Molecular Pathways: JAK/STAT Pathway: Mutations, Inhibitors, and Resistance

Alfonso Quintás-Cardama and Srdan Verstovsek

Abstract

Aberrant activation of the JAK/STAT pathway has been reported in a variety of disease states, including inflammatory conditions, hematologic malignancies, and solid tumors. For instance, a large proportion of patients with myeloproliferative neoplasms (MPN) carry the acquired gain-of-function JAK2 V617F somatic mutation. This knowledge has dramatically improved our understanding of the pathogenesis of MPNs and has facilitated the development of therapeutics capable of suppressing the constitutive activation of the JAK/STAT pathway, now recognized as a common underlying biologic abnormality in MPNs. Ruxolitinib is an oral JAK1 and JAK2 inhibitor that has recently been approved for the treatment of myelofibrosis and has been tested against other hematologic malignancies. A series of agents with different specificities against different members of the JAK family of proteins is currently undergoing evaluation in clinical trials for patients with MPNs, lymphoma, and solid tumors such as breast or pancreatic cancer. Despite the significant clinical activity exhibited by these agents in myelofibrosis, some patients fail to respond or progress during JAK kinase inhibitor therapy. Recent reports have shed light into the mechanisms of resistance to JAK inhibitor therapy. Several approaches hold promise to overcome such resistance.

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Disclosure of Potential Conflicts of Interest

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CME Staff Planners Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

On completion of this activity, the participant should have a better understanding of the role of the JAK/STAT pathway in cancer, the new development of therapeutics targeting JAK kinases, and the mechanisms of resistance to JAK inhibitor therapy.

Acknowledgment of Financial or Other Support

This activity does not receive commercial support.

Background

The JAK/STAT pathway is critical in normal hematopoiesis. The JAK family of kinases includes JAK1, JAK2, JAK3, and TYK2. Homozygous germline deletion of JAK2 alleles in mice results in embryonic lethality due to ineffective erythropoiesis (1, 2). JAK kinases are activated through tyrosine phosphorylation of the cytoplasmic domains of cytokine receptors upon cytokine binding (3). Erythropoietin, thrombopoietin, granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3, and IL-5, among others, signal through JAK2, whereas IL-6, IL-10, IL-11, IL-19, IL-20, IL-22, and IFN-γ signal through both JAK1 and JAK2. JAK2 activation promotes recruitment to the receptor complex of the transcription factors STAT3 and STAT5 (3). JAK2-mediated STAT phosphorylation leads to the formation of stable homodimers and heterodimers, which leads to their nuclear translocation (Fig. 1). Once in the nucleus, STAT molecules bind specific promoter DNA sequences that result in the transcription of genes that regulate cell proliferation, differentiation, and apoptosis (e.g., Bcl-xl, cyclin D1, and PIM1; refs. 3, 4). The myeloproliferative neoplasms (MPN) polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (MF) are clonal malignancies that arise from hematopoietic stem or progenitor cells and are characterized by unchecked proliferation of terminally differentiated myeloid cells (5). Despite certain idiosyncratic features,
these MPNs have remarkable phenotypic and clinical commonalities, such as their proclivity to develop thrombotic and hemorrhagic complications and to progress to acute myeloid leukemia (AML; refs. 6–9). The molecular pathogenesis of MPNs remained elusive for decades, which has proven detrimental for the development of effective therapies, particularly for primary myelofibrosis, an MPN associated with high morbidity and mortality. In fact, none of the standard approaches for the treatment of MPNs (e.g., hydroxyurea, growth factors, splenectomy) has been shown to improve the survival of patients with primary myelofibrosis, which is estimated to be only about 5 years (10). Allogeneic stem cell transplantation has proved the only curative strategy in myelofibrosis but at the expense of a very high morbidity and mortality (11, 12). A nexus linking polycythemia vera, essential thrombocythemia, and myelofibrosis was revealed in 2005 with the discovery of a recurrent somatic point mutation in the pseudokinase domain of the Janus kinase 2 (JAK2) gene, which is present in a large proportion of patients with these MPNs. In addition, overactivation of the JAK/STAT pathway with or without JAK protein mutations has been reported in subsets of patients with certain solid tumors and hematologic malignancies. Somatic mutations in the JAK3 gene, including JAK3A572V, JAK3V722I, and JAK3P132T, and chimeric fusion transcripts involving JAK2, such as ETV6-JAK2, PCM1-JAK2, TEL-JAK2, and PLCG1-JAK2, have been reported in acute lymphoblastic leukemia (ALL) and AMLs (13, 14), as well as in multiple myeloma and non–Hodgkin lymphoma (NHL; ref. 15). JAK2R683 mutations have been reported in 7% of patients with high-risk B-cell ALLs and in 25% of cases of ALLs associated with Down syndrome (16–18). Overall, mutated JAK1, JAK2, and JAK3 proteins have been reported in approximately 10% of children with high-risk...
Philadelphia chromosome-negative ALLs (16) and anecdotally in AMLs (19–22). Activation of the JAK/STAT pathway is also present in chronic myeloid leukemia, in which inhibition of JAK2-mediated extrinsic survival signals restores sensitivity to BCR-ABL1 kinase inhibitors (23). JAK2 mutations have not been reported in NHLs, but the JAK/STAT pathway is frequently activated through JAK2 amplification via 9p24 copy number gain, which has been reported in 30% to 50% of cases of Hodgkin lymphoma and primary mediastinal B-cell lymphoma (24–26). In solid tumors, persistent phosphorylation of STAT1, STAT3, and STAT5 has been shown in breast, lung, and head and neck cancers, mediated by an increase in cytokine levels, in both an autocrine and paracrine manner, and by enhanced expression of cytokine receptors (27).

**Molecular biology of the JAK/STAT pathway**

In 2005, a gain-of-function acquired somatic mutation was described in the JAK2 gene in a significant proportion of patients with MPNs (28–32). JAK2^V617F^ mutation arises from a single base G\(-\)T transversion in the pseudokinase domain of JAK2, resulting in a valine-to-phenylalanine substitution at codon 617 that putatively disrupts the autoinhibitory activity of the pseudokinase domain (H12), thus constitutively activating the kinase domain (H11) of JAK2 (33). As a consequence, hematopoietic cells carrying the JAK2^V617F^ mutation exhibit cytokine hypersensitivity and cytokine-independent growth (34). JAK2^V617F^ is present in 50% to 60% of patients with primary myelofibrosis or essential thrombocytosis and in more than 95% of those with polycythemia vera (28, 31). Although most bone marrow erythroid colonies obtained from patients with essential thrombocytosis bear JAK2^V617F^ heterozygous (3, 30, 31), virtually all patients with polycythemia vera carry JAK2^V617F^ homozygous erythroid colonies as a result of uniparental disomy at the JAK2 locus (3, 30, 31). Several somatic gain-of-function mutations at exon 12 of JAK2 have been described in patients with polycythemia vera without the JAK2^V617F^ mutation (35–38). Therefore, JAK2 mutations are present virtually in all patients with polycythemia vera. In addition, somatic mutations at exon 10 of the MPL gene, which encodes the transmembrane–juxtamembrane junction of MPL (W515L/K/A), are present in approximately 5% of patients with essential thrombocytosis or myelofibrosis, resulting in downstream signaling activation similar to that mediated by JAK2^V617F^ (39–42).

LNK, a negative regulator of JAK/STAT signaling, has been found mutated in a subset of patients with MPNs, providing a mechanism of JAK/STAT activation in patients carrying wild-type JAK2 alleles (43). The presence of the JAK2^V617F^ mutation in CD34^+^CD38^-^ hematopoietic stem cells in all mature blood cell lineages of patients with MPNs (44–46) disrupts the autoregulatory activity of JH2, resulting in constitutive activation of the JAK2/STAT pathway and cell growth in the absence of cytokine stimulation (29, 31). JAK2^V617F^ also activates the phosphoinositide 3-kinase (PI3K)/Akt/mTOR/forkhead transcription factors (FoxO) signaling proteins as well as the Ras pathway that promotes survival and proliferation, thereby preventing apoptosis of hematopoietic progenitor cells (3). Furthermore, enforced expression of JAK2^V617F^ in human hematopoietic stem cells and myeloid progenitors steers differentiation toward the erythroid lineage, which is accompanied by decreased expression of PU.1 and enhanced expression and phosphorylation of GATA-1 (47–49). JAK2 signaling is negatively regulated by suppressor of cytokine signaling (SOCS) proteins, most importantly SOCS1. JAK2 inhibitor treatment (50, 51), or overexpression of a dominant-negative form of STAT5, abrogates the growth of polycythemia vera erythroid progenitors in vitro (52), thus implicating JAK2 in the pathogenesis of MPNs and providing the rationale to use JAK2 inhibitors for the treatment of patients with MPNs.

The role of JAK2^V617F^ in the pathogenesis of MPNs has been validated in transgenic mouse models of JAK2^V617F^-driven disease in which low levels of JAK2^V617F^ rendered an essential thrombocytosis-like phenotype, whereas high levels were associated with a polycythemia vera-like phenotype (53, 54). A mouse model in which JAK2^V617F^ is expressed from its endogenous promoter displays a phenotype resembling human polycythemia vera and is transplantable to recipient mice (55), indicating that the resulting JAK2^V617F^-induced MPN is cell autonomous in nature. In this model, JAK2 inhibitor therapy ameliorated the phenotype but failed to fully eradicate MPN-initiating cells (55). To definitely confirm these results, a similar knockin approach in a different mouse model produced a phenotype characterized by erythrocytosis, leukocytosis, thrombocytosis, splenomegaly, reduced serum erythropoietin, and erythroid-independent erythroid colonies in both heterozygous and homozygous mice for the mutation, although most significantly in the latter (56).

**STAT-independent JAK2 oncogenic signaling**

In addition to modulating cytokine-mediated signaling via activation of STAT transcription factors, JAK2 kinase also renders oncogenic effects through epigenomic alterations (Fig. 1). Wild-type JAK2 as well as JAK2^V617F^ proteins have been found in the cytoplasm and the nucleus of human leukemic cell lines and primary CD34^+^ hematopoietic progenitors (57). In the nucleus, JAK2 phosphorylates histone H3 at tyrosine 41 (H3Y41). The levels of phosphorylated H3Y41 correlate with JAK2 activity in vivo. Notably, JAK2 appears to be the only kinase responsible for H3Y41 phosphorylation as treatment with JAK2 inhibitors such as TG101209 or AT9283 abrogates H3Y41 phosphorylation in the nucleus (57). In *Drosophila*, JAK2 kinase activation disrupts the binding of the transcriptional repressor heterochromatin protein 1 alpha (HP1\(\alpha\)) from chromatin. Interestingly, the affinity of HP1\(\alpha\) for histone H3 is dependent on the phosphorylation status of H3Y41. H3Y41 phosphorylation decreases the affinity of H3 to HP1\(\alpha\). JAK2 inhibitors abrogate H3Y41 phosphorylation and enhance chromatin-bound HP1\(\alpha\) in cells, thus repressing HP1\(\alpha\)-regulated genes. Most JAK2-regulated genes do not contain a predicted STAT5-binding site, suggesting that these genes...
are regulated by signals other than JAK2/STAT5 pathway (57). One such gene is lmo2, which is involved in normal hematopoiesis and in leukemogenesis. JAK2 inhibitors decrease H3Y41 phosphorylation and promote HP1α binding at the lmo2 transcriptional start site, which results in downregulation of lmo2 expression. Therefore, the JAK2/ H3Y41/HP1α pathway interconnects JAK2 kinase activity, histone phosphorylation, aberrant gene expression, and genome instability.

Recently, JAK2V617F was found to bind and phosphorylate the protein arginine methyltransferase 5 (PRMT5) much more efficiently than JAK2 (58). Such modification impairs the activity of PRMT5 and impedes its interaction with methylosome protein 50 (MEP50). The net result is a marked decrease in global arginine methylation of histones H2A and H4, both targets of MEP50. The gain-of-function mechanism of JAK2V617F that contribute to tumorigenesis through epigenomic alterations (58).

Clinical–Translational Advances

Clinical trials with JAK tyrosine kinase inhibitors

Various JAK2 inhibitors have been tested in clinical trials for patients with intermediate- or high-risk myelofibrosis. An account of the in vitro activity of the agents that are further in clinical development is presented in Table 1 (59). Ruxolitinib potently inhibits the phosphorylation of JAK1, JAK2, JAK3V617F, STAT5, and ERK1/2 in vitro, which is coupled with induction of apoptosis (60). In a phase I/II study in 153 patients with myelofibrosis, the dose-limiting toxicity (DLT) was grade 4 thrombocytopenia (61). Ruxolitinib marked reduction in multiple fibrogenic and pro-inflammatory cytokines (e.g., IL-6, IL-8, TNF-α) and pro-inflammatory cytokines (e.g., IL-6, IL-8, TNF-α) regardless of JAK2 mutational status, suggesting that the clinical activity of ruxolitinib may be partly due to its JAK1 inhibitory activity (61). In the phase III study COMFORT-I, patients with myelofibrosis were randomized to placebo (n = 154) or ruxolitinib (n = 155). The primary endpoint, ≥35% volumetric reduction of the spleen at week 24, occurred in 41.9% versus 0.7% (P < 0.001) of patients receiving ruxolitinib or placebo, respectively (62). Similarly, ruxolitinib improved total symptom score by at least 50% in 45.9% of patients versus 5.3% with placebo (62). After a median follow-up of 24 months, fewer deaths were observed in the ruxolitinib arm (27 vs. 41 for placebo; P = 0.028; ref. 63). The most common grade 3 and 4 adverse events with ruxolitinib were anemia (45.2% vs. 19.2% with placebo) and thrombocytopenia (12.9% vs. 1.3% with placebo; ref. 62). COMFORT-II randomized patients with intermediate- or high-risk myelofibrosis 2:1 to ruxolitinib (n = 146) or best available therapy (BAT; n = 73; ref. 64). Reduction of spleen volume ≥35% at 48 weeks occurred in 28.5% of patients with ruxolitinib and 0% in those receiving BAT (P < 0.001). After a median follow-up of 28 months, the percentage of deaths was lower in the ruxolitinib arm (14% vs. 22% for BAT; P = 0.041; ref. 65). In November 2011, ruxolitinib was approved by the U.S. Food and Drug Administration for treating intermediate- and high-risk myelofibrosis based on the results of the COMFORT trials.

<table>
<thead>
<tr>
<th>Compound</th>
<th>JAK1</th>
<th>JAK2</th>
<th>JAK3</th>
<th>TYK2</th>
<th>Stage of development</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruxolitinib</td>
<td>3.3</td>
<td>2.8</td>
<td>428</td>
<td>19</td>
<td>Approved in myelofibrosis, phase III in polycythemia vera, breast cancer, acute leukemia</td>
<td>(50, 61)</td>
</tr>
<tr>
<td>SAR302503</td>
<td>105</td>
<td>3</td>
<td>1,002</td>
<td>405</td>
<td>Phase III in myelofibrosis, phase I in solid tumors</td>
<td>(79)</td>
</tr>
<tr>
<td>Pacritinib</td>
<td>23</td>
<td>23</td>
<td>56</td>
<td>2</td>
<td>Phase I in hematologic malignancies, phase II in myelofibrosis</td>
<td>(80, 72)</td>
</tr>
<tr>
<td>CYT387</td>
<td>11</td>
<td>18</td>
<td>155</td>
<td>NA</td>
<td>Phase II</td>
<td>(81, 82)</td>
</tr>
<tr>
<td>AZD1480</td>
<td>1.3</td>
<td>&lt;0.4</td>
<td>3.9</td>
<td>NA</td>
<td>Phase I in solid tumors, phase II in myelofibrosis</td>
<td>(83)</td>
</tr>
<tr>
<td>Tasocitinib</td>
<td>1.7</td>
<td>1.8</td>
<td>0.75</td>
<td>260</td>
<td>Approved in rheumatoid arthritis</td>
<td>(84)</td>
</tr>
<tr>
<td>INC028050</td>
<td>5.9</td>
<td>5.7</td>
<td>560</td>
<td>53</td>
<td>Phase II in rheumatoid arthritis</td>
<td>(85)</td>
</tr>
</tbody>
</table>

NOTE: Values expressed as K_d (dissociation constant).

\( ^a \)Never tested in MPNs.
neutropenia occurred in 35%, 24%, and 10% of patients, respectively. After 12 cycles, 47% of patients in the MTD cohort achieved a ≥50% decrease in splenomegaly that was sustained for ≥8 weeks (66, 67). In a phase II study, patients were randomized to SAR302503 at 300, 400, or 500 mg daily. Reductions of spleen volume ≥35% at the end of cycle 3 were dose-dependent: 30%, 50%, and 64% for the 300-, 400-, and 500-mg daily groups, respectively, which correlated with inhibition of STAT3 phosphorylation (68). A phase III placebo-controlled study of SAR302503 in myelofibrosis is under way.

CYT387 is a JAK1/2 inhibitor that is being studied in a phase I/II study that includes 166 patients with myelofibrosis in the core portion of the study and 120 patients in the multicenter extension portion (69). The MTD was determined to be 300 mg/d, and the DLT was reached at 400 mg/d (70). After a median follow-up of 16.9 months, 13% of patients in the core study had an increase in hemoglobin level of at least 2 g/dL, and 37% had durable reduction in spleen length by palpation of at least 50%. On the basis of these encouraging results, the dose of 300 mg/d was selected for a phase III study that will be conducted as a strategy for the approval of this agent.

Pacritinib is a selective JAK2 inhibitor that has been tested in a phase II study involving 34 patients with myelofibrosis. Eleven (32%) patients had ≥35% reduction in spleen volume at 24 weeks (71). The most frequent toxicity was gastrointestinal, although it was generally mild and manageable, without significant myelosuppression (71). Two patients had clinical improvement of hemoglobin, and a significant proportion of patients had improvement of constitutional symptoms. A phase III study of pacritinib for patients with low platelet levels is being planned. Of note, pacritinib has been recently tested in a phase I study in 34 patients with relapsed/refractory Hodgkin lymphoma or NHL. Pacritinib was well-tolerated, and the MTD was not reached. Three patients had a partial response, and 15 had stable disease (72).

Overcoming JAK2 inhibitor resistance

Although JAK2 inhibitors have proved effective in patients with MPNs, a fraction of them will have suboptimal responses and overall will experience significant reductions in JAK2V617F allele burden, indicating persistence of the malignant clones. It has been recently shown that such resistance to JAK2 inhibitor therapy is not mediated by secondary mutations in the JAK2V617F kinase. Rather, it is the consequence of the reactivation of the JAK/STAT pathway via heterodimerization of activated JAK2 and JAK1 or TYK2 that promotes resistance to JAK2 inhibitor–induced apoptosis (73). Chronic JAK2 inhibitor therapy results in stabilization of activated JAK2 and an increase in JAK2 mRNA expression, which facilitates the formation of heterodimers. Notably, removal of JAK2 inhibitor treatment results in resistant JAK2V617F-positive cell resensitization, suggesting that patients resistant to JAK2 inhibitors might respond to retreatment with the same JAK2 inhibitor or with others after a period of therapy discontinuation.

It has been shown that Hsp90 inhibitors or histone deacetylase inhibitors (HDACi) promote JAK2 degradation, which suggests a potential role for these agents in the treatment of JAK2 inhibitor–resistant MPNs (74, 75). Treatment of cells with persistent JAK2 inhibitor–induced JAK/STAT pathway activation with the Hsp90 inhibitor PU-H71 resulted in JAK2 degradation and decreased activation of the JAK/STAT pathway. The combination of panobinostat, which is known to inhibit the chaperone function of Hsp90 and promote proteasomal degradation of JAK2V617F, with the JAK2 inhibitor TG101209 synergistically induced apoptosis of HEL and Ba/F3-JAK2V617F cells and exerted greater cytotoxicity against primary CD34+ MPN cells than normal CD34+ hematopoietic progenitor cells (74). Murine models suggest that the combination of a HDACi such as panobinostat with ruxolitinib exhibits markedly improved anticancer activity compared with either agent alone in a JAK2V617F bone marrow transplantation mouse model of MPN (76).

An alternative strategy to eliminate resistance cells is the use of type II JAK2 inhibitors, such as BBT-594, which, unlike available JAK2 inhibitors, retain the ability to bind the inactive conformation of JAK2 and inhibit signaling emanating from mutant JAK2 in persistent JAK2V617F-positive cells.

In addition to cell-autonomous mechanisms of resistance, extrinsic humoral factors secreted by the bone marrow microenvironment have been shown to protect MPN clones from JAK2 inhibitor therapy. We have recently shown the potent growth suppression exerted by the JAK2 inhibitor atiprimod on murine FDCP-EpoRV617F and JAK2V617F-positive human SET-2 cells while causing minimal effects on stromal cells (77). However, culture of JAK2V617F-positive cells on monolayers of stromal cells markedly impaired the ability of atiprimod to inhibit the phosphorylation of the JAK2/STAT3/5 pathway and the proliferation of JAK2V617F-positive cells. These effects are not due to direct interactions between the malignant clones and the marrow stroma. Rather, they are mediated by a network of cytokines secreted by the stromal cells, such as IL-6, fibroblast growth factor, and IP-10 (77). Blocking such cytokines with specific neutralizing antibodies restored JAK2 inhibitor sensitivity, thus showing the importance of non–cell autonomous mechanisms of resistance against JAK2 inhibitors and the therapeutic potential of strategies targeting the bone marrow niche in MPNs.

Conclusions

Our understanding of the pathogenesis of MPNs has markedly improved in recent years. The critical importance of the JAK2V617F mutation has been validated in vitro as well as in vivo by means of murine models of MPNs driven by JAK2V617F. In addition, several novel mutations in other genes have since been described in patients with MPNs, including TET2 mutations, which can appear before the acquisition of JAK2V617F, or IKZF1, EZH2, and ASXL1, which appear to contribute to leukemic transformation (78).
Despite this wealth of information, several questions remain to be answered. First, the precise role of other mutated alleles in the pathogenesis of MPNs and potentially in the resistance to JAK kinase inhibitor therapy remains unknown. Second, mounting data indicate that mutant JAK2 induces epigenetic deregulation, which, coupled with the fact that some of the mutant alleles found in MPNs outside of the JAK2 locus encode important epigenetic regulators, suggests the possibility that therapeutic epigenetic modifiers might play a role in the management of these malignancies. Third, available evidence indicates that JAK2 inhibition produces very modest effects on JAK2V617F allele burden, cytopenias, and bone marrow fibrosis, which suggests that such an approach alone cannot significantly correct the malignant phenotype observed in patients with myelofibrosis. Fourth, the therapeutic role of JAK1 inhibition versus JAK2 inhibition in myelofibrosis is unclear. Ongoing efforts to identify new therapeutic epigenetic modifiers might play a role in the management of these malignancies. Third, available evidence indicates that JAK2 inhibition produces very modest effects on JAK2V617F allele burden, cytopenias, and bone marrow fibrosis, which suggests that such an approach alone cannot significantly correct the malignant phenotype observed in patients with myelofibrosis. Fourth, the therapeutic role of JAK1 inhibition versus JAK2 inhibition in myelofibrosis is unclear. Ongoing efforts to identify new therapeutic epigenetic modifiers might play a role in the management of these malignancies.

**References**


