blood® Perspective

The role of JAK2 inhibitors in MPNs 7 years after approval

Francesco Passamonti[1,2] and Margherita Maffioli[2]

[1]Dipartimento di Medicina e Chirurgia, University of Insubria, Varese, Italy; and [2]Hematology, Dipartimento di Medicina Specialistica, ASST Settelaghi Varese, Ospedale di Circolo, Varese, Italy

Myeloproliferative neoplasms (MPNs) include essential thrombocythemia, polycythemia vera (PV), and primary myelofibrosis (MF). Phenotype-driver mutations of *JAK2*, *CALR*, and *MPL* genes are present in MPNs and can be variably combined with additional mutations. Driver mutations entail a constitutive activation of the JAK2/STAT pathway, the key signaling cascade in MPNs. Among JAK2 inhibitors (JAKis), ruxolitinib (RUX) has been approved for the treatment of intermediate and high-risk MF and for PV inadequately controlled by or intolerant of hydroxyurea. Other JAKis, such as fedratinib and pacritinib, proved to be useful in MF. The primary end points in MF trials were spleen volume response (SVR) and symptom response, whereas in PV trials they were hematocrit control with or without spleen response. In advanced MF, RUX achieved a long lasting SVR of >35% in ~60% of patients, establishing a new benchmark for MF treatment. RUX efficacy in early MF is also remark-able and toxicity is mild. In PV, RUX achieved hematocrit control in ~60% of cases and SVR in 40%. Symptom relief was evident in both conditions. In the long-term, however, many MF patients lose their SVR. Indeed, the definition of RUX failure and the design of new trials in this setting are unmet needs. Decrease of hemoglobin/platelet levels and increased infection rates are the most common side effects of RUX, and nonmelanoma skin tumors need to be monitored while on treatment. In conclusion, the introduction of JAKis raises the bar of treatment goals in MF and PV. (*Blood*. 2018; 131(22):2426-2435)

Introduction

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders including 3 main diseases: essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (MF).[1] Blast phase[2] and post-PV and post-ET MF[3] can occur. The same driver mutations of *JAK2*, *CALR*, and *MPL* genes are variably present and are mostly mutually exclusive in MPNs.[1] In PV, *JAK2* mutations cover almost the whole mutational profile, with *JAK2*V617F being present in 95% to 97% of patients[4] and exon 12 mutations in the remaining.[5] In ~5% of ET and 5% to 10% of primary MF, *MPL* mutations have been identified.[6] Almost 70% of *JAK2*/*MPL*-negative ET and primary MF patients carry *CALR* mutations.[7]

JAK2, *MPL*, and *CALR* mutations have been functionally validated and are sufficient to engender an MPN phenotype in mice.[8,9] All these mutations have a gain-of-function effect on JAK2/signal transducer and activator of transcription (STAT) signaling, and studies of gene expression profile unequivocally showed that activated JAK2 signaling is seen in all MPN patients, irrespective of the driver mutation.[10] The JAK/STAT pathway is the key signaling cascade in MPNs.

After the discovery of these mutations, pharmaceutical companies and investigators developed small-molecule inhibitors of JAK2 (JAKis) for the treatment of MPNs.[11] JAKis inhibit JAK2 and STAT phosphorylation, resulting in reduced cellular proliferation and induction of apoptosis in cell lines.[11] On 16 November, 2011, the US Food and Drug Administration (FDA) approved ruxolitinib (RUX) for the treatment of intermediate and high-risk MF, including primary MF and post-PV/post-ET MF. Subsequently, on 4 December, 2014, the FDA approved RUX to treat patients with PV having an inadequate response to or being intolerant of hydroxyurea (HU).

Perspective on MPN molecular complexity and JAK inhibition

In clinical trials, responses obtained with JAKis are independent of the underlying driver mutation, as expected on the basis of the mutations' effect on the JAK/STAT pathway and of JAKis' mechanism of action per se.[12,13] However, the efficacy of JAKis in MPNs is partial, partly because the targeted pathway is required for normal hematopoiesis and because of the absence of specificity of JAKis for the mutated counterpart. A correlation between different genotypes and clinical phenotype has been shown in MPNs,[3,14-17] with a potential effect on JAKi efficacy. Furthermore, the *JAK2*V617F mutation has been described in cases of clonal hematopoiesis of indeterminate potential,[18,19] bearing clinical relevance. Healthy individuals (ie, not MPN diagnosed) carrying *JAK2*V617F have an increased risk of thrombosis.[18] This potentially has implications on prognosis and therapy of MPNs and needs to be assessed in the future. Additional mutations acting outside the JAK-STAT pathway and, as a consequence, outside the direct JAKi effect, can affect gene expression, thus contributing to an aberrant transcriptional

output. These can occur at diagnosis or be acquired during the disease course as a sign of genetic instability.[20,21] Evidence indicates that having >1 high-molecular-risk mutation (ie, mutations in *ASXL1*, *EZH2*, *IDH1/2*, or *SRSF2*) confers a detrimental prognosis in MPNs.[22,23] RUX has proven effective in MF patients carrying a high-molecular-risk profile[23,24] but, despite this, does not seem to fully overcome its negative impact on survival. Finally, host effect[25] and multiple pathways, including phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR and RAF/MEK/extracellular signal-regulated kinase, cooperate and intersect with JAK-STAT, carrying weight in MPN pathogenesis and constituting potential therapeutic targets.

Early results of JAKis in MPNs

JAKis developed in MF include RUX,[26,27] fedratinib (FED),[28,29] pacritinib (PAC),[30,31] and momelotinib (MMB),[32,33] whose activity in early clinical trials is reported in Table 1 for comparison. Among these, only RUX entered clinical practice in MF.[34] All randomized MF studies with JAKis included patients with intermediate and high-risk primary MF and post-PV and post-ET MF. The primary end points were spleen volume response (SVR) (35% reduction by MRI) and symptom response (50% reduction using different symptom scores).

PV and ET studies with RUX (Tables 2 and 3) included patients with HU intolerance/resistance with the aim to define the activity as second-line treatment.[35-39] No other JAKis were studied on a large scale in the PV/ET setting.

Can we personalize the use of JAKis in MF patients?

While acknowledging the impossibility of performing direct comparisons of trial results, we believe that confronting patient characteristics across trials can be of interest. Median age is similar between studies (ranging from 63 to 69 years). The rate of post-PV and post-ET MF (vs primary MF) is variable, ranging from 35% in JAKARTA-1 (FED vs PBO)[28] to 55% in COMFORT-1 (RUX vs PBO).[26] This must be taken into account when comparing results, because survival of post-PV and post-ET MF is different from that of primary MF.[40] Splenomegaly is a key feature in MF (and its reduction is the primary end point of all studies); median baseline spleen size varied from 12 (PAC, PERSIST-1)[30] to 16 cm (RUX, COMFORT-1)[26] from the left costal margin. The median baseline value of hemoglobin is ~10 to 11 g/dL across studies. Some studies, such as the COMFORT trials, included only patients with platelet counts >100 × 10^9/L; thrombocytopenia below this threshold is a known negative prognostic factor in primary MF. Differently, PAC was studied also in patients with a platelet count <100 × 10^9/L (PERSIST-2).

Overall, patients entering these trials were in advanced phases of MF, and most received HU before enrollment. Looking at trial results (Table 1), RUX, FED, and MMB seem very active on splenomegaly, all JAKis tackle symptomatology, PAC appears to be particularly attractive for cytopenic patients, MMB alleviates anemia, and FED is extremely active as a second line after RUX failure.

Some considerations on the use of JAKis as second-line therapy in PV and ET

On the basis of a 60% hematocrit control rate and a 40% SVR rate, RUX is an effective second-line therapy in PV.[35,36,39,41] The RESPONSE trials included mainly HU-failing patients; however, RUX efficacy/safety data have also been captured in interferon-failing patients.[42] Despite these very positive results, some words of caution are needed regarding PV.

First, hematocrit is a surrogate end point of thrombosis.[43] Nonetheless, it is of interest that in the 80-week follow-up analysis of the RESPONSE study, the rate of all thrombotic events was 1.8 × 100 patient-years of exposure to RUX and 8.2 × 100 patient-years of exposure to standard care.[39] At the last 4-year follow-up update, the incidence of thrombosis was reduced to 1.2 × 100 patient-years, reverting the natural trend in PV.[44]

Second, transferring the definition of HU resistance/intolerance[45] into clinical practice is problematic. For instance, in a patient receiving few phlebotomies along with HU to maintain hematocrit levels <45%, is RUX necessary? A significantly higher rate of thrombosis was found in patients treated with HU plus ≥3 phlebotomies per year compared with HU plus 0 to 2 phlebotomies per year (20.5% vs 5.3% at 3 years) in a retrospective analysis.[46] Conversely, phlebotomy intensity did not affect thrombosis in a prospective clinical trial that was, however, designed with a different end point.[47] Hence, there is no definitive evidence that the number of phlebotomies is an issue in PV. On the other hand, patients with progressive spleen enlargement, symptomatology, or signs of myeloproliferation despite HU, or those who are intolerant of HU, are candidates for RUX, as these aspects impact events and quality of life.[48,49]

Third, the design of available trials cannot address the possible role of RUX in delaying or preventing post-PV MF, although progression to MF has been described under RUX without an exceedingly long follow-up.[44,50]

In HU-resistant/intolerant ET, RUX achieved clinically meaningful and durable reductions in platelet and leukocyte counts and improvements in ET-related symptoms, as reported in a phase 2 trial with a long follow-up.[37] However, when compared with current second-line treatments, RUX was equivalent to standard therapy (MAJIC-ET trial).[38] It has thus become questionable to use RUX in this setting, as ET (especially if diagnosed according to the WHO 2017 criteria)[1] is a benign disease, the risk of MF evolution being quite low, and other opportunities such as interferons or anagrelide being an option for these patients.[51,52]

Achievements of and perspectives for RUX in clinical practice

A 50% palpatory SVR rate with RUX was documented in ~60% of patients with advanced MF and was fairly long-lasting (median duration of response 3.2 years).[53] A debate on the role of spleen size in MF was consequently opened. The prospective COMFORT trials revealed a 1.14-fold higher risk of death for each additional 5 dL of spleen volume at baseline and a possible role for SVR under RUX as surrogate end point for survival.[54] In fact, any SVR upon RUX was associated with a better prognosis compared with a <10% reduction. Patients who achieved reductions of ≥25% had a prolonged survival compared with those who had no change or an increase in spleen volume.[54] To achieve this end point, patients should receive

Table 1. Selected JAKi trials in MF

Agent	Target(s)	Clinical trial	Numerosity and study-specific features	Key results	Toxicities	Comments
RUX	JAK1/2	COMFORT-1,[64] RUX vs PBO	RUX (n = 155), PBO (n = 154)	SVR ≥35% at 24 wk: 41.9% (RUX) vs 0.7% (PBO); reduction in TSS ≥50% at 24 wk: 45.9% (RUX) vs 5.3% (PBO); med. spleen response duration: 3.2 y (RUX); med. OS at 5 y: NR (RUX) vs 3.8 y (PBO)	G3/4 anemia, 45.2%; G3/4 thrombocytopenia, 12.9%; G3/4 neutropenia, 7.1% Rate of nonhematologic toxicities similar between RUX and PBO	67%-75% of enrolled patients had failed HU prior to enrollment in COMFORT-1/-2 Led to FDA/EMA approval for MF
		COMFORT-2,[53] RUX vs BAT	RUX (n = 146), BAT (n = 73)	SVR ≥ 35% at 48 wk: 28% (RUX) vs 0% (BAT) (at 24 wk: 32% [RUX]); med. spleen response duration: 3.2 y (RUX); med. OS at 5 y: NR (RUX) vs 4.1 y (BAT)	Similar to COMFORT-1 Any grade diarrhea, 23%	
FED	JAK2	JAKARTA-1,[28] FED vs PBO	FED 400 mg (n = 96), FED 500 mg (n = 97), PBO (n = 96), JAKi naive	SVR ≥ 35% at 24 wk: 36% (FED 400 mg) and 40% (FED 500 mg); reduction in TSS ≥ 50% at 24 wk: 36% (FED 400 mg) and 34% (FED 500 mg)	G3/4 anemia, 43% FED 400 mg, 60% FED 500 mg; G3/4 thrombocytopenia, 17% FED 400 mg, 27% FED 500 mg); G3/4 neutropenia, 8% FED 400 mg, 18% FED 500 mg; frequent GI toxicity (mostly G1/2) Frequent elevations of liver/ pancreatic enzymes and creatinine (mostly G1/2) Encephalopathy in 4/97 patients in the FED 500 mg group	Suspected cases of Wernicke's encephalopathy led to early study termination; recently, however, the FDA decided to lift the clinical hold based on updated clinical data[98]
		JAKARTA-2,[29] single arm, phase 2	FED 400 mg, RUX resistant/intolerant	SVR ≥35% at 24 wk: 55%; reduction in TSS ≥50% at 24 wk: 26%	G3/4 anemia, 38%; G3/4 thrombocytopenia, 22%	
PAC	JAK2/FLT3	PERSIST-1[30] PAC vs BAT (excl. JAKi)	PAC 400 mg QD (n = 220), BAT (n = 107), JAKi naïve No exclusions for cytopenias	SVR ≥35% at 24 wk: 19% (PAC) vs 5% (BAT); symptom response: no significant benefit of PAC (ITT); transfusion need: 25% of TD patients achieved transfusion-independence	G3/4 anemia, 17%; G3/4 thrombocytopenia, 12%; G3/4 diarrhea, 5%; heart failure, 2%	PAC was on full clinical hold Feb. 2016/Jan. 2017 for fatal toxicity concerns; further dose-finding studies are now ongoing. Overall, PAC seems potentially attractive for cytopenic MF patients.
		PERSIST-2,[31] PAC vs BAT (incl. RUX)	PAC 400 mg QD (n = 104), PAC 200 mg BID (n = 107), BAT (n = 100); previously treated or JAKi naive; PLT $<100 \times 10^9$/L; 48% had prior RUX and BAT included RUX in 45%	SVR ≥35% at 24 wk: 18% (PAC) vs 3% (BAT); reduction in TSS ≥50% at 24 wk: 25% (PAC) vs 14% (BAT)	Toxicities less frequent in PAC BID dosing than QD dosing; cardiac AEs in 7% (PAC BID), 13% (PAC QD), and 9% (BAT); intracranial hemorrhage, 1% (PAC QD)	
MMB	JAK1/2	SIMPLIFY-1,[32] MMB vs RUX	MMB (n = 215), RUX (n = 217), JAKi naive	SVR ≥35% at 24 wk: 26.9% (MMB) vs 29% (RUX); reduction in TSS ≥50% at 24 wk: MMB inferior to RUX; transfusion need: MMB associated with reduced transfusion requirement	G3/4 thrombocytopenia, 7%; G3/4 anemia, 6%; all grade PN 10% (MMB) vs 5% (RUX)	MMB seems attractive for anemic MF patients in need of spleen/ symptom control; however, due the results of SIMPLIFY-1 and 2, MMB development has been discontinued. MMB improves anemia likely because of reduced hepcidin production by the liver.

AE, adverse event; BAT, best-available therapy; BID, twice daily; EMA, European Medicines Agency; G, grade; ITT, intention-to-treat analysis; med., median; NR, not reached; OS, overall survival; PBO, placebo; PLT, platelets; PN, peripheral neuropathy; QD, once daily; TD, transfusion dependence; TSS, total symptom score.

Table 1. (continued)

Agent	Target(s)	Clinical trial	Numerosity and study-specific features	Key results	Toxicities	Comments
		SIMPLIFY-2,[99] MMB vs BAT (incl. RUX)	MMB (n = 104), BAT (n = 52); previously treated with RUX; BAT included RUX in 88%	SVR ≥ 35% at 24 wk: MMB not superior to BAT (including RUX) in improving spleen size in patients previously treated with RUX; reduction in TSS ≥ 50% at 24 wk: MMB 26.2% (MMB) vs 5.9% (BAT); transfusion need: MMB associated with reduced transfusion requirement	G3/4 anemia, 13%; G3/4 thrombocytopenia, 7%; all-grade PN, 11% (MMB) vs 0% (BAT)	

AE, adverse event; BAT, best-available therapy; BID, twice daily; EMA, European Medicines Agency; G, grade; ITT, intention-to-treat analysis; med., median; NR, not reached; OS, overall survival; PBO, placebo; PLT, platelets; PN, peripheral neuropathy; QD, once daily; TD, transfusion dependence; TSS, total symptom score.

the most appropriate dose of RUX, taking into account that dose modifications are routinely performed especially in the first 4 to 6 months of treatment. In advanced MF, the magnitude of SVRs achieved with RUX is what we currently expect, meaning that new drugs or combinations must overcome this result. An international consensus will help shape what will be required of upcoming therapies in terms of spleen response, such as the abrogation of splenomegaly in those with a huge spleen and longer duration of spleen response.

The second achievement of RUX is symptom control. Beyond the obvious benefit in terms of patients' quality of life (well documented with ad hoc questionnaires in clinical trials), reducing symptoms means eliminating variables impacting on outcomes.[55,56] In addition, symptom burden is guided by cytokine activation.[57] One of the most relevant JAKi-derived insights into MPN pathobiology is the understanding of the role of inflammation. First, a huge variety of cytokines are activated by driver mutations and induce MF complications, eventually affecting survival.[58] Second, RUX reverses proinflammatory cytokines.[59] Third, clinical improvement with RUX correlates with a reduction in plasma levels of several proinflammatory cytokines.[60] Although this anticytokine effect occurs mainly through JAK1 inhibition, itacitinib, a selective JAK1 inhibitor, achieved symptom reduction in 28% to 35% of advanced MF patients (60% intermediate-2 and high risk Dynamic International Prognostic Scoring System [DIPSS]),[60] similarly to RUX, thus not producing an additional advantage at that stage. The future challenge will be to switch off cytokines, using JAKis or itacitinib, or to minimize their pathogenetic relevance, using PRM-151 (a recombinant form of pentraxin-2 acting as anti-fibrotic agent),[61] earlier in disease development when the overt effect of cytokines is not yet dominant.

Beyond the effect on symptoms, cytokine activation induces progressive bone marrow fibrosis, which is relevant for outcomes in MF and PV.[62,63] RUX induces stabilization and reduction of bone marrow fibrosis in 32% and 16% of MF, respectively.[64] Abrogation of bone marrow fibrosis has been proposed as a potential end point for new agents or combinations. Although this is clearly intriguing, such an end point will require the evaluation of many biopsy samples, and we argue that the sole reduction of fibrosis, without the achievement of a normalization of blood counts and splenomegaly, is not a critical end point for advanced MF patients.

Concerning the effect of RUX on *JAK2*V617F mutational load, the majority of MF patients obtained a mild effect, with one-third having a 20% reduction.[65] In PV, the mean changes from baseline ranged from −12% to −40%, with very few obtaining a complete molecular response and 32% a partial one.[66] The net advantage of reducing the *JAK2*-mutated clone in MPNs is a challenging question for future research. To date, in MF, there is no clear evidence that reducing the clone implies a control of clinical parameters. In PV, on the contrary, 80% of patients with significant allele burden reductions had a spleen response without, however, a significant correlation with cell count improvements. This limits the potential usefulness of monitoring molecular dynamics and suggests a slow or absent correlation between genotype and clinical phenotype improvements. Another similar discrepancy has been described with imetelstat (a telomerase inhibitor) in ET.[67] This molecule obtained 100% hematological responses and 88% partial molecular responses without affecting vascular events (4 severe episodes in 18 ET patients treated), which still remain the most relevant problem in PV and ET.

The effect of RUX on survival generated some debate. These are the available data on this topic: the 5-year update of the COMFORT trials showed a median survival of 5.3 years in RUX-treated patients, which was significantly improved compared with control data.[53] Furthermore, standard therapies in historical matched cohorts allowed lower median survival rates compared with RUX in advanced primary MF.[68,69]

Managing RUX therapy: old and new issues deserving consideration

The widespread use of RUX in MF and, to a lesser extent, HU-resistant/intolerant PV has highlighted some clinically noteworthy aspects complementing the data emerging from clinical trials.

Worsening of anemia and transfusion burden during RUX treatment

Anemia and thrombocytopenia are among the expected dose-dependent on-target effects of RUX. Both COMFORT studies

Table 2. Selected JAKi trials in PV

Agent	Target(s)	Clinical trial	Numerosity and study-specific features	Key results	Toxicities	Comments
RUX	JAK1/2	RESPONSE,[35] RUX vs BAT	RUX (n = 110), BAT (n = 112); HU resistant/ intolerant, in need of phlebotomies, with splenomegaly	Hematocrit control without phlebotomy at wk 32: 60% (RUX) vs 18.8% (BAT); SVR ≥35% at wk 32: 40% (RUX) vs 0.9% (BAT); composite end-point: 22.7% (RUX) vs 0.9% (BAT)	All grade anemia, 27.2 per 100 p-y; G3/4, 0.9 per 100 p-y; all-grade thrombocytopenia, 14.9 per 100 p-y; G3/4, 2.6 per 100 p-y	Led to FDA/EMA approval for HU resistant/ intolerant PV
		RESPONSE-2,[36] RUX vs BAT	RUX (n = 74), BAT (n = 75); HU resistant/ intolerant, in need of phlebotomies, without splenomegaly	Hematocrit control at wk 28: 62% (RUX) vs 19% (BAT)	G1/2 anemia, 14%; G1/2 thrombocytopenia, 3%; no G3/4 anemia/ thrombocytopenia	

p-y, patient-years of exposure.

in advanced MF have shown a drop in mean hemoglobin levels in virtually all patients during the first weeks/months of treatment, with 51% receiving at least 1 transfusion of packed red blood cells and ~5% requiring RUX discontinuation for this reason.[53,64] Among 163 IPSS intermediate-1 MF patients treated with RUX, anemia (any grade) was documented in 54% of patients (grade 3/4 in 24.5%), suggesting a somewhat milder hematologic toxicity profile in patients with early disease.[70] Although disease-related anemia negatively impacts survival and is a risk factor included in most prognostic scoring systems, a post hoc analysis showed that new or worsening postbaseline RUX-induced anemia did not affect survival.[71] Other JAKis such as PAC, MMB, or NS-018 (a JAK2/Src inhibitor currently under investigation showing 56% SVR)[34] seem to be less toxic for hematopoiesis, with a lower rate of anemia and thrombocytopenia.

How to improve anemia in MF (disease or RUX induced) is still not clear. Transfusions and appropriate iron chelation (envisaged for allogeneic stem cell transplant [SCT] candidates) might be a good option, and erythropoiesis-stimulating agents have produced some results.[72] Ongoing phase 2 trials with sotatercept (NCT01712308)[73] and luspatercept (NCT03194542), acting on the late-stage maturation of erythroblasts, are designed to evaluate anemia responses in MF patients with and without concomitant RUX and could prove instrumental in the cure of anemia in MF patients, regardless of JAKi treatment. An alternative approach under investigation combines RUX and itacitinib in MF patients in whom cytopenias preclude dose optimization (NCT03144687). Preliminary information on these different approaches will become available in 2018/2019 and will help in the design of appropriate randomized phase 3 trials.

Infections in MF and RUX treatment

Evidence before JAKi development indicated that infections accounted for ~10% of deaths in MF patients,[55] with higher IPSS risk category and splenomegaly being the most impactful variables for infections.[74] In COMFORT-2, infections often involved the urinary tract (25%) or lung (13%). Frequent cases of herpes zoster infection (11%) and septic shock (8%) and few cases of tuberculosis (1%) were reported. A list of case reports or series concerning infections under RUX has recently been published.[75] In PV, the RESPONSE trials reported similar and relatively low rates of bacterial infections in the RUX and in the control arm.[35,36] Recently, SIE-ELN guidelines on RUX therapy have been published,[76] without any restriction to the use of RUX but recommending caution, specific monitoring, or prophylactic measures in patients with at least 1 risk factor.

Neoplasms in MPNs with or without concomitant RUX treatment

Prospective trials in PV and MF have disclosed a significant rate of nonmelanoma skin cancers (NMSCs) in RUX-treated patients.[35,36,65,77] Importantly, both COMFORT and RESPONSE trials excluded enrollment of patients with active malignancy within the preceding

Table 3. Selected JAKi trials in ET

Agent	Target(s)	Clinical trial	Numerosity and study-specific features	Key results	Toxicities	Comments
RUX	JAK1/2	MAJIC-ET,[100] RUX vs BAT	RUX (n = 58), BAT (n = 52); HU resistant/ intolerant	Complete response rate within 1 y: RUX (46.6%) vs BAT (44.2%); at 2 y, rates of thrombosis, hemorrhage, and transformation were not significantly different	G3/4 anemia, 21% RUX vs 0% BAT (2 patients discontinued RUX for anemia); G3/4 thrombocytopenia, 3.4% RUX vs 0% BAT; infections, 15.5% G3 RUX vs 3.5% G3/4 BAT	RUX is not superior to current second-line treatments for ET

Table 4. RUX-based combination trials currently under investigation in MF

Class	Agent (combined with RUX)	Target(s)	Phase	Status	ClinicalTrials.gov identifier
Epigenetic agents	Azacitidine	DNA methylation	2	Recruiting	NCT01787487
	Decitabine	DNA methylation	1/2	Ongoing, not recruiting	NCT02076191
	Pracinostat	HDAC	2	Ongoing, not recruiting	NCT02267278
	Panobinostat	HDAC	1	Ongoing, not recruiting	NCT01433445
	Panobinostat	HDAC	1/2	Ongoing, not recruiting	NCT01693601
Hedgehog pathway inhibitors	Sonidegib	SMO	1/2	Ongoing, not recruiting	NCT01787552
JAKis	Itacitinib	JAK1	2	Recruiting	NCT03144687
Immunomodulators	Thalidomide	Immunomodulation	2	Recruiting	NCT03069326
	Lenalidomide	Immunomodulation	2	Ongoing, not recruiting	NCT01375140
	Pomalidomide	Immunomodulation	1/2	Recruiting	NCT01644110
PI3K/AKT/mTOR pathway inhibitors	INCB050465	PI3K-δ	2	Recruiting	NCT02718300
	TGR-1202	PI3K-δ	1	Recruiting	NCT02493530
Other agents	Ribociclib/PIM447	CDK4/6 inhibitor Pan-PIM kinases	1	Ongoing, not recruiting	NCT02370706
	PU-H71	HSP90	1	Recruiting	NCT03373877
	Pevonedistat	NAE	1	Not yet recruiting	NCT03386214
	Sotatercept	ActRIIA ligands	2	Recruiting	NCT01712308
	Luspatercept	ActRIIB ligands	2	Recruiting	NCT03194542
	Peg-IFN α-2a		1/2	Recruiting	NCT02742324

ActR, activin receptor; HDAC, histone deacetylase; HSP90, heat-shock protein 90; IFN, interferon; NAE, NEDD8-activating enzyme; Peg-IFN α-2a, Peg-interferon alpha-2a.

5 years, with the exception of specific skin cancers. The incidence rates for skin basal and squamous cell carcinoma were 2.7 vs 3.9 (COMFORT-1) and 6.1 vs 3.0 (COMFORT-2) per 100 patient-years of exposure in the RUX-randomized group and control arm, respectively. In PV, the exposure-adjusted rate of NMSCs in RUX-randomized patients was 5.1 (week 208) vs 4.4 (week 80) per 100 patient-years and 2.6 (week 208) vs 2.0 (week 80) per 100 patient-years in the crossover group. Rates were higher among patients with a history of NMSCs.[39,44] In a small series of RUX-treated patients, B-cell lymphomas developed in 4 out of 69 patients (5.8%) with RUX compared with only 2 of 557 (0.4%) in the control group.[78]

Several studies performed before the introduction of JAKis found that MPN patients are at increased risk of developing second malignancies. A study on 1915 consecutive patients with MPNs found that the risk of developing a lymphoid neoplasm was 2.79-fold higher than that of the control population.[79] Similar evidence derives from a Danish population-based study (n = 7,229),[80] and from the Surveillance, Epidemiology, and End Results Program registry (n = 20 250), the latter reporting an incidence of second cancers (all sites) of 15.2% at 10 years.[81] In the Surveillance, Epidemiology, and End Results Program series, second malignancies overrepresented with respect to the general population were both hematological (myeloid and lymphoid) and nonhematological, the latter including the following sites: oral cavity, brain, esophagus, lung and bronchus, skin (melanoma), uterus and ovary, prostate, kidney, and thyroid. Neoplasms found in patients treated with RUX seem to be mostly related to the skin. However, careful monitoring and data collection within international registries would be useful. In the RUX-treated patients we follow, a dermatological visit is prescribed at baseline and regularly during follow-up to detect early lesions. Furthermore, we educate our patients with respect to sun-protective behavior. In case of multiple skin cancer recurrence, we evaluate treatment discontinuation (especially in case of suboptimal response).

Resistance to JAKis: something beyond the consequence of dose reduction?

Primary resistance to JAKis is a clinical issue for few patients (<2% to 5%),[26,27] whereas losing a spleen response is frequent during follow-up.[53] The explanation for this is unclear. We offer some considerations: (1) The dose is of importance, and we

recommend using the highest safe dose to control splenomegaly, since reducing RUX dose results in a loss of benefit in terms of SVR. (2) Some patients have progressive disease in terms of evolution into accelerated or blast phase, and in many of these cases, splenomegaly increases. (3) The biology of JAKi resistance is of great interest but remains without a clear translation into clinical decision-making to date. Some recently reviewed mechanisms of resistance have been identified in MPN cell lines and murine models.[82] These can be recapitulated as acquired *JAK2* kinase mutations, persistence of JAK-STAT signaling due to JAK family heterodimer formation, or protective cytokine effects.

Treatment sustainability for the health care system

A big challenge for the health care system is treatment sustainability, especially considering the cost of new therapies. In the pre-JAKi era, HU, steroids, and red blood cell transfusions were relatively cheap, whereas splenectomy was a risky surgical intervention with a high economic impact. SCT has huge health care costs, and its indication is currently unchanged with respect to the pre-JAKi era. Some pharmacoeconomic analyses on RUX have been recently reviewed.[83] Overall, studies reported similar incremental efficacy of RUX with a plus of 1.04-2.51 quality-adjusted life years (QALYs). The incremental cost per QALY ranged from $40 000 to $54 000 per QALY saved.

Rising MPN patient awareness and its consequences

The great interest within the scientific community, the widespread dissemination of information, and the availability of a drug rapidly and tangibly affecting quality of life has increased patient awareness with regards to MPNs, resulting in the creation of patient associations and awareness groups that may possibly affect health care policies with regards to MPNs in individual countries.

MPNs in 2018: when to use RUX and when to transplant

Today, there is evidence for the use of RUX in advanced phases of MF disease, ie, higher risk primary MF and post-PV and post ET MF according to the (D)IPSS[55,84] and the MF secondary to PV and ET prognostic model (MYSEC-PM),[56] respectively. Responses are mostly obtained within the first 6 months of therapy; afterwards, any patient permanently failing RUX should be considered for investigative clinical trials. With regards to this, prediction of RUX response would be of relevance, especially in a precision medicine era. Subgroup analysis of the COMFORT studies suggested that any MF patient can benefit from RUX.[85] However, harboring ≥3 mutations was inversely correlated with spleen response and time to treatment discontinuation.[24] A large retrospective study of 408 patients found that higher IPSS risk categories and delay in RUX initiation are predictors of lower RUX response rates.[86] This could suggest starting RUX earlier in the disease course, as conveyed by the National Comprehensive Cancer Network guidelines that advocate for the use of RUX in low and intermediate-1 risk MF patients with troublesome symptoms.[87] Prospective, single-arm trials evaluating the earlier use of RUX are available.[70,88] The JUMP study disclosed a high level of RUX efficacy (64% SVR at week 24) and a good safety profile in intermediate-1 risk MF.[70] In the management of intermediate-1 risk MF, we follow the recent European LeukemiaNet guidelines,[51] using RUX as first line in the case of highly symptomatic splenomegaly.

SCT indications in MPNs remain unchanged in the JAKi era; ie, patients with intermediate-2 and high risk MF and with intermediate-1 risk disease and high-risk features are to be considered for a SCT.[89,90] Because SCT and RUX indications tend to coincide and RUX can improve transplant-specific risk factors, such as splenomegaly and symptomatology, most patients are treated with RUX before undergoing SCT. Several studies on small numbers of patients have confirmed the feasibility of this approach.[91,92] Results of an ongoing prospective transplantation-based trial in MF are awaited. This recently initiated study (NCT03333187) sets out to assess the relative benefit of RUX without subsequent SCT vs RUX as a "bridge" to SCT, according to donor availability. The impact of RUX-induced responses at the time of SCT has been recently studied retrospectively.[93] A favorable outcome of MF patients experiencing clinical improvement with JAKi prior to SCT has been observed. This suggests a prognostic relevance of achieving a good "JAKi-inducible" response before SCT with RUX or alternative JAKis. Finally, in the case of RUX failure, outcome is dismal but can be improved by including SCT in the therapeutic program.[94]

Perspective on new clinical needs in MF

The "from now on" issue in MF is the treatment of patients after RUX failure. We have no definition of this condition, and we need a consensus for the future development of clinical trials. If we consider 35% reduction of spleen size by magnetic resonance imaging a satisfactory end point in clinical practice, then every patient who does not reach such a SVR or loses it during follow-up should be defined as RUX failure. On the basis of the already provided numbers, 60% to 70% of RUX-treated patients end up failing treatment at one point during follow-up. All patients in this condition are potentially candidates for other JAKis such as FED or PAC (if approved) or clinical trials (other JAKis, combinations, or other targeted therapies) or SCT. Among these patients, a subset discontinuing RUX undergoes clonal evolution (mostly acquiring *ASXL1* mutations) with a very dismal outcome of 6 months.[95] Another condition to be considered as RUX failure is the progressive increase of blast cells in the peripheral blood or bone marrow. Having 5% to 9% blast cells confers a similar outcome to having accelerated phase disease (10% to 19% circulating blast cells).[96] However, RUX seems to improve survival in patients with 5% to 9% blast cells and not in those with 10% to 19% blast cells, a difference that needs to be substantiated in prospective trials.

How to cure, delay, or prevent RUX failure should ideally be answered by upcoming trials. The progressively improving understanding of the pathobiology of MF has prompted further exploration of alternative therapeutic targets (eg, those addressing epigenetics, bone marrow fibrosis, checkpoint inhibition, and cell cycle regulation/apoptosis) recently reviewed.[97] A number of RUX-based combination therapies have been successfully evaluated in preclinical models and are now being actively pursued in the clinic (ongoing JAKi-based combination trials listed in Table 4).

Conclusions

The introduction of JAKis in clinical practice has raised the bar of treatment goals in MF and PV. Spleen/symptom responses and impact on survival are the most relevant achievements in MF, similarly to hematocrit control in PV. Careful safety monitoring is useful in clinical practice with skin neoplasms and infectious disease screening protocols. Patients, physician-investigators, basic scientists, and pharmaceutical companies must collaborate to improve currently obtained results.

Acknowledgements

This work was supported by the Associazione Italiana per la Ricerca sul Cancro (Milan, Italy), Special Program Molecular Clinical Oncology 5x1000 (grant to Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative, project 1005). The study was also funded by Fondazione Regionale Ricerca Biomedica (Milan, Italy; project 2015-0042; Genomic profiling of rare hematologic malignancies, development of personalized medicine strategies, and their implementation into the Rete Ematologica Lombarda clinical network), Fondazione Matarelli (Milan, Italy), Fondazione Rusconi (Varese, Italy), and AIL (Associazione Italiana contro le Leucemie-linfomi e mieloma) Varese ONLUS (Organizzazione Non Lucrativa di Utilità Sociale).

Authorship

Contribution: F.P. and M.M. contributed to the conception, writing, and revision of the work.

Conflict-of-interest disclosure: F.P. serves on advisory boards for Sanofi, Gilead, Janssen Pharmaceuticals, Celgene, Novartis, Bristol-Myers Squibb, and Roche. M.M. declares no competing financial interests.

Correspondence: Francesco Passamonti, Dipartimento di Medicina e Chirurgia, University of Insubria, 21100 Varese, Italy; e-mail: francesco.passamonti@uninsubria.it.

Footnote

Submitted 10 January 2018; accepted 7 April 2018. Prepublished online as *Blood* First Edition paper, 12 April 2018; DOI 10.1182/blood-2018-01-791491.

REFERENCES

1. Passamonti F, Maffioli M. Update from the latest WHO classification of MPNs: a user's manual. *Hematology (Am Soc Hematol Educ Program)*. 2016;2016(1):534-542.
2. Passamonti F, Rumi E, Arcaini L, et al. Leukemic transformation of polycythemia vera: a single center study of 23 patients. *Cancer*. 2005;104(5):1032-1036.
3. Passamonti F, Mora B, Giorgino T, et al. Driver mutations' effect in secondary myelofibrosis: an international multicenter study based on 781 patients. *Leukemia*. 2017;31(4):970-973.
4. Passamonti F, Rumi E, Pietra D, et al. A prospective study of 338 patients with polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. *Leukemia*. 2010;24(9):1574-1579.
5. Passamonti F, Elena C, Schnittger S, et al. Molecular and clinical features of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations. *Blood*. 2011;117(10):2813-2816.
6. Rumi E, Pietra D, Guglielmelli P, et al; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative. Acquired copy-neutral loss of heterozygosity of chromosome 1p as a molecular event associated with marrow fibrosis in MPL-mutated myeloproliferative neoplasms. *Blood*. 2013;121(21):4388-4395.
7. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med*. 2013;369(25):2391-2405.
8. Mullally A, Lane SW, Ball B, et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. *Cancer Cell*. 2010;17(6):584-596.
9. Elf S, Abdelfattah NS, Chen E, et al. Mutant calreticulin requires both its mutant C-terminus and the thrombopoietin receptor for oncogenic transformation. *Cancer Discov*. 2016;6(4):368-381.
10. Rampal R, Al-Shahrour F, Abdel-Wahab O, et al. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood*. 2014;123(22):e123-e133.
11. Quintás-Cardama A, Vaddi K, Liu P, et al. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. *Blood*. 2010;115(15):3109-3117.
12. Passamonti F, Caramazza D, Maffioli M. JAK inhibitor in CALR-mutant myelofibrosis. *N Engl J Med*. 2014;370(12):1168-1169.
13. Guglielmelli P, Biamonte F, Rotunno G, et al; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative (AGIMM) Investigators. Impact of mutational status on outcomes in myelofibrosis patients treated with ruxolitinib in the COMFORT-II study. *Blood*. 2014;123(14):2157-2160.
14. Passamonti F, Rumi E. Clinical relevance of JAK2 (V617F) mutant allele burden. *Haematologica*. 2009;94(1):7-10.
15. Passamonti F, Rumi E, Caramella M, et al. A dynamic prognostic model to predict survival in post-polycythemia vera myelofibrosis. *Blood*. 2008;111(7):3383-3387.
16. Rumi E, Pietra D, Ferretti V, et al; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. *Blood*. 2014;123(10):1544-1551.
17. Pietra D, Rumi E, Ferretti VV, et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. *Leukemia*. 2016;30(2):431-438.
18. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-2498.
19. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-2487.
20. Lundberg P, Karow A, Nienhold R, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood*. 2014;123(14):2220-2228.
21. Grinfeld J, Nangalia J, Green AR. Molecular determinants of pathogenesis and clinical phenotype in myeloproliferative neoplasms. *Haematologica*. 2017;102(1):7-17.
22. Vannucchi AM, Lasho TL, Guglielmelli P, et al. Mutations and prognosis in primary myelofibrosis. *Leukemia*. 2013;27(9):1861-1869.
23. Guglielmelli P, Lasho TL, Rotunno G, et al. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients. *Leukemia*. 2014;28(9):1804-1810.
24. Patel KP, Newberry KJ, Luthra R, et al. Correlation of mutation profile and response in patients with myelofibrosis treated with ruxolitinib. *Blood*. 2015;126(6):790-797.
25. Olcaydu D, Rumi E, Harutyunyan A, et al. The role of the JAK2 GGCC haplotype and the TET2 gene in familial myeloproliferative neoplasms. *Haematologica*. 2011;96(3):367-374.
26. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med*. 2012;366(9):799-807.
27. Harrison C, Kiladjian JJ, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med*. 2012;366(9):787-798.
28. Pardanani A, Harrison C, Cortes JE, et al. Safety and efficacy of fedratinib in patients

with primary or secondary myelofibrosis: a randomized clinical trial. *JAMA Oncol*. 2015; 1(5):643-651.

29. Harrison CN, Schaap N, Vannucchi AM, et al. Janus kinase-2 inhibitor fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study. *Lancet Haematol*. 2017;4(7):e317-e324.

30. Mesa RA, Vannucchi AM, Mead A, et al. Pacritinib versus best available therapy for the treatment of myelofibrosis irrespective of baseline cytopenias (PERSIST-1): an international, randomised, phase 3 trial. *Lancet Haematol*. 2017;4(5):e225-e236.

31. Mascarenhas J, Hoffman R, Talpaz M, et al. Pacritinib vs best available therapy, including ruxolitinib, in patients with myelofibrosis: a randomized clinical trial [published online ahead of print 8 March 2018]. *JAMA Oncol*. doi:10.1001/jamaoncol.2017.5818.

32. Mesa RA, Kiladjian JJ, Catalano JV, et al. SIMPLIFY-1: a phase III randomized trial of momelotinib versus ruxolitinib in janus kinase inhibitor-naïve patients with myelofibrosis. *J Clin Oncol*. 2017;35(34):3844-3850.

33. Harrison CN, Vannucchi AM, Platzbecker U, et al. Phase 3 randomized trial of momelotinib (MMB) versus best available therapy (BAT) in patients with myelofibrosis (MF) previously treated with ruxolitinib (RUX). *J Clin Oncol*. 2017;35(15 suppl):7001.

34. Verstovsek S, Talpaz M, Ritchie E, et al. A phase I, open-label, dose-escalation, multicenter study of the JAK2 inhibitor NS-018 in patients with myelofibrosis. *Leukemia*. 2017; 31(2):393-402.

35. Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. *N Engl J Med*. 2015;372(5):426-435.

36. Passamonti F, Griesshammer M, Palandri F, et al. Ruxolitinib for the treatment of inadequately controlled polycythaemia vera without splenomegaly (RESPONSE-2): a randomised, open-label, phase 3b study. *Lancet Oncol*. 2017;18(1):88-99.

37. Verstovsek S, Passamonti F, Rambaldi A, et al. Ruxolitinib for essential thrombocythemia refractory to or intolerant of hydroxyurea: long-term phase 2 study results. *Blood*. 2017;130(15):1768-1771.

38. Harrison CN, Mead AJ, Panchal A, et al. Ruxolitinib vs best available therapy for ET intolerant or resistant to hydroxycarbamide. *Blood*. 2017;130(17):1889-1897.

39. Verstovsek S, Vannucchi AM, Griesshammer M, et al. Ruxolitinib versus best available therapy in patients with polycythemia vera: 80-week follow-up from the RESPONSE trial. *Haematologica*. 2016;101(7):821-829.

40. Masarova L, Bose P, Daver N, et al. Patients with post-essential thrombocythemia and post-polycythemia vera differ from patients with primary myelofibrosis. *Leuk Res*. 2017;59:110-116.

41. Verstovsek S, Passamonti F, Rambaldi A, et al. A phase 2 study of ruxolitinib, an oral JAK1 and JAK2 inhibitor, in patients with advanced polycythemia vera who are refractory or intolerant to hydroxyurea. *Cancer*. 2013.

42. Kiladjian JJ, Guglielmelli P, Griesshammer M, et al. Efficacy and safety of ruxolitinib after and versus interferon use in the RESPONSE studies. *Ann Hematol*. 2018;97(4):617-627.

43. Marchioli R, Finazzi G, Specchia G, et al; CYTO-PV Collaborative Group. Cardiovascular events and intensity of treatment in polycythemia vera. *N Engl J Med*. 2013;368(1):22-33.

44. Kiladjian J-J, Verstovsek S, Griesshammer M, et al. Results from the 208-week (4-Year) follow-up of RESPONSE trial, a phase 3 study comparing ruxolitinib (Rux) with best available therapy (BAT) for the treatment of polycythemia vera (PV) [abstract]. *Blood*. 2017;130(suppl 1). Abstract 322.

45. Barosi G, Birgegard G, Finazzi G, et al. Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet consensus conference. *Blood*. 2009;113(20):4829-4833.

46. Alvarez-Larrán A, Pérez-Encinas M, Ferrer-Marín F, et al; Grupo Español de Neoplasias Mieloproliferativas Filadelfia Negativas. Risk of thrombosis according to need of phlebotomies in patients with polycythemia vera treated with hydroxyurea. *Haematologica*. 2017;102(1):103-109.

47. Barbui T, Carobbio A, Ghirardi A, Masciulli A, Rambaldi A, Vannucchi AM. No correlation of intensity of phlebotomy regimen with risk of thrombosis in polycythemia vera: evidence from European Collaboration on Low-Dose Aspirin in Polycythemia Vera and Cytoreductive Therapy in Polycythemia Vera clinical trials. *Haematologica*. 2017;102(6):e219-e221.

48. Alvarez-Larrán A, Kerguelen A, Hernández-Boluda JC, et al; Grupo Español de Enfermedades Mieloproliferativas Filadelfia Negativas (GEMFIN). Frequency and prognostic value of resistance/intolerance to hydroxycarbamide in 890 patients with polycythaemia vera. *Br J Haematol*. 2016; 172(5):786-793.

49. Barbui T, Masciulli A, Marfisi MR, et al. White blood cell counts and thrombosis in polycythemia vera: a subanalysis of the CYTO-PV study. *Blood*. 2015;126(4):560-561.

50. Verstovsek S, Passamonti F, Rambaldi A, et al. A phase 2 study of ruxolitinib, an oral JAK1 and JAK2 Inhibitor, in patients with advanced polycythemia vera who are refractory or intolerant to hydroxyurea. *Cancer*. 2014;120(4):513-520.

51. Barbui T, Tefferi A, Vannucchi AM, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet [published online ahead of print 27 February 2018]. *Leukemia*. doi:10.1038/s41375-018-0077-1.

52. Passamonti F, Brusamolino E, Lazzarino M, et al. Efficacy of pipobroman in the treatment of polycythemia vera: long-term results in 163 patients. *Haematologica*. 2000;85(10): 1011-1018.

53. Verstovsek S, Gotlib J, Mesa RA, et al. Long-term survival in patients treated with ruxolitinib for myelofibrosis: COMFORT-I and -II pooled analyses. *J Hematol Oncol*. 2017;10(1):156.

54. Vannucchi AM, Kantarjian HM, Kiladjian JJ, et al; COMFORT Investigators. A pooled analysis of overall survival in COMFORT-I and COMFORT-II, 2 randomized phase III trials of ruxolitinib for the treatment of myelofibrosis. *Haematologica*. 2015;100(9):1139-1145.

55. Passamonti F, Cervantes F, Vannucchi AM, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood*. 2010;115(9):1703-1708.

56. Passamonti F, Giorgino T, Mora B, et al. A clinical-molecular prognostic model to predict survival in patients with post polycythemia vera and post essential thrombocythemia myelofibrosis. *Leukemia*. 2017;31(12):2726-2731.

57. Tefferi A, Vaidya R, Caramazza D, Finke C, Lasho T, Pardanani A. Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study. *J Clin Oncol*. 2011;29(10):1356-1363.

58. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood*. 2017; 129(6):667-679.

59. Verstovsek S, Kantarjian H, Mesa RA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *N Engl J Med*. 2010;363(12):1117-1127.

60. Mascarenhas JO, Talpaz M, Gupta V, et al. Primary analysis of a phase II open-label trial of INCB039110, a selective JAK1 inhibitor, in patients with myelofibrosis. *Haematologica*. 2017;102(2):327-335.

61. Verstovsek S, Mesa RA, Foltz LM, et al. PRM-151 in myelofibrosis: durable efficacy and safety at 72 weeks. *Blood*. 2015;126(23):56.

62. Guglielmelli P, Lasho TL, Rotunno G, et al. MIPSS70: mutation-enhanced international prognostic score system for transplantation-age patients with primary myelofibrosis. *J Clin Oncol*. 2018;36(4):310-318.

63. Barbui T, Thiele J, Passamonti F, et al. Initial bone marrow reticulin fibrosis in polycythemia vera exerts an impact on clinical outcome. *Blood*. 2012;119(10):2239-2241.

64. Harrison CN, Vannucchi AM, Kiladjian JJ, et al. Long-term findings from COMFORT-II, a phase 3 study of ruxolitinib vs best available therapy for myelofibrosis. *Leukemia*. 2017;31(3):775.

65. Harrison CN, Vannucchi AM, Kiladjian J-J, et al. Long-term efficacy and safety in COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for the treatment of myelofibrosis: 5-year final study results. *Blood*. 2015;126(23):59.

66. Vannucchi AM, Verstovsek S, Guglielmelli P, et al. Ruxolitinib reduces JAK2 p.V617F allele burden in patients with polycythemia vera enrolled in the RESPONSE study. *Ann Hematol*. 2017;96(7):1113-1120.

67. Baerlocher GM, Oppliger Leibundgut E, Ottmann OG, et al. Telomerase inhibitor imetelstat in patients with essential thrombocythemia. *N Engl J Med*. 2015;373(10): 920-928.

68. Verstovsek S, Kantarjian HM, Estrov Z, et al. Long-term outcomes of 107 patients with myelofibrosis receiving JAK1/JAK2 inhibitor ruxolitinib: survival advantage in comparison to matched historical controls. *Blood*. 2012; 120(6):1202-1209.

69. Passamonti F, Maffioli M, Cervantes F, et al. Impact of ruxolitinib on the natural history of primary myelofibrosis: a comparison of the DIPSS and the COMFORT-2 cohorts. *Blood*. 2014;123(12):1833-1835.

70. Al-Ali HK, Griesshammer M, le Coutre P, et al. Safety and efficacy of ruxolitinib in an open-label, multicenter, single-arm phase 3b expanded-access study in patients with myelofibrosis: a snapshot of 1144 patients in the JUMP trial. *Haematologica*. 2016;101(9): 1065-1073.

71. Gupta V, Harrison C, Hexner EO, et al. The impact of anemia on overall survival in patients with myelofibrosis treated with ruxolitinib in the COMFORT studies. *Haematologica*. 2016;101(12):e482-e484.

72. McMullin MF, Harrison CN, Niederwieser D, et al. The use of erythropoiesis-stimulating agents with ruxolitinib in patients with myelofibrosis in COMFORT-II: an open-label, phase 3 study assessing efficacy and safety of ruxolitinib versus best available therapy in the treatment of myelofibrosis. *Exp Hematol Oncol*. 2015;4(1):26.

73. Bose P, Daver N, Pemmaraju N, et al. Sotatercept (ACE-011) alone and in combination with ruxolitinib in patients (pts) with myeloproliferative neoplasm (MPN)-associated myelofibrosis (MF) and anemia [abstract]. *Blood*. 2017;130(suppl 1). Abstract 255.

74. Polverelli N, Breccia M, Benevolo G, et al. Risk factors for infections in myelofibrosis: role of disease status and treatment. A multicenter study of 507 patients. *Am J Hematol*. 2017;92(1):37-41.

75. Dioverti MV, Abu Saleh OM, Tande AJ. Infectious complications in patients on treatment with ruxolitinib: case report and review of the literature. *Infect Dis (Lond)*. 2017;50(5):381-387.

76. Marchetti M, Barosi G, Cervantes F, et al. Which patients with myelofibrosis should receive ruxolitinib therapy? ELN-SIE evidence-based recommendations. *Leukemia*. 2017;31(4):882-888.

77. Verstovsek S, Mesa RA, Gotlib J, et al; COMFORT-I investigators. Efficacy, safety, and survival with ruxolitinib in patients with myelofibrosis: results of a median 3-year follow-up of COMFORT-I. *Haematologica*. 2015;100(4):479-488.

78. Tripolt S, Porpaczy EA, Hoelbl-Kovacic A, et al. JAK2-inhibitor therapy associated lymphomas in myelofibrosis: distinct clinico-pathological features recapitulated in a mouse model [abstract]. *Blood*. 2017;130(suppl 1). Abstract 1643.

79. Rumi E, Passamonti F, Elena C, et al. Increased risk of lymphoid neoplasm in patients with myeloproliferative neoplasm: a study of 1,915 patients. *Haematologica*. 2011;96(3):454-458.

80. Frederiksen H, Farkas DK, Christiansen CF, Hasselbalch HC, Sørensen HT. Chronic myeloproliferative neoplasms and subsequent cancer risk: a Danish population-based cohort study. *Blood*. 2011;118(25):6515-6520.

81. Brunner AM, Hobbs G, Jalbut MM, Neuberg DS, Fathi AT. A population-based analysis of second malignancies among patients with myeloproliferative neoplasms in the SEER database. *Leuk Lymphoma*. 2016;57(5): 1197-1200.

82. Meyer SC. Mechanisms of resistance to JAK2 inhibitors in myeloproliferative neoplasms. *Hematol Oncol Clin North Am*. 2017;31(4): 627-642.

83. Marchetti M. Cost-effectiveness of kinase inhibitors for hematologic malignancies: a systematic and critical review. *Expert Rev Pharmacoecon Outcomes Res*. 2017;17(5): 469-480.

84. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood*. 2009; 113(13):2895-2901.

85. Verstovsek S, Mesa RA, Gotlib J, et al. The clinical benefit of ruxolitinib across patient subgroups: analysis of a placebo-controlled, Phase III study in patients with myelofibrosis. *Br J Haematol*. 2013;161(4):508-516.

86. Palandri F, Palumbo GA, Bonifacio M, et al. Baseline factors associated with response to ruxolitinib: an independent study on 408 patients with myelofibrosis. *Oncotarget*. 2017;8(45):79073-79086.

87. Mesa RA, Jamieson C, Bhatia R, et al. NCCN Guidelines Insights: Myeloproliferative Neoplasms, Version 2.2018. *J Natl Compr Canc Netw*. 2017;15(10):1193-1207.

88. Mead AJ, Milojkovic D, Knapper S, et al. Response to ruxolitinib in patients with intermediate-1-, intermediate-2-, and high-risk myelofibrosis: results of the UK ROBUST Trial. *Br J Haematol*. 2015;170(1):29-39.

89. Kröger NM, Deeg JH, Olavarria E, et al. Indication and management of allogeneic stem cell transplantation in primary myelofibrosis: a consensus process by an EBMT/ELN international working group. *Leukemia*. 2015;29(11):2126-2133.

90. Kröger N, Giorgino T, Scott BL, et al. Impact of allogeneic stem cell transplantation on survival of patients less than 65 years of age with primary myelofibrosis. *Blood*. 2015; 125(21):3347-3350, quiz 3364.

91. Jaekel N, Behre G, Behning A, et al. Allogeneic hematopoietic cell transplantation for myelofibrosis in patients pretreated with the JAK1 and JAK2 inhibitor ruxolitinib. *Bone Marrow Transplant*. 2014; 49(2):179-184.

92. Stübig T, Alchalby H, Ditschkowski M, et al. JAK inhibition with ruxolitinib as pretreatment for allogeneic stem cell transplantation in primary or post-ET/PV myelofibrosis. *Leukemia*. 2014;28(8):1736-1738.

93. Shanavas M, Popat U, Michaelis LC, et al. Outcomes of allogeneic hematopoietic cell transplantation in patients with myelofibrosis with prior exposure to janus kinase 1/2 inhibitors. *Biol Blood Marrow Transplant*. 2016;22(3):432-440.

94. Kuykendall AT, Shah S, Talati C, et al. Between a rux and a hard place: evaluating salvage treatment and outcomes in myelofibrosis after ruxolitinib discontinuation. *Ann Hematol*. 2018;97(3):435-441.

95. Newberry KJ, Patel K, Masarova L, et al. Clonal evolution and outcomes in myelofibrosis after ruxolitinib discontinuation. *Blood*. 2017;130(9):1125-1131.

96. Masarova L, Bose P, Pemmaraju N, et al. Characteristics and survival of patients with chronic phase myelofibrosis and elevated blasts (5-9%), and the effect of therapy with JAK2 inhibitor ruxolitinib [abstract]. *Blood*. 2017;130(suppl 1). Abstract 201.

97. Bose P, Verstovsek S. JAK2 inhibitors for myeloproliferative neoplasms: what is next? *Blood*. 2017;130(2):115-125.

98. Harrison CN, Mesa RA, Jamieson C, et al. Case series of potential Wernicke's encephalopathy in patients treated with fedratinib [abstract]. *Blood*. 2017; 130(suppl 1). Abstract 4197.

99. Harrison CN, Vannucchi AM, Platzbecker U, et al. Momelotinib versus best available therapy in patients with myelofibrosis previously treated with ruxolitinib (SIMPLIFY 2): a randomised, open-label, phase 3 trial. *Lancet Haematol*. 2018;5(2):e73-e81.

100. Harrison CN, Mead AJ, Panchal A, et al. Ruxolitinib versus best available therapy for ET intolerant or resistant to hydroxycarbamide in a randomized trial. *Blood*. 2017;130(17): 1889-1897.