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REVIEW ARTICLE

TYROSINE-PROTEIN KINASE JAK2 INHIBITORS FOR THE TREATMENT OF MYELOPROLIFERATIVE NEOPLASMS

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SUMMARY

Tyrosine-protein kinase JAK2, a cytoplasmic tyrosine kinase, plays a critical role in hematopoiesis and activating mutations in this kinase are associated with a number of hematological disorders. Recently, several somatic point mutations, such as JAK2 V617F, that constitutively activate the JAK/STAT signaling pathway have been identified in the majority of patients with myeloproliferative neoplasms (MPNs). This discovery made JAK2 an attractive molecule for therapeutic targeting in MPNs and has been the driving force behind the development of small-molecule inhibitors that specifically target JAK2. Since then, many different JAK2 inhibitors have been identified using a variety of screening techniques and have been characterized preclinically, as well as clinically. This review article discusses the efficacy of small-molecule inhibitors for therapeutic targeting of JAK2 in MPNs, with special focus on inhibitors with recently reported results from preliminary phase I/II clinical trials. Based on the analysis of reports from these initial studies, we attempt to identify potential problems and issues that stand in the way of the development of a successful targeted therapy for JAK2-mediated disorders.

INTRODUCTION

Tyrosine-protein kinase JAK2, a cytosolic protein of approximately 130 kDa in mass, is a member of the Janus family of cytoplasmic tyrosine kinases. Other known members of this family are TYK2, JAK1 and JAK3. These kinases are ubiquitously expressed in a variety of different cell types, with the exception of JAK3, which is predominantly expressed in cells of hematopoietic origin. The unique structure of the JAK kinases clearly distinguishes them from other members of the tyrosine-protein kinase family. JAK kinases have seven highly conserved JAK homology (JH) domains, JH1 through JH7, numbered from the *C*- to the *N*-terminus, respectively (1) (Fig. 1). A hallmark feature of all Janus kinases is the presence of two domains (JH1 and JH2) in the carboxyl half of the protein that both have extensive homology with the kinase domain of other tyrosine-protein kinases. However, only the JH1 domain has functional kinase activity, whereas the JH2 or pseudokinase domain has an important role in limiting the activity of the kinase domain. The JH2 domain regulates both the basal and the ligand-induced levels of phosphotransferase activity (2, 3). The JH3-JH4 region is called the SH2-like domain, but its function has yet to be elucidated (4). The JH4-JH7 domains are collectively called the FERM (4.1, Ezrin, Radixin, Moesin) domain and are involved in binding to and crosstalk with other cellular proteins (5). For example, regions within the JH6 and JH7 domains mediate interactions with cell-surface receptors by associating with conserved Box 1 structural motifs on the cytokine receptors (6, 7).

JAK2 is an important downstream signaling molecule activated by a variety of cytokines, growth factors and G protein-coupled receptor (GPCR) ligands. An overview of the canonical JAK/STAT (signal transducer and activator of transcription) signaling pathway is shown in Figure 2. Briefly, signaling is initiated by binding of the ligand to its cognate cell-surface receptor, resulting in receptor dimerization. Receptor dimerization brings the receptor-associated JAK proteins in close proximity to each other, allowing them to transphosphorylate one another on specific tyrosine residues: Tyr 1007/1008 in the case of JAK2 (8). An activated JAK can in turn phosphorylate specific tyrosine residues on the cytoplasmic tails of the receptors, thereby creating docking sites for SH2 domain-containing proteins such as the STAT proteins. Receptor-bound STAT monomers are then phosphorylated by JAK2 on specific tyrosine residues. Phosphorylated STATs form homo- or heterodimers, which translocate to the nucleus, where they bind to gene promoter elements to modulate gene transcription. Thus, JAK/STAT signaling results in a signal cascade from extracellular binding and activation of a cell-surface receptor to changes in gene transcription in the nucleus.

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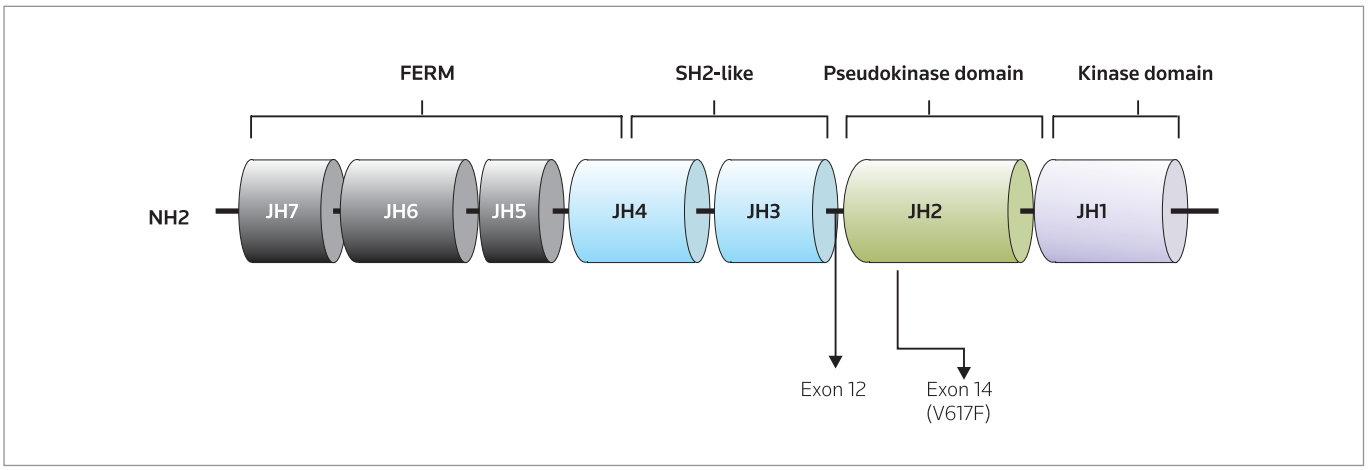


Figure 1. Schematic representation of the JAK2 protein showing the regions where the somatic mutations identified in patients with myeloproliferative neoplasms (MPNs) are commonly found. JAK2 has seven highly conserved JAK homology, or JH, domains, JH1-JH7. The *JAK2* V617F point mutation, the predominant disease-associated allele in MPNs, is on exon 14 of the JH2, or pseudokinase domain of JAK2. Other exon 14 mutations have also been identified in MPN patients who lack the V617F mutation. Additionally, about 16 different exon 12 mutations have been detected in *JAK2* V617F-negative polycythemia vera patients. These mutations cluster in a region spanning from amino acids 538 to 543.

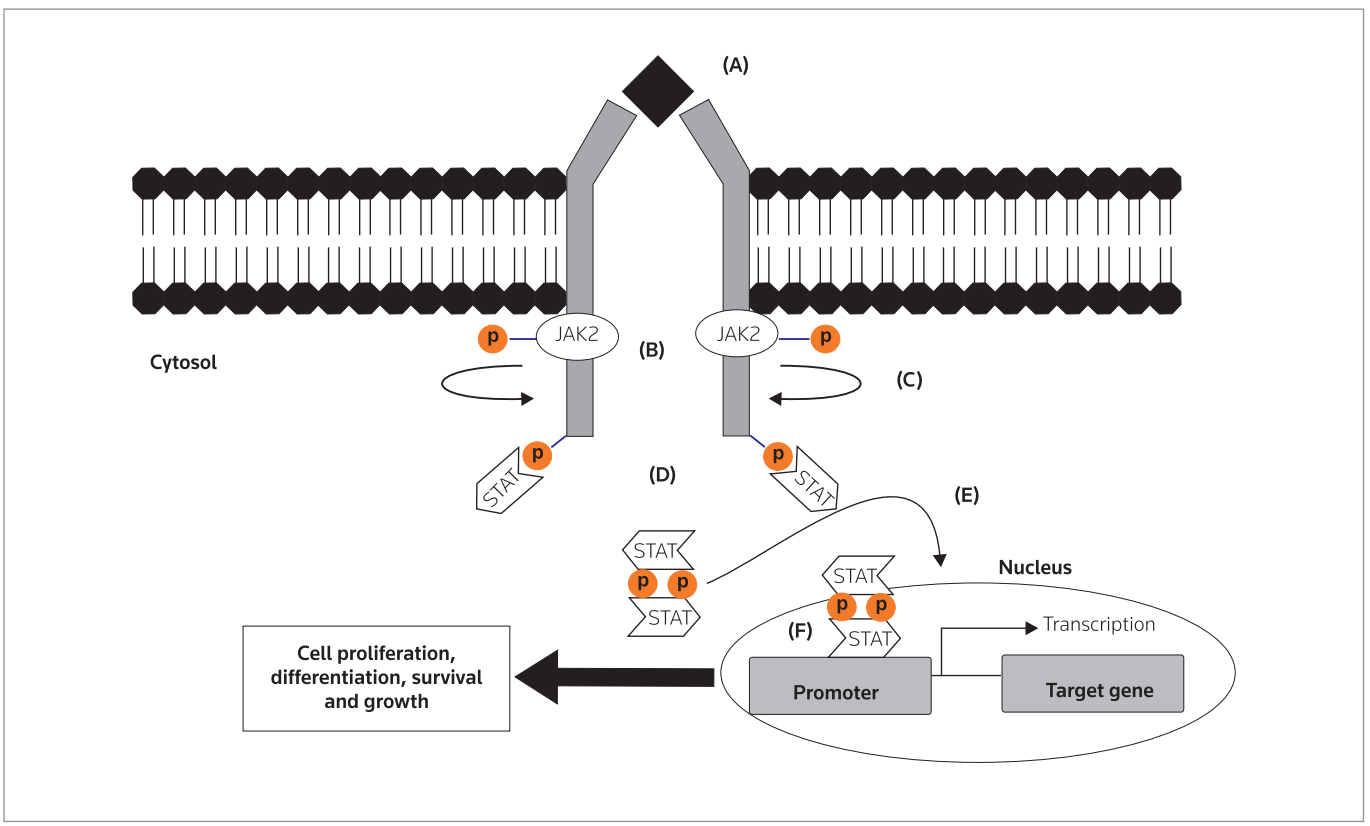


Figure 2. Schematic representation of the JAK/STAT signaling paradigm. (**A**) Binding of the ligand to its cognate cell-surface receptor causes receptor dimerization. (**B**) Receptor dimerization brings the receptor-associated JAK molecules in close proximity, such that they can transphosphorylate one another on specific tyrosine residues (**C**) Active JAKs can now phosphorylate tyrosine residues on the cytoplasmic tail of the receptor. (**D**) STAT proteins bind to these phosphorylated tyrosine residues on the receptor and are also phosphorylated by receptor-associated active JAKs. (**E**) Phosphorylated STAT proteins form dimers which translocate to the nucleus. (**F**) STAT dimers bind to the promoter region of specific target genes and modulate gene transcription, producing biological effects such as cell proliferation, differentiation, survival and growth.

JAK2 IN HEMATOLOGICAL DISORDERS

Hematopoiesis, the process of generating blood cells from a self-renewing population of multipotent hematopoietic stem cells, is regulated by a group of soluble factors called cytokines. The binding of cytokines to their cognate receptors initiates signal transduction pathways that govern survival, proliferation, differentiation and apoptosis of the hematopoietic cells. A deregulation of these signaling pathways can lead to the development of different hematological disorders, such as leukemias and myeloproliferative neoplasms (MPNs). Cytokine receptors lack an integral cytoplasmic kinase domain and hence signal via association with cytoplasmic tyrosine kinases, such as the JAK kinase family members (9, 10). JAK2 is widely involved in cytokine signaling. Cytokines, such as erythropoietin, thrombopoietin, growth hormone, prolactin and granulocyte–macrophage colony-stimulating factor (GM-CSF), signal exclusively through JAK2 (11, 12). Hence, it is not surprising that *Jak2* knockout mice die during embryonic development due to a lack of definitive erythropoiesis (13, 14). Although JAK2 has a critical role to play in normal physiology and development, excessive JAK2 kinase activity can also have detrimental effects, as a deregulated JAK/STAT signaling pathway, which promotes aberrant cell growth and prevents apoptosis, has been implicated in a variety of neoplastic disorders.

Several studies have linked specific JAK2 chromosomal translocations to human neoplastic growth. For example, an abnormal fusion protein that has the helix–loop–helix dimerization domain of TEL, an ETS family transcription factor, fused to the catalytic domain of JAK2 was detected in a patient with early B-precursor acute lymphobastic leukemia (ALL) and another diagnosed with atypical chronic myeloid leukemia (CML) (15, 16). The phenotypes of these two patients were diverse because of the fact that the TEL-JAK2 fusion proteins had resulted from two distinct translocation events: a t(9:12)(p24;p13) in the former case and a t(9:15:12)(p24;q15;p13) in the latter. However, in both cases, the translocation event resulted in constitutive activation of the JAK2 kinase domain and its downstream signaling pathways (17). Recent studies have also reported the presence of a BCR-JAK2 fusion protein arising from a t(9:22)(p24;q11.2) translocation in patients with typical and atypical CML (18-20). In other instances, a t(8:9) translocation event results in a PCM-1-JAK2 fusion protein that contains the coiled coil domains of PCM-1 and the tyrosine kinase domain of JAK2 (21-23). This fusion protein has been implicated in several hematological disorders, including acute erythroid leukemia, atypical CML, T-cell lymphoma and MPNs.

In addition to chromosomal translocations, point mutations in the *JAK2* allele also cause constitutive JAK2 activation and subsequent activation of its downstream signaling pathways. Substitution and deletion mutations in *JAK2* have been detected in many patients with different hematological disorders. For example, JAK2 amino acids 682-686 were found to be deleted (*JAK2*-Δ*IREED*) in a patient with Down syndrome and B-cell precursor ALL (24). In another instance, the Val617-to-Phe substitution mutation (*JAK2* V617F) was identified in a large number of patients with MPNs. Myeloproliferative neoplasms are a group of heterogeneous diseases arising from a transformed hematopoietic stem cell and characterized by excessive numbers of one or more terminally differentiated blood cells of the myeloid lineage, such as erythrocytes, thrombocytes or white blood cells. These differentiated cells accumulate due to excess proliferation and/or decreased apoptosis of hematopoietic progenitors. Excess production of red blood cells is a hallmark of polycythemia vera (PV) and too many platelets is a classical characteristic of essential thrombocythemia (ET), whereas primary myelofibrosis (PMF) is characterized by bone marrow fibrosis and impaired hematopoietic stem cell function. Specifically, five groups working independently and using different approaches identified the *JAK2* V617F mutation in a significant proportion of patients with PV, ET and PMF (25-29). These groundbreaking studies, as well as other subsequent studies, reported the presence of the *JAK2* V617F mutation in over 90% of patients with PV and about 50% of patients with ET and PMF. A guanine-to-thymine mutation results in a substitution of valine to phenylalanine at codon 617 in exon 14 which encodes the JH2 pseudokinase domain of JAK2 (Fig. 1). It is believed that this mutation in the autoinhibitory pseudokinase domain of JAK2 allows the kinase to evade inhibition and leads to a constitutively active JAK/STAT signaling pathway. The presence of this mutation thereby confers a cytokine-independent growth property to cells (25, 28). In vivo studies have demonstrated that expression of the *JAK2* V617F mutation in murine bone marrow is sufficient for the development of fully penetrant MPNs (30-34). These data strongly suggest that the *JAK2* V617F mutation plays a causative role in the pathogenesis of MPNs.

Although the *JAK2* V617F mutation on exon 14 is the predominant disease-associated allele in MPNs, several other *JAK2* exon 14 mutations, such as C616Y (35) and D620E (36), have been identified in V617F-negative MPN patients. Moreover, about 16 different *JAK2* exon 12 mutations have been detected to date in *JAK2* V617F-negative PV patients (37). These exon 12 mutations are usually insertions, deletions or substitutions in the *JAK2* sequence spanning amino acids 538-543 (Fig. 1). This region is highly conserved and links the SH2-like and the pseudokinase domains of JAK2. A recent study suggested that this linker region acts as a switch in relaying signals from receptor binding to JAK2 kinase activation by flexing the pseudokinase domain hinge (38). Finally, a subset of patients with *JAK2* V617F-negative ET and PMF were found to carry MPLW515L/K mutations in the thrombopoietin receptor, which also leads to the deregulation of the JAK/STAT signaling pathway via activation of wild-type JAK2 protein (39, 40).

Collectively, these studies clearly demonstrate that hyperkinetic JAK2 plays a crucial role in the pathogenesis of MPNs, as well as in a number of hematological malignancies. Therefore, the clinical development of JAK2 inhibitors is of potential value for patients with these hematological disorders and is currently an area of active research.

JAK2 INHIBITORS FOR THE TREATMENT OF MYELOPROLIFERATIVE NEOPLASMS

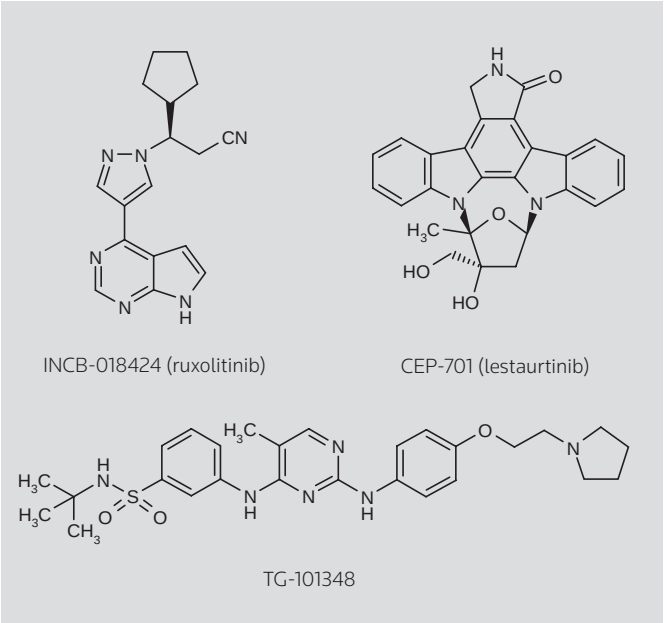
Currently available treatment options for MPN patients are limited. For PV/ET patients, the available therapies aim to reduce myeloproliferation and associated thrombotic/hemorrhagic complications. As such, a PV/ET patient is mostly treated with phlebotomy/thrombapheresis or myelosuppressive agents such as hydroxyurea (41-43). On the other hand, in PMF patients, treatment is directed towards management of symptoms such as anemia and splenomegaly.

Therefore, agents that can improve cytopenia (erythropoietin, androgens) or induce myelosuppression (hydroxyurea, thalidomide) are routinely used for treating these individuals (43, 44). In certain cases, more invasive techniques, such as splenectomy or radiation, are used to treat severe splenomegaly in MPN patients. Unfortunately, these treatment strategies are more palliative and not curative in nature. The only therapy that has been shown to be curative in patients with MPNs is stem cell transplantation. Although this approach is potentially curative, risks associated with it, such as graft versus host disease and nonrelapse mortality, severely limit its application for therapy (44).

Given the critical role that constitutive JAK2 kinase activity plays in the pathophysiology of MPNs, it became an attractive molecule for therapeutic targeting and prompted researchers to start the search for potent and selective small-molecule JAK2 inhibitors. Interestingly, the first widely available JAK2 inhibitor dates back to 1995, when Meydan et al. identified AG-490 via a high-throughput screen of potential tyrosine-protein kinase inhibitors (45). However, it was subsequently reported that, although AG-490 was a potent JAK2 inhibitor, it had poor specificity (46). The discovery of the *JAK2* V617F mutation in 2005 and its identification in the vast majority of patients with MPNs provided a new thrust to the search for small-molecule inhibitors that specifically target JAK2. Given the fact that the *JAK2* V617F mutation leads to sustained kinase signaling, similar to the effect of the BCR-ABL fusion protein in CML, it was expected that a JAK2 inhibitor would be as effective in MPN therapy as imatinib is in CML (47). Moreover, in 2006, the resolution of a crystal structure encoding a portion of the JAK2 kinase domain provided a valuable tool for designing inhibitors that could be highly specific for JAK2 (48).

Several pharmaceutical companies have developed JAK2 inhibitors that are currently under study for the treatment of MPNs. The clinical trials conducted thus far have focused on patients with PMF because it is the most serious condition among the different MPNs. Primary myelofibrosis patients are characterized by fibrous scar tissue in their bone marrow, impaired hematopoiesis and high levels of proinflammatory cytokines and growth factors. These symptoms significantly reduce the quality of life of these patients and, as mentioned above, effective therapies are limited. The results from four clinical trials in these patients are publicly available; the inhibitors tested were INCB-018424, CEP-701, TG-101348 and XL-019.

INCB-018424 (ruxolitinib) is a potent JAK2 inhibitor which has an in vitro IC_{50} of 2.8 nM (49). In primary cultures this inhibitor has the ability to suppress erythroid progenitor colony formation from *JAK2* V617F-positive PV patients, with an IC_{50} of 67 nM vs. > 400 nM in healthy donors. In a mouse model of *JAK2* V617F-mediated MPN, treatment with 180 mg/kg/day of INCB-018424 significantly decreased splenomegaly, reduced levels of some circulating cytokines, preferentially eliminated neoplastic cells, and increased animal survival, without myelosuppressive or immunosuppressive effects. In clinical phase I/II trials this drug was generally well tolerated; the maximum tolerated dose (MTD) was 25 mg p.o. b.i.d. (50). INCB-018424 treatment reduced splenomegaly in over 93% of the patients irrespective of *JAK2* mutational status (51). Treatment with this drug inhibited the production of proinflammatory cytokines in all PMF patients (52). There was also significant improvement in the quality of life and weight of the patients with treatment (50). However, the *JAK2* V617F allele burden showed only a modest decrease of 13% in the marrow and 9% in the peripheral blood (53), suggesting that this drug might be inhibiting downstream JAK/STAT signaling in cells with hyperkinetic JAK2 rather than altering the mutant allele burden. There were also no reports of improvement or normalization of either the diseased bone marrow or the spleen tissues in any of the treated patients. No significant changes in fibrosis score, bone marrow cellularity or circulating $CD34^+$ cells were observed (54). Finally, thrombocytopenia and myelosuppression were the most common side effects of drug treatment (50). Phase III clinical studies for this drug have recently been initiated (www.clinicaltrials.gov).



CEP-701 (lestaurtinib), an indolocarbazole alkaloid, is an orally available tyrosine-protein kinase inhibitor that has anti-JAK2 kinase activity. This drug is a known inhibitor of Fms-like tyrosine kinase 3 (FLT3) and is currently being evaluated in clinical trials for treating AML patients with *FLT3* mutations (55, 56). Lestaurtinib inhibits JAK2 in vitro, ex vivo and in vivo (57). It potently inhibits JAK2 kinase activity in vitro, with an IC_{50} of 1 nM, and suppresses ex vivo erythroid colony formation from primary $CD34^+$ cells isolated from MPN patients at a concentration of 100 nM. CEP-701 treatment is also able to reduce phosphorylation of STAT3, STAT5 and other downstream signaling molecules of the JAK/STAT signaling pathway in vitro. With respect to in vivo studies, the drug was able to inhibit the proliferation of *JAK2* V617F-bearing human erythroleukemia HEL 92.1.7 cells xenografted into nude mice at a dose of 30 mg/kg b.i.d. The results of a phase II clinical study of CEP-701 in 22 *JAK2* V617F-positive PMF patients were recently published (58). The patients were treated with an oral dose of 80 mg twice a day and 27% of the patients (6 of 22) showed clinical improvement (i.e., the lowest level of response as per the International Working Group for Myelofibrosis Research and Treatment criteria). The responding patients included three who only showed a reduction in spleen size, two who achieved transfusion independency, but only one who had a reduction in

spleen size in conjunction with improvement in cytopenia. Median time for response was about 3 months and the duration of the response was 14 months or more. A decrease in the levels of phosphorylated STAT3 from baseline levels was observed in the responding patients. Most critically, no improvements were seen either in marrow fibrosis or the *JAK2* V617F allele burden in any of the treated patients. Finally, 36% of the treated patients (8 of 22) experienced toxic effects, principally myelosuppression (anemia and thrombocytopenia) and gastrointestinal problems such as diarrhea and nausea.

TG-101348 is a potent and selective inhibitor of JAK2. It is an ATP-competitive inhibitor that shows high selectivity for JAK2 over other JAK family members. Among all the JAK2 inhibitors currently in clinical trials, TG-101348 appears to be the most selective. Previous studies with this compound have shown that it inhibits JAK2 kinase activity with an IC_{50} of 3 nM in vitro (59, 60). Furthermore, it was found to suppress erythroid colony formation from primary progenitor cells with an IC_{50} of about 300-600 nM (61). The in vivo therapeutic efficacy of TG-101348 in a murine model of *JAK2* V617F-mediated PV was also evaluated in a study which found that mice treated with 120 mg/kg b.i.d. showed a decrease in *JAK2* V617F mutant allele burden, hematocrit values, leukocyte counts and spleen sizes (59). TG-101348 was also evaluated in a phase I/II dose-escalation study in 28 myelofibrosis patients (62, 63). The MTD was found to be 680 mg once daily. A decrease in spleen size of over 50% was seen in 64% of the patients. Fourteen patients who initially had leukocytosis experienced improvement in their white blood cell counts post-treatment. Mutant allele burden was found to decrease in 32% of the *JAK2* V617F-positive patients enrolled in the study. The most frequent toxicities observed were nausea/vomiting (64%) and diarrhea (50%) (62). No changes in levels of plasma cytokines were detected in patients treated with this drug (63). Thrombocytopenia, neutropenia and anemia were the other side effects related with drug treatment (62).

XL-019 inhibits JAK2 kinase activity with an IC_{50} of 2 nM. In ex vivo erythropoietin-stimulated primary erythroid cultures, STAT5 phosphorylation was inhibited with an IC_{50} of 64 nM. In in vivo studies using the HEL cell xenograft model, treatment with this inhibitor reduced tumor growth in a dose-dependent manner, with 60% inhibition at a dose of 200 mg/kg b.i.d. and 70% inhibition at a dose of 300 mg/kg b.i.d. relative to vehicle control-treated animals (64). The effect of XL-019 administration was tested in a phase I/II clinical study in patients with PMF post-PV or post-ET. To date, 21 patients have been given multiple doses ranging from 25 mg to 300 mg using different schedules of administration (either once daily or three times weekly). Adverse neurotoxicity was observed in patients receiving doses > 100 mg. However, the drug was well tolerated at the lower doses of 25-50 mg/day or 25 mg three times weekly. A > 50% reduction in spleen size was achieved in 42% of the patients. Reduction in leukocytosis and circulating blast cells and improvement in anemia, pruritus and fatigue were also observed (65). The drug-associated toxicities were mostly related to adverse neurological effects such as peripheral neuropathy, formication, balance disorder and confusion (64). Adverse hematological side effects were, however, not seen in treated patients. Although XL-019 showed some beneficial effects, it has been withdrawn from clinical trials due to neurotoxicity concerns.

A summary of the preclinical data pertaining to INCB-018424, CEP-701, TG-101348 and XL-019 is shown in Table I and a summary of the clinical trial regimens for the same four drugs is displayed in Table II.

There are also a number of other JAK2 inhibitors that are currently being evaluated in early clinical trials, such as SB-1518 and CYT-387 (www.clinicaltrials.gov). SB-1518 is a potent, selective, orally active, ATP-competitive JAK2 inhibitor that inhibits *JAK2* V617F activity with an IC_{50} of 19 nM. This compound was found to inhibit the proliferation of Ba/F3 cells that had been transfected with erythropoietin receptor (EPO-R) and mutant *JAK2* V617F with an IC_{50} of 81 nM. In a mouse model of myeloproliferative diseases, established by injection of Ba/F3-*JAK2* V617F cells into nude mice, SB-1518 was found to produce therapeutic effects such as normalization of elevated white blood cell counts, reduction of GFP-labeled Ba/F3 cells in the peripheral blood, resolution of hepatosplenomegaly, reduction of phospho-STAT5 in diseased organs, prolonged survival and alleviation of terminal-stage anemia and thrombocytopenia (66).

CYT-387, an aminopyrimidine derivative, inhibits JAK1 and JAK2 with IC_{50} values of 11 and 18 nM, respectively. It inhibited the growth of Ba/F3-*JAK2* V617F and HEL cells (IC_{50} ~ 1500 nM). The drug selectively suppressed the in vitro growth of erythroid colonies harboring *JAK2* V617F from PV patients, an effect that was attenuated by exogenous erythropoietin (67). In a murine MPN model, CYT-387 normalized white cell counts, hematocrit and spleen size, and restored normal levels of inflammatory cytokines. Furthermore, treatment with this drug was able to reduce the *JAK2* V617F allelic burden, but did not eliminate the mutant cells (68).

With a number of other putative JAK2 inhibitors currently in various stages of preclinical studies, it is hoped that the list of JAK inhibitors in clinical trials will grow significantly in the years to come.

Table I. *Preclinical characterization of JAK2 inhibitors.*

Drug	JAK2 IC_{50} (nM)	HEL cell IC_{50} (nM)	Ex vivo IC_{50} (nM)	In vivo dose (mg/kg) (mouse model used)
INCB-018424	2.8	186	67	180 daily (Ba/F3-*JAK2* V617F xenograft)
CEP-701	1	30-100	100	30 twice daily (HEL xenograft)
TG-101348	3	300	300-600	120 twice daily (*JAK2* V617F-induced PV bone marrow transplant)
XL-019	2	60-200	64	200-300 twice daily (HEL xenograft)

The JAK2 IC_{50} was derived from cell-free assays using recombinant JAK2 protein. The HEL cell IC_{50} was determined using in vitro cultures of *JAK2* V617F-expressing HEL cells. The ex vivo IC_{50} values were measured in primary cultures isolated from patients with myeloproliferative neoplasms. The in vivo drug dose (mg/kg) is shown along with the animal model that was employed for that specific study. PV, polycythemia vera.

Table II. *Clinical characterization of JAK2 inhibitors.*

Drug	Company	Phase of development	Clinical trial dose	Route of administration	Patient population studied	Number of patients	Clinical/molecular responses	Observed toxicities
INCB-018424	Incyte	Phase I/II; phase III planned	25 mg twice daily	Oral	PMF and post-PV/ET MF	> 100	Decrease in splenomegaly irrespective of *JAK2* mutational status; improved quality of life and weight; reduced levels of inflammatory cytokines; modest decrease in *JAK2* V617F allele burden; no change in bone marrow fibrosis	Thrombocytopenia, myelosuppression
CEP-701	Cephalon	Phase II	80 mg twice daily	Oral	*JAK2* V617F-positive PMF	22	Reduced spleen size; improvement in cytopenia; 2 patients achieved transfusion independency; decrease in phospho-STAT3; no improvement in bone marrow fibrosis; no significant improvement in allele burden	Myelosuppression (anemia and thrombocytopenia, gastrointestinal problems (diarrhea and nausea)
TG-101348	TargeGen	Phase I/II	680 mg once daily	Oral	PMF and post-PV/ET MF	28	More than 50% decrease in spleen size; reduction in *JAK2* V617F allele burden; marked reduction in leukocytosis; improvement in constitutional symptoms like pruritus; no change in levels of plasma cytokines	Nausea/vomiting, diarrhea, thrombocytopenia, neutropenia, anemia
XL-019	Exelixis	Phase I/II (discontinued)	25-50 mg once daily	Oral	Primary or post-PV/ET MF	21	Decrease in splenomegaly; reduction in leukocytosis and circulating blast cells; improvement of anemia, pruritus and fatigue	Neurotoxicities such as peripheral neuropathy, formication, balance disorder and confusion

Shown is a list of the four drugs tested in clinical trials and an overview of the treatment regimen. PMF, primary myelofibrosis; post-PV/ET MF, post-polycythemia vera (PV)/essential thrombocythemia (ET) myelofibrosis.

DISCUSSION

The discovery of JAK2-activating mutations in the majority of MPN patients has spurred the development of small-molecule inhibitors that specifically target JAK2. To date, a limited number of JAK2 inhibitors have been evaluated in clinical trials. Interestingly, while these inhibitors exhibited moderate to good preclinical efficacy, including murine models of human MPNs, those results have not translated well at the level of clinical trials. In these trials, the primary clinical benefits observed have been significant reductions in splenomegaly, patient weight gain and, in some cases, reductions in the *JAK2* mutant allele burden and/or reduced levels of some inflammatory cytokines. However, none of the inhibitors has yet been able to reverse the disease by providing any improvement in bone marrow fibrosis of the treated patients. The reasons as to why these inhibitors have been disappointing in clinical trials are now an area of investigation. For example, one theory is that the genetics underlying MPNs are more complex than first thought. For instance, it is unclear how a single point mutation such as *JAK2* V617F can lead to the development of three phenotypically distinct disorders,

namely, PV, ET and PMF. Some hypothesize that there are additional genetic and/or epigenetic events that contribute to the pathogenesis of these disorders and in turn determine which of the three disorders a specific patient will manifest (69).

Recently, three independent groups showed that the presence of a genetic haplotype leads to a predisposition for acquiring the *JAK2* V617F mutation and consequently developing MPNs (70-72). Results from recent epigenetic studies suggest that hypermethylation of the suppressor of cytokine signaling (SOCS) family proteins, negative regulators of JAK/STAT signaling, may also play a role in the pathogenesis of MPNs (73). The presence of such genetic and epigenetic factors may determine how a specific MPN manifests itself in a given individual and, in turn, how that individual responds to a specific treatment. This also raises the possibility that we might have to consider the use of combination therapies for effective treatment of these disorders instead of merely JAK2 monotherapy. Lastly, although this runs contrary to current theories on the development of JAK2-specific inhibitors, perhaps inhibitors of the future need to be less specific for JAK2 and instead have some off-target properties as well. In other words, dirty might be better.

Another important factor to be considered when judging the limited success of the JAK2 inhibitor clinical trials is the patient population being studied. These early clinical trials have all been performed in patients with PMF because it is the most severe of the three classical MPNs. However, it must be kept in mind that a drug that does not show significant improvement in these patients might still be effective if given to a patient with a less severe MPN, such as PV, ET or even early-stage PMF. Hence, it will be important to design more appropriate clinical trials that can address this important issue before final conclusions are drawn regarding the efficacy of these drugs in humans.

Most of the efforts to identify JAK2 inhibitors have been focused on screening for small molecules that target the ATP-binding pocket within the JAK2 kinase domain. However, since this pocket is highly conserved among all kinases, developing a JAK2 inhibitor that does not inhibit other kinases is a challenge. The ATP pocket-targeted inhibitors have another limitation as well. Specifically, they are unable to distinguish between wild-type and mutant JAK2 kinases. Hence, it is not surprising that these inhibitors, due to their potency against wild-type JAK2, cause myelosuppression and anemia in treated patients. The occurrence of such adverse side effects imposes an upper limit on the dose of the drug that can be administered to these patients.

Another dilemma is that the JAK2-activating mutations identified in MPN patients, including V617F, are mostly present in the JH2 or pseudokinase domain of JAK2 and not the kinase domain. Unfortunately, the crystal structure of JAK2 encoding both the JH1 (kinase) and JH2 (pseudokinase) domains has not yet been resolved. As such, it is currently not possible to design inhibitors that specifically target mutant forms of JAK2 over the wild-type protein. To complicate matters even further, one wonders whether an inhibitor that is specifically designed against *JAK2* V617F will be effective against the dozens of other JAK2 mutations that are known to exist. That said, in the future, solving the crystal structure of full-length JAK2, or at least the JH1-JH2 domains, will be very critical for the design of future JAK2 inhibitors.

Attempts are also being made to design more specific JAK2 inhibitors by exploiting the subtle differences between the kinase domain structures of JAK2 and other protein kinases. Crystal structure analyses have shown that the ATP-binding site of the JAK kinases is much more constricted when compared to the other tyrosine-protein kinases (48, 74). It has also been shown that the electrostatic surface potential around the active site within JAK2 is more positively charged than other JAK family members, such as JAK3 (74). Moreover, several residues in the hinge region (such as M929, Y931, P934, R938), the glycine loop (such as K857), the catalytic loop (such as I982) and the activation loop (such as E1015) may potentially confer inhibitor selectivity to JAK2 over other tyrosine kinases, including other JAK family members (48). That said, only completion of such studies will determine whether these structure–activity relationships (SAR) do in fact exist.

In summary, the causative nature of JAK2 somatic mutations in the pathogenesis of MPNs was established in 2005. The first clinical trial testing a putative small-molecule JAK2 inhibitor for the treatment of MPNs was initiated less than 3 years later. While the initial data from these limited clinical trials have not lived up to expectations, it is evident that these inhibitors, even if not curative, may still have great therapeutic benefit for MPN patients due to their ability to reduce some clinical symptoms, as well as improve the overall quality of life. However, one of the challenges to implementing these JAK2 inhibitor therapies for MPN patients will be their prohibitive costs. The symptoms of MPN patients are effectively managed by existing therapeutic options, such as venesection/cytoreductive agents. Therefore, a shift towards the use of more expensive JAK2 inhibitor therapy will be warranted only if these inhibitors show some curative effects as opposed to just palliative effects. The issue of acquired resistance may also be a potential problem with the use of JAK2 inhibitors, similar to that seen with other types of inhibitor-based therapeutics, such as BCR-ABL and FLT3 inhibitors.

In this time of rapid expectations, we must be mindful that this area of research is still in its infancy. Current avenues of investigation continue to expand our knowledge of JAK2 kinase and its potential inhibitors. With an increased knowledge of: 1) the molecular pathogenesis of MPNs; 2) the structural properties of wild-type and mutant JAK2 proteins; 3) the continued identification of SAR between JAK2 and putative inhibitors; and 4) the development of better animal models that more closely resemble the complex nature of MPNs observed in humans, we are confident that one day JAK2 inhibitors will be a viable therapy for the treatment of MPNs.

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DISCLOSURES

The authors state no conflicts of interest.

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