Clinical potential of pacritinib in the treatment of myelofibrosis

Ana B. Duenas-Perez and Adam J. Mead

Abstract: Myelofibrosis (MF) is a myeloid disorder caused by a clonal hematopoietic stem-cell proliferation associated with activation of the Janus kinase (JAK) signal transducer and activator of transcription (STAT) signaling pathways. Patients with MF often develop severe splenomegaly, marked symptom burden and significant cytopenias, with a consequent marked negative impact on quality of life and survival. The management of MF patients has dramatically improved with the development of a group of drugs that inhibit JAK signaling. The first of these agents to be approved was ruxolitinib, a JAK1/JAK2 inhibitor, which has been shown to improve both spleen size and symptoms in patients with MF. However, myelotoxicity, particularly of the platelet lineage, significantly limits the patient population who can benefit from this agent. Thus, there is an unmet need for novel agents with limited myelotoxicity to treat MF. Pacritinib, a JAK2 and FMS-like tyrosine kinase 3 (FLT3) inhibitor, has shown promising results in early phase trials with limited myelotoxicity and clinical responses that are comparable with those seen with ruxolitinib, even in patients with severe thrombocytopenia. Currently there are two large phase III clinical trials of pacritinib in MF, including patients with thrombocytopenia, and those previously treated with ruxolitinib. If the encouraging results observed in early phase clinical trials are confirmed, pacritinib will represent a new and exciting treatment option for patients with MF and particularly patients with significant cytopenias.

Keywords: JAK2 inhibitors, myelofibrosis, pacritinib, thrombocytopenia

Introduction

The therapeutic landscape for the treatment of patients with myelofibrosis (MF) has changed markedly over the past few years. In this review, we outline the clinical features and current treatment options for patients with MF, with particular emphasis on recent advances following the emergence of Janus activated kinase 2 (JAK2) inhibitor treatments. In the context of this present day management of MF patients, we outline the clinical potential of pacritinib, a novel JAK2 inhibitor, with potential meet a current unmet need, particularly for MF patients with baseline or treatment-emergent cytopenias.

MF: clinical features, diagnosis and treatment goals

The Philadelphia-negative myeloproliferative neoplasms (MPNs), encompassing primary

myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocythemia (ET), are hematologic disorders characterized by clonal hematopoietic stem-cell (HSC) proliferation and excessive production of one or more of the myeloid lineages with relatively preserved hematopoietic maturation. PMF is generally regarded as a more advanced stage of MPN, and both PV and ET can progress to a secondary MF stage, classified as PPV-MF and PET-MF, respectively.

Diagnosis of MF is based on a combination of morphologic and histological features together with presence of markers of clonal hematopoiesis. Diagnostic criteria have been established by both the World Health Organization (WHO) and the British Committee for Standards in Haematology (BCSH) (Table 1). In the bone marrow, hyperplasia of morphologically abnormal megakaryocytes is present in almost all patients, although the

Ther Adv Hematol

2015, Vol. 6(4) 186–201

DOI: 10.1177/

2040620715586527

© The Author(s), 2015.

Reprints and permissions:

[http://www.sagepub.co.uk/](http://www.sagepub.co.uk/journalsPermissions.nav)

[journalsPermissions.nav](http://www.sagepub.co.uk/journalsPermissions.nav)

Correspondence to:

Adam J. Mead, MD, PhD

Haematopoietic Stem

Cell Biology, Weatherall

Institute of Molecular

Medicine, University of

Oxford, Oxford OX3 9DS,

UK

NIHR Oxford Biomedical

Research Centre, Oxford

University Hospitals NHS

Trust, Oxford, UK

adam.mead@imm.ox.ac.uk

Ana B. Duenas-Perez, MD

Haematopoietic Stem

Cell Biology, Weatherall

Institute of Molecular

Medicine, University of

Oxford, Oxford, UK

Table 1. Diagnostic criteria for myelofibrosis according to the World Health Organization (WHO) and the British Committee for Standards in Haematology (BCSH).

2008 WHO diagnostic criteria	BCSH criteria
<p><i>Major criteria:</i>*</p> <ol style="list-style-type: none"> 1. Presence of megakaryocyte proliferation and atypia[§], usually accompanied by reticulin and/or collagen fibrosis, or in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (e.g. prefibrotic cellular phase disease) 2. Not meeting WHO criteria for PV, BCR-ABL-positive CML, myelodysplasia, or other myeloid disorders 3. Demonstration of JAK2 V617F or other clonal marker (e.g. MLPW515K), or in the absence of clonal markers, no evidence that bone marrow fibrosis or other changes are secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasms, metastatic malignancies, or toxic (chronic) myelopathies <p><i>Minor criteria:</i></p> <ol style="list-style-type: none"> 1. Leukoerythroblastosis 2. Increase in serum lactate dehydrogenase level 3. Anemia 4. Palpable splenomegaly 	<p><i>Diagnostic criteria for primary fibrosis</i>[‡]</p> <ol style="list-style-type: none"> A1. Bone marrow fibrosis ≥ 3 (on scale 0–4) A2. Pathogenetic mutations (e.g. in JAK2, CALR or MPL), or absence of both BCR-ABL and reactive causes of bone marrow fibrosis <ol style="list-style-type: none"> B1. Palpable splenomegaly B2. Unexplained anemia B3. Leuco-erythroblastic blood film B4. Tear-drop red cells B5. Constitutional symptoms[§] B6. Histological evidence of extramedullary hematopoiesis <p><i>Diagnostic criteria for post-PV and post-ET myelofibrosis</i>[‡]</p> <ol style="list-style-type: none"> A1. Bone marrow fibrosis ≥ 3 (on scale 0–4) A2. Previous diagnosis of ET or PV <ol style="list-style-type: none"> B1. New palpable splenomegaly or increase in spleen size of ≥ 5 cm B2. Unexplained anemia with 20 g/l decrease from baseline hemoglobin B3. Leucoerythroblastic blood film B4. Tear-drop red cells B5. Constitutional symptoms[§] B6. Histological evidence of extramedullary hematopoiesis
<p>*Diagnosis requires meeting all three major criteria and two minor criteria.</p> <p>§Small to large megakaryocytes with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei and dense clustering.</p> <p>‡Diagnosis requires A1 + A2 and any two B criteria.</p> <p>§Constitutional symptoms: Drenching night sweats, weight loss >10% over 6 months, unexplained fever (>37.5°C) or diffuse bone pains.</p> <p>CML, chronic myeloid leukemia; ET, essential thrombocythemia; JAK2, Janus activated kinase 2; PV, polycythemia vera.</p>	

requirement for presence of advanced fibrosis in the bone marrow is somewhat controversial as in the WHO, but not BCSH classification, some patients without marrow fibrosis can be diagnosed with PMF, based on distinct histologic features which in certain cases can be challenging to reliably distinguish from ET [Wilkins *et al.* 2008]. As secondary bone marrow (BM) fibrosis can be present in a number of other hematologic and nonhematologic disorders, careful integration of clinical, molecular and pathological features is necessary to make an accurate diagnosis.

Clinically, patients can present with a variety of different problems, which can be categorized as those relating to excessive proliferation (leukocytosis, thrombocytosis, constitutional symptoms and splenomegaly) as well as those relating to marrow fibrosis (anemia, neutropenia and thrombocytopenia). The symptomatic burden in patients

with MF is heterogeneous, with presence and intensity of different symptoms showing high variability between patients [Geyer and Mesa, 2014]. Accordingly, specific questionnaires to assess symptoms and also the impact of disease on quality of life (QOL) in patients with MPNs have been developed, most notably the MPN symptom assessment form total symptom score (MPN-SAF TSS) which has been validated in large cohorts of patients with MPN and is now routinely used to assess symptom responses in MPN clinical trials [Emanuel *et al.* 2012]. One of the most frequent symptoms reported by patients with MF is fatigue, which can appear at diagnosis or in more advanced phases of disease, and although this can be related to anemia, other systemic consequences of the disease also contribute. Extramedullary hematopoiesis in MF also causes a range of clinical problems, most notably abdominal symptoms related to massive splenomegaly with consequent spleen

Table 2. Available treatments for myelofibrosis before JAK2 inhibitors.

Group of symptoms	Splenomegaly and extramedullar hematopoiesis	Anemia	Myelosuppressive therapy	Constitutional symptoms	Life expectancy
Treatment options	Hydroxycarbamide Thalidomide + prednisolone Lenalidomide Splenectomy Spleen radiation	Blood transfusion Epo Androgens	Hydroxycarbamide Anagrelide INF- α	Corticosteroids	HSCT
Epo, erythropoietin; INF- α , interferon- α ; HSCT, hematopoietic stem-cell transplant; JAK2, Janus activated kinase 2.					

ischemia, portal hypertension and mechanical obstruction. Presence of severe constitutional symptoms of fever, weight loss and night sweats have also been shown to correlate with poor prognosis [Cervantes *et al.* 2009].

MF is associated with a significant negative impact on life expectancy, with a median survival of between 4 and 5 years. The principal causes of death are leukemic transformation, thrombosis, consequences of cytopenias (infections and bleeding) and portal hypertension. Prognosis is heterogeneous, and a number of factors that are associated with worse prognosis have been identified including advanced age (≥ 65), the presence of constitutional symptoms, low hemoglobin (< 10 g/dl), high leukocyte count ($\geq 25 \times 10^9/l$) and the presence of blasts ($\geq 1\%$) in peripheral blood [Cervantes *et al.* 2009], together forming the basis of the International Prognostic Scoring System (IPSS). The IPSS identifies four distinct prognostic groups, with a median life expectancy from diagnosis of 135, 95, 48 and 27 months for patients with low, intermediate-1, intermediate-2 and high-risk PMF, respectively. However, MF is a chronic disease and prognostic scoring systems predicting survival from diagnosis can be problematic for patients who might stochastically develop disease progression events over their disease course. Consequently, dynamic prognostic scoring systems have been developed which can be applied at any stage of disease. For example, the Dynamic International Prognostic Scoring System (DIPSS) plus system which incorporates the above prognostic factors and also includes a number of new factors; transfusion dependence, platelet count below $100 \times 10^9/l$ or unfavorable karyotype (complex karyotype, or single or specific abnormalities +8, -7/7q, i(17q), 5/-5q, 12p-, inv(3) or 11q23 rearrangements) [Gangat *et al.* 2011].

As might be expected for a heterogeneous disorder such as MF, treatment goals are distinct for each patient. For some, anemia is the main clinical problem; for others, the massive splenomegaly is disabling and other patients experience severe constitutional symptoms. Some develop combinations of these problems. Table 2 summarizes conventional treatment options up to 2011 in each of these settings, prior to the availability of JAK2 inhibitors [Reilly *et al.* 2012]. It should be noted that most of these treatments are based on small case series or clinical trials without robust phase III clinical trials data. Furthermore, none of the listed conventional treatments are approved by the US Food and Drug Administration (FDA) and in general the response rates are unsatisfactory and duration of response is limited. With regards to improving survival in MF, no conventional treatment option for MF has been shown to confer a survival benefit. The only curative option is allogeneic hematological stem-cell transplantation (HSCT), but high morbidity and mortality rates make this treatment unsuitable for the majority of patients, even when using reduced intensity conditioning approaches [McLornan *et al.* 2012]. Furthermore, allogeneic stem-cell transplantation in patients with MF is particularly high risk, probably due to the frequent presence of marked splenomegaly and high levels of inflammatory cytokines leading to increased rates of graft failure and graft versus host disease, respectively [McLornan *et al.* 2012].

Dysregulation of JAK2 signaling in MF

JAK2 is a member of a family of intracellular tyrosine kinases that are activated via a range of cytokine receptors expressed at the cell surface. In the hematopoietic system, the key cytokine receptors associating with JAK2 are erythropoietin,

thrombopoietin, granulocyte macrophage colony-stimulating factor (GM-CSF) and granulocyte macrophage colony-stimulating factor (G-CSF) receptors, key mediators of myeloid lineages. Downstream, JAK2 phosphorylates a number of key targets, including signal transducer and activator of transcription 3 (STAT3) and STAT5, that once activated translocate to the nucleus and initiate transcription of genes involved in proliferation, differentiation and apoptosis. Furthermore, JAK2 has also been shown to have a direct impact on transcription by modification of histone acetylation marks [Dawson *et al.* 2009]. In contrast, the rest of the members of the Janus kinase family, JAK1, JAK3 and TYK2, have a predominant effect on lymphopoiesis and immune responses [Meyer and Levine, 2014].

Aberrant JAK2 signaling has been long recognized in patients with MPNs [Komura *et al.* 2003]. However, the discovery of the *JAK2V617F* somatic mutation at the pseudokinase domain of JAK2 in 2005 led to a major advance in understanding of the pathophysiology of these diseases [Baxter *et al.* 2005; James *et al.* 2005; Kralovics *et al.* 2005; Levine *et al.* 2005]. This activating *JAK2* mutation was found to occur across the spectrum of MPNs, with frequencies of approximately 97% in patients with PV and 50–60% of patients with ET and PMF, representing a common link between these disorders with shared clinical and pathological features [Goldman, 2005]. The mutation causes a disruption of the autoinhibitory activity of the pseudokinase domain (JH2) of JAK2, with consequent autophosphorylation, constitutively activation of the JAK/STAT pathway and marked cytokine hypersensitivity [Vainchenker *et al.* 2011]. Exactly how the mutation leads to a clonal advantage in HSCs remains unclear, although studies of isolated germline *JAK2* mutations support the theory that increased JAK2 signaling can increase the HSC pool in humans [Mead *et al.* 2013]. Besides the JAK/STAT pathway, JAK2 is also involved in the activation of other important pathways for cytokine signaling such as phosphoinositidine-3-kinase (PI3K)/Akt/mTOR and MAPK signaling intermediates [Vainchenker and Constantinescu, 2013].

Other mutations leading to activation of the JAK/STAT pathway have also been described in patients with PMF. For example, activating mutations involving exon 10 of the thrombopoietin receptor (*MPL*) have been identified in 5–10% of *JAK2V617F* negative ET and PMF patients

[Pardanani *et al.* 2006]. Mutations in negative regulators of the JAK/STAT pathway like *LNK* and *CBL* have been described in approximately 5% of patients with PMF [Vainchenker *et al.* 2011]. Furthermore, two different groups recently identified mutations in exon 9 of the calreticulin (*CALR*) gene [Klampfl *et al.* 2013; Nangalia *et al.* 2013] which are mutually exclusive with mutations in *JAK2* and *MPL*, and are therefore only very rarely present in patients with PV. As *CALR* mutations are present in the majority of patients with ET or MF without *JAK2* or *MPL* mutations, this has been incorporated into the diagnostic criteria for ET in a BCSH modification (Table 1) [Harrison *et al.* 2014]. Ba/F3 cells expressing the *CALR* mutation show cellular growth independent of interleukin 3, associated with an increased phosphorylation of STAT5 [Klampfl *et al.* 2013]. Together, the presence of multiple different mutations, all causing activation of the JAK/STAT signaling pathway, highlights the central importance of this pathway in the pathogenesis of MPNs.

JAK2 inhibitors

Following the discovery of *JAK2V617F* mutation in PV, ET and PMF, several molecules targeting the JAK2 tyrosine kinase have been developed (Table 3). In 2010, the first phase I/II trial was conducted with ruxolitinib (INCB018424), a potent and selective inhibitor of JAK1 and JAK2 [Verstovsek *et al.* 2010]. This pivotal study revealed improvement of constitutional symptoms and exercise tolerance, decrease in proinflammatory cytokines and phosphorylated STAT3, and impressive reductions in spleen size in patients with MF. Subsequently, two randomized phase III trials were conducted comparing ruxolitinib with placebo and best available therapy (BAT) in patients with MF; the COMFORT-I and COMFORT-II trials, respectively [Harrison *et al.* 2012; Verstovsek *et al.* 2012]. In the COMFORT-I study, 41.9% of patients on ruxolitinib achieved the primary endpoint of a reduction of spleen volume $\geq 35\%$ (approximately equivalent to a 50% reduction in spleen length) at week 24 compared with 0.7% of placebo treated patients. Moreover, a 2-year follow-up report from COMFORT-I showed that, of the patients that achieved a reduction of the spleen volume of at least 35%, the majority (64%) maintained this response at 2 years [Verstovsek *et al.* 2013c]. Furthermore, 45.9% in the ruxolitinib group achieved a reduction of 50% or more in the total symptom score from baseline at week 24

Table 3. JAK2 inhibitors investigated for myeloproliferative neoplasms (MPNs).

	IC50 (nanomolar)				Adverse events	Current study (phase)
	JAK1	JAK2	JAK3	TYK2		
Ruxolitinib	2.8	4.5	322	30	Cytopenias (anemia and thrombocytopenia), infection	3
Pacritinib	1280	6	18.3	27	Gastrointestinal (diarrhea, nausea)	3
Momelotinib	11	18	155	17	Increase amylase/lipase, thrombocytopenia, peripheral neuropathy	3
Fedratinib	105	3	>1000	405	Wernicke's encephalopathy	†
LY2784544	NA	2	NA	8	Elevated creatinine (healthy volunteers)	2
NS018	33	22	0.72	22	NA	1–2
BMS911453	356	1	73	66	Cytopenias especially anemia	1–2
XL019	134	2	214	344	NA	*
AZD1480	0.26	NA	NA	NA	NA	1–2
CEP701	0.9	NA	NA	NA	Gastrointestinal	\$
IC50, half-maximal inhibitory concentration; NA, not available.						
*Stopped after phase I.						
\$Stopped after phase II.						
†Stopped in phase III.						

(assessed with the MFSAF) compared with 5.3% for placebo. In the COMFORT-II trial, 28% of patients met the primary endpoint of $\geq 35\%$ spleen volume reduction at week 48, significantly greater than observed with BAT (0%). Similarly, the group of patients treated with ruxolitinib experienced improvement in MF associated symptoms, whereas patients in the BAT arm had worsening of symptoms. Furthermore, although only of borderline statistical significance, ruxolitinib was associated with a 52% decrease in the risk of death compared with BAT in the COMFORT-II trial [Cervantes *et al.* 2013], with similar findings also for the placebo controlled COMFORT-I study. Importantly, both COMFORT studies showed that responses occur independently of the JAK2 mutation status, illustrating the benefit of JAK1/2 inhibition in patients regardless of the mechanism of activation of the JAK/STAT pathway.

In both studies, ruxolitinib was well tolerated, and most of the adverse events observed were hematologic. In COMFORT-I, all grades of anemia, thrombocytopenia and neutropenia were reported in 96%, 70% and 19% of patients, respectively [Verstovsek *et al.* 2012]. Both anemia and thrombocytopenia mostly occurred in the first 6–8 weeks of treatment with ruxolitinib. Furthermore,

whilst the anemia associated with ruxolitinib treatment improved in some patients with more prolonged therapy, the thrombocytopenia persisted, necessitating frequent dose reduction and a lower starting dose of ruxolitinib in patients with a lower baseline platelet count. Importantly, patients requiring a dose reduction to less than 10 mg twice a day average dose (approximately 20% of patients on the COMFORT-I study) showed significantly lower rates of spleen and symptom responses than patients who were able to maintain a higher dose level [Verstovsek *et al.* 2013c]. Moreover, patients with significant baseline thrombocytopenia ($<100 \times 10^9/l$), accounting for 28% of MF patients [Gangat *et al.* 2011], were excluded from the COMFORT studies, restricting interpretation of data from these studies for this challenging patient group.

With longer experience of using ruxolitinib, some important additional side effects have emerged. In 2012 the Mayo Clinic published a report on five patients who presented with serious side effects after ruxolitinib discontinuation. The presenting symptoms in most cases were a rebound of the splenomegaly and constitutional symptoms, worsening of cytopenias, occasional hemodynamic decompensation or septic shock like syndrome [Tefferi and Pardanani, 2011]. The

speculated mechanism for this ‘ruxolitinib withdrawal syndrome’ is a cytokine rebound, the risk of which can be reduced by tapering the dose of ruxolitinib, and treating with corticosteroids and/or restarting treatment with ruxolitinib. The JAK1 inhibitory effect of ruxolitinib could be important for this cytokine rebound; however, data from clinical trials with other JAK inhibitors is necessary to explore whether withdrawal of specific inhibition of JAK2 might have the same effect. It should be noted that in the phase III COMFORT studies, no reproducible pattern of a withdrawal syndrome was observed and it remains controversial whether a true ‘withdrawal syndrome’ exists, or whether many of these cases simply reflect a rapid return of symptoms to baseline levels [Verstovsek *et al.* 2013c].

An immunosuppressive impact of ruxolitinib now also seems clear. In the COMFORT studies, herpes zoster and urinary tract infections were common with ruxolitinib occurring in 4.3% and 12.3% of patients, respectively. Furthermore, tuberculosis reactivation [Colomba *et al.* 2012], hepatitis B reactivation [Shen *et al.* 2014], pneumonia caused by *Cryptococcus neoformans* [Wysham *et al.* 2013], pneumonitis caused by *Pneumocystis jiroveci* [Lee *et al.* 2014], toxoplasmosis retinitis [Goldberg *et al.* 2013] and progressive multifocal leukoencephalopathy (PML) [Wathes *et al.* 2013], have all been reported to occur during ruxolitinib treatment. Given that such opportunistic infections are rare, and might also occur in MF patients not receiving ruxolitinib, it is difficult to establish the exact role of ruxolitinib in such events. Nevertheless, preclinical studies have observed that ruxolitinib causes a rapid and prolonged decrement of T regulatory cells, and impairs the cytokine production and the normal function of dendritic cells [Heine *et al.* 2013; Massa *et al.* 2014]. These effects might, in part, be caused by the JAK1 inhibitory effect of ruxolitinib, but further studies are needed to fully understand the mechanisms.

Fedratinib (SAR302503/TG101348) is a JAK2 inhibitor that reached phase III clinical trials and showed significant improvements in spleen size [Pardanani *et al.* 2013a]. Although initially the safety profile of fedratinib was acceptable, with diarrhea, anemia and thrombocytopenia as the most important side effects, during the phase III clinical trial (JAKARTA) a few cases of Wernicke encephalopathy were reported, and subsequently

the study and clinical development of fedratinib was terminated. One hypothesis is that fedratinib inhibits the human thiamine transporter hTHT2, significantly decreasing the oral absorption of thiamine [Zhang *et al.* 2014]. Mometinib (CYT387) is a potent inhibitor of JAK1, JAK2 and TYK2, and is currently being assessed in phase III clinical trials, with preliminary results from a phase I/II trial showing spleen responses of $\geq 35\%$ in 46.6% of patients and, interestingly, anemia responses in 26% of patients [Gupta *et al.* 2014]. Notable adverse events were grade 3–4 thrombocytopenia in 28% and peripheral neuropathy in 46% of patients with 17 of 61 patients discontinuing treatment due to adverse events [Gupta *et al.* 2014]. Other JAK2 inhibitors currently in development in phase I/II trials are BMS-911543 [Pardanani *et al.* 2013b], LY2784594 [Verstovsek *et al.* 2013d], NS-018 [Nakaya *et al.* 2013], AZD1480 [Scuto *et al.* 2011] and INCB039110 [Quintas-Cardama *et al.* 2010]. CEP701 [Santos *et al.* 2010] and XL019 [Verstovsek *et al.* 2014] are JAK2 inhibitors no longer under clinical evaluation for PMF because of toxicity or lack of efficacy. The selectivity profile of the JAK inhibitors against members of the Janus kinase family and the stage of clinical development are summarized in Table 3.

Pacritinib

Mechanism of action

Pacritinib (previously known as SB1518) is a low-molecular-weight macrocycle, with limited conformational options conferring a stable structure with potent and selective JAK2 inhibitory activity [William *et al.* 2011]. Pacritinib is a type I JAK2 inhibitor, preferentially binding to an ‘activated’ form of JAK2 through the formation of hydrogen bonds with residues Leu932 and Ser936 and salt bridge to Asp939, and blocking phosphorylation at the Y221 site [Zuccotto *et al.* 2010; Hart *et al.* 2011; William *et al.* 2011], an effect that is independent of the JAK2 mutational status. As a result of the inhibition of STAT3 and STAT5, JAK2-signaling dependent cell lines treated with pacritinib suffer cell cycle arrest at G1 phase and undergo apoptosis [Hart *et al.* 2011]. The chemical structures of pacritinib, ruxolitinib, fedratinib and mometinib are quite different as shown in Figure 1, which might decrease the chance of undesirable neurological side effects observed with other agents [Zhang *et al.* 2014].

Figure 1. Different chemical structure of pacritinib, ruxolitinib, fedratinib and momelotinib.

Pacritinib has a potent and selective inhibitory activity against both wild-type and JAK2V617F mutant with IC₅₀s of 6 nM and 9.4 nM, respectively. Inhibition of other JAK family members is distinct from ruxolitinib and momelotinib with, JAK1, JAK3 and TYK2 inhibited by pacritinib with IC₅₀s of 1280 nM, 18.3 nM and 27 nM, respectively. It remains unclear whether the lack of JAK1 inhibitory activity will result in reduced efficacy and/or reduced toxicity. Additionally, pacritinib inhibits FMS-related tyrosine kinase 3 (FLT3) wildtype as well as FLT3 tandem duplications and kinase domain mutations with IC₅₀s of 6.4 nM, 12 nM and 8.3 nM, respectively. This is of potential relevance for MF, but has also prompted development of this agent in the treatment of patients with acute myeloid leukemia. Pacritinib also inhibits FMS with an IC₅₀ of 39.5 nM. These distinct molecular targets of pacritinib, in comparison with ruxolitinib, are potentially of interest

in the context of MF therapy. For example, increased plasma levels of FL, the ligand for FLT3, together with an increased expression of FLT3 and its phosphorylated form have been observed in megakaryocyte progenitors in JAK2 wildtype and JAK2V617F patients with MF [Desterke *et al.* 2011]. Furthermore, IRAK1 is inhibited by pacritinib with an IC₅₀ of 13.6 nM, representing a novel therapeutic target of potential interest in myeloid malignancies [Rhyasen *et al.* 2013].

Pharmacokinetics (PK) and pharmacodynamics (PD)

PK following single administration of pacritinib was first evaluated in mice, rats and dogs, establishing a T_{max} of 1, 4 and 2 hours, respectively, following oral administration with oral bioavailability of 24% [William *et al.* 2011]. Pacritinib is overwhelmingly eliminated by biliary excretion

with minimal involvement of metabolic or renal excretion. In human studies, including 3 phase I clinical trials, pacritinib has high oral bioavailability, with a T_{max} of 3–9 hours independent of food intake and a terminal half-life of 1–4 days [Verstovsek *et al.* 2009; Seymour *et al.* 2010; Younes *et al.* 2012]. In one study, peak plasma concentrations at all dose levels exceeded 5 µg/ml, well above the level required for JAK2 inhibition [Younes *et al.* 2012]. Furthermore, inhibition of phosphorylated STAT3 and STAT5 was observed at all dose levels in this study [Younes *et al.* 2012].

Safety profile

Results of a phase I dose escalation study of pacritinib in acute and chronic myeloid malignancies were first presented in 2009 [Verstovsek *et al.* 2009]. Data were presented from 36 patients who were treated at 6 dose levels, including 31 patients with MF and 5 with acute myeloid leukemia. The study found that 3 out of 6 patients treated at 600 mg daily experienced dose limiting gastrointestinal toxicity, which was reversible at a lower dose level. In this study, the most common side effect was diarrhea, occurring in 33% of the patients (4% grade 3) and nausea in 13% of patients. A second phase I study was reported in 2010 [Seymour *et al.* 2010], including 20 patients with MF. No dose limiting toxicities were seen between 100 and 400 mg once daily dose levels (each $n = 3$). At 600 mg, 2 out of 4 patients experienced grade 3 gastrointestinal toxicity. Cohort expansion at 500 mg once daily did not result in dose-limiting toxicity, but 3 out of 4 patients required dose interruptions due to diarrhea, abnormal liver function and dizziness respectively. A further phase I study of pacritinib in patients with relapsed lymphoma was reported in 2012 [Younes *et al.* 2012]. This trial once again reported gastrointestinal toxicity as the most frequent treatment-related adverse event with diarrhea occurring in 32% of patients, nausea and vomiting in 29%, and constipation in 26%.

Therefore, taken together, the above phase I dose escalation studies established 400 mg once daily as the appropriate dose to be taken forward for the phase II component of these studies. However, recently an exposure–response analysis utilizing PK data from phase I/II trials ($n = 129$) and healthy volunteers ($n = 42$) predicted that 200 mg twice daily dosing would result in a mean systemic exposure that is 41% higher than with 400 mg once daily, probably by higher drug accumulation

and reduced effect of saturable absorption [Al-Fayoumi *et al.* 2013]. Additionally, the twice a day regimen might be associated with lower concentration in the gastrointestinal tract, thus decreasing the proportion of gastrointestinal adverse events, especially diarrhea, and thus needs further evaluation in phase III trials.

In a safety report presented at the 18th European Hematology Association (EHA) Congress in 2013, results from 3 phase I and II studies were collated, including 191 patients with AML ($n = 7$), advanced lymphoid malignancies ($n = 62$) and MF ($n = 122$) [Verstovsek *et al.* 2013b]. Again, the most frequent side effects associated with pacritinib were gastrointestinal, with all grades of diarrhea, nausea, vomiting, constipation and abdominal pain occurring in 73%, 48%, 30%, 24% and 21% of patients, respectively. Grades 3/4 diarrhea occurred in 8% of patients with the onset of diarrhea usually occurring in the first 30 days. Overall rates of adverse events requiring dose interruption or reduction was 56.6%, including 31.8% due to gastrointestinal toxicity. Adverse events requiring study drug discontinuation occurred in 20.9% of patients, but gastrointestinal toxicity requiring permanent study drug discontinuation only occurred in 3.9% of patients. It should be noted, however, that use of routine gastrointestinal side effect prophylaxis was not implemented in these early phase studies, unlike ongoing phase III studies. To date, withdrawal reactions have not been reported with pacritinib.

With regard to hematologic toxicity, despite the fact that nearly half of the patients included in these studies had a baseline platelet count below $100 \times 10^9/l$, no dose reductions were required due to thrombocytopenia in the subgroup of patients with MF [Verstovsek *et al.* 2013a]. Indeed, there was no clinically significant decline in mean hemoglobin or platelet count levels from baseline values. Even in the group of patients with myeloid malignancy and a baseline platelet count $<50 \times 10^9/l$, the median reduction of platelet count was only $3 \times 10^9/l$, in contrast to the myelotoxicity associated with ruxolitinib treatment and of considerable interest for the management of patients with low platelets at baseline.

Taken together, data from these early phase trials support that pacritinib is well tolerated, with minimal myelotoxicity and the most frequent recurrent adverse events being gastrointestinal. Whilst low grade gastrointestinal toxicity might hamper

tolerability of pacritinib, the early phase studies suggest that these side effects rarely result in treatment discontinuation and might be manageable with careful use of supportive measures.

Efficacy of pacritinib in MF

Preclinical studies with pacritinib demonstrated a dose-dependent reduction in phosphorylated STAT5 and STAT3 with pacritinib treatment in Ba/F3 cells modified to carry the *JAK2V617F* mutation [William *et al.* 2011]. In a Ba/F3JAK2^{V617F} *in vivo* model, with doses of 150 mg/kg twice a day, normalization rates of 42% and 99% of spleen and liver weight, respectively, were observed. Furthermore, pacritinib was shown to induce dose-dependent inhibition of STAT5 phosphorylation in primary erythroid progenitor cells from PV patients [Hart *et al.* 2011], including inhibition of endogenous erythroid colony formation.

Subsequently, the efficacy of pacritinib in MF has been studied in two early phase clinical trials, both of which included phase I and phase II components (Table 4). The SB1518-001 trial enrolled patients with advanced myeloid malignancies. Data from the phase I, dose finding component of this trial, were reported in 2009 and included data from 31 cases of MF and 5 cases of AML. Of 21 cases evaluable for response, 7 out of 17 (41%) patients with palpable splenomegaly at diagnosis had a decrease in palpable spleen length of $\geq 35\%$ by palpation, and 4 of 17 (24%) had a reduction of $>50\%$ [Verstovsek *et al.* 2009]. Data from the phase II component were presented in 2011, including data from 33 MF patients with a median palpable spleen length at baseline of 18 cm who were treated at a dose of 400 mg once daily. Of 30 patients assessed by magnetic resonance imaging (MRI), 17 (57%) had a spleen volume reduction of $\geq 25\%$, corresponding in this trial to a reduction palpable spleen length by 50%, including 7 patients in whom the spleen became nonpalpable. Intensity of MF related symptoms also decreased by 40–65% in patients treated for 6 months or more [Deeg *et al.* 2011].

The second phase I/II trial in MF patients (SB1518-003) enrolled 35 patients in the phase II component of the study. Patients were eligible if they had clinical splenomegaly, poorly controlled with standard therapies, or were newly diagnosed with intermediate- or high-risk Lille Score, including 15 (43%) with platelet counts below

$100 \times 10^9/l$ and 7 with platelet counts below $50 \times 10^9/l$ at baseline. The median baseline spleen size was 14 cm (range 4–30 cm) assessed by palpation. Spleen length reduction on treatment was observed in 88% of patients with MRI and confirmed reductions in spleen volume $\geq 35\%$ in 31% of the patients from baseline up to week 24, irrespective of the baseline platelet count. Furthermore, improvements in hemoglobin levels were also observed in two of the patients treated with pacritinib, including one patient who achieved transfusion independence. Up to week 24, $\geq 50\%$ reduction in total symptom score from baseline was observed in 15 (48%) of 31 patients. In this study, half of the patients ($n = 17$) discontinued treatment, including 5 for disease progression, 2 for lack of response and 9 due to adverse events [Komrokji *et al.* 2011, 2015].

In a combined analysis of all patients with MF included in the above phase I and phase II clinical trials and treated at a dose of 400 mg once daily of pacritinib ($n = 65$), patients were stratified according to baseline platelet count of $>100 \times 10^9/l$ ($n = 37$) or $\leq 100 \times 10^9/l$ ($n = 28$). Of 49 evaluable patients, spleen volume reductions of $\geq 35\%$ at or before 36 weeks on treatment, as assessed by MRI, were observed in 35% of the total cohort and in 43% of patients with a baseline platelet count of $\leq 100 \times 10^9/l$, with no statistically significant difference between the groups stratified according to baseline platelet count [Verstovsek *et al.* 2013a].

The above results of early phase studies were sufficiently encouraging for the clinical development of pacritinib to be taken forward in two phase III clinical trials. PERSIST-1 is a randomized phase III study comparing pacritinib at a dose of 400 mg once a day with BAT. This trial, which is now fully recruited (322 patients), randomized MF patients (2:1) to receive pacritinib or BAT. For the PERSIST-1 study, patients with prior JAK2 inhibitor exposure were excluded and JAK2 inhibitor treatment was not allowed as part of the BAT arm. This trial includes patients with intermediate-1, intermediate-2 or high-risk PMF, PPV-MF and PET-MF with palpable splenomegaly (≥ 5 cm) and a significant symptom burden measured by a Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF-TSS) of ≥ 13 . Patients with significant cytopenias were included, with no platelet count cutoff and patients with an absolute neutrophil count above $0.5 \times 10^9/l$ allowed. The primary endpoint of the PERSIST-1

study is the frequency of a $\geq 35\%$ reduction in spleen volume from baseline to week 24, assessed by MRI or computerized tomography (CT). The study also includes a key secondary endpoint of a reduction in total symptom score (TSS) of at least 50% using MPN-SAF TSS at week 24 compared with baseline. A subgroup analysis of the patients stratified by platelet count is planned. Preliminary results from this study are expected in mid-2015.

PERSIST-2 is the second randomized phase III clinical trial with pacritinib in MF patients. In contrast with PERSIST-1, this study is designed to compare three treatment options (pacritinib 400 mg once a day, pacritinib 200 mg twice a day, and BAT), including a total of 300 patients (1:1:1 randomization). Patients must have a diagnosis of intermediate-1, intermediate-2 or high risk PMF, PPV-MF or PET-MF (by DIPSS) with a platelet count $< 100 \times 10^9/l$, palpable splenomegaly and a TSS of at least 13 on the MPN-SAF-TSS; patients previously exposed to other JAK2 inhibitors other than pacritinib are allowed. The efficacy co-endpoints for this analysis are the proportion of patients achieving a $\geq 35\%$ reduction in spleen volume from baseline to week 24, and the proportion of patients achieving a $\geq 50\%$ reduction in TSS from baseline to week 24 as measured by the MPN-SAF-TSS. The main secondary endpoint is an analysis of each pacritinib schedule compared with BAT, including approved JAK2 inhibitors as part of the options for this arm. There is also a key secondary PK and PD objective to assess exposure response relationships on the safety and efficacy of pacritinib using two dosing schedules.

Possible combination therapies with pacritinib

Although available JAK2 inhibitors have shown encouraging responses in terms of spleen volume reduction and symptom control, one of the disappointing features of the responses reported is the lack of a marked disease modifying effect as might have been evidenced through reversal of BM fibrosis or clonal responses with major reductions in mutant allele burden [Verstovsek *et al.* 2012]. Formation of heterodimers between other members of the Janus kinase family might help explain the disease persistence observed in patients receiving JAK2 inhibitor therapies [Koppikar *et al.* 2012]. Additionally, secondary

mutations in the JAK2 kinase domain have been detected *in vitro* [Deshpande *et al.* 2012], although as yet such mutations have not been observed in patients. Whilst, therefore, it appears that current JAK2 inhibitor therapy might not result in complete JAK2 inhibition, this might also be important to limit toxicity as, unlike tyrosine kinase inhibitor therapy in CML, JAK2 is indispensable for normal hematopoiesis, so complete inhibition of this pathway would likely be associated with severe hematologic toxicity [Akada *et al.* 2014]. For these reasons, several studies are investigating additional therapeutic targets that might synergize with JAK2 inhibitor therapy to enhance therapeutic responses and minimize resistance/persistence of the malignant clone without excessive toxicity. Figure 2 shows alternative pathways that are currently under investigation, as potential targets in combination with JAK inhibitors.

Preclinical studies have identified the PI3K/AKT/mTOR pathway as a potential target for combination therapy with JAK2 inhibitors. For example, BEZ235 is a pan-class I PI3K, mTORC1/2 inhibitor with *in vitro* capacity to induce cell cycle arrest and apoptosis of MPN cells as monotherapy, and also showing synergy with JAK2 inhibitors [Fiskus *et al.* 2013]. Additionally, there is a phase I study currently underway that is investigating the combination of BKM120, another PI3K/mTOR inhibitor, and ruxolitinib in patients with MF. Another example is everolimus, a potent mTOR inhibitor which has shown promising responses in terms of spleen size reduction and symptomatic improvement in a phase I/II study in patients with MF [Guglielmelli *et al.* 2011]. Inhibitors of the heat shock protein 90 (HSP90) can also target JAK2-driven malignancies [Weigert *et al.* 2012]. HSP90 is a chaperone protein that acts to stabilize JAK2 and its mutant V617F. Inhibition of HSP90 causes disruption of the chaperone association between JAK2 and HSP90, promoting degradation of JAK2V617F. For example, AUY922 is a HSP90 inhibitor that has been used in combination with JAK2 inhibitors to enhance the capacity to induce apoptosis of cells expressing JAK2V617F [Fiskus *et al.* 2011].

As the other key group of mutations occurring in MF affect epigenetic machinery [Vainchenker *et al.* 2011], there is a strong rationale for combination therapy with JAK2 inhibitors and agents

Figure 2. Potential alternative pathways in myelofibrosis and possible treatment combinations with pacritinib. Pacritinib inhibits JAK2 (Janus activated kinase 2) and FLT3 (FMS-like tyrosine kinase). The binding of FLT3 (FL) and other ligands inducing JAK2 signaling (JL) phosphorylate and activate different shared pathways, including signal transducer and activator of transcription 5 (STAT5), phosphoinositide-3-kinase (PI3K)/AKT/mTOR and RAS/MAPK. Potential combinations with pacritinib are PI3K-, mTOR-, HSP90-, histone deacetylase (HDAC) or mitogen-activated protein kinase kinase (MEK) inhibitors. Additionally, DNA methyltransferase (DNMT) inhibitors could be potentially combined with pacritinib, as DNMT inhibitors could reverse inhibition of suppressor of cytokine signaling (SOCS-1), a negative regulator of the JAK/STAT pathway. HSP90, heat shock protein 90; RAS, renin-angiotensin system.

targeting epigenetic pathways [Mascarenhas *et al.* 2011]. Histone deacetylase (HDAC) inhibitors are a group of agents that have shown considerable promise in this regard. As single agents, HDAC inhibitors have demonstrated substantial differential cytotoxicity against JAK2V617F positive cell lines and primary human MPN cells *in vitro* [Guerini *et al.* 2008; Akada *et al.* 2012] and in mouse models [Akada *et al.* 2012]. This effect is mediated, in part, through the disruption of the chaperone association of JAK2V617F with HSP90 leading to proteasomal degradation of JAK2V617F expressing cells [Wang *et al.* 2009]. Interestingly, HDAC inhibitor cytotoxicity shows synergism with JAK2-inhibitor induced cytotoxicity in MPN cells [Wang *et al.* 2009]. Early phase clinical studies have demonstrated some activity of HDAC inhibitors in patients with MPNs, including some cases of MF [Rambaldi *et al.* 2010; Mascarenhas *et al.* 2011; Deangelo *et al.* 2013].

The combination of pracinostat (SB939) and pacritinib has been studied using *in vitro* models with cells expressing JAK2WT or JAK2V617F mutant. When pracinostat was used alone, important reductions in phosphorylation of STAT5 and JAK2 were observed only in the subgroup of cells with JAK2V617F. Interestingly, the combination of pacritinib and pracinostat had a synergistic effect with a potent inhibition of JAK2-autophosphorylation [Novotny-Diermayr *et al.* 2012]. It has also been proposed that DNMT inhibitors might reverse the epigenetic mechanism that silences genes, for example, *SOCS* genes, involved in negative regulation of the JAK/STAT pathway [Mascarenhas, 2014]. Indeed, both 5-azacytidine and decitabine are currently under investigation in phase I/II trials in combination with ruxolitinib. There is also interest in the possibility of combining JAK2 inhibitors with allogeneic transplantation in order to reduce spleen size

Table 4. Early phase clinical trials with pacritinib in myelofibrosis.

Group carrying out study	Responses	Hematological toxicity, % (grade 3/4)	Frequent grade 3/4 nonhematological toxicity
Seymour (phase I) (n = 20)	Not reported	Not reported	Diarrhea 11% Nausea and vomiting 6% Abdominal pain 11% Fatigue 11%
Verstovsek (phase I) (n = 31)	7/17 (41%) decrease in spleen size $\geq 35\%^*$ 4/17 (24%) decrease in spleen size $\geq 50\%^*$	Thrombocytopenia 4%	Diarrhea 4%
Komrokji (phase II) (n = 34)	30 (88%) decrease in spleen size* 15 (44%) decrease in spleen size $\geq 50\%^*$ 6 (18%) resolution of splenomegaly* 11 (32%) spleen volume reduction $\geq 35\%^{\S}$ 14 (41%) spleen volume reduction $> 25\%^{\S}$ Reduction in intensity of MF related symptoms after 6 months of treatment 2 patients showed improvement in hemoglobin	Not reported	Grade 3 diarrhea 6%
Deeg (phase II) (n = 33)	12/31 (39%) decrease in spleen size $\geq 50\%^*$ 7/31 (23%) decrease in spleen size of 100%* 17/30 (57%) spleen volume reduction $\geq 25\%$ 29/30 any spleen volume reduction § 40–65% reduction on intensity of MF-related symptoms after 6 months of treatment	No grade 3/4 neutropenia or thrombocytopenia	Diarrhea 6%
*Assessed with physical examination. § Assessed with magnetic resonance imaging.			

and inflammatory cytokine levels pre transplant, with a number of ongoing clinical trials [McLornan *et al.* 2012; Stubig *et al.* 2014]. Other novel investigational combination therapies include combination with antifibrosis agents, e.g. PRM-151 and hedgehog pathway inhibition [Mascarenhas, 2014]. Finally, conventional agents like immunomodulatory agents, interferon- α (INF- α) and danazol could be useful, in combination with JAK2 inhibitors, to manage anemia or other disease manifestations.

Conclusion

MF is a clinically and biologically heterogeneous disease with high symptom burden and impaired QoL. Traditional treatments using cytoreductive drugs or corticosteroids have limited efficacy, at best only offering a transient relief of symptoms. Other curative options like allogeneic transplant are only suitable for a limited number of patients. Thus, treatment with JAK2 inhibitors provides an important new option for MF patients offering the previously unattainable goal of rapid control of

symptoms and reduction in spleen size. However, myelosuppression seems to be an important and limiting toxicity with ruxolitinib and a number of other JAK2 inhibitors. Pacritinib is a potent and selective JAK2 inhibitor with limited myelosuppression and low inhibitory activity against JAK1, whilst offering activity against other targets of interest in MF such as FLT3 and IRAK1. Pacritinib is relatively well tolerated, with gastrointestinal side effects representing the most frequently observed toxicity. The distinct kinase inhibition profile likely underlies the reduced myelosuppressive impact of pacritinib, but whether this might also translate through to lower levels of immunosuppressive effects than seen with ruxolitinib remains to be seen. Results of current randomized phase III trials are eagerly awaited as, if the primary endpoints are met, pacritinib will represent an exciting new treatment addressing a major unmet need for patients with MF, particularly those frequent patients with disease-related or treatment-emergent thrombocytopenia.

Acknowledgements

The authors thank Dr Jack Singer and Dr Theocharous Panteli for information about the pacritinib kinoma.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

References

- Akada, H., Akada, S., Gajra, A., Bair, A., Graziano, S., Hutchison, R. *et al.* (2012) Efficacy of vorinostat in a murine model of polycythemia vera. *Blood* 119: 3779–3789.
- Akada, H., Akada, S., Hutchison, R., Sakamoto, K., Wagner, K. and Mohi, G. (2014) Critical role of JAK2 in the maintenance and function of adult hematopoietic stem cells. *Stem Cells* 32: 1878–1889.
- Al-Fayoumi, S., Wang, L., Li, H., Wanda, R. and Dean, J. (2013) Exposure-response analysis for pacritinib (SB1518), a novel oral JAK2/FLT3 inhibitor, in patients with myelofibrosis. *Blood* 122: 4080–4080.
- Baxter, E., Scott, L., Campbell, P., East, C., Fourouclas, N., Swanton, S. *et al.* (2005) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 365: 1054–1061.
- Cervantes, F., Dupriez, B., Pereira, A., Passamonti, F., Reilly, J., Morra, E. *et al.* (2009) New prognostic scoring system for primary myelofibrosis based on a study of the international working group for myelofibrosis research and treatment. *Blood* 113: 2895–2901.
- Cervantes, F., Vannucchi, A., Kiladjian, J., Al-Ali, H., Sirulnik, A., Stalbovskaya, V. *et al.* (2013) Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. *Blood* 122: 4047–4053.
- Colomba, C., Rubino, R., Siracusa, L., Lalicata, F., Trizzino, M., Titone, L. *et al.* (2012) Disseminated tuberculosis in a patient treated with a JAK2 selective inhibitor: a case report. *BMC Res Notes* 5: 552.
- Dawson, M., Bannister, A., Gottgens, B., Foster, S., Bartke, T., Green, A. *et al.* (2009) JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature* 461: 819–822.
- Deangelo, D., Mesa, R., Fiskus, W., Tefferi, A., Paley, C., Wadleigh, M. *et al.* (2013) Phase II trial of panobinostat, an oral pan-deacetylase inhibitor in patients with primary myelofibrosis, post-essential thrombocythaemia, and post-polycythaemia vera myelofibrosis. *Br J Haematol* 162: 326–335.
- Deeg, H., Odenike, O., Scott, B., Estrov, Z., Cortes, J., Thomas, D. *et al.* (2011) Phase II study of SB1518, an orally available novel JAK2 inhibitor, in patients with myelofibrosis. *J Clin Oncol* 29: abstract 6515.
- Deshpande, A., Reddy, M., Schade, G., Ray, A., Chowdary, T., Griffin, J. *et al.* (2012) Kinase domain mutations confer resistance to novel inhibitors targeting JAK2V617F in myeloproliferative neoplasms. *Leukemia* 26: 708–715.
- Desterke, C., Bilhou-Nabera, C., Guerton, B., Martinaud, C., Tonetti, C., Clay, D. *et al.* (2011) FLT3-mediated P38-MAPK activation participates in the control of megakaryopoiesis in primary myelofibrosis. *Cancer Res* 71: 2901–2915.
- Emanuel, R., Dueck, A., Geyer, H., Kiladjian, J., Slot, S., Zweegman, S. *et al.* (2012) Myeloproliferative neoplasm (MPN) symptom assessment form total symptom score: prospective international assessment of an abbreviated symptom burden scoring system among patients with MPNs. *J Clin Oncol* 30: 4098–4103.
- Fiskus, W., Verstovsek, S., Manshouri, T., Rao, R., Balusu, R., Venkannagari, S. *et al.* (2011) Heat shock protein 90 inhibitor is synergistic with JAK2 inhibitor and overcomes resistance to JAK2-TKI in human

- myeloproliferative neoplasm cells. *Clin Cancer Res* 17: 7347–7358.
- Fiskus, W., Verstovsek, S., Manshouri, T., Smith, J., Peth, K., Abhyankar, S. *et al.* (2013) Dual PI3K/AKT/MTOR inhibitor BEZ235 synergistically enhances the activity of JAK2 inhibitor against cultured and primary human myeloproliferative neoplasm cells. *Mol Cancer Ther* 12: 577–588.
- Gangat, N., Caramazza, D., Vaidya, R., George, G., Begna, K., Schwager, S. *et al.* (2011) DIPSS plus: a refined dynamic international prognostic scoring system for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. *J Clin Oncol* 29: 392–397.
- Geyer, H. and Mesa, R. (2014) Assessing disease burden in patients with classic MPNs. *Best Pract Res Clin Haematol* 27: 107–119.
- Goldberg, R., Reichel, E. and Oshry, L. (2013) Bilateral toxoplasmosis retinitis associated with ruxolitinib. *N Engl J Med* 369: 681–683.
- Goldman, J. (2005) A unifying mutation in chronic myeloproliferative disorders. *N Engl J Med* 352: 1744–1746.
- Guerini, V., Barbui, V., Spinelli, O., Salvi, A., Dellacasa, C., Carobbio, A. *et al.* (2008) The histone deacetylase inhibitor ITF2357 selectively targets cells bearing mutated JAK2(V617F). *Leukemia* 22: 740–747.
- Guglielmelli, P., Barosi, G., Rambaldi, A., Marchioli, R., Masciulli, A., Tozzi, L. *et al.* (2011) Safety and efficacy of everolimus, a MTOR inhibitor, as single agent in a phase 1/2 study in patients with myelofibrosis. *Blood* 118: 2069–2076.
- Gupta, V., Mesa, R., Deininger, M., Rivera, C., Sirhan, S., Kawashima, J. *et al.* (2014) A Phase I/II, open-label study evaluating twice-daily administration of momelotinib (GS-0387, CYT387) in primary myelofibrosis or post-polycythemia vera or post-essential thrombocythemia myelofibrosis. *Haematologica* 99: 530–531.
- Harrison, C., Butt, N., Campbell, P., Conneally, E., Drummond, M., Green, A. *et al.* (2014) Modification of British Committee for Standards in haematology diagnostic criteria for essential thrombocythaemia. *Br J Haematol* 167: 421–423.
- Harrison, C., Kiladjian, J., Al-Ali, H., Gisslinger, H., Waltzman, R., Stalbovskaya, V. *et al.* (2012) JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med* 366: 787–798.
- Hart, S., Goh, K., Novotny-Diermayr, V., Hu, C., Hentze, H., Tan, Y. *et al.* (2011) SB1518, a novel macrocyclic pyrimidine-based JAK2 inhibitor for the treatment of myeloid and lymphoid malignancies. *Leukemia* 25: 1751–1759.
- Heine, A., Held, S., Daecke, S., Wallner, S., Yajnanarayana, S., Kurts, C. *et al.* (2013) The JAK-inhibitor ruxolitinib impairs dendritic cell function *in vitro* and *in vivo*. *Blood* 122: 1192–1202.
- James, C., Ugo, V., Le Couedic, J., Staerk, J., Delhommeau, F., Lacout, C. *et al.* (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 434: 1144–1148.
- Klampfl, T., Gisslinger, H., Harutyunyan, A., Nivarthi, H., Rumi, E., Milosevic, J. *et al.* (2013) Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med* 369: 2379–2390.
- Komrokji, R., Seymour, J., Roberts, A., Wadleigh, M., To, L., Scherber, R. *et al.* (2015) Results of a phase 2 study of pacritinib (SB1518), a JAK2/JAK2(V617F) inhibitor, in patients with myelofibrosis. *Blood* 125: 2649–2655.
- Komrokji, R., Wadleigh, M., Seymour, J., Roberts, A., To, L., Zhu, H. *et al.* (2011) Results of a phase 2 study of pacritinib (SB1518), a novel oral JAK2 inhibitor, in patients with primary, post-polycythemia vera, and post-essential thrombocythemia myelofibrosis. *ASH Annu Meet Abstr* 118: 282.
- Komura, E., Chagraoui, H., Mansat De Mas, V., Blanchet, B., De Sepulveda, P., Larbret, F. *et al.* (2003) Spontaneous STAT5 activation induces growth factor independence in idiopathic myelofibrosis: possible relationship with FKBP51 overexpression. *Exp Hematol* 31: 622–630.
- Koppikar, P., Bhagwat, N., Kilpivaara, O., Manshouri, T., Adli, M., Hricik, T. *et al.* (2012) Heterodimeric JAK-STAT activation as a mechanism of persistence to JAK2 inhibitor therapy. *Nature* 489: 155–159.
- Kralovics, R., Passamonti, F., Buser, A., Teo, S., Tiedt, R., Passweg, J. *et al.* (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 352: 1779–1790.
- Lee, S., Feenstra, J. and Georgiou, P. (2014) Pneumocystis jiroveci pneumonitis complicating ruxolitinib therapy. *BMJ Case Rep*. DOI:10.1136/bcr-2014-204950.
- Levine, R., Wadleigh, M., Cools, J., Ebert, B., Wernig, G., Huntly, B. *et al.* (2005) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 7: 387–397.
- Mascarenhas, J. (2014) Rationale for combination therapy in myelofibrosis. *Best Pract Res Clin Haematol* 27: 197–208.
- Mascarenhas, J., Roper, N., Chaurasia, P. and Hoffman, R. (2011) Epigenetic abnormalities in

- myeloproliferative neoplasms: a target for novel therapeutic strategies. *Clin Epigenetics* 2: 197–212.
- Massa, M., Rosti, V., Campanelli, R., Fois, G. and Barosi, G. (2014) Rapid and long-lasting decrease of T-regulatory cells in patients with myelofibrosis treated with ruxolitinib. *Leukemia* 28: 449–451.
- McLornan, D., Mead, A., Jackson, G. and Harrison, C. (2012) Allogeneic stem cell transplantation for myelofibrosis in 2012. *Br J Haematol* 157: 413–425.
- Mead, A., Chowdhury, O., Pecquet, C., Dusa, A., Woll, P., Atkinson, D. *et al.* (2013) Impact of isolated germline JAK2V617I mutation on human hematopoiesis. *Blood* 121: 4156–4165.
- Meyer, S. and Levine, R. (2014) Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. *Clin Cancer Res* 20: 2051–2059.
- Nakaya, Y., Shide, K., Naito, H., Niwa, T., Horio, T., Miyake, J. *et al.* (2013) NS-018, a selective JAK2V617F inhibitor, improves JAK2V617F-induced murine myelofibrosis without decreasing the erythrocyte or platelet count. *Blood* 122: 3847–3847.
- Nangalia, J., Massie, C., Baxter, E., Nice, F., Gundem, G., Wedge, D. *et al.* (2013) Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med* 369: 2391–2405.
- Novotny-Diermayr, V., Hart, S., Goh, K., Cheong, A., Ong, L., Hentze, H. *et al.* (2012) The oral HDAC inhibitor pracinostat (SB939) is efficacious and synergistic with the JAK2 inhibitor pacritinib (SB1518) in preclinical models of AML. *Blood Cancer J* 2: e69.
- Pardanani, A., Harrison, C., Cortes, J., Cervantes, F., Mesa, R., Milligan, D. *et al.* (2013a) Results of a randomized, double-blind, placebo-controlled phase III study (Jakarta) of the JAK2-selective inhibitor fedratinib (SAR302503) in patients with myelofibrosis (MF). *Blood* 122: 393.
- Pardanani, A., Levine, R., Lasho, T., Pikman, Y., Mesa, R., Wadleigh, M. *et al.* (2006) MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood* 108: 3472–3476.
- Pardanani, A., Roberts, A., Seymour, J., Burbury, K., Verstovsek, S., Kantarjian, H. *et al.* (2013b) BMS-911543, a selective JAK2 inhibitor: a multicenter phase 1/2a study in myelofibrosis. *Blood* 122: 664–664.
- Quintas-Cardama, A., Vaddi, K., Liu, P., Manshouri, T., Li, J., Scherle, P. *et al.* (2010) Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. *Blood* 115: 3109–3117.
- Rambaldi, A., Dellacasa, C., Finazzi, G., Carobbio, A., Ferrari, M., Guglielmelli, P. *et al.* (2010) A pilot study of the histone-deacetylase inhibitor givinostat in patients with JAK2V617F positive chronic myeloproliferative neoplasms. *Br J Haematol* 150: 446–455.
- Reilly, J., McMullin, M., Beer, P., Butt, N., Conneally, E., Duncombe, A. *et al.* (2012) Guideline for the diagnosis and management of myelofibrosis. *Br J Haematol* 158: 453–471.
- Rhyasen, G., Bolanos, L., Fang, J., Jerez, A., Wunderlich, M., Rigolino, C. *et al.* (2013) Targeting IRAK1 as a therapeutic approach for myelodysplastic syndrome. *Cancer Cell* 24: 90–104.
- Santos, F., Kantarjian, H., Jain, N., Manshouri, T., Thomas, D., Garcia-Manero, G. *et al.* (2010) Phase 2 study of CEP-701, an orally available JAK2 inhibitor, in patients with primary or post-polycythemia vera/essential thrombocythemia myelofibrosis. *Blood* 115: 1131–1136.
- Scuto, A., Krejci, P., Popplewell, L., Wu, J., Wang, Y., Kujawski, M. *et al.* (2011) The novel JAK inhibitor AZD1480 blocks STAT3 and FGFR3 signaling, resulting in suppression of human myeloma cell growth and survival. *Leukemia* 25: 538–550.
- Seymour, F., To, B., Goh, A., Meadows, L., Ethirajulu, A. and Wood, A. *et al.* (2010) First report of the phase-I study of the novel oral JAK2 inhibitor SB1518 in patients with myelofibrosis. *Haematologica* 95: abstract 1144.
- Shen, C., Hwang, C., Chen, Y. and Chen, C. (2014) Hepatitis B virus reactivation associated with ruxolitinib. *Ann Hematol* 93: 1075–1076.
- Stubig, T., Alchalby, H., Ditschkowski, M., Wolf, D., Wulf, G., Zabelina, T. *et al.* (2014) JAK inhibition with ruxolitinib as pretreatment for allogeneic stem cell transplantation in primary or post-ET/PV myelofibrosis. *Leukemia* 28: 1736–1738.
- Tefferi, A. and Pardanani, A. (2011) Serious adverse events during ruxolitinib treatment discontinuation in patients with myelofibrosis. *Mayo Clin Proc* 86: 1188–1191.
- Vainchenker, W. and Constantinescu, S. (2013) JAK/STAT signaling in hematological malignancies. *Oncogene* 32: 2601–2613.
- Vainchenker, W., Delhommeau, F., Constantinescu, S. and Bernard, O. (2011) New mutations and pathogenesis of myeloproliferative neoplasms. *Blood* 118: 1723–1735.
- Verstovsek, S., Dean, J., Cernohous, P., Komrokji, R., Seymour, J., Mesa, R. *et al.* (2013a) Pacritinib, a dual JAK2/FLT3 inhibitor: an integrated efficacy and safety analysis of phase II trial data in patients

- with primary and secondary myelofibrosis (MF) and platelet counts $\leq 100,000/\mu\text{L}$. *Blood* 122: 395.
- Verstovsek, S., Kantarjian, H., Mesa, R., Pardanani, A., Cortes-Franco, J., Thomas, D. *et al.* (2010) Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *N Engl J Med* 363: 1117–1127.
- Verstovsek, S., Liang, S., Komrokji, R., Mesa, R., Seymour, J., Dean, J. *et al.* (2013b) Safety overview of phase I-II studies of pacritinib, a non-melosuppressive JAK2/FLT3 inhibitor, in patients with hematological malignancies. *Haematologica* P278: 119–120.
- Verstovsek, S., Mesa, R., Gotlib, J., Levy, R., Gupta, V., Dpersio, J. *et al.* (2012) A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med* 366: 799–807.
- Verstovsek, S., Mesa, R., Gotlib, J., Levy, R., Gupta, V., Dpersio, J. *et al.* (2013c) Efficacy, safety and survival with ruxolitinib in patients with myelofibrosis: results of a median 2-year follow-up of comfort-I. *Haematologica* 98: 1865–1871.
- Verstovsek, S., Mesa, R., Salama, M., Giles, J., Pitou, C., Zimmermann, A. *et al.* (2013d) Phase I study of LY2784544, a JAK2 selective inhibitor, in patients with myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET). *Blood* 122: 665–665.
- Verstovsek, S., Odenike, O., Scott, B., Estrov, Z., Cortes, J., Thomas, D. *et al.* (2009) Phase I dose-escalation trial of SB1518, a novel JAK2/FLT3 inhibitor, in acute and chronic myeloid diseases, including primary or post-essential thrombocythemia/polycythemia vera myelofibrosis. *ASH Ann Meet Abstr* 114: 2809.
- Verstovsek, S., Tam, C., Wadleigh, M., Sokol, L., Smith, C., Bui, L. *et al.* (2014) Phase I evaluation of XL019, an oral, potent, and selective JAK2 inhibitor. *Leuk Res* 38: 316–322.
- Wang, Y., Fiskus, W., Chong, D., Buckley, K., Natarajan, K., Rao, R. *et al.* (2009) Cotreatment with panobinostat and JAK2 inhibitor TG101209 attenuates JAK2V617F levels and signaling and exerts synergistic cytotoxic effects against human myeloproliferative neoplastic cells. *Blood* 114: 5024–5033.
- Wathes, R., Moule, S. and Milojkovic, D. (2013) Progressive multifocal leukoencephalopathy associated with ruxolitinib. *N Engl J Med* 369: 197–198.
- Weigert, O., Lane, A., Bird, L., Kopp, N., Chapuy, B., Van Bodegom, D. *et al.* (2012) Genetic resistance to JAK2 enzymatic inhibitors is overcome by HSP90 inhibition. *J Exp Med* 209: 259–273.
- Wilkins, B., Erber, W., Bareford, D., Buck, G., Wheatley, K., East, C. *et al.* (2008) Bone marrow pathology in essential thrombocythemia: interobserver reliability and utility for identifying disease subtypes. *Blood* 111: 60–70.
- William, A., Lee, A., Blanchard, S., Poulsen, A., Teo, E., Nagaraj, H. *et al.* (2011) Discovery of the macrocycle 11-(2-pyrrolidin-1-yl-ethoxy)-14,19-dioxo-5,7,26-triaza-tetracyclo[19.3.1.1(2,6).1(8,12)]heptacos-1(25),2(26),3,5,8,10,12(27),16,21,23-decaene (SB1518), a potent Janus kinase 2/FMS-like tyrosine kinase-3 (JAK2/FLT3) inhibitor for the treatment of myelofibrosis and lymphoma. *J Med Chem* 54: 4638–4658.
- Wysham, N., Sullivan, D. and Allada, G. (2013) An opportunistic infection associated with ruxolitinib, a novel Janus kinase 1,2 inhibitor. *Chest* 143: 1478–1479.
- Younes, A., Romaguera, J., Fanale, M., McLaughlin, P., Hagemeister, F., Copeland, A. *et al.* (2012) Phase I study of a novel oral Janus kinase 2 inhibitor, SB1518, in patients with relapsed lymphoma: evidence of clinical and biologic activity in multiple lymphoma subtypes. *J Clin Oncol* 30: 4161–4167.
- Zhang, Q., Zhang, Y., Diamond, S., Boer, J., Harris, J., Li, Y. *et al.* (2014) The Janus kinase 2 inhibitor fedratinib inhibits thiamine uptake: a putative mechanism for the onset of Wernicke's encephalopathy. *Drug Metab Dispos* 42: 1656–1662.
- Zuccotto, F., Ardini, E., Casale, E. and Angiolini, M. (2010) Through the 'gatekeeper door': exploiting the active kinase conformation. *J Med Chem* 53: 2681–2694.