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JAK2 inhibitors: are they the solution?

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Abstract

The discovery of the JAK2V617F mutation in patients with Philadelphia-negative myeloproliferative neoplasms (Ph-negative MPNs) started the era of targeted therapy for these diseases. Until now, patients had few treatment options available, usually restricted to hydroxyurea, interferon preparations, and chemotherapy in more aggressive cases. JAK2 inhibitors have been developed over the past 5 years, and the results of the first clinical trials with JAK2 inhibitors for patients with myelofibrosis were recently published. Current results suggest that JAK2 inhibitors have a potential to decrease disease burden and its activity, as manifested by a decrease in splenomegaly and improvement in systemic disease-related symptoms, but they do not seem to be able to eradicate the malignant clone. On the other hand, JAK2 inhibitors help patients regardless of their mutation status as patients without JAK2V617F mutation benefit to the same extent as patients with JAK2V617F mutation. A greater understanding of the pathophysiology of MPNs is needed before we can cure myelofibrosis with drug therapy. Currently, several new JAK2 inhibitors are in clinical trials for patients with MF and clinical trials for patients with PV and ET have also started. We review recent data on JAK2 inhibitors for the management of patients with Ph-negative MPNs.

Keywords

Polycythemia vera; Essential thrombocythemia; Myelofibrosis; JAK2 inhibitor; JAK2 V617F

Introduction

In 2005, four independent groups reported on the discovery a novel point mutation in the JAK2 gene in patients with Philadelphia-negative myeloproliferative neoplasms (Ph-negative MPNs), polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF; either primary [PMF] or post-PV/post-ET) (1–4). This mutation (JAK2V617F) induces constitutive activation of the JAK2 tyrosine kinase (TK) and animal models have confirmed that it plays a major role in the pathogenesis of these disorders (5–15). JAK2V617F was the first mutation to be described in patients with Ph-negative MPNs,

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and the fact that it can be detected in patients with all three classic subtypes of MPNs attest to their interrelationship, which was recognized in the 1950s by Dameshek in a classic Blood editorial (16). After discovery of JAK2V617F, other investigators discovered mutations in related genes of hematopoietic growth factor signaling pathways, including exon 12 mutations in JAK2, mutations of MPL (thrombopoietin [TPO] receptor) and more recently mutations in the adapter protein LNK (17–20). It appears that mutations of the signaling pathways responsible for response to hematopoietic growth factors are a common feature of these disorders.

Besides providing important insight into the pathophysiology of Ph-negative MPNs, the JAK2V617F mutation also represents a potential therapeutic target. In fact, since mid-2007 several clinical trials were started exploring the therapeutic potential of JAK2 inhibitors in patients with PV, ET and MF. In this article we review the pre-clinical and clinical data of these compounds for therapy of patients with Ph-negative MPNs.

The JAK2V617F Mutation

The JAK2 TK belongs to the JAK family of TKs, along with JAK1, JAK3 and TYK2. JAKs were first described in 1989 by employing degenerate oligonucleotide probes corresponding to amino acid sequences found in the catalytic domain of all known TKs in a polymerase chain reaction of cDNA from hematopoietic cell lines (21). JAKs have two TK domains, and hence their name derives from the two-faced Roman god Janus (22). The domain JH1 (JAK-homology 1) is the active TK domain; domain JH2 is adjacent to the JH1 domain and is catalytically inactive, being called “pseudo-kinase domain” (23). It is believed that the JH2 domain purpose is to regulate activity of the JH1 domain, as deletion of the JH2 domain leads to increased TK activity of JAK2 and JAK3 with increased phosphorylation of intracellular signaling molecules such as STAT5 (Signal Transducer and Activator of Transcription) (24).

JAKs associate with the intracellular portion of cytokine receptors, such as MPL, erythropoietin (EPO) receptor (EPOR), G-CSF receptor and Interferon (IFN)- $\alpha/\beta/\gamma$ receptors (25). Binding of the putative ligand to the receptor induces receptor dimerization and JAK activation, with consequent phosphorylation of target proteins and activation of downstream pathways, including STATs, PI3K/Akt/mTOR (Phosphatidylinositol 3-kinase/Akt/mammalian Target of Rapamycin) and MAPKs (Mitogen Activated Protein Kinases) (25–29). Activation of these pathways leads to increased cellular proliferation and resistance to apoptosis. Different JAKs associate with distinct receptors and play diverse roles in cytokine signaling, findings which were elucidated with knock-out animal models. Neubauer et al. reported that JAK2 is essential for erythropoiesis, as JAK2-deficient mice embryo die after 12.5 days due to absence of definitive erythropoiesis (30). In another study reported at the same time, it was demonstrated that fetal liver myeloid progenitors from JAK2-deficient embryos did not respond to EPO, TPO, interleukin-3 (IL-3), granulocyte-macrophage colony stimulating factor (GM-CSF) and IFN- γ (31). This indicates that JAK2 is essential for signaling through EPOR, MPL and cytokine receptors harboring the common β -chain (*CSF2RB*; CD131) (31).

The JAK2V617F mutation is believed to cause constitutive activation of the JAK2 kinase. Indeed, cells from patients with Ph-negative MPNs positive for the JAK2V617F mutation display increased phosphorylation of JAK2 and downstream targets such as STAT5 and Akt (2). Ba/F3 cells engineered to express EPOR and JAK2V617F display EPO-independent growth and hypersensitivity to EPO (4). Residue V617 is located in a loop of the JH2 domain which interacts with the JH1 domain (32). Residue V617F, possibly by interaction with the phenylalanine residue F595, disrupts this interaction and leads to increased activity of the JH1 domain (33). Besides the canonical pathway of increased activation of cytokine induced signaling cascades, JAK2 V617F may also modulate gene transcription through a non-canonical pathway. Dawson et al. recently demonstrated that JAK2 is present in the nucleus of hematopoietic cells and that it phosphorylates histone H3 at residue Y41 (34). This prevents binding of protein Heterochromatin Protein 1 α (HP1 α), which mediates gene silencing through epigenetic mechanisms (35, 36). Through this mechanism JAK2V617F leads to increased expression of the oncogene *LMO2*, and inhibition of JAK2 activity increases binding of HP1 α and inhibits expression of *LMO2* (34). In another publication, Rinaldi et al. reported that nuclear JAK2 was preferentially detected in the CD34+ fraction of hematopoietic cells of patients with Ph-negative MPNs, but not in the granulocytic, erythrocytic and megakaryocytic cell population (37). It thus appears that epigenetic modulation of gene transcription by JAK2V617F occurs predominantly in the immature hematopoietic stem cell population. This probably has an important role in the pathogenesis of JAK2V617F-positive MPNs, as a recent mouse model has demonstrated that JAK2V617F only initiates disease when expressed in immature hematopoietic stem cells (10).

Several case series have reported on the prevalence of JAK2V617F, which is more common in PV (97%) as compared to ET (50–60%) and MF (50%) (1–4). The burden of mutated JAK2 appears to be associated with distinct clinical and prognostic features. Patients with PV are usually homozygous for the JAK2 mutation, and those with high mutation burden have more frequent splenomegaly and thrombosis (38–40). In ET, JAK2V617F is usually present in heterozygosity (38), and patients with JAK2V617F-positive ET display some “PV-like” clinical features, such as increased hematocrit and may eventually evolve into PV, suggesting that both are part of the same disease spectrum (41, 42). Interestingly, one recent paper suggested that the benefit of anti-platelet agents in patients with ET was restricted to those patients who were JAK2V617F-positive, which suggests that in the future the presence of this mutation may guide therapy for patients with ET (43). In MF the prognostic significance of JAK2 mutation burden is unclear, as some reports have suggested that low mutation burden is associated with decreased survival and others have reported that a high mutated burden is associated with splenomegaly and a higher rate of leukemic transformation (44–46).

Besides JAK2V617F, other mutations have been described in patients with Ph-negative MPNs. JAK2 exon 12 mutations are detected in 3% of PV patients, mostly those who are negative for the JAK2V617F mutation (19). MPL mutations are found in 10% of MF patients and 8.5% of ET patients, and are correlated with older age and anemia (17, 18, 47, 48). Recently, Oh et al. reported on mutations of the adapter protein LNK, which negatively regulates activity of the JAK2 TK (20). Mouse models have revealed that LNK suppresses

activity of both wild-type and mutated JAK2, and knock-out of LNK accelerates disease phenotype in mice harboring the JAK2V617F mutation (49). Mutations of LNK are preferentially located in the pleckstrin homology domain and are not exclusive of other MPN-associated mutations, including JAK2V617F (50).

JAK2 Inhibitors in development for Myelofibrosis (Table 1 and Table 2)

Most clinical studies with JAK2 inhibitors have been undertaken in patients with MF. Some common features have emerged. The majority of responses consist of improvement in spleen size and systemic symptoms; responses are seen in patients with and without JAK2V617F mutation. Some patients with leukocytosis and thrombocytosis achieve normalization of blood counts. No improvement in BM fibrosis and no significant decrease in JAK2 allele burden are usually seen. In clinical trials with a dual JAK1/JAK2 inhibitor, normalization of pro-inflammatory cytokines has been observed, a finding which has not been replicated in other clinical trials with more selective JAK2 inhibitors (51–53). MF is a pro-inflammatory disease, with elevated levels of several cytokines including tumor necrosis factor- α , IL-6, IL-8, basic fibroblast growth factor, transforming growth factor-beta1, TPO and vascular endothelial growth factor (54–59). Cytokines might lead to resistance to apoptosis induced by JAK2 inhibitors (60). By reducing the levels of pro-inflammatory cytokines, JAK2 inhibitors might improve splenomegaly and systemic symptoms (58). The effect of JAK2 inhibitors might not be restricted to malignant cells, but may also effect normal cells (61). Finally, recent evidence from animal models suggest that JAK2 inhibitors might not eradicate the most primitive leukemic stem cell in MF, which is one possible explanation why these drugs are not able to cure this disease, only to slow its progression (10).

a) INCB018424

The compound INCB018424 is a potent, orally available JAK1 and JAK2 inhibitor (62). It is being currently evaluated in clinical trials for patients with MF and PV/ET. In kinase assays INCB018424 inhibited JAK1 and JAK2 at similar potencies (half-maximal inhibitory concentrations [IC50] 3.3 nM and 2.8 nM, respectively), while being selective against TYK2 and JAK3 (Table 1). Cytokine stimulated whole blood assays demonstrated that INCB018424 inhibited phosphorylation of STAT3, a known downstream messenger of JAK1/JAK2, after stimulation with IL-6 and TPO (62). INCB018424 reduced viability of Ba/F3 cells transduced with EPOR and JAK2V617F (IC50 126 nM), and the same effect was observed on HEL cell line which endogenously express JAK2V617F (IC50 186 nM). This was accompanied by a reduction in levels of phosphorylated JAK2 and downstream messengers STAT5 and ERK1/2, indicating that the reduction in cell viability is due to kinase inhibition (62). Studies conducted with erythroid (burst-forming-units-erythroid [BFU-E]) and myeloid (colony-forming-units-myeloid [CFU-M]) progenitor cells demonstrated that INCB018424 inhibited proliferation and cell colony formations of progenitors cells from patients with PV with a greater potency when compared to cells from normal donors. In an animal model of MPN, INCB018424-treated mice had a reduction in spleen size and decrease in proinflammatory cytokines levels in peripheral blood, including IL-6 and TNF- α (62).

A phase I/II study evaluated INCB018424 in patients with MF (53). One hundred and fifty-three patients were recruited, and the results were recently reported. In the phase I portion of the trial, patients were treated with INCB018424 at different dose schedules, ranging from 25 mg twice daily to 50 mg twice daily and from 25 mg once daily to 200 mg once daily. The dose limiting toxicity (DLT) was thrombocytopenia, and the maximum tolerated dose (MTD) was determined to be 25 mg twice daily and 100 mg once daily (this last dose cohort was not expanded due to late development of significant thrombocytopenia). In the phase 2 portion of the trial, the cohort of 25 mg twice daily and 50 mg once daily were expanded. Further exploration of alternative dose schedules demonstrated that an individualized dose schedule of 15 mg twice daily represented the best balance between efficacy and safety. In this schedule, patients start therapy with 15 mg twice daily (10 mg if platelet counts $< 100 \times 10^9/L$), and after one month the dose is escalated to 20 mg twice daily if there is no response and no toxicity; the dose is further escalated to 25 mg twice daily if no response/toxicity is observed at 2 months.

Among the 140 patients who entered study with splenomegaly, 61 (44%) patients had a greater than 50% reduction in spleen size (53), consistent with a clinical improvement (CI) by the International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) response criteria (63). Response rates by dose received were 52% (15 mg twice daily), 49% (25 mg twice daily), 41% (50 mg once daily) and 30% (10 mg twice daily). Responses were durable, and at 12 months 73% and 78% of patients in the 15 mg twice daily and 25 mg twice daily cohorts maintained response. Evaluation of spleen size with magnetic resonance imaging confirmed that INCB018424 reduced patient's spleen volume by 33% and length by 52%. Importantly, response rate was not affected by JAK2 mutational status or prior diagnosis of PV/ET, indicating that INCB018424 is effective in all subgroups of MF. The drug also led to transfusion independency in 14% of transfusion-dependent patients and decreased platelet counts in 16 of 17 patients who presented with thrombocytosis. No improvement in bone marrow fibrosis was seen, and mean decrease in JAK2 V617F allele burden among mutated patients was 13%.

Therapy with INCB018424 had rapid and long-lasting effects on patients well being and functional capacity (53). The majority of patients reported a greater than 50% improvement in their Myelofibrosis Symptom Assessment Form (MFSAF) after only 1 month of therapy. Patients also had improvement in the ability to walk, a decrease in chronic symptoms such as pruritus and reported weight gain as early as 2 months after starting treatment with INCB018424. Correlative studies demonstrated an increase in the serum levels of erythropoietin and leptin and a decrease in the levels of proinflammatory cytokines, which correlated with patient's improvement in symptoms.

Side effects of INCB018424 were mainly related to myelosuppression and included thrombocytopenia and anemia (53). Grade 3–4 thrombocytopenia was more common in patients who started therapy with 25 mg twice daily compared to patients in the individualized dose schedule who started with 15 mg twice daily (23.4% vs. 2.9%). Incidence of new onset anemia was also more frequent in patients who were treated with 25 mg twice daily (26.7% vs. 8.3%). Non-hematological side effects were usually mild and

uncommon, the most frequent included diarrhea (all grades 5.9%, grade 3–4 0%), fatigue (all grades 4.3%, grade 3–4 1.3%) and headache (all grades 3.3%, grade 3–4 0%).

b) TG101348

TG101348 is an orally available JAK2 inhibitor currently being evaluated in a phase I/II clinical trial in patient with MF (64, 65). TG101348 has selective activity against JAK2 (IC₅₀=3nM) when compared to JAK1 (35-fold) and JAK3 (334-fold). TG101348 inhibited proliferation and induced apoptosis of JAK2V617F-positive HEL cells and Ba/F3 cells transduced with JAK2V617F (64, 65). In a murine model of polycythemia vera, mice were treated with placebo or TG101348 at doses of 60–120 mg/Kg orally twice daily (64). Mice treated with TG101348 had a decrease in Ht, improvement in splenomegaly and extramedullary hematopoiesis and reduction in bone marrow fibrosis. Flow cytometry analysis revealed a decrease in the number of JAK2 V617F-positive erythroid CD71 cells and BFU-E progenitors, as well as CFU-GM and CFU-M myeloid progenitors (64, 65).

A phase I clinical trial explored different doses of TG101348 in patients with primary (75%) or post-PV/ET (25%) MF (51). Overall, 59 patients have been enrolled, and the MTD was 680 mg. DLT was asymptomatic grade 3–4 hyperamylasemia/hyperlipasemia. The MTD cohort was expanded and 40 patients have received 680 mg/day. JAK2 V617F was positive in 86% of patients and 98% had palpable splenomegaly, with a median spleen size of 18 cm below left costal border. Forty-nine percent of patients had a clinical improvement (CI) based on reduction of palpable splenomegaly. Among patients with leukocytosis and thrombocytosis, therapy with TG101348 normalized blood counts in 73% and 100% of patients, respectively. Patients also reported better symptom control, including fatigue, early satiety, night sweats and pruritus. In 22 patients with baseline JAK2 V617F allele burden 20%, a decrease in allele burden was seen in 59% of cases, and allele burden remained stable in 36%. Some patients also had improvement in BM fibrosis and BM cellularity. Interestingly, no change in pro-inflammatory cytokines (IL-2, IL-6, IL-8, TNF-α) was seen.

TG101348 most common side effects were related to myelosuppression and GI disturbances (51). Among patients treated at the MTD, hematological side effects included grade 3–4 neutropenia (15%), thrombocytopenia (33%) and new-onset transfusion requiring anemia (67%). Non-hematologic adverse events included diarrhea (all grades 76%; grade 3–4 13%), nausea (all grades 70%; grade 3–4 5%) and vomiting (all grades 71%; grade 3–4 3%). Asymptomatic grade 3–4 elevations of amylase and lipase were seen in 6% and 13% of patients, respectively. Laboratory abnormalities associated with therapy with TG101348 were usually asymptomatic and either resolved spontaneously or following drug reduction/interruption.

c) CEP-701

CEP-701 (also known as lestaurtinib) is a potent FLT3 and JAK2 inhibitor (66). Pre-clinical studies have demonstrated that CEP-701 is a potent JAK2 inhibitor, inhibiting both wild type and JAK2 V617F (66). At a concentration of 100nM CEP-701 decreases proliferation of primary cells from patients with JAK2V617F-positive ET and MF and reduces level of

phosphorylated STAT5. In a xenograft mouse model, CEP-701 decreased proliferation of JAK2V61F-positive HEL 92.1 cells (66).

Two clinical trials have evaluated CEP-701 for therapy of patients with MF. In the first report, 22 patients with JAK2V617F-positive MF were treated with a liquid formulation of CEP-701 at an initial dose of 80 mg twice daily (based on clinical studies conducted in patients with AML) (52). Median spleen size was 19 cm, and 36% were transfusion dependent. Responses were seen in 6 patients (27%) and consisted in improvement in splenomegaly (N=3), transfusion independency (N=2) and reduction in spleen size with increase in blood counts (N=1). Median response duration was 14+ months. Grade 3–4 toxicity was experienced by 8 patients (36%) and most common side effects included anemia (grade 3–4 14%), thrombocytopenia (grade 3–4 23%), diarrhea (all grades 72%; grade 3–4 9%), nausea (grade 1–2 only 50%) and vomiting (grade 1–2 only 21%). Interestingly, decreased phosphorylation levels of STAT3 were observed in responders compared to non-responders. This suggests that the clinical activity of CEP-701 in these patients was due to inhibition of JAK2 V617F and downstream signaling pathways.

In another clinical trial of CEP-701 in MPN conducted by the MPD research consortium, the investigators sought to evaluate the MTD of CEP-701 in patients with MF, and the efficacy and tolerability of a capsule formulation of CEP-701 as compared to the standard liquid formulation (67). Twenty-six patients with JAK2V671F-positive MF were recruited, and received CEP-701 at doses ranging from 80–100 mg twice daily (liquid formulation cohort; N=7) and 100–160 mg twice daily (capsule formulation cohort; N=19). Mean spleen size was 16.7 cm (range 2–30 cm) and median JAK2V671F allele burden was 78.4%. Transfusion dependency was present in 50% of cases. Similar to other clinical studies with CEP-701, most common non-hematological side effects were gastrointestinal toxicities. Among patients receiving the liquid formulation, early (within 28 days of starting study drug) toxicity included diarrhea (Grade 1–2 71%; one patient had grade 3 diarrhea [DLT]), nausea (grade 1–2 29%), vomiting (grade 1–2 29%) and pancreatitis (grade 1–2 14%). For patients who received the capsule formulation, the drug was better tolerated, and side effects were diarrhea (grade 1–2 37%) and nausea (grade 1–2 37%). Late (beyond 28 days of starting study drug) included diarrhea (grade 1–2 in 6 patients and grade 3–4 in one patient), nausea (grade 1–2 in 5 patients), neutropenia (grade 3–4 in 1 patient) and thrombocytopenia (grade 3–4 in 2 patients). Regarding efficacy, there was no change in WBC, platelet count, Hb or transfusion dependency. Spleen size decreased by a median of 5.8 cm. Among 6 patients with paired baseline/12 weeks blood samples, JAK2V617F decreased in 3 patients (range of decrease 4.3–12.9%), was stable in one patient and increased in 2 patients. Among 4 patients with paired baseline/24 weeks samples, 3 patients had a decrease in JAK2V617F allele burden (range of decrease 6–41.5%).

Overall, both studies demonstrate that CEP-701 has at best modest efficacy in therapy of JAK2V617F positive MF. Pharmacological issues may hamper the efficacy of CEP-701 by limiting its ability to inhibit target kinases. In a recently presented clinical trial of patients with relapsed FLT3-mutated AML who were randomized between salvage chemotherapy and salvage chemotherapy plus CEP-701, correlative studies demonstrated that in only 58% of patients at day 15 of treatment there was inhibition of FLT3 activation (68). These

patients had significantly superior rates of complete remission (39% vs. 9% [no FLT3 inhibition]). Lack of FLT3 inhibition was due to variable levels of CEP-701 and binding by circulating proteins such as α 1-glycoprotein acid. Thus, limited activity of CEP-701 against both FLT3 and JAK2 may be related to the pharmacokinetics of the compound.

d)SB1518

SB1518 is a JAK2 inhibitor that has activity against both wild-type and mutated JAK2 (IC₅₀=23 nM [wild-type] and 19 nM [mutated]), being selective against JAK1 (IC₅₀=1280 nM) and JAK3 (IC₅₀=520 nM) (69). Similar to CEP-701, SB1518 also inhibits FLT3 (IC₅₀=22nM) (69). In pre-clinical studies, SB1518 inhibited proliferation of murine Ba/F3 cells transduced with EPOR and JAK2V617F (IC₅₀=140–460 nM) and reduced JAK2 and STAT5 phosphorylation. In a mouse model of JAK2V617F-positive MPN, oral administration of 150 mg/Kg twice daily of SB1518 led to normalization of WBC, reduction in spleen size and improvements in splenomegaly and animal survival, confirming SB1518 activity in vivo (69).

Two phase I studies evaluated SB1518 for therapy of patients with MPN. In the first study reported, 43 patients (MF=43; AML=6) were recruited (70). Patients received SB1518 at doses ranging from 100–600 mg/daily. JAK2 V617F mutation was positive in 78% of cases, and median spleen size for the 28 patients with a baseline spleen >5 cm was 13 cm. At the time of last report, median time on study drug was 111 days and 16 patients had discontinued therapy. The majority of adverse events were grade 1–2. GI toxicity was the most frequent side effect observed, including diarrhea (all grades: 33%; grade 3: 4%) and nausea (grade 1–2 only: 13%). Dose limiting grade 3 toxicities were observed at the cohort that received 600 mg (diarrhea, abdominal pain and syncope) and the MTD was determined to be 500 mg/daily. Grade 3–4 myelosuppression was observed in a few patients (grade 3–4 thrombocytopenia: 14%), but didn't appear to be dose related and the drug could be safely given at full doses in patients with platelet counts < 100×10⁹/L. Response data was available for 30 patients, and 7 (23%) achieved CI by IWG-MRT criteria. CI consisted mainly in spleen size reduction. Among 25 MF patients who had baseline splenomegaly and follow-up visits, 7 (28%) had spleen size reduction greater than 50%. Responses were seen both in JAK2V617F-negative and JAK2V617F-positive patients. Among patients with AML, 3 of 7 had evidence of clinical benefit (reduction in splenomegaly=1; stable disease=2). Correlative studies demonstrated a decrease in phospho-STAT3 and phospho-STAT5 in blood samples taken 2 hours after drug dosing. The dose of 400 mg/daily of SB1518 was recommended for further clinical trials.

In the second reported phase I study of SB1518, 20 patients with MF were recruited (71). Eighty-five percent were positive for the JAK2V617F mutation, and median spleen size as 17 cm below left costal margin. The MTD was determined to be 500 mg daily, and DLTs observed were diarrhea, nausea, fatigue and dehydration. Most common side effects were diarrhea (grade 1–2 75%; grade 3–4 10%), nausea (grade 1–2 30%, grade 3–4 5%) and vomiting (grade 1–2 35%). No major myelosuppression was observed in this trial, apart from anemia (all grades 25%; grade 3–4: 5%). Platelet transfusions were only needed during study for patients who presented with baseline thrombocytopenia and in one patient who

transformed to AML. Pharmacokinetics studies revealed that drug levels reached a plateau with doses above 400 mg/day, and this was selected as the dose to be tested in future phase 2 studies. A greater than 50% reduction in spleen size was seen in two patients and nine other patients had decrease in splenomegaly, but final response data is still pending. Other beneficial effects observed included transfusion independency (reached in 2 of 9 transfusion-dependent patients), normalization of WBC (seen in 2 patients with leukocytosis and 1 patient with leucopenia) and normalization of platelet count (seen in 2 patients with thrombocytosis and 1 patient with thrombocytopenia).

e) XL019

XL019 is a potent, reversible and selective inhibitor of both wild-type and mutated JAK2 (IC₅₀=2 nM) (72). XL019 does not inhibit JAK1 (IC₅₀=132 nM) or JAK3 (IC₅₀=250 nM). Pre-clinical studies demonstrated that XL019 had activity both *in vitro* and *in vivo* in a xenotransplantation model of HEL 92.1 cells in nude mice. A phase I clinical trial of XL019 in patients with MF demonstrated clinical activity of the compound (73). Thirty patients were recruited and received XL019 at doses ranging from 25–300 mg using different schedules of administration. Initial dose escalation started with 100 mg daily for 3 weeks every month. However, reversible peripheral neuropathy was observed at dose levels 100 mg/day. The protocol was amended, and patients received 25–50 mg once daily or 25 mg thrice weekly. Clinical activity was seen at this dose schedule, with reduction in spleen size, improvement in systemic symptoms, hemoglobin and peripheral blood blast count. Even though myelosuppression was not a major side effect of XL019, neurotoxicity still continued to be a problem even at lower doses, with patients developing peripheral neuropathy, weakness, paresthesia, formication and unsteady gait. Due to the high frequency of these symptoms, XL019 is no longer being developed.

f) CYT387

CYT387 is a novel aminopyrimidine compound which inhibits JAK1 and JAK2 at the low nanomolar range. CYT387 was discovered through enzyme- and cell-based high throughput screening of small molecule libraries (74). *In vitro* kinase assays revealed that CYT387 inhibits JAK1, JAK2 and TYK2 with IC₅₀ values of 11, 18 and 17 nM, respectively (75). CYT387 doesn't inhibit JAK3 (IC₅₀=155 nM). CYT387 inhibits proliferation of cell lines which depend on signaling by JAK kinases for proliferation, including Ba/F3 cells engineered to express both EPOR and JAK2V617F (IC₅₀=500 nM) (75). Inhibition of proliferation was accompanied by apoptosis and decreased JAK2, ERK1/2 and STAT5 phosphorylation in EPOR-JAK2V617F positive Ba/F3 cells. CYT387 also inhibits proliferation of Ba/F3 cells transduced with MPLW515L (IC₅₀=200nM) and endogenous erythroid colonies from patients with JAK2 V617F positive PV (76). In a mouse model of JAK2V617F-positive MPN, mice were lethally irradiated and transplanted with BM cells transduced with JAK2 V617F retrovirus (75). Mice developed splenomegaly, erythrocytosis, leukocytosis, splenomegaly and BM fibrosis. Therapy with CYT387 improved Hb levels, normalized WBC counts and reduced spleen size. In mice treated with the highest dose of CYT387 (50 mg/Kg twice daily) there was a decrease in JAK2V617F allele burden, but mutated cells remained indicating that the compound does not eradicate malignant cells, similar to what has been observed with other drugs in clinical trials. Mice with MPN had

elevated levels of several pro-inflammatory cytokines compared to normal mice, and therapy with CYT387 normalized levels of cytokines, an effect which has also been observed with the other dual JAK1/JAK2 inhibitor in development, INCB018424 (58). A phase I clinical trial with CYT387 in patients with MF is currently underway and results will be presented in the near future.

g) AZD1480

AZD1480 is a pyrazolyl pyrimidine compound that selectively and potently inhibits JAK2 (77). Pre-clinical studies demonstrated that AZD1480 inhibits JAK2 activity and phosphorylation and STAT5 phosphorylation in Ba/F3 cells transduced with the activated oncogene TEL-JAK2 (77). However, no inhibition of STAT5 phosphorylation was observed in Ba/F3 cells expressing TEL-JAK1, TEL-JAK3 and TEL-TYK2, confirming that AZD1480 has selective inhibitory activity against JAK2. In a murine fibroblast cell line expressing yellow fluorescent protein-STAT3 fusion protein (MEF-YFP-STAT3), AZD1480 inhibited JAK2 activity, STAT3 phosphorylation and translocation of STAT3 to the nucleus (77). MEF-YFP-STAT3 cells were subcutaneously injected in athymic mice and formed tumor masses. Therapy with AZD1480 inhibited tumor growth and tumor cell lysates had reduced levels of phospho-STAT3 (77). In xenograft models of solid tumors cell lines, AZD1480 reduced tumor cell growth and this was associated with inhibition of STAT3 phosphorylation (77). A phase I clinical trial is currently evaluating the activity of AZD1480 in patients with MPNs.

JAK2 Inhibitors in development for Polycythemia Vera and Essential Thrombocythemia (Table 3)

a) INCB018424

A clinical trial for patients with hydroxyurea-refractory PV and ET is currently underway (78). Inclusion criteria include hematocrit (Ht) $\geq 45\%$ and/or phlebotomy dependency for patients with PV and platelet count $\geq 600 \times 10^9/L$ for patients with ET. In the phase I portion of the trial, the best dose schedule was determined to be 10 mg twice daily (PV) and 25 mg twice daily (ET). The MTD cohort was then expanded and further patients were recruited. Response criteria were evaluated by both standard and European LeukemiaNet criteria (table 4) (79, 80). At the time of last report, 73 patients had been enrolled (PV=34, ET=39), and median follow-up was 10.4 months.

Patients with PV were JAK2V617F-positive (100%), and majority had hydroxyurea-refractoriness (74%) and/or phlebotomy dependency (76%) (78). Median WBC was $13.4 \times 10^9/L$ and 74% of patients had splenomegaly, with a median spleen size of 9 cm below left costal margin. Therapy with INCB018424 led to rapid normalization of Ht in patients with PV. Mean Ht was 46% at baseline, 43% with 1 month of therapy and 39% with 2 and 6 months of treatment. After starting therapy, only 2 patients required phlebotomies in the first 2 weeks of treatment, and none ever since. INCB018424 also normalized WBC (mean $15 \times 10^9/L$ at baseline; $10 \times 10^9/L$ at 6 months), platelet count (mean $553 \times 10^9/L$ at baseline; $324 \times 10^9/L$ at 6 months) and reduced splenomegaly by 50% in 80% of patients who presented with enlarged spleens at baseline. Patients also reported improvement in pruritus,

bone pain and night sweats. By standard criteria, overall response rate (ORR) was 100% (complete response [CR]=62%; partial response [PR]=38%). By LeukemiaNet criteria, ORR was 97% (CR=45%; PR=52%). Among patients who achieved PR by LeukemiaNet criteria, 12% did not achieved CR due to palpable spleen; however, in all of these cases spleen size had reduced by 50%.

In patients with ET, there was rapid and sustained reduction in platelet counts (78). Median platelet count at baseline was $884 \times 10^9/L$ and it went down to $558 \times 10^9/L$ at 6 months of therapy. Among 11 patients with baseline WBC $10 \times 10^9/L$, all normalized WBC. There was also improvement in spleen size (100%), pruritus (75%), bone pain (55%), night sweats (46%) and peripheral numbness (40%). Response by standard criteria was 62% (CR=41%, PR=21%) and by LeukemiaNet criteria was 90% (CR=13%; PR=77%).

INCB018424 was very well tolerated in patients with PV/ET (78). Main side effects were related to myelosuppression and included anemia (grade 1–2 12% [PV]; grade 1–2 18% [ET]), thrombocytopenia (grade 1–2 3% and grade 3–4 3% [PV]), leucopenia (grade 1–2 9% [PV]; grade 3–4 5% [ET]). So far, only 6 patients have discontinued the study drug, 3 of them due to side effects (atrial flutter, GI toxicity and renal insufficiency in one each).

b) CEP-701

A trial with high-risk JAK2V617F-positive PV and ET patients is investigating the activity of CEP-701 in this clinical scenario (81). Thirty-nine patients (PV=27, ET=12) were recruited. Nineteen percent and 25% of PV and ET patients, respectively, had prior thrombotic events. Median JAK2 V617F allele burden was 70% (PV) and 40% (ET). The primary end point of the study was reduction of the JAK2 V617F neutrophil allele burden. To date, 15 patients completed 18 weeks of therapy with CEP-701, and responses observed included decrease in spleen size (83%) and improvement in pruritus (100%). Decrease in phlebotomy requirements was seen in 3 of 5 eligible patients. No improvement in WBC and platelet counts was observed, and in fact in many patients there was an increase in WBC or platelets during therapy with study drug. Reduction of JAK2V617F allele burden 15% was seen in 20% of evaluable patients. As expected, side effects were mainly related to GI toxicity. Serious adverse events included venous thrombotic events (N=3), arterial thrombotic events (N=2) and one non-serious episode of deep venous thrombosis. It seems that the activity of CEP-701 is limited in patients with PV/ET, and may not be sufficient for reducing the risk of thrombosis.

Conclusions

JAK2 inhibitors are a new class of drugs being actively developed for patients with Ph-negative MPNs. While they are far from being a solution to all clinical issues in patients with MPNs, great clinical benefit can be obtained from the use of these drugs. In patients with MF, therapy with JAK2 inhibitors can lead to improvement in spleen size, WBC count, platelet count and systemic symptoms, including weight loss, fatigue and night sweats. This is associated with improved quality of life. Importantly, patients with MF have few options for treating splenomegaly and systemic symptoms, and thus these drugs might fill an unmet need in the therapy of these patients. Normalization of pro-inflammatory cytokines levels is

also observed with some compounds, particularly with dual JAK1/JAK2 inhibitors. In patients with hydroxyurea-refractory PV/ET, JAK2 inhibitors can lead to improvement in Ht, WBC count, platelet count and systemic symptoms, but their role in frontline therapy of these disorders still needs to be defined. There is still a lot to be learned from these compounds. There are several of them in current clinical trial, with subtle differences in activity and side effect profile, and we need to understand more how they are producing clinical responses and whether there is any difference in their mechanism of action. Which cells are they targeting, normal cells, malignant cells, or both. Why some patients fail to respond to JAK2 inhibitors is unknown, and biomarkers could help physicians to better select patients to be treated with these drugs. Interestingly, the JAK2V617F mutation does not discriminate which patient will respond to a JAK2 inhibitor. JAK2 inhibitors fail to eradicate the malignant clone in patients with MF, likely due to its complexity and the presence of multiple mutations or cytogenetic alterations contributing to the disease presence. In fact, it is known that multiple different malignant clones exist in a patient with MF. Preliminary data suggests that some JAK2 inhibitors may delay risk of transformation to AML, which might suggest that they can reduce levels, or activity, of MF progenitor cells which are prone to leukemic transformation (53). Phase III randomized studies should compare the activity of JAK2 inhibitors against classical agents used in Ph-negative MPNs, like hydroxyurea, interferon formulations and thalidomide, to document properly their clinical benefit, and the possibility of combination therapy should be explored.

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Table 1

JAK2 inhibitors in current development

Drug	Current Phase	Target(s)	IC50 Value (nM)		
			JAK2	JAK1	JAK3
CEP-701	I/II	JAK2, FLT3	1	?	?
INCB018424	III	JAK2, JAK1	2.8	3.3	322
SB1518	I	JAK2, FLT3	23	1280	520
TG101348	II	JAK2, FLT3	3	105	996
CYT387	I	JAK1, JAK2	18	11	155
AZD1480	I	JAK1, JAK2	<0.4*	1.3*	3.9*

* assays conducted with Km concentrations of ATP

Table 2

Clinical results with JAK2 inhibitors in MF

Study	Therapy	Dose Level/MTD	N	Responses/Toxicity
Verstovsek et al. (53)	INCB018424 (Phase I/II)	10–25 mg twice daily and 25 mg- 100 mg once daily/25 mg twice daily	153	CI by IWG criteria: 44% (reduction in splenomegaly), improvement in exercise ability, systemic symptoms; cytopenias (thrombocytopenia, anemia)
Pardanani et al. (51)	TG101348 (Phase I/II)	30–800 mg once daily/680 mg once daily	59	CI by IWG criteria: 28%, improvement in systemic symptoms, leukocytosis, thrombocytosis; some patients with improvement in BM fibrosis; cytopenias, GI toxicity
Santos et al. (52)	CEP-701 (Phase II)	80 mg twice daily/NE	22	CI by IWG criteria: 27% (reduction in splenomegaly and improvement in cytopenias); GI toxicity, cytopenias
Hexner et al. (67)	CEP-701 (Phase I)	80–160 mg twice daily/160 mg twice daily	26	Reduction in splenomegaly; GI toxicity, cytopenias
Verstovsek et al. (70)	SB1518 (Phase I)	100–600 mg daily/500 mg daily	43	CI by IWG criteria: 28% (reduction in splenomegaly); GI toxicity, cytopenias
Seymour et al. (71)	SB1518 (Phase I)	100–600 mg daily/500 mg daily	20	Reduction in splenomegaly, improvement in transfusion dependency; GI toxicity

MTD: maximum tolerated dose; CI: Clinical improvement; IWG: international working group on myelofibrosis research and therapy; BM: bone marrow; GI: gastrointestinal; NE: not established

Table 3

Clinical results with JAK2 Inhibitors in Polycythemia Vera and Essential Thrombocythemia

Study	Therapy	Dose Level/MTD	N	Responses
Moliterno et al. (81)	CEP-701 80mg twice daily (Phase II)	80 mg twice daily/NE	39	Reduction in spleen size: 83%; reduction in phlebotomy requirements: 3/5 (60%); GI toxicity, 5 thrombotic episodes
Verstovsek et al. (78)	INCB018424 10–25mg twice daily (Phase II)	10–25 mg twice daily/NE	73	Standard criteria- PV: 100% (CR 62%; PR 38%); ET: 62% (CR: 41%; PR: 21%) LeukemiaNet criteria- PV: 97% (CR 45%; PR 52%); ET: 90% (CR: 13%; PR: 77%); Cytopenias

NE: not established; MTD: maximum tolerated dose; PV: polycythemia vera; ET: essential thrombocythemia; GI: gastrointestinal

Table 4

Response Criteria for PV and ET used in JAK2 inhibitor trials

Response	Definition	
	Standard criteria (PV/ET)(80)	LeukemiaNet (PV/ET)(79)
Complete Response	PV	PV
	<ul style="list-style-type: none"> • Normalization of Ht (< 45% males, < 42% females), WBC counts, platelets, spleen size • No use of hydroxyurea, anagrelide, phlebotomies • Absence of thrombotic events 	<ul style="list-style-type: none"> • Ht < 45% (without phlebotomies) • Platelet count $400 \times 10^9/L$ • WBC $10 \times 10^9/L$ • Normal spleen size • No disease related symptoms
	ET	ET
	<ul style="list-style-type: none"> • Normalization of platelet counts ($< 400 \times 10^9/L$) • No use of hydroxyurea, anagrelide • Absence of thrombotic events 	<ul style="list-style-type: none"> • Platelet count $400 \times 10^9/L$ • WBC $10 \times 10^9/L$ • Normal spleen size • No disease related symptoms
	Partial Response	Partial Response
	PV	PV
Partial Response	<ul style="list-style-type: none"> • At least 50% reduction in phlebotomy requirements and spleen size 	<ul style="list-style-type: none"> • In patients who do not fulfill the criteria for CR, Hct < 45% without phlebotomies OR • Response in 3 or more of the other criteria
	ET	ET
	<ul style="list-style-type: none"> • At least 50% reduction in platelet counts (but still $> 400 \times 10^9/L$) 	<ul style="list-style-type: none"> • In patients who do not fulfill the criteria for CR, platelet count $600 \times 10^9/L$ OR decrease $> 50\%$ from baseline

PV: polycythemia vera; ET: essential thrombocytemia; CR: complete response; Ht: hematocrit; WBC: white blood cell count