2</i> (0.5)</li> <li>• Triple negativity (1.5)</li> </ul>	<ul style="list-style-type: none"> <li>• Age &gt;60 y (2)</li> <li>• Very high-risk karyotype (3)</li> <li>• High-risk karyotype (1)</li> <li>• <i>JAK2</i> (2)</li> <li>• <i>MPL</i> (2)</li> <li>• <i>CALR</i> type 2/2-like (2)</li> <li>• <i>ASXL1/SRSF2</i> (1)</li> <li>• Triple negativity (2)</li> </ul>
Risk groups, scores (OS)					
• low	0 (11.3 y)	0 (not reached)	0 (185 mo)	0-0.5 (26.4 y)	0 (not reached)
• int-1	1 (7.9 y)	1-2 (14.2 y)	1 (78 mo)	1-1.5 (9.7 y)	1-2 (9 y)
• int-2	2 (4 y)	3-4 (4 y)	2-3 (35 mo)	2-3.5 (6.4 y)	2-3 (5 y)
• high	≥3 (2.3 y)	≥5 (1.5 y)	≥4 (16 mo)	≥4 (1.9 y)	≥5 (2.2 y)

C Sx, constitutional symptoms; DIPSS, Dynamic International Prognostic Scoring System; GPSS, Genetics-Based Prognostic Scoring System; Hb, hemoglobin; int, intermediate; IPSS, International Prognostic Scoring System; mo, months; MIPSS, Mutation-Enhanced International Prognostic Scoring System; OS, median overall survival; PB, peripheral blood; Plt, platelet count; WCC, white cell count; y, years.

<sup>a</sup>Proposed scoring system, not used in standard practice.

<sup>b</sup>Monosomal karyotype, isochromosome of the long arm of chromosome 17 and inversion of chromosome 3.

years, hemoglobin (Hb) level below 100 g/L, white cell count below 25 × 10<sup>9</sup>/L, peripheral blood (PB) blast level of at least 1%, and constitutional symptoms (Table 1). The Dynamic International Prognostic Scoring System (DIPSS)<sup>21</sup> can be applied at any time during the disease course by using the same risk factors, although the presence of anemia raises the score. DIPSS Plus<sup>22</sup> additionally includes transfusion dependency, karyotype, and platelet count (Table 1).<sup>23</sup> Patients in the DIPSS Plus high-risk category can be further stratified into a very high-risk group, with a 2-year mortality rate of more than 80%, by the presence of a monosomal karyotype, inv(3)/i(17q) abnormalities, or any 2 of the following 3 features: circulating blast level above 9%, leukocyte level of at least 40 × 10<sup>9</sup>/L, and another unfavorable karyotype.<sup>23</sup>

Regarding the potential effect of driver mutations, patients who had a *CALR* mutation were younger and had a favorable prognosis, with a median overall survival of 17.7 years.<sup>24</sup> *MPL* and *JAK2* mutations are associated with similar median survivals of approximately 9 years. Triple-negative status is associated with a shortened median survival of 3.2 years<sup>25</sup> and poorer leukemia-free survival. A *JAK2* mutation is linked with an increased propensity to thrombosis.<sup>22</sup> A large multicenter analysis found that *ASXL1*, *SRSF2*, and *EZH2* mutations adversely affected survival, but only *ASXL1* mutations held significance independently of IPSS and DIPSS

Plus.<sup>26</sup> Patients with *ASXL1* mutations had a median survival of less than 2.5 years, but an association of *CALR* mutations with *ASXL1* mutations attenuated to a degree the poor prognosis of *ASXL1* mutations. Leukemic transformation was more likely in those with *IDH1/2*, *ASXL1*, or *SRSF2* mutations.

A Mutation-Enhanced IPSS (MIPSS) for patients with PMF<sup>27</sup> has been proposed that includes *JAK2*, *MPL*, *ASXL1*, and *SRSF2* mutation status in addition to clinical and full blood count parameters. A similar scoring system, the Genetics-Based Prognostic Scoring System (GPSS),<sup>28</sup> which incorporates both cytogenetic and mutational factors, has also been proposed (Table 1). Recently, a more simplified scoring system was suggested<sup>29</sup> that comprises *CALR* or *MPL* mutation status, *JAK2* allele burden, and age. Patients with a high *JAK2* V617F allele burden and an *MPL* or *CALR* mutation who were 65 years of age or younger had a median survival of 126 months, whereas those older than 65 years who had triple-negative disease or a low *JAK2* V617F allele burden had a median survival of 35 months. Although promising, these newer prognostic scores need to be validated and are not in routine use.

Until recently, no prognostic model was specifically designed for MF after PV or ET. In the Myelofibrosis Secondary to PV and ET-Prognostic Model (MYSEC-PM),<sup>30</sup> points are assigned for the following: Hb level below 110 g/L, PB blast level of at least 3%, platelet count below

$150 \times 10^9/L$ , absence of a *CALR* mutation, presence of constitutional symptoms, and any year of age. Patients are stratified into 4 risk groups—low, intermediate-1 (int-1), int-2, and high—with corresponding median survivals of not reached, 9.3 years, 4.4 years, and 2 years.

## Treatment

The treatment of MF aims to reduce patients' symptom burden and improve survival by reducing the risk for leukemic transformation and/or thrombosis. As discussed, prognostication serves to guide treatment decisions. Therapy requires an individualized approach; awareness of patients' comorbidities is necessary when considering toxicities of drug therapies and the option of AHSCT. JAK inhibitors have transformed treatment options and the first-in-class JAK inhibitor, ruxolitinib (Jakafi, Incyte Corporation), is now approved for treatment of MF in the United States and in Europe. They are also recommended for the treatment of MF-related hepatomegaly and portal hypertension. This section elaborates on JAK inhibitors, older therapies, newer targeted therapies in ongoing studies, and the role of AHSCT in the management of this condition.

### JAK Inhibitors

**Ruxolitinib.** The first and only drug approved in this class, ruxolitinib is an oral selective JAK1/JAK2 inhibitor. Phase 1/2 studies<sup>31</sup> established its safety profile, and it subsequently received approval (in the United States in 2011 and in Europe in 2012) following the results of 2 large phase 3 multicenter randomized double-blind trials known as COMFORT-I and COMFORT-II. Patients with IPSS int-2 or high-risk MF were enrolled in COMFORT-I<sup>32</sup> (n=309) or COMFORT-II<sup>33</sup> (n=219), which compared ruxolitinib with placebo or best available therapy (BAT), respectively. Crossover to the study arm was permissible at 6 months for those on placebo and at 12 months for those on BAT. The primary endpoint of spleen volume reduction (SVR) was attained significantly more often with ruxolitinib than with placebo or BAT in both trials—41.9% vs 0.7% ( $P<.001$ ) in COMFORT-I and 28.5% vs 0% ( $P<.001$ ) in COMFORT-II. Spleen responses were sustained in both trials at 3-year follow-up,<sup>34,35</sup> with a median duration of spleen response of 3.2 years.<sup>36</sup> Patient-reported symptom decrease of more than 50% on the modified Myelofibrosis Symptom Assessment Form (MFSAF) was obtained in the study arms in both trials.

Conventional markers of disease response, such as reduced bone marrow fibrosis, cytogenetics or molecular markers, and reduced allele burden, have not been correlated with decrease in symptoms or increase in survival and thus are not valid primary endpoints. Nonetheless,

as exploratory endpoints, the COMFORT trials reviewed bone marrow fibrosis and *JAK2* allele burden. In the ruxolitinib arm of the COMFORT-II trial,<sup>33</sup> bone marrow fibrosis was reduced in 15.8% of patients and stable in 32.2%, and approximately one-third of patients had a reduction in *JAK2* allele burden. Cytokine markers were also studied in COMFORT-II; patients receiving ruxolitinib had reduced levels of proinflammatory cytokines (interleukin-6, tumor necrosis factor alpha, and C-reactive protein).

Independent analysis of each COMFORT trial<sup>34,35</sup> and a pooled analysis<sup>37</sup> of both, with correction for the effect of crossover from the control arms, showed ruxolitinib to be associated with an overall survival benefit at 3 years. This survival benefit correlated with a reduction in spleen size of at least 10%. Longer-term 5-year follow-up of COMFORT-II found a 33% reduction in risk for death in the patients treated with ruxolitinib.<sup>36</sup> However, a Cochrane review of JAK inhibitors, including the COMFORT trials, concluded that there was insufficient evidence to draw conclusions regarding efficacy and survival owing to small sample size.<sup>38</sup> The survival benefit was found across all subgroups,<sup>39</sup> and of particular interest were the patients with high-risk mutations (*EZH2*, *SRSF2*, *IDH1/2*, and *ASXL1*), who still demonstrated improved survival in comparison with the patients who received BAT in COMFORT-II.<sup>10</sup>

Ruxolitinib is well tolerated; grade 3/4 anemia (~20%) and thrombocytopenia (~15%) are the most frequently reported adverse events,<sup>32,33</sup> with few patients discontinuing because of these. The adverse events manifest early, during the first 12 weeks of treatment, usually decrease by 24 weeks, and are effectively managed with dose alterations. Drug cessation is rarely required. Erythropoiesis-stimulating agents may be used to mitigate anemia.<sup>40</sup> Analysis has shown ruxolitinib-induced anemia to have no negative effect on overall survival—unlike disease-related anemia.<sup>41</sup> Furthermore, responses achieved in those patients receiving ruxolitinib offset the poor prognostic implication of disease-related anemia.<sup>42</sup>

Nonhematologic toxicities in both ruxolitinib studies were largely grade 1/2, usually consisted of diarrhea and peripheral edema, and rarely led to treatment cessation. Ruxolitinib is more immunosuppressive than was initially appreciated, with an augmented risk for infections such as urinary tract infections and pneumonia (24.6% and 13.1%, respectively).<sup>33</sup> Atypical and opportunistic infections may also occur, including toxoplasmosis retinitis, hepatitis B reactivation,<sup>43</sup> disseminated tuberculosis, and *Cryptococcus neoformans* pneumonia. In vitro and in vivo studies have shown ruxolitinib to disturb the normal immune system milieu, with a reduction in T regulatory cell and T helper cell function,<sup>44</sup> inhibition of natural killer cell differentiation and function,<sup>45</sup> and impairment

of dendritic cell development.<sup>46</sup> Screening for certain infections is recommended,<sup>47</sup> including hepatitis B, hepatitis C, and tuberculosis, and if detected, appropriate prophylaxis should be administered concomitantly.<sup>48</sup> Conversely, this anti-inflammatory and immunosuppressive effect has been used advantageously to treat diseases such as graft-versus-host disease (GVHD).<sup>49</sup>

Nonmelanoma skin cancers were reported at a slightly increased rate in the ruxolitinib arm of COMFORT-II at 5-year follow-up: 6.1 per 100 patient-years with ruxolitinib vs 3 per 100 patient-years with BAT. Although this increase may be explained by previous exposure to hydroxyurea, skin surveillance is recommended in these patients. No increased risk for leukemia was reported in those who received ruxolitinib in the COMFORT trials. A single-center review of patients on ruxolitinib reported extramedullary acute myeloid leukemia in 4 of 40 patients.<sup>50</sup>

In early studies,<sup>31</sup> rebound of symptoms occurred during drug suspension, but this was not replicated in the COMFORT trials. However, disease symptoms did reappear shortly after the drug had been discontinued.

Ruxolitinib has also been shown to be effective in int-1 patients enrolled in the following 2 trials: the phase 3b expanded-access JUMP study<sup>51</sup> and the UK ROBUST trial.<sup>52</sup> A subgroup of particular interest comprised those with earlier MF and high-risk mutations; evaluation in a prospective study called ReTHINK was undertaken, but unfortunately the study was terminated early.

To mitigate treatment-associated anemia and thrombocytopenia or to improve overall disease response, studies have evaluated ruxolitinib in combination with other agents (Table 2). Thus far, none of these studies have moved forward into phase 3 testing.

**Other JAK inhibitors.** Several other JAK inhibitors have been examined in ongoing studies: fedratinib, pacritinib, momelotinib, and most recently itacitinib. Fedratinib, a selective JAK2 inhibitor, was withdrawn from development in 2013. Although it achieved splenic responses in a phase 3 placebo-controlled trial, cases of Wernicke encephalopathy were reported.<sup>53</sup> Fedratinib structurally resembles thiamine and thus inhibits human thiamine transporter 2 (hTHTR-2), reducing thiamine absorption.<sup>54</sup> This effect is compound-specific, is not class-specific, and has not been reported with other JAK inhibitors. Data showing efficacy of second-line fedratinib were recently published.<sup>55</sup>

Pacritinib, a selective inhibitor of JAK2, Fms-like tyrosine kinase (FLT) 3, interleukin-1 receptor-associated kinase 1 (IRAK1), and colony stimulating factor 1 receptor (CSF1R), has also come under intense scrutiny. In early studies, it appeared to be less myelosuppressive than ruxolitinib, a feature possibly explained by its selective

JAK2 inhibition.<sup>56</sup> Subsequently, a phase 3 trial called PERSIST-1 randomly assigned patients with higher-risk MF (including those with cytopenias) to pacritinib or BAT (excluding JAK inhibitors). Spleen size reduction at 24 weeks was better in the pacritinib arm than in the BAT arm (19.1% vs 4.7%, respectively;  $P=.0003$ ), including in patients with severe cytopenias.<sup>57</sup> PERSIST-2 is a phase 3 trial that opened in 2014. Patients with higher-risk MF and platelet counts no higher than  $100 \times 10^9/L$  were randomly assigned to 200 mg of pacritinib twice daily, 400 mg once daily, or BAT (including ruxolitinib).<sup>58</sup> Regardless of prior JAK inhibitor use, responses in both pacritinib arms were better than those with BAT. Patients on pacritinib were significantly more likely to have SVR (18.1% vs 2%;  $P=.001$ ), with a trend toward a greater likelihood of symptom decrease that was not statistically significant (50% vs 4%;  $P=.079$ ). Unfortunately, the US Food and Drug Association (FDA) put a full clinical hold on pacritinib in February 2016<sup>59</sup> owing to concerns about cardiac failure, cardiac arrest, and intracerebral hemorrhage in those patients randomized to pacritinib. The clinical hold was lifted in January 2017.<sup>60</sup> In response to an FDA request, CTI BioPharma designed a new trial that began enrollment in August 2017 (NCT03165734). It is evaluating the safety and efficacy of lower pacritinib doses of 100 mg once daily, 100 mg twice daily, and 200 mg once daily.

Momelotinib, a potent JAK1/JAK2 inhibitor, has shown a notable effect of mitigating anemia. The drug was well tolerated in early studies, with anemia becoming less severe in 53% of patients and SVR occurring in 39%.<sup>61</sup> Grade 1/2 peripheral neuropathy was reported in 38% of patients, however, and resulted in treatment discontinuation in 2 patients. The neuropathy was dose-independent and appeared to be irreversible.<sup>62</sup> In the phase 3 SIMPLIFY 1 trial,<sup>63</sup> momelotinib was noninferior to the comparator ruxolitinib for achieving an SVR response at 24 weeks and superior in achieving transfusion independence, but inferior in symptom score response (28.4% for momelotinib vs 42.2% for ruxolitinib;  $P=.98$ ). In the SIMPLIFY 2 trial,<sup>64</sup> patients with MF and previous exposure to ruxolitinib who were transfusion-dependent or had required dose adjustments owing to grade 3 or higher hematologic toxicities were randomly assigned to momelotinib or BAT (including ruxolitinib). Momelotinib did not reach the primary endpoint of noninferiority in achieving SVR but was superior to BAT for transfusion independence (43.3% vs 21.2%, respectively;  $P=.001$ ). Peripheral neuropathy was described in 11% of patients in the momelotinib arm but no patients in the BAT arm. These results are varied but suggest that momelotinib may have a place in the treatment of patients with MF—potentially those with difficult-to-manage anemia or with disease refractory to ruxolitinib.

**Table 2.** Ruxolitinib Combination Studies

Drug Combination	Study	Disease (No. Patients Recruited)	Toxicities	Outcomes
Ruxolitinib + azacitidine	Phase 2, ongoing (NCT01787487)	MDS/MPN (35 pts) <sup>105</sup> and int- to high-risk MF (39 pts) <sup>106</sup>	MDS/MPN: grade 3/4 anemia 51% (18 pts), thrombocytopenia 19% (19 pts)  MF: Grade 3/4 hematologic AEs in 16 pts, grade 3/4 nonhematologic AEs in 4 pts	MDS/MPN: ORR 49% (17/35)  MF: ORR 72% (28/39), spleen reduction >50% in 59% (23/39), reduction of BM fibrosis grade in 31% (12/39)
Ruxolitinib + lenalidomide	Phase 2 (NCT01375140) <sup>107</sup>	int-1, int-2, or high-risk MF (31 pts)	Dose interruptions in 23 pts owing to AEs (in 14 pts owing to cytopenia)	Responses in 55% (17/31), no complete or partial responses  Terminated early because of failure to meet efficacy targets
Ruxolitinib + danazol	Phase 2 <sup>108</sup>	int-1, int-2, or high-risk MF with anemia (14 pts)	Grade 3/4 hematologic AEs in 71.4% (10 pts), nonhematologic AEs in 14.3% (2 pts)	Clinical improvement in 21.4% (3 pts), stable disease in 64.2% (9 pts), Hb increase in 55.5% (5/9) of pts with prior JAKi use and in 80% (4/5) of JAKi-naive pts
Ruxolitinib + panobinostat	Phase 1b escalation and phase 2 expansion <sup>109</sup>	int-1, int-2, or high-risk MF and spleen ≥5 cm by palpation (61 pts)	Discontinuation in 21% owing to AEs  Grade 3/4 hematologic AEs: anemia (32%) and thrombocytopenia (29%)  Grade 3/4 nonhematologic AEs: diarrhea (18%), asthenia (12%), and fatigue (9%)	Spleen responses in expansion phase in 87% (20/23) at wk 24, in 74% (17/23) at wk 48  BM fibrosis (in those evaluable) decreased in 4/12, unchanged in 6, and increased in 2 at wk 48  <i>JAK2</i> V617F allele burden (17 pts evaluable): ≥20% reduction in 29% by wk 48
Ruxolitinib + interferon	Phase 2 (COMBI) <sup>73</sup>	PV (20 pts) or primary MF (10 pts, evidence of active disease)	Anemia (15 pts, grade 3 in 2 pts), thrombocytopenia (6 pts, grade 3 in 1 pt), or neutropenia (13 pts, grade 3 in 2 pts)  Hospitalization required in 11 pts with SAEs; pneumonia most frequent, occurring in 3 pts	Complete response in 63.3% (19 pts), partial or major response in 26.7% (8 pts)  Hct control without phlebotomy achieved by wk 4 in 78% of those who had elevated Hct at baseline

AE, adverse event; BM, bone marrow; Hb, hemoglobin; Hct, hematocrit; int, intermediate; JAKi, JAK inhibitors; MDS/MPN, myelodysplasia/myeloproliferative neoplasm; MF, myelofibrosis; ORR, overall response rate; pts, patients; PV, polycythemia rubra vera; SAE, serious adverse event; wk, week.

Itacitinib (INCB039110) is a selective JAK1 inhibitor. In a phase 2 study<sup>65</sup> that assessed 3 doses, itacitinib showed clinical activity in higher-risk MF and was less myelosuppressive than previously discussed JAK inhibitors. A study evaluating itacitinib in combination with low-dose ruxolitinib or itacitinib alone has opened for recruitment (NCT03144687).

### Older Treatments

Hydroxyurea, an oral ribonucleotide reductase inhibitor,

was the mainstay of the medical treatment of MF before the introduction of JAK inhibitors. Hydroxyurea may be helpful for symptomatic splenomegaly and symptoms of hyperproliferation,<sup>66</sup> but limited data support its efficacy. In the COMFORT-II trial,<sup>33</sup> 47% of the patients assigned to BAT received hydroxyurea, with no sustained responses. The predominant toxicities were cytopenias, gastrointestinal upset, and ulcers (leg and oral), but skin cancers were also described. There is an unproven risk for progression to leukemia with long-term use, but this chiefly pertains

to those who have had additional exposure to alkylating agents such as oral busulfan and melphalan. Owing to their leukemogenicity, alkylating agents are reserved for the treatment of older patients whose disease has failed to respond to other treatments.<sup>67</sup>

Recombinant interferon alfa-2b (rIFN alfa-2b) is increasingly used in the treatment of MPN, principally in patients with PV or ET, in whom it is indicated for first-line use in younger patients.<sup>68</sup> It suppresses hematopoietic stem cell growth by way of MAPK pathway activation.<sup>69,70</sup> It is administered subcutaneously, can be given safely during pregnancy, and has no leukemic potential. In a small prospective study<sup>71</sup> of 17 patients with lower-risk MF who were treated with rIFN alfa-2b or pegylated IFN alfa-2a, 80% showed clinical improvement or disease stability. In addition, 4 patients had a reduction in bone marrow fibrosis. Treatment was well tolerated, with most toxicities graded 1 or 2. A more recent, albeit retrospective, study of 62 patients treated with pegylated IFN alfa-2a<sup>72</sup> showed a reduction in splenomegaly in 46.5% and a reduction in constitutional symptoms in 82%. In a phase 2 nonrandomized trial in which patients received a combination of ruxolitinib and pegylated IFN alfa-2a,<sup>73</sup> a clinical response was obtained in approximately 90% of patients (Table 2).

The immunomodulatory agents thalidomide, lenalidomide (Revlimid, Celgene), and pomalidomide (Pomalyst, Celgene) have been studied in MF, with modest responses seen—mostly in patients with anemia. Agents in this drug class act by reducing proinflammatory cytokines, inhibiting vascular endothelial growth factor<sup>74</sup> and fibroblast growth factor (resulting in an antiangiogenic effect), and enhancing natural killer cell and T-cell activity.<sup>75</sup> Thalidomide was studied with a tapered dose of prednisolone.<sup>76</sup> Modest reductions in anemia were noted in 62% of patients and reductions in splenomegaly in 19%, but neurotoxicity was a limiting complication. Lenalidomide has shown similar moderate responses,<sup>77</sup> but with less neurotoxicity. Pomalidomide was compared with placebo but did not meet the primary endpoint of transfusion independence at 6 months.<sup>78</sup>

Palliative treatments are predominantly for symptomatic splenomegaly and anemia. Splenectomy carries a significant risk for morbidity and mortality, and it should be considered only for patients who have massive splenomegaly refractory to drug therapies. In a single-center review of 223 patients with MF who underwent splenectomy over a 20-year period,<sup>79</sup> the 3-month postoperative morbidity and mortality rates were 31% and 9%, respectively. Palliative radiotherapy may be considered for those with symptomatic splenomegaly and a platelet count of at least  $50 \times 10^9/L$  who have failed other treatments and are not surgical candidates,<sup>80</sup> but its effect is short-lived with a risk for long-term severe cytopenias.

Further strategies to manage anemia that provide symptom control but have no disease-modifying effects include red cell transfusions, erythropoiesis-stimulating agents, and anabolic steroids such as danazol. This semisynthetic androgen achieved an anemia response in 30% of patients,<sup>81</sup> but its use is restricted owing to toxicities: weight gain, hirsutism, hepatotoxicity, and hepatic tumors. Monitoring with liver function tests and screening for hepatic tumors and prostate adenocarcinoma are recommended in male patients.

### *Allogeneic Hematopoietic Stem Cell Transplant*

AHSCT, the only curative treatment for MF, is an option for just a small subset of patients owing to high rates of transplant-related morbidity and mortality. The decision to proceed with transplant, along with the determination of optimal timing, is increasingly difficult in the current era of JAK inhibitors. Those categorized as having int-2 or high-risk disease, with a projected survival of less than 5 years, should be considered for AHSCT if they are deemed fit according to British Society for Haematology guidelines<sup>82</sup> and the European Society for Blood and Marrow Transplantation/European LeukemiaNet (EBMT/ELN) International Working Group.<sup>83</sup> No randomized controlled trials have compared AHSCT with alternative options; the vast majority of data are retrospective, with substantial heterogeneity in all aspects among these studies. A retrospective series of PMF patients younger than 65 years compared 190 patients who received AHSCT with 248 patients who received non-AHSCT therapies.<sup>84</sup> Those with DIPSS int-2 or high-risk disease had superior survival if they received AHSCT, but the risk of AHSCT outweighed the benefit in those with low-risk disease. This review did not include patients with post-PV or post-ET MF or those treated with JAK inhibitors, making it difficult to extrapolate the results to these subgroups of patients.

Optimal conditioning remains to be defined. Myeloablative conditioning (MAC) regimens are associated with an unacceptably high mortality risk, especially in those older than 45 years.<sup>85</sup> After adjustment for patient age, reduced-intensity conditioning has been shown to be associated with superior survival compared with MAC.<sup>86</sup> Similar outcomes have been described for matched related and unrelated donors, and stem cell source does not appear to affect outcome.<sup>87</sup> Patients with MF may be at higher risk for hepatotoxicity after transplant, such as sinusoidal obstructive syndrome,<sup>88</sup> which is thought to be related to pretransplant hepatic dysfunction (from extramedullary hematopoiesis and drugs). Routine pre-AHSCT splenectomy is not usually recommended and requires an individualized approach.<sup>83,85</sup>

The place of JAK inhibitors in AHSCT warrants discussion. First, the decision of whether to proceed to AHSCT in suitable patients or continue JAK inhibition

when there is evidence of an ongoing clinical response is problematic. No data are available to guide this decision, so at present the question remains unanswered. AHSCT may now be a valid option for a larger proportion of patients, particularly those deemed not to be fit, once ruxolitinib treatment has improved performance status and reduced spleen size.<sup>89</sup> When ruxolitinib is used as a bridge to transplant, the reduction in spleen size may also improve engraftment rates. In addition, the reduction in proinflammatory cytokines may reduce the risk for post-AHSCT GVHD. Adverse events of tumor lysis syndrome and cardiogenic shock have been reported in a single prospective study,<sup>90</sup> but this finding has not been reproduced in other small studies. The largest multicenter retrospective study of JAK inhibitor use in the peritransplant period<sup>91</sup> suggests continuing JAK inhibitor treatment to the time of conditioning. Survival was better and transplant-related mortality rates were lower in patients who responded to a JAK inhibitor than in those with stable/progressive disease, which may be explained by favorable disease biology in the former group or by selection bias. A prospective phase 2 trial examining ruxolitinib use before transplant is using this method (NCT01790295).

### **Leukemic Transformation**

Leukemic transformation has been reported to occur in 8% to 23% of patients in the first 10 years.<sup>92</sup> The previously described prognostic models are not adequate to predict who is at highest risk for leukemic transformation. Multivariate analysis of 649 patients<sup>93</sup> with MF identified a bone marrow blast level of 10% or greater and high-risk karyotype (17p-, -5, -7, or complex karyotype) as independent predictors of leukemic transformation; the risk at 1 year was 13% for those with one or both risk factors vs 2% for those with neither of these. Certain somatic mutations (*TET2*, *ASXL1*, *IDH1/2*, *EZH2*, *DNMT3A*, and *TP53*) are associated with transformation to acute myeloid leukemia (AML). *ASXL1* mutations result in loss of polycomb repressive complex 2 (PRC2)-mediated histone methylation, which promotes leukemogenesis.<sup>94</sup>

Leukemic transformation carries a bleak prognosis; one retrospective study<sup>92</sup> found that median survival was 2.6 months and mortality was 98%. No treatment option—AML-type induction chemotherapy, low-intensity chemotherapy, or supportive care—demonstrated superior outcomes. However, induction chemotherapy followed by AHSCT offers a potential viable option for this extremely poor-risk group, with a single-center review (n=14) finding a long-term survival rate of 49%.<sup>95</sup> In the United Kingdom, a phase 1b study called PHAZAR is currently recruiting patients. This study is assessing the safety and tolerability of ruxolitinib in combination with the hypomethylating agent azacitidine in accelerated or blast-phase myeloproliferative neoplasms or myelodysplasia.

### **Non-JAK Inhibitor Targeted Therapies**

Multiple non-JAK inhibitor targeted therapies are being investigated in ongoing studies, in some cases in combination with ruxolitinib, as previously discussed.

PRM-151 is a recombinant analogue of pentraxin-2 that induces macrophage differentiation to act at areas of tissue damage to prevent and reduce fibrosis. This agent has displayed clinical benefit,<sup>96</sup> with increased hemoglobin levels and platelet counts, reduced symptoms, and SVR; 54% of patients showed a morphologic reduction in bone marrow fibrosis.

Histone deacetylase (HDAC) is involved in the epigenetic regulation of gene expression and nonhistone effects, such as in DNA repair. Dysfunction of HDAC activity has been established in MPNs, rationalizing the investigation of HDAC inhibitors in MPN.<sup>97</sup> Early-phase studies of a pan-deacetylase inhibitor,<sup>98,99</sup> panobinostat (Farydak, Novartis), showed clinical responses with some significant reduction in bone marrow fibrosis; however, treatment was limited by toxicities (fatigue, diarrhea, and thrombocytopenia), leading to dose reductions and some drug discontinuation. Better responses were seen when panobinostat was combined with ruxolitinib (Table 2).

Imetelstat is a telomerase inhibitor that shows activity in patients with MF.<sup>100</sup> In one study, 21% of patients had complete or partial responses, with some morphologic and molecular remissions. These responses were not associated with a change in telomere length. The exact mechanism has not been elucidated. IMbark (NCT02426086) is an ongoing phase 2 trial of imetelstat in patients with int-2 or high-risk PMF refractory to or relapsed after JAK inhibitor treatment.

Heat shock protein (HSP) inhibitors have a potentially synergistic effect if combined with ruxolitinib. Loss of response or resistance to JAK inhibition may be related to heterodimer formation between JAK2 and other JAK kinases (JAK1/TYK2), which results in the reactivation of JAK2 and active JAK-STAT signaling but is reversible on cessation of JAK inhibition.<sup>101</sup> HSP90 inhibitors can degrade JAK2, abrogating this effect. A phase 2 study (NCT01668173) was undertaken in patients with MF but was terminated early.

Hedgehog pathway genes have been shown to be upregulated in MPNs, but their role in MF has not been precisely unravelled.<sup>101</sup> The combination of sonidegib (Odomzo, Sun Pharmaceutical Industries), an oral smoothened inhibitor, and ruxolitinib was superior to ruxolitinib alone in decreasing bone marrow fibrosis in a mouse model. This drug combination has been assessed in a multicenter phase 1b/2 study,<sup>102</sup> with approximately 50% of patients exhibiting a reduction in spleen size of at least 35%. Adverse effects were significant, however, with anemia the most common toxicity (grade 3/4 in 33% of patients). Dose adjustment or interruptions were required in 63% of patients overall.



The Aurora A kinase pathway activity is dysregulated in MPNs. A selective Aurora A kinase inhibitor, MLN8237, promoted megakaryocyte polyploidization and differentiation.<sup>103</sup> Fibrosis and allele burden (*JAK2* and *MPL*) were reduced, suggesting a therapeutic potential in PMF. These agents are being investigated in ongoing phase 1 studies.

## Discussion

The management of MF is ever advancing, especially in this genomic era, with a move toward targeted therapies. Ruxolitinib remains the only approved JAK inhibitor, and although it has changed the treatment of MF by mitigating symptoms and potentially prolonging survival in comparison with other non-AHSCT treatments, it has not manifested clear disease-modifying effects or negated the risk for leukemic transformation. This is understandable because MF is genetically complex; in contrast, a single driver mutation in *BCR-ABL*-positive chronic myeloid leukemia can be eloquently targeted with the tyrosine kinase inhibitor imatinib, reducing the malignant clone to a minimally detectable level. Furthermore, the off-target effects of myelosuppression (JAK2 inhibition) and opportunistic infections (JAK1 inhibition) pose challenges during treatment, although they are generally manageable. Other JAK inhibitors in varying stages of development show promise, but to date all share the limitations of ruxolitinib. Therefore, a rational next approach in improving the treatment paradigm would be to combine other targeted therapies—such as HDAC inhibitors and hedgehog inhibitors—with JAK inhibitors to induce synergistic responses. Various combinations in ongoing trials (Table 2) have yet to exhibit superiority to ruxolitinib alone, but these are early studies, and larger phase 3 trials are needed.

AHSCT will continue for now to have a legitimate place in the treatment algorithm, and ruxolitinib has undoubtedly expanded the group of patients who may be transplant-eligible by way of improving performance status, reducing splenomegaly, and decreasing constitutional symptoms.

A subgroup worthy of mention comprises patients with lower-risk MF and high-risk mutations such as *ASXL1*, whose best treatment strategy remains undefined. AHSCT is not advocated for those with low-risk or int-1 disease, and a reduced disease-free survival has been described in those with certain somatic mutations (*ASXL1*, *U2AF1*, *IDH2*, and *DNMT3A*), although this was in a single small retrospective study.<sup>104</sup> IFN has shown potential in early MF, and its conceivable benefit in those with high-risk mutations awaits investigation in further studies.

In conclusion, ruxolitinib continues to be the mainstay in the realm of targeted therapies for MF. Multiple small-molecule inhibitors are emerging and under investigation, either as monotherapy or in combination with JAK inhibitors. Ongoing efforts to improve our understanding of MF pathogenesis and further the development of rational drug targets ensure that the treatment of MF will continue to advance.

## Disclosures

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