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Leukemia (PH Wiernik, Section Editor)

# New Concepts of Treatment for Patients with Myelofibrosis

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## Opinion statement

Seven years after the approval of the Janus kinase 1/2 (JAK1/2) inhibitor ruxolitinib, it remains the only drug licensed for the treatment of myelofibrosis. Patients who discontinue ruxolitinib have a dismal outcome, and this is, therefore, an area of significant unmet need. Given the central role that JAK-signal transducer and activator of transcription (STAT) activation plays in disease pathogenesis, there have been many other JAK inhibitors tested, but most have been abandoned, for a variety of reasons. The JAK2-selective inhibitor fedratinib has recently been resurrected, and there has been a resurgence of interest in the failed JAK1/2 inhibitor momelotinib, which possibly improves anemia. Pacritinib, a non-myelosuppressive JAK2-selective inhibitor, is currently in a dose-ranging study mandated by regulatory authorities. A plethora of other targeted agents, most backed by preclinical data, are in various stages of investigation. These include epigenetic and immune therapies, agents targeting cellular survival, metabolic and apoptotic pathways, the cell cycle, DNA repair, and protein folding and degradation, among others. However, at this time, none of these is close to registration or even in a pivotal trial, illustrating the difficulties in recapitulating the clinical disease in preclinical models. Most current clinical trials are testing the addition of a novel agent to ruxolitinib, either in the frontline setting or in the context of an insufficient response to ruxolitinib, or attempting to study new drugs in the second-line, "ruxolitinib failure" setting. Emerging data supports the addition of azacitidine to ruxolitinib in some patients. Other strategies have focused on improving cytopenias, through amelioration of bone marrow fibrosis or other mechanisms. This is important, because cytopenias are the commonest reason for ruxolitinib interruption and/or dose reduction, and dose optimization of ruxolitinib is tied to its survival benefit. The activin receptor ligand trap, sotatercept, and the anti-fibrotic agent, PRM-151, have shown promise in this regard.

## Introduction

While the Janus kinase (JAK) 1/2 inhibitor ruxolitinib represents a major therapeutic advance in the myeloproliferative neoplasm (MPN) field, bringing substantial benefits to patients with myelofibrosis, the drug has several limitations. Median duration of spleen response is about 3 years [1••, 2••], and median survival after discontinuation of ruxolitinib among patients treated at large academic medical centers has been reported to be 13–14 months [3, 4•]. Doubts persist over ruxolitinib's anti-clonal activity [5, 6], and dose-limiting anemia and thrombocytopenia could represent one reason for this. The drug is generally contraindicated in individuals with platelet counts $< 50 \times 10^9$/L, making this a subgroup of patients with high unmet need. In routine clinical practice, cytopenias often hinder dose optimization of ruxolitinib, or even lead to its premature discontinuation. Spleen responses to ruxolitinib have been shown to be dose-dependent and to correlate with survival in patients with myelofibrosis [7–9]. For all these reasons, much work remains to be done in the area of drug development for myelofibrosis, and there is tremendous interest in looking beyond JAK inhibition to find other active agents that could complement ruxolitinib or be used in patients who fail ruxolitinib. Equally, efforts are underway to develop alternative JAK inhibitors that are less myelosuppressive, may improve anemia, and/or display activity in patients that have to discontinue ruxolitinib.

# New approaches to treat anemia and thrombocytopenia

This topic has recently been reviewed by the authors [10] and will only briefly be discussed here. Anemia and thrombocytopenia are well-recognized poor prognostic features in myelofibrosis [11, 12] and also the two most common adverse events (AEs) associated with ruxolitinib use [13, 14]. Although ruxolitinib-induced anemia does not share the deleterious impact on survival of disease-associated anemia [15] and ruxolitinib may, in fact, overcome this adverse prognostic impact [16], anemia represents a major problem in everyday practice, often leading to ruxolitinib discontinuation and/or dose reduction. Current management of anemia involves the use of danazol, erythroid-stimulating agents (ESAs), or immunomodulatory drugs (Imids), but remains unsatisfactory [10]. The observation that ESAs, which signal through JAK2, are effective in counteracting anemia in some patients on ruxolitinib [17–19] suggests that at the doses currently employed (up to 25 mg twice daily), ruxolitinib only leads to incomplete inhibition of JAK2. The combination of lenalidomide and ruxolitinib is difficult to deliver because of excessive myelosuppression [20]. In updated results from an ongoing study of the combination of ruxolitinib and pomalidomide (0.5 mg daily), only 3 of 37 (8%) patients had an anemia response, although 12 (32%) remained on study beyond 12 cycles because of either response or stable disease (SD) with clinical benefit [21]. Thalidomide is a non-myelosuppressive Imid that can additionally improve thrombocytopenia. The first results from an ongoing study (NCT03069326, Table 1) of the combination of ruxolitinib and low-dose thalidomide (50 mg daily) were released at the 2018 annual meeting of the American Society of Hematology (ASH), and showed platelet responses in the majority of patients [29].

The activin receptor ligand traps, sotatercept and luspatercept, administered subcutaneously every three weeks, have generated considerable enthusiasm as novel agents in development for anemia of lower risk myelodysplastic

**Table 1. Ruxolitinib-based combination trials in myelofibrosis.**

| Drug | Number of pts | Drug class | Phase | Key eligibility/methods | Main results | Major toxicities | Reference |
|---|---|---|---|---|---|---|---|
| Panobinostat | 23 pts in expansion phase at RP2D (rux 15 mg bid + pan 25 mg tiw × 3 wks q4wks) | HDAC inhibitor | 1b | Risk: interm/high<br>Splenomegaly ≥ 5 cm by palpation | At RP2D, 57% and 39% had ≥ 35% SVR at wk 24 and 48<br>29% had ≥ 20% decrease in JAK2 V617F allele burden at wk 48. | Anemia, thrombocytopenia, diarrhea, asthenia | [22] |
| Pracinostat | 20 patients analyzed (received both drugs) | HDAC inhibitor | 2 | Risk: interm/high (if newly diagnosed)<br>ANC ≥ 1 and platelets ≥ 50<br>Splenomegaly ≥ 5 cm by palpation, pracinostat (60 mg tiw × 3 wks q4wks) initiated after 12 wks of rux | 80% had CI in spleen, symptoms or both at any time.<br>High D/C rate, mostly due to AEs; most responses preceded pracinostat introduction. | Anemia and thrombocytopenia | [23] |
| Buparlisib | 22 pts in arm A and 20 pts in arm B treated at MTD (rux 15 mg bid + buparlisib 60 mg daily) | Pan-PI3K inhibitor | 1b | Risk: interm/high<br>Splenomegaly ≥ 5 cm by palpation<br>2 arms: A, JAK inhibitor-naïve; B, JAK inhibitor--pretreated | 82% and 55% of pts in arms A and B achieved ≥ 50% reduction in palpable splenomegaly at any time; 55% and 20% at wk 24. 45% and 18% achieved ≥ 35% SVR at wk 24 in expansion phase. | Anemia, thrombocytopenia, anxiety, depression | [24] |
| Azacitidine | 54 pts analyzed | HMA | 2 | Risk: interm/high<br>ANC ≥ 1 and platelets ≥ 50 | 72% achieved IWG-MRT responses, 23% after addition of AZA | Anemia, thrombocytopenia, and neutropenia | [25•] |

**Table 1.** (Continued)

| Drug | Number of pts | Drug class | Phase | Key eligibility/methods | Main results | Major toxicities | Reference |
|---|---|---|---|---|---|---|---|
| | | | | Palpable spleen ≥ 5 cm required; AZA added in cycle 4; AZA dose could be increased gradually from 25 to 50 to 75 mg/m$^2$/day. Spleen response assessed by palpation | 60% had improved bone marrow fibrosis by ≥ 1 grade<br>64% had ≥ 50% reduction in palpable spleen length at any time, 57% at wk 24, 82% had molecular responses. | | |
| Umbralisib | 23 pts (17 analyzed for spleen response) | PI3Kδ inhibitor | 1 | RUX-experienced MF Grade ≥ 1 bone marrow fibrosis<br>Stable RUX dose for ≥8 wks with no, sub-optimal, or lost response | 2 pts (9%) achieved durable complete remission. Another 48% achieved IWG-MRT-defined CI (anemia, spleen, symptoms) at any time. | Anemia, thrombocytopenia, neutropenia, liver/pancreatic enzyme elevation, colitis, dyspnea, diarrhea | [26] |
| Parsaclisib | 31 pts (19 analyzed for spleen and symptom response at wk 24) | PI3Kδ inhibitor | 2 | MF with insufficient response to RUX, treated with RUX for ≥ 6 months with stable dose for ≥ 8 wks, spleen palpable ≥ 10 cm or 5–10 cm with active MF symptoms | 63% achieved SVR at wk 24 (median change – 8.8%), median change in TSS – 35.9% at wk 24 | Some rash and transaminitis seen, but no colitis reported | [27] |
| Sonidegib | 27 pts treated at RP2D (rux 20 mg bid + sonidegib | Hedgehog (smooth-ened) inhibitor | 1b/2 | Risk: interm/high Palpable spleen ≥ 5 cm required | 55.6% achieved ≥ 35% SVR at any time; 44.4% at wk 24<br>92.6% had ≥ 50% reduction in | Anemia and muscle spasms, increased creatine kinase, myalgias | [28] |

**Table 1.** (Continued)

| Drug | Number of pts | Drug class | Phase | Key eligibility/methods | Main results | Major toxicities | Reference |
|---|---|---|---|---|---|---|---|
| | 400 mg daily) | | | | palpable splenomegaly at any time; 55.6% at wk 24 | | |
| Thalidomide | 21 pts enrolled but 10 evaluable for response | Imid | 2 | Previously untreated or those on RUX for ≥ 3 months with stable dose for ≥ 4 wks. ANC ≥ 1 and platelets ≥ 50 | CI in 40% and platelet responses in 60% | Grade 3/4 limb edema, diarrhea, neutropenia, and DVT occurred in 1 pt each (6.7%). | [29] |

Abbreviations: *pts* patients, *interm* intermediate, *wk* week, *tiw* three times a week, *ANC* absolute neutrophil count, *RUX* ruxolitinib, *PAN* panobinostat, *AZA* azacitidine, *JAK* Janus kinase, *HMA* hypomethylating agent, *HDAC* histone deacetylase, *PI3K* phosphoinositide 3-kinase, *SVR* spleen volume reduction, *RP2D* recommended phase 2 dose, *MTD* maximum tolerated dose, *IWG-MRT* International Working Group for Myeloproliferative Neoplasms Research and Treatment, *CI* clinical improvement, *MF* myelofibrosis, *Imid* immunomodulatory drug, *DVT* deep vein thrombosis

syndromes (MDS) [30, 31]. These agents sequester bone marrow stroma-derived ligands belonging to the transforming growth factor beta (TGF-β) superfamily (targeting TGF-β itself has not proven very successful thus far in myelofibrosis) [32] and prevent their binding to activin receptors IIA (sotatercept) and IIB (luspatercept), through which these ligands signal to inhibit the terminal stages of erythropoiesis [33, 34]. In an ongoing investigator-initiated trial, sotatercept produced an anemia (including RBC transfusion independence) response in 7 of 18 (39%) evaluable subjects when given as a single agent, and in 3 of 10 (30%) evaluable subjects on concomitant ruxolitinib [35•]. Luspatercept, which met the primary and key secondary endpoints in a pivotal trial in anemic patients with lower risk MDS with ringed sideroblasts [36•], is now being studied in a multi-center phase 2 clinical trial (NCT03194542) in anemic patients with myelofibrosis, both transfusion-dependent and transfusion-independent, and both alone and in conjunction with a stable dose of ruxolitinib.

## Novel concepts in targeting bone marrow fibrosis

Although traditionally considered a reactive phenomenon [37], recent evidence suggests that bone marrow fibrosis in myelofibrosis is driven by clonal, neoplastic, monocyte-derived fibrocytes [38•]. The grade of bone marrow fibrosis has been shown to negatively influence prognosis in primary myelofibrosis (PMF) [39] and has now been incorporated into a new prognostic model [40•]. Furthermore, patients with pre-fibrotic PMF (pre-PMF) who, by definition, have lower grades of bone marrow fibrosis than those with overt PMF, have a lower prevalence of cytopenias [41•]. The anti-fibrotic agent, PRM-151, is an intravenously administered (every 4 weeks), recombinant form of human pentraxin-2, also known as serum amyloid protein. This agent has yielded responses in patients with myelofibrosis, both when administered as monotherapy and in combination with ruxolitinib [42]. Sustained improvements in anemia and thrombocytopenia were observed among 13 patients who remained on the drug for > 72 weeks [43]. In the most recent update, results on 18 patients, 9 of whom received PRM-151 alone and 9 in combination with ruxolitinib in an open-label extension study, were presented [44]. The median time on study was 30.9 months, and the drug was extremely well-tolerated. The mean and median changes in palpable spleen length and MPN-symptom assessment form total symptom score (MPN-SAF TSS) were – 37% and – 26% (for spleen) and – 54% and – 64% (for symptoms). Interestingly, even the patients on PRM-151 monotherapy derived substantial benefit in terms of spleen size and symptom burden. Reticulin grade improvements were observed in 50% of patients, and collagen in 44%; in general, these coincided with improvements in cytopenias [44]. Results from a larger, more definitive study of different doses of this agent in myelofibrosis are eagerly anticipated.

Other investigators have shown that $Gli1^{+}$ mesenchymal stromal cells (MSCs) are recruited from endosteal and perivascular niches to become myofibroblasts that then drive bone marrow fibrosis [45•]. The Gli family of transcription factors is located downstream of the hedgehog signaling pathway, but the results of therapeutic hedgehog (smoothened) inhibition in myelofibrosis have been disappointing thus far, with only inconsistent effects seen on

Gli1 messenger RNA and protein levels and degree of bone marrow fibrosis [46]. These researchers demonstrated that direct pharmacological targeting of Gli proteins inhibited myofibroblast differentiation and attenuated the severity of bone marrow fibrosis [45]. Another approach shown preclinically in *JAK2*$^{V617F}$ and *MPLW*$^{515L}$ mouse models to block excessive collagen production by MSCs is through the use of galunisertib, a small-molecule inhibitor of the TGF-β receptor 1 kinase ALK5 [47•]. There is also preclinical evidence suggesting that leptin receptor-expressing MSCs are the fibrogenic cells in PMF, and that these could potentially be targeted using imatinib to block platelet-derived growth factor receptor alpha (PDGFRA) signaling and ameliorate bone marrow fibrosis [48•]. Finally, work from Japanese investigators has demonstrated that activation of the thrombopoietin receptor, MPL, induces fibrocyte differentiation and leads to bone marrow fibrosis [49]. Furthermore, they found that circulating monocytes with high expression of SLAMF7 and MPL were possible precursors of fibrocytes, providing a possible rationale to test the anti-SLAMF7 monoclonal antibody, elotuzumab, in myelofibrosis [49].

# Newer JAK2 inhibitors

The clinical development of JAK2 inhibitors for myelofibrosis has been challenging, with ruxolitinib, approved in 2011, still being the only one licensed for use. Several have been abandoned due to toxicity. Development plans for NS-018, a JAK2-selective inhibitor in phase 2 of clinical testing [50], remain unclear at present. See ref. [51] for a detailed review of this topic. A brief overview of the three investigational agents that have been tested in phase 3 randomized controlled trials (RCTs) follows.

## Fedratinib

Development of the JAK2-selective inhibitor fedratinib (formerly Sanofi, Paris, France, now Celgene Corporation, Summit, NJ) was halted owing to concerns regarding Wernicke's encephalopathy (WE) in a small number of patients, and there exists some preclinical data showing that the drug might inhibit neuronal thiamine uptake [52, 53]. A more recent analysis found the incidence of WE to be only 0.4–0.7% (3–5 cases across 9 trials enrolling 670 patients), with only 1 confirmed case, 2 unconfirmed cases, and 2 where the diagnosis was felt to be unlikely [54•]. Development of fedratinib for myelofibrosis has now been resumed in earnest. In the placebo-controlled, phase 3 JAKARTA RCT, ≥ 35% spleen volume reduction (SVR) at week 24 was achieved by 36% and 40% of patients in the fedratinib 400 mg/day and 500 mg/day groups, respectively, and ≥ 50% reduction in total symptom score (TSS) by 36% and 34%, respectively [55•]. These primary and key secondary endpoints were achieved by only 1% and 7%, respectively, of the placebo-treated patients [55•]. While JAKARTA was conducted in a JAK inhibitor-naïve population ($n$ = 289), the single-arm, open-label JAKARTA-2 study evaluated fedratinib, 400 mg daily, in ruxolitinib-exposed (≥ 2 weeks) patients with myelofibrosis [56•]. Of 83 evaluable patients at week 24, 46 (55%) achieved ≥ 35% SVR (per-protocol analysis) and 23 of 90 evaluable patients (26%) experienced a ≥ 50% reduction in TSS [56]. Grade 3/4 anemia (38%) and thrombocytopenia (22%) were common. This trial was

terminated early because concerns regarding WE in other fedratinib trials. The relatively small number of patients and the lack of a rigorous definition of ruxolitinib failure for eligibility are important limitations of this trial. Fedratinib will next be studied in a phase 3b trial in patients with intermediate- or high-risk myelofibrosis that have previously received ruxolitinib (the FREEDOM trial, NCT03755518).

## Pacritinib

Like fedratinib, pacritinib (CTI BioPharma, Seattle, WA) is a JAK2-selective inhibitor that also inhibits *fms*-like tyrosine kinase 3 (FLT3; the gastrointestinal AEs common with both agents are attributed to this), but is believed to be non-myelosuppressive [57]. Accordingly, the trials of this agent have not specified a minimum platelet count for eligibility. The PERSIST-1 RCT, like JAKARTA, was conducted in a JAK inhibitor-naïve population ($n = 327$), and met its primary endpoint of superiority for ≥ 35% SVR (19% with pacritinib compared to 5% with best available therapy (BAT)) at week 24 [58•]. Approximately a third of patients had baseline platelet counts $< 100 \times 10^9$/L (i.e., would not have been eligible for the COMFORT trials of ruxolitinib) and were evenly distributed between the two arms of the trial. The benefit of pacritinib in terms of ≥ 35% SVR was preserved among these patients. There was no significant difference between pacritinib and BAT in terms of ≥ 50% TSS reduction at week 24 in the intention to treat (ITT) population, but pacritinib beat BAT in the evaluable population, with 36% achieving ≥ 50% TSS reduction at week 24 versus 14% ($p = 0.029$) [58•]. The PERSIST-2 RCT was conducted in thrombocytopenic patients (baseline platelet count $\leq 100 \times 10^9$/L) who could have had prior ruxolitinib (48%) and compared two doses of pacritinib (400 mg once daily, the dose evaluated in PERSIST-1, and 200 mg twice daily) to BAT (which could include ruxolitinib, and did in 45% of the patients) [59•]. Although 311 patients were recruited, only 221 (75, 74, and 72 in the three arms) could be included in the ITT efficacy population as accrual to this trial was affected by the imposition of a "full clinical hold" on the pacritinib development program by the Food and Drug Administration (FDA) in February 2016 [60] because of concerns over excess mortality in pacritinib-treated patients in the two RCTs. The rate of ≥ 35% SVR was significantly higher in the pooled pacritinib arms (18%) than the BAT arm (3%), but that of ≥50% TSS reduction was only numerically higher (25% versus 14%, $p = 0.08$) [59]. However, twice daily pacritinib was significantly superior to BAT for both co-primary endpoints (22% versus 3% for ≥ 35% SVR and 32% versus 14% for ≥ 50% TSS reduction) [59]. The FDA has since lifted the full clinical hold [61], and at present, pacritinib is being studied in an international, dose-ranging (100 mg daily, 100 mg twice daily, and 200 mg twice daily) phase 2 clinical trial, PAC203 (NCT03165734), in 150 patients with myelofibrosis who have failed ruxolitinib.

## Momelotinib

Momelotinib (formerly Gilead, Foster City, CA, now Sierra Oncology, Vancouver, BC) is a JAK1/2 inhibitor that has the unique attribute of improving anemia [62, 63]. In a rodent model of anemia of chronic disease, momelotinib normalized hemoglobin and RBC via direct inhibition of the type I activin A

**Table 2. Selected single agent early phase trials in myelofibrosis.**

| Drug [ref.] | Number of pts | Drug class | Phase | Key eligibility/methods | Main results | Major toxicities | Reference |
|---|---|---|---|---|---|---|---|
| ImetelstaT [81] | 107 pts, all JAK inhibitor-exposed. 59 in 9.4 mg/kg dosing arm; 48 in 4.7 mg/kg dosing arm (closed to enrollment due to insufficient activity) | Telomerase inhibitor | 2 | Risk: interm-2/high<br>Relapsed/refractory to JAK inhibitor therapy<br>Active MF symptoms<br>Spleen palpable ≥ 5 cm below LCM or ≥ 450 $cm^3$ by MRI.<br>Administered IV every 3 wks | In 9.4 mg/kg dosing arm ($n = 59$), 10% achieved ≥ 35% SVR at wk 24 and 37% achieved ≥ 10% SVR. 32% had ≥ 50% reduction in TSS at wk 24. BM fibrosis improved in 18%. Median OS 19.9 m in 4.7 mg/kg arm; 29.9 m in 9.4 mg/kg arm; median f/u 27.4 m | Thrombocytopenia, neutropenia, anemia, N/V/D, cough, fatigue, peripheral edema, fever, dyspnea, abdominal pain, asthenia | Ayalew Tefferi et al. |
| LCL-161 [82] | 44 pts | Smac Mimetic | 2 | Risk: interm/high<br>ANC ≥ 0.5, any platelet count<br>Intolerant of/ineligible for or failed JAK inhibitor therapy<br>Dose 1500 mg orally weekly | Overall response rate 32% by IWG-MRT criteria at any time<br>Most responses CI in symptoms and anemia | Fatigue, nausea, vomiting, pain, pruritus, dizziness, vertigo, syncope, rash, fever, diarrhea | Naveen Pemmaraju et al. |
| Everolimus [83] | | mTOR inhibitor | 1/2 | Risk (Lille): interm/high or low | 20% had ≥ 50% reduction in | Stomatitis, fatigue, skin and | |

**Table 2.** (Continued)

| Drug [ref.] | Number of pts | Drug class | Phase | Key eligibility/methods | Main results | Major toxicities | Reference |
|---|---|---|---|---|---|---|---|
| | 39 pts; 30 (phase 2) pts analyzed for response | | | risk with palpable spleen length > 10 cm<br>Treatment-naïve or previously treated<br>Dose 10 mg orally daily | spleen length by palpation at any time.<br>69% had complete resolution of systemic symptoms.<br>23% had IWG-MRT responses. | musculoskeletal disorders, and dyslipidemia | Paola Guglielmelli et al. |
| Itacitinib [84] | 87 pts treated at 100 mg bid (10), 200 mg bid (45), or 600 mg daily (32); 83 (10 + 42 + 31) evaluable for primary endpoint (≥ 50% reduction in TSS from baseline to wk 12) | JAK1 inhibitor | 2 | Risk: interm/high<br>Platelet ≥ 50, ANC ≥ 1, Hgb ≥ 8<br>Palpable spleen not required<br>Active MF-related symptoms<br>Treatment naïve or previously treated<br>Simon 2-stage design | 35.7% and 32.3% had ≥ 50% reduction in TSS in the 200 mg bid and 600 mg daily cohorts at wk 12.<br>53.8% had ≥ 50% reduction in RBC transfusion requirements on-study.<br>Only 10 pts achieved ≥ 35% SVR at wk 24. | Thrombocytopenia, anemia, infections, and fatigue | John Mascarenhas et al. |
| Alisertib [85] | 22 pts (different numbers of evaluable pts for different efficacy parameters) | Aurora kinase inhibitor | Pilot | Risk: interm/high<br>Intolerant of/ineligible for or failed JAK inhibitor therapy<br>Dose 50 mg bid on days 1–7 q21days | 29% spleen response rate (by palpation), 11% erythroid response rate, 54% | Neutropenia, FN, lymphopenia, anemia, thrombocytopenia, diarrhea, cellulitis | Naseema Gangat et al. |

**Table 2.** (Continued)

| Drug [ref.] | Number of pts | Drug class | Phase | Key eligibility/methods | Main results | Major toxicities | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | symptom response rate. Megakaryocyte morphology improved, correlating with BM fibrosis improvement | | |
| Glasdegib [86] | 21 pts in phase 1 portion | Hedgehog (smoothened) inhibitor | 1/2 | Symptomatic MF<br>Previously treated with ≥ 1 JAK inhibitor<br>Dose 100 mg orally daily | No pt with ≥ 35% SVR<br>1 anemia response<br>1 pt with prolonged lack of RBC transfusion requirement<br>Only 10% and 5% at wks 12 and 24 had ≥ 50% symptom reduction | Dysgeusia, muscle spasms, increased lipase, alopecia, decreased appetite, weight loss, and fatigue | Aaron Gerds et al. |
| NS-018 [50] | 48 pts in completed phase 1 (23 JAK inhibitor--exposed); 29 pts enrolled in phase 2 portion | JAK2 inhibitor | 1/2 | Risk: interm/high<br>All pts with prior JAK inhibitor therapy in phase 2. RP2D from phase 1: 300 mg daily | In phase 1, 56% had ≥ 50% reduction in spleen length by palpation at any time (39% CI-spleen by | Thrombocytopenia, nausea, and anemia | Srdan Verstovsek et al. |

**Table 2.** (Continued)

| Drug [ref.] | Number of pts | Drug class | Phase | Key eligibility/methods | Main results | Major toxicities | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | IWG-MRT criteria) Only 12% rate of ≥ 35% SVR at wk 24 in phase 2 | | |

Abbreviations: *interm* intermediate, *pt* patient, *wk* week, *m* month, *bid* twice daily, *JAK* Janus kinase, *Smac* second mitochondrial activator of caspases, *mTOR* mammalian target of rapamycin, *RP2D* recommended phase 2 dose, *CI* clinical improvement, *FN* febrile neutropenia, *MF* myelofibrosis, *SVR* spleen volume reduction, *TSS* total symptom score, *IWG-MRT* International Working Group for Myeloproliferative Neoplasms Research and Treatment, *LCM* left costal margin, *MRI* magnetic resonance imaging, *BM* bone marrow, *IV* intravenous, *N/V/D* nausea/vomiting/diarrhea, *OS* overall survival, *ANC* absolute neutrophil count

receptor and subsequent reduction of hepcidin production from the liver [64•]. Ruxolitinib had no effect on this pathway. The development of momelotinib was ceased after somewhat disappointing results from two phase 3 RCTs, but the drug has very recently been acquired by Sierra Oncology. SIMPLIFY-1 was a head-to-head comparison of momelotinib, 200 mg daily, and ruxolitinib in 432 JAK inhibitor-naïve patients with myelofibrosis [65•]. The trial met its primary endpoint of non-inferiority of momelotinib for ≥ 35% SVR at week 24 (26.5% with momelotinib versus 29% with ruxolitinib) but not the key secondary endpoint of non-inferiority for ≥ 50% TSS reduction (28.4% with momelotinib and 42.2% with ruxolitinib). However, rates of RBC transfusion and transfusion independence and dependence were all improved with momelotinib (nominal $p \leq 0.019$ for all) [65•]. SIMPLIFY-2 compared momelotinib, 200 mg daily to BAT (2:1) in 156 patients with myelofibrosis who had failed prior ruxolitinib (≥ 28 days) [66•]. The BAT turned out to be ruxolitinib in 46 of the 52 patients (89%). The primary endpoint of ≥ 35% SVR at week 24 was achieved by only 7% of patients in the momelotinib group and 6% in the BAT group [66•]. Because the primary endpoint was not met, the secondary endpoints were again assessed for nominal significance only. The rate of ≥ 50% TSS reduction was significantly higher in the momelotinib group (26% versus 6%, nominal $p = 0.0006$) [66•]. Like in SIMPLIFY-1, the rates of RBC transfusion and transfusion independence and dependence until or at week 24 all favored momelotinib (nominal $p$ values 0.39, 0.0012, and 0.10, respectively) [66•]. Peripheral sensory neuropathy is an important AE of momelotinib, affecting nearly one half of patients, usually grade 1/2, but generally irreversible [67, 68].

## Targeting epigenetic writers, erasers, and readers

Aberrant methylation is frequently encountered in myeloproliferative neoplasms (MPN) [69], and the combination of ruxolitinib (splenomegaly, symptoms) and azacitidine (bone marrow blasts, dysplasia) may co-target distinct pathophysiologic aspects of the disease. An overall response rate (ORR) by International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) criteria of 73% was recently reported in a single-center trial in 54 patients with myelofibrosis in which azacitidine was added after 12 weeks of ruxolitinib alone and the dose of azacitidine stepped up from 25 to 50 to 75 mg/m$^2$/day over 3 cycles [25•]. With the caveat that spleen response (≥ 50% reduction in spleen length) was measured by palpation in this trial, the same (57% at 24 weeks, 64% at any time point) appeared higher than would be expected with ruxolitinib alone (Table 1), and a substantial number of patients responded after the addition of azacitidine. Of particular interest, the combination led to improvement of bone marrow fibrosis in a significant proportion (60%, 21 of 35 sequentially evaluable) of patients with a median time to response of 12 (6–18) months [25•]. Molecular responses (decline in *JAK2*$^{V617F}$ allele burden) were also observed, albeit in a small number of serially evaluable patients (14 of 17, 82%).

Among the pleiotropic actions of histone deacetylase inhibitors (HDACi) in neoplastic cells is their ability to downregulate oncoproteins of importance in leukemia, e.g., JAK2, BCR-ABL, and FLT3, by disrupting the chaperone function

of heat shock protein 90 (HSP90) through acetylation [70]. Synergism against *JAK2*$^{V617F}$ MPN cells and in mouse models has been shown between the HDACi panobinostat and ruxolitinib [71]. HDACi clearly have single agent activity in myelofibrosis, but are difficult to deliver over long periods because of chronic, low-grade toxicities, and disease-modifying effects appear to emerge only after prolonged exposure [72–74]. A trial of ruxolitinib and panobinostat was stopped after the recommended phase 2 doses (RP2D) of the combination had been determined [22], and even though the spleen response rates appeared higher than would be expected with ruxolitinib alone (Table 1). We have reported on the combination of ruxolitinib and pracinostat: all patients discontinued pracinostat, mostly because of AEs, and the contribution of pracinostat to clinical efficacy was difficult to ascertain (Table 1) [23].

Pharmacologic targeting of bromodomain extraterminal (BET) proteins has the potential to correct the dysregulated transcriptome (e.g., c-Myc, nuclear factor kappa B (NF-κB) overexpression) characteristic of diverse hematologic malignancies [75]. Synergism between ruxolitinib and the small-molecule BET protein inhibitor JQ1 against *JAK2*$^{V617F}$/*MPL*$^{W515L}$ preclinical models of myelofibrosis and post-MPN acute myeloid leukemia (AML) has been demonstrated [76•, 77], both in vitro and in vivo, with reduction of inflammatory cytokine production and disease burden and reversal of bone marrow fibrosis shown in vivo [77•]. CPI-0610 is a BET protein inhibitor being studied in the clinic specifically in patients with myelofibrosis, both with and without ruxolitinib (NCT02158858). Another epigenetic target of interest in myelofibrosis is lysine-specific demethylase 1 (LSD1, also called KDM6A), although none of the current LSD1 inhibitor clinical trials are focusing exclusively on myelofibrosis.

## Targeting the PI3K/Akt/mTOR axis

The phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) cascade integrates cellular growth and proliferation signals downstream of JAK-STAT, and constitutive activation of this pathway is central to MPN pathogenesis [78]. Preclinical studies have shown activity of inhibitors of this pathway, both alone and synergistically in conjunction with ruxolitinib or fedratinib, in reducing proliferation and inducing apoptosis of *JAK2*$^{V617F}$/*MPL*$^{W515L}$ MPN cell lines and primary cells [78–80]. In a pre-ruxolitinib era phase 1/2 study of the mTOR inhibitor everolimus (Table 2), an ORR of 23% by IWG-MRT criteria was reported among the 30 patients treated at 10 mg daily [83]. Ruxolitinib was combined with the pan-PI3K inhibitor buparlisib in a phase 1b trial (Table 1), but this trial was subsequently terminated [24]. In the expansion phase at week 24 in patients treated at the maximum tolerated doses (MTDs) of the combination, the rate of ≥ 35% SVR was 45% in the JAK inhibitor-naïve patients and 18% in the group that had received JAK inhibitors previously [24]. Updated results from a trial in which umbralisib, a selective inhibitor of the delta isoform of PI3K with a superior tolerability profile, was "added on" to ruxolitinib (stable dose for ≥ 8 weeks) in patients with an insufficient response to the latter, were recently presented [26]. Two patients achieved complete response (CR), and spleen, anemia, and symptom responses were all seen (Table 1). Of note, determination of sub-optimal response to ruxolitinib for patient eligibility for this trial was left up to physician discretion.

Parsaclisib is another PI3K delta isoform-specific inhibitor that is also being studied in combination with ruxolitinib in an ongoing "add on" trial (NCT02718300), but sub-optimal response to ruxolitinib is clearly defined in this trial (palpable spleen ≥ 10 cm or 5–10 cm with active symptoms of myelofibrosis after at least 6 months of ruxolitinib with a stable dose over the preceding 8 weeks or longer) [27]. Parsaciclib exhibited a good tolerability profile in this trial (Table 1), but a switch from daily to weekly dosing after 8 weeks of combination therapy (to mitigate toxicities) appeared to correlate with some loss of response. A similar phase 1 trial of idelalisib added to ruxolitinib (stable dose for ≥ 4 weeks) has been completed and results are awaited (NCT02436135).

## Targeting hedgehog signaling

Excess hedgehog signaling and loss of "patched" have been implicated in the pathogenesis and leukemic transformation of MPN [87•]. As alluded to above, the results of hedgehog (smoothened) inhibition in the clinic have, however, been relatively disappointing [46]. In a recently reported phase 1/2 trial (Table 2), 21 patients with myelofibrosis, 11 of whom had exhibited an inadequate response to previous JAK inhibitor treatment, received glasdegib [86]. Five patients achieved some SVR and one an anemia response (RBC transfusion independence), but toxicity was high [86]. Based on laboratory evidence of synergism between ruxolitinib and the smoothened inhibitor sonidegib in a murine bone marrow transplant model of myelofibrosis [88], a phase 1b/2 clinical trial of the combination was conducted [28]. The rate of ≥ 35% SVR at week 24 among the 27 patients treated at the RP2D for the combination was 44.4% (Table 1), and the trial was subsequently terminated by the sponsor. The FDA-approved (for advanced basal cell carcinoma) smoothened inhibitor, vismodegib, was also studied in combination with ruxolitinib with disappointing results [89]; this combination will also not be developed further in myelofibrosis.

## Overcoming JAK2 inhibitor persistence through HSP90 inhibition

JAK2 inhibitor "persistence" is a phenomenon in which JAK2 is activated *in trans* via heterodimerization with other members of the JAK family such as JAK1 or tyrosine kinase 2 (TYK2) despite the presence of an inhibitor that binds to the JAK2 kinase in its active conformation (as all JAK2 inhibitors tested in the clinic to date do) [90]. This can be overcome by therapeutic blockade of HSP90, the chaperone protein of which JAK2 is a client, leading to proteasomal degradation of mutant JAK2 [91]. Based on this work, a phase 1 clinical trial combining ruxolitinib and the HSP90 inhibitor PU-H71 is currently enrolling patients with myelofibrosis who have had at least six months of ruxolitinib and still have active disease (NCT03373877). Other investigators have reported synergism between JAK2 inhibitors and the HSP90 inhibitor AUY922 against human MPN cells, demonstrating marked reduction in p-JAK2, p-STAT5, p-Akt, and the anti-apoptotic protein Bcl-xL with combination treatment [92]. However,

AUY922 turned out to have unexpected toxicities in a phase 2 clinical trial (night blindness, gastrointestinal bleeding) that were deemed to be specific to this agent and led to study termination after only seven patients had been treated, despite evidence of clinical activity (reduction in palpable spleen length in all five patients with palpable splenomegaly at baseline and clinical improvement (CI) in anemia by IWG-MRT criteria in one patient) [93].

## Targeting anti-apoptotic proteins

The anti-apoptotic protein Bcl-xL is regulated by JAKs, and combined targeting of JAK2 and Bcl-2/-xL (using ABT-737) has been shown to be synergistic in preclinical *JAK2*$^{V617F}$ MPN models and to overcome acquired resistance to ruxolitinib, while the Bcl-2-selective inhibitor venetoclax failed to induce apoptosis of ruxolitinib-resistant MPN cells [94]. These findings form the basis of an ongoing phase 2 clinical trial (NCT03222609) investigating the addition of navitoclax, the clinical equivalent of ABT-737, in patients with myelofibrosis who have been on ruxolitinib for ≥ 24 weeks (stable dose of ≥ 10 mg twice daily for ≥ 8 weeks). The second mitochondrial activator of caspases (Smac)-mimetic LCL-161 targets a different arm of the anti-apoptotic machinery by antagonizing the function of inhibitor of apoptosis (IAP) proteins. This group of drugs may be particularly effective in the tumor necrosis factor alfa (TNF-α)-rich environment promoted by *JAK2*$^{V617F}$ [95, 96]. In a phase 2, investigator-initiated clinical trial of LCL-161 in mostly ruxolitinib-pretreated or ruxolitinib-ineligible patients ($n = 44$) with myelofibrosis, CI in anemia, splenomegaly and symptoms were all reported in several patients (Table 2), and one patient experienced resolution of cytogenetic abnormalities, for an ORR of 32% [82].

## Targeting the cell cycle, mitosis, and DNA repair

Synergistic interactions between ruxolitinib, the cyclin-dependent kinase 4/6 inhibitor ribociclib, and the pan-PIM kinase inhibitor PIM447 both in vitro and in vivo in *JAK2*$^{V617F}$ and *MPL*$^{W515L}$ murine xenograft models [97] led to this triple combination being investigated in a phase 1 trial (NCT02370706), but the study enrolled only 15 participants and no results have been presented or published yet. Other investigators have reported synergism between ruxolitinib and other PIM kinase inhibitors against MPN cell lines and primary cells [98, 99], as well as in murine xenograft models [100].

Megakaryocytes in PMF exhibit impaired maturation associated with down-regulation of the transcription factor GATA1, which is accompanied by an RPS14-deficient gene expression profile, similar to MDS with 5q deletion, where the defective ribosomal protein function is restricted to the erythroid lineage [101•]. These atypical megakaryocytes may contribute to bone marrow fibrosis through the release of cytokines such as TGF-β. The aurora kinase A inhibitor alisertib has been shown to promote polyploidization and differentiation of PMF megakaryocytes, with potent anti-fibrotic and anti-tumor effects in vivo in mouse models of PMF [102•]. These findings led to a multi-center, pilot study of alisertib in 24 patients with myelofibrosis, 63% of whom had received prior JAK inhibitor therapy [85]. Of evaluable patients, two (11%) had

erythroid responses, seven (54%) had ≥ 50% reduction in their MPN-SAF score at cycle 6, and four (29%) had IWG-MRT spleen responses (Table 2). Correlative studies showed normalization of megakaryocyte morphology and restoration of GATA1 staining, which appeared to correlate with reduction in bone marrow fibrosis [85].

Finally, hypersensitivity to small-molecule inhibitors of poly-ADP-ribose polymerase (PARP) has been demonstrated in circulating myeloid cells from MPN patients [103] and ruxolitinib has recently been shown to inhibit both major mechanisms of DNA double strand break (DSB) repair—homologous recombination and non-homologous end joining (NHEJ)—sensitizing MPN stem and progenitor cells to PARP inhibition [104•]. *JAK2*$^{V617F}$, *MPL*$^{W515L}$, and *CALR*$^{del52}$ MPN cell lines accumulated reactive oxygen species (ROS)-induced DSBs, a phenomenon greatly enhanced by co-treatment with ruxolitinib and the PARP inhibitor olaparib. This resulted in elimination of most primary MPN cells, including disease-initiating cells, and the combination plus hydroxyurea was highly effective in vivo against primary MPN xenograft models [104]. To our knowledge, these findings have not been translated into a clinical trial yet, although the PARP inhibitor veliparib has been studied in conjunction with topotecan and carboplatin in patients with aggressive MPN and post-MPN AML [105]. Another novel agent that generates ROS, induces DNA damage, and inhibits NF-κB activity is the first-in-class inhibitor of NEDD8 activating enzyme (NAE), pevonedistat [106]. While this agent is mainly being developed in AML [107], an investigator-initiated phase 1 trial adding pevonedistat in patients with myelofibrosis who have been on ruxolitinib for ≥ 3 months (stable dose for ≥ 8 weeks) and have not achieved a complete response (CR) by IWG-MRT criteria is underway (NCT03386214).

## Targeting JAK1

Given the critical role of JAK2 in hematopoiesis [108], there has been interest in selectively targeting JAK1 to suppress pro-inflammatory cytokine signaling and alleviate myelofibrosis-associated symptoms without causing or worsening cytopenias. However, it was recently shown that conditional loss of JAK1 in hematopoietic stem cells (HSCs) reduces their self-renewal capacity and markedly alters lymphoid/myeloid differentiation in vivo [109]. JAK1-deficient HSCs were unable to rescue hematopoiesis in the setting of myelosuppression, exhibiting increased quiescence, an inability to enter the cell cycle in response to hematopoietic stress, and a marked reduction in cytokine sensing [109]. Itacitinib is a selective JAK1 inhibitor that was studied in a phase 2 trial in 87 patients with myelofibrosis and baseline platelets ≥ 50 × $10^9$/L, most of whom had not received prior JAK inhibitor therapy [84]. Approximately a third of patients in the higher dose cohorts achieved ≥ 50% reduction in TSS at week 12 (primary endpoint), but rates of ≥ 35% SVR were much lower (Table 2). Over half the RBC transfusion-dependent patients were able to lower their transfusion requirements by ≥ 50% during weeks 1–24 [84]. This agent is now being studied in combination with low-dose ruxolitinib (stable dose for ≥ 8 weeks of < 20 mg/day) in a phase 2 trial (NCT03144687) geared towards patients who are unable to have their ruxolitinib dose optimized because of cytopenias. The trial also has an itacitinib monotherapy cohort for patients who have

experienced spleen growth after initial response to ruxolitinib or had to discontinue it due to hematologic toxicities.

## Some final concepts

The telomerase inhibitor imetelstat generated much enthusiasm when several durable complete and partial responses, unusual with ruxolitinib monotherapy, with reversal of bone marrow fibrosis in all four patients who achieved a CR, were reported in a pilot study, although responses did not correlate with baseline telomere length [110•]. The development of imetelstat in myelofibrosis was then taken over by Janssen, and the much-awaited results from the phase 2 IMbark™ trial (Table 2) in patients with intermediate-2/high-risk myelofibrosis who have failed therapy with a JAK inhibitor (NCT02426086) were released at the 2018 ASH annual meeting [81]. Patients were required to have worsening of splenomegaly-related abdominal pain and either worsening splenomegaly on JAK inhibitor therapy or no reduction in spleen volume after 12 weeks of the same, and active symptoms of myelofibrosis. The 4.7 mg/kg dosing arm ($n$ = 48) was closed to new patient entry due to insufficient activity after an interim analysis, and the patients permitted to dose-escalate. One hundred and seven patients in all were treated for a median of 26.9 weeks. Importantly, after a median follow-up of 27.4 months, median survival was 19.9 months in the 4.7 mg/kg dosing arm and 29.9 months in the 9.4 mg/kg dosing arm [81]. However, Janssen has recently announced the termination of their involvement in the development of imetelstat [111].

Based on preclinical studies showing that $JAK2^{V617F}$ leads to overexpression of murine double minute 2 (MDM2) in MPN [112] and preliminary evidence that the small-molecule MDM2 antagonist, idasanutlin, is active in patients with polycythemia vera and essential thrombocythemia [113], there is interest in targeting MDM2 to activate wild-type p53 in myelofibrosis. Preclinical studies in xenograft models of $CALR^{del52}$, $MPL^{W515L}$, and $JAK2^{V617F}$ MPN also support testing mitogen activated protein kinase kinase (MEK) inhibitors [114, 115], and the selective inhibitor of nuclear export, selinexor [116], in patients with myelofibrosis. Finally, our enhanced understanding of how mutant calreticulin binds to the thrombopoietin receptor, MPL (requirement for the lectin-dependent function of mutant calreticulin to bind to the extracellular domain of MPL) to drive oncogenesis in MPN [117•] has led to the development of novel, vaccine-based approaches to target this immunogenic [118, 119•] mutant protein, but these have not entered the clinic yet. Although barring a few reports in early disease [120, 121], interferons have mostly been disappointing in myelofibrosis; early data on the combination of ruxolitinib and pegylated interferon-alfa from the ongoing RUXOPEG study look promising in terms of safety and efficacy [122], with no clinical evidence of antagonism due to JAK1 (important for interferon signaling) inhibition by ruxolitinib.

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## Compliance with Ethical Standards

**Conflict of Interest**

Prithviraj Bose has received research funding from Incyte Corporation, Celgene, Blueprint Medicines, Constellation Pharmaceuticals, Kartos Therapeutics, CTI BioPharma, Astellas, and Pfizer, and has received compensation from Incyte Corporation, Celgene, and Blueprint Medicines for service as a consultant.

Mansour Alfayez declares that he has no conflict of interest.

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**Human and Animal Rights and Informed Consent**

This article does not contain any studies with human or animal subjects performed by any of the authors.

## Publisher's Note



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- •• Of major importance

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