Preclinical and Clinical Activity of ATP Mimetic JAK2 Inhibitors

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Abstract: The discovery of a common Janus kinase 2 (JAK2) point mutation, JAK2V617F, in myeloproliferative neoplasms has generated enormous interest in the development and therapeutic use of small molecule JAK2 inhibitor–targeted therapy in these diseases. A handful of compounds are currently in clinical development in primary myelofibrosis or post-polycythemia vera (PV)/essential thrombocythemia (ET) myelofibrosis. To date, clinical benefit has been demonstrated in terms of reduction of splenomegaly, improvement in constitutional symptoms, and control of leukocytosis. Some of the drugs have also been evaluated in PV and ET, with demonstrated activity against erythrocytosis, thrombocytosis, pruritus, and splenomegaly. However, drug effect on bone marrow fibrosis or JAK2 allele burden has been modest so far. Regardless, it is important to keep in mind that current anti-JAK2 treatment trials constitute only the beginning of many upcoming similar clinical trials, and that it is premature to make generalizations or any form of comparative conclusions regarding drug activity or toxicity.

Keywords
JAK2 inhibitors, myelofibrosis, myeloproliferative neoplasms

Introduction: The Promise of Targeted Therapy

In 2005, the discovery of a common point mutation (*JAK2V617F*) in the Janus kinase 2 (JAK2 kinase) in more than 90% of patients with polycythemia vera (PV) and nearly one-half of those with essential thrombocythemia (ET) or primary myelofibrosis (PMF) led to a greater understanding of the pathogenesis of the *BCR-ABL*-negative classic myeloproliferative neoplasms (MPNs).[1-5] PV, ET, and PMF are characterized by clonal expansion of hematopoietic precursors and panhyperplasia of all myeloid lineages.[6-8] PV and ET are more indolent than PMF; patients with PMF have an average survival of approximately 69 months from diagnosis.[9] The natural history of these disorders is progression to a fibrotic phase, characterized by bone marrow failure and extramedullary hematopoiesis, with ultimate progression to acute leukemia in a percentage of patients. Medical treatment options for MPNs are palliative in nature; phlebotomy or anti-proliferative agents such

as hydroxyurea are used to decrease thrombotic events, blood counts, and spleen size. When patients develop bone marrow failure, transfusions are often used. Allogeneic stem cell transplantation is an option available to very few patients secondary to donor availability, patient age, and comorbidity. Consequently, there is a need for better therapies for these disorders, which ideally would be targeted to their causal molecular events. The hope was that the *JAK2V617F* mutation in MPNs would be akin to *BCR-ABL* in chronic myelogenous leukemia (CML). As a result, there has been a great deal of enthusiasm and investigation into targeted therapy through JAK2 inhibition with ATP-mimetic small molecules, which would be analogous to the successful treatment of CML by imatinib mesylate (Gleevec, Novartis). In this review we discuss the current preclinical and clinical data on this emerging field.

Role of JAK2

Bone marrow cells from PV patients form endogenous erythroid colonies (EECs) in the absence of exogenous erythropoietin, indicating that erythropoiesis in PV patients is erythropoietin independent.[10] Likewise, erythroid and myeloid cells from ET and PMF patients also demonstrate hypersensitivity to various growth factors.[11-13] Mutations in the erythropoietin receptor were seen in cases of congenital erythropoiesis; however, no mutations were observed in PV patients. This led investigators to speculate that the hypersensitivity to growth factors was downstream of the growth factor receptor, but involved common signaling pathways.

The JAK family of kinases includes the cytoplasmic protein kinases JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2). The JAKs mediate the signaling for cytokine, hormone, and growth factor receptors, and the pathway is essential for cytokine-mediated cell proliferation, survival, and apoptosis.[14,15] Ligand binding to cognate cytokine receptors results in activation of the respective JAK kinase and phosphorylation of specific tyrosine residues on the cytokine receptor scaffold. These in turn serve as docking sites for downstream effectors, in particular the signal transducer and activator of transcription (STAT) family of proteins.[16] This results in further activation of the pathway through translocation of STAT proteins into the nucleus to initiate gene transcription.[15] JAK2 plays a central role in the signaling pathway of many hematopoietic growth factor receptors including erythropoietin, thrombopoietin, granulocyte-macrophage colony stimulating factor, interleukin (IL)-3, IL-5, and growth hormone.[17] In addition to playing a direct role in signal transduction, JAK2 promotes the cell surface expression of the erythropoietin and MPL receptors.[18,19]

JAK2V617F

In 2005, 5 independent labs reported on the identification of a *JAK2* somatic mutation in MPNs, which resulted in a valine to phenylalanine substitution at codon 617 of *JAK2* (*JAK2V617F*).[1-5] This mutation in the JH2, or pseudokinase domain, results in constitutive activation of the JAK2 enzyme. The psuedokinase domain is thought to serve as a negative regulator of the kinase domain. It is hypothesized that the V617F mutation prevents the pseudokinase domain from inhibiting the kinase, resulting in aberrant JAK2 tyrosine kinase activity. Using highly sensitive allele-specific assays, this mutation has been found in more than 90% of patients with PV and approximately 50% of patients with ET and PMF.[20,21] This mutation occurs in a multipotent hematopoietic progenitor cell, which gives rise to erythroid and myeloid lineages and is thought to confer a growth and survival advantage over nonmutated cells.[22] In vitro studies have demonstrated that erythroid progenitors carrying the mutation are able to form colonies in the absence of exogenous erythropoietin and are hypersensitive to the effects of erythropoietin.[3,5] Using a murine bone marrow transplantation model, the expression of *JAK2V617F* produced a phenotype resembling PV.[23-26] Not all patients with PV, ET, or PMF have a *JAK2V617F* mutation, suggesting that other alleles may be involved. Other mutations have since been found in the thrombopoietin receptor *MPL* (*MPLW515L* and *MPLW515K*) in ET and PMF patients.[27-29] Most commonly these are found in *JAK2V617F*-negative patients. Expression of the *MPLW515* mutation similarly results in factor-independent growth and constitutive activation of downstream pathways, including the STAT and MAP kinase pathways.[28,29] In vivo expression of *MPLW515L* in a murine bone marrow transplantation model results in marked thrombocytosis and increased bone marrow reticulin fibrosis.[23,25,26,29] Mutations in exon 12 of *JAK2* have also been identified in *JAK2V617F*-negative PV.[30]

JAK2 Inhibitors

Since the discovery of the *JAK2V617F* mutation in MPNs, several groups have been actively developing orally available small molecule JAK2 kinase inhibitors. JAK2 inhibitors can be categorized as class I, or JAK2 selective, and class II, or JAK2-nonselective, inhibitors. Class I inhibitors have been rationally designed based upon the molecular structure of JAK2 and JAK3 to selectively bind to JAK2 relative to JAK3.[31] This strategy yields compounds that inhibit both wild type and mutated JAK2, and thus will likely suppress normal hematopoiesis, given the importance of JAK2 in normal hematopoiesis. As a result, efforts are ongoing to develop allele-specific inhibitors of *JAK2V617F*. Based on their selectivity for

Table 1. JAK2 Inhibitors Currently in Development

Compound (Company)	Stage of Development	JAK2 IC_{50} (nM)	Disease	Clinical Benefit	Toxicities
INCB018424 (Incyte)	III	4.5	MF (primary and secondary), ET, and PV	Splenomegaly, constitutional symptoms, pruritus, cachexia, erythrocytosis (PV)	Thrombocytopenia (DLT), anemia, cytokine rebound phenomenon
TG101348 (TargeGen)	II	3	MF (primary and secondary)	Splenomegaly, constitutional symptoms, pruritus, leukocytosis, thrombocytosis, JAKV617F burden	Increased amylase/lipase (DLT), increased transaminases, diarrhea, nausea/vomiting, thrombocytopenia, anemia
XL019 (Exelixis)	I (development halted)	2	MF (primary and secondary)	Splenomegaly, constitutional symptoms, leukocytosis	Neuropathy (DLT)
CEP-701 (Cephalon)	I/II	1	MF (primary and secondary), ET, and PV	Splenomegaly, anemia, pruritus	Diarrhea nausea/vomiting, anemia (MF), thrombocytopenia (MF), thrombosis (PV/ET), leukocytosis (PV/ET), thrombocytosis (PV/ET)
SB1518 (S*Bio)	II	260	MF (primary and secondary)	Splenomegaly	GI symptoms (DLT), diarrhea, nausea, thrombocytopenia
CYT387 (Cytopia)	I/II	18	MF (primary and secondary)	Ongoing	Ongoing

DLT=dose-limiting toxicity; ET=essential thrombocythemia; GI=gastrointestinal; IC_{50}=half maximal inhibitory concentration; MF=myelofibrosis; PV=polycythemia vera.

JAK2V617F, these compounds would have a more desirable therapeutic window since only the disease allele would be inhibited. Class II compounds were developed primarily for other indications, and *JAK2* inhibition is an "off-target" effect. Table 1 details some of the JAK2 inhibitors in development.

JAK2 Inhibitors in Myelofibrosis

The JAK2 inhibitors were first introduced in patients with either PMF or post-ET or post-PV MF who required treatment or who had high- or intermediate-risk disease. This population of patients has an overall shortened survival, and treatment options are limited.

INCB018424

INCB018424 (Incyte Corporation) is the furthest along in its clinical development. This compound is a potent, orally bioavailable JAK1 and JAK2 inhibitor with more than 80-fold selectivity for JAK2 over JAK3.[32] In vitro, INCB018424 inhibited the proliferation of factor-dependent cell progenitor cells and BA/F3 cells expressing *JAK2V617F* with a half maximal inhibitory concentration (IC_{50}) of 100–130 nM, but not the proliferation of cell lines expressing activating mutations in either *BCR-ABL* or *KIT*.[33] The effect on cell proliferation correlated with reduced levels of phosphorylated JAK2 and STAT5.[33] In colony-forming assays using cells harvested from patients with *JAK2V617F*, INCB018424 inhibited the cytokine-independent formation of erythroid progenitor cells at a low IC_{50} compared to normal colony formation from healthy donors.[33] In a murine model of MPN, implantation of BA/F3 cells expressing *JAK2V617F* resulted in rapid organomegaly and reduced survival. In this model, oral administration of INCB018424 reduced splenomegaly, eliminated neoplastic cells from the liver, spleen, and bone marrow, and significantly prolonged survival.[33]

Phase I/II studies of INCB018424 in patients with primary or post-PV or post-ET MF with or without a *JAK2V617F* mutation explored daily (25–200 mg/day)

as well as twice daily (10–25 mg twice daily) regimens of INCB018424.[34] The phase I study established 25 mg twice daily as the maximum tolerated dose (MTD). Treatment with INCB018424 was associated with a rapid reduction in splenomegaly, which typically occurred within the first month of therapy, with slower decreases observed thereafter. The dose-limiting toxicity was thrombocytopenia, likely secondary to inhibition of thrombopoietin signaling through JAK2.[32,34] In an optimized dosing trial, dosing was started at 10 mg or 15 mg twice daily depending on the baseline platelet count, and the dose was titrated after 1–2 months to 15 mg or 20 mg twice daily.[35] Over 150 patients have been enrolled to date. Eleven of 23 (48%) patients at the optimized dose level achieved a 35% or higher reduction in spleen volume at 6 months (equivalent to International Working Group [IWG] clinical improvement criteria of 50% reduction by palpation).[35] Patients also reported an improvement in constitutional symptoms including night sweats, fatigue, and pruritis.[32,34,35] In a separate report, which examined 34 patients treated for at least 2 months with 25 mg twice a day, INCB018424 was associated with weight gain, improved appetite, and increased cholesterol and leptin levels, which in turn reflects the improved nutritional status of subjects after treatment.[36] Symptomatic improvements coincided with a rapid and sustained reduction in the pro-inflammatory cytokines IL-1b, IL-1ra, IL-6, and tumor necrosis factor (TNF)-α.[35] Unfortunately, despite the promising clinical benefit seen in patients treated with INCB018424, the *JAK2V617F* allele burden did not change in a substantial manner, indicating that the clinical activity of INCB018424 may be the result of inhibition of downstream signaling of JAK2 and JAK1 or reductions in cytokine levels.[37] Also, probably because of the latter effect, some patients have experienced severe "cytokine rebound" symptoms including acute enlargement of spleen size, relapse of constitutional symptoms, and, occasionally, life-threatening hemodynamic disturbances, especially in those patients with baseline cardiopulmonary disease.[38] Therefore, drug discontinuation should be gradual and be done under close supervision. Phase III approval studies of INCB018424 are currently ongoing in the United States and Europe.

TG101348

TG101348 (TargeGen) is a class I potent inhibitor of JAK2 with an IC_{50} of 3 nM, and 35- and 334-fold selectivity for JAK2 compared to JAK1 and JAK3, respectively.[39] In vitro, TG101348 treatment of HEL and BA/F3 cells expressing *JAK2V617F* induced apoptosis at very low concentrations of 305 nM and 270 nM, respectively.[39,40] In a murine bone marrow transplant assay of PV, TG101348 was administered by oral gavage at 60 mg/kg or 120 mg/kg twice daily for 42 days. There was a dose-dependent reduction in hematocrit compared to vehicle controls. Furthermore, there was a dose-dependent reduction of splenomegaly, extramedullary hematopoiesis, and evidence for reduction of myelofibrosis. In vivo responses correlated with reduction of *JAK2V617F* allele burden as assessed by quantitative genomic polymerase chain reaction, suppression of EEC formation, and in vivo inhibition of the JAK-STAT signal transduction pathway as assessed by phosphorylated STAT5.[39]

A phase I dose escalation study of TG101348 was conducted in symptomatic patients with primary or post-PV and post-ET MF with intermediate- or high-risk disease.[31,41,42] This study identified an MTD of 680 mg daily, with a dose-limiting toxicity of asymptomatic grade 3/4 increase in serum amylase/lipase, which was reversible. Results of this trial were updated and presented at the 2009 meeting of the American Society of Hematology (ASH).[42] At the time of the presentation, 59 patients had been enrolled—28 in the dose escalation phase and 31 in the dose confirmation phase. The majority of patients had PMF, and 86% of patients were *JAK2V617F* positive. Median follow-up was 12 weeks (range, <1–76). TG101348 was well tolerated, with grade 3/4 neutropenia and thrombocytopenia observed in 14% and 25% of participants, respectively. Nonhematologic toxicities were grade 1/2 nausea, vomiting, and diarrhea, which tended to be self-limited. Responses included reduction of splenomegaly, with 22 patients experiencing a greater than 50% decrease in spleen size, including 9 whose spleen became nonpalpable. There was a marked reduction in leukocyte count for the 21 patients who had elevated leukocyte counts at baseline. *JAK2V617F* allele burden was measured in 48 patients who had completed 1 cycle of therapy, and the median granulocyte mutant allele burden decreased by 48%. Similar to the results of INCB018424, clinical benefits included resolution of constitutional symptoms of early satiety, fatigue, pruritus, and night sweats.

XL019

XL019 (Exelixis) is a potent and reversible inhibitor of the JAK2 enzyme with a Ki of 2 nM and 50-fold selectivity for JAK2 against more than 120 protein kinases, including JAK1 and JAK3.[43] In vitro assays demonstrated that XL019 inhibits proliferation of cell lines harboring activated or overexpressed JAK2. In tumor xenograft models, twice daily dosing led to substantial tumor grown inhibition with increases in tumor cell apoptosis. In phase I data presented at the 2008 ASH meeting, 30 patients with primary or post-PV or post-ET MF had been enrolled, with 21 treated at 50 mg or less.[44] Initially, the starting dose was 100 mg daily for 21 days of a 28-day cycle,

and escalated to 300 mg daily.[44] Unfortunately, reversible low-grade peripheral neuropathy was observed at doses of higher than 100 mg, so the study was amended. Subsequent doses studied were 25 mg and 50 mg daily or 25 mg 3 times a week. All patients with a *JAK2V617F* (n=11) or a *MPLW515F* (n=1) mutation experienced a 50% or higher decrease in spleen size from baseline, but no patients with wild-type JAK2 had a spleen response. Patients reported improvements in constitutional symptoms of pruritus and fatigue. No treatment-related hematologic adverse events were observed. Other adverse events observed were neurologic in nature, including formication, balance disorder, paresthesias, confusional state, and peripheral neuropathy. Although reversible and mild, these toxicities prevented further clinical development of this compound.

CEP-701

CEP-701 (lestaurtinib, Cephalon) is a staurosporine analog initially developed clinically as an oral FLT3 inhibitor for the treatment of acute myeloid leukemia. It is a potent class II JAK2 inhibitor with an IC_{50} of 1 nM.[45] CEP-701 inhibits proliferation of mutant JAK2-dependent HEL cells in vitro and in xenograft models. At concentrations of 100 nM or more, CEP-701-inhibited erythroid cells expanded from primary CD34-positive cells from MPN patients and inhibited phosphorylation of STAT5 as well as other downstream effectors of JAK2. In contrast, growth of erythroid cells derived from healthy controls was not significantly inhibited.[45]

Studies of CEP-701 in AML established 80 mg twice a day as the recommended dose. In these trials, incomplete target inhibition secondary to gastrointestinal toxicity, as well as low free drug levels related to in vivo plasma protein binding, were observed. Therefore, an MTD for CEP-701 was not reached in these studies. In a dose escalation trial conducted by the Myeloproliferative Disorders Research Consortium (MPD-RC), CEP-701 was administered orally twice a day at doses of 80–160 mg for a minimum of 28 consecutive days.[46] Nineteen patients were enrolled with primary, post-PV, or post-ET MF. The most common toxicities were diarrhea (in 5 of 7 patients treated with the liquid formulation and 2 of 12 patients receiving the capsule formulation) and nausea. All patients had splenomegaly at baseline, and CEP-701 resulted in a median decrease in spleen size of 6.4 cm. There was a modest reduction in JAK2 allele burden with a median decrease of 8.6%.[46] In a phase II study conducted at the M.D. Anderson Cancer Center, 22 patients with *JAK2V617F*-positive high- or intermediate-risk MF were treated with CEP-701 80 mg twice a day.[47] There was clinical improvement in 6 (27%) patients as defined by the IWG criteria.[48] Three patients only had a reduction in spleen size; 2 became transfusion independent; and 1 patient had a reduction in spleen size along with an improvement in neutrophil and platelet counts. Median time to response was 3 months and median duration of response was 14 months. Despite the clinical improvement seen with this compound, similar to other JAK2 inhibitors, no reduction in marrow fibrosis or *JAK2V617F* allele burden was seen. In this study, the majority of side effects were hematologic (grade 3/4 anemia and thrombocytopenia in 13% and 23% of patients, respectively) and gastrointestinal (diarrhea of any grade in 72% of patients and grade 3/4 nausea in 9%).[47]

SB1518

SB1518 (S*Bio) is a potent inhibitor of both wild-type JAK2 (IC_{50} of 22 nM) and the *JAK2V617F* mutation (IC_{50} of 19 nM).[49] It has 58- and 24-fold selectivity for JAK2 compared to JAK1 and JAK3, respectively.[50] In preclinical studies, SB1518 inhibited phosphorylated STAT5 levels in a dose-dependent manner and reduced the viability of expanded erythroid progenitors from normal volunteers with only wild-type JAK2 as well as from PV patients with *JAK2V617F* mutations.[51] This suggests that clinical benefit with this compound will be seen in patients regardless of JAK2 mutational status. Mouse models demonstrated antitumor activity in JAK2-dependent BA/F3-*JAK2V617F* leukemia.[50,51] In a phase I dose escalation trial of SB1518 in patients with acute and chronic myeloid diseases including primary or post-ET and post-PV MF, the dose-limiting toxicity was gastrointestinal related, with 33% experiencing diarrhea (4% grade 3) and 13% experiencing nausea (grade 1 and 2). Other toxicities included thrombocytopenia in 4% of patients (all grade 3/4). Of the 31 patients with MF, 21 were evaluable for response. Of 17 patients with palpable splenomegaly, 7 had a greater than 35% decrease in spleen size by physical examination; 4 of these patients had a decrease of 50%. Inhibition of phosphorylated STAT3 and STAT5 was demonstrated within 4–6 hours of the first dose. Based on this trial, 400 mg daily is being tested in ongoing phase II studies.

CYT387

CYT387 (Cytopia) is now in early clinical trials. Preclinical studies have demonstrated that it is a potent JAK1 and JAK2 inhibitor with IC_{50} of 11 nM and 18 nM, respectively.[52] It has significantly less activity for other kinases including JAK3. CYT387 inhibits the growth of BA/F3 cells expressing *JAK2V617F* or *MPLW515L* and HEL cells at nanomolar and near micromolar concentrations. CYT387 treatment of HEL cells resulted in a dose-dependent decrease in the phosphorylation of JAK2 downstream effectors, STAT5, and STAT3. CYT387

selectively suppresses the in vitro growth of erythroid colonies harboring *JAK2V617F* from PV patients. Phase I/II trials with this compound are ongoing.

JAK2 Inhibitors in Refractory PV/ET

Some of the above-listed compounds are currently being evaluated in patients with PV and ET who are refractory to standard treatment. INCB018424 is being tested in a phase II trial in advanced PV and ET patients.[53] Starting doses of 10 mg twice daily in PV and 25 mg twice daily in ET were chosen based on efficacy and tolerability in an initial dose-finding phase. In the 2009 update of this trial presented at the ASH meeting, 45% of patients with PV and only 13% of those with ET had achieved a complete response, as defined by a European consensus conference.[54] Although the drug was effective in eliminating the need for phlebotomy, its effect on thrombocytosis was only modest, which was the main reason for the low 13% complete response rate in ET. As was the case in MF, PV patients with splenomegaly or pruritus also benefitted. Grade 2 toxicities in PV patients were anemia (12%) and thrombocytopenia (6%), which were reversible upon dose interruption or modification. Grade 2 toxicities in ET were anemia (18%) and neutropenia (6%), which were also reversible with dose interruption or modification.

CEP-701 has also been tested in advanced PV and ET. The results of the first 39 patients enrolled were updated at the 2009 ASH meeting.[55] The primary endpoint was reduction in the *JAK2V617F* neutrophil allele burden, and secondary endpoints included reduction in phlebotomy rates, improvement in cell counts, and reduction in hydroxyurea dose and spleen size. Enrollment included 27 patients with PV and 12 with ET aged 38–80 years. Within 18 weeks, responses included a reduction of spleen size of more than 5 cm or to nonpalpable in 15 of 18 (83%) subjects and improvement of pruritis in 5 of 5 patients. A number of phlebotomy-dependent patients had a reduction in their phlebotomy requirements (3/5 evaluable patients at the time of report); however, this effect was not seen until after 6 months of therapy, and concomitant reductions in white cell or platelet counts were not seen. Interestingly, many patients had increases in platelet and white cell counts on therapy. At the 18-week assessment, 3 of 15 patients had a reduction in the *JAK2V617F* allele burden by 15% or more. Dose-related gastrointestinal adverse events were common and improved over time. Serious adverse events included thrombosis in 5 patients (3 venous and 2 arterial), which has not been frequently seen with CEP-701 therapy for other malignancies or with other JAK2 inhibitors.

Conclusion

The expectation that the discovery of *JAK2V617F* would lead to the next success story in targeted therapy has yet to be realized. At this time, the small molecule JAK2 inhibitors have demonstrated modest clinical benefit in terms of shrinkage of splenomegaly and improvement in constitutional symptoms in MF subjects. There has not been a consistent decrease in JAK2 allele burden or reversal of marrow fibrosis with these compounds in this disease. There are substantial inter-drug differences in their toxicity and efficacy profiles, which is in part due to their variable activity against other JAK and non-JAK kinase targets. Given that all of these drugs are early in the development phase, it would be premature to make generalizations as to one drug's efficacy compared to another. The value of these drugs in PV or ET is highly questionable since there are currently-available alternative drugs that induce a much higher CR rate in hydroxyurea-refractory patients with PV or ET, including interferon-α, busulfan, and pipobroman. However, their unique and impressive effect against pruritus should be helpful for patients with intractable PV-associated pruritus.

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