Therapeutic Potential of Janus-activated Kinase-2 Inhibitors for the Management of Myelofibrosis

Srdan Verstovsek

Abstract

Myelofibrosis (either primary or postpolycythemia vera/essential thrombocytopenia) is a chronic and debilitating myeloproliferative neoplasm for which there is no well-accepted standard of care. Clinical manifestations of this disease (e.g., cytopenias, splenomegaly, bone marrow fibrosis) and constitutional symptoms (e.g., hypercatabolic state, fatigue, night sweats, fever) create significant treatment challenges. For example, progressive splenomegaly increases the risk for more serious clinical sequelae (e.g., portal hypertension, splenic infarction). Myelofibrosis arises from hematopoietic stem cells or early progenitor cells. However, the molecular mechanisms underlying its pathogenesis and clinical presentation are poorly understood, delaying the development of effective and targeted treatments. Recent studies have implicated mutations that directly or indirectly lead to the deregulated activation of Janus-activated kinase 2 (JAK2). Appreciation for the activation of JAK2 and the importance of increased levels of circulating proinflammatory cytokines in the pathogenesis and clinical manifestations of myelofibrosis has led to novel therapeutic agents targeting JAKs. This review will briefly discuss the origins of the JAK2 hypothesis, the clinical relevance of JAK2 mutations in myelofibrosis, and recent clinical progress in targeting JAKs as a therapeutic intervention for patients with this chronic and debilitating disease.

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Translational Relevance

Primary myelofibrosis and postpolycythemia vera and post-essential thrombocythemia myelofibrosis are chronic and debilitating myeloproliferative neoplasms for which there is no accepted standard of care. Recent studies have implicated a somatic gain-of-function mutation in the Janus-activated kinase 2 (JAK2), namely JAK2V617F, in the pathophysiology and clinical manifestations of myelofibrosis and spurred the development of molecularly targeted agents (JAK2 inhibitors) as therapy for this condition. This review explores the origins of the JAK2 hypothesis, the central role that alterations in signaling through the expression of the JAK2V617F mutation play in clinical manifestations of myelofibrosis, and recent clinical progress in targeting JAK2s as a therapeutic intervention for patients with this disease. Available clinical evidence provides a strong rationale for targeted modulation of dysregulated JAK signaling as an effective treatment approach in patients with myelofibrosis.

Table 1. MF diagnostic criteria

<table>
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<th>Criteria for PMF*</th>
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<tr>
<td><strong>Major criteria</strong></td>
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<tr>
<td>1. Megakaryocyte proliferation and atypical morphology,* typically accompanied by bone marrow fibrosis (i.e., increased collagen or reticulin staining)†</td>
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<tr>
<td>2. Does not meet WHO criteria for PV, chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasm</td>
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<td>3. Presence of JAK2 V617F or other clonal marker (e.g., MPL W515L&gt;K)¶</td>
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<tr>
<td><strong>Minor criteria</strong></td>
</tr>
<tr>
<td>1. Leukoerythroblastosis**</td>
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<td>2. Serum level of lactate dehydrogenase increased**</td>
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<td>3. Anemia**</td>
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<td>4. Palpable splenomegaly**</td>
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*Revised WHO criteria for PMF modified from (13); diagnosis requires meeting all 3 major criteria and 2 minor criteria.
†Dense clustered megakaryocytes with abnormally increased nuclear/cytoplasmic ratio and hyperchromatic and round or irregularly folded nuclei. In the absence of overt reticulin fibrosis, increased megakaryocyte proliferation must be accompanied by granulocyte proliferation, with or without erythropoiesis.
¶In the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis.
**May present as borderline to marked abnormality.

May present as borderline to marked abnormality.

infection) and mortality (approximately 28% and 7%, respectively; ref. 20).

Current treatment strategies for MF include experimental drug therapy, stem cell transplantation, and conventional drugs that were approved for other indications but not for MF (21). Conventional medications are largely palliative and rarely provide durable benefits, whereas stem cell transplantation is restricted to a small percentage of patients. These limitations underscore the need to develop more effective disease-targeted therapeutic approaches in patients with MF.

Origins of the JAK2 Hypothesis in MF

The first evidence implicating mutated JAK2 in patients with Ph-negative MPNs came from genetic and functional
studies of hematopoietic progenitor cells, which frequently contained a mutation (V617F) of somatic origin (22–26). The acquired mutation in JAK2 implicated in the pathogenesis of Ph-negative MPNs is a point mutation in the negative autoregulatory pseudokinase domain (V617F) that lies adjacent to the tyrosine kinase domain (22): this mutation results in constitutive activation of the kinase and increased sensitivity to cytokine signaling (24). It promotes increased cell survival, cell cycle transition, and differentiation in progenitor cells (Fig. 2). Additional strong evidence supporting a role of JAK2 in MF comes from several independent studies using mouse models. Transgenic mice that constitutively expressed JAK2V617F in hematopoietic progenitor cells exhibited leukocytosis, erythroblastosis, marked thrombocytosis, and hemoglobin levels consistent with anemia when examined at 1 month of age (27). These mice developed marked splenomegaly with the severity of the phenotype correlated with the level of expression of the mutant transgene. Transgenic mice expressing low levels of JAK2V617F (i.e., low mutant allele burden) mimicked ET and those with higher mutant allele burden developed PV (28). Aged mice expressing high levels of JAK2V617F eventually developed MF (27, 29). Although JAK2V617F is expressed by >50% of patients with ET and >90% of patients with PV, the role of this mutation in the progression of ET or PV to MF in patients is not well understood. In PV and ET, the risk of developing MF increases with the duration of disease (e.g., in patients with ET progression to MF occurs in 4%, 20%, and 29% at 10, 20, and 30 years, respectively), and the risk is greater in patients who harbor mutated compared with wild-type JAK2 (5, 24, 30).

Mutated JAK2V617F is not the only genetic alteration, however, associated with the development of MF. Some MF patients who are JAK2V617F negative harbor a point mutation (tryptophan to leucine substitution) in the thrombopoietin receptor (i.e., MPLW515L) that confers increased sensitivity to thrombopoietin through the activation of wild-type JAK2/signal transducers and activators of transcription (STAT) signaling and induces a spectrum of features of MF when expressed in a mouse model (31). Additional mutations have recently been identified in the MPL receptor including S204P, S505N, K39N, and W515K/L, and in exon 12 (H538-K539delinsL) of JAK2 in patients negative for V617F, suggesting that there are multiple paths that may lead to elevated JAK2 signaling and the development of MPN (32, 33). These results suggest that drugs in development that target JAK2 may be beneficial for MF patients with different molecular/genetic background. JAK2 is a member of the JAK family of cytosolic tyrosine kinases that also includes JAK1, JAK3, and tyrosine kinase 2 (TYK2); for an in-depth review, see Yamaoka et al. (34). The ligands for these receptors include erythropoietin, thrombopoietin, granulocyte-macrophage...
colony-stimulating factor, growth hormone, IFNs, ILs, and many other cytokines. JAKs are used singly or in combination by different cytokine receptor systems to activate specific STATs that regulate gene transcription (35). For example, in response to erythropoietin or thrombopoietin receptor binding, JAK2-mediated activation of STAT3 or STAT5 supports the survival, proliferation, and differentiation of erythroid cells (36) or megakaryocyte-erythroid progenitor cells, respectively (37).

Clinical Relevance of JAK2V617F in MF

Acquisition of the gain-of-function JAK2V617F somatic mutation in patients with Ph-negative MPNs is harbored by ~50% of patients with ET and MF, and 95% of patients with PV (38). The presence of JAK2V617F may be a risk factor for morbidity and survival in PMF. Univariate analysis showed that patients with JAK2V617F mutation compared with JAK2-wild-type had a significantly increased risk of all-cause mortality and splenomegaly, and the majority of patients who required splenectomy were homozygous for the V617F mutation (39). In a more recent analysis, JAK2V617F allele burden (ratio between JAK2V617 DNA and total JAK2 DNA) was significantly and directly correlated with splenomegaly and constitutional symptoms but inversely correlated with overall survival, and the relationship to survival was manifested in a multivariate analysis (40). A third study revealed a significantly shorter overall survival for PMF patients in the lowest-quartile JAK2V617F allele burden group, and such patients were at increased risk for transformation compared with patients in upper quartiles (41).

JAK2 Inhibitors in Clinical Development

The discovery of JAK2 mutations coupled with the known biology of JAK signaling provided a great deal of enthusiasm for the development of JAK2 inhibitors for the treatment of Ph-negative MPNs. Drugs currently in clinical development exhibit differential inhibitory activity against JAK family members (Table 2), and some exhibit effects on other receptor kinases (e.g., fibroblast growth factor receptor, platelet-derived growth factor receptor, Trk, vascular endothelial growth factor receptor, and FLT3; refs. 42–44).
INCB018424. INCB018424 is the first JAK2 inhibitor to enter phase III clinical trials in patients with PMF and post-ET or post-PV MF. It is a potent and selective inhibitor of JAK1 and JAK2 (IC_{50} of 3.3 and 2.8 nmol/L, respectively), and it shows modest selectivity against TYK2 (≈6-fold) and excellent selectivity (≥130-fold) against JAK3 (Table 2; ref. 45). In preclinical studies, INCB018424 inhibited the proliferation of FDCP and BaF/3 cells expressing mutated JAK2 at IC_{50} values of 100 to 130 nmol/L (46). Selectivity of INCB018424 was further shown by the lack of inhibitory effect in TF-1 cells transformed with BCR-ABL or cell lines expressing activating mutations in c-KIT (45). In a murine model of JAK2V617F-driven malignancy, INCB018424-treated mice exhibited decreased spleen growth and increased survival compared with control mice, outcomes that were accompanied by significant decreases in the circulating levels of IL-6 and tumor necrosis factor-α (45), proinflammatory cytokines that have been implicated in the pathogenesis of Ph-negative MPNs. Results of a phase I/II clinical study with 153 MF patients show that INCB018424 produces a profound reduction in splenomegaly and improvement of constitutional symptoms regardless of JAK2V617F mutational status (47). Clinical responses have been maintained over the entire duration of treatment, and most patients (115 of 153 patients; 74%) remain on therapy after a median follow-up of 16 months. Thrombocytopenia was identified as the dose-limiting toxicity occurring in ≈30% of patients at the 25-mg twice daily dose, which may be related to the inhibition

<table>
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<tr>
<th>Inhibitor</th>
<th>Structure</th>
<th>Relative inhibitory activity, nmol/L</th>
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<tr>
<td>CEP701</td>
<td></td>
<td>JAK1 1 JAK2 NA JAK3 NA TYK2 NA</td>
</tr>
<tr>
<td>INCB018424</td>
<td></td>
<td>3.3 2.8 428 19</td>
</tr>
<tr>
<td>TG101348</td>
<td></td>
<td>105 3 996 405</td>
</tr>
<tr>
<td>XL019</td>
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<td>130 2 250 340</td>
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Table 2. Comparative kinase activity of JAK2 Inhibitors in clinical development (45, 51, 62, 66, 67)

Abbreviation: NA, not available.
of JAK2-dependent thrombopoietin signaling. Treatment resulted in a significant reduction in several proinflammatory cytokines (e.g., IL-1, tumor necrosis factor-α, and IL-6), and angiogenic and fibrogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor (48). Further optimization of the dosing regimen used baseline platelet count to determine the starting dose (10 or 15 mg twice daily) and allowed dose titration after 1 and 2 months of therapy; most patients were optimized to 15 or 20 mg twice daily (47). This approach significantly reduced the incidence of thrombocytopenia (<5%, n = 33) while providing equivalent efficacy to higher dose regimens. With an optimized dosing regimen, spleen volume reduction was evident in as early as 1 month, and durable over 6 months of therapy (33% median decrease after 6 months, n = 23). Based on magnetic resonance imaging, 11 of 23 (48%) of patients achieved a 35% spleen volume reduction (equivalent to the International Working Group clinical improvement criteria of 50% reduction by palpation) from baseline at month 6. Progression to acute myeloid leukemia occurred in three patients, which is below the expected frequency based on published data (39). Treatment with INCB018424 improved the exercise capacity of MF patients as measured by the 6-minute walk test, with median increase from baseline of 33, 58, and 70 meters after 1, 3, or 6 months of therapy, respectively. Rapid and durable reduction of a total symptom score based on key symptoms (fatigue, abdominal discomfort/pain, bone/muscle pain, night sweats, and pruritus) was noted (51% and 58% of patients achieving 50% reduction in the total score following 1 and 6 months of treatment, respectively). Improvement in symptoms coincided with a rapid and sustained reduction in proinflammatory cytokines, including IL-1β, IL-1ra, IL-6, and tumor necrosis factor-α, without tachyphylaxis to therapy (47, 49). Although significant clinical improvements have consistently been observed, only modest reductions in the JAK2V617F allele burden have been noted (13% in the marrow and 9% in the peripheral blood; ref. 50). These results suggest that the clinical benefits of INCB018424 treatment may be the result of the inhibition of aberrant JAK2 signaling and possibly the inhibition of JAK1 signaling, rather than the elimination of mutant cell clones. Phase III registration studies of INCB018424 recently initiated in the United States and Europe should lead to increased understanding of the therapeutic role for INCB018424 in the clinical management of MF.

**CEP-701.** CEP-701, also known as lestaurtinib, a staurosporine analogue derived from the indolocarbazole K252, is a multikinase inhibitor that targets JAK2 (51) in addition to FLT-3, platelet-derived growth factor receptor, vascular endothelial growth factor receptor 2, and Trk-A with an IC_{50} in the nmol/L range (Table 2; refs. 42–44, 52, 53). CEP-701 at concentrations of 50 to 100 nmol/L caused nearly complete inhibition of RET autophosphorylation (54). The activity of CEP-701 has been well characterized in preclinical studies. Clinical trials in patients with acute myeloid leukemia established a recommended CEP-701 oral dose of 80 mg twice daily (55), and this inhibitor is currently being tested in patients with JAK2V617F-positive MPNs (56, 57). In a study of previously treated patients (n = 22) who were mostly transfusion dependent at study entry, had splenomegaly (median spleen size of 19 cm below costal margin), had a median JAK2V617F allele burden of 53%, and had undergone treatment with CEP-701 for a median duration of 4 months, 6 of 22 (27%) experienced clinical improvement as defined by the International Working Group for Myelofibrosis Research and Treatment (56, 58). Responses included a reduction in spleen size alone (three patients), transfusion independence (two patients), and a reduction in spleen size together with improvement in neutrophil counts and platelets (one patient). The median time to response was 3 months, and the median duration of response was 14+ months (range, 3-17+). No changes were observed in JAK2V617F allele burden, bone marrow fibrosis, or cytogenetics during therapy. Drug-induced adverse events included anemia (grades 3-4, 14%), thrombocytopenia (grades 3-4, 23%), and diarrhea (all grades, 72%; grades 3-4, 9%).

However, it should be emphasized that results described here with CEP-701 used a liquid formulation because of issues with the solid dosage formulation. A new capsule formulation is currently being tested in a phase 1 dose-escalation study in MF (59).

**XLO19.** XLO19 is a potent and selective inhibitor of JAK2 kinase (IC_{50}, 2 nmol/L) that showed a >10-fold selectivity for inhibition of STAT5 phosphorylation following erythropoietin stimulation of erythroid cells (IC_{50}, 64 nmol/L) and suppressed STAT phosphorylation by 50% at a dose of 42 mg/kg in a mouse xenograft model (60). A phase I/II study of XLO19 was conducted in patients with MF. Doses of XLO19 from 25 to 300 mg (provided daily for 21 days in 28-day cycles) were evaluated in the phase I portion. Therapy caused adverse neurotoxicity in most patients at doses >100 mg (61), and as a result, XLO19 doses of 25 to 50 mg once daily or 25 mg every Monday, Wednesday, and Friday were selected for further evaluation. This study enrolled 30 patients, 21 of whom received a dose of ≤50 mg. Treatment resulted in a ≥50% reduction in splenomegaly in 5 of 12 evaluable patients (61). A minority of patients showed improvement in anemia, WBC counts, pruritus, and fatigue, and three of four preleukemic patients showed a reduction in blast counts (circulating and/or bone marrow). Drug-related adverse events associated with XLO19 were nonhematologic in nature and included neurotoxicity manifested as formication, peripheral neuropathy, confusional state, balance disorder, and paresthesia. Although reversible in nature, the occurrence of mild neurotoxicity precluded long-term therapy in most patients, and no additional studies of XLO19 are planned.

**TG101348.** TG101348 was synthesized using a structure-based drug design to inhibit JAK2 (IC_{50}, 3 nmol/L) and showed 300-fold selectivity against JAK3, and 35- and 135-fold selectivity against JAK1 and TYK2, respectively (Table 2; ref. 62). Studies in HEL cells and BaF/3 cells expressing JAK2V617F showed that TG101348 induced dose-dependent apoptosis and inhibited proliferation with an IC_{50} of ~300 nmol/L for either line (63), consistent...
with the notion that JAK2 activity was required for both proliferation and survival (64). In a mouse model of JAK2V617F-induced PV, TG101348 decreased hematocrit and spleen size and increased overall survival (63). TG101348 is being evaluated in a phase I/II study in patients with MF (65). Patients were given escalating doses of TG101348 ranging from 30 to 800 mg daily (n = 28), or maximum tolerated dose of 680 mg daily (n = 31). The most frequent nonhematologic toxicities have been grade 1/2 nausea/vomiting and diarrhea, which were seen in the majority of patients and were either self-limiting or easily treated. Other nonhematologic toxicities included grade 1/2 transaminitis (38%), grade 1/2 serum creatinine elevation (38%), and asymptomatic hyperlipasemia (33%). Hematologic toxicities included grade 3/4 thrombocytopenia and neutropenia (30% and 15%, respectively) and grade 3/4 anemia in nontransfusion-dependent patients (50%; ref. 66). The dose-limiting toxicity at 800 mg was asymptomatic amylasemia and lipasemia, and maximum tolerated dose was established at 680 mg/d. Efficacy data in patients who started at ≥680 mg/d showed that a total of 22 patients (67%) have experienced a ≥50% decrease in spleen size compared with baseline (median spleen size, 18 cm; range, 6-32 cm), including 9 whose spleen became nonpalpable. In addition, 44% of patients who were JAK2V617F positive had >50% reduction in JAK2V617F allele burden, and patients with leukocytosis at baseline had a marked reduction in their WBC counts (67).

**Perspective and Future Clinical Direction for JAK2 Inhibitors in MF**

The typical clinical features of MF include marked splenomegaly, progressive anemia, and constitutional symptoms (18). Discoveries including the identification of mutations such as JAK2V617F and high-circulating cytokine levels in MF have improved our understanding of this complex disease and allowed the development of targeted therapies. Given that the current clinical management of MF patients is largely palliative and minimally effective, significant improvement in two of the three most important clinical manifestations of MF seen with JAK2 inhibitors is believed to be a major milestone in the development of new therapies for MF.

Before clinical evaluation of JAK2 inhibitors, and based on imatinib experience in chronic myelogenous leukemia and the fact that the BCR-ABL translocation and JAK2V617F mutation both result in kinase activation, a molecular response rendering JAK2V617F alleles undetectable was expected in response to JAK2 inhibitor therapy. However, despite significant clinical benefits observed with JAK2 inhibitors, significant decreases (>50% of baseline) were noted only in a minority of patients. Although it is possible that none of JAK2 inhibitors evaluated clinically have been tested at doses high enough to suppress JAK2 around-the-clock to be able to achieve deeper molecular responses, there may be additional factors that limit such a therapeutic goal. First, because JAK2V617F mutation is not in the ATP binding pocket of the enzyme, this mutation does not lend itself to allow inhibition of mutant JAK2 without impacting wild-type JAK2. Administration of JAK2 inhibitors at high doses will invariably result in the significant suppression of wild-type JAK2, which is essential for normal hematopoiesis. Second, the mutation is in the hematopoietic progenitor cells and hence administration of JAK2 inhibitors at high enough doses to eliminate this clone will likely be met with intolerable myelosuppression. The improvement in the quality of life of MF patients observed with INC8018424, which is JAK1 and JAK2 inhibitor, has not been reported to that extent with other investigational agents to date. It is possible that the level of JAK2 inhibition achieved with tolerable doses of CEP-701 and XL019 was lower than needed to show symptom improvement. Results from ongoing clinical studies with TG101348 and other JAK2 inhibitors, which may be better tolerated, are needed to further understand potential differences between JAK2-selective inhibitors and inhibitors of both JAK1 and JAK2 with regard to symptomatic benefits.

Nonhematologic adverse event profiles also seem to be different among different JAK inhibitors. Although neutropathy was only reported with XL019, gastrointestinal adverse events such as diarrhea and vomiting were observed with CEP-701 and TG101348. In addition, liver and pancreatic enzyme elevations were reported with TG101348 but not with other JAK2 inhibitors. Exact mechanisms of nonpharmacologic adverse events are difficult to rationalize with small-molecule drugs and are typically related to the inherent properties of the chemical scaffolds from which they are derived. Although improvement in anemia was reported only in a handful of patients taking JAK2 inhibitors, lack of consistent beneficial effect is likely due to the fact that JAK2 inhibitors, at the doses currently used, interfere with growth factor signaling through wild-type JAK2, an integral component of bone marrow function. Additional studies are needed to determine whether lower doses or alternative schedules of JAK2 inhibitors, with less effect on erythropoietin and/or thrombopoietin signaling, can improve hematologic parameters while maintaining the clinical benefits noted thus far. Alternatively, if bone marrow fibrosis is the primary cause of cytopenias in advanced MF, resolution of bone marrow fibrosis will be required to achieve meaningful improvements in cytopenias.

Longer term results are required to determine the full potential of JAK2 inhibitors in MF and to determine whether they will have an effect on patients’ survival. Ongoing studies will improve understanding of the pathophysiology of MF and the role of JAK inhibitors in clinical management of this chronic life-threatening disorder.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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