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REVIEW

Standard care and investigational drugs in the treatment of myelofibrosis

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Abstract

Myelofibrosis (MF) is a heterogeneous disorder characterized by splenomegaly, constitutional symptoms, ineffective hematopoiesis, and an increased risk of leukemic transformation. The ongoing research in understanding the pathophysiology of the disease has allowed for the development of targeted drugs optimizing patient management. Furthermore, disease prognostication has significantly improved. Current therapeutic interventions are only partially effective with only allogeneic stem cell transplant potentially curative. Ruxolitinib is the only approved therapy for MF by the US Food and Drug Administration. However, despite efficacy in reducing splenomegaly and controlling symptomatology, it is not associated with consistent molecular or pathologic responses. Drug discontinuation is associated with a dismal outcome. The therapeutic landscape in MF has significantly improved, and emerging drugs with different target pathways, alone or in combination with ruxolitinib, seem promising.

Keywords: anemia, JAK inhibitors, myelofibrosis, survival, treatment.

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Introduction

Myelofibrosis (MF) is *a BCR-ABL1*-negative chronic myeloproliferative neoplasm (MPN) that includes primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (PPV-MF), and post-essential thrombocythemia myelofibrosis (PET-MF), characterized by stem cell-derived clonal proliferation[1] that is often, but not always, accompanied by somatic mutations, which are classified into driver mutations (i.e., a mutation of either *JAK2*, *CALR*, or *MPL*) and subclonal mutations.

MF is a heterogeneous disease with up to 30% of patients being initially asymptomatic[2]; in 83–89% of newly diagnosed PMF patients, a palpable splenomegaly is present, approximately one-third have grade 2 anemia (i.e., hemoglobin [Hb] values <10 g/dL), and about one-fourth are burdened by constitutional symptoms.[2–4] Thrombocytopenia, and specifically a platelet count $\leq 100 \times 10^9$/L, is present in approximately 15% of MF patients at diagnosis and is less common in secondary myelofibrosis (SMF).[5,6] Furthermore, all these features tend to be progressive over time and, at least with regards to anemia, systemic symptomatology, and thrombocytopenia, carry an independent negative prognostic weight.[2,3,7–9] Both PMF and SMF negatively affect patients' life expectancy and can transform into blast phase (BP).[2]

The considerable heterogeneity in clinical presentation and disease evolution results in widely variable patient outcomes, which are being captured with increasing accuracy by continuously evolving prognostic scoring systems, such as the PMF-specific International Prognostic Scoring System (IPSS),[2] its dynamic version (dynamic IPSS [DIPSS]),[3] and its augmented version (DIPSS-Plus),[8] which increases the number of risk factors considered. Recent attempts at including molecular characteristics into prognostic models have resulted in the Mutation-Enhanced International Prognostic Scoring System (MIPSS70),[9] which, however, requires that patients be characterized for subclonal mutations. Even more recently, a transplant-specific clinical-molecular model has been put forward.[10]

Progressive splenomegaly and anemia are distinctive in the evolution from PV to SMF,[11] while genetic[12] and molecular[6] characteristics, as well as gender,[13] impact disease outcomes in SMF. Differences in terms of genetics, phenotype, and prognosis between PMF and SMF[4] have recently been acknowledged and have resulted in the development of a

prognostic tool specific for SMF: the Myelofibrosis Secondary to PV and ET Collaboration-Prognostic Model (MYSEC-PM).[14]

The unraveling, albeit partial, of cellular pathways underlying disease occurrence and progression has led the way to the development of targeted treatments; however, the only curative strategy is allogeneic stem cell transplant (ASCT). This approach is recommended for transplant-eligible patients with higher risk disease (and intermediate-1 risk MF with an adverse cytogenetic or molecular profile) and is associated with a significant transplant-related mortality and relapse rate.[15–18] Current recommendations and guidelines suggest a problem-based approach for transplant-ineligible patients and for those with lower risk disease.[19–21] Available drugs are often unable to concurrently address different problems that can coexist within the same patient, such as cytopenias and myeloproliferative features. In such a scenario, drug combinations targeting different clinical issues may be of value.[22] This review is focused on problem-based treatment options for MF patients, discriminating in standard care and novel therapeutic approaches still under investigation. Published literature was reviewed using available databases (PubMed, Medline) and web pages (clinicaltrials.gov).

Management of MF-related cytopenias

Currently, there are no US Food and Drug Administration (FDA) or European Medicines Agency (EMA)-approved drugs for the treatment of anemia, with or without red blood cell (RBC) transfusion dependence, and thrombocytopenia in MF, but commonly used drugs include corticosteroids, androgenic steroids, erythropoietin, and immunomodulatory drugs (such as thalidomide, lenalidomide, and pomalidomide). Furthermore, given the detrimental effects of anemia and transfusion-related iron overload, a proper iron chelation therapy is crucial and seems to be effective on outcome of disease,[23] in particular in terms of hematologic improvement and survival.[24–26]

Corticosteroids

The rationale for the use of corticosteroids is the suppression of inflammatory stimuli and immune-mediated mechanism at the basis of pathogenesis of MF-anemia. Clinical data on the use of corticosteroids are scant and mostly derive from case studies.

More recently in a retrospective study including 30 MF patients with severe anemia who had failed conventional therapies, treatment with oral prednisone, at an initial daily dose of 0.5–1 mg/kg, allowed the achievement of anemia response in 40% of them, with the median response duration of 12 months, within the first 4 months of treatment. A durable platelet response in a quarter of thrombocytopenic patients was also obtained.[27]

Androgens

Androgens have been largely used to treat the anemia of MF with responses ranging from 30% to 60% of patients.[28,29] In a recent retrospective analysis on 50 patients treated with danazol, 30% achieved anemia response (defined as transfusion independence or an Hb increase >2 g/dL in patients without transfusion requirements, both maintained for at least 12 weeks) with a median response duration of 14 months and a response rate higher in transfusion-independent patients.[30] Thrombocytopenia response was observed in almost a quarter of patients with moderate thrombocytopenia (platelet count $<100 \times 10^9$/L).[30] The initial dose to achieve the desired effect is 600 mg daily; treatment should be maintained for at least 6 months before stopping for lack of efficacy. Once a response is obtained, the danazol dose must be progressively reduced to the minimum necessary to maintain efficacy. The therapy is well tolerated. Androgenic steroids are associated with risks of liver toxicity and liver and prostate cancers: before starting danazol, prostate cancer must be excluded, and a regular monitoring of liver function, prostatic-specific antigen levels, and ultrasound imaging to screen for hepatic tumors has been recommended.[31] In one retrospective study, history of treatment with danazol was associated with an increased risk of leukemic transformation.[32] Treatment with danazol led to telomere elongation in patients with telomere diseases[33]: how this mechanism of action is involved in the improvement of MF-related cytopenias is unknown. A recent multicenter phase 2 study of combination therapy with ruxolitinib and danazol showed clinical improvement in terms of spleen reduction in 21.4%, and most patients had a sustained response without hematological deterioration.[34]

Erythropoiesis-stimulating agents

Erythropoiesis-stimulating agents (ESAs) are widely used for the treatment of anemia associated with both lymphoid and myeloid neoplasms. Anemia response rates of approximately 40% have been reported with ESAs.[35,36] Lower baseline erythropoietin levels (EPO <125 U/L) and transfusion-independent mild anemia are generally associated with a favorable response.[31] Responses usually are seen within a few weeks, and a lack of response after 3 months is a criterion to discontinue the treatment. The treatment is well tolerated. Safety concerns concerning the possible worsening of splenomegaly[35–37] and an association of leukemic transformation with ESA use[32] have led investigators to recommend a careful use of ESA.

Despite potential antagonistic mechanisms of action on Janus kinase 2, some responses on anemia have been reported with the addition of ESAs to ruxolitinib in a small subset of patients in the COMFORT II trial[38] and more recently in an Italian study.[39] An observational trial aimed to better assess the combination of ESAs and ruxolitinib is ongoing (NCT03208803).

Immunomodulatory drugs

Thalidomide, lenalidomide, and pomalidomide are immunomodulatory agents (IMiDs) with clinical activity in a variety of disorders, including other hematologic diseases. They are characterized by anti-angiogenic, anti-TNF-α, and T-cell costimulatory activity in addition to inhibition of T-regulatory cell proliferation.[40,41]

The standard dose of thalidomide, at 100–200 mg/day, is associated with anemia response of 29% and improvement of platelet count in 35% of cases; however, it is characterized by an unfavorable dose-related toxicity profile composed of constipation, fatigue, paresthesiae, sedation, hematologic toxicity, and myeloproliferative acceleration.[42]

To minimize the drug toxicity, several trials were performed with lower dose of thalidomide. Mesa and colleagues performed an analysis on 21 patients treated with low-dose thalidomide (50 mg/day) and prednisone: 20 patients (95%) were able to complete 3 months of treatment. An objective clinical response was demonstrated in 13 (62%) patients: all improvements in anemia; 40% became transfusion-independent, and among patients with thrombocytopenia, 75% experienced an increase of 50% or higher in platelet count. Marchetti and colleagues demonstrated that doses of thalidomide starting at 50 mg and going up to 400 mg/d allowed to achieve RBC transfusion independence and an increase of platelet count $>50 \times 10^9$/L in 39% and 22% of patients, respectively.[43] A combination of ruxolitinib and thalidomide is now under investigation (NCT03069326).

Lenalidomide yielded overall response rates of 22% for anemia and 50% for thrombocytopenia with high discontinuation rate because of side effects, mainly due to excessive myelosuppression.[44]

To reduce toxicity, lenalidomide has been combined with a low-dose prednisone taper. In the study conducted at the MD Anderson Cancer Center, anemia response was 30%, and an improvement on bone marrow reticulin fibrosis was noted: there were no neutropenia or thrombocytepenia responses.[45] By contrast, in another study the anemia response remained 23%, and there was a high rate of discontinuation due to hematologic and nonhematologic toxicity, whereas no regression of the marrow fibrosis was seen.[46]

A trial evaluating the combination ruxolitinib and lenalidomide has been performed, and it was characterized by a high rate of early discontinuations due to hematologic side effects.[47]

Pomalidomide is a potent second-generation IMiD with a superior toxicity and safety profile than thalidomide and lenalidomide. Several MF trials with pomalidomide showed encouraging anemia and platelet responses.[48–50] However, the only phase 3 placebo-controlled randomized trial of pomalidomide yielded disappointing results: the study randomized 252 patients with MF in a 2:1 double-blinded fashion to pomalidomide 0.5 mg daily or placebo. Unfortunately, the rate of RBC transfusion independence was 16% in both arms. However, platelet response was significantly more in the pomalidomide group: 22% pomalidomide *versus* 0% in the placebo arm.[51] A recent German study of pomalidomide in 103 cytopenic patients with MF showed that an erythroid response was observed in 20% of patients, seven patients showed a platelet response, and two patients a neutrophil response, with three patients responding in ≥1 lineage.[52] A pomalidomide dose of 2 mg/d and mutated *TET2* were significantly associated with response, and receipt of prednisolone did not affect the rate or duration of response (median duration of responses was 13.0 months).

An ongoing phase 1b/2 trial to assess the efficacy of oral drug combination ruxolitinib and pomalidomide is now under investigation (NCT01644110).

Novel agents for MF-related cytopenias

There are ongoing studies evaluating agents targeting various key mediators involved in the pathogenesis of MF such as transforming growth factor-β (TGF-β; sotatercept, luspatercept, and fresolimumab) and bone marrow fibrosis reticulin (PRM-151), as promising options in the management of cytopenias in MF.

Sotatercept and luspatercept are ligand traps consisting of the extracellular domain of ActRII A linked to the human IgG1 Fc domain. These agents sequester bone marrow stroma-derived ligands belonging to the TGF-β superfamily and prevent their binding to activin receptors IIA (sotatercept) and IIB (luspatercept), relieving blockade of terminal stages of erythropoiesis. Both drugs have been tested in myelodysplastic syndrome (MDS), MF, and beta-thalassemia. Preliminary results from a phase 2 trial in MF patients showed that 6/17 (35%) and 1/8 (12.5%) patients treated with sotatercept (as a subcutaneous injection 0.75 or 1 mg/kg) alone and combined with ruxolitinib, respectively, achieved erythroid response, with good tolerance and safety profile.[52] Most adverse events were grade 1 or 2: the only adverse events possibly related to this drug were hypertension, myalgia, bone pain, and pain in extremity.

In a recent randomized, placebo-controlled phase 3 trial (MEDALIST) in 229 transfusion-dependent MDS with ringed sideroblasts, 38% and 53% of patients who received luspatercept (1–1.75 mg/kg/3 weeks) achieved transfusion independence and erythroid response, respectively, with a median response duration to luspatercept of 30.6 weeks and a favorable safety profile.[52]

Luspatercept is now being studied in a multicenter phase 2 clinical trial in anemic patients with MF, both transfusion-dependent and transfusion-independent, and both alone and in conjunction with a stable dose of ruxolitinib (NCT03194542).

Fresolimumab, a monoclonal antibody neutralizing all isoforms of TGF-β, induced reduction in TGF-β levels in two evaluable

patients, improvements of anemia, but no significant changes in fibrosis were documented.[53]

PRM-151 is a recombinant intravenous form of pentraxin-2, an acute phase response protein involved in tissue repair and prevention and/or reversal of fibrosis in preclinical models.[54] A phase 2 study of PRM-151 (intravenously administration every 28 days) alone or in combination with ruxolitinib showed a clinical benefit in terms of spleen 26% and cytopenias: anemia response in 40% and platelet response in 62% of treated subjects; six patients had response of at least one grade reduction in bone marrow fibrosis. Improvement in symptoms, spleen, cytopenias, and fibrosis were also noted to be durable. In long-term follow-up of 13 patients who continued the drug up to 72 weeks, 69% and 38% of treated patients had >50% or 100% improvement in their symptoms (MPN-SAF total symptom score), respectively. Three of five patients who were transfusion-dependent at baseline achieved transfusion independence. In general, PRM-151 was well tolerated.[36]

Management of splenomegaly and constitutional symptoms

Drugs such as hydroxyurea, widely used in MPNs, are associated with only partial and transient responses in MF.[55] The recent advent of JAK (Janus kinase) inhibitors has dramatically impacted our ability to treat splenomegaly and constitutional symptoms.[22] A limited number of JAK inhibitors has been studied in randomized clinical trials and will be reported in this section.

Ruxolitinib

Ruxolitinib, a JAK1/2 inhibitor, is currently the only drug licensed for the treatment of PMF and SMF, following the benefits in terms of spleen volume reduction (SVR) and symptom improvement demonstrated by the pivotal phase 3 COMFORT trials conducted in patients with IPSS intermediate-2 and high-risk disease (COMFORT-1, ruxolitinib compared with placebo, and COMFORT-2, ruxolitinib compared with best available therapy).[56,57]

The primary endpoint (SVR ≥35% at week 24, COMFORT-1, and week 48, COMFORT-2) was reached in 41.9% and 28.5% of ruxolitinib-treated patients in COMFORT-1 and -2, respectively (*versus* approximately 0% in the control arms of both studies). MF-related symptoms and other means of assessing quality of life were also significantly improved during ruxolitinib treatment. Response occurred irrespective of underlying driver mutation, reflecting the constitutive activation of the JAK/STAT pathway common to all MF patients. Furthermore, *post hoc* analyses of the COMFORT trials have shown that responses are also to be expected in patients with so-called high-risk additional mutations (i.e., mutations of *ASXL1*, *EZH2*, *SRSF2*, *IDH1/2*). The negative outcome prediction associated with such mutations is however not fully abrogated by ruxolitinib.[58,59]

Data from COMFORT-2 showed that ruxolitinib seems to induce stabilization and reduction of bone marrow (BM) fibrosis in 32% and 16% of MF patients.[60] A retrospective analysis of BM data from patients participating in a phase 1/2 ruxolitinib study (INCB18424-251) and from patients treated with best available therapy at a large referral center showed that worsening of BM fibrosis was more common with best available therapy, whereas improvement of BM fibrosis occurred more frequently with long-term ruxolitinib therapy and was associated with SVR.[61] Complete resolution of BM fibrosis is however uncommon with ruxolitinib treatment. Reductions in *JAK2*V617F allele burden, mostly relatively mild (one-third of patients having a >20% allele burden reduction) and of uncertain clinical significance, were documented in COMFORT-2.[60] Furthermore, current evidence suggests that ruxolitinib is not effective in preventing leukemic transformation in MF.[62]

As the primary endpoint of the available randomized clinical trials was SVR, attempts of defining predictors of SVR are underway. A subgroup analysis of COMFORT-1 has confirmed that efficacy is maintained across all analyzed baseline factors (i.e., MF subtype, age, IPSS risk group, baseline Eastern Cooperative Oncology Group performance status, *JAK2*V617F mutational status, baseline hemoglobin level, baseline platelet count, baseline palpable spleen size, and baseline quartile of spleen volume and total symptom score).[63] A greater or equal to 50% *JAK2*V617F allele burden has also been identified as a predictor of spleen response in a retrospective study on 69 patients published a few years ago.[64] Furthermore, harboring ≥3 mutations seems to be inversely correlated with spleen response and with time to treatment discontinuation.[59] Information deriving from a multicenter, retrospective collection of ruxolitinib-treated patient data pointed to a better SVR at 6 months when treatment was started in an earlier disease phase, that is, in the presence of splenomegaly palpable at <10 cm from the left costal margin, of a platelet count ≥200 × 10^9/L, and when the patient is not transfusion-dependent. Patients with intermediate-2/high-risk IPSS disease also seemed to have a worse chance at obtaining an SVR with respect to intermediate-1 risk patients, as did those starting treatment more than 2 years after diagnosis.[65] Even though splenomegaly is not included in prognostic scores outside the transplant setting, a pooled analysis of the COMFORT trials revealed a 1.14-fold increased risk of death for each additional 5 dL of spleen volume at baseline. Furthermore, any degree of SVR ≥10% during ruxolitinib treatment was associated with a better prognosis with respect to achieving a less than 10% SVR from baseline.[66] The 5-year update of the COMFORT trials revealed a median duration of spleen response of approximately 3 years.[60,67]

The most common adverse effects were anemia (grade 3/4, 42–45%) and thrombocytopenia (grade 3/4, 13–31%), which often resulted in dose reduction and treatment discontinuation.[56,57] The development of drug-induced anemia, which occurs predominantly early during therapy, does however not seem to

bear the same negative prognostic weight as disease-related anemia.[68,69] Ruxolitinib affects immunologically relevant cytokines and T- and NK-cell repertoire/function, leading to variable degrees of immunosuppression. Accordingly, patients treated with ruxolitinib need to be screened at baseline for previous exposure to infectious agents (e.g., *M. tuberculosis*, HBV, VZV) and monitored throughout treatment.[70,71] In the phase 4 postmarketing study (JUMP study), the incidence of the most frequent infections was 8% for herpes zoster, 6.1% for bronchitis, and 6% for urinary tract infections.[62]

A multicenter retrospective analysis has suggested that patients with a previous infectious event and those pertaining to the high-risk IPSS category have a higher risk of developing infections under ruxolitinib. Conversely, an SVR by palpation of ≥50% after 3 months of treatment was found to reduce the risk of infections.[72] A recent publication raised considerable concern regarding the possibly increased risk of developing aggressive lymphoproliferative disorders in MPN patients treated with JAK inhibitors, especially in the presence of a pre-existing B-cell clone.[73] Subsequent analyses of large academic datasets did however not confirm such data.[74,75] A nested case–control study including 37 ruxolitinib-treated MF patients experiencing a second cancer, revealed an increased risk of developing a non-melanoma skin cancer with respect to ruxolitinib-naive patients, without however disclosing an increased risk of developing a lymphoid neoplasm.[76]

None of the available randomized clinical trials was designed to demonstrate a survival benefit with ruxolitinib therapy. However, a *post hoc* analysis of 5-year data pooled from COMFORT-1 and -2, with the aid of a statistical tool that takes into account the crossover design of the trials, shows a 30% reduction in the risk of death among patients randomized to ruxolitinib compared with the control group (median overall survival [OS], 5.3 *versus* 3.8 years, respectively; hazard ratio [HR], 0.70 [95% CI: 0.54–0.91]; p=0.0065). This benefit was even more pronounced after correction for crossover (median OS, 5.3 *versus* 2.3 years; HR [ruxolitinib *versus* control group with crossover correction], 0.35 [95% CI: 0.23–0.59]).[77] Its effect on survival has also been demonstrated by matching ruxolitinib-treated patients within the COMFORT-2 cohort and patients conventionally treated within the DIPSS cohort.[78]

Of note, the strength of evidence provided by the COMFORT trials, notwithstanding their randomized design, is determined by many factors, such as comparator choice and outcome and population indirectness.[79–81]

Median survival after ruxolitinib discontinuation was 13–14 months.[82,83] Clonal evolution was documented in approximately one-third of patients after ruxolitinib discontinuation (of whom 61% acquired a mutation of *ASXL1*) and was associated with a particularly dismal outcome (median overall survival of 6 months). Low platelet counts at the start or end of therapy were associated with worse survival after discontinuation and RBC transfusion dependence at baseline was associated with clonal evolution (48 *versus* 15%; p=0.001).[82]

A list of ruxolitinib-based combination trials is reported in Table 1.

Fedratinib

Fedratinib is a JAK2-selective inhibitor tested against placebo in intermediate-2/high IPSS risk, JAK-inhibitor naive MF patients in the phase 3 randomized clinical trial, JAKARTA. The study assessed the efficacy of two different fedratinib doses (400 and 500 mg once daily). The primary endpoint (SVR ≥35% at week 24) was reached in 36% (400 mg QD) and 40% (500 mg QD) of fedratinib-treated patients, *versus* 1% in the placebo arm. Symptom response rates at week 24 were 36%, 34%, and 7% in the fedratinib 400 mg QD, 500 mg QD, and placebo groups, respectively (p<.001). Myelotoxicity was common, with grade 3/4 anemia, thrombocytopenia, and neutropenia occurring in 43%, 17%, and 8% of the patients treated with fedratinib 400 mg QD, and in 60%, 27%, and 18% of those in the 500 mg QD arm. Furthermore, mostly low-grade gastrointestinal toxicity (probably due to *fms*-like tyrosine kinase 3 [FLT3] inhibition), increased levels of liver transaminases, and elevated pancreatic enzyme levels were relatively common. Encephalopathy was reported in 4 out of 97 patients who received fedratinib at the highest dose of 500 mg QD,[84] not applied in subseequent studies. A subsequent phase 2 study, JAKARTA-2, evaluated the 400 mg QD schedule in ruxolitinib resistant or intolerant MF patients. In such a difficult patient population, results in terms of efficacy were overall encouraging, as an SVR of ≥35% at week 24 was reached in 55% of patients and the symptom response rate at the same timepoint was 26%.[85] The study was however prematurely terminated due to neurologic toxicity concerns based on outcomes of other fedratinib trials. Specifically, safety reviews alerted to an increased incidence of Wernicke's encephalopathy in fedratinib-treated patients. A retrospective evaluation of nine fedratinib trials enrolling 670 MPN or solid tumor subjects identified between three and five patients who experienced Wernicke's encephalopathy (0.4–0.7%), which in one case occurred in the setting of malnutrition and in two cases resolved without drug interruption, suggesting that fedratinib does not inhibit thiamine absorption.[86] Fedratinib has consequently been resumed, and the drug is currently being tested in a phase 3b trial including MF patients previously treated with ruxolitinib (NCT03755518).

Pacritinib

Pacritinib is a JAK2-selective inhibitor also active against FLT3. Two phase 3 randomized clinical trials of pacritinib *versus* best available therapy have been conducted either in JAK-inhibitor naive patients (PERSIST-1) or both in JAK-inhibitor naive and pretreated patients (PERSIST-2).[87,88] Of note, PERSIST-2 tested the efficacy of two different pacritinib schedules (400 mg QD and 200 mg twice a day), and 45% of patients in the control arm of the trial were treated with ruxolitinib as best available therapy. The primary endpoint (SVR ≥35% at week 24) was

Table 1. Ruxolitinib-based combination trials in myelofibrosis

Class	Agent (combined with Ruxolitinib)	Target(s)	Phase	Status	Previous JAK-inhibitor	Preliminary efficacy data	Major toxicities	NCT.gov identifier	Ref.
Epigenetic agents	Azacitidine	DNA methylation	2	Recruiting	RUX-treated excluded	- 72% achieved IWG-MRT responses, 23% after addition of azacitidine - 60% had improved BM fibrosis by ≥1 grade - 64% had ≥50% reduction in palpable spleen length at any time, 57% at wk 24 - 82% had molecular responses	Myelosuppression	NCT01787487	96
	Pracinostat	HDAC	2	Completed	Excluded (except for RUX for <3 months and ongoing at the time of screening)	- 80% clinical improvement in spleen, symptoms or both at any time - Most responses preceded pracinostat introduction	- Anemia and thrombocytopenia - High discontinuation rate, mostly due to adverse events	NCT02267278	97
	Panobinostat	HDAC	1b	Active, not recruiting	Allowed	- At RP2D, 57% and 39% had ≥35% SVR at wk 24 and 48 - 29% had ≥20% decrease in *JAK2*V617F AB at wk 48	Anemia, thrombocytopenia, diarrhea, asthenia	NCT01433445	98
	Panobinostat	HDAC	1/2	Completed	Allowed	-	-	NCT01693601	
	CPI-0610	BET bromo-domain	2	Recruiting	Allowed	-	-	NCT02158858	
Hedgehog pathway inhibitors	Vismodegib	SMO	1b	Completed	Excluded	- 30% achieved a ≥35% SVR at week 24 - 50% symptom response - No anemia responses - No improvements in BM fibrosis	- All pts experienced grade 1/2 muscle spasm - Grade 1/2 dysgeusia (50%), alopecia (50%), and nausea (40%) - Grade 1/2/3 thrombocytopenia (50%)	NCT02593760	99

(*Continued*)

Table 1. (*Continued*)

Class	Agent (combined with Ruxolitinib)	Target(s)	Phase	Status	Previous JAK-inhibitor	Preliminary efficacy data	Major toxicities	NCT.gov identifier	Ref.
	Sonidegib	SMO	1b/2	Completed	Excluded	- 55.6% achieved ≥35% SVR any time, 44.4% at wk 24 - 92.6% had a ≥50% reductions in palpable splenomegaly at any time, nonpalpable in 55.6% - Mean change in *JAK2*V617F AB was -9.0% (range, -56.5% to 7.0%) at wk 24	Anemia and muscle spasms, increased creatinine kinase, myalgias	NCT01787552	100
JAK inhibitors	Itacitinib	JAK1	2	Recruiting	- RUX-treated allowed - JAK1-inhibitors excluded	-	-	NCT03144687	
Immuno-modulators	Thalidomide	Immuno-modulation	2	Recruiting	RUX-treated allowed (except if in conjunction with thalidomide)	- Clinical improvement in 40% - Plt responses in 60%	Grade 3/4 limb edema, diarrhea, neutropenia, and deep vein thrombosis occurred in 1 pt each (6.7%)	NCT03069326	101
	Lenalidomide	Immuno-modulation	2	Completed	RUX-treated excluded	Failure to meet the predetermined efficacy rules for treatment success resulted in early termination of the study	- Myelosuppression, GI symptoms - High rate of early lenalidomide interruptions	NCT01375140	47
	Pomalidomide	Immuno-modulation	1/2	Recruiting	Allowed	- 3/37 (8%) anemia response, although 12 pts (32%) remained on study beyond 12 cycles because of either response or stable disease with clinical benefit - Mean Hb increased from 8.6 g/dL at baseline to 9.3 g/dL at the end of cycle 12	- Worsening anemia within the first 6 cycles in 13 subjects (35%) - Fatigue in 11 (30%)	NCT01644110	102

(*Continued*)

Table 1. (*Continued*)

Class	Agent (combined with Ruxolitinib)	Target(s)	Phase	Status	Previous JAK-inhibitor	Preliminary efficacy data	Major toxicities	NCT.gov identifier	Ref.
PI3K/AKT/mTOR pathway inhibitors	Buparlisib	Pan-PI3K	1b	Terminated	Allowed	- At RP2D. 75% and 86.7% of JAKi naive patients and 35.3% and 36.4% of JAKi pretreated pts achieved a ≥50% reduction in palpable splenomegaly at wk 24 and 48 - Approximately, 40% of pts had a spleen volume reduction of ≥35% at wk 24 in the expansion phase - Modest effect on *JAK2*V617F allele burden and rare improvement or stabilization in BM fibrosis	Anemia, thrombocytopenia, anxiety, depression	NCT01730248	103
	Idelalisib	PI3K-delta	1	Completed	Allowed, RUX-treatment at study entry mandatory	-	-	NCT02436135	
	Parsaclisib	PI3K-delta	2	Recruiting	Allowed	63% achieved an SVR at wk 24 (median change -8.8%), median change in TSS -35.9% at wk 24	Rash and increased liver transaminases, no colitis reported	NCT02718300	104
	Umbralisib	PI3K-delta	1	Recruiting	Only RUX-treated pts allowed	- 2 patients (9%) achieved durable CR - 48% achieved IWG-MRT-definied clinical improvement at any time	Myelosuppression, liver/pancreatic enzyme elevation, colitis, dyspnea, diarrhea	NCT02493530	105
Other agents	Ribociclib/PIM447	CDK4/6 inhibitor Pan-PIM kinases	1	Active, not recruiting	Allowed	-	-	NCT02370706	

(*Continued*)

Table 1. (*Continued*)

Class	Agent (combined with Ruxolitinib)	Target(s)	Phase	Status	Previous JAK-inhibitor	Preliminary efficacy data	Major toxicities	NCT.gov identifier	Ref.
	PU-H71	HSP90	1	Active, not recruiting	Allowed, RUX-treatment at study entry mandatory	-	-	NCT03373877	
	PU-H71	HSP90	1	Not yet recruiting	Allowed, RUX-treatment at study entry mandatory	-	-	NCT03935555	
	Pevonedistat	NAE	1	Recruiting	Allowed, RUX-treatment at study entry mandatory	-	-	NCT03386214	
	Sotatercept	ActRIIA ligands	2	Recruiting	Allowed	- Anemia (including transfusion independence) response in 3/10 (30%) of pts - Response duration ranged between three and 15 months	No sotatercept-specific toxicity concerns	NCT01712308	106
	Luspatercept	ActRIIB ligands	2	Recruiting	Allowed	-	-	NCT03194542	
	Peg-IFN alpha-2a		1/2	Recruiting	JAK2-inhibitor treated pts excluded	-	-	NCT02742324	
	Navitoclax	Bcl-2/-xL	2	Active, not recruiting	Allowed, RUX-treatment at study entry mandatory	-	-	NCT03222609	

AB, allele burden; ActRIIA, activin receptor type IIA; ActRIIB, activin receptor type IIB; BET, bromodomain and extra-terminal; BM, bone marrow; CR, complete remission; GI, gastrointestinal; Hb, hemoglobin; HDAC, histone deacetylase; HSP90, heat-shock protein 90; IWG-MRT, International Working Group for Myeloproliferative Neoplasms Research and Treatment; JAKi, JAK-inhibitor; NAE, NEDD8-activating enzyme; Peg-IFN alpha-2a, Peg-interferon alpha-2a; PI3K/AKT/mTOR, phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin; Plt, platelet; pt(s), patient(s); RP2D, recommended phase 2 dose; RUX, ruxolitinib; SMO, smoothened homolog; SVR, spleen volume response; wk, week.

reached in 19% and 18% of pacritinib-treated patients in PERSIST-1 and -2, respectively (*versus* approximately 3–5% in the control arms of both studies). Symptom response rate at week 24 was higher in pacritinib-treated patients in the evaluable population, but not in the intention-to-treat population in PERSIST-1, and in pacritinib 200 mg twice daily treated patients in PERSIST-2. Pacritinib was expected to be less myelosuppressive and, accordingly, both trials did not specify a minimum platelet count for eligibility, at variance with the previously mentioned COMFORT and JAKARTA trials. Interestingly, responses were seen irrespective of platelet count.

Furthermore, one quarter of pacritinib-treated transfusion-dependent patients in the PERSIST-1 study became transfusion-independent. A temporary clinical hold, due to concerns over excess mortality due to cardiovascular events and bleeding, cut back the drug's development. As the hold has been lifted, a dose-finding (100 mg once daily *versus* 100 mg twice daily *versus* 200 mg twice daily) phase 2 study in ruxolitinib-pretreated patients has been conducted, without available results to date (NCT03165734). Pacritinib is also being studied in the pretransplant setting (NCT03645824) and in conjunction with sirolimus-based immune suppression for the treatment of graft *versus* host disease (NCT02891603).

Momelotinib

Momelotinib is a JAK1/2 inhibitor capable of alleviating inflammatory anemia through inhibition of ACVR1-mediated hepcidin expression in the liver, eventually resulting in stimulation of erythropoiesis.[89] Phase 3 randomized clinical trial data for momelotinib *versus* ruxolitinib (in JAK-inhibitor naive patients, SIMPLIFY-1) or *versus* best available therapy (in ruxolitinib-pretreated patients, SIMPLIFY-2) are available.[90,91] Of note, in SIMPLIFY-2, 89% of patients in the control arm of the trial were treated with ruxolitinib as best available therapy. Results of the SIMPLIFY-1 trial showed that, in JAK-inhibitor naive patients, SVR ≥35% at 24 weeks was non-inferior for momelotinib (26.5%) with respect to ruxolitinib (29%). On the contrary, symptom response rate was reduced in momelotinib-treated patients. Grade 3/4 anemia and thrombocytopenia, respectively, occurred in 13% and 7% of momelotinib-treated patients and momelotinib treatment was associated with reduced transfusion requirement. In the setting of ruxolitinib resistant or intolerant patients (SIMPLIFY-2), momelotinib proved not superior to best available therapy (which included ruxolitinib in 89% of cases) in obtaining an SVR ≥35% at 24 weeks. Similarly to what occurred in SIMPLIFY-1, momelotinib treatment was associated with reduced transfusion requirement. In both trials, treatment-emergent, generally irreversible, peripheral neuropathy occurred in 10–11% of patients who received momelotinib (all but one grade ≤2). Currently, there are no actively recruiting momelotinib trials listed in ClinicalTrials.gov.

Other, selected, single-agent treatments

NS-018

NS-018 is a selective JAK2-inhibitor evaluated in a phase 1/2 clinical trial in both JAK-inhibitor naive (52%) and pretreated (48%) patients. Spleen responses did not occur at the lowest doses (75 and 125 mg QD), whereas in all other instances (all NS-018 doses, both in JAK-inhibitor naive and pretreated patients), a ≥50% reduction in spleen length by palpation at any time was registered in at least 25% of patients. Overall, 56% of patients experienced such a response, whereas approximately one-third reached a 100% reduction in spleen length at any time. In the phase 2 part of the study, in which almost 80% of individuals were previously treated with a JAK-inhibitor, an SVR ≥35% at 24 weeks was however obtained only by 12% of patients. Grade 3/4 anemia and thrombocytopenia, respectively, occurred in 6% and 17% of patients (no cases of grade 4 anemia were reported, whereas thrombocytopenia was more frequent in patients with lower baseline platelet count). Treatment-emergent nonhematologic events were typically grade 1/2 neurological and gastrointestinal disorders, most commonly dizziness (23%) and nausea (19%).[92] Currently, there are no actively recruiting NS-018-based trials listed in ClinicalTrials.gov.

Alisertib

Alisertib is an aurora kinase A inhibitor capable of promoting polyploidization and differentiation of PMF megakaryocytes in preclinical murine models and patient samples. A pilot phase 1 trial was conducted in 24 JAK-inhibitor resistant/intolerant (63%) or ineligible (37%), intermediate-1 (33%), intermediate-2 (46%), and high (21%) DIPSS risk MF patients (NCT02530619).[93] It has to be underlined that patients could be deemed JAK-inhibitor-ineligible if transfusion-dependent (6/9, 67%), without splenomegaly (2/9, 22%), and in the presence of a high risk genotype (1/9, 11%). Alisertib reduced splenomegaly on physical exam and symptom burden in 29% and 32% of patients, respectively. Myelosuppression was frequent: grade 3 neutropenia, thrombocytopenia, and anemia occurred each in 21% of patients, whereas grade 4 neutropenia, thrombocytopenia, and anemia were recorded in 21%, 8%, and 0% of patients, respectively. Treatment-emergent non-hematologic events occurring in >10% patients included grade 1/2 gastrointestinal disorders. Unfortunately, the drug's discontinuation rate was quite high (17/24 patients discontinued, of whom 11 due to progressive disease or lack of response – of note, 2/5 patients with progressive disease transformed to acute leukemia). Correlative studies demonstrated that alisertib restored normal morphology and GATA1 expression to atypical megakaryocytes and reduced the degree of bone marrow fibrosis. Currently, there are no actively

recruiting, MF-restricted, alisertib-based trials listed in ClinicalTrials.gov.

Imetelstat

The telomerase inhibitor imetelstat seemed to hold much promise when impressive rates of partial and complete responses (with BM fibrosis reversal) were reported in a pilot study in MF.[94] A subsequent, phase 2 randomized study of two dose levels was conducted in intermediate-2/high risk DIPSS MF patients having failed treatment with a JAK-inhibitor (NCT02426086). The 4.7 mg/kg dosing arm was closed after an interim analysis due to insufficient activity. Six (10.2%) patients in the 9.4 mg/kg arm had an SVR ≥35% at 24 weeks, whereas 19 (32%) patients in the same arm had a symptom response. BM fibrosis improved in 18%. Imetelstat showed myelosuppression with grade 3/4 neutropenia, thrombocytopenia, and anemia occurring in 32%, 41%, and 39% of patients, respectively, in the 9.4 mg/kg cohort. Grade 3/4 infections and hemorrhagic events ensued in 10% and 5% of patients, respectively. Low-grade gastrointestinal toxicity was recorded in up to one-third of patients, whereas imetelstat-related hepatic toxicities were not observed. After a median follow-up of 27.4 months, median survival in the 9.4 mg/kg dosing arm was 29.9 months. Intriguingly, an association was observed between triple negativity and better outcome in the 9.4 mg/kg arm.[95] Currently, there are no actively recruiting imetelstat-based trials listed in ClinicalTrials.gov, and future development of the drug is uncertain.

KRT-232

Growing interest in small molecule inhibitors of MDM2, capable of activating wild type p53, in the field of MPNs has resulted in an ongoing multicenter, international phase 2 study to determine efficacy and safety of KRT-232 in patients with MF who have failed previous treatment with JAK inhibitor or ruxolitinib (NCT03662126). The study just started, and the estimated primary completion date is in August, 2021.

Conclusion

Management of MF is challenging. In the past decades, discovery of MPN driver mutations and a better understanding of pathophysiology of disease have led to great advances modifying the therapeutic algorithms and improvement of disease-related symptoms, quality of life, and survival. However, a therapeutic option for transplant-ineligible patients that can fully modify the natural history of the disease and prevent evolution to blast-phase is still lacking.

Anemia and thrombocytopenia in MF, in terms of negative prognostic and unmodifiable factors by conventional drugs, are the contemporary challenge in the present-day therapeutic landscape in MF. Furthermore, clinical data obtained by COMFORT trials and long-term follow-up analysis highlighted both the benefits obtained with ruxolitinib treatment in MF and the areas of persistent unmet clinical need, in particular with regard to (i) optimization of response to ruxolitinib, for example through improved patient selection based on the identification of reliable predictors of response, optimal timing of treatment intervention, and combination with drugs acting synergistically or capable of mitigating ruxolitinib's expected myelotoxicity, and (ii) identifying active second-line treatments for those patients who fail JAK inhibitor. Multiple different targeted therapies have been recently evaluated or are undergoing evaluation in MF. Most of the current clinical trials are finalized to improve overall response rate, targeting cytopenias, complete resolution of splenomegaly and bone marrow fibrosis. Target populations include suboptimal responders to ruxolitinb or ruxolitinib-failing patients. A better understanding of disease, treatment-resistance mechanisms, and pharmacodynamics of novel drugs is required.

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