

Update on JAK2 inhibitors in myeloproliferative neoplasm

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Abstract: Since the discovery of mutant Janus Kinase 2 (JAK2), JAK2V617F, in a major proportion of myeloproliferative neoplasm (MPN) patients, there has been a flurry of activity in the development of JAK2 inhibitors. Pan-JAK, predominantly JAK2 and off-target JAK2 inhibitors have been developed in the short span of the past 5 years. These compounds have since been tested to varying success in both *in vitro* and *in vivo* settings with several proceeding on to advanced clinical trials. Although it was hoped that these inhibitors would be the silver bullet in the manner than imatinib was to chronic myeloid leukemia, it is becoming apparent that this is not the case for various reasons, chief of which is that a significant reduction of the underlying pathogenic clone is not achieved. In fact, the very notion that the target of JAK2 inhibitors (be it pan-JAK or JAK2 specific) is the mutant JAK2V617F is being challenged with findings from several clinical trials showing a poor correlation between the reduction in JAK2V617F mutant allele burden and clinical response. In view of this, it is not surprising that several groups are now investigating combinations of JAK2 inhibitors and other agents in MPN. Although much knowledge has been added in this short span of time, it is apparent that our understanding of the role of JAK2 inhibitors in the treatment scheme of MPN is only beginning.

Keywords: JAK2 inhibitors, JAK2V617F, myeloproliferative neoplasm

Introduction

BCR-ABL negative myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). In 2005 novel mutations, the most common being the V617F mutation in the tyrosine kinase Janus Kinase 2 (JAK2), were described in most patients with PV and approximately 50% of patients with ET and PMF by several groups [Baxter *et al.* 2005; James *et al.* 2005; Kralovics *et al.* 2005; Levine *et al.* 2005]. It is no wonder, then, that a rapid development of kinase inhibitors was soon undertaken. The adenosine triphosphate (ATP)-mimetic tyrosine kinase inhibitors (TKIs) have so far been the focus and those that have made it into advanced clinical trials include CEP-701, CYT387, INCB018424, SB1518 and TG101348. Initial results from these trials suggest that these drugs are diverse in their toxicity and efficacy profiles. This phenomenon might be linked to their variable *in vitro* activity against other JAK family members, for example JAK1 and JAK3, as well as non-JAK kinase targets (e.g. FLT3

and JNK1). Although there is ample evidence to suggest that JAK2 mutations are driving the MPN phenotype [Delhommeau *et al.* 2007; Dupont *et al.* 2007; Jamieson *et al.* 2006], they do not necessarily represent the primary clonogenic event [Nussenzweig *et al.* 2007; Kralovics *et al.* 2006] further complicating the interpretation of the benefits from JAK2 inhibitors and perhaps is manifested by the poor correlation between clinical response and the eradication of the JAK2 mutant. Furthermore, the anti-inflammatory properties of JAK2 inhibitors in rheumatological diseases [Ghoreschi *et al.* 2009] is opening up the paradigm that these inhibitors may in some part be working through an inflammatory modulation pathway rather than by JAK2 inhibition *per se*.

The aim of this review article is to summarize the developments in the understanding of MPN pathogenesis that were published in the past year and that may influence the therapeutic approach. We also provide an update of both the clinical trial and *in vitro* data on JAK2

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inhibitors that have recently been reported with a focus on exploring the differences among the various JAK2 inhibitors.

Background

The classic *BCR-ABL* negative MPNs are characterized by the clonal overproduction of normally differentiated hematopoietic lineages. Cytokine-independent growth had been noted for many years to be a hallmark of these diseases and the phenomenon was finally explained with the discovery of the gain of function *JAK2*V617F mutation in nearly all PV and a large subset of ET and PMF patients [Baxter *et al.* 2005; James *et al.* 2005; Kralovics *et al.* 2005; Levine *et al.* 2005].

The Janus family of nonreceptor protein tyrosine kinases (JAK) consists of JAK1, JAK2, JAK3 and TYK2. JAKs are constitutively associated with the membrane-proximal regions of cytokine receptors, activated by autophosphorylation of tyrosine residues upon ligand-induced receptor aggregation and subsequently trigger downstream signaling events such as the phosphorylation of signal transducers and activators of transcription (STATs) [Schindler *et al.* 2007; Yamaoka *et al.* 2004]. JAK2 is widely expressed and is essential for signaling through a variety of cytokine receptors including erythropoietin (EPO) and thrombopoietin (MPL) receptors. In addition to the canonical JAK-STAT signaling pathway, JAK2 has been recently shown to enter the nucleus, phosphorylate histone H3 and modify chromatin structure. These findings identify a previously unrecognized nuclear role for JAK2 in the phosphorylation of Histone H3 at Tyrosine 41 (Y41) and reveals a possible direct mechanistic link between two genes, *JAK2* and *LIM* Domain only 2 (*LMO2*), involved in normal hematopoiesis and leukemia [Dawson *et al.* 2009].

The most common mutation in the *JAK2* allele in MPN patients, *JAK2*V617F, occurs in the JH2 pseudokinase domain of JAK2, releasing JAK2 from an auto-inhibitory effect and leading to constitutive phosphorylation and downstream signaling from JAK2 in a noncytokine-dependent manner [Levine *et al.* 2007; Shannon and Van Etten, 2005].

It remains unclear, however, how a single mutation *JAK2*V617F can generate the different MPN phenotypes. Recently, Chen and colleagues, by comparing clonally derived mutant

and wild-type cells from individual patients, demonstrated that *JAK2*V617F-heterozygous erythroid cells from ET and PV patients exhibit differential interferon signaling and STAT1 phosphorylation [Chen *et al.* 2010]. Increased STAT1 activity in normal CD34-positive progenitors produces an ET-like phenotype, whereas a decrease of STAT1 activity in *JAK2*V617F-heterozygous ET progenitors produces a PV-like phenotype. This is suggestive that the phenotypic consequences of *JAK2*V617F could possibly be due to a balance between STAT5 and STAT1 activations. Whether JAK2 targeting affects STAT5 and STAT1 signaling in a similar fashion remains to be determined.

Although the *JAK2*V617F mutation is the dominant JAK2 mutation associated with *BCR-ABL* negative MPN, other *JAK2*-activating mutations, such as *JAK2*T875N in the kinase domain [Mercher *et al.* 2006], JAK2DIREED in the JH2 pseudokinase domain [Malinge *et al.* 2007], and the *JAK2* exon 12 mutations [Scott *et al.* 2007], have been found in a small group of MPN patients lacking *JAK2*V617F [Levine and Gilliland, 2008]. It has also been reported that other non-*JAK* activating alleles are involved in these disorders (e.g. *MPL*W515L, *MPL*W515K, *MPL*S505N) [Pikman *et al.* 2006]. These mutations of the thrombopoietin receptor gene (*MPL*) are found in approximately 5% of PMF and 2% of ET patients, activate the JAK-STAT pathway and produce an MPN phenotype in a mouse model. These mutations can be present together with or without *JAK2*V617F [Pardanani *et al.* 2006].

More recently, mutations in *TET2*, *ASXL1*, *IDH1* and 2, *IKZF1*, *CBL*, *LNK* and *EZH2* were reported in MPN and are usually not mutually exclusive [Ernst *et al.* 2010; Oh *et al.* 2010; Pardanani *et al.* 2010b; Tefferi *et al.* 2010; Carbuccion *et al.* 2009; Delhommeau *et al.* 2009; Grand *et al.* 2009; Jager *et al.* 2009]. Evidence that the *JAK2*V617F induces a PV-like phenotype in mouse transplantation models [James *et al.* 2005] and the fact that virtually all patients with PV harbor a *JAK2*-activating mutation [Wang *et al.* 2008] initially ignited interest that there might be a direct cause and effect relationship between *JAK2*V617F and MPN. However, clonal heterogeneity observed within the progenitor cell pool in patients with *BCR-ABL* negative MPN harboring the *JAK2*V617F mutation

[Nussenzveig *et al.* 2007; Kralovics *et al.* 2006] suggests that unlike CML where *BCR-ABL* is the causative event; *JAK2* V617F may not be the disease-initiating event. As such, the phenomenal efficacy seen with imatinib in CML may not be repeated by *JAK2* inhibitors in *BCR-ABL* negative MPNs.

What should be the goal of *JAK2* inhibitor therapy?

While PV, ET and PMF are grouped together, the goal of treatment in these disorders is vastly different.

PV and ET

PV and ET are disorders with a prolonged life expectancy [Cervantes *et al.* 2009; Tefferi, 2008]. They can be reasonably well controlled by current medical measures including phlebotomy, antiplatelet therapy and cytoreductive therapy with hydroxycarbamide or anagrelide, interferon and more recently pegylated interferon [Quintas-Cardama *et al.* 2009]. Patients are mainly at risk for thrombo-hemorrhagic complications of the disease itself, minor complications of phlebotomy and the side effects of cytoreductive therapy.

Some issues that are not solved with current therapeutic options in PV and ET include the treatment of pruritus, the highly controversial roles of hydroxycarbamide in the late transformation to acute leukemia [Noor *et al.* 2010; Chim and Ma, 2005] and anagrelide to promote myelofibrosis [Hultdin *et al.* 2007], and the treatment of choice for hydroxycarbamide-resistant or intolerant patients. *JAK2* inhibitor therapy in this regard could be implemented in the above subgroups of treatment-resistant or intolerant patients, and responses to these agents should be evaluated according to clinical response criteria developed recently [Barosi *et al.* 2009]. Furthermore, by targeting the malignant clone, *JAK2* inhibition can potentially reduce the (albeit low) risk of late complications of myelofibrosis and blastic transformation.

PMF and post-PV/ET myelofibrosis

Myelofibrosis is the most serious condition among the MPNs and is characterized by bone marrow failure, symptomatic splenomegaly and debilitating constitutional symptoms including fatigue, fever and weight loss. The latter are often attributed to a pro-inflammatory cytokine

milieu that is associated with the bone marrow stromal cells in PMF [Ho *et al.* 2007]. Patients with PMF also have a substantial risk of blastic transformation and have a significant reduction in life expectancy [Cervantes *et al.* 2009; Tefferi, 2008]. In contrast to PV/ET, up to 20% of PMF patients will have leukemic blast transformation [Mesa *et al.* 2005]. Unfortunately, current PMF therapies are largely palliative, primarily focusing on anemia and splenomegaly; other than allogeneic stem cell transplant, none have an impact on its natural progression [Guardiola *et al.* 1999]. Even amongst therapeutic approaches under development, the efficacy of agents such as interferons in MPNs is limited mainly to that of ET and PV with little/no activity on PMF [Jabbour *et al.* 2007; Kiladjan *et al.* 2006; Langer *et al.* 2005]. Other novel agents (e.g. lenalidomide, pomalidomide) have been tried in MF with only limited success. Lenalidomide was shown in a phase II trial to have only modest activity in a subset of myelofibrosis with myeloid metaplasia (MMM) patients [Tefferi *et al.* 2006a]. In another phase II trial, low-dose pomalidomide was shown to modestly improve anemia in MF patients [Begna *et al.* 2010].

The goal of therapy in myelofibrosis should first focus on amelioration of the debilitating symptoms and should be evaluated according to suggested response criteria of the IWG-MRT [Tefferi *et al.* 2006b]. In view of the fact that there are no disease-modifying agents available for myelofibrosis, the initial promising results with *JAK2* inhibition therapy ideally would translate to an alteration in the disease's natural course, reducing transformation risk and prolonging life.

Inhibitors of JAK2 tyrosine kinase

The term *JAK2* inhibitors is possibly a misnomer as a number of *JAK2* inhibitors also inhibit *JAK1* or *JAK3* (i.e. pan-*JAK* inhibitors) with varying degrees of potency. Furthermore, many *JAK2* inhibitors were actually designed as non-*JAK* kinase inhibitors (e.g. CEP701, a FLT3 inhibitor) and were discovered to have an anti-*JAK2* activity as an off-target effect. In addition, although the majority of *JAK2* inhibitors are small ATP-mimetic agents, there are several agents in development and in preclinical testing which are non-ATP-mimetic small molecule inhibitors (e.g. LS104) [Lipka *et al.* 2008].

Overall, one of the most dramatic effects of JAK2 inhibitor therapy is the reduction in splenomegaly. The effect of JAK inhibitors against splenomegaly is rapid and often evident within the first month of treatment, with best responses observed in the first 3 months of therapy. JAK inhibitor-induced spleen responses are usually dose-dependent but a significant number of patients undergo dose reductions because of drug-related anemia or thrombocytopenia [Verstovsek *et al.* 2010b], therefore limiting the maximum effect that could have been achieved by therapy.

JAK2 kinase inhibitors in clinical trials

Details of clinical trials of JAK2 kinase inhibitors are given in Table 1.

INCB018424 (Ruxolitinib). In a landmark phase I/II trial reported recently [Verstovsek *et al.* 2010b], the JAK1 and JAK2 inhibitor INCB018424 was tested in 153 PMF and post-PV/ET MF patients (both *JAK2V617F* positive and negative). Doses evaluated ranged from 10–50 mg twice daily to 25–200 mg once daily. The majority of patients had a rapid, significant and durable reduction in splenomegaly within the first month. After 1 month of therapy, the majority of patients who received INCB018424 at a dose of 10 mg twice daily to 25 mg twice daily

had a more than 50% improvement in total or individual symptom scores according to the Myelofibrosis Symptom Assessment Form (MFSAF). This was in association with decreased levels of cytokines including MIP-1 β , interleukin (IL)-1ra, IL-6, tumor necrosis factor α (TNF- α), and C-reactive protein but with only a modest reduction in the *JAK2V617F* allele burden. Overall, the agent was well tolerated with no significant nonhematological toxicities. However, grade 3/4 thrombocytopenia and anemia developed in approximately 20% and 15%, respectively. INCB018424 was similarly effective in patients either with or without the *JAK2V617F* mutation suggesting that some of the activity of the inhibitor may be due to other upstream alterations that activate the STAT pathway in these diseases [Levine *et al.* 2007]. In addition, the activity of INCB018424 in alleviating constitutional symptoms has been correlated with a marked reduction in serum pro-inflammatory cytokines and therefore possibly related to its primarily anti-JAK1 activity suggesting that its clinical benefits are from a cytokine modulation role. In keeping with this hypothesis, the relapse of splenomegaly with INCB018424 discontinuation occurs on a timescale that is too short to be explained by regrowth of the malignant clone; consequently, it may instead

Table 1. JAK2-inhibiting agents in clinical trials.

Drug	JAK activity	Stage of development	Remarks
INCB018424	JAK1 JAK2	Phase III: COMFORT-I (placebo controlled)/COMFORT II (best available oral/parenteral therapy controlled) [PMF, post-ET/PV MF] Phase III: RESPONSE (best available care controlled) [HU-resistant PV]	Ongoing ⁵ (Phase I/II published ¹)
TG101348	JAK2	Phase I/II: PMF, post-PV/ET MF	Published ²
CYT387	JAK1 JAK2 JAK3	Phase I/II: PMF, post-PV/ET MF (included subjects with prior JAK2 inhibitor therapy)	Reported ³
CEP701	JAK2*	Phase II: PV, ET, PMF, post-PV/ET MF	Published ⁴
SB1518	JAK2	Phase I/II: PMF	Reported ³
AZD1480	JAK2	Phase I/II: PMF, post-PV/ET MF	Ongoing ⁵
Erlotinib	JAK2**	Phase II: PV (<i>JAK2V617F</i> mutant only)	Ongoing ⁵
AT9283	JAK2****	Phase I/II: PMF included	Ongoing ⁵
ITF2357	JAK2***	Phase II: GIVINOSTAT (in combination with HU-resistant PV; <i>JAK2V617F</i> mutant only)	Ongoing ⁵
LY2784544	Uncertain	Phase I: PV, ET, PMF (<i>JAK2V617F</i> mutant only)	Ongoing ⁵

*FLT3 inhibitor with JAK2 off-target effect.

**EGFR inhibitor with JAK2 off-target effect.

***HDAC inhibitor with JAK2 off-target effect.

****Aurora kinase inhibitor with JAK2 off-target effect.

¹Verstovsek *et al.* [2010b].

²Pardanani *et al.* [2011].

³ASH annual meeting 2010.

⁴Santos *et al.* [2010].

⁵Source: www.ClinicalTrials.gov

JAK, janus kinase; PV, polycythemia vera; ET, essential thrombocytosis; MF, myelofibrosis; PMF, primary myelofibrosis; HU, hydroxyurea.

reflect vascular or angiogenic phenomena associated with loss of INCB018424's anticytokine effect [Vannucchi, 2009].

Long-term follow-up data of INCB018424 in PV and ET patients refractory or intolerant to hydroxyurea were reported recently [Verstovsek *et al.* 2010c]. This is an open-label phase II study that had a previously established dose of 10 mg and 25 mg twice daily as starting doses for PV and ET, respectively. Data from 34 PV patients at a median of 108 months from diagnosis were presented. After a median follow up of 15 months, almost all subjects achieved good hematocrit control without the need for phlebotomy. Splenomegaly was present in around three quarters of subjects at entry and more than half of the subjects achieved $\geq 50\%$ reduction in palpable spleen length. Leukocytosis and thrombocytosis, present in a half and a third of the subjects at study entry respectively, improved or normalized in the majority of subjects. Data from 39 ET subjects at a median of 84 months from diagnosis was presented. After a median follow up of 15 months, half of the subjects achieved normalization of white blood cell (WBC) and platelet counts in the presence of nonpalpable splenomegaly. Both PV and ET subjects demonstrated reductions in patient-reported symptom scores for pruritus, night sweats and bone pain. These responses were unrelated to the presence/absence of *JAK2*V617F mutation at study entry or to the allele burden changes following treatment. As with PMF, the decrease in the *JAK2*V617F burden was only modest.

In view of these encouraging results in both MF and ET/PV patients, phase III clinical trials including a trial in PV (COMFORT I/II and RESPONSE trial) with INCB018424 are about to begin.

CYT387. CYT387 is a pan-JAK inhibitor. It inhibits JAK1 and JAK2 to a similar and JAK3 to a lesser extent with an additional off target activity against JNK1 and CDK2 [Pardanani *et al.* 2009]. Early clinical data for CYT387 has shown that it is capable of reducing splenomegaly and controlling the constitutional symptoms of PMF and, importantly, may also provide the additional benefit of improving anemia in these patients. Detailed results from the first 60 patients treated in the phase I and early phase II portion of this trial were reported at the American Society of Hematology (ASH) conference in 2010

[Pardanani *et al.* 2010a] (and personal communication). At the time of reporting, the trial had accrued 36 of a target of 120 patients (81% of whom were *JAK2*V617F mutant positive) and the maximum tolerated dose (MTD) had been determined to be 300 mg/day. Of note, several of the patients had previously been treated with another JAK2 inhibitor, INCB018424 (18%) or TG101348 (5%). In addition to good control of constitutional symptoms (night sweat, pruritus, fever and bone pain) and splenomegaly (47%), the drug also showed significant improvement of anemia in myelofibrosis patients. The median time to onset of improvement in splenomegaly and anemia was 2 weeks and 4 weeks, respectively. Interestingly, the anemia and splenomegaly responses were higher in the *JAK2*V617F negative than in the positive group.

TG101348. Preclinical data indicates that this is one of the most JAK2-specific inhibitors in current trials, with inhibition of FLT3 and RET kinases as off targets [Apostolidou *et al.* 2009; Wernig *et al.* 2008]. In a phase I/II study of TG101348 [Pardanani *et al.* 2011], 59 PMF and post-PV/ET MF were treated at eight dose levels from 30 mg to 800 mg daily, in which the MTD was determined as 680 mg with an additional 40 patients treated at the MTD. A dose-dependent myelosuppression was observed, in particular anemia that was more common in transfusion-dependent patients. Approximately 50% of patients had a reduction in splenomegaly and patients with leukocytosis and thrombocytosis had significant reductions in their counts. Mutant *JAK2*V617F allele burden was modestly reduced, however cytokine levels (IL2, IL-6, IL-8 and TNF α) were not significantly changed. This is in contradistinction to the effect of INCB018424 where cytokine levels were reduced. This would seem to suggest that TG101348 works primarily by an anti-JAK2 mechanism rather than by modifying the pro-inflammatory milieu.

CEP-701 (Lestaurtinib). CEP-701 is a FLT3 and JAK2 inhibitor which is structurally related to staurosporine [Hexner *et al.* 2008]. In an open-label phase II trial, CEP-701 was tested in 40 advanced phase PV and ET patients resulting in a reduction of splenomegaly [Molitero *et al.* 2008]. In an open-label phase II study of CEP-701 in PMF and post-PV/ET MF [Santos *et al.* 2010; Verstovsek *et al.* 2007], patients were started at 80 mg twice daily with dose adjustments according to a previous phase II/III trial

as a FLT3 inhibitor in AML. Out of 22 patients, only 6 patients (27%) responded according to International Working Group (IWG) criteria. Median time to response was 3 months. There was no change in the *JAK2*V617F allele burden, bone marrow fibrosis, nor cytogenetic responses. Grade 3/4 myelosuppression was the main side effect. These results of CEP-701 in PMF and post-PV/ET MF are thus rather modest.

SB1518. SB1518 is a potent inhibitor of both JAK2 kinase and the adjustments mutant in the nanomolar range [Goh *et al.* 2007]. It also potently inhibits FLT3 and its mutant D835Y [Verstovsek, 2009]. SB1518 is currently being evaluated in phase I and I/II clinical trials in the United States for the treatment of PMF as well as advanced lymphoid malignancies and advanced myeloid malignancies including AML, chronic myelomonocytic leukemia (CMML) and CML. Preliminary results of the phase I/II trial of SB1518 in PMF was reported in ASH 2010 [Verstovsek *et al.* 2010a]. In the phase I portion of the trial 400 mg orally daily was selected as the recommended phase II dose based on the response rate and safety/tolerability profile. At the time of reporting, 33 patients with PMF were enrolled onto the phase II portion of the trial with 32 evaluable for safety. All patients entered the study with splenomegaly and all had been treated previously. The side effect profile was relatively benign with Grade 3 adverse events of diarrhea (6%) and rash (3%). Neutropenia and thrombocytopenia were uncommon. Significant reductions in spleen size were observed by both MRI and physical exam and a trend for reduction in MF-associated symptoms was noted.

LY2784544. The *in vitro* data suggests that mutant adjustments is more sensitive to LY2784544 than cytokine-activated wild-type JAK2, thus possibly conferring it a wider therapeutic window advantage [Ma *et al.* 2010]. Consistent with this observation, in MPN disease model testing, LY2784544 selectively reduced adjustments cell burden with no effect on the normal erythroid progenitor cells. These results supported the advancement of LY2784544 into an ongoing phase I trial in MPN for which there is scant reported data at this point in time.

AZD-1480. AZD-1480 is a potent JAK2 inhibitor and in a mouse model it reduced the proliferation

of stem cells transfected with the JAK2 mutant [Napper, 2008]. The compound is currently being tested in Hodgkin's disease and a phase I/II trial with MF patients.

XL019. A phase I study of the JAK2-specific inhibitor XL019 showed reduction in splenomegaly in PV patients [Paquette *et al.* 2008] but due to neurotoxicity the drug did not proceed to further trials.

Off-target JAK2 inhibitors in clinical trials

Details of clinical trials of off-target JAK2 inhibitors are given in Table 1.

ITF2357 (Givinostat)

ITF2357 is a histone-deacetylase (HDAC) inhibitor that also inhibits *JAK2*V617F-positive cell lines in the nanomolar range. *In vitro* data suggests that it preferentially inhibits proliferation of cells bearing the *JAK2*V617F mutation through a specific downmodulation of the *JAK2*V617F protein and inhibition of its downstream signaling [Guerini *et al.* 2008].

In a phase IIA study [Rambaldi *et al.* 2010], 13 PMF and post-PV/ET MF, 12 PV, and 1 ET patients were started on 50 mg twice daily of ITF2357, increasing to 50 mg three times a day if tolerated. Of the 13 MF patients, 2 patients had major responses and 2 patients had moderate responses by European Myelofibrosis Network (EUMNET) criteria. Three complete responses (CRs) and eight partial responses were reported. Overall the drug was well tolerated, with no grade 4 toxicities and only one patient developing grade 3 hematological toxicity. Nonhematological toxicities included diarrhea, fatigue, nausea and abdominal pain. A trial of ITF2357 in combination with hydroxyurea in PV patients is ongoing (GIVINOSTAT).

AT9283. AT9283, an Aurora kinase as well as a potent JAK2 inhibitor, is in phase I/II clinical trials for the treatment of acute leukemias, CML, and PMF [Ravandi *et al.* 2007]. The phase I portion of the trial has been terminated as the recommended phase II dose has been determined.

Erlotinib. Erlotinib (originally developed as an epidermal growth factor receptor TKI), which is used for treating patients with metastatic non-small cell lung cancer, inhibited the growth and expansion of *JAK2*V617F-expressing PV hematopoietic progenitor cells and the human

erythroleukemia HEL cells while having little effect on normal cells [Li *et al.* 2007]. A trial of erlotinib in the treatment of *JAK2*V617F mutant positive PV patients is ongoing.

Selected JAK2 inhibitors in pre-clinical trials/in vitro studies

Details of selected JAK2 inhibitors in preclinical trials and *in vitro* studies are given in Table 2.

LS104. LS104 is a novel non-ATP mimetic JAK2 inhibitor. In preclinical studies, it inhibited JAK2 kinase activity *in vitro* and this effect was irreversible even in the presence of elevated ATP concentrations. A combination of LS104 plus an ATP-competitive JAK2 inhibitor led to synergistically increased apoptosis in *JAK2*V617F-positive cells. In addition, LS104 strongly inhibited cytokine-independent growth of endogenous erythroid colonies isolated from patients with *JAK2*V617F-positive MPN *in vitro*, whereas there was no significant effect on the growth of myeloid colonies obtained from normal controls [Lipka *et al.* 2008].

NS-018. NS-018 was reported at ASH 2010 to be a novel JAK2 inhibitor that inhibits JAK2 kinase activity with an IC₅₀ value of less than 1 nM and has a 30–50-fold selectivity for JAK2 over the other JAK-family kinases. NS-018 demonstrated therapeutic efficacy in a murine model of MPN induced by *JAK2*V617F with improvement in MPN features, that is body weight loss, hepatosplenomegaly, leukocytosis and anemia progression and a significantly improved survival rate [Nakaya *et al.* 2010; Shide *et al.* 2010].

BMS-911543. BMS-911543 was reported at ASH 2010 [Purandare *et al.* 2010] to be a

reversible inhibitor of JAK2 with a biochemical IC₅₀ of 0.001 μM. It has over 74- and 350-fold selectivity against the other JAK family members (JAK3 and JAK1, respectively). BMS-911543 displayed potent antiproliferative and pharmacodynamic effects in mutant JAK2-STAT signaling and had little activity in cell types dependent upon other pathways such as JAK1 and JAK3.

TG101209. TG101209 potently inhibits JAK2 tyrosine kinase, with markedly less activity against JAK3 [Pardanani *et al.* 2007]. It suppresses the proliferation of human erythroleukemia cells that express the *JAK2*V617F mutation, effectively treats *JAK2*V617F-induced hematopoietic disease in mice and reduces the growth of hemopoietic colonies from primary progenitor cells harboring *JAK2*V617F mutations. Of note, there have been two studies reporting on novel *in vitro* synergism of TG101209 with other agents. In a study reported at ASH 2010 [Fiskus *et al.* 2010], HEL92.1.7, Ba/F3-*JAK2*V617F and primary human MPN cells were cotreated with TG101209 and the MEK inhibitor AZD6244, the dual PI3K/mTOR inhibitor BEZ235 or the PIM1 kinase inhibitor SGI-1776. Each of the combination regimens resulted in an enhanced anti-*JAK2*V617F activity of TG101209 compared with single therapy. In another *in vitro* combination study, LBH589 (Panobinostat) was found to attenuate *JAK2*V617F levels and downstream signaling when combined with TG101209 with synergistic cytotoxic effects against human myeloproliferative neoplastic cells [Wang *et al.* 2009]. The authors showed that treatment with LBH589 disrupted the chaperone association of *JAK2*V617F with HSP90, promoting proteasomal degradation of *JAK2*V617F and suggested this

Table 2. Selected JAK2-inhibiting agents in preclinical studies.

Drug	JAK activity	<i>In vitro/in vivo</i> studies (–no, +yes)	Comments
LS014	JAK2	+/-	Non-ATP mimetic
NS018	JAK2	+/+	
CP690550	JAK2, JAK3	+/-	
BMS911543	JAK2	+/-	Combination with MEK, PIM1, PI3K/mTOR inhibitors or LBH589 tested
TG101209	JAK2	+/+	
WP1066	JAK2	+/-	PI3K, STAT3 with off-target JAK2

JAK, janus kinase; ATP, adenosine tri-phosphate; MEK, mitogen-activated protein kinase; PIM1, PIM-1 oncogene; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; STAT3, signal transducer and activator of transcription 3.

mechanism as a possible explanation for the synergistic effect with the JAK2 inhibitor.

WP1066. WP1066 is a potent PI3K and STAT3 inhibitor with the off-target effect as a JAK2 inhibitor [Verstovsek *et al.* 2008]. It has been shown to be effective in xenograft models of renal cell carcinoma [Horiguchi *et al.* 2010]. In hematological malignancy models, it potently suppresses proliferation and induces apoptosis in human erythroid cells containing the *JAK2V617F* mutation. WP1066 also inhibits the expansion of peripheral blood hematopoietic progenitors of PV patients who are positive for the *JAK2V617F* mutation. WP1066 was previously shown to inhibit phosphorylation of JAK2 in acute myelogenous leukemia cells and also causes degradation of the JAK2 protein [Ferrajoli *et al.* 2007].

CP690550 (Tasocitinib). CP690550 is a selective JAK3 inhibitor that also exhibits JAK2-inhibitory properties [Manshouri *et al.* 2008]. It demonstrates potent antiproliferative and proapoptotic activity against cells expressing the *JAK2V617F* mutation with a higher efficacy against the mutant compared with the wild-type JAK2. Furthermore, CP-690550 selectively inhibited the growth of *JAK2V617F*-positive progenitors from PV patients, suggesting that CP-690550 is a putative inhibitor of *JAK2V617F*.

Conclusions

In spite of all of the exciting developments in the field of JAK2-inhibiting agents, we need to be reminded that whether these agents will alter the natural history of MPN is as yet undetermined. Furthermore, the role of these agents in PV and ET (as opposed to MF) is still unclear especially since the long-term side effects of this relatively new class of agents are not fully known. In considering the use of anti-JAK2 therapy in MPN, we have to recognize the fact that life expectancy in the majority of patients with PV or ET is near-normal and disease complications are effectively managed by relatively harmless medical treatment. Therefore, further studies are needed to show added value and justify the possible risk of unknown long-term side effects associated with these agents in PV and ET. In the case of PMF (where current treatment options are limited in variety and efficacy) the case to use these agents may be more compelling. As has been shown in other malignancies, blockade of a single (kinase) pathway is often

insufficient to abrogate any overactive signaling pathway. This is all the more important in *JAK2* mutant MPN considering that ‘oncogene addiction of *JAK2V617F*’ is unlikely to be as marked as in CML. It would thus be interesting to see if there are subsequent reports of resistance to these agents in a similar vein to TKI resistance in CML.

As such we await more mature clinical trial data of these agents, clinical trials using combinations of these agents with other compounds (e.g. HDAC inhibitors) and clinical trials of novel non-ATP mimetic JAK2-inhibiting agents in MPN.

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Conflict of interest statement

None declared.

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