Myelofibrosis: Genetic Characteristics and the Emerging Therapeutic Landscape

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ABSTRACT

Primary myelofibrosis (PMF) is one of three myeloproliferative neoplasms (MPN) that are morphologically and molecularly interrelated, the other two being polycythemia vera (PV) and essential thrombocythemia (ET). MPNs are characterized by JAK-STAT–activating *JAK2*, *CALR*, or *MPL* mutations that give rise to stem cell–derived clonal myeloproliferation, which is prone to leukemic and, in case of PV and ET, fibrotic transformation. Abnormal megakaryocyte proliferation is accompanied by bone marrow fibrosis and characterizes PMF, while the clinical phenotype is pathogenetically linked to ineffective hematopoiesis and aberrant cytokine expression. Among MPN-associated driver mutations, type 1–like *CALR* mutation has been associated with favorable prognosis in PMF, while *ASXL1*, *SRSF2*, *U2AF1*-Q157, *EZH2*, *CBL*, and *K/NRAS* mutations have been shown to be prognostically detrimental. Such information has enabled development of exclusively genetic (GIPSS) and clinically integrated (MIPSSv2) prognostic models that facilitate individualized treatment decisions. Allogeneic stem cell transplantation remains the only treatment modality in MF with the potential to prolong survival, whereas drug therapy, including JAK2 inhibitors, is directed mostly at the inflammatory component of the disease and is therefore palliative in nature. Similarly, disease-modifying activity remains elusive for currently available investigational drugs, while their additional value in symptom management awaits controlled confirmation. There is a need for genetic characterization of clinical observations followed by *in vitro* and *in vivo* preclinical studies that will hopefully identify therapies that target the malignant clone in MF to improve patient outcomes.

Historical Prelude

William Dameshek (1900–1969) is credited for coining the concept of myeloproliferative disorders (1951; ref. 1), which are now referred to as myeloproliferative neoplasms (MPN; ref. 2). The original MPN members included primary myelofibrosis (PMF), polycythemia vera (PV), essential thrombocythemia (ET), chronic myeloid leukemia, and Di Guglielmo's syndrome (erythroleukemia; ref. 1). Pre-Dameshek PMF luminaries include Gustav Heuck (1854–1940) who first described PMF (1879; ref. 3) and recognized its association with bone marrow fibrosis (BMF), extramedullary hematopoiesis (EMH), and osteosclerosis; Max Askanazy (1865–1940; ref. 4); and Herbert Assmann (1882–1950; ref. 5). Pseudonyms for MF used in the past include agnogenic myeloid metaplasia (6), chronic idiopathic myelofibrosis (7), and myelofibrosis with myeloid metaplasia. In 2006, the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) selected "PMF" as the preferred term (8). In 1939 (9), Vaughan and Harrison underscored the relationship between PMF, PV, and ET, in terms of their origination from a common mesenchymal reticulum cell, a view subsequently shared by others (10, 11) and ultimately led to the formal description of the MPN concept by Dameshek (1).

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Pathogenetic Insights

Dameshek's aforementioned concept of MPN was genetically ratified in 2005 by the seminal discovery of a Janus kinase 2 (*JAK2* located on chromosome 9p24) gain of-function mutation (*JAK2*V617F; a G to T somatic mutation at nucleotide 1849, in exon 14, resulting in the substitution of valine to phenylalanine at codon 617) in PV, ET, and PMF (12–15). Additional MPN driver mutations have since been described and include *JAK2* exon 12 described in *JAK2*V617F-negative PV (16); *CALR* (calreticulin; located on chromosome 19p13.2; refs. 17, 18) and *MPL* (myeloproliferative leukemia virus oncogene; located on chromosome 1p34; ref. 19). Among these mutations, *JAK2* is the most frequent with frequencies of approximately 98% in PV (95% *JAK2*V617F and 3% *JAK2* exon 12), 50% to 60% in ET, and 55% to 65% in PMF (20, 21). *CALR* and *MPL* mutations are often absent in PV, except rare exceptions (22, 23), while their frequencies in ET are estimated at 20% to 25% and 3% to 4%, respectively, and in PMF at 20% to 25% and 6% to 7% (21). Approximately 10% to 15% of patients with PMF or ET do not express any one of the three MPN driver mutations and are operationally referred to as being triple-negative, although that might not be the case during higher sensitivity testing (21, 24). MPN driver mutations have also been reported in other myeloid malignancies, including myelodysplastic syndromes with ring sideroblasts associated with marked thrombocytosis (MDS-RS-T; 50% frequency; refs. 25, 26).

JAK2 and *MPLW515K/L/A/R* and S505N mutations are believed to directly activate JAK2-STAT, resulting in clonal myeloproliferation that is cytokine independent or hypersensitive (19, 27). Frameshift *CALR* mutations mostly include type 1 (52-bp deletion in exon 9) or type 2 (5-bp insertion in exon 9), and less frequently a myriad of type 1-like or type 2-like variants (28). The precise mechanism of mutant *CALR*-induced myeloproliferation is less clear but one possibility includes mutant CALR binding to the extracellular domain of MPL in the endoplasmic reticulum, leading to dimerization and transfer to cell surface and activation of JAK-STAT (29). Mutant *CALR*-induced mouse models have suggested a primary effect on platelet production

that is followed by development of MF (30). Although the central role of JAK-STAT activation in MPN has been highlighted (29, 31), the particular concept is confounded by the coexistence of an inflammatory state in MF with aberrant cytokine expression and the fact that activated JAK-STAT is a nonspecific phenomenon in cancer (32). Furthermore, "targeted therapy" with JAK inhibitors has so far failed to induce selective suppression of the disease clone in MF (33).

The pathogenetic role of MPN driver mutations is highlighted by their origin at the stem cell level and the demonstration of persistent JAK-STAT activation and induction of mutant *JAK2/CALR/MPL*–driven MPN phenotype in mice (13, 34). Recent studies have highlighted the central pathogenetic role of persistent MPL-driven JAK-STAT activation in MPN; this is presumably accomplished, in addition to activating *MPL* mutations, by direct activation and altered trafficking of MPL by JAK2 V617F or binding of extracellular domain of MPL by mutant CALR that begins in the endoplasmic reticulum and leads to dimerization of immature receptors and their translocation to the cell surface for activation (29, 35, 36). It is currently assumed that the phenotypic differences between MPN variants are in part contributed by differences in the conformations of specific cytokine receptors that lead to distinct signaling outcomes for EPOR and MPL and further modified by interactions with other cooccurring mutations, including those of epigenetic regulators, and their order of acquisition (29, 34). Additional mechanisms of phenotype diversity associated with MPN-driving mutations include variations in signal intensity (often related to *JAK2*V617F allele burden) and specificity of downstream signals, including STAT5, STAT1, and STAT3 activation (37). Phenotype-wise, *JAK2*V617F, versus mutant *CALR*, is associated with older age, higher hemoglobin level, leukocytosis, lower platelet count and increased risk of thrombosis, while the latter was associated with younger age, male sex, higher platelet count, lower hemoglobin level, lower leukocyte count and lower incidence of thrombotic events (17, 18, 38, 39); type 2 versus type 1 *CALR* mutations were associated with higher platelet count (40). Patients with *CALR*-mutated PMF are also younger and present with higher platelet count and lower frequencies of anemia, leukocytosis, and spliceosome mutations (41). Recently published mouse studies appear to recapitulate the phenotypic differences seen among patients with different driver mutations (42, 43). Regardless, the underlying mechanisms that enable single mutations to result in different MPN phenotypes remain not fully understood (44–47).

Other mutations in PMF with frequencies of 10% or higher include *ASXL1* (additional sex combs-like 1), *TET2* (TET oncogene family member 2), *SRSF2* (serine/arginine-rich splicing factor 2), and *U2AF1* (U2 small nuclear RNA auxiliary factor 1; refs. 48–50); the latter has been associated with severe anemia and thrombocytopenia in PMF (50) and treatment response to imetelstat (51) while all three mutations (*ASXL1*, *SRSF2*, *U2AF1*) have been associated with inferior survival (52–54). Other mutations who are less frequent in chronic phase disease but with significantly higher frequency in blast phase MPN include *IDH1*/*IDH2* (isocitrate dehydrogenase 1 and 2; refs. 55, 56), *TP53* (tumor protein p53; refs. 56, 57), *DNMT3A* (DNA cytosine methyltransferase 3a; ref. 58), and *LNK* mutation (59, 60). Other infrequent mutations reported in MPN include *SF3B1* (splicing factor 3B subunit 1), which are characteristic and more frequent in MDS-RS and MDS-RS-T (25), *EZH2* (enhancer of zeste homolog 2), which were shown to be prognostically relevant (61), Casitas B-lineage lymphoma proto-oncogene (*CBL*), which are more frequent in juvenile and chronic myelomonocytic leukemia (62) and *SETBP1* (SET binding protein 1), which are more frequent in chronic neutrophilic leukemia and atypical chronic myeloid leukemia (63, 64).

The pathogenetic role of mutations other than *JAK2/CALR/MPL* in MPN is much less understood but believed to involve cooperation with driver mutations, leading to downstream disruption of epigenetic (e.g., *ASXL1*, *TET2*, *EZH2*, *IDH1*, *IDH2*, *DNMT3A*), RNA splicing (e.g., *SRSF2*, *U2AF1*, *SF3B1*), or transcriptional (*TP53*, *IKZF1*, *NF-E2*, *CUX1*) regulation, which might facilitate disease progression and leukemic transformation (56, 65). The recent demonstration of *TET2*, *ASXL1*, and *DNMT3A* mutations in "normal" elderly individuals has added to the complexity regarding their precise pathogenetic contribution (66, 67). More recent observations in MF include identification of STK11 as a tumor suppressor and that loss of LKB1/STK11 leads to stabilization of HIF1α and thus might promote disease progression (68), impaired megakaryocyte maturation associated with reduced *GATA1* expression that might stem from ribosomal deficiency (69), and identification of aurora kinase A (70, 71), peroxisome proliferator-activated receptor gamma (PPARγ; ref. 72) and cyclin-dependent kinase 6 (CDK6; ref. 73), as therapeutic targets in MF.

Among the *JAK2* mutation–related MPNs (MF, PV, ET), MF is uniquely characterized by more intense bone marrow and splenic tissue stromal changes, including reticulin/collagen fibrosis and neoangiogenesis (74). The mechanisms of such intense stromal reaction are not fully understood but might involve abnormal proliferation of fibroblasts, endothelial, and other mesenchymal cells mediated by inflammatory cytokines derived from clonal megakaryocytes or mononuclear cells, as well as *in situ* cell interactions involving microvascular endothelial cells and mesenchymal cells (75–81). Whether or not some of the latter stromal cells are part of the underlying disease clone is currently debated although the presence of their increased proliferation has been demonstrated and in certain cases phenotypically characterized (82–85). The potential role of fibroblasts in bone marrow fibrosis is supported by their morphologic and phenotypic resemblance to hematopoietic stem cells and monocytes and their participation in the production of extracellular matrix components (86). Several studies in MF have suggested increased expression of fibroblast growth factors or their receptors and pathogenetic interaction between megakaryocytes and fibroblasts (75–77). The reactive nature of bone marrow fibrosis in MF is further supported by clinical observations that document its complete resolution under effective targeted therapy of the disease-initiating clone (51, 87).

Mutation-Enhanced Morphologic Diagnosis

Over the last several years, we have been involved in the development of both the 2008 (88) and 2016 (89) World Health Organization (WHO) classification system for MPNs. According to these consensus guidelines, PMF is operationally subclassified into "prefibrotic" and "overtly fibrotic" variant (90–92). Diagnosis of overt PMF requires the presence of three major criteria and at least one minor criterion: major criteria include (i) presence of grade ≥2 reticulin/collagen bone marrow fibrosis, associated with megakaryocyte proliferation and dysmorphia, (ii) presence of *JAK2*/*CALR*/*MPL* or other clonal markers, or absence of evidence for reactive bone marrow fibrosis, and (iii) absence of evidence for another myeloid neoplasm; minor criteria include the presence of (i) anemia, (ii) leukocytosis, (iii) palpable splenomegaly, (iv) increased serum level of lactate dehydrogenase (LDH), and (v) leukoerythroblastosis; the latter indicates presence of nucleated red cells, immature granulocytes and/or dacryocytes (teardrop-shaped erythrocytes) in the peripheral blood. The diagnostic criteria for prefibrotic PMF are almost identical to those of overt PMF, with two exceptions: (i) the first major criterion does not require the

presence of grade ≥2 reticulin/collagen bone marrow fibrosis, and (ii) leukoerythroblastosis was not listed as a minor criterion. Because more than 80% of patients with PMF harbor mutations other than *JAK2*/*CALR*/*MPL*, additional mutation screening by next-generation DNA sequencing (NGS) might help confirm clonality in triple-negative cases (93). About 15% of patients with ET or PV develop a PMF-like phenotype over time, referred to as post-ET or post-PV MF. The diagnosis of post-PV or post-ET MF should adhere to criteria published by the IWG-MRT; these criteria require documentation of (i) an antecedent WHO-compliant diagnosis of PV or ET, and (ii) presence of grade ≥2 reticulin/collagen bone marrow fibrosis, as well as presence of at least 2 minor criteria, including (i) anemia, (ii) leukoerythroblastosis, (iii) increasing splenomegaly, (iv) development of constitutional symptoms, and, in case of post-ET MF, (v) increased LDH.

Clinical Phenotype

Prominent clinical features in MF include anemia (attributed primarily to ineffective erythropoiesis), hepatosplenomegaly (attributed to EMH and cytokine-mediated organ congestion), constitutional symptoms including fatigue, night sweats, and low-grade fever, progressive cachexia with loss of muscle mass, bone pain, splenic infarct, pruritus, nonhepatosplenic EMH, thrombosis and bleeding (94). Consequences of hepatosplenic EMH include portal hypertension that might lead to variceal bleeding or ascites and those of nonhepatosplenic EMH include spinal cord compression, ascites, pleural effusion, pulmonary hypertension or extremity pain. It is currently assumed that aberrant cytokine production by clonal cells and host immune reaction contribute to PMF-associated bone marrow stromal changes, ineffective erythropoiesis, EMH, cachexia, and constitutional symptoms (95). Causes of death in PMF include leukemic progression that occurs in approximately 20% of patients (96).

Mutation and Karyotype-Enhanced Prognostication

Beginning in 2009, a number of prognostic models in PMF have been described and summarized in **Table 1** (52–54, 97–99). The most recently developed prognostic models in PMF incorporate mutations and/or cytogenetic information: MIPSS70, MIPSS70+ version 2.0 (MIPSS*v2*) and GIPSS (52–54). MIPSS70 (mutation-enhanced international prognostic scoring system for transplant-age patients) utilizes mutations and clinical variables (54); MIPSS*v2* (the karyotype-enhanced MIPSS70) utilizes mutations, karyotype and clinical variables (52); GIPSS (the genetically-inspired prognostic scoring system) is based exclusively on mutations and karyotype (53).

MIPSS*v2* incorporates 5 genetic and 4 clinical risk factors (52); the five genetic risk factors include very high risk (VHR) karyotype (4 adverse points), unfavorable karyotype (3 points), ≥2 high molecular risk (HMR) mutations (3 points), presence of one HMR mutation (2 points), absence of type 1/like *CALR* mutation (2 points); the four clinical variables in MIPSS*v2* include constitutional symptoms (2 points), severe anemia, defined by hemoglobin levels of <8 g/dL in women and <9 g/dL in men (2 points), moderate anemia, defined by hemoglobin levels of 8–9.9 g/dL in women and 9–10.9 g/dL in men (one point) and circulating blasts ≥2% (one point; **Fig. 1**). MIPSS*v2* includes five risk categories: very high risk (≥9 points); high risk (5–8 points); intermediate risk (3–4 points); low risk (1–2 points); and very low risk (zero points); in patients age 70 years or younger, the corresponding median survivals (10-year survival rates) were 1.8 years (<5%), 4.1 years (13%), 7.7 years (37%), 16.4 years (56%) and "median not reached" (92%; **Fig. 1**). In instances where cytogenetic information is not available, MIPSS70 (54) performs as well as MIPSSv2, whereas GIPSS offers a prognostic model that is exclusively dependent on mutations and karyotype (53). Most recently, *RAS*/*CBL* mutations in PMF were associated with poor response to ruxolitinib therapy, poor prognostic features and inferior survival; in the latter regard, however, incorporation of such information to MIPSS*v2* did not show additional value (100, 101).

Risk-Adjusted Treatment Approach

Allogeneic hematopoietic stem cell transplant (AHSCT) currently remains the only treatment modality in MF that can prolong life and, in some cases, cure the disease (102). However, transplant-related complications are not trivial and might result in death or substantial morbidity in about half of the cases (103). Therefore, for the individual patient, the risk of AHSCT must be balanced against expected survival without AHSCT. On the other hand, current drug therapy in MF, including use of JAK2 inhibitors, is mostly palliative and has not been shown to modify disease natural history or prolong survival (**Table 2**; refs. 33, 104–108). **Figure 1** outlines our current treatment algorithm that is based on aforementioned risk stratification model, MIPSS*v2*.

There is no evidence to support the value of specific drug therapy in asymptomatic patients with MIPSS*v2* "low" or "very low" risk disease, whose expected 10-year survival rates were reported at 50% and 86%, respectively; expected survival in such patients was retrospectively estimated to be superior in the absence of AHSCT as first-line therapy (109, 110). Therefore, observation alone is a reasonable treatment strategy in such patients, in the absence of treatment-requiring symptoms. AHSCT is the preferred treatment of choice for MIPSS*v2* "high" or "very high" risk disease, where 10-year expected survival rates without transplant might be as low as 10% and <3%, respectively (**Fig. 1**; refs. 109, 110). In transplant-ineligible patients with high/very high-risk disease, as well as in those with symptomatic low/intermediate risk disease, clinical trial participation might be the most appropriate treatment choice, considering the current dearth of disease-modifying drugs.

Symptom-directed conventional therapy

Choice of drugs for combatting MF symptoms is based on the primary clinical indication and is outlined in **Table 2** and **Fig. 1**. For anemia, we consider androgen preparations, prednisone, immunomodulatory drugs (IMiDs; thalidomide, lenalidomide, pomalidomide), or danazol, depending on tolerability of potential side effects for the individual patient (111). For symptomatic splenomegaly, our first-line drug of choice is hydroxyurea (112) and secondarily JAK2 inhibitors (e.g., ruxolitinib, fedratinib). Ruxolitinib is our first-line drug of choice for constitutional symptoms while involved-field radiotherapy is most effective for symptomatic non-hepatosplenic EMH or localized bone pain. Splenectomy might be necessary for drug-refractory spleen-associated symptoms. Anemia response rates to each one of the aforementioned drugs are in the vicinity of 15 to 25% and response durations average about one to two years; lenalidomide works best in the presence of del(5q31) (87). We are less enthusiastic about the use of radiotherapy for hepatosplenic EMH (danger of treatment-induced protracted thrombocytopenia; ref. 113) and the use of erythropoiesis stimulating agents (ESA; refs. 114, 115) or luspatercept (116), because of inadequate efficacy (112).

Ruxolitinib and fedratinib are now FDA approved for use in MF and are considered effective treatment options for countering splenomegaly and constitutional symptoms in hydroxyurea-refractory MF, with

Table 1. Contemporary prognostic scoring systems for primary myelofibrosis.

Models	Variables	Risk categories					
		Very low	Low	Intermediate-1	Intermediate-2	High	Very high
IPSS (97)[a]	Age >65 years (1 point) Constitutional symptoms[b] (1 point) Hemoglobin <10 g/dL (1 point) Leukocytes >25 x 10(9)/L (1 point) Circulating blasts ≥1% (1 point)	NA	(0 points) 11.3 years	(1 point) 7.9 years	(2 points) 4 years	(≥3 points) 2.3 years	NA
DIPSS (98)[c]	Age >65 years (1 point) Constitutional symptoms (1 point) Hemoglobin <10 g/dL (2 points) Leukocytes >25 x 10(9)/L (1 point) Circulating blasts ≥1% (1 point)	NA	(0 points) Not reached	(1–2 points) 14.2 years	(3–4 points) 4 years	(5–6 points) 1.5 years	NA
DIPSS-plus (99)[d]	Age >65 years (1 point) Constitutional symptoms[b] (1 point) Hemoglobin <10 g/dL (1 point) Leukocytes >25 x 10(9)/L (1 point) Circulating blasts ≥1% (1 point) Unfavorable karyotype[e] (1 point) Platelet count <100 x 10(9)/L (1 point) Transfusion needs (1 point)	NA	(0 points) 15.4 years	(1 point) 6.5 years	(2–3 points) 2.9 years	(≥4 points) 1.3 years	NA
MIPSS70 (54)[c]	≥2 HMR mutations[f] (2 points) Leukocytes >25 × 10^9/L (2 points) Platelets <100 × 10^9/L (2 points) Hemoglobin <10 g/dL (1 point) Circulating blasts ≥2% (1 point) BM fibrosis grade ≥2 (1 point) Constitutional symptoms[a] (1 point) Type 1/like *CALR* absent (1 point) One HMR mutation[f] (1 point)	NA	(0–1 point) Not reached	(2–4 points) 6.3 years		(≥5 points) 3.1 years	NA
MIPSS70+v2 (52)[d]	Very high-risk karyotype[g] (4 points) Unfavorable karyotype[h] (3 points) ≥2 HMR mutations[f] (3 points) One HMR mutation[f] (2 points) Type 1/like *CALR* absent (2 points) Constitutional symptoms[b] (2 points) Severe anemia[i] (2 points) Moderate anemia[j] (1 point) Circulating blasts ≥2% (1 point)	(0 points) Not reached	(1–2 points) 16.4 years	(3–4 points) 7.7 years		(5–8 points) 4.1 years	(≥9 points) 1.8 years
GIPSS (53)[d]	Very high-risk karyotype[g] (2 points) Unfavorable karyotype[h] (1 point) *ASXL1* mutation (1 point) *SRSF2* mutation (1 point) *U2AF1*Q157 mutation (1 point) Type 1/like *CALR* absent (1 point)	NA	(0 points) 26.4 years	(1 point) 8 years	(2 points) 4.2 years	(≥3 points) 2 years	NA

Abbreviations: DIPSS, Dynamic International Prognostic Scoring System; GIPSS, Genetically Inspired International Prognostic Scoring System; IPSS, International Prognostic Scoring System; MIPSS, Mutation-enhanced International Prognostic Scoring System (age ≤ 70); NA, not applicable.

[a]HMR for MIPSSv2 and GIPSS include *ASXL1*, *SRSF2*, and *U2AF1*Q157.

[b]Constitutional symptoms include weight loss, fever, drenching night sweats.

[c]Parameters used at the time of diagnosis.

[d]Parameters used at any time in the clinical course.

[e]Unfavorable karyotype in the context of DIPSS-plus = complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), inv(3), −5/5q-, 12p- or 11q23 rearrangement.

[f]High molecular risk (HMR) mutations for MIPSS70 include *ASXL1*, *SRSF2*, *EZH2*, *IDH1*, *IDH2*.

[g]Very high-risk (VHR) karyotype = single/multiple abnormalities of -7, inv(3)/3q21, i(17q), 12p-/12p11.2 or 11q-/11q23, single/multiple autosomal trisomies other than +9 and +8.

[h]Unfavorable karyotype in the context of GIPSS/MIPSSv2 = any abnormal karyotype other than VHR karyotype, normal karyotype or sole abnormalities of 20q-, 13q-, +9, chr. 1 translocation/duplication, -Y, or sex chromosome abnormality other than -Y.

[i]Severe anemia: Hemoglobin <8 g/dL in women and <9 g/dL men.

[j]Moderate anemia: Hemoglobin 8–9.9 g/dL in women and 9–10.9 g/dL men.

response rates estimated at about 30% to 50% (117–120). However, treatment with these two drugs is complicated by treatment-emergent anemia and thrombocytopenia (120). Longer-term experience with ruxolitinib therapy in MF has disclosed a high rate of treatment discontinuation rate (92% after a median time of 9.2 months) and the occurrence of severe withdrawal symptoms during treatment discontinuation ("ruxolitinib withdrawal syndrome"), characterized by acute relapse of disease symptoms, accelerated splenomegaly,

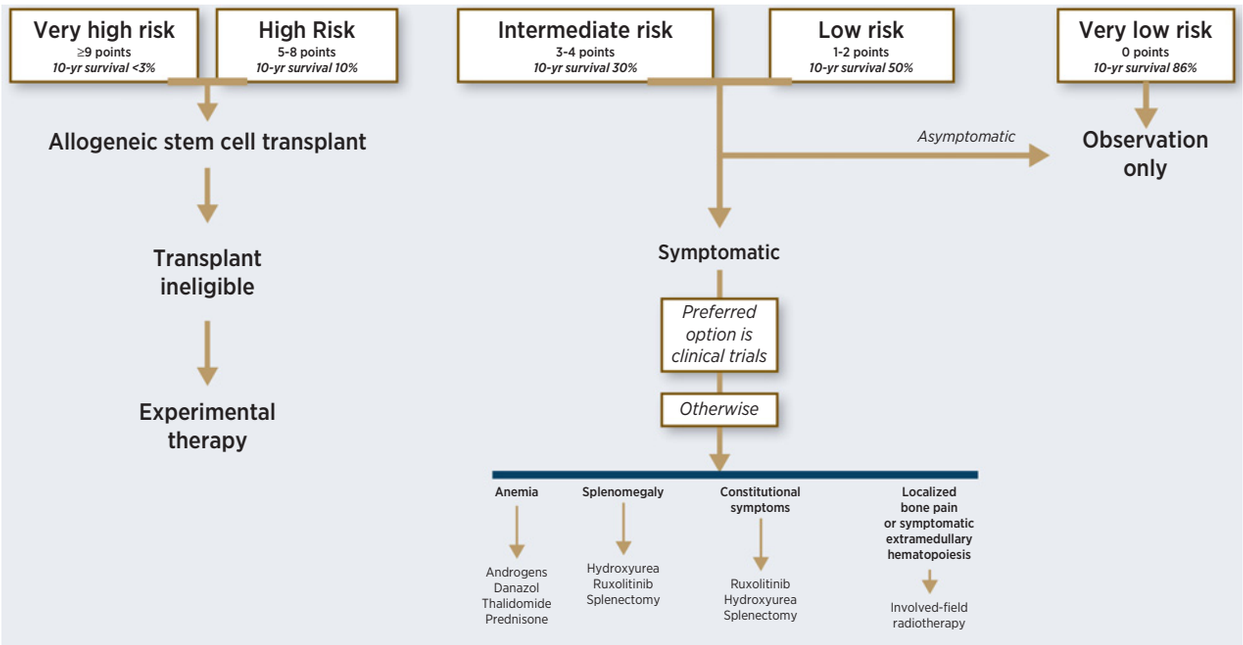

Figure 1.
Current treatment algorithm in myelofibrosis based on risk stratification according to the mutation enhanced international prognostic scoring system (MIPSS70+ version 2.0). Genetic risk factors: very high-risk karyotype, 4 points; unfavorable karyotype, 3 points; ≥2 high risk mutations, 3 points; one high risk mutation, 2 points; type 1 CALR absent, 2 points. Clinical risk factors: constitutional symptoms, 2 points; severe anemia, 2 points; moderate anemia, 1 point; ≥2% circulating blasts, 1 point.

worsening of cytopenias and occasional hemodynamic decompensation, including a septic shock-like syndrome (105, 121, 122). In addition, several reports have now associated ruxolitinib with sometimes fatal serious opportunistic infections (123–125), and as of late, poor immune antibody response to COVID-19 vaccination (126). It is important to underscore the fact that neither ruxolitinib nor other JAK2 inhibitors in MF possess antitumor activity and none of them has been shown to reverse bone marrow fibrosis or induce cytogenetic or molecular remissions. Instead, their mechanism of action is based on their broad activity in suppressing inflammatory cytokines.

JAK2 inhibitors other than ruxolitinib that have undergone phase III clinical trials include fedratinib, momelotinib, and pacritinib (127). Fedratinib was recently approved for use in patients intolerant or resistant to ruxolitinib, with approximately a third of patients responding, regardless of the reason for ruxolitinib discontinuation (128). Compared with placebo, the spleen response rate with fedratinib 400 mg/day (36% vs. 1%) was similar to that seen with ruxolitinib (JAKARTA-1; ref. 129); spleen response rates for pacritinib were 19% versus 5% and 18% versus 3%, compared with BAT, which excluded (PERSIST-1) (130) or included (PERSIST-2; ref. 131) JAK inhibitor therapy, the latter involving patients with platelet count of $<100 \times 10^6$/L. Momelotinib was compared with ruxolitinib (SIMPLIFY-1) with similar spleen response rates (26.5% vs. 29%; ref. 132) and compared with BAT (SIMPLIFY-2) in patients failing treatment with ruxolitinib with meager spleen response rates (7% vs. 6%; ref. 133). The toxicity profiles were noticeably different for the aforementioned JAK inhibitors: fedratinib (Wernicke's encephalopathy, anemia, thrombocytopenia, gastrointestinal distress and elevations in serum liver function tests and pancreatic enzymes); pacritinib (cardiac events, severe diarrhea, nausea, thrombocytopenia, anemia and hemorrhage); momelotinib (peripheral neuropathy, thrombocytopenia, first-dose effect including dizziness, nausea, hypotension, headache, and flushing; refs. 107, 108). Among the currently available JAK inhibitors, momelotinib is uniquely identified as being potentially useful in alleviating anemia, in addition to the expected responses in spleen size and symptoms, and the particular activity has been ascribed to the drug's inhibitory activity against activin receptor type 1 (ACVR1), which upregulates hepcidin synthesis (134).

AHSCT in Myelofibrosis

MIPSSv2-stratifed outcome analysis of AHSCT in MF was assessed in a retrospective study of 110 patients with a median follow-up of 64 months (110); approximately 89% of evaluable patients were MIPSSv2 high or very high risk and the remaining intermediate risk. The 5-year overall survival rate for the entire patient cohort was 65%, progression-free survival 60%, relapse rate 17%, nonrelapse mortality 24%, grade 2–4 acute graft versus host disease (GVHD) 45%, and chronic extensive GVHD 59%. In the particular study (110), somatic mutations did not affect outcome after AHSCT, whereas survival in patients with intermediate or high risk MIPSSv2 risk category (median not reached at last follow-up) was superior to that of patients with very high risk MIPSSv2 risk category (median 25 months; ref. 110). The better than 50% five-year survival was also apparent in our own institutional experience where neither very high risk nor unfavorable karyotype affected post-transplant survival (102). In a much larger study of 2,224 patients with MF who underwent AHSCT, 781 received myeloablative (MAC; median age 53 years) and 1,443 reduced intensity (RIC; median age 58 years) conditioning; outcome in the two groups were mostly similar in regards to engraftment period, rates of grade 2–4 acute GVHD (MAC 28% and RIC 31%), 5-year nonrelapse mortality (MAC 35% and RIC 34%), and 5-year survival (MAC 53% and RIC 51%; ref. 135). More recent studies have confirmed the value

Table 2. Conventional drug treatment strategies in myelofibrosis.

Indications	Treatment options	Reported response rates	Side effects include
Anemia	Thalidomide 50 mg QD + Prednisone 20 mg QD (171, 172)	46%–62%	Peripheral neuropathy Constipation Fatigue Cutaneous reactions CNS symptoms/sedation Sleep disturbances
	Anabolic steroids (173, 174) (dose depends on preparation)	44%–57%	Virilizing effects Liver toxicity
	Danazol 200 mg BID (175)	30% (175)	Liver toxicity LFT abnormalities
	Lenalidomide 10 mg QD ± Prednisone 20 mg QD (176, 177)	20%–25%	Myelosuppression
	ESAs (114, 178)	0% in Tx-dependent (114) 37% in Tx-independent (114)	Unremarkable
Splenomegaly	Hydroxyurea 500 mg BID (112) (starting dose)	40%[b]	↓hemoglobin; ↓platelet count Skin changes including ulcers Oral ulcers Nail discolorations
	Ruxolitinib 15 mg BID (117, 120) (starting dose)	32%[a]	↓hemoglobin; ↓platelet count Herpes zoster (180) Reactivation of tuberculosis (180) Other opportunistic infections (180) ↓COVID-19 vaccine response (126)
	Fedratinib 400 mg QD (129, 179)	47%[a]	↓hemoglobin; ↓platelet count ↑LFTs; ↑pancreatic enzymes Gastrointestinal symptoms Encephalopathy[b]
Constitutional symptoms	Ruxolitinib 15 mg BID (117, 120) Fedratinib 400 mg QD (129, 179)	Majority of patients Majority of patients	See above See above

Abbreviations: BID, twice-daily; ESAs, erythropoiesis-stimulating agents including darbepoetin 150–300 g every 2 weeks; LFT, liver function test; Tx, red blood cell transfusion; QD, once-daily.
[a]Seen at 500 mg/day dose.
[b]Response rates are not comparable because of reference to different patient populations and different methods and timing of spleen response assessment.

of AHSCT in older patients (136), the possibility of using family mismatched donors (137) and newer effective therapies for GVHD (e.g., ruxolitinib, ibrutinib; refs. 138–140). In a recent study of 556 transplanted patients with MF and age ≥65 years (median 67, range 65–76), followed for a median of 3.4 years, 5-year survival, nonrelapse mortality, and relapse rates were 40%, 37%, and 25%, respectively (136). Similarly favorable outcome data have also been reported in patients receiving alternative donor grafts (141). Unresolved issues with AHSCT in MF include the role of splenectomy (142–144), involved field radiotherapy (145, 146), and use of JAK2 inhibitors before and after AHSCT (147–151). A number of recent publications have addressed the issue of pretransplant management of splenomegaly in MF, including splenectomy, splenic irradiation, and use of JAK2 inhibitors (142, 144, 145, 147, 152). There is currently no consensus regarding either the need or specific treatment option in this regard and decisions are best made on a case-by-case basis (152).

New Drugs

In this section, we provide a synopsis of selected novel agents under active clinical investigation in patients with MF (**Table 3**).

Pelabresib (CPI-0610)

CPI-0610, is an oral inhibitor of bromodomain and extraterminal domain (BET) proteins that inhibits cytokine production and promotes megakaryocytic and erythroid differentiation. In the phase II (MANIFEST) study, CPI-0610 was administered as (i) monotherapy in MF patients that were refractory or intolerant to JAK inhibitors (Arm 1, $n = 43$; ref. 153), (ii) add on therapy in those with suboptimal response to ruxolitinib (Arm 2, $n = 78$; ref. 154), or (iii) in combination with ruxolitinib in JAK inhibitor–naïve patients (Arm 3, $n = 64$; ref. 155); 3/14 (21%) and 13/36 (36%) transfusion-dependent patients achieved transfusion independence in Arm 1 (CPI-0610 monotherapy) and Arm 2 (CPI-0610 with ruxolitinib) cohorts, respectively (153, 154). Correspondingly, among nontransfusion-dependent patients, 13 of 22 (59%) in Arm 1 and 4 of 23 (17%) in Arm 2 had a rise in hemoglobin level of >1.5 g/dL (153, 154). Notably, none of the transfusion dependent patients in Arm 1 versus 21% in Arm 2 demonstrated spleen response. CPI-0610 in combination with ruxolitinib yielded spleen and symptom response in 63% and 59% of JAK inhibitor–naïve patients, respectively (155). Commonly reported adverse events included gastrointestinal toxicity (46%), thrombocytopenia (40%), respiratory infections (32%),

Table 3. Summary of selected novel therapies in clinical trials in patients with myelofibrosis.

Drug/mechanism	Dose	Spleen response	Anemia response	Symptom response	Correlative studies	Toxicity	Current status
Pelabresib (CPI-0610) (153–155, 181) Bromodomain and Extraterminal Domain Inhibitor (BETi). Phase II trial (MANIFEST) Arm 1 (refractory/intolerant to ruxolitinib). Non-TD ($n = 27$) TD ($n = 19$)	125 mg daily on days 1–14 of a 21-day cycle	Week 24 SVR≥35% Non-TD cohort 5/21(23.8%) TD cohort none	Week 24 TD cohort 3/14 (21%) TD-TI Non-TD cohort 13/22 (59%), hemoglobin improved ≥1.5 g/dL over 12 weeks	Week 24 TSS≥50% Non-TD cohort 9/19 (47.4%) TD cohort 1/12 (8.3%)	Non-TD cohort Improvement in fibrosis	Thrombocytopenia (25.6%), Anemia (11.6%), Nausea (32.6%), Diarrhea (30.2%), Respiratory tract infections (27.9%)	
Arm 2 (suboptimal response or progression on ruxolitinib) TD ($n = 52$), Non-TD ($n = 26$).	CPI-0610 + ruxolitinib	Week 24 SVR≥35% TD cohort: 5/24 (20.8%) Non-TD cohort: 4/18 (22.2%)	TD cohort 13/36 (36%) TD-TI Non-TD cohort 4/23 (17%) Hb improved ≥1.5 g/dL over 12 weeks.	Week 24 TSS ≥ 50% TD cohort: 12/26 (46.2%) Non-TD cohort: 7/19 (36.8%)	Not provided	Thrombocytopenia (40%), Asthenia (32%), Diarrhea (46%), Nausea (36%), Respiratory infections (32%)	
Arm 3: JAKi-naïve patients ($n = 64$)	CPI-0610 + ruxolitinib 10 mg, bid	Week 24 SVR≥35% 19/30 (63.3%)	None	Week 24 TSS≥50% 17/29 (58.6%)	Not provided	Anemia (23.4%), Thrombocytopenia (20.3%), Diarrhea (26.6%), Respiratory infections (18.8%), Nausea (18.8%)	Phase 3 study CPI-0610 + ruxolitinib vs. placebo + ruxolitinib in JAKi-naïve MF (MANIFEST-2)
Navitoclax (157, 182) Antiapoptotic, binds to B-cell lymphoma 2 (BCL2) Family, including BCL-X_L, BCL2, and BCL-W Phase II trial in pts with suboptimal response to ruxolitinib ($n = 34$)	50 mg to 300 mg daily + ruxolitinib (>10 mg, bid)	Week 24 SVR ≥35% 9 (27%) Anytime SVR ≥35% 15 (44%) Median duration of SVR- 13.8 months	7/11 (64%) with Hb < 10 g/dL or TD improved ≥ 2 g/dL ($n = 6$) or TI ($n = 1$)	Week 24 TSS≥50% 6/20 (30%)	Fibrosis reduction ≥1 grade in 11/33 (33%). 12/26 (46%) with >10% driver gene VAF reduction. Median overall survival- not reached 24-month survival- 84%	AEs ≥20% Thrombocytopenia (88%) Diarrhea Fatigue Anemia Nausea Dizziness Confusion Vomiting	Phase III study navitoclax + ruxolitinib vs. placebo + ruxolitinib in JAKi naïve (TRANSFORM-1) vs. BAT in relapsed/refractory to JAKi. (TRANSFORM-2)

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Table 3. Summary of selected novel therapies in clinical trials in patients with myelofibrosis. (Cont'd)

Drug/mechanism	Dose	Spleen response	Anemia response	Symptom response	Correlative studies	Toxicity	Current status
Imetelstat (161) Telomerase inhibitor Phase II trial (MYF2001) in relapsed/refractory to JAKi, ($n = 107$)	Imetelstat (9.4 mg/kg or 4.7 mg/kg) i.v. every 3 weeks	Week 24 SVR ≥35% 6 (10.2%) in 9.4 mg/kg arm with no responses in 4.7 mg/kg arm	None	Week 24 TSS≥50% 19 (32%) in 9.4 mg/kg arm and 3 (6%) in 4.7 mg/kg arm.	41% with ≥1 grade reduction in fibrosis. 5/24 (20.8%) with ≥50% reduction in abnormal cytogenetic clones (all with sole 13q) 12/26 (46.2%) in 9.4 mg/kg vs. (4/23) 17.4% in 4.7 mg/kg arm with ≥20% VAF reduction of any mutated gene Median overall survival 29.9 months with 9.4 mg/kg.	Thrombocytopenia (49%), Anemia (44%), Neutropenia (36%), Nausea (34%).	Phase 3 study 9.4 mg/kg of imetelstat vs. BAT with the exclusion of JAKi in refractory MF
Luspatercept (159, 160) Binds to TGFβ superfamily ligand, reduces aberrant Smad2/3 signaling and enhances late-stage erythropoiesis. Phase II trial Non-TD without ruxolitinib ($n = 22$) (Cohort 1) With ruxolitinib ($n = 14$) (Cohort 3a) TD Without ruxolitinib ($n = 21$) (Cohort 2) With ruxolitinib ($n = 22$) (Cohort 3b)	1–1.75 mg/kg s.c. every 21 days	Not provided	Week 24 Cohort 1 (14%) Cohort 3a (21%)) Cohort 2 2/21 (10%) Cohort 3b 6/22 (27%) achieved TI. Overall Cohort 2 4/21 (19%) Cohort 3b 8/22 (36%) achieved TI.	Not provided	Not provided	Treatment-related AEs; Hypertension (13%), Bone pain (9%), Diarrhea (5%). 8 (10%) pts had ≥ 1 AE leading to drug discontinuation	Phase 3 study in MF-associated anemia with concomitant JAKi therapy and transfusion needs
Parsaclisib (164) PI3Kδ inhibitor Phase II trial in MF with suboptimal response to ruxolitinib ($n = 53$)	10 or 20 mg QD oral for 8 wks QW thereafter) or parsaclisib QD (5 or 20 mg QD for 8 wks/5 mg QD thereafter) with stable ruxolitinib dose (5–25 mg bid)	Week 12 median % change, −2.3 and −15.4 Week 24 −2.5 and −25.4 with QD/QW and QD dosing	Hemoglobin remained stable	Week 12 median % change −14.0 in QD/QW −39.6 in QD	Not provided	18%/30% grade 3 thrombocytopenia. 21%/0% grade 4 thrombocytopenia, grade 3/4 events; tuberculosis, enteritis, fatigue, hypertension, abnormal liver tests, stomatitis	Phase 3 randomized study ruxolitinib + parsaclisib in JAK- and PI3K-inhibitor treatment-naïve myelofibrosis. Phase 3 randomized study ruxolitinib + parsaclisib vs. ruxolitinib + placebo in patients with suboptimal responseto ruxolitinib.

(Continued on the following page)

Table 3. Summary of selected novel therapies in clinical trials in patients with myelofibrosis. (Cont'd)

Drug/mechanism	Dose	Spleen response	Anemia response	Symptom response	Correlative studies	Toxicity	Current status
Bomedemstat (IMG-7289) (163) Lysine-specific demethylase, LSD1 inhibitor Phase II trial in MF resistant to approved therapies. ($n = 62$)	Dose uptitrated based on platelet count targeting 50–100k/μL (QD orally).	Week 24 81% ($n = 19$) reduction in spleen volume (mean SVR: -8%).	72% of evaluable patients ($n = 36$) with stable or improved hemoglobin >1 g/dl.	Week 24 85% ($n = 13$), TSS reduction (mean change -31%). 31% with TSS >50% reduction.	Improvement in fibrosis > grade 1 in 26%. Reduction in driver/HMR mutation VAF in 42%	8 SAEs possibly related: painful splenomegaly, rectal bleeding, heart failure, headache, vertigo, GI hemorrhage, anemia, pyoderma gangrenosum	Ongoing.
Sotatercept (ACE-011) (183) activin receptor type IIA ligand trap Phase II trial in MF pts with anemia, ($n = 53$)	Single agent 0.75 or 1 mg/kg S/C every 3 weeks ($n = 31$) and in combination with a stable dose of ruxolitinib ($n = 21$)	Not provided	Sotatercept alone. 4 anemia responses (29%); 3 TD to TI. combination cohort 5/17 (29%) pts responded, all non-TD pts	Not provided	Not provided	Hypertension ($n = 7$), limb pain ($n = 3$), headache ($n = 1$)	Ongoing
Tagraxofusp (SL-401) (184) CD123-directed antibody Phase I/II trial in relapsed/refractory MF ($n = 35$)	Stage 1 (dose escalation), at 7, 9, or 12 mcg/kg i.v. on days 1–3 every 21 days (cycle 1–4), 28 days (cycles 5–7), 42 days (cycles 8^{+}). Stage 2 (expansion), recommended phase II dose (12 mcg/kg).	Week 24 Spleen response by palpation ($n = 10$, 45%), 2 pts with >50% reduction.	None	3 patients by IWG-MRT 12/26 (46%) with symptom reduction.	Not provided	hypoalbuminemia (23%), headache (17%), elevated ALT (17%). Capillary leak syndrome in 3 pts (1 grade 4).	Ongoing

Abbreviations: ALT, alanine transferase; AEs, adverse events; BAT, best available therapy; BID, twice daily; HMR, high molecular risk mutation; IWG-MRT, International working group-myeloproliferative neoplasms research and treatment; JAKi- Janus kinase inhibitor; MF, myelofibrosis; SAE, serious adverse event; SVR, spleen volume reduction;TSS, total symptom score; TI, transfusion independent; TD, transfusion dependent; VAF, variant allele frequency; QD/QW, once daily/once weekly.

and anemia (12%; refs. 153–155). Based on these findings, a randomized placebo-controlled phase III study (MANIFEST-2) with CP-0610 in combination with ruxolitinib in JAK inhibitor–naïve patients is ongoing (156).

Navitoclax

Navitoclax is an oral small-molecule inhibitor of antiapoptotic B-cell lymphoma 2 (BCL2) family proteins, including BCL-X_L, BCL2, and BCL-W. In a phase II study with navitoclax in combination with ruxolitinib in patients with MF with suboptimal response to ruxolitinib, 34 patients were enrolled, with ongoing therapy in 17 patients (157, 158). Spleen and symptom response were reported in 27% and 30% of patients, respectively (157). Commonly reported adverse events included thrombocytopenia in 88%, which led to dose reduction in 56% of patients, diarrhea (71%), and fatigue (62%). As a next step, two phase III studies are ongoing, which include a placebo-controlled study of navitoclax in combination with ruxolitinib in JAK inhibitor treatment–naïve patients (TRANSFORM-1), and navitoclax in combination with ruxolitinib versus best available therapy in patients relapsed following JAK inhibitor therapy (TRANSFORM-2).

Luspatercept

Luspatercept, an erythropoiesis maturating agent that binds to TGFβ superfamily ligand, reduces aberrant Smad2/3 signaling and enhances late-stage erythropoiesis, is FDA approved for myelodysplastic syndromes with ringed sideroblasts. In an ongoing phase II investigation in MF, four cohorts that included transfusion-dependent and -independent patients that were either receiving or not on ruxolitinib were enrolled (159). Among transfusion-dependent patients, 36% and 19% of patients receiving or not receiving ruxolitinib were rendered transfusion independent with median duration of response of 55 and 59 weeks, respectively (160). Most common adverse event of hypertension was noted in 13% of patients. Currently, luspatercept is undergoing phase III investigation in the INDEPENDENCE study, which includes patients with MF with transfusion requiring anemia and receiving JAK inhibitor therapy.

Imetelstat

Imetelstat, a telomerase inhibitor, has proven safety and efficacy in a pilot study in patients with treatment-naïve and relapsed MF (51). Recently published data from the randomized phase II study in patients relapsed/refractory to JAK inhibitors showed modest activity in terms of spleen (10%) and symptom (32%) response in patients receiving imetelstat 9.4 mg/kg i.v. every 3 weeks (161). However, improvement in bone marrow fibrosis was noted in 41%, with driver mutation variant allele frequency reduction in 42% of patients, which correlated with superior overall survival (median survival; 29.9 months; ref. 161). Major toxicities included thrombocytopenia recorded in half of patients, followed by anemia (44%) and neutropenia in one-third of patients (161). Based on its selective impact on the malignant clone, a confirmatory phase II study of imetelstat 9.4 mg/kg vs. best available therapy excluding JAK inhibitors in relapsed MF is ongoing.

Bomedemstat (IMG-7289)

An oral inhibitor of lysine-specific demethylase-1 inhibitor (LSD1), which is involved in differentiation and maturation of megakaryocytes, has been shown to improve blood counts, splenomegaly, cytokine profile, mutant allele burden and fibrosis in murine models (162). In a phase II study of IMG-7289 monotherapy in patients with MF resistant to approved therapies, 62 patients have been enrolled, majority had received prior ruxolitinib ($n = 56$), one-third were transfusion-dependent, and 94% harbored *ASXL1* mutation (163). Eighty-one percent of patients demonstrated spleen response, one-third reported symptom response, and 72% of patients had stable or >1 g/dL improvement in hemoglobin levels (163). In addition, 26% of patients demonstrated at least grade 1 improvement in fibrosis, 42% with reduction, and 50% with stability of driver and high-risk mutation allele burden.

Parsaclisib

An oral highly selective PI3Kδ inhibitor is under phase II investigation in patients with MF with suboptimal response to ruxolitinib that may arise due to persistent PI3K/AKT activation (164). Patients with MF underwent randomization to add-on parsaclisib once daily/once weekly or parsaclisib once daily while remaining on a stable ruxolitinib dose. Fifty-three patients have been treated; 33 and 20 patients received parsaclisib once daily/once weekly and once daily, respectively. At 12- and 24-week assessments, median percent spleen change was −2.3 and −15.4 at week 12; and −2.5 and −25.4 at week 24 with once daily/once weekly and once daily dosing, respectively, while median percent change in symptom score at week 12 was −14.0 in once daily/once weekly, and −39.6 in once daily (164). With once daily/once weekly and once daily dose, 18% and 30% developed grade 3 thrombocytopenia; 21% and 0% had grade 4 thrombocytopenia, while hemoglobin levels remained stable. Additional grade 3/4 treatment-related, adverse events included disseminated tuberculosis, enteritis, fatigue, hypertension, abnormal liver tests and stomatitis. Importantly, half of patients interrupted parsaclisib due to adverse events (164). On the basis of the above findings, a randomized study of add-on parsaclisib versus placebo in patients with suboptimal response to ruxolitinib is underway (165), together with first-line phase III investigation in patients with JAK and PI3K inhibitor—naïve MF (166).

Concluding Remarks

At present, AHSCT remains the only treatment modality in MF that secures disease-free remission state and prolonged survival; furthermore, palliative value beyond ruxolitinib has remained out of reach for most of the drugs that are currently under investigation, and the possibility of incremental value is likely to be countered by additional side effects (**Table 1**). The scenario warrants urgent attention to newer therapeutic targets and, more importantly, identification of repurposable drug candidates, to accelerate the discovery process (167). In a recent edition of *Cancer Research*, Dutta and colleagues described their observations from *JAK2*V617F knock-in mice, which included upregulation of CDK6 expression in hematopoietic progenitors and *in vivo* therapeutic activity for CDK4/6 inhibitor palbociclib alone or in combination with ruxolitinib, including reversal of bone marrow fibrosis and reduction of spleen size (73). The observations regarding PPARγ and CDK6 as therapeutic targets are particularly noteworthy considering the current availability of FDA-approved drug antagonists (168, 169). A similar approach might be needed to address the unmet need in the treatment of blast-phase MF, which occurs in approximately

10%–20% of patients during their clinical course and where outcome under current therapy, including AHSCT, is dismal (170). Now more than ever, there is a need for genetic characterization of clinical observations followed by the relevant *in vitro* and *in vivo* animal studies that hopefully lead to development of novel therapies that target the malignant clone, and not only its secondary constitutional effects.

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