New Strategies in Myeloproliferative Neoplasms: The Evolving Genetic and Therapeutic Landscape

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Abstract

The classical BCR-ABL1-negative myeloproliferative neoplasms (MPN) include essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF). Although these clonal disorders share certain clinical and genetic features, MF in particular is distinct for its complex mutational landscape, severe disease phenotype, and poor prognosis. The genetic complexity inherent to MF has made this disease extremely challenging to treat. Pharmacologic JAK inhibition has proven to be a transformative therapy in MPNs, alleviating symptom burden and improving survival, but has been hampered by off-target toxicities and, as monotherapy, has shown limited effects on mutant allele burden. In this review, we discuss the genetic heterogeneity contributing to the pathogenesis of MPNs, focusing on novel driver and epigenetic mutations and how they relate to combination therapeutic strategies. We discuss results from ongoing studies of new JAK inhibitors and report on new drugs and drug combinations that have demonstrated success in early preclinical and clinical trials, including type II JAK inhibitors, antifibrotic agents, and telomerase inhibitors.

Disclosure of Potential Conflicts of Interest

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CME Staff Planners' Disclosures

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Learning Objectives

Upon completion of this activity, the participant should have improved understanding of the similarities and differences between the various classical myeloproliferative disorders from both a clinical and biologic perspective. The participant should appreciate that the molecular alterations that drive myeloproliferative neoplasms (MPN) occur not only in growth signaling pathways such as JAK-STAT, but also encompass epigenetic and transcriptional processes as well, providing a rationale for combination therapy.

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Background

Myeloproliferative neoplasms (MPN) are hematopoietic stem cell diseases characterized by expansion of one or more myeloid lineage with largely intact cellular differentiation.

William Dameshek first recognized the unifying features of chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and agnogenic myeloid metaplasia, now termed myelofibrosis (MF; ref. 1). Of these original MPNs, CML was separated with the discovery of the Philadelphia chromosome, while the term "classical MPN" came to be used for PV, ET, and MF. In 2010, the prevalences of ET, PV, and MF in the United States were 134,000, 148,000, and 13,000, respectively (2). PV and MF are more common in males, while there is a small preponderance for females in ET (3). Risk factors for the development of MPNs include white race and older age (4). Constitutional symptoms are common due to elevated circulating proinflammatory cytokines (5). The MPN Symptom Assessment Form (MPN-SAF) and Total Symptom Score (TSS) help assess symptom severity (Table 1; ref. 6). MPN patients are at 5- to 7-fold higher risk for thrombosis compared...
with the general population (7). Patients with ET, characterized by thrombocytosis, and PV, characterized by erythrocytosis, exhibit microvascular symptoms such as erythromelalgia and Raynaud syndrome. MF patients are typically more debilitated, with marked splenomegaly, profound constitutional symptoms, and severe cytopenias. The risk of transformation to acute myelogenous leukemia (AML) in PV and ET is approximately 8% (8, 9), but nearly 20% in MF. Retrospective analysis of 826 MPN patients from the Mayo Clinic demonstrated median overall survival durations (OS) of 19.8 years for ET, 13.5 years for PV, and 5.8 years for PMF, all shorter than those in control populations (10).

Pathogenesis

In 2005, a somatic mutation in Janus kinase 2 (JAK2) was identified and revolutionized MPN diagnosis, classification, and treatment. The JAK2<sup>V617F</sup> mutation occurs in 95% of PV, 65% of PMF, and 55% of ET cases, respectively (6, 11). Many other somatic mutations have since been identified.

**Growth-factor signaling.** Janus kinases (JAK1, JAK2, JAK3, and TYK2) mediate cytokine signaling via downstream activation of the STAT family of transcriptional regulators (12). Activated STATs promote transcription of genes that regulate multiple cellular functions, including proliferation, apoptosis, migration, and differentiation (13). JAK2 is critical for hematopoiesis and cellular functions, including proliferation, apoptosis, migration, and differentiation (13). JAK2 is critical for hematopoiesis and mediates erythropoietin, GM-CSF, thrombopoietin, growth hormone, leptin, IL3, and IL5 signaling (14, 15). Ligand-binding induces conformational changes to cytokine receptors that result in activation of JAKs, which phosphorylate tyrosine residues on the intracellular receptor domain (12). These residues recruit downstream effectors bearing Src homology-2 or phosphotyrosine-binding domains, which leads to activation of STAT, Ras-MAPK, and PI3K-AKT signaling pathways (12). The pseudokinase domain (IH2) of JAK2 inhibits the catalytic domain (IH1) and prevents activation in the absence of ligand binding. The substitution of valine for phenylalanine at codon 617 (JAK2<sup>V617F</sup>) in IH2 generates a constitutively active ligand-independent kinase by compromising the autoinhibitory function of IH2 (15).

Mutations in JAK2 exon 12 have been identified in approximately 4% of PV cases and are associated with lower platelet and leukocyte counts compared with JAK2<sup>V617F</sup>, while thrombosis and leukemic transformation risk is similar (16).

Approximately 5% of JAK2<sup>V617F</sup>-negative cases of ET, and 10% of JAK2<sup>918T200I</sup>-negative cases of PMF, have activating mutations in the myeloproliferative leukemia virus oncogene (MPL), which encodes the thrombopoietin receptor (17). MPL muta-
tions correlate with lower hemoglobin in ET and MF and higher platelet counts in ET (18). MPL mutations do not increase risk of thrombosis or fibrotic and leukemic transformation (18).

Mutations in the calreticulin gene (CALR), which encodes a calcium-binding endoplasmic reticulum protein, have been identified in up to 80% of ET and MF patients without JAK2 or MPL mutations and entail either a 52-bp deletion (type I) or a 5-bp insertion (type II) in exon 9 that causes a 1-bp frameshift (19). CALR mutations induce JAK–STAT signaling via thrombopoietin receptor activation, are mutually exclusive with JAK2 and MPL mutations, and in MF are associated with improved outcome compared with JAK2 mutations (10, 20–23), although this may be confined to type I mutants (24). CALR mutations in ET are associated with reduced thrombosis risk, though OS and risk of fibrotic transformation are not different from that of JAK2-mutant patients (10, 25–27). Triple-negative MF patients, without JAK2, MPL, or CALR mutations, have the worst outcome (6, 10, 22).

Inactivation of negative regulators of growth-factor signaling is another mechanism of JAK–STAT activation in MPNs. Mutations in the adaptor protein LNK, a negative regulator of JAK2 signaling, have been described in JAK2<sup>918T200I</sup>-negative MF and ET (28, 29). Mutations in Casitas B-cell lymphoma (CBL), a ubiquitin ligase that mediates proteasomal degradation of cytokine receptors, are found in approximately 6% of MF cases (30).

**mRNA splicing.** Loss-of-function mutations in spliceosome genes that regulate mRNA processing have downstream effects similar to those in loss-of-function mutations in cell-cycle regulatory genes and are frequent in MF, but rare in ET and PV (31). Mutations in SR5F2 are reported in 17% of MF cases and associated with advanced age, high-risk disease, and shorter leukemia-free and OS (32). Mutations in U2AF1 are reported in 15% of PMF patients and associated with anemia and thrombocytopenia (33, 34). Spliceosome mutations are more prevalent in PMF than in secondary MF (35).

### Table 1. Assessing symptom burden in MPNs

<table>
<thead>
<tr>
<th>MPN-SAF TSS&lt;sup&gt;a&lt;/sup&gt; Score</th>
<th>PV (538 pts)</th>
<th>ET (594 pts)</th>
<th>MF (293 pts)</th>
<th>ET-PV-MF (1,425 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue in last 24 hours</td>
<td>(None) 0–10 (extreme)</td>
<td>88 (4.4)</td>
<td>87 (4.1)</td>
<td>96 (5.0)</td>
</tr>
<tr>
<td>Early satiety</td>
<td>(None) 0–10 (extreme)</td>
<td>64 (2.5)</td>
<td>59 (2.2)</td>
<td>77 (3.2)</td>
</tr>
<tr>
<td>Trouble with concentration</td>
<td>(None) 0–10 (extreme)</td>
<td>65 (2.7)</td>
<td>59 (2.3)</td>
<td>69 (2.6)</td>
</tr>
<tr>
<td>Inactivity</td>
<td>(None) 0–10 (extreme)</td>
<td>61 (2.4)</td>
<td>56 (1.9)</td>
<td>74 (3.1)</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>(None) 0–10 (extreme)</td>
<td>51 (1.6)</td>
<td>50 (1.7)</td>
<td>66 (2.5)</td>
</tr>
<tr>
<td>Night sweats</td>
<td>(None) 0–10 (extreme)</td>
<td>52 (2.1)</td>
<td>50 (2.0)</td>
<td>62 (2.6)</td>
</tr>
<tr>
<td>Pruritus/itching</td>
<td>(None) 0–10 (extreme)</td>
<td>62 (2.8)</td>
<td>46 (1.7)</td>
<td>50 (2.0)</td>
</tr>
<tr>
<td>Generalized joint pain</td>
<td>(None) 0–10 (extreme)</td>
<td>50 (2.0)</td>
<td>46 (1.7)</td>
<td>52 (2.2)</td>
</tr>
<tr>
<td>Unintentional weight loss</td>
<td>(None) 0–10 (extreme)</td>
<td>31 (1.0)</td>
<td>24 (0.8)</td>
<td>42 (1.7)</td>
</tr>
<tr>
<td>Fever &gt;100°F</td>
<td>(None) 0–10 (daily)</td>
<td>18 (0.4)</td>
<td>17 (0.3)</td>
<td>22 (0.5)</td>
</tr>
<tr>
<td>TSS score (0–100)</td>
<td>(None) 0–10 (extreme)</td>
<td>21.8 (10)</td>
<td>18.7 (10)</td>
<td>25.3 (10)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Myeloproliferative Symptom Assessment Form Total Symptom Score.

Epigenetic modifiers. Mutations in epigenetic modifiers often accompany growth-factor signaling mutations and complicate MPN risk-stratification and prognostication. DNA methyltransferases, such as DNMT3A, attach methyl groups to gene promoter or enhancer sequences, suppressing transcription. DNMT3A mutations occur in 15% of MF patients, 7% of PV patients, and 3% of ET patients (36). Murine models show that DNMT3A mutations confer a proliferative advantage to myeloid cells (37).

TET proteins convert 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) and subsequently oxidation products that are differentially recognized by epigenetic regulators. Conversion of 5mC to 5hmC prohibits the maintenance of existing DNA methylation patterns and leads to passive DNA demethylation in proliferating cells (38). TET2 mutations occur in 12% to 14% of MPN cases (39, 40).

Polycomb-repressive-complex-2 (PRC2) governs gene expression through post-translational modification of histones (41). Loss-of-function mutations in additional-of-sex-combs-like-1 (ASXL1), which encodes a protein that interacts with PRC2, cause loss of PRC2-mediated gene repression, resulting in enhanced oncogene activity (42). ASXL1 mutations are present in approximately 4% of ET, 7% of PV and 20% of MF patients and are associated with a poor prognosis in MF (39, 43). Loss-of-function mutations in enhancer-of-zeste homolog-2 (EZH2), which encodes the catalytic subunit of PRC2, occur in 13% of MF patients and are associated with poor leukemia-free and OS (44). Isocitrate dehydrogenase (IDH) gain-of-function mutations occur in 4% of MF, 2% of PV, and 1% of ET patients (45). IDH1 and IDH2 catalyze the conversion of isocitrate to α-ketoglutarate. Mutant IDH enzymes are associated with an adverse prognosis in MF and produce the oncometabolite 2-hydroxyglutarate, which inhibits the function of epigenetic modifiers (43).

Management: PV and ET

Therapy goals in PV and ET are to minimize thrombomelbic and bleeding events, prevent development of secondary MF, and mitigate constitutional symptoms. The International Prognostic Score for Thrombosis in Essential Thrombocythemia (IPSET) predicts survival and risk of thrombosis in ET (46). In PV, a risk score based on age, leukocytosis, and history of venous thrombosis predicts survival (8).

Antiplatelet therapy. Low-dose aspirin significantly reduces vascular events in PV and is regarded as standard-of-care therapy across all risk categories (47). In ET, aspirin is of little benefit for the majority of low-risk patients, with the exception of those with JAK2 mutations, cardiovascular risk factors, or vasomotor symptoms (48, 49). High-risk ET patients should receive aspirin, unless there are contraindications. Microvascular symptoms are highly responsive to aspirin therapy.

Cytoreduction. In low-risk PV patients, phlebotomy remains an important and benign option to reduce vascular events. The CYTO-PV trial demonstrated that maintaining hematocrit <45% decreases mortality from cardiovascular causes or major thrombosis by 4-fold (50). Cytoreduction with hydroxyurea (HU), in combination with low-dose aspirin, lowers thrombotic risk in high-risk PV and ET (6). The leukemogenicity of single-agent HU is controversial, although multiple studies suggest this concern is unsubstantiated (51–53). The ANAHYDRET study demonstrated noninferiority of anagrelide to HU in lowering thrombotic risk in WHO-classified high-risk ET, but the larger UK-FT1 trial found anagrelide is more often associated with arterial thrombosis, hemorrhage, and myelofibrotic transformation (2, 6, 54, 55).

IFNα is the only drug capable of inducing profound reductions in JAK2V617F allele burden in MPN patients (56). A phase II trial of PegIFNα in PV and ET reported complete hematologic responses (CHR) in 70% of patients and complete molecular responses in 18% (57). Prolonged exposure to PegIFNα is necessary for achievement of deep molecular responses, whereas hematologic responses are observed in the first 3 months of therapy (58). Two phase III randomized trials (NCT01259856 and NCT01949805) will directly compare PegIFNα versus HU in PV and ET.

JAK2 inhibitors. The RESPONSE trial randomized phlebotomy-dependent PV patients with resistance to or intolerance of HU to ruxolitinib versus best available therapy (BAT; ref. 59). CHR was achieved in 24% of patients in the ruxolitinib group and 9% in the control group. Forty-nine percent of patients in the ruxolitinib group had 50% reduction in TSS at week 32 compared with 5% with BAT. Spleen volume was reduced in 38% of ruxolitinib patients compared with 1% with standard therapy. Ruxolitinib is now approved as salvage therapy for PV. Results from a phase II study of ruxolitinib in ET patients intolerant of or unresponsive to HU demonstrated efficacy in reducing spleen size, improving symptoms, and decreasing leukocyte and platelet counts, suggesting that the drug may be a salvage option in ET as well (60).

Management: MF

Goals of therapy in MF include reducing excessive myeloid proliferation and constitutional symptoms, ameliorating cytopenias, and minimizing risk of leukemic transformation. Prognostic tools have evolved to encompass the International Prognostic Scoring System (IPSS), the Dynamic International Scoring System (DIPSS), and the DIPSS-plus (61–63).

Cytoreduction. Before JAK inhibitors, treatment of myelofibrosis was reliant upon HU, which effectively controls splenomegaly, but exacerbates cytopenias. Alkylators have been used with success in HU-refractory patients, but are associated with myelosuppression and increased risk of AML (52). Splenectomy relieves symptoms associated with massive splenomegaly and improves cytopenias from splenic sequestration, but perioperative mortality ranges from 3% to 10% (64–66). Splenic irradiation is an option for poor surgical candidates, but causes profound cytopenias.

Anemia. Drugs to improve MF-associated anemia include immunomodulatory drugs (IMiD), erythropoietin-stimulating agents (ESA), androgens and steroids. Phase II studies of thalidomide have reported anemia response rates of 20% to 30% (67–69). Lenalidomide can improve anemia, thrombocytopenia, and splenomegaly, and is effective in patients with del(5q), in whom cytogenetic and molecular responses have been reported (70). A phase II trial of low-dose pomalidomide demonstrated a disappointing 17% anemia response rate (71). ESAs, danazol, and single-agent corticosteroids have yielded response rates between 20% and 40% (72–74).

Ruxolitinib. In 2011, two randomized trials, COMFORT-I and COMFORT-II, led to FDA approval of ruxolitinib, an oral
JAK1/2 kinase inhibitor, to treat intermediate- and high-risk MF. In COMFORT-I, ruxolitinib was compared with placebo in intermediate-2 or high-risk MF. The primary endpoint, reduction in spleen volume by ≥35% at 24 weeks, was achieved by 41.9% of patients in the ruxolitinib group compared with 0.7% in the control arm (75). There was suggestion of survival benefit, although the study was not designed to analyze survival. Grade 3/4 anemia and thrombocytopenia occurred in 45% of patients on ruxolitinib compared with 13% on placebo. COMFORT-II compared ruxolitinib with BAT and reported superior results with ruxolitinib in improving symptoms and decreasing spleen size, with diarrhea being the most frequent nonhematologic toxicity. Three-year efficacy and survival data from COMFORT-II showed durable reductions in spleenomegaly and 52% reduction in mortality with ruxolitinib compared with BAT (76, 77). Pooled analysis of COMFORT-I and COMFORT-II findings confirmed improved survival for ruxolitinib-treated patients compared with controls (78). Nevertheless, ruxolitinib’s effect on JAK2V617F allele burden is modest. Three-year follow-up data from COMFORT-II revealed a median 8% decrease in allele burden from baseline after 72 weeks of ruxolitinib compared with no change with BAT (76). A recent update of COMFORT-I revealed a more substantial reduction in a subset of patients (79). Ruxolitinib’s survival benefit may result from improved performance status due to reduced cytokine levels (80).

**Newer JAK inhibitors.** Ruxolitinib’s dose-limiting myelosuppression and lack of profound molecular responses have stimulated development of alternative JAK inhibitors. The attraction of newer JAK inhibitors resides in their improved selectivity for particular JAK isoforms and for JAK2V617F over JAK2WT, with the goal of minimizing off-target effects from wild-type JAK inhibition. Development of the JAK2 inhibitor fedratinib was discontinued after reports of encephalopathy in the phase III JAKARTA study. AZD1480, CEP-701, BMS-911543, LY2784544, and INCBO39110 have all been phased out of clinical development for MPNs due to various toxicities and, in some cases, insufficient efficacy (Table 2).

A phase II trial of the JAK 1/2 inhibitor momelotinib in MF reported reductions in spleenomegaly and symptoms comparable with those for ruxolitinib (81). Anemia responses were impressive, with 70% of transfusion-dependent patients achieving independence by the International Working Group-Myelofibrosis Research and Therapy (IWG-MRT) criteria. Low-grade irreversible peripheral neuropathy occurred in 44% of patients and Grade 3/4 thrombocytopenia in 30% (82). Momelotinib’s effect on JAK2 mutant allele burden appears to be similar to that for ruxolitinib. A phase III trial directly comparing momelotinib and ruxolitinib in JAK-inhibitor–naïve MF patients is ongoing (NCT01969838).

Pacritinib is selective for JAK2 over other JAKs, but has activity against FLT3. Modest myelosuppression was noted in a phase II study in MF with no eligibility restrictions on thrombocytopenia or anemia (83). PERSIST-1 and PERSIST-2 are phase III trials investigating pacritinib’s efficacy compared with that of BAT in MF patients with baseline thrombocytopenia. Preliminary data from PERSIST-1 show anemia responses and good tolerance in patients with baseline thrombocytopenia <50,000/μL (84). Spleen volume reduction ≥35% at week 24 was 19% in the pacritinib arm and 5% in the BAT arm.

NS-018 is a JAK2–Src inhibitor undergoing phase II testing in MF. In preclinical studies NS-018 exhibited 4.3-fold selectivity for JAK2V617F over JAK2WT. In a murine model of JAK2V617F–positive myelofibrosis, NS-018 reduced leukocytosis and splenomegaly, reversed marrow fibrosis, and improved survival (85). Phase I testing demonstrated an acceptable safety profile, although over 30% of patients discontinued treatment due to adverse effects or progressive disease (86).

**Transplant.** Allogeneic stem cell transplant is the only curative option for MF and should be considered in patients with intermediate-2 or high-risk disease, who have an expected survival of less than 5 years. Following transplant, the long-term OS rate for these patients generally ranges from 30% to 50% (87–91). Nonmyeloablative regimens may further improve survival (87, 92). Low-risk MF patients do not benefit from transplant, as transplant-related mortality exceeds disease-related mortality with conventional therapies (93).

**On the Horizon**

**Type II JAK2 inhibitors.** Although ruxolitinib has revolutionized treatment of MF and other MPNs, its ability to decrease mutant JAK2 allele burden has been underwhelming (94). Persistence of JAK2-mutant clones despite JAK2 inhibition does not involve mutations of the target kinase, but activation of JAK2 in trans by other JAK kinases, allowing for reactivation of JAK–STAT signaling via heterodimerization between activated JAK2 and JAK1 or TYK2 (95). Although mutant clones are functionally impaired, the JAK2 inhibitor therapy, they remain dependent on JAK2 expression. These findings have been confirmed not only with ruxolitinib, but also with other JAK2 inhibitors, including CYT387 (momelotinib), BMS911543, and SAR302503 (96). It is noteworthy that all JAK2 inhibitors in clinical development are type I inhibitors, competing with ATP for the drug-binding pocket while JAK2 is in its active conformation (Fig. 1). The ability of JAK2V617F-type I inhibitor complexes to interact with other JAK kinases presents a mechanistic limitation for these drugs. NVP-CHZ868, a novel type II JAK inhibitor that binds the inactive kinase, completely suppressed JAK–STAT signaling in type I JAK inhibitor–resistant cells and resulted in significant reductions in mutant allele burden in murine models of PV and MF (96). Attrition of the mutant clone occurred in tandem with normalization of blood counts, splenomegaly, and bone marrow fibrosis, suggesting that type II JAK inhibitors have clinical potential.

**Antifibrotic agents.** Pentraxin-2 (PTX-2) is a plasma protein that acts at sites of tissue damage via FcγRs to inhibit differentiation of monocytes into fibrocytes, reduce neutrophil adhesion, and promote phagocytosis (97). PTX-2 levels are lower in MF patients compared with age-matched healthy controls, and PTX-2 levels further decline with increasing fibrosis grade. Preliminary results from a phase II trial in MF investigating PRM-151, a recombinant human pentraxin-2, in combination with ruxolitinib reported a 35% response rate with 4 IWG-MRT symptom clinical improvements and 6 bone marrow fibrosis responses in 26 evaluable patients (98). Responses were also seen with single-agent PRM-151. The FDA granted PRM-151 fast-track designation for MF in late 2014.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Off-targets</th>
<th>Phase</th>
<th>Current status</th>
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<tbody>
<tr>
<td>Momelotinib (CYT387)</td>
<td>JAK1, JAK2</td>
<td>TYK2</td>
<td>3</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Pacritinib (SB1518)</td>
<td>JAK2, FLT3</td>
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</tr>
<tr>
<td>NS-018</td>
<td>JAK2, Src family</td>
<td></td>
<td>1/2</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Fedratinib (SAR302503)</td>
<td>JAK2, FLT3, RET</td>
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<td>Drug development terminated due to adverse neurologic sequelae, including Wernicke encephalopathy</td>
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<tr>
<td>AZD1480</td>
<td>JAK1, JAK2</td>
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<td>Study terminated following observed low-grade neurologic toxicity</td>
</tr>
<tr>
<td>Lestaurtinib (CEP701)</td>
<td>JAK2, FLT3, TrkA</td>
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<td>Phased out of development in MF due to unacceptable gastrointestinal toxicity and narrow therapeutic window</td>
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<tr>
<td>BMS-911543</td>
<td>JAK2</td>
<td></td>
<td>1/2</td>
<td>No longer in clinical development for MF due to high rate of drug discontinuation rate and questionable efficacy</td>
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<tr>
<td>Gandotinib (LY2784544)</td>
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<td>No longer in clinical development for MF; high rate of renal insufficiency, tumor lysis</td>
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<td>INCB039110</td>
<td>JAK1</td>
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<td>No longer in clinical development for MF</td>
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</table>
Inhibition of TGFβ1 signaling in the GATA-1<sub>low</sub> mouse model of MF restores normal hematopoiesis and reduces bone marrow fibrosis, implicating TGFβ as a target in MF (99). A phase I trial in MF using fresolimumab, a monoclonal antibody against TGFβ, was terminated early due to drug supply issues despite producing significant reductions in TGFβ1 levels in the 2 evaluable patients (100, 101).

Telomerase inhibitors

The telomerase inhibitor imetelstat selectively inhibits megakaryocytic colonies from ET patients. A phase II study of imetelstat in refractory ET showed CHR in 89% of patients, with molecular responses demonstrated in 88% of JAK2<sup>V617F</sup> positive patients (102). Results from a phase I/II trial examining imetelstat in MF demonstrated a 21% overall response rate, including several complete responses by IWG-MRT (103). Complete molecular responses and reversal of bone marrow fibrosis were noted in the 75% and 100% of patients achieving CR, respectively. Grade 4 neutropenia and thrombocytopenia are common with imetelstat, suggesting that myelotoxicity may limit use of this agent.

Immunologic targets

JAK2<sup>V617F</sup>-allele burden positively correlates with TNFα expression, and TNFα selectively stimulates colony formation by JAK2<sup>V617F</sup>-positive progenitor cells (104). Etanercept, a soluble TNF receptor 2 (TNFR2), has been shown to ameliorate constitutional symptoms in MF (105). Preliminary data show that JAK2<sup>V617F</sup> clonal dominance in MF may be mediated via TNFR2, suggesting that TNFR2 blockade may have therapeutic potential in MPNs (106).

Eph receptors are tyrosine kinases which interact with their ligands to modulate cell adhesion, motility, and shape. The Eph receptor EPHA-3 is overexpressed in various hematologic malignancies, including MPNs. KB004, a monoclonal EPHA-3 antibody, is in early clinical testing for hematologic malignancies, including MF (107).

Ruxolitinib combinations

There is considerable interest in combining JAK inhibitors with other agents to improve responses.

Hedgehog inhibitors. Hedgehog (Hh) proteins activate signaling by binding to patched homolog 1 (PTCH1), releasing the G-protein coupled receptor Smoothened (SMO) from PTCH1 inhibition. Hh targets regulate stem cell survival, differentiation, and proliferation (108). Hh activation through gain-of-function mutations in SMO or loss-of-function mutations in PTCH1 occurs in various solid tumors (108). Hh signaling has been demonstrated in MPN granulocytes and is probably due to extrinsic signals (109). Several SMO inhibitors are in clinical development, including sonidegib (LDE225), PF-04449913, and saridegib (IPI-926; ref. 110). On the basis of preclinical activity in a murine model, ruxolitinib was combined with sonidegib in a phase 1b study of MF; the combination was well tolerated and reduced spleen size by ≥50% in 65% of patients (111, 112). A clinical trial of PF-04449913 in MF is ongoing, while a phase II trial of saridegib was discontinued after failing prespecified activity criteria (109).
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**Epigenetic modifiers.** Mutations in epigenetic modifiers alter gene expression and contribute to MPN pathogenesis and transformation. Histone deacetylase (HDAC) inhibitors and hypomethylating agents target epigenetic dysregulation. In a murine MPN model the combination of the HDAC inhibitor panobinostat with ruxolitinib had superior effects on spleen volume and bone marrow histology than either agent alone (115). A phase II trial investigating this combination reported spleen volume reductions similar to those from single-agent ruxolitinib in the two COMFORT trials (116). JAK2V617F allele burden decreased ≥20% in 29% of patients, and bone marrow fibrosis improved in 3 patients. Hypomethylating agents such as 5-azacitidine and decitabine have been used as monotherapy for MF, but response rates tend to be low (117, 118). As 5-azacitidine and decitabine have been used as monotherapy for MF, but response rates tend to be low (117, 118). A phase II study of ruxolitinib and 5-azacitidine in MF and MDS/MPN patients is under way (NCT01787487). Decitabine and ruxolitinib are being investigated in a phase I/II trial examining their use in accelerated-phase MPN and post-MPN AML (NCT01787487). Decitabine and ruxolinitob in the two COMFORT trials (116). JAK2V617F allele burden decreased ≥20% in 29% of patients, and bone marrow fibrosis improved in 3 patients. Hypomethylating agents such as 5-azacitidine and decitabine have been used as monotherapy for MF, but response rates tend to be low (117, 118). A phase II study of ruxolitinib and 5-azacitidine in MF and MDS/MPN patients is under way (NCT01787487). Decitabine and ruxolitinib are being investigated in a phase I/II trial examining their use in accelerated-phase MPN and post-MPN AML (NCT02076191). Aneima requiring transfusion developed in 5 of 6 MF patients treated with pomalidomide and ruxolitinib in a phase Ib trial (120). Other efforts to minimize ruxolitinib-induced myelosuppression include combination treatment with erythropoiesis-stimulating agents (ESA) and androgens. Post hoc analysis of COMFORT-II patients who received ESAs with ruxolitinib demonstrated 23% of patients actually had an increase in transfusion requirement with concomitant ESA (121). Interim results from a phase II trial of ruxolitinib and danazol in MF demonstrated minimal benefit (122).

**Conclusions**

Ruxolitinib has changed the treatment paradigm in MF and represents the first standard-of-care treatment for intermediate- and high-risk patients. However, the effect of ruxolitinib and presumably other type I JAK2 inhibitors on mutant allele burden is modest, and most of the therapeutic benefit likely results from control of inflammatory cytokines as drivers of morbidity. Whether there is a true disease-modifying effect and whether greater and more consistent suppression of JAK–STAT signaling with type II inhibitors will overcome these limitations remains to be seen. Ultimately, therapeutic strategies engaging single transduction pathways may not be effective in classical MPNs, particularly the genetically complex MF. Combination therapy appears to be promising, but long-term safety and efficacy data are needed.

**Authors’ Contributions**

Conception and design: M.W. Deininger

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