Looking forward: novel therapeutic approaches in chronic and advanced phases of myelofibrosis

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Myelofibrosis (MF) is complex at the pathobiologic level and heterogeneous at the clinical level. The advances in molecular characterization of MF provide important insight into the mechanisms driving this chronic myeloid malignancy, refine risk stratification, offer novel therapeutic targets, and serve to measure therapeutic response. Although JAK2 inhibition has been the focus of laboratory and clinical efforts over the last decade, current experimental therapeutic approaches have broadened to include inhibitors of key alternative signaling pathways, epigenetic modulators, anti-fibrotics, and immunotherapies. Based on compelling preclinical rationale, a number of JAK2 inhibitor based combination therapies are now actively being evaluated in the clinic with the goal of disease course modification. The role and timing of hematopoietic stem cell transplant (HSCT) for MF has been challenged with the availability of commercial ruxolitinib and the plethora of experimental treatment options that exist. Integration of preconditioning JAK2 inhibition, reduced intensity conditioning regimens, and alternative donor sources are all being explored in an attempt to optimize this potentially curative modality. This review will summarize modern MF risk stratification, current clinical research approaches to chronic and advance phase MF focusing on novel agents alone and in combination, and update the reader on new directions in HSCT.

Learning Objectives

- To understand the evolution of prognostication in myelofibrosis
- To identify novel therapeutic approaches for the treatment of myelofibrosis

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Myelofibrosis (MF) is a term that encompasses primary myelofibrosis (PMF), post-essential thrombocythemia (ET) and post-polycythemia (PV) MF (PET-MF, PPV-MF), and is considered the most severe subtype of the classic BCR-ABL1–negative myeloproliferative neoplasms (MPNs). MF is recognized by the World Health Organization (WHO) as a clonal hematopoietic stem cell (HSC) disorder characterized by myeloproliferation within the bone marrow, extramedullary hematopoiesis (EMH) leading to progressive organomegaly, anemia, and a leukoerythroblastic peripheral blood picture. MF is associated with a propensity to evolve into acute leukemia (predominantly of myeloid lineage) in 10%-20% of cases in the first decade after diagnosis. Generally speaking, chronic phase (CP) can be characterized by either a peripheral blood or bone marrow blast count <10%, accelerated phase (AP) of 10%-19%, and blast phase (BP) ≥20%. The prognosis for patients with MPN-BP is dismal (estimated at 3-5 months) and is tragically not improved with the use of induction regimens commonly used for acute myeloid leukemia (AML), unless followed immediately by hematopoietic stem cell transplantation (HSCT).[1]

It is also increasingly appreciated that MF is an intensely inflammatory state mediated by hyperactive JAK-STAT signaling (importantly, irrespective of *JAK2* mutation status), which has been linked to the considerable symptom burden that results in reduced performance status and poor quality-of-life (QOL). The discovery of a point activating mutation in the *JAK2* gene in 2005, renewed interest in both the academic and pharmaceutical communities in the study of MPNs and rapidly resulted in the first and only Food and Drug Administration (FDA)-approved therapeutic for MF, and more recently PV. However, realization of the importance of *JAK2*V617F in diagnosis, prognosis, and treatment of MPNs was tempered by the reality that much remained unknown of the molecular pathophysiology governing these myeloid malignancies. Subsequent studies have identified other driver mutations (MPL515L/K, *CALR* exon 9) associated with hyperactive JAK-STAT signaling, as well as numerous additional molecular events that influence signal transduction (*CBL,LNK*), chromatin modification (*TET2, EZH2, IDH1/2, ASXL1*), and RNA splicing (*SF3B1, SRSF2, U2AF1*), tumor suppressor function (*TP53*) and collectively contribute to disease progression and evolution. Moreover, recent investigations suggest that order of mutational acquisition can further influence MF phenotype and even response to ruxolitinib therapy in vitro.[2] The current scientific appreciation of the complex clonal architecture of MPNs has provided the clinical basis for the current and future evaluation of single and combinations of agents targeting signaling pathways outside of the JAK-STAT pathway and epigenetic alterations. It is essential that current MF clinical trials incorporate mutational profiling at baseline to assess whether a mutational signature can predict therapeutic response.

Prior to discussing modern therapeutic advances in MF, it is worth updating the reader on the advances in risk stratification of MF

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patients in order to assess benefit/risk determination for experimental therapies and/or HSCT. The successes and deficiencies of current JAK2 inhibitor therapy for the treatment of MF must also be adequately reviewed before discussing ongoing trials evaluating agents directed at novel targets. Subsequently, this report will focus on next generation trials evaluating the combination of active agents from different therapeutic classes. Lastly, it is important to also acknowledge advances being made in HSCT as the only proven therapy to offer the possibility of cure in MF.

Refining risk stratification in MF

The first widely used risk stratification tool for MF was the Lille Classification which incorporated the following variables at time of diagnosis: white blood cell count (WBC) $>30 \times 10^9$/L or $<4 \times 10^9$/L earning 1 point and a hemoglobin <10 g/dL earning 1 point.[3] Low risk (0 points), intermediate risk (1 point), and high risk (2 points) had associated median overall survivals of 93, 26, and 13 months, respectively. This risk stratification tool was simple and could be used universally as it only required a complete blood count. However, it was also clear that in so much as it was easy to utilize, it did not incorporate other important prognostic disease features that could better distinguish between groups of patients with differing outcomes. In 2009, Cervantes et al, on behalf of the International Working Group for MF Research and Treatment (IWG-MRT), set out to develop a PMF prognostication tool at time of diagnosis that would better discriminate prognosis and therefore provide more confidence in therapeutic decision making.[4] The International Prognostic Scoring System (IPSS) incorporated five clinical features at the time of diagnosis that were found to have prognostic significance in multivariate analysis; age >65 years, presence of constitutional symptoms, hemoglobin <10g/dL, leukocytes $>25 \times 10^9$/L, peripheral blood blasts ≥1%. The IPSS risk groups of low (0 points), intermediate-1 (1 point), intermediate-2 (2 points), and high (3+ points) provided nonoverlapping survival curves with median OS of 135, 95, 48, and 27 months, respectively. Shortly after the advent of the IPSS, Passamonti et al proposed the dynamic IPSS (DIPSS) which allowed application of a risk stratification system at any point during PMF disease course.[5] The DIPSS risk groups used the same clinical variables as the IPSS, but more weight was assigned to anemia due to the higher prognostic power in time-dependent analysis. The DIPSS groups included low (0 points), intermediate-1 (1-2 points), intermediate-2 (3-4 points), and high risk (5-6 points) with a median OS not yet reached, 170, 48, 18 months, respectively. An age adjusted (aa)DIPSS was also created for PMF patients <65 years of age that would be traditionally considered appropriate for definitive therapy with HSCT. The DIPSS was later modified by the IWG-MRT to also include three additional negative prognostic risk factors including platelet count $<100 \times 10^9$/L, the need for red blood cell transfusions, and the presence of an unfavorable karyotype (complex karyotype or sole or two abnormalities that include +8, −7/7q−, i(17q), −5/5q−, 12p−, inv(3), or 11q23 rearrangement).[6] The DIPSS-plus stratifies patients into 4 risk groups with median OS of 185, 78, 35, and 16 months, respectively. Additionally, it was shown that an unfavorable karyotype and thrombocytopenia predicted leukemia-free survival (LFS) in this analysis with a 10 year risk of developing MPN-BP of 31% versus 12% in the absence of either risk factor.

Most recently, efforts to integrate cytogenomic information with the clinical prognostication systems have led to the development of the Genetics-Based Prognostic Scoring System (GPSS) and Mutation-Enhanced International Prognostic Scoring System (MIPSS; Figure 1.[7,8] The mutational status of the three key drivers (*JAK2*, *MPL*,

			Prognostic scoring system					
			Lille (1996)	IPSS (2009)	DIPSS (2010)	DIPSS+ (2011)	MIPSS (2014)	GPSS (2014)
Patient specific variable		Age		⊙	⊙	⊙	⊙	
Disease specific variables	clinic	Constitutional symptoms		⊙	⊙	⊙	⊙	
	laboratory	WBC[1]	⊙	⊙	⊙	⊙		
		Hemoglobin <10 g/dL	⊙	⊙	⊙	⊙		
		Peripheral blood blasts >1%		⊙	⊙	⊙		
		Platelet count[2]				⊙	⊙	
		RBC Transfusional support				⊙		
	genetic	Karyotype[3]				⊙		⊙
		Mutational[4] status					⊙	⊙

Figure 1. WBC indicates white blood cell; RBC, red blood cell; IPSS, International Prognostic Score System; DIPSS, dynamic IPSS; DIPSS+, DIPSS plus; MIPSS, Molecular International Prognostic Score System; and GPS, Genetics-Based Prognostic Scoring System. [1]WBC $<4 \times 10^9$/L or $>30 \times 10^9$/L in Lille; WBC $>25 \times 10^9$/L in IPSS, DIPSS, DIPSS+. [2]Platelets $<100 \times 10^9$/L in DIPSS+ and $<200 \times 10^9$/L in MIPSS. [3]DIPSS unfavorable karyotype: complex karyotype or 1 or 2 abnormalities including +8, −7/7q−, i(17q), −5/5q−, 12p−, inv(3) or 11q23 rearrangement; GPSS high-risk: complex without MK, 2 abnormalities not included in very high-risk category, 5q−, +8, other autosomal trisomies except +9, and other sole abnormalities not included in other risk categories; very high-risk: MK, inv(3), i(17q), −7/7q−, 11q or 12p. [4]Includes driver mutation status (JAK2/MPL/CALR), as well as ASXL1 and SRSF2.

CALR) in addition to the presence of other somatic mutations (*ASXL1*, *SRSF2*, *EZH2*, and *IDH1/2*) that have shown to influence outcome were included in the construction of these newer prognostication systems.[9,10] Age >60 years, hemoglobin <10g/dL, constitutional symptoms, platelets $<200 \times 10^9$/L, "triple-negativity", *JAK2/MPL* mutant, *ASXL1* mutant, and *SRSF2* mutant were all determined to have prognostic significance in multivariate analysis and used to develop 4 risk categories in a learning cohort. Low, intermediate-1, intermediate-2, and high-risk patients by MIPSS had OS of 26.4, 9.7, 6.4, and 1.9 years, respectively. When compared (using the Akaike information criterion) to the IPSS, the MIPSS provided refinement of the prognostic score and allowed the identification of subgroups of patients with a worse prognosis within an IPSS category. The genetics-based prognostic scoring system (GPSS) was developed by the Mayo group in a large cohort of PMF patients and validated in an independent patient cohort from Italy. The GPSS incorporates only cytogenetic and mutational prognostic data to create 4 risk groups of low, intermediate-1, intermediate-2, and high risk with corresponding OS of >17, 9, 5, and 2.2 years, respectively.

It is important to point out that the dizzying array of prognostication tools that have been created in the last few years have not been validated in PET-MF and PPV-MF and are still best utilized in the setting of determining eligibility for clinical trial enrolment and determining optimal benefit/risk ratio in the pursuit of HSCT. Presently, the DIPSS is most practical risk stratification tool

Table 1. JAK2 inhibitors tested in clinical trials in patients with myelofibrosis

	Agent	JAK family target	Non-JAK target*	Heme toxicity	Non-heme toxicity†	Heme response	BMR‡	CMR§	Trial phase	NCT no.
Approved	Ruxolitinib (INCB18424)	JAK1/2		Hg ↓		None	No	No	III	NCT00952289
				Plts ↓					III	NCT00934544
FDA new drug application	Pacritinib (SB1518)	JAK2	FLT3	Hg ↓	GI	None	No	No	III	NCT01773187
									III	NCT02055781
	Momelotinib (CYT387)	JAK1/2	JNK1 CDk2	Plts ↓	Neuro	Anemia	No	No	III	NCT01969838
									III	NCT02101268
Continued investigation	Gandotinib (LY2784544)	JAK2 V617F		Hg ↓	Renal TLS	None	No	No	II	NCT01594723
	BMS-911543	JAK2		Hg ↓ Plts ↓	Lipase	None	No	No	I/II	NCT01236352
	NS018	JAK2 V617F	SRC	Hg ↓ Plts ↓	Neuro	None	No	No	I/II	NCT01423851
Development halted	Fedratinib (SAR302503)	JAK2	FLT3 RET	Hg ↓ Plts ↓	Neuro	None	No	No	II	NCT01523171
									III	NCT01437787
	Lestaurtinib (CEP701)	JAK2	FLT3	Hg ↓ Plts ↓	GI	None	No	No	I/II	NCT00668421
									II	NCT00494585
	AZD1480	JAK1/2	Aurora-A	Hg ↓ Plts ↓	Neuro	None	No	No	I	NCT00910728
	XL019	JAK2			Neuro	None	No	No	I	NCT00522574

Hg indicates hemoglobin; Plts, platelets; TLS, tumor lysis syndrome; BMR, bone marrow response; and CMR, complete molecular response.

* Non-JAK targets inhibited at administered doses of drug with potentially relevant clinical effect.

† Notable and clinically relevant or distinct, in some cases leading to study termination or halted drug development.

‡ Bone marrow response either in terms of CR of histopathologic abnormalities and resolution of reticulin/collagen fibrosis.

§ Complete molecular response of JAK2V617F, MPL, or CALR.

available for the community practitioner in treatment decision making for their individual patient with MF.

A brief update on JAK2 inhibitors in MF

As a class of agents, JAK2 inhibitors have been very successful in reducing splenomegaly and ameliorating disease related symptoms. Patients treated with JAK2 inhibitors often feel better, attain an increase in physical mobility, and achieve a reversal of MF-related cachexia. Aspects of MF-associated biologic features such as potent reduction in levels of circulating inflammatory cytokines have been linked to the clinical improvement in constitutional symptoms following JAK2 inhibitor therapy.[11] Although this may be a form of disease modification, the absence of molecular responses, and the lack of reproducible and convincing resolution of bone marrow fibrosis and MPN histopathologic features suggest that the malignant HSC clone itself is not sufficiently impacted by JAK-STAT inhibition alone.

The preclinical evaluation of the novel type II JAK2 inhibitor NVP-CHZ868 (Novartis), has been shown to inhibit JAK2 in the inactive conformation thereby inhibiting JAK-STAT signaling in JAK2 persistent MPN cells that have previously seen a type I JAK2 inhibitor.[12] The proposed mechanism for this biologic phenomenon is discussed briefly in the combination therapy section below. NVP-CHZ868 appears highly active in an MPN murine model and may offer more potent inhibition of JAK-STAT signaling than currently tested type I inhibitors and deserves further evaluation in the clinic.

The 3 oral, small molecule, type I (competitive inhibitor of the adenosine triphosphate-binding site), JAK2 inhibitors with clear clinical activity and sufficient safety data to warrant discussion in 2015 are ruxolitinib (INCB18424, Incyte), pacritinib (SB1518, CTI biopharma), and momelotinib (CYT387, Gilead). A number of other JAK2 inhibitors (Table 1) have also been evaluated in clinical trials conducted in MF patients, some of which are no longer in

development due to issues surrounding neurotoxicity (Fedratinib, Sanofi Aventis; XL019, Exelixis; AZD1480, AstraZeneca) and gastrointestinal toxicity (Lestaurtinib, Cephalon).[13-15]

Pacritinib is a JAK2/FLT3 kinase inhibitor that has completed phase I/II trials in patients with intermediate/high-risk MF.[16-18] In a multicenter, single-arm, open-label, phase II trial of pacritinib in 35 patients with MF conducted in the United States, all patients had intermediate-1 or worse disease by DIPSS and 43% and 20% had baseline platelets <100 and $<50 \times 10^9$/L, respectively. Grade 1-2 diarrhea (69%) and nausea (49%) were the most common nonhematologic toxicities, and led to treatment discontinuation in a only a single patient. Spleen responses were seen in 31% of patients by imaging and 42% by exam, and 58% of treated patients achieved symptom response. Grade 3/4 anemia and thrombocytopenia were seen in 26% and 20%, respectively. Importantly, drug interruption/discontinuation due to thrombocytopenia occurred in only 3 subjects. PERSIST-1 (NCT01773187) and 2 (NCT02055781) are randomized phase III trials comparing pacritinib to a best available therapy (BAT) arm aiming to provide efficacy and safety results in MF patients with low platelets as this remains a serious unmet clinical need. The results of PERSIST-1 were recently presented by Mesa et al as a late-breaking abstract at ASCO 2015. In this pivotal, randomized (2:1 pacritinib, BAT) phase III trial of 327 intermediate/high-risk MF patients, 32% and 15% had platelet counts <100 and $<50 \times 10^9$/L. After a median duration of 16 months of pacritinib therapy, grade 3/4 adverse events were mainly restricted to nausea, vomiting, and diarrhea in <5%, 1%, and 1%, respectively. Hematologic toxicity was not different between the 2 arms and 25% of RBC transfusion dependent patients at baseline became independent with pacritinib therapy. Importantly, response rates were not diminished in the thrombocytopenic patients, with spleen volume reduction of ≥ 35% obtained in 17% versus 0% and 23% versus 0% in the pacritinib arm versus BAT arm with baseline platelets <100 and $<50 \times 10^9$/L, respectively. Symptom improvement was also statistically significantly superior in the pacritinib treatment arm.

Momelotinib is another potent JAK1/2 inhibitor that was evaluated in a phase I/II trial in which long-term follow-up is available.[19,20] This drug was effective in achieving combined spleen and symptom responses in 39% of the treated subjects. With a median treatment duration of 507 days, most interestingly and strikingly, 68% of transfusion dependent patients at baseline achieved independence for a minimum of 12 weeks and 26% of patients with baseline hemoglobin <10g/dL and not receiving transfusional support achieved at least a 2 g/dL increase in hemoglobin level. The median duration of the anemia response had not yet been reached at the time of this report. The pivotal phase III randomized trial comparing momelotinib to ruxolitinib in JAK2 inhibitor naïve patients is ongoing (NCT01969838), as well the phase III randomized study with BAT comparator arm in MF patients with cytopenias and prior ruxolitinib therapy (NCT02101268).

Ruxolitinib (Jakafi, Incyte) has been approved in the United States for the treatment of patients with intermediate/high-risk MF since 2011 and 3-year follow-up data from the 2 pivotal phase III trials, COMFORT-1 and 2 is available.[21,22] It is essential to realize this indication includes patients with PMF as well as PET/PPV-MF and as of December 2014 has also been expanded to include patients with PV based on the results of the RESPONSE trial.[23] Additionally, the package insert was subsequently updated to allow for the treatment of MF patients with a baseline platelet count as low as 50×10^9/L based on convincing safety data.[24] The use of ruxolitinib in the treatment of PV is beyond the scope of this paper and is addressed elsewhere.[25]

There are 4 facets of ruxolitinib treatment in MF which need to be addressed and these include: (1) long-term safety, (2) durability of spleen and symptom response, (3) histopathologic changes in the bone marrow, and (4) improvement in overall survival. In terms of overall safety to date, with 3-year follow-up from the COMFORT studies reported, the emergence of late onset hematologic toxicity does not appear to be a major concern. The clinical relevance of maintaining a maximally reduced spleen volume for the entire duration of ruxolitinib treatment is unclear, as most patients will continue to report benefit from spleen related symptoms even if not at their maximum spleen volume reduction. However, the loss of symptom response with time will likely be clinically meaningful and prompt a change in therapy to either a next generation JAK2 inhibitor or JAK2 inhibitor-based combination therapy. It has even been suggested that ruxolitinib-treated MF patients with loss of response (either primary or secondary resistance) have a particularly poor outcome with a median survival of only 6 months.[26] Exploratory analysis suggests that ruxolitinib induced regression/stabilization of bone marrow fibrosis in a subset of treated patients after prolonged exposure (60 months) is associated with downregulation of certain inflammatory cytokines and modulation of the bone marrow microenvironment, but importantly, the actual clinical implication and significance of this finding is not entirely clear.[27]

A question that often arises, and for which no evidence-based data exists, is the use of ruxolitinib in MF patients with low-risk disease. Although there is a substantial amount of data to support the use of this agent in patients with intermediate/high-risk disease and even some suggestion of risk-score downgrading by some groups,[28,29] there is simply no data to support earlier use in the disease course. Only a limited number of patients in the world have been treated beyond 5 years and the potential for infectious complications, disease progression, and loss of response at later time points is unknown and is therefore a serious concern in the low-risk group

with an expected median survival that can exceed 15 years in some cases.

Non-JAK2 inhibitor therapy in MF

Based on an expanding understanding of the complex biologic mechanisms of MF pathogenesis, several novel therapeutic targets have emerged and multiple agents have already demonstrated preclinical as well as clinical activity. Agents that target alternative signaling pathways, epigenetic modifications, immune deregulation, HSC cell cycling, and bone marrow fibrosis are in various phases of clinical development (Table 2). In the last few years, the number of agents has grown substantially and precludes detailing each in this paper, as this has been done expertly in previous years.[30] Instead, it is worth updating the reader of those with the most mature and otherwise remarkable results.

The first-in-class telomerase inhibitor, Imetelstat (GRN163L, Janssen), is a covalently-lipidated 13-mer thiophosphoramidate oligonucleotide that competitively binds the RNA component of human telomerase reverse transcriptase (hTERT).[31] Rationale for the use of imetelstat in MPNs stems from observations that: (1) telomerase activity is increased in clonal granulocytes of patients with ET/PV, (2) telomere length is shortened in MPN patients and is directly correlated to the allelic burden of JAK2V617F, (3) imetelstat selectively induces apoptosis of MF CD34+ cells, and (4) selectively reduces malignant megakaryocyte progenitor cells.[32-35] The investigator-initiated, single institution, pilot study of infusional imetelstat given every 3 weeks or weekly ×3 followed by every 3 weeks in 33 patients with intermediate/high-risk MF was first reported by Tefferi et al in 2013 and then updated in 2014 (NCT01731951).[36,37] Remarkably, although 18 subjects terminated treatment for reasons of suboptimal response or progression, 7 patients responded (CR=4; PR=3) at a median treatment duration of 11 months. These responses included resolution of BM fibrosis and histopathologic abnormalities, as well as molecular responses. The investigators proposed that responses may be linked to mutational status as those patients with *JAK2V617F*, or *SF3B1/U2AF1*, and lacking *ASXL1* mutation were more likely to attain a response. The numbers of treated patients were too few to conclude a clear connection between mutational status and treatment response, but do reinforce the importance of mutational profiling at baseline and at time of clinical response as a necessary correlative component of future trials. Grade 3/4 myelosuppression was frequent and transaminitis was an initial cause for concern prompting a brief clinical hold and FDA review. A highly anticipated company sponsored, multicenter, phase II trial in patients with MF as second-line therapy after ruxolitinib treatment will begin this year.

Another innovative approach that has attracted considerable attention is the Simon 2 stage, phase II trial of PRM-151 (Promedior) in patients with at least grade 2 bone marrow fibrosis (BMF) by European consensus classification. PRM-151 is a recombinant human pentraxin-2 (PTX-2) analogue, an acute phase response protein (serum amyloid P) that acts at sites of tissue damage, promoting wound healing, reducing inflammation, and inducing monocyte differentiation away from a fibrocyte and toward regulatory macrophage, resulting in reduction in the deposition of pathologic reticulin fibrosis.[38] Serum PTX-2 levels have been shown to be reduced in patients with MF (as well as fibrotic diseases of kidney, lungs, and liver) and are inversely proportional to grade of BMF. Twenty-seven intermediate/high-risk MF patients with measureable BMF at baseline were treated on 4 study arms (PRM-151 at 2 different schedules with and without concurrent

Table 2. Novel non-JAK2 inhibitors in clinical trials for patients with myelofibrosis

	Target	Agent	Trial phase	NCT no.
Epigenetic	HDAC	Panobinostat	I/II	NCT01298934
			II	NCT00931762
		Givinostat	II	NCT00606307
		Vorinostat	I	NCT00357305
	DNMT	Azacytidine	II	NCT00569660
		Decitabine	II	NCT00630994
			II	NCT00095784
Signaling pathway	JAK1	INCB39110	II	NCT01633372
	mTOR	Everolimus	I/II	NCT00081874
	SMO	PF-04449913	II	NCT02226172
	Wnt	PRI-724	I/II	NCT01606579
	Aurora-B	TAK-901	I	NCT00807677
	TGF-B/Activin	Sotatercept	II	NCT01712308
Anti-fibrotic	PTX-2	PRM-151	II	NCT01981850
	TGF-β	Fresolimumab	I	NCT01291784
	LOXL2	Simtuzumab	II	NCT01369498
Leukemic stem cell	Epha3	KB004	I/II	NCT01211691
Chaperone	HSP90	AUY922	II	NCT01668173
Checkpoint inhibitor and immunomodulator	PD-1	Nivolumab	II	NCT02421354
	Immune functions	IFN-α2a	II	NCT00452023
		IFN-α2b	II	NCT01758588
			II	NCT02370329
		Pomalidomide	III	NCT01178281
Pro-apoptosis	Telomerase	Imetelstat	I	NCT01731951
			II	NCT02426086
	IAP	LCL161	II	NCT02098161
	Peptide toxin	SL-401	I/II	NCT02268253

HDAC indicates histone deacetylase; DNMT, DNA methyltransferase; HSP90, heat shock protein 90; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositide 3-kinase; PTX-2, pentraxin-2; IMiD, immunomodulatory; TGF-β, transforming growth factor beta; PD-1, programmed cell death-1; Epha3, ephrin type-A receptor 3; and IAP, inhibitor of apoptosis protein.

ruxolitinib).[39] A total of 11 treated patients had documented reduction of BMF by at least 1 grade from baseline as assessed by central, blinded, adjudicated review. This infusional agent was well tolerated without any related grade 3/4 adverse events reported. Modest reduction in symptoms (as measured by the TSS), spleen size, and improvements in hemoglobin and platelet levels were observed and appeared to be independent of coadministration of the JAK1/2 inhibitor. A borderline association ($p = 0.09$) between reduction in bone marrow fibrosis and platelet response was appreciated with computer-assisted image analysis (CIA) performed on serial bone marrow slides.[40] The second stage of this trial will be recruiting MF patients to PRM-151 monotherapy that are either ruxolitinib intolerant/resistant or naïve due to thrombocytopenia and will be conducted at multiple sites across the US and Europe (NCT01981850).

Ephrin ligands and their cognate receptor tyrosine kinases are bi-directional signalling pathways integral to cell positioning and tissue organization during normal embryonic development and their expression is lost in adult tissue. The ephrin receptor, EphA3, is selectively expressed in a broad spectrum of hematologic malignancies. EphA3 has been shown to be expressed specifically by leukemic stem cells, tumor neovasculature, and stroma, thus providing a novel therapeutic target for the treatment of hematologic malignancies.[40,41] KB004 (KaloBios) is a recombinant, non-fucosylated IgG1k monoclonal antibody targeting the extracellular domain of EphA3 receptor kinase resulting in receptor activation, rounding of the cell and induction of apoptosis. KB004 exerts anti-leukemic activity via 4 distinct mechanisms: (1) antibody-dependent cell-mediated cytotoxicity (ADCC), (2) direct apoptosis, (2) disruption of the tumor vasculature, and (4) as an anti-fibrotic

through a mechanism of action that remains unclear at present. The first-in-man dose escalation phase I/II of the trial in a broad spectrum of hematologic malignancies has concluded and the results were recently presented.[42] Fifty patients with advanced hematologic malignancies were treated and other than grade 1/2 infusion reactions that were managed with premedication and slowing of the infusion rate, the agent appears to be well tolerated. Three responses (AML, MDS/MPN, and MF) were reported with the MF patient achieving a reduction in blast count, spleen size, BMF, and improvement in transfusion independence. Correlation between Epha3 expression level in the bone marrow and response remains exploratory. The dose expansion phase in the MF cohort at 250 mg IV weekly is currently underway at multiple centers (NCT01211691).

Focus on combination therapy in MF

The need to improve upon the current success of JAK2 inhibition in MF has also led to the evaluation of combination therapy in MF. The use of combinations of agents from different therapeutic classes to achieve deeper clinical responses is routine in other hematologic malignancies and is now an active strategy being pursued in MF. Unlike chronic myelogenous leukemia (CML) in which *BCR-ABL1* as the primary disease driver can be effectively targeted by a single agent, MF will likely require polytherapy to achieve similar success. Presently, agents with proven biologic activity alone are being combined with ruxolitinib in an attempt to more effectively target the MPN HSC and achieve durable disease control. The specific rationale for each partner agent in combination is based either on preclinical laboratory investigation or in some cases, as an antidote to JAK2 inhibitor therapy induced myelosuppression.

Table 3. Double- and triple-combination therapy trials in chronic and advanced phases of myelofibrosis

Agent 1 class	Agent 1	Agent 2 class	Agent 2	Agent 3 class	Agent 3	Phase of study	NCT identifier
JAK 1/2 inhibitor	Ruxolitinib	Epigenetic	Panobinostat			I/II	NCT01693601
						Ib	NCT01433445
			Pracinostat			II	NCT02267278
			Azacytidine			II	NCT01787487
			Decitabine			I/II	NCT02076191
		IMiD	Lenalidomide			II	NCT01375140
			Pomalidomide			Ib/II	NCT01644110
		Androgen	Danazol			II	NCT01732445
		Anti-fibrosing agent	PRM-151*			II	NCT01981850
			GS-6624			II	NCT01369498
		HH Pathway inhibitor	Sonidegib			I/II	NCT01787552
		PI3K inhibitor	Buparlisib			Ib	NCT01730248
		Chaperone inhibitor	PU-H71			I	NCT01393509
		PIM kinase inhibitor	PIM447	CDK4/6 inhibitor	LEE011	I	NCT02370706

* Stage 2 of the phase II trial will only evaluate PRM-151 as monotherapy.

Combination therapies that are currently being evaluated in MF clinical trials and are based on scientific rationale include ruxolitinib and epigenetic modifiers [panobinostat (NCT01693601, NCT01433445), azacitidine (NCT01787487), decitabine (NCT02076191); Table 3]. Combination therapy of ruxolitinib with immunomodulatory agents (IMiDs), such as lenalidomide (NCT01375140) and pomalidomide (NCT01644110) or the synthetic androgen, danazol (NCT01732445) or erythropoiesis-stimulating agents (darbopoeitin) are examples of partner agents chosen to counterbalance the therapy-related myelosuppression that is expected with ruxolitinib treatment. The first known attempt to formally evaluate the safety and clinical activity of triple-agent therapy in MF is being tested in a phase I trial with ruxolitinib in combination with the pan-PIM kinase inhibitor, PIM447, and the CDK4/6 inhibitor LEE011 (NCT02370706). This section will highlight several ongoing combination trials with both preclinical rationale and available clinical data.

The combination of ruxolitinib and panobinostat is based on documented single-agent clinical activity in MF and synergistic activity in combination in a murine model of MF.[43-45] The final results of the Novartis sponsored, multicenter, phase Ib trial conducted in Europe and involving 61 patients with intermediate/high-risk MF was reported at ASH 2014.[46] The two oral drugs were very well tolerated in combination (diarrhea was the most common nonhematologic adverse event, grade 3/4 in 13%) and a maximal tolerated dose (MTD) was not achieved. The recommended phase II dose (RPTD) of ruxolitinib 15 mg twice daily continuously and concurrent oral panobinostat 25 mg thrice weekly every other week was established. At a median duration of ~1 year exposure to the dual therapy at the RPTD, grade 3/4 anemia and thrombocytopenia was 32%, and 27%, respectively, and was the reason for discontinuation in 2 patients for each cytopenia. 85% of patients treated at the RPTD achieved a spleen reduction ≥50% and 59% attained nonpalpable splenomegaly. Reductions in JAK2V617F allele burden were modest, (29% of patients with mutation present at baseline had a ≥20% decrease from baseline). Three patients had reduction in BMF grade by blinded central review. An ongoing investigator initiated, single institution, phase I/II trial with a slightly different design of 1 month induction with ruxolitinib followed by combination therapy in patients with MF that can have had previous ruxolitinib exposure is currently enrolling in the US (NCT01433445).

Levine et al have postulated that chronic ruxolitinib exposure results in JAK2 transactivation allowing for continued JAK-STAT signaling. This mechanism for JAK2-dependent disease persistence in the presence of a JAK2 inhibitor provides rationale for additional or alternative targeting of JAK2.[47] The concept that JAK2, an essential pharmacologic target in MF, is not effectively silenced with current type I JAK2 inhibitors has led to the preclinical evaluation of combination partner agents that alternatively target the protein. An example of this approach is combination therapy with inhibitors of heat shock protein 90 (HSP90) chaperone, in which JAK2 is a known client protein.[48] JAK2 gene deletion studies in murine transplant models of MPL-driven MF have demonstrated significant reductions in clinical, bone marrow pathologic, and mutational burden when compared to JAK2 inhibitor therapy alone. Combined JAK1/2 inhibitor + the HSP90 inhibitor, PU-H71, in this murine model resulted in potent reduction of total JAK2 and phospho-JAK2 levels as opposed to increased phospo-JAK2 levels seen with JAK1/2 inhibitor therapy alone. Combination therapy led to more potent reduction in downstream JAK-STAT pathway mediator expression and provides the rationale for clinical evaluation of this combinatorial approach (NCT01393509). The single-agent HSP90 inhibitor, AUY922 (Novartis), is currently being evaluated in MF at a single institution, phase II trial in the US (NCT01668173), and future combination trials with this agent are also planned.

The Hedgehog (Hh) signaling pathway is active in various stages of normal hematopoiesis and has been shown to be up-regulated in primary MF granulocytes with significant increase in target gene expression of the transcription factor Gli1 and the tumor suppressor Ptch1.[49] Additionally, the Hh-Gli1 pathway has also been implicated in the pathogenesis of fibrotic diseases and inhibition of this pathway is a therapeutic target in MF that was recently evaluated in a phase II trial of 14 patients with MF.[50] Single-agent IPI-926 (saridegib; Infinity Pharmaceuticals), a smoothened (SMO) inhibitor was generally well tolerated without significant myelosuppression. However, only limited clinical responses and improvements in correlative biomarkers was observed and continued evaluation of this agent as monotherapy was terminated. In a murine transplant model of MF, the combination of ruxolitinib and the selective small molecule SMO inhibitor, sonidegib (LDE225, Novartis) resulted in synergistic activity in reducing leukocytosis, splenomegaly, BMF and mutant allele burden.[49] A multicenter, phase Ib/II, combination trial of ruxolitinib and sonidegib in JAK2 inhibitor naïve patients

Table 4. Current HSCT clinical trial approaches in myelofibrosis

Donor source	Preconditioning regimen	Conditioning regimen	Primary endpoint	NCT no.
MRD, MUD	None	Flu-Mel ± ATG	PFS at 2 y	NCT00572897
MRD, MUD	None	Flu-Bu ± ATG	NRM at day 100	NCT00475020
MRD, MUD	None	Flu-Bu vs Flu-Thio	PFS at 1 y	NCT01814475
MRD, MUD	JAK2 inhibition	Flu-Bu	Alive and engrafted at day 100	NCT01790295
MRD, MUD	JAK2 inhibition	Flu-Mel	DFS at 1 y	NCT01795677
MRD, MUD, CB	JAK2 inhibition	Flu-Mel	Survival at 2 y	NCT02251821
Haploidentical	None	Flu-Mel	Alive and engrafted at day 100	Pending
Cord blood	None	TBD	TBD	Planned
Autologous	JAK2 inhibition	BU	Graft failure at day 30	Pending

Flu indicates fludarabine; Mel, melphalan; ATG, anti-thymocyte globulin; Bu, busulfan; PFS, progression free survival; DFS, disease free survival; NRM, non-relapse mortality; MRD, matched/mismatched related donor; MUD, matched/mismatched unrelated donor; CB, cord blood; and TBD, to be determined.

with intermediate/high-risk MF is currently underway (NCT01787552).[51] Results from the phase Ib portion of this trial have been presented and demonstrate tolerability and signal of clinical activity with grade 3/4 CPK elevation occurring in 10% of treated patients and 70% achieving ≥50% reduction palpable spleen.[52] Sonidegib 400 mg daily in combination with ruxolitinib 20 mg twice daily is being explored for efficacy endpoints in the phase II portion currently.

Activity of phosphoinositide-3-kinase (PI3K)/AKT/ mammalian target of rapamycin (mTOR) pathway in MPN cells has been well documented and serves as a therapeutic target.[53,54] Single-agent everolimus (RAD001, Afinitor, Novartis) was evaluated in a multicenter, phase I/I trial of 39 patients with MF and demonstrated a 23% response rate by IWG-MRT.[55] Responses in symptomatology, spleen reduction and anemia were reported in the absence of changes in the *JAK2*V617F allele burden. Overall, everolimus was well tolerated and myelosuppression was modest and only reversible anemia was notable in this trial. Compelling preclinical studies utilizing the dual PI3K/AKT and mTOR inhibitor BEZ235 in combination with JAK2 inhibitors have demonstrated synergistic activity in a *JAK2*V617F-driven murine transplant model as well as against cultured and primary MPN CD34+ cells while sparing normal CD34$^+$ hematopoietic cells.[56,57] The pan-PI3K inhibitor buparlisib (BKM 120, Novartis) is being evaluated in combination with ruxolitinib in a multicenter phase Ib trial of patients with intermediate/high-risk MF (NCT01730248). Initial results were recently presented of this combination approach in 33 patients (ruxolitinib naïve and ruxolitinib resistant) in which the RPTD was determined to be ruxolitinib 15 mg twice daily and buparlisib 60 mg daily.[58] The combination appears well tolerated with expected modest myelosuppression noted and reduction in spleen size in the majority of treated patients was observed. Perhaps most importantly, clinical activity was shown in patients who previously failed ruxolitinib monotherapy. Updated results with long-term follow-up are anticipated.

Role of HSCT in MF

HSCT remains the only proven therapeutic approach in MF that offers the potential for cure in those intermediate/high-risk individuals that are typically below 65-70 years of age, have a reasonable performance status, lack significant competing comorbid conditions, and importantly have an available related or unrelated HLA-matched donor. Considering these requisites, only a fraction of MF patients are appropriate candidates for HSCT. Efforts to address a high rate of non-relapse mortality (NRM) owing to the advanced age and high comorbid index of MF patients has been
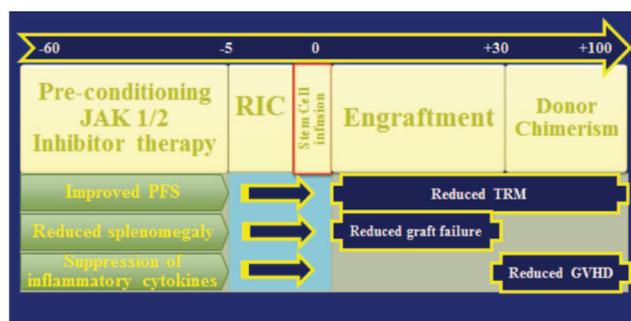

Figure 2. Rationale for JAK2 inhibition prior to HSCT in myelofibrosis. Splenomegaly contributing to hypersplenism-mediated sequestration of donor graft leading to primary graft failure, poor performance status and cachexia contributing to increased TRM, and hyper-inflammatory state contributing to increased risk of GVHD have all been identified as potential obstacles to successful outcome in HSCT of patients with MF. The use of a JAK2 inhibitor immediately prior to conditioning chemotherapy should result in reduction of splenomegaly, improvement in performance status, reversal of cachexia, suppression of circulating inflammatory cytokines. Therefore, the effects of JAK2 inhibition prior to HSCT is hypothesized to result in a decrease in rate of graft failure, TRM, and possibly GVHD.

achieved through the use of reduced intensity conditioning (RIC) regimens. However, the success of reduction in transplant related mortality (TRM) associated with RIC preparative regimens can be compromised by an increase in risk of disease relapse.

A recent review from the Center for International Blood and Marrow Transplant Research (CIBMTR) of 233 MF patients who received RIC-HSCT revealed that the only independent predictor of poor outcome in multivariable analysis was the use of an unrelated donor source.[59] This finding was also appreciated in the prospective, global, phase II MPD-RC 101 study of fludarabine/melphalan ± ATG-based RIC-HSCT in 66 MF patients. The rate of overall graft failure, acute graft versus host disease (aGVHD) grade II-IV, NRM, and 2-yr-OS was 6% versus 36%, 12% versus 21%, 22% versus 59%, and 75% versus 32% in the related versus unrelated arms, respectively.[60] The exact reasons for such discrepant outcomes in these 2 arms is not readily apparent and was not due to differences in *JAK2*V617F status, age, degree of donor HLA match, or diagnosis (PMF or PET/PPV-MF). Unrelated donor source was also significantly associated with poor HSCT outcome due to a high rate of relapse and nonrelapse mortality in a large retrospective study conducted by the European Group for Blood and Marrow Transplantation.[61]
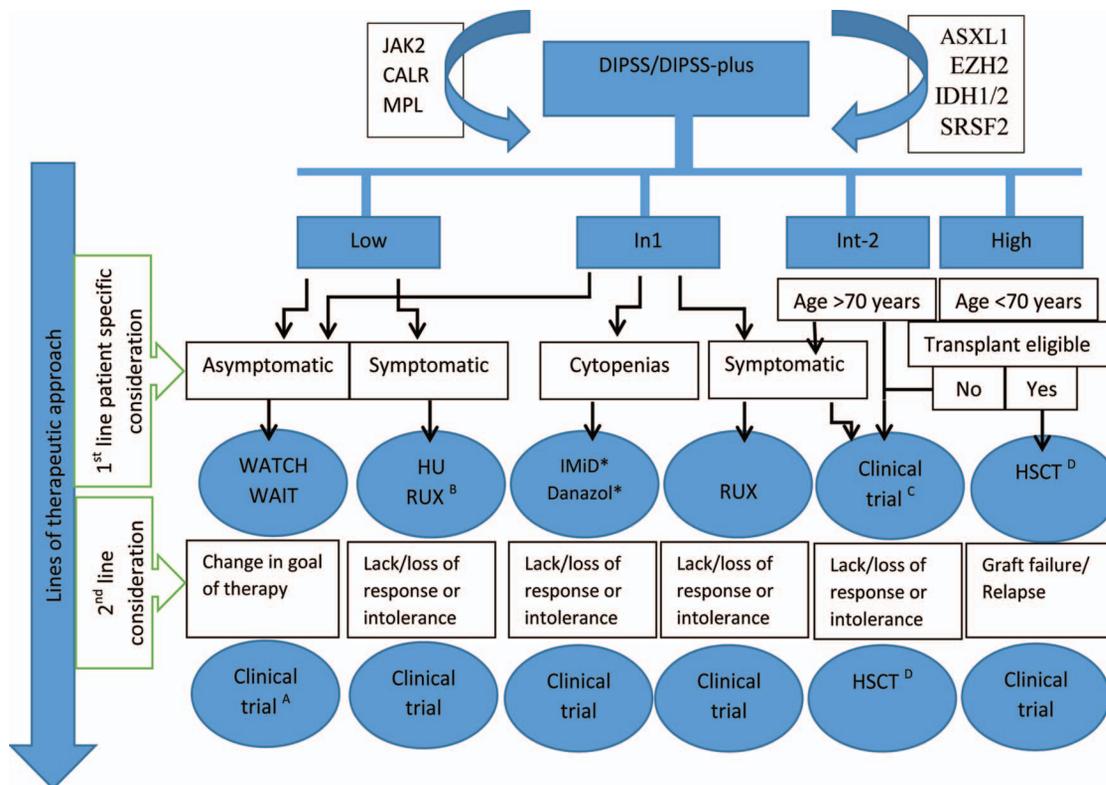
Figure 3. Modern risk-adapted treatment algorithm for myelofibrosis. The use of the dynamic international prognostic scoring system (DIPSS) and DIPSS-plus (DIPSS+) is the most comprehensive risk stratification tool currently available to guide therapeutic decision-making. The influence of driver mutation status as well the acquisition of additional somatic mutations may also prove to further refine this process. Ultimately, therapeutic approach is based on patient specific (age, performance status) and disease specific (symptom burden, presence of cytopenias) considerations in conjunction with an individual's personal goal of therapy. It should be stressed that whenever possible, clinical trial should be considered and hematopoietic stem cell transplantation remains the only potential curative treatment option available to a subset of patients. A indicates interferon-α; B, not FDA-approved for low-risk patients; C, second-generation JAK2 inh, combination therapy, imetelstat, PRM151, KB004, PF-04449913; D, HSCT on clinical trial when available; HU, hydroxyurea; RUX, ruxolitinib; and IMID, immunomodulator. *Erythropoiesis-stimulating agent can also be considered if baseline erythropoietin level is low.

Given that 70% of patients do not have a matched related-donor option for HSCT, combined with the poor outcome reported in the unrelated arm of the MPD-RC 101 study, alternative donor sources need to be explored in MF, such as umbilical cord blood (UCB) and haploidentical (HI) donors. Although, prospective trials of HI-HSCT are currently lacking for patients with MF, a limited number of advanced phase MF patients have received this treatment at MD Anderson with encouraging results in terms of engraftment, graft versus host disease, and overall survival (Stefan Ciurea, MD Anderson, oral communication, June 11, 2015). The MPD-RC will be opening a phase II trial of RIC-HSCT using haploidentical donor grafts in transplant eligible MF and MPN-BP patients lacking HLA-matched related/unrelated donors (Table 4). A retrospective study of outcomes of UCBT for 35 MF/MPN-BP patients from European centers support the potential of this approach to improve survival in advanced phase MF, particularly in the subset of patients that received double-cord grafts in the setting of RIC regimen without the use of ATG.[62]

Preconditioning therapy with a JAK1/2 inhibitor would be expected to improve performance status which may in turn reduce TRM, reduce splenomegaly which may improve donor cell engraftment, and suppress inflammatory cytokines which may modify GVHD.[63] Based on this hypothesis, three prospective HSCT clinical trials are currently evaluating ruxolitinib therapy immediately in advance of

conditioning for RIC-HSCT (NCT01790295, NCT02251821, NCT01795677) (Figure 2). The French Goelams-FIM/Sfgmtc group reported a preliminary description of their ongoing phase II JAK ALLO trial in which, alarmingly, cardiogenic shock ($n = 2$) and tumor lysis syndrome (TLS) complicated by acute renal failure ($n = 1$) occurred within 21 days of stopping ruxolitinib.[64] The protocol was subsequently amended to allow for JAK1/2 inhibitor taper with prednisone overlay immediately prior to conditioning regimen and the use of TLS prophylaxis. Despite these measures, additional reports of 2 more cases of TLS (no renal failure) and 1 case of cardiogenic shock occurred within 2 weeks of ruxolitinib discontinuation. Conversely, retrospective analyses by others have not raised issues related to ruxolitinib withdrawal in the setting of HSCT.[65,66] The MPD-RC 114 phase II RIC-HSCT trial is currently evaluating the safety and efficacy of at least 2 months of preconditioning JAK1/2 inhibition prior to fludarabine-based RIC regimen in intermediate/high-risk MF patients with related/unrelated donor options (Figure 2).

In the era of JAK2 inhibitors, the precise timing of HSCT is now unclear as the sense of urgency (patient and physician) can be delayed when responding to JAK2 inhibitor therapy and may result in a paradigm shift of delayed referral for evaluation of transplant only after progressive disease on a JAK2 inhibitor. Should HSCT be offered only upon loss of response to ruxolitinib, worsening

treatment related cytopenias, or signs of progressive disease are important questions that still need to be answered.

Evaluating approaches to accelerated/blast phase MPN

MF in accelerated/blast phase (AP/BP) appears to be molecularly distinct from de novo AM, clinically more aggressive, and less responsive to traditional induction-remission chemotherapy.[1,67] Although, an intensive therapy approach of induction chemotherapy followed by HSCT offers a reasonable potential of success for those that are appropriate candidates, alternative strategies that are less intensive, yet effective, are strongly desired.[68] Due to the recognized role of hyperactive JAK-STAT signaling in MPN pathogenesis, a phase II trial of ruxolitinib monotherapy at doses of 25-50 mg twice daily was conducted in 38 AML patients in which 18 were MPN-BP and 3 CR/CRi were achieved in this group.[69] More recently, higher doses of 50, 100, and 200 mg twice daily were tested in 26 patients with relapsed/refractory AML and a single CRi was reported with an increased rate of infectious complications observed.[70] DNA methyltransferase inhibitors (decitabine and azacytidine) have demonstrated clinical activity in retrospective series of MPN-BP.[68,71,72] Using a JAK2V617F+/TP53 knock-out murine transplant model of MPN leukemic transformation, Rampal et al showed that ruxolitinib and decitabine inhibit colony formation in a dose-dependent fashion that was maximally enhanced in combination.[67] This preclinical data in combination with previous documented clinical activity of decitabine has led to the MPD-RC 109 phase I/II trial of combination decitabine at the fixed dose of 20 mg/m2 × 5 days in combination with continuous ruxolitinib therapy in 28 day cycles, escalated in dose cohorts from 10 mg twice daily to 50 mg twice daily in patients with MPN-AP/BP (NCT02076191).

Conclusion

In the last 10 years since the discovery of *JAK2*V617F, significant advances have been made in terms of improved understanding and characterization of the molecular underpinning of the MPNs. It is clear that MF is a clinically heterogeneous disease process that is driven in most cases by at least three genetic alterations (*JAK2V617F*, *CALR*, *MPL*) and is further diversified by the acquisition of other genomic and epigenomic aberrations (*ASXL1*, *TET2*, *EZH2*, *IDH1/2*, *SRSF2*). The complex molecular pathogenesis of MF has thus far prohibited the successful use of a single targeted-agent approach to achieve molecular and histopathologic remissions, which are presumed to underlie achievement of improved PFS and OS. Rational therapies selectively targeting the malignant HSC and sparing normal hematopoiesis are currently being developed and evaluated in early phase clinical trials with the prospect of inducing remissions or "functional cures." The evolution of MF risk-stratification tools integrating cytogenomic data will likely refine therapeutic decision making as available treatment options increase and particularly in calculating the benefit–risk ratio of HSCT (Figure 3). The next generation of combination therapy protocols in MF, novel treatment approaches to MF and MPN-BP, and development of more widely applicable HSCT options will undoubtedly serve to benefit our patients.

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References

1. Rampal R, Mascarenhas J. Pathogenesis and management of acute myeloid leukemia that has evolved from a myeloproliferative neoplasm. *Curr Opin Hematol*. 2014;21:65-71.
2. Ortmann CA, Kent DG, Nangalia J, et al. Effect of mutation order on myeloproliferative neoplasms. *N Engl J Med*. 2015;372:601-612.
3. Dupriez B, Morel P, Demory JL, et al. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. *Blood*. 1996;88:1013-1018.
4. 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;113:2895-2901.
5. 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;115:1703-1708.
6. Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. *J Clin Oncol*. 2011;29:392-397.
7. Tefferi A, Guglielmelli P, Finke C, et al. Integration of mutations and karyotype towards a genetics-based prognostic scoring system (GPSS) for primarymyelofibrosis. *Blood*. 2014;124:406.
8. Vannucchi A, Guglielmelli P, Rotunno G, et al. Mutation-enhanced international prognostic scoring system (MIPSS) for primarymyelofibrosis: an AGIMM & IWG-MRT project. *Blood*. 2014;124(21):405.
9. Vannucchi AM, Lasho TL, Guglielmelli P, et al. Mutations and prognosis in primary myelofibrosis. *Leukemia*. 2013;27:1861-1869.
10. Rumi E, Pietra D, Pascutto C, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. *Blood*. 2014;124:1062-1069.
11. 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;363:1117-1127.
12. Meyer SC, Keller MD, Koppikar P, et al. Type II inhibition of JAK2 with NVP-CHZ868 reverses type I JAK inhibitor persistence and demonstrates increased efficacy in MPN models. *Blood*. 2014;124. Abstract 160.
13. Hexner EO, Mascarenhas J, Prchal J, et al. Phase I dose escalation study of lestaurtinib in patients with myelofibrosis. *Leuk Lymphoma*. 2015: 1-9.
14. Verstovsek S, Tam CS, Wadleigh M, et al. Phase I evaluation of XL019, an oral, potent, and selective JAK2 inhibitor. *Leuk Res*. 2014;38:316-322.
15. Zhang Q, Zhang Y, Diamond S, et al. The Janus kinase 2 inhibitor fedratinib inhibits thiamine uptake: a putative mechanism for the onset of Wernicke's encephalopathy. *Drug Metab Dispos.* 2014;42:1656-1662.
16. Verstovsek S, Odenike O, Scott B, et al. Phase I dose-escalation trial of SB1518, a Novel JAK2/FLT3 inhibitor, in acute and chronic myeloid diseases, including primary or post-essential thrombocythemia/ polycythemia vera myelofibrosis. *ASH Annual Meeting Abstracts*. 2009;114. Abstract 3905.
17. Seymour JF, To B, Goh A, et al. First report of the phase I study of the novel oral JAK2 inhibitor SB1518 in patients with myelofibrosis. *Haematologica*. 2010;95(Suppl 2):472. Abstract 1144.
18. Komrokji RS, Seymour JF, Roberts AW, et al. Results of a phase 2 study of pacritinib (SB1518), a JAK2/JAK2(V617F) inhibitor, in patients with myelofibrosis. *Blood*. 2015;125:2649-2655.
19. Pardanani A, Laborde RR, Lasho TL, et al. Safety and efficacy of CYT387, a JAK1 and JAK2 inhibitor, in myelofibrosis. *Leukemia* 2013;27:1322-1327.
20. Pardanani AD, Gotlib J, Gupta V, et al. Update on the long-term efficacy

and safety of momelotinib, a JAK1 and JAK2 inhibitor, for the treatment of myelofibrosis. *Blood*. 2013;122(21):108.

21. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med*. 2012;366: 799-807.

22. Harrison C, Kiladjian JJ, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med*. 2012;366:787-798.

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

24. Talpaz M, Paquette R, Afrin L, et al. Interim analysis of safety and efficacy of ruxolitinib in patients with myelofibrosis and low platelet counts. *J Hematol Oncol*. 2013;6:81.

25. Griesshammer M, Gisslinger H, Mesa R. Current and future treatment options for polycythemia vera. *Ann Hematol*. 2015;94(6):901-910.

26. Jabbour E, Kantarjian H, Garcia-Manero G, et al. Outcome of patients (pts) with myelofibrosis (MF) after ruxolutinib (Rux) therapy [abstract]. *Blood*. 2013;122(21):abstract 1584.

27. Kvasnicka HM, Thiele J, Bueso-Ramos CE, et al. Ruxolitinib-induced modulation of bone marrow microenvironment in patients with myelofibrosis is associated with inflammatory cytokine levels [abstract]. *Blood*. 2014;124(21):abstract 3182.

28. 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;120:1202-1209.

29. Vannucchi A, Kantarjian H, Kiladjian JJ, et al. A Pooled overall survival analysis of the COMFORT studies: 2 randomized phase 3 trials of Ruxolitinib for the treatment of myelofibrosis. *Blood*. 2013;39:138-139.

30. Odenike O. Beyond JAK inhibitor therapy in myelofibrosis. *Hematology Am Soc Hematol Educ Program*. 2013;2013:545-552.

31. Asai A, Oshima Y, Yamamoto Y, et al. A novel telomerase template antagonist (GRN163) as a potential anticancer agent. *Cancer Res*. 2003;63:3931-3939.

32. Ferraris AM, Mangerini R, Pujic N, et al. High telomerase activity in granulocytes from clonal polycythemia vera and essential thrombocythemia. *Blood*. 2005;105:2138-2140.

33. Bernard L, Belisle C, Mollica L, et al. Telomere length is severely and similarly reduced in JAK2V617F-positive and -negative myeloproliferative neoplasms. *Leukemia*. 2009;23:287-291.

34. Wang X, Hu CS, Qiu J, Lam M, Eng K, Hoffman R. Effects of Imetelstat on CD34+ cells of patients with myelofibrosis. *Blood*. 2014;124(21):1879

35. Iancu-Rubin C, Mosoyan G, Parker CC, Eng K, Hoffman R. Imetelstat (GRN163L), a telomerase inhibitor selectively affects malignant megakaryopoiesis in myeloproliferative neoplasms (MPN) [abstract]. *Blood*. 2014;124(21):abstract 4582.

36. Tefferi A, Begna K, Laborde RR, et al. Imetelstat, a telomerase inhibitor, induces morphologic and molecular remissions in myelofibrosis and reversal of bone marrow fibrosis. *Blood*. 2013;122(21):662.

37. Tefferi A, LaPlant B, Begna K, et al. Imetelstat, a telomerase inhibitor, therapy for myelofibrosis: a pilot study. *Blood*. 2014;124(21):634.

38. Crawford JR, Pilling D, Gomer RH. FcgammaRI mediates serum amyloid P inhibition of fibrocyte differentiation. *J Leukoc Biol*. 2012;92:699-711.

39. Verstovsek S, Mesa R, Foltz LM, et al. Phase 2 trial of PRM-151, an anti-fibrotic agent, in patients with myelofibrosis: stage 1 results. *Blood*. 2014;124(21):713.

40. Pozdnyakova O, Hasserjian R, Salama M, et al. Bone marrow fibrosis by WHO grade and quantitative image analysis is reduced by PRM-151 in patients with myelofibrosis and associated with improved bone marrow morphology and increased platelet counts. *Haematologica*. 2015;100:677.

41. Guan M, Liu L, Zhao X, et al. Copy number variations of EphA3 are associated with multiple types of hematologic malignancies. *Clin Lymphoma Myeloma Leuk*. 2011;11:50-53.

42. Keane N, Freeman C, Swords R, Giles FJ. EPHA3 as a novel therapeutic target in the hematological malignancies. *Expert Rev Hematol*. 2012;5: 325-340.

43. Swords R, A.H. W, Durrant S, et al. KB004, a novel non-fucosylated Humaneered antibody, targeting EphA3, is active and well tolerated in a phase I/II study of advanced hematologic malignancies. *Blood*. 2014; 124(21):3756.

44. Evrot E, Ebel N, Romanet V, et al. JAK1/2 and Pan-deacetylase inhibitor combination therapy yields improved efficacy in preclinical mouse models of JAK2V617F-driven disease. *Clin Cancer Res*. 2013;19: 6230-6241.

45. DeAngelo DJ, Mesa RA, Fiskus W, et al. Phase II trial of panobinostat, an oral pan-deacetylase inhibitor in patients with primary myelofibrosis, post-essential thrombocythaemia, and post-polycythaemia vera myelofibrosis. *Br J Haematol*. 2013;162:326-335.

46. Mascarenhas J, Lu M, Li T, et al. A phase I study of panobinostat (LBH589) in patients with primary myelofibrosis (PMF) and post-polycythaemia vera/essential thrombocythaemia myelofibrosis (post-PV/ET MF). *Br J Haematol*. 2013;161:68-75.

47. Kiladjian JJ, Heidel F, Vannucchi A, et al. Efficacy, safety, and confirmation of the recommended phase 2 dose of ruxolitinib plus panobinostat in patients with intermediate or high-risk myelofibrosis. *Blood*. 2014;124(21):711.

48. Koppikar P, Bhagwat N, Kilpivaara O, et al. Heterodimeric JAK-STAT activation as a mechanism of persistence to JAK2 inhibitor therapy. *Nature*. 2012;489:155-159.

49. Bhagwat N, Koppikar P, Keller M, et al. Improved targeting of JAK2 leads to increased therapeutic efficacy in myeloproliferative neoplasms. *Blood*. 2014;123:2075-2083.

50. Bhagwat N, Keller MD, Rampal R, et al. Improved efficacy of combination of JAK2 and hedgehog inhibitors in myelofibrosis. *Blood*. 2013;122(21):666.

51. Sasaki K, Gotlib JR, Mesa RA, et al. Phase II evaluation of IPI-926, an oral Hedgehog inhibitor, in patients with myelofibrosis. *Leuk Lymphoma*. 2015:1-6.

52. Zingariello M, Martelli F, Ciaffoni F, et al. Characterization of the TGF-beta1 signaling abnormalities in the Gata1low mouse model of myelofibrosis. *Blood*. 2013;121:3345-3363.

53. Gupta V, Koschmieder S, Harrison C, et al. Phase 1b dose-escalation study of Sonidegib (LDE225) in Combination with Ruxolitinib (INC424) in patients with myelofibrosis. *Blood*. 2014;124(21):712.

54. Grimwade LF, Happerfield L, Tristram C, et al. Phospho-STAT5 and phospho-Akt expression in chronic myeloproliferative neoplasms. *Br J Haematol*. 2009;147:495-506.

55. Vicari L, Martinetti D, Buccheri S, et al. Increased phospho-mTOR expression in megakaryocytic cells derived from CD34+ progenitors of essential thrombocythaemia and myelofibrosis patients. *Br J Haematol*. 2012;159:237-240.

56. Guglielmelli P, Barosi G, Rambaldi A, et al. Safety and efficacy of everolimus, a mTOR inhibitor, as single agent in a phase 1/2 study in patients with myelofibrosis. *Blood*. 2011;118:2069-2076.

57. Bartalucci N, Tozzi L, Bogani C, et al. Co-targeting the PI3K/mTOR and JAK2 signalling pathways produces synergistic activity against myeloproliferative neoplasms. *J Cell Mol Med*. 2013;17:1385-1396.

58. Fiskus W, Verstovsek S, Manshouri T, et al. Dual PI3K/AKT/mTOR inhibitor BEZ235 synergistically enhances the activity of JAK2 inhibitor against cultured and primary human myeloproliferative neoplasm cells. *Mol Cancer Therapeut*. 2013;12:577-588.

59. Durrant S, Koren-Michowitz M, Lavie D, et al. HARMONY: an open-label, multicenter, 2-arm, dose-finding, phase 1b study of the combination of ruxolitinib and buparlisib (BKM120) in patients with myelofibrosis (MF). *Blood*. 2014;124(21):710.

60. Gupta V, Malone AK, Hari PN, et al. Reduced-intensity hematopoietic cell transplantation for patients with primary myelofibrosis: a cohort analysis from the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant*. 2014;20:89-97.

61. Rondelli D, Goldberg JD, Isola L, et al. MPD-RC 101 prospective study

of reduced-intensity allogeneic hematopoietic stem cell transplantation in patients with myelofibrosis. *Blood*. 2014;124:1183-1191.

62. Lussana F, Rambaldi A, Finazzi MC, et al. Allogeneic hematopoietic stem cell transplantation in patients with polycythemia vera or essential thrombocythemia transformed to myelofibrosis or acute myeloid leukemia: a report from the MPN Subcommittee of the Chronic Malignancies Working Party of the European Group for Blood and Marrow Transplantation. *Haematologica*. 2014;99:916-921.

63. Robin M, Giannotti F, Deconinck E, et al. Unrelated cord blood transplantation for patients with primary or secondary myelofibrosis. *Biol Blood Marrow Transplant*. 2014;20:1841-1846.

64. Spoerl S, Mathew NR, Bscheider M, et al. Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. *Blood*. 2014;123:3832-3842.

65. Robin M, Francois S, Huynh A, et al. Ruxolitinib before allogeneic hematopoietic stem cell transplantation (HSCT) in patients with myelofibrosis : a preliminary descriptive report of the JAK ALLO study, a phase ii trial sponsored by Goelams-FIM in collaboration with the Sfgmtc. *Blood*. 2013;122(21):2111.

66. 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;49:179-184.

67. Stubig T, Badbaran A, Luetkens T, et al. 5-azacytidine promotes an inhibitory T-cell phenotype and impairs immune mediated antileukemic activity. *Mediators Inflamm*. 2014;2014:418292.

68. Rampal R, Ahn J, Abdel-Wahab O, et al. Genomic and functional analysis of leukemic transformation of myeloproliferative neoplasms. *Proc Nat Acad Sci U S A*. 2014;111:E5401-E5410.

69. Kennedy JA, Atenafu EG, Messner HA, et al. Treatment outcomes following leukemic transformation in Philadelphia-negative myeloproliferative neoplasms. *Blood*. 2013;121:2725-2733.

70. Eghtedar A, Verstovsek S, Estrov Z, et al. Phase 2 study of the JAK kinase inhibitor ruxolitinib in patients with refractory leukemias, including postmyeloproliferative neoplasm acute myeloid leukemia. *Blood*. 2012;119:4614-4618.

71. Pemmaraju N, Kantarjian H, Kadia T, et al. A phase I/II study of the Janus kinase (JAK)1 and 2 inhibitor ruxolitinib in patients with relapsed or refractory acute myeloid leukemia. *Clin Lymphoma Myeloma Leuk*. 2015;15:171-176.

72. Mascarenhas J, Navada S, Malone A, Rodriguez A, Najfeld V, Hoffman R. Therapeutic options for patients with myelofibrosis in blast phase. *Leuk Res*. 2010;34:1246-1249.

73. Thepot S, Itzykson R, Seegers V, et al. Treatment of progression of Philadelphia-negative myeloproliferative neoplasms to myelodysplastic syndrome or acute myeloid leukemia by azacitidine: a report on 54 cases on the behalf of the Groupe Francophone des Myelodysplasies (GFM). *Blood*. 2010;116:3735-3742.