Janus kinase inhibitors: jackpot or potluck?

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Abstract

The reports of a unique mutation in the Janus kinase-2 gene (*JAK2*) in polycythemia vera by several independent groups in 2005 quickly spurred the development of the Janus kinase inhibitors. In one of the great victories of translational research in recent times, the first small-molecule Janus kinase inhibitor ruxolitinib entered a phase I trial in 2007. With the approval of ruxolitinib by the US Federal Drug Administration in November 2011 for high-risk and intermediate-2 risk myelofibrosis, a change in paradigm has occurred in the management of a subset of myeloproliferative neoplasms (MPN): primary myelofibrosis, post-polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis. Whereas the current evidence for ruxolitinib only covers high-risk and intermediate-2 risk myelofibrosis, inhibitors with greater potency are likely to offer better disease control and survival advantage in patients belonging to these categories, and possibly to the low-risk and intermediate-1 risk categories of MPN as well. But use of the Janus kinase inhibitors also probably has certain disadvantages, such as toxicity, resistance, withdrawal phenomenon, non-reversal of histology, and an implausible goal of disease clone eradication, some of which could offset the gains. In spite of this, Janus kinase inhibitors are here to stay, and for use in more than just myeloproliferative neoplasms.

Introduction

The Philadelphia-negative myloproliferative neoplasms (MPN), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), are characterized by a long and progressive course. The median survival of PMF is less than five years[1] whereas, with the best available therapy, ET patients have close to normal survival[2] and the median survival of patients with PV is 19 years.[3] Two inter-related processes are responsible for the symptoms in PV, ET and PMF, and myelofibrosis (MF) primary, post-ET or post-PV: i) clonal proliferation; and ii) a reactive inflammatory state. Since the reports of the discovery of the Janus kinase-2 gene $(JAK2)^{V617F}$ mutation in PV in 2005,[4-8] the last seven years have witnessed several advances in the understanding of these disorders. The observation that the $JAK2^{V617F}$ mutation is found in 95% PV and more than 50% in ET and MF quickly prompted the development of JAK2 protein inhibitors, which marks one of the great advances in translational research of recent times.

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Normal JAK2-mediated signaling

The Janus kinase family of non-receptor tyrosine kinases includes four proteins: JAK1, JAK2, JAK3 and Tyk2. Growth factors such as erythropoietin, thrombopoietin and granulocyte colony stimulating factor bind to their cognate receptors at the cell membrane; JAK2 associates with these receptors, like an adapter, for the downstream transduction of signals to the nucleus through the JAK-STAT pathway. The binding of cytokine ligand to the cytokine receptors results in activation of the receptors and consequent autophosphorylation of JAK2. The phosphorylation of tyrosines serves as docking sites for the recruitment and assembly of downstream signaling proteins. This in turn results in activation of specific cascades involving STAT, MAPK, ERK and P-I-3 Kinase-AKT. Negative feedback mechanisms involving silencer of cytokine signaling (SOCS), CBL, LNK and other proteins attenuate the signaling.[9]

$JAK2^{V617F}$ mutation in myloproliferative neoplasms

In 2005, four groups reported a specific mutation of the *JAK2* gene, *i.e.* $JAK2^{V617F}$ in PV, ET and PMF. The mutation is observed with a frequency of over 95% in PV, 32-57% in ET and 35-50% in PMF.[4,7] The $JAK2^{V617F}$ results from a guanine-to-thymidine transversion at nucleotide 1849 on exon 14 of *JAK2* which translates into the substitution of valine by phenylalanine at position 617 in the pseudokinase domain of the JAK2 protein.[4-8] The gain-of-function mutation leads to a constitutive activation of $JAK2^{V617F}$ and an uncontrolled activation of the downstream pathway called JAK-STAT pathway, in addition to some other *pleotropic effects* of $JAK2^{V617F}$.

Of the seven domains of the JAK2 protein, the mutation hits the pseudokinase JH2 domain. JH2 has an auto-inhibitory effect on JH1 which is the kinase domain of JAK2. The substitution of phenylalanine

at position 617 in JH2 domain results in a *pi-stacking of phenylalanine residues* and changed physical characteristics of JAK2.[10] This relieves the kinase domain of the inhibition from JH2, which is rendered perpetually *switched-on*. JAK2^{V617F} hyperphosphorylates attenuating SOCS3 protein and exploits it in ensuring the intense signal transduction duration is prolonged.[11] When *JAK2^{V617F}* is expressed in hematopoietic cells, several signaling pathways, including STAT3, STAT5, MAPK, ERK and PI3K-AKT, are overactivated. The net effect is proliferation, survival and differentiation in hematopoietic cells leading to the MPN phenotype.

In addition to the two gains referred to above, there is at least one additional epigenetic effect conferred by the *JAK2^{V617F}* mutation: JAK2^{V617F} translocates to the nucleus and phosphorylates PRMT5 incapacitating it from methylating histone H2A and H4 (on specific arginine residues). Abrogation of PRMT5 may also contribute to the MPN phenotype.[9]

Exon 12 and other mutations

Recurring mutations on *JAK2* other than in exon 14 have been observed in exon 12. Exon 12 mutations are observed roughly in one-third of patients with *JAK2^{V617F}*-negative PV; the overall incidence in PV is 3%. The frequency of other mutations such as *MPL, LNK, CBL, IDH1, IDH2, TET2, EZH2, DNMT3A, ASXL1, SF3B1, IKZF1, TP53, CUX1* and others[12,13] is less than 20% and often below 10%[13] (Table 1).

Targeting the Janus kinase in myelofibrosis to bridge the gap between the need and the availability

All MPNs, irrespective of the frequency of *JAK2^{V617F}* mutation, have the same basic underlying pathophysiology: clonal myeloproliferation and hyperreactiveness to cytokines.[9] ET, PV, PMF and post-ET/PV MF, all harbor the same somatic mutations albeit with variable frequencies. Since ET and PV have a very long natural history with median survival of decades, and are amenable to control with the best available therapy (Table 2), there does not seem to be an urgent need of a drug for these diseases. However, PMF and post-PV/ET MF are characterized by shorter median survival and a greater severity of symptoms, including constitutional symptoms. There was, therefore, an urgent need for a new therapy in these patients, and in particular in the worse subset. Hence, though the activity of Janus kinase inhibitors in PV and ET has been and is being tested, for the moment, the main focus of the Janus kinase inhibitor trials is on MF.

The Janus kinase inhibitors are small-molecule ATP inhibitors. They exert their effect by competing with the ATP-binding site on the kinase.[14] The first among these, ruxolitinib (Jakafi, Incyte Corp., Wilmingtom, DE, USA), which was introduced in trials in 2007, was approved by the US Federal Drug Administration for use in high-risk and intermedicate-2 risk MF in November 2011 on the basis of phase III data.[15] There are a number of Janus kinase inhibitors currently in clinical trials at various stages of development (Table 3).

Janus kinase inhibitors differ from one another with respect to: i) their ability to inhibit one or the other Janus kinase to different degrees; ii) their specificity for mutated rather than wild-type kinase; iii) hematologic and non-hematologic toxicities; iv) off-target activity; v) potential for reducing the *JAK2^{V617F}* allele burden; and vi) activity against other kinases such as FLT.

Table 1. Mutations associated with myloproliferative neoplasms, and likely targeted therapy.

Pathway	Mutations	Targeted therapy
JAK/STAT PI3K-AKT-Mtor	*JAK2^{V617F}*, *JAK2* exon 12, *MPL, CBL, LNK*	Ruxolitinib and others Everolimus
Epigenetic	*TET2, ASXL1, EZH2*	Givinostat, Panobinostat, vorinostat
Oncogenic	*IDH*	-

Ruxolitinib (JAKAFI)

Pre-clinical evidence of ruxolitinib activity in myloproliferative neoplasms

Ruxolitinib has potent inhibitory activity against JAK 1 and 2, moderate activity against TYK2 and negligible activity against JAK 3. In Ba/F3 cells expressing *JAK2^{V617F}*, ruxolitinib induced dramatic inhibition of phosphorylation of JAK2^{V617F}, STAT 5 and ERK 1 and 2, along with reduced cellular proliferation and induction of apoptosis. Ruxolitinib potently inhibited the proliferation of *ex vivo* expanded erythroid progenitors obtained from patients with *JAK2^{V617F}*-positive PV. In a murine model of *JAK2^{V617F}*-driven malignancy, ruxolitinib reversed the disease with normalization of histology and reduction of spleen, and prolonged survival. Circulating levels of pro-inflammatory cytokines, such as IL-6 and TNF-alpha, believed to be responsible for the constitutional symptoms in PMF, were dramatically reduced in this model.[16]

Table 2. Traditional therapies for myloproliferative neoplasms.

	Objective	Options
PV	To avoid hemorrhagic and thrombotic complications	Low risk: phlebotomy frontline (Target hct <45 in men and <42 in women) High risk: phlebotomy+ Aspirin HU in patients exhibiting poor response to aspirin HU+Aspirin
ET	To avoid hemorrhagic and thrombotic complications	Low risk: no therapy Intermediate risk: Aspirin High risk: HU standard, Aspirin
PMF	Improvement of cytopenias	Corticosteroids, danazol, erythropoietic stimulating agents
	Reduction in splenomegaly	HU, irradiation or splenectomy Lenalidomide-Prednisolone combination
	Curative intent	Allogeneic stem-cell transplant

PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; HU, hydroxyurea; hct, hematocrit.

Clinical evidence of ruxolitinib efficacy in myelofibrosis: phase I/II

Safety and efficacy of ruxolitinib was tested in a phase I/II trial of 153 patients of PMF, post-ET or post-PV myelofibrosis.[17] The dose-limiting toxicity was grade 4 thrombocytopenia. After 28 days of therapy, there were dramatic reductions in fibrogenic, angiogenic and pro-inflammatory growth factors independent of *JAK2* mutational status.[17] Mean leukocyte count after three months of treatment (15 or 25 mg twice-daily) decreased from 29.8×10^9/L to 16.0×10^9/L, and 7 (44%) of 16 patients with baseline thrombocytosis normalized their platelet count. After a median follow up of approximately 15 months, the anemia, spleen (>50% reduction on palpation) and constitutional-symptoms (scored by Myelofibrosis Symptoms Assessment Form) response rates were 14%, 44% and more than 50%, respectively.[17] Ruxolitinib was found to have little effect on *JAK2*^{V617F} allele burden or bone marrow fibrosis. The improvements with ruxolitinib were independent of mutational status or origin of myelofibrosis (primary or post-PV/ET). Non-hematologic toxicity occurred in less than 10% of patients and was commonly grade 1 or 2. Thrombocytopenia (grade 3,17%; grade 4,3%) and treatment-emergent anemias (23%) were the most common adverse effects.[18]

Clinical evidence of ruxolitinib efficacy in polycythemia vera and essential thrombocythemia: phase II

Ruxolitinib activity was also tested in a phase II study of PV (n=34) and ET (n=39) patients refractory or intolerant to hydroxyurea (HU). In patients with PV, significant reductions in hematocrit, leukocyte count and platelet count were observed at six months, with a complete response rate of 45% and partial response rate of 52% according to European Leukemia Net response criteria.[19,20] In patients with ET, up to 75% patients experienced a reduction in pruritus, bone pain, night sweats, and an at least 50% reduction in peripheral neuropathy scores. The complete and partial response rates were 13% and 77%, respectively. Toxicity in this study was less than grade 4: grade 2 anemia in 12% PV and 18% ET, and grade 3 leukopenia in 9% PV and 5% ET.[19,20]

Clinical evidence of ruxolitinib superiority in high- and intermedicate-2 risk myelofibrosis: phase III (COMFORT I)

Ruxolitinib was tested in two phase III trials: COMFORT I and II (controlled myelofibrosis study with oral JAK inhibitor treatment). COMFORT I compared ruxolitinib (n=155) with placebo (n=154) in International Prognostic Scoring System[21] high-risk or intermediate-2 risk MF.[22] Continuous oral administration of ruxolitinib was given at doses of 15 mg twice a day (platelets $100\text{-}200\times10^9$/L) or 20 mg twice a day (platelets $>200\times10^9$/L).

The primary end point was regression of spleen volume by 35% or more with computed tomography or magnetic resonance imaging (MRI) at the end of 24 weeks. The secondary end point was symptom improvement. In addition, there were some exploratory end points. In the ruxolitinib arm, approximately 45% had PMF, 32% had post-PV MF and 23% had post-ET MF, whereas the respective percentages in the control arm were 55%, 32% and 14%. The main patient distribution data of the two COMFORT trials (COMFORT I and II) have been summarized in Table 4.

At 24 weeks, spleen response (primary end point) was 41.9% with ruxolitinib *versus* 0.7% on placebo. Almost all patients on ruxolitinib had some spleen response and the majority of patients receiving placebo had growth in spleen size (Figure 1). In addition, 45.9% of patients on ruxolitinib *versus* 5.3% on placebo experienced a 50% or more improvement in constitutional symptoms. Most patients on ruxolitinib had improvement in symptoms and the majority of patients receiving placebo had a worsening of symptoms. The benefits were apparent among all subtypes of MF irrespective of *JAK2* mutational status, reflecting data obtained from a phase II study. Ten deaths occurred in the ruxolitinib arm and 14 in the placebo arm. The authors reported a statistically significant survival benefit with ruxolitinib.[22]

Grade 3 and grade 4 anemia with ruxolitinib was 45.2% compared with 19.2% with placebo; and grade 3 and grade 4 thrombocytopenia

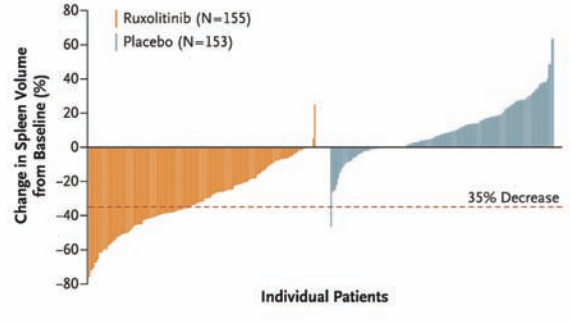

Figure 1. Waterfall diagram showing the percent change in spleen volume from baseline (*with permission:* COMFORT I, Verstovsek *et al.*, New England Journal of Medicine 2012).

Table 3. The Janus kinase inhibitors: selectivity and phase of development.

Compound	Selectivity against JAKs			Stage of development
	JAK2 *vs.* JAK3	JAK2 *vs.* JAK1	JAK2 *vs.* TYK2	
Ruxolitinib	153	1.1	6.7	Phase 3
TG101348 (SAR302503)	332	35	135	Phase 3
Lestaurtinib	3	N/A	N/A	Phase 2
XL019	125	65	170	Halted
CYT387	8.6	0.6	N/A	Phase 1/2
AZD1480	15	5	N/A	Phase 1/2
SB1518	24	58	N/A	Phase 1/2
LY2784544	N/A	N/A	N/A	Phase 1/2

JAK, Janus kinase; N/A, not applicable or not available.

was reported in 12.9% patients with ruxolitinib and 1.3% with placebo. The most frequent grade 3 and 4 non-hematologic toxicities in the study arm were abdominal pain, fatigue and dyspnea; these were more frequent in the placebo arm.

There was no difference in rate of withdrawal due to adverse events between the ruxolitinib (11%) the placebo (10.6%) arms. There was no clear pattern of any syndrome following withdrawal of ruxolitinib.

Clinical evidence of ruxolitinib superiority in high- and intermediate-2 risk myelofibrosis: phase III COMFORT II

This European study comparing ruxolitinib with best available treatment (BAT) examined spleen volume at the end of 48 weeks.[23] Patients eligible had high- or intermediate-2 risk disease. Ruxolitinib was administered at doses of 15 mg twice a day (platelets <200×10^9/L) or 20 mg twice a day (platelets >200×10^9/L). The principal end point was reduction in spleen volume by 35% or more (spleen response). Secondary end points were duration of spleen response and improvement in symptom score.

Spleen response was 32% at 24 weeks and 28% at 48 weeks *versus* 0% for BAT. At the time of reporting of the trial, the median duration of response had not reached and 80% were still holding the spleen response at a median follow up of 12 months. All subgroups derived benefit from ruxolitinib. Spleen response was seen in both mutation-positive and negative patients, but some numerical differences emerged. In *JAK2^{V617F}*-positive patients, the spleen response rate for ruxolitinib was 33% (*vs* 0% with BAT), whereas in *JAK2^{V617F}*-negative patients the spleen response was 14% (*vs* 0% with BAT).

At week 48, patients receiving ruxolitinib had marked reductions in myelofibrosis-associated symptoms (anorexia, dyspnea, fatigue, insomnia and pain) whereas patients receiving BAT had worsening symptoms measured with EORTC QLQ-C30 scores (Figure 2). The authors did not suggest any survival benefit with ruxolitinib. Whether survival benefit will be demonstrable at a longer follow up remains to be seen.

The most frequent combined grade 3 and 4 non-hematologic toxicity was diarrhea (1%). Combined grade 3 and 4 anemia was 42% with ruxolitinib and 31% with best available therapy. Grade 3 and 4 thrombocytopenia was seen in 8% with ruxolitinib *versus* 7% with BAT.[23] Overall, serious adverse events were infrequent in the ruxolitinib arm and were more frequent in patients receiving BAT. The number of patients discontinuing the study medication due to all-grade adverse events was small (8%) and did not differ greatly from the number discontinuing BAT (5%).

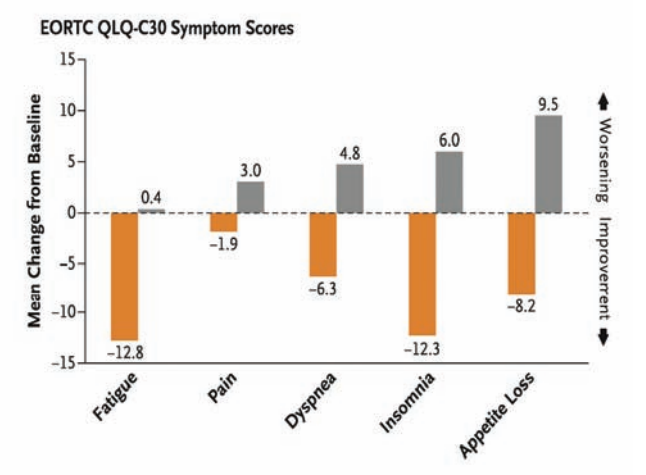

Figure 2. Mean changes in EORTC QLQ-C30 scores from baseline, showing improvement all symptoms with ruxolitinib and worsening of all symptoms with BAT. (*with permission:* COMFORT II, Harrison et al, New England Journal of Medicine 2012).

Table 4. Comparison of patient distribution and end points of COMFORT I and II.

		COMFORT I		COMFORT II	
Design		Randomized, blinded *vs* placebo		Randomized, open, *vs* BAT	
Randomization		1:1		2:1	
Location		North America and Australia		Europe	
Number		309 (155 in ruxolitinib arm, 154 in placebo arm)		219 (146 in ruxolitinib arm, 73 in BAT arm)	
		Ruxolitinib	*Placebo*	*Ruxolitinib*	*BAT*
Patient distribution by subtype of MF%	PMF	45%	55%	53%	53%
	Post-PV	32%	31%	33%	27%
	Post-ET	23%	14%	14%	19%
Dose 1		15 mg BID if platelet count 100-200×10^9/L 20 mg BID if platelet count >200×10^9/L Dose titration as required			
Primary end point		Spleen volume reduction by ≥35% by CT or MRI at 24 weeks		Spleen volume reduction by ≥35% by CT or MRI at 48 weeks	
Secondary end points		≥50% reduction in MFSAF		Duration of spleen response, symptom improvement (EORTC QLQ-C30 Scores)	

COMFORT, COntrolled MyeloFibrosis Study with ORal JAK Inhibitor Treatment; BAT, Best Available Therapy; CT, computed tomography; MRI, magnetic resonance imaging; PV, polycythemia vera; ET, essential thrombocythemia; MF, myelofibrosis; PMF, primary myelofibrosis; MFSAF, Myelofibrosis Symptom Assessment Form; EORTC QLQ, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire; BID, twice a day.

Clinical evidence of ruxolitinib efficacy in polycythemia vera: phase III

A phase III trial of ruxolitinib in patients with HU resistant or HU–intolerant PV is currently underway.[15]

Long-term follow up of ruxolitinib a phase I/ II in myelofibrosis: the Mayo experience

The follow up of Mayo Clinic patients of MF (one of the two participant centers, the other being MD Anderson Cancer Center) in a phase I/II ruxolitinib trial (n=51) was updated in July 2011. The overall response rate was reported to be 29% for spleen, 21% for anemia and 63% for constitutional symptoms. Most frequent grade 2, 3 and 4 toxicities were: thrombocytopenia (26%) and anemia (33%). Cumulative treatment discontinuation rate at one year was 51%, at two years 72% and at three years 89%. The most common causes for discontinuation were loss of treatment response and toxicity. A rapid rebound in symptoms following drug discontinuation, sometimes requiring hospitalization, was seen in almost all such cases. After comparison of the risk-adjusted survival of the Mayo cohort of ruxolitinib-treated patients with 410 untreated patients, no survival advantage was seen.[24]

Long-term follow up of ruxolitinib phase I/ II: the MD Anderson experience

Of 107 patients in a phase I/II ruxolitinib trial at the MD Anderson Cancer Center, 54% were still continuing treatment at a median follow up of 32 months. After comparison with historic patients, authors concluded that there was a survival benefit with ruxolitinib.[25]

Other Janus kinase inhibitors in trials

There are many other JAK inhibitors which have undergone phase I/II trials in humans. One has entered phase III (SAR302503) while the development of another trial (XL019) was halted following drug-induced neuropathy.

SAR302503 (TG101348)

This is the only Janus kinase inhibitor after ruxolitinib to have entered into a phase III trial in high-risk and intermediate-2 risk MF patients against placebo. At the time of writing, phase III interim data have still not been.

In a phase I/II experience, DLT was hyperamylasemia. Independent of *JAK2* mutational status, 6 cycles of treatment with SAR302503 yielded a 39% palpable spleen response (defined as ≥50% regression) and constitutional symptom response (defined as ≥50% reduction). Leukocytosis response (defined as normalization) was 72% and thrombocytosis response was 90%. Common side effects were nausea and diarrhea (majority of patients), transfusion dependency (35%), grade 3 or 4 thrombocytopenia (24%), and asymptomatic increases in serum lipase (27%) and transaminases (27%).[26] After 24 cycles of treatment, there was a statistically significant decrease in *JAK2*^{V617F} allele burden from a baseline of median 20% (n=51; range 3-100%) to median 9% (n=21; range 0-100%).[27]

CYT387

In a phase I/II multi-center study CYT387, 163 patients with high- or intermediate-2 risk MF had been enrolled at the time of the most recent study report.[28] DLT included grade 3 hyperlipasemia and grade 3 headaches. For the initial 60 patients completing at least 3 cycles of treatment with CYT387, spleen, anemia and constitutional symptom response rates (by conventional criteria)[29] were 45%, 50% and 50% or over, respectively, irrespective of *JAK2*^{V617F} mutational status.[30] Interestingly, 58% of transfusion-dependent patients became transfusion-independent.[30] The most common toxicity included transient lightheadedness and hypotension seen only with the first dose, and grade 3 or 4 thrombocytopenia seen in 16% of subjects.[30] Grade 1 toxicities included peripheral sensory neuropathy whose incidence and natural history is currently being studied. A unique divergence in toxicity with respect to ruxolitinib and SAR302503 was the lack of treatment-related grade 3 or 4 anemia, which was less than 1%. At the time of the most recent report, 25 (15%) of the 163 study subjects had discontinued treatment.

Lestaurtinib

In a phase II study,[31] lestaurtinib (CEP-701) at 80 mg twice daily was given to 22 *JAK2*-mutated MF patients. Overall response by International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) criteria[29] was 27%. Among 8 transfusion-dependent patients, 2 (25%) became transfusion-independent. Treatment had no impact on bone marrow histopathology, *JAK2*^{V617F} allele burden or inflammatory cytokine levels. Side effects included diarrhea (73%), nausea (50%), and grade 3 or 4 anemia (14%) and thrombocytopenia (23%). After a median follow up of less than 1.5 years, 21 patients (91%) discontinued therapy mostly because of lack of response and 6 (27%) deaths were reported.

A phase I study of lestaurtinib with a new capsule formulation (as opposed to liquid formulation) to circumvent excess plasma protein binding is currently ongoing in patients with *JAK2*^{V617F}-positive MF.[32]

Results were disappointing in another phase II study of lestaurtinib in high-risk patients of *JAK2*^{V617F}-positive PV (n=27) or ET (n=12). Five patients experienced a worsening of leukocytosis and thrombocytosis, and thrombosis.[33]

SB1518 (pacritinib)

The dose limiting-toxicity (DLT) during a phase I study of SB1518 diarrhea was an adverse event at a dose of 600 mg/day.[34] In the phase II part of the same study, MF patients not suitable for standard therapy were enrolled. Of 30 patients assessed by MRI, 17 (57%) had a reduction in spleen-volume of 25% or more. There was a reduction in intensity of MF-related symptoms of 40-65% in patients treated for six months. The most common treatment-related toxicities were diarrhea (81%; 6% grade 3), nausea (41%; all grade 1/2), vomiting (22%; all grade 1/2), and fatigue (9%; all of grade 1/2). At six months, 21 patients remain on therapy. There was no grade 3/4 neutropenia or thrombocytopenia. SB1518 was tolerated equally well by patients with normal platelet counts and those with thrombocytopenia.[35]

In another phase II study in 34 patients of MF (PMF, post-ET/PV MF), spleen response rate was 44% by physical examination and 32% by MRI (≥35% reduction in splenic volume). Two patients met anemia response. Seventeen (50%) had discontinued treatment, mainly because of adverse effects or disease progression.[36]

LY2784544

Among the 19 patients enrolled so far in a phase I study of LY2784544 in patients with PMF (n=17), PV (n=1) or ET (1), DLT included increases in uric acid and creatinine at a dose of 200-240 mg/day related to tumor lysis syndrome (TLS). The patient with PV was reported to experience a 100% reduction in spleen size. In MF, 4 (22%) patients have so far achieved spleen response. No responses have so far been observed in terms of *JAK2*^{V617F} allele burden reduction. Toxicity has mainly involved diarrhea (42%), nausea (37%), anemia (21%) and transient increases in serum creatinine, uric acid, and potassium, some of which has been attributed to TLS.[37]

XL019

In a phase I study involving 21 MF patients, 7 of 9 patients receiving XL019 at daily doses of 100 mg or more experienced grade 1 or 2 peripheral or central neuropathy. At lower doses (≤50 mg/day), used in the remaining patients, favorable effects were seen in *JAK2* or *MPL* mutated patients but not in unmutated patients. However, because of neurological toxicities, further development of XL019 in trials has been halted.[38]

BMS911543, NS-018 and AZD1480

At present, no information is available on the studies undertaken with these drugs.[15]

Janus kinase inhibitors: beyond myloproliferative neoplasms

Inhibitors of one or the other type of Janus kinase are likely to be of use in hematologic malignancies and in benign disorders such as rheumatoid arthritis and other autoimmune diseases.[14]

Targeting in myloproliferative neoplasms: beyond Janus kinase inhibitors

Histone deacetylase inhibitors givinostat, panobinostat and vorinostat were documented to down-regulate the levels of phosphorylated JAK2^{V617F} in *in vitro* studies which led to the phase I/II studies with these agents in MPN.

Givinostat

In a phase IIA study of givinostat, a novel histone-deacetylase inhibitor, in patients with PV (n=12), ET (n=1) and MF (n=16) bearing the *JAK2^{V617F}* mutation, givinostat was given orally for 24 weeks at a starting dose of 50 mg twice daily. Ten patients discontinued treatment mainly due to disease, minor toxicity and withdrawal of consent. Among 13 PV/ET patients, one complete, 6 partial and 4 no responses were documented at study end while 2 patients went off-study prematurely. Three major responses were registered among 16 MF patients. Pruritus disappeared in most patients, and reduction of splenomegaly was observed in 75% of PV/ET and 38% of MF patients. Reverse transcription polymerase chain reaction identified a trend towards reduction of the JAK2^{V617F} allele burden. Givinostat was well tolerated and could induce hematologic response in most PV and some MF patients.[39]

Panobinostat

A phase I trial identified reversible thrombocytopenia as the DLT.[40] A phase II trial by the same investigator group is ongoing, and had enrolled 14 patients at the time of the last reports.[40]

Interim data of another phase II trial was presented in abstract form in 2010. The authors reported that the majority of patients required dose reduction. However, specific adverse events, their frequency, and response rates were not reported in the 2010 abstract and no further reports of data fromthis study have been published.[41]

Eeverolimus

In addition to dysregulated JAK/STAT signaling, activation of the AKT/mTOR pathway occurs in MPN. In a phase I/II study with everolimus, an mTOR inhibitor, in 39 high- or intermediate-risk PMF or post-PV/ET myelofibrosis subjects, responses were evaluated in 30 patients of the phase II group.[42] No dose-limiting toxicity was observed in phase I up to 10 mg/d. When this dose was used in phase II, grade 3 or more toxicities were infrequent; the most common toxicity was grade 1-2 stomatitis. Rapid and sustained reduction in splenomegaly of more than 50% and more than 30% occurred in 20% and 44% of subjects, respectively. A total of 69% and 80% patients experienced complete resolution of systemic symptoms and pruritus, respectively. Response in leukocytosis, anemia, and thrombocytosis occurred in 15-25%. Clinical responses were not associated with reduced *JAK2^{V617F}* burden or cytokine levels. Response rate was 60% when European Network for Myelofibrosis criteria were used (8 major, 7 moderate, 3 minor responses) or 23% when IWG-MRT criteria were used (one partial response, 6 clinical improvements). These results provide proof-of-concept that targeting mTOR pathway in myelofibrosis may be clinically relevant.

Pomalidomide

In a study of long-term follow-up data on patients from the Mayo Clinic who had participated in three consecutive clinical trials using single-agent pomalidomide for MF, the authors reported their observations on 82 patients with MF (primary and post-PV/ET) enrolled in phase I and II clinical trials. Forty-five (55%), 24 (29%), 7 (9%), and 2 (2%) patients remained on pomalidomide therapy for at least 6, 12, 24 and 36 months, respectively. The overall anemia response rate per IWG-MRT criteria was 27% (22 of 82). There was no report of spleen or symptom response in their abstract. The authors concluded that anemia response to pomalidomide therapy in myelofibrosis often occurs in the first six months of treatment and is more likely to occur in the presence of *JAK2^{V617F}* and absence of marked splenomegaly. Sensory peripheral neuropathy was reported to be likely with long-term therapy with pomalidomide.[43]

Skepticism over Janus kinase inhibitors in myloproliferative neoplasms

Though a clear palliative benefit with ruxolitinib has been established in MF by means of two phase III randomized controlled clinical trials, there are reasons to suspect shortcomings associated with the use of ruxolitinib, in particular, and all the Janus kinase inhibitors in general and, therefore, a few notes of caution are in order.

Currently, there is evidence of benefit from ruxolitinib only in high- and intermediate-2 risk MF patients.[22,23] The low- and intermediate-1 risk categories may not derive any benefit from ruxolitinib. The role of ruxolitinib needs to be verified in phase III trials for these patients; data for the high- and intermediate-2 risk patients cannot be overzealously extrapolated to low- and intermediate-1 risk categories to treat them with ruxolitinib.

Ruxolitinib therapy has toxicity and transfusion requirement.[22,23] In intermediate-2 and high-risk cases, it is worth it's worth trading off the adverse effects with desease symptoms, but this is not the case in the relatively quiescent risk categories of the disease.

The salutary effect of ruxolitinib and other Janus kinase inhibitors comes from their ability to inhibit JAK1 thus reducing the effects of pro-inflammatory cytokines.[14] Therefore, the more JAK2-specific and JAK2^{V617F}-specific inhibitors may not reduce the constitutional symptoms with the same intensity as that of ruxolitinib.

None of the Janus kinase inhibitors in development, including ruxolitinib, have a disease-modifying property in MPN nor do they lead to the eradication of the clone of MPN.[44] None of these agents specifically targets the mutated JAK2^{V617F}. There has been no clearance of mutated *JAK2^{V617F}* allele from the MPN patients with ruxolitinib. SAR302503

did reduce the *JAK2*^{V617F} allele burden but there was no radical change.[45] Though *JAK2*^{V617F} as a sole mutation can cause the MPN phenotype in mouse, in humans, whether it is the initiating mutation or not still awaits clarification; several MPN-specific mutations such as *TET2* have often, but not always, been demonstrated to have appeared in MPN clone cells before the appearance of *JAK2*^{V617F}. Hence *JAK2*^{V617F} is not a *founding mutation* in MPN. Therefore, eradication of the disease-causing clones seems implausible with JAK2 inhibitors.[46,47]

There has been no reversal of fibrosis with any of the Janus kinase inhibitors, including ruxolitinib, in human disease within phase I/II or phase III trials, although ruxolitinib did yield histopathological responses in mouse models of MF.[16] For this reason, Janus kinase inhibitors cannot be looked upon as a panacea for MF.

The Mayo experience of extended follow up of phase II patients on ruxolitinib may not have been reproduced in phase III trials, but it is extremely interesting. There was a progressive escape from response to ruxolitinib over time.[24] Ruxolitinib withdrawal syndrome was frequent and occasionally required hospitalization,[48] although no such phenomenon has been ratified by the COMFORT studies. Loss of response and discontinuation of treatment was a common occurrence with ruxolitinib and other Janus kinase inhibitors in all the phase II trials of the other Janus kinase inhibitors across the board, which is quite alarming.

Spleen rate is only a *soft* end point for high-risk and intermediate-2 risk patients with MF. In the pre-statins era, a number of medications were introduced for hyperlipidemia, including nicotinic acid to reduce total and LDL cholesterol; only statins were seen to reduce mortality. Ruxolitinib performed well in terms of the *soft* end points and was shown to have a statistically significant survival benefit in a North American-Australian phase III trial (COMFORT I).[22] The European trialists (COMFORT II) have failed to replicate this observation at least at the time of last publication of their results.[23] Likewise, when the phase II data of ruxolitinib were presented by the two participating institutions, the MD Anderson experience suggested, by way of a proxy comparison, a survival advantage with ruxolitinib[25] that was not reflected in the Mayo experience.[24] It is, therefore, clear that we need robust data from a phase III study, with probably a higher power, to document definite survival benefit with ruxolitinib in the intermediate-2 and high-risk patients.

The ruxolitinib resistant mutations have already been documented in wild-type and V617F-mutated *JAK2*.[49] A suspicion that can be raised is that an otherwise low-grade MF with a median survival of 3-5 years, after treatment with Janus kinase inhibitors might be converted to a high-proliferation phenotype due to genotypic variation. This could make the disease even more unamenable to control even with conventional medications.

Finally, whereas the non-leukemogenicity of hydroxyurea is time tested and amply testified in literature,[50-51] the precise rate of leukemogenesis in MF has yet to be studied with ruxolitinib by way of a long-term follow up of non-responders.

Conclusions

Inhibitors of one or the other type of Janus kinase are likely to be of use in hematologic malignancies and in benign disorders such as rheumatoid arthritis and other autoimmune diseases. In MPN, the success of ruxolitinib in phase III trials and that of many other small-molecule JAK2 inhibitors in phase II trials in terms of spleen and constitutional response has given rise to the hope that an imatinib-like phenomenon could be repeated in MPN in the time to come with more JAK2^{V617F}-specific inhibitors. It remains to be seen what consistent impact these agents will have on survival of the high-risk and intermediate-2 risk patients with MF, whether resistant mutations will lead to the effect attrition with time, what natural history the non-responders will have, what effect these agents will have on evolution to leukemia, whether a rebound in resistant phenotype will offset the gains during sensitive stage, whether these agents will find a place in low-risk and intermediate-1 risk patients of MF, and whether any meaningful health outcomes and replacement of hydroxyurea and aspirin will be possible with these drugs in non-myelofibrosis PV and ET.

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