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- Provide an overview on the classification, classification and pathogenesis of primary myelofibrosis
- Provide an update on prognostication and management
- Provide an update on novel drug therapies

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ANNUAL CLINICAL UPDATES IN HEMATOLOGICAL MALIGNANCIES: A CONTINUING MEDICAL EDUCATION SERIES

Primary myelofibrosis: 2013 update on diagnosis, risk-stratification, and management

Ayalew Tefferi*

Disease overview: **Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by stem cell-derived clonal myeloproliferation, abnormal cytokine expression, bone marrow fibrosis, anemia, splenomegaly, extramedullary hematopoiesis (EMH), constitutional symptoms, cachexia, leukemic progression, and shortened survival.**

Diagnosis: **Diagnosis is based on bone marrow morphology. The presence of fibrosis, *JAK2*/*MPL* mutation, or +9/13q− cytogenetic abnormality is supportive but not essential for diagnosis. Prefibrotic PMF mimics essential thrombocythemia in its presentation and the distinction is prognostically relevant. Differential diagnosis of myelofibrosis should include chronic myeloid leukemia, myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia.**

Risk stratification: **The Dynamic International Prognostic Scoring System-plus (DIPSS-plus) prognostic model for PMF can be applied at any point during the disease course and uses eight independent predictors of inferior survival: age >65 years, hemoglobin <10 g/dL, leukocytes >25 × 10^9/L, circulating blasts ≥1%, constitutional symptoms, red cell transfusion dependency, platelet count <100 × 10^9/L, and unfavorable karyotype (i.e., complex karyotype or sole or two abnormalities that include +8, −7/7q−, i(17q), inv(3), −5/5q−, 12p−, or 11q23 rearrangement). The presence of 0, 1, "2 or 3," and ≥4 adverse factors defines low, intermediate-1, intermediate-2, and high-risk disease with median survivals of approximately 15.4, 6.5, 2.9, and 1.3 years, respectively. A >80% two-year mortality is predicted by monosomal karyotype, inv(3)/i(17q) abnormalities, or any two of circulating blasts >9%, leukocytes ≥40 × 10^9/L or other unfavorable karyotype. Most recently, mutations involving *ASXL1*, *SRSF2*, *EZH2*, and *IDH1/2* or increased plasma IL-2R, IL-8, or serum-free light chain levels have been shown to adversely affect survival.**

Risk-adapted therapy: **Observation alone is adequate for asymptomatic low/intermediate-1 risk disease. Allogeneic stem cell transplantation (ASCT) is often considered for high risk disease. Conventional or experimental drug therapy is reasonable for symptomatic intermediate-1 or intermediate-2 risk disease; however, ASCT is an acceptable treatment option for such patients in the presence of *ASXL1* or other prognostically adverse mutations. Splenectomy and low-dose radiotherapy are used for drug-refractory splenomegaly. Radiotherapy is also used for the treatment of non-hepatosplenic EMH, PMF-associated pulmonary hypertension, and extremity bone pain. Am. J. Hematol. 88:142–150, 2013. © 2013 Wiley Periodicals, Inc.**

Disease Overview

The World Health Organization (WHO) classification system for hematopoietic tumors recognizes five categories of myeloid malignancies including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), MDS/MPN overlap, and *PDGFR*/*FGFR1*-rearranged myeloid/lymphoid neoplasm with eosinophilia (Table I) [1]. "*BCR-ABL1*-negative MPN" is an operational sub-category of MPN that includes polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [2]. These three disorders are characterized by stem cell-derived clonal myeloproliferation and presence of somatic mutations involving primarily *JAK2* and to a smaller extent *MPL*, *LNK*, *CBL*, *TET2*, *ASXL1*, *IDH*, *IKZF1*, *EZH2, DNMT3A, TP53, SF3B1*, or *SRSF2* mutations (Table II) [3–9]. The pathogenetic relevance of these mutations is currently under investigation (Table II) but none of them appear to garner the disease specificity or pathogenetic relevance otherwise displayed by *BCR-ABL1*. However, the phenotype of clonal erythrocytosis might require the presence of a mutation in *JAK2* (*JAK2*V617F or *JAK2* exon 12 mutation) [10] or its negative regulators such as *LNK* [11]. Similarly, there appears to be a close association between *SF3B1* mutations and presence of bone marrow ring sideroblasts in MPN [6].

In PMF, clonal myeloproliferation is associated with reactive bone marrow fibrosis, osteosclerosis, angiogenesis, extramedullary hematopoiesis (EMH), and an abnormal cytokine expression [12,13]. Clinical manifestations in PMF include severe anemia, marked hepatosplenomegaly, constitutional symptoms (e.g., fatigue, night sweats, fever), cachexia, bone pain, splenic infarct, pruritus, thrombosis, and bleeding [14]. Ineffective erythropoiesis and EMH are the main causes of anemia and organomegaly, respectively. Other disease complications include symptomatic portal

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TABLE I. World Health Organization (WHO) Classification of Myeloid Malignancies

1. Acute myeloid leukemia (AML) and related precursor neoplasms[a]
2. Myeloproliferative neoplasms (MPN)
   2.1. Classic MPN
      2.1.1. Chronic myelogenous leukemia, *BCR-ABL1* positive (CML)
      2.1.2. Polycythemia vera (PV)
      2.1.3. Primary myelofibrosis (PMF)
      2.1.4. Essential thrombocythemia (ET)
   2.2. Non-classic MPN
      2.2.1. Chronic neutrophilic leukemia (CNL)
      2.2.2. Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
      2.2.3. Mastocytosis
      2.2.4. Myeloproliferative neoplasm, unclassifiable (MPN-U)
3. Myelodysplastic syndromes (MDS)
   3.1. Refractory cytopenia[b] with unilineage dysplasia (RCUD)
      3.1.1. Refractory anemia (ring sideroblasts<15% of erythroid precursors)
      3.1.2. Refractory neutropenia
      3.1.3. Refractory thrombocytopenia
   3.2. Refractory anemia with ring sideroblasts
      (RARS; dysplasia limited to erythroid
      lineage and ring sideroblasts≥15% of bone marrow erythroid precursors)
   3.3. Refractory cytopenia with multi-lineage dysplasia
   (RCMD; ring sideroblast count does not matter)
   3.4. Refractory anemia with excess blasts (RAEB)
      3.4.1. RAEB-1 (2–4% circulating or 5–9% marrow blasts)
      3.4.2. RAEB-2 (5–19% circulating or 10–19%
         marrow blasts or Auer rods present)
   3.5. MDS associated with isolated del(5q)
   3.6. MDS, unclassifiable
4. MDS/MPN
   4.1. Chronic myelomonocytic leukemia (CMML)
   4.2. Atypical chronic myeloid leukemia, *BCR-ABL1* negative
   4.3. Juvenile myelomonocytic leukemia (JMML)
   4.4. MDS/MPN, unclassifiable
      4.4.1. Provisional entity: Refractory anemia with ring
         sideroblasts associated with marked thrombocytosis (RARS-T)
5. Myeloid and lymphoid neoplasms with eosinophilia and
   abnormalities of *PDGFRA*,[c] *PDGFRB*,[c] or *FGFR1*[c]
   5.1. Myeloid and lymphoid neoplasms with *PDGFRA* rearrangement
   5.2. Myeloid neoplasms with *PDGFRB* rearrangement
   5.3. Myeloid and lymphoid neoplasms with *FGFR1* abnormalities

[a] Acute myeloid leukemia-related precursor neoplasms include "therapy-related myelodysplastic syndrome" and "myeloid sarcoma."

[b] Either mono- or bi-cytopenia: hemoglobin level<10 g/dL, absolute neutrophil count<1.8 $\times$ 10^9/L, or platelet count<100 $\times$ 10^9/L. However, higher blood counts do not exclude the diagnosis in the presence of unequivocal histological/cytogenetic evidence for myelodysplastic syndrome.

[c] Genetic rearrangements involving platelet-derived growth factor receptor α/β (*PDGFRA/PDGFRB*) or fibroblast growth factor receptor 1 (*FGFR1*).

hypertension that might lead to variceal bleeding or ascites and non-hepatosplenic EMH that might lead to cord compression, ascites, pleural effusion, pulmonary hypertension, or diffuse extremity pain. It is currently assumed that aberrant cytokine production by clonal cells and host immune reaction contributes to PMF-associated bone marrow stromal changes, ineffective erythropoiesis, EMH, cachexia, and constitutional symptoms [12]. Causes of death include leukemic progression that occurs in approximately 20% of patients but many patients also die of comorbid conditions including cardiovascular events and consequences of cytopenias including infection or bleeding [15].

Diagnosis

Current diagnosis of PMF is based on WHO-criteria and involves a composite assessment of clinical and laboratory features (Table III) [16]. The diagnosis of post-PV or post-ET MF should adhere to criteria recently published by the International Working Group for MPN Research and Treatment (IWG-MRT) (Table IV) [17]. Peripheral blood leukoerythroblastosis (i.e., presence of nucleated red cells, immature granulocytes, and dacryocytes) is a typical but not invariable feature of PMF; prefibrotic PMF might not display overt leukoerythroblastosis [20]. Bone marrow fibrosis in PMF is usually associated with *JAK2*V617F, trisomy 9, or del(13q) [21]. The presence of these genetic markers, therefore, strongly supports a diagnosis of PMF, in the presence of a myeloid neoplasm associated with bone marrow fibrosis.

PMF should be distinguished from other closely related myeloid neoplasms including chronic myeloid leukemia (CML), PV, ET, MDS, chronic myelomonocytic leukemia (CMML), and "acute myelofibrosis." The presence of dwarf megakaryocytes raises the possibility of CML and should be pursued with *BCR-ABL1* cytogenetic or molecular testing. Patients who otherwise fulfill the diagnostic criteria for PV should be labeled as "PV" even if they display substantial bone marrow fibrosis [16]. Prefibrotic PMF can mimic ET in its presentation and careful morphologic examination is necessary for distinguishing the two; megakaryocytes in ET are large and mature-appearing whereas those in prefibrotic PMF display abnormal maturation with hyperchromatic and irregularly folded nuclei [20]; the distinction between ET and pre-fibrotic PMF is prognostically relevant [22]. MDS should be suspected in the presence of dyserythropoiesis or dysgranulopoiesis [23]. CMML is a possibility in the presence of peripheral blood monocyte count of greater than 1 $\times$ 10^9/L. Patients with acute myelofibrosis (either acute panmyelosis with myelofibrosis or acute megakaryoblastic leukemia) usually present with severe constitutional symptoms, pancytopenia, mild or no splenomegaly, and increased circulating blasts [24].

Risk Stratification

Robust prognostic modeling in PMF started with the development of the International Prognostic Scoring System (IPSS) in 2009 [25]. The IPSS for PMF is applicable to patients being evaluated at time of initial diagnosis and uses five independent predictors of inferior survival: age >65 years, hemoglobin <10 g/dL, leukocyte count >25 $\times$ 10^9/L, circulating blasts ≥1%, and presence of constitutional symptoms [25]. The presence of 0, 1, 2, and ≥3 adverse factors defines low, intermediate-1, intermediate-2, and high-risk disease. The corresponding median survivals were 11.3, 7.9, 4, and 2.3 years [25].

The IWG-MRT subsequently developed a dynamic prognostic model (Dynamic International Prognostic Scoring System, DIPSS) that utilizes the same prognostic variables used in IPSS but can be applied at any time during the disease course [26]. DIPSS assigns two, instead of one, adverse points for hemoglobin <10 g/dL and risk categorization is accordingly modified: low (0 adverse points), intermediate-1 (1 or 2 points), intermediate-2 (3 or 4 points), and high (5 or 6 points). The corresponding median survivals were not reached, 14.2, 4, and 1.5 years [26].

More recently, IPSS- and DIPSS-independent risk factors for survival in PMF were identified and included unfavorable karyotype (i.e., complex karyotype or sole or two abnormalities that include +8, −7/7q−, i(17q), inv(3), −5/5q−, 12p−, or 11q23 rearrangement) [27,28], red cell transfusion need [29,30], and platelet count <100 $\times$ 10^9/L [31] Accordingly, DIPSS was recently modified into DIPSS-plus by incorporating these three additional DIPSS-independent risk factors: platelet count <100 $\times$ 10^9/L, red cell transfusion need, and unfavorable karyotype [32]. The four DIPSS-plus risk categories based on the aforementioned eight risk factors (Table V; Fig. 1) are low (no risk factors), intermediate-1 (one risk factor), intermediate-2 (two or 3 risk factors), and high (four or more risk factors) with respective median survivals of 15.4, 6.5, 2.9, and 1.3 years [32]. Furthermore, a >80% two-year mortality was predicted by monosomal karyotype, inv(3)/i(17q) abnormalities, or any two of circulating blasts >9%, leukocytes ≥40 $\times$ 10^9/L, or other unfavorable karyotype [34]. Patients with the latter characteristics are

TABLE II. Somatic Mutations in Primary Myelofibrosis (PMF) and the Closely Related *BCR-ABL1*-Negative Myeloproliferative Neoplasms (MPN) Including Polycythemia Vera (PV) and Essential Thrombocythemia (ET)

Mutations	Chromosome location	Mutational frequency	Pathogenetic relevance
JAK2 (Janus kinase 2) *JAK2V617F exon 14 mutation*	9p24	PV ~96% ET ~55% PMF ~65% BP-MPN ~50%	Contributes to abnormal myeloproliferation and progenitor cell growth factor hypersensitivity
JAK2 exon 12 mutation	9p24	PV ~3%	Contributes to primarily erythroid myeloproliferation
MPL (Myeloproliferative leukemia virus oncogene) *MPN-associated MPL mutations involve exon 10*	1p34	ET ~3% PMF ~10% BP-MPN ~5%	Contributes to primarily megakaryocytic myeloproliferation
LNK (as in Links) *a.k.a. SH2B3 (*a membrane-bound adaptor protein) *MPN-associated mutations were monoallelic and involved exon 2*	12q24.12	PV ~rare ET ~rare PMF ~rare BP-MPN ~10%	Wild-type LNK is a negative regulator of JAK2 signaling
TET2 (TET oncogene family member 2) *Mutations involve several exons*	4q24	PV ~16% ET ~5% PMF ~17% BP-MPN ~17%	TET proteins catalyze conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), which favors demethylated DNA. Both TET1 and TET2 display this catalytic activity. *IDH* and *TET2* mutations might share a common pathogenetic effect, which might include abnormal DNA hypermethylation and impaired myelopoiesis.
ASXL1 (Additional Sex Combs-Like 1) *Exon 12 mutations*	20q11.1	ET ~3% PMF ~13% BP-MPN ~18%	Wild-type ASXL1 is needed for normal hematopoiesis and might be involved in co-activation of transcription factors and transcriptional repression.
IDH1/IDH2 (Isocitrate dehydrogenase) *Exon 4 mutations*	2q33.3/15q26.1	PV ~2% ET ~1% PMF ~4% BP-MPN ~20%	*IDH* mutations induce loss of activity for the conversion of isocitrate to 2-ketoglutarate (2-KG) and gain of function in the conversion of 2-KG to 2-hydroxyglutarate (2-HG). 2-HG might be the mediator of impaired TET2 function in cells with mutant *IDH* expression.
EZH2 (enhancer of zeste homolog 2) *Mutations involve several exons*	7q36.1	PV ~3% PMF ~7% MDS ~6%	Wild-type EZH2 is part of a histone methyltransferase (polycomb repressive complex 2 associated with H3 Lys-27 trimethylation). MPN-associated *EZH2* mutations might have a tumor suppressor activity, which contrasts with the gain-of-function activity for lymphoma-associated *EZH2* mutations.
DNMT3A (DNA cytosine methyltransferase 3a) *Most frequent mutations affect amino acid R882*	2p23	PV ~7% PMF ~7% BP-MPN ~14%	DNA methyl transferases are essential in establishing and maintaining DNA methylation patterns in mammals
CBL (Casitas B-lineage lymphoma proto-oncogene) *Exon 8/9 mutations*	11q23.3	PV ~rare ET ~rare MF ~6%	CBL is an E3 ubiquitin ligase that marks mutant kinases for degradation. Transforming activity requires loss of this function.
IKZF1 (IKAROS family zinc finger 1) *Mostly deletions including intragenic*	7p12	CP-MPN ~rare BP-MPN ~19%	IKZF1 is a transcription regulator and putative tumor suppressor
TP53 (tumor protein p53) *Exons 4 through 9*	17p13.1	PMF ~ 4% BP-MPN ~27%	A tumor suppressor protein that targets genes that regulate cell cycle arrest, apoptosis and DNA repair.
SF3B1 (splicing factor 3B subunit 1) *Exons 14 and 15, mostly*	2q33.1	PMF ~7%	SF3B1 is a component of the RNA spliceosome, whose dysfunction promotes global abnormalities in RNA splicing and increased apoptosis. *SF3B1* mutations are closely associated with the ring sideroblast phenotype.
SRSF2 (serine/arginine-rich splicing factor 2) *Exon 2*	17q25.1	PMF ~17%	SRSF2 is a component of the RNA spliceosome, whose dysfunction promotes defects in alternative splicing.

MPN, myeloproliferative neoplasms; ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; MF includes both PMF and post-ET/PV myelofibrosis; BP-MPN, blast phase MPN; CP-MPN, chronic phase MPN; See text for references.

Mutational frequencies in blast phase (BP) disease are also provided.

operationally assigned a "very high risk" category and might be better served by immediate consideration for allogeneic stem cell transplantation (ASCT) (Fig. 2) [34].

More recent data suggest inferior survival in PMF associated with nullizygosity for *JAK2* 46/1 haplotype [35], low *JAK2*V617F allele burden [36,37], or presence of *IDH* [38,39], *EZH2* [40], *SRSF2* [9], or *ASXL1* [41] mutations. In contrast, the presence or absence of *JAK2*V617F [36,37], *MPL* [42], or *TET2* [43] mutations did not appear to affect survival. Survival in PMF was also affected by increased serum IL-8 and IL-2R levels as well as serum-free light chain levels, both independent of DIPSS-plus [44,45].

Risk factors for leukemia-free survival include $\geq$3% circulating blasts, platelet count $<100 \times 10^9$/L, and presence of unfavorable karyotype [46,47]. Although DIPSS has been shown to predict leukemia-free survival [48] in the aforementioned DIPSS-plus study of 793 patients with PMF, the only two risk factors for leukemic transformation were unfavorable karyotype and platelet count $<100 \times 10^9$/L [33]; 10-year risk of leukemic transformation were 12% in the absence of these two risk factors and 31% in the presence of one or both risk factors. As is becoming evident for overall survival, leukemia-free survival is also significantly compromised in patients carrying certain mutations including *IDH* and *SRSF2* [9,38,39].

Risk-Adapted Therapy

Current drug therapy for PMF is not curative and has not been shown to prolong survival. ASCT for PMF is potentially curative but dangerous; transplant-related death or severe morbidity occurs in about half of transplanted patients, regardless of the intensity of conditioning regimens used [49]. As a result, more and more patients with PMF (or post-PV/ET MF) are seeking treatment with novel drugs. However, it should be noted that many patients can be observed without any therapeutic intervention and some can be effectively managed by conventional drug therapy (Fig. 2).

Management of low or intermediate-1 risk patients

There is no evidence to support the value of specific therapy in asymptomatic patients with low or intermediate-1

TABLE III. World Health Organization (WHO) Diagnostic Criteria for Polycythemia Vera, Essential Thrombocythemia, and Primary Myelofibrosis

	2008 WHO Diagnostic Criteria					
		Polycythemia vera*		Essential thrombocythemia[a]		Primary myelofibrosis[a]
Major criteria	1	Hgb>18.5 g/dL (men) > 16.5 g/dL (women) or[b]	1	Platelet count≥450 × 10^9/L	1	Megakaryocyte proliferation and atypia[c] accompanied by either reticulin and/or collagen fibrosis, or[d]
	2	Presence of *JAK2*V617F or *JAK2* exon 12 mutation	2	Megakaryocyte proliferation with large and mature morphology.	2	Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm
			3	Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm	3	Demonstration of *JAK2*V617F or other clonal marker or no evidence of reactive marrow fibrosis
			4	Demonstration of *JAK2*V617F or other clonal marker or no evidence of reactive thrombocytosis		
Minor criteria	1	BM trilineage myeloproliferation			1	Leukoerythroblastosis
	2	Subnormal serum Epo level			2	Increased serum LDH level
	3	EEC growth			3	Anemia
					4	Palpable splenomegaly

[a] PV diagnosis requires meeting either both major criteria and one minor criterion **or** the first major criterion and 2 minor criteria. ET diagnosis requires meeting all 4 major criteria. PMF diagnosis requires meeting all 3 major criteria and two minor criteria.

[b] **or** Hgb or Hct>99th percentile of reference range for age, sex, or altitude of residence **or** red cell mass>25% above mean normal predicted **or** Hgb>17 g/dL (men)/>15 g/dL (women) if associated with a sustained increase of≥2 g/dL from baseline that can not be attributed to correction of iron deficiency

[c] Small to large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.

[d] **or** In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (i.e. pre-fibrotic PMF).

BM, bone marrow; Hgb, hemoglobin; Hct, hematocrit; Epo, erythropoietin; EEC, endogenous erythroid colony; WHO, World Health Organization; CML, chronic myelogenous leukemia; PV, polycythemia vera; PMF, primary myelofibrosis; MDS, myelodysplastic syndromes; LDH, lactate dehydrogenase.

TABLE IV. International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) Recommended Criteria for Post-Polycythemia Vera and Post-Essential Thrombocythemia Myelofibrosis [17]

Criteria for post-polycythemia vera myelofibrosis
- Required criteria:
  1. Documentation of a previous diagnosis of polycythemia vera as defined by the WHO criteria (see table II)
  2. Bone marrow fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale) (see footnote for details)
- Additional criteria (two are required):
  1. Anemia or sustained loss of requirement for phlebotomy in the absence of cytoreductive therapy
  2. A leukoerythroblastic peripheral blood picture
  3. Increasing splenomegaly defined as either an increase in palpable splenomegaly of≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly
  4. Development of≥1 of three constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5 °C)

Criteria for post-essential thrombocythemia myelofibrosis
- Required criteria:
  1. Documentation of a previous diagnosis of essential thrombocythemia as defined by the WHO criteria (see Table II)
  2. Bone marrow fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale) (see footnote for details)
- Additional criteria (two are required):
  1. Anemia and a≥2 g/dL decrease from baseline hemoglobin level
  2. A leukoerythroblastic peripheral blood picture
  3. Increasing splenomegaly defined as either an increase in palpable splenomegaly of≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly
  4. Increased lactate dehydrogenase
  5. Development of≥1 of three constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5 °C)

Grade 2–3 according to the European classification [18]: diffuse, often coarse fiber network with no evidence of collagenization (negative trichrome stain) or diffuse, coarse fiber network with areas of collagenization (positive trichrome stain). Grade 3–4 according to the standard classification [19]: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis or diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis.

TABLE V. Risk Stratification and Risk-Adapted Therapy in Primary Myelofibrosis

DIPSS-plus [48] risk groups PMF	Median survival	Management PMF
Low-risk (No risk factors[a])	~15.4 years	Observation or Conventional drugs[b]
Intermediate-1 risk (1 risk factor[a])	~6.5 years	Observation or Conventional drugs[b] or Experimental drugs
Intermediate-2 risk (2 or 3 risk factors[a])	~2.9 years	Allo-SCT or Experimental drugs
High-risk (≥ 4 risk factors[a])	~1.3 years	Allo-SCT or Experimental drugs

DIPSS, Dynamic International Prognostic Scoring System [33].

[a] DIPSS-plus [33] uses 8 risk factors for inferior survival: age>65 years, hemoglobin<10 g/dL, leukocyte count>25 × 10^9/L, circulating blasts≥1%, presence of constitutional symptoms, presence of unfavorable karyotype, platelet count<100 × 10^9/L and presence of red cell transfusion need. Please note that a transfusion-dependent patient automatically has 2 risk factors because of transfusion need (one risk point) and hemoglobin<10 g/dL (one risk point).

[b] Androgen preparations or thalidomide with prednisone for anemia; hydroxyurea for symptomatic splenomegaly.

risk disease [33]. It is conceivable that some low or intermediate-1 risk patients might require therapy for symptomatic anemia, splenomegaly, non-hepatosplenic EMH, bone pain, EMH-associated pulmonary hypertension, or constitutional symptoms (e.g., fatigue, night sweats, and pruritus). In addition, cytoreductive therapy is reasonable but not mandated in the presence of extreme leukocytosis or thrombocytosis.

MF-associated anemia is usually treated with androgens (e.g., testosterone enanthate 400–600 mg IM weekly, oral fluoxymesterone 10 mg three-times-a-day (TID)), prednisone (0.5 mg/kg/day), danazol (600 mg/day) [50], thalidomide (50 mg/day)±prednisone [51–53], or lenalidomide (10 mg/day)±prednisone [54,55] (10 mg/day). I do not use erythropoiesis stimulating agents (ESAs) because they are ineffective in transfusion-dependent patients and could exacerbate splenomegaly [56]. Response rates to each one of the aforementioned drugs are in the vicinity of 15–25% and response durations average about one to two years. Lenalidomide works best in the presence of del(5q31) [57]. Drug side effects include hepatotoxicity and virilizing effects for androgens, peripheral neuropathy for thalidomide, and myelosuppression for lenalidomide.

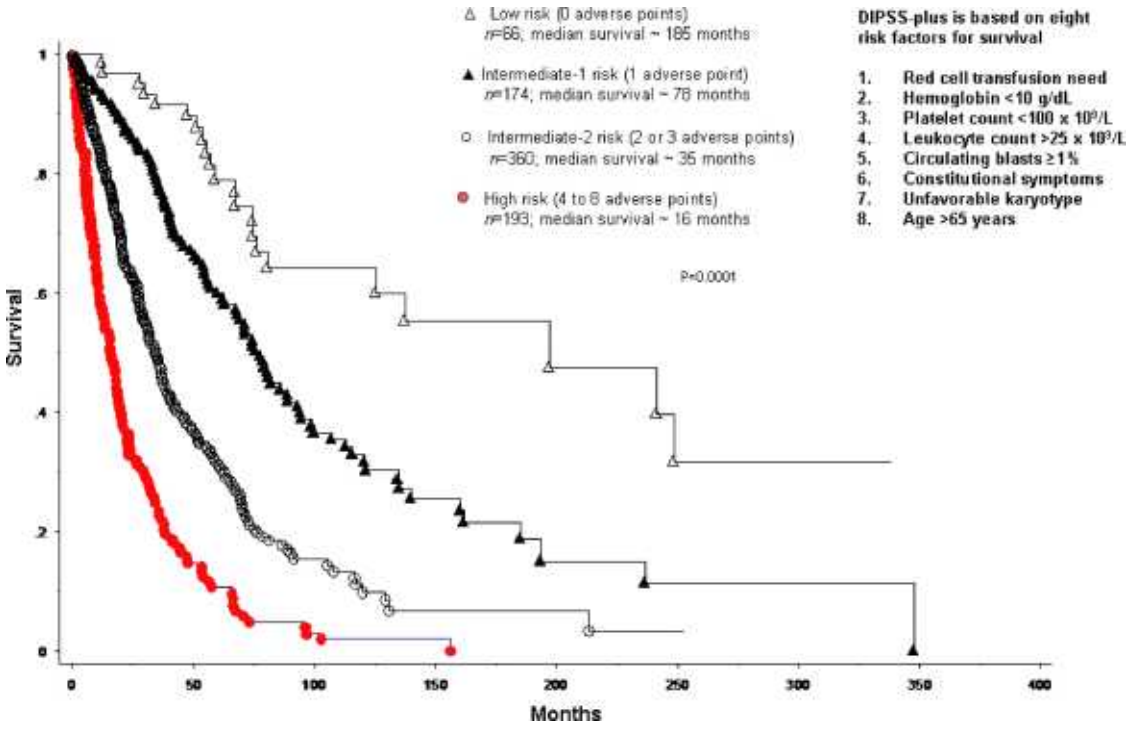

Figure 1. DIPSS-plus (Dynamic International Prognostic Scoring System+karyotype+platelet count+transfusion status) risk stratification in 793 patients with primary myelofibrosis seen at Mayo Clinic Rochester (with permission from Gangat et al. (Reproduced from Ref. [32] with permission from American Society of Clinical Oncology)). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

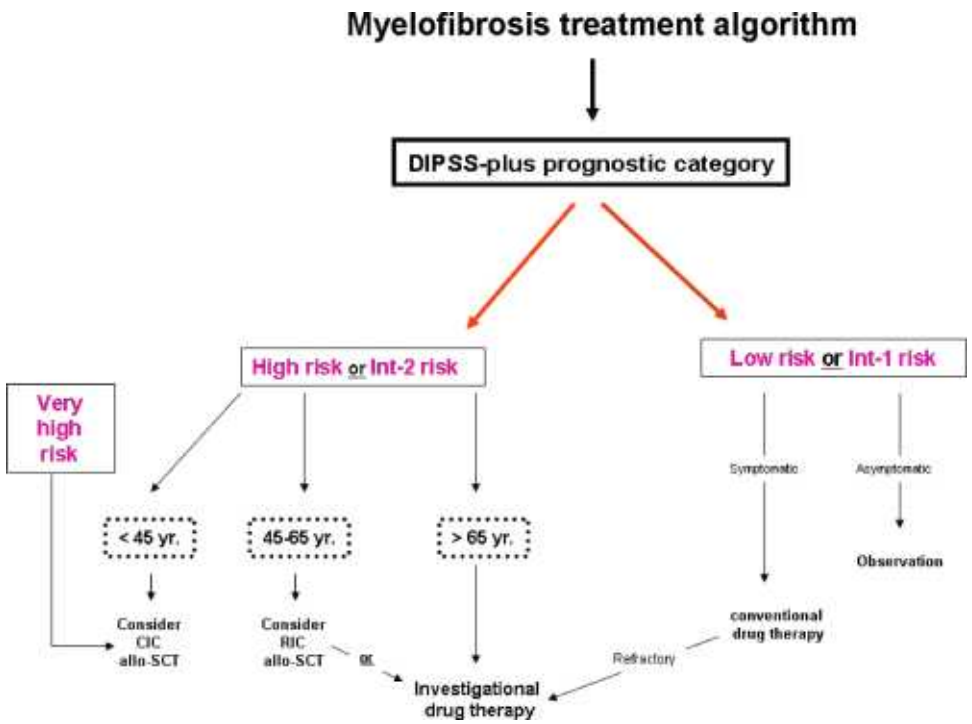

Figure 2. Contemporary treatment algorithm for PMF. High, intermediate-2, intermediate-1 and low risk categories are according to the Dynamic International Prognostic Scoring System (DIPSS)-plus (see figure 1) [32]. Very high risk group includes patients with monosomal karyotype, inv(3)/i(17q) abnormalities, or any two of circulating blasts >9%, leukocytes $\geq 40 \times 10^9$/L or other unfavorable karyotype [33]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

First-line therapy for MF-associated splenomegaly is hydroxyurea, which is effective in reducing spleen size by half in approximately 40% of patients [58]. Spleen response to hydroxyurea lasts for an average of one year and treatment side effects include myelosuppression and mucocutaneous ulcers. Both thalidomide and lenalidomide might improve splenomegaly and thrombocytopenia in some patients [51–53,55]. In contrast, interferon (IFN)-α is of limited value in the treatment of MF-associated splenomegaly [59]. The degree of splenomegaly in low or intermediate-1

TABLE VI. JAK2 Inhibitor ATP Mimetics With Phase-2/3 Clinical Trial Information

Anti-JAK2 ATP mimetic	Targets other than JAK2	Disease features shown to be favorably affected	Side effects
Ruxolitinib (Phase 1/2/3 study)	JAK1	Splenomegaly Constitutional symptoms Pruritus Cachexia	Thrombocytopenia (DLT) Anemia Diarrhea "Ruxolitinib withdrawal syndrome" (see text)
SAR302503 (Phase 1/2 study)	FLT3 RET	Splenomegaly Constitutional symptoms Pruritus Leukocytosis Thrombocytosis *JAK2*V617F burden	Increased amylase/lipase (DLT) Anemia Thrombocytopenia Nausea/vomiting Diarrhea Increased transaminases
CEP-701 (Lestaurtinib) (Phase 2 study)	FLT3 TrkA	Splenomegaly Anemia Pruritus	Diarrhea Nausea/vomiting Anemia Thrombocytopenia
CYT387 (Phase 1/2 study)	JAK1 TYK2 JNK1 CDK2	Anemia Splenomegaly Constitutional symptoms Pruritus	Increased amylase/lipase (DLT) Headache (DLT) Thrombocytopenia Increased transaminases Peripheral neuropathy "First dose-effect characterized by transient hypotension and lightheadedness
SB1518 (Phase 1/2 study)	FLT3	Splenomegaly	(DLT=GI symptoms) Diarrhea Nausea Thrombocytopenia

Abbreviations: DLT, dose-limiting toxicity; GI, gastrointestinal; See text for references.

risk patients is often not severe enough to require either splenectomy or radiotherapy.

Recommendations: Low or intermediate-1 risk asymptomatic patients with PMF can be observed without any therapeutic intervention. Specific therapy is considered only in the presence of symptoms. First-line drugs of choice for anemia include thalidomide+prednisone, an androgen preparation or danazol. Prostate cancer screening in men and monitoring of liver function tests are necessary when considering treatment with androgen preparations. I use lenalidomide in the presence of del(5q) or in case of treatment failure with thalidomide, danazol, or androgens. First-line drug of choice for symptomatic splenomegaly is hydroxyurea (starting dose 500 mg TID).

Management of intermediate-2 or high risk disease

PMF patients with high or intermediate-2 risk disease should be considered for investigational drug therapy or ASCT. Based on the associated extremely poor prognosis (i.e., >80% two-year mortality), the presence of monosomal karyotype, inv(3)/i(17q) abnormalities, or any two of circulating blasts >9%, leukocytes $\geq 40 \times 10^9$/L or other unfavorable karyotype warrant immediate consideration of (ASCT) (Fig. 2) [34].

Transplant. In considering allo-SCT as a treatment modality, one should be acutely aware of the risks involved. In one of the largest studies of allo-SCT in PMF [60], 5-year disease-free survival (DFS) and treatment-related mortality (TRM) were 33% and 35% for matched related and 27% and 50% for unrelated transplants, respectively. Of note, outcome did not appear to be favorably affected by reduced intensity conditioning (RIC) [60]. In another RIC transplant study, 5-year DFS was estimated at 51% [61]; chronic graft-versus-host disease (cGVHD) occurred in 49% of the patients and relapse (29%) was predicted by high-risk disease and prior splenectomy [61]. In the earlier study [60], the respective cGVHD and relapse rates for matched related transplants were 40% and 32% and history of splenectomy did not affect outcome [60]. More recent outcome reports on ASCT in MF were more encouraging: 100-day mortality 13%, a relapse rate of 11%, and a 7-year survival of 61% [62].

Investigational drug therapy. Several experimental drugs are currently being evaluated in PMF, post-PV/ET MF, and other related MPN [63]. So far, pomalidomide, JAK2 inhibitor ATP mimetics (Table VI), and mammalian target of rapamycin (mTOR) inhibitors have shown the most promise [64–68]. Although not further elaborated in the current review, it is important to note that JAK-STAT can be inhibited by many other classes of drugs, which have been evaluated for the treatment of MF and related MPN; these include histone deacetylase inhibitors, such as panobinostat (LBH589) and givinostat (ITF2357) [69,70].

Pomalidomide. Pomalidomide is a second generation immunomodulatory drug and in a Phase-2 randomized study, 25% of patients with anemia responded to the drug used alone (2 mg/day) or in combination with prednisone (0.5 or 2 mg/day) [64]. In a subsequent Phase-2 study of single agent pomalidomide (0.5 mg/day) [71], anemia response was documented only in the presence of *JAK2*V617F (24% vs. 0%) and predicted by the presence of pomalidomide-induced basophilia (38% vs. 6%) or absence of marked splenomegaly (38% vs. 11%). Platelet response was seen in 58% of patients but the drug had limited activity in reducing spleen size [71]. Drug-associated neuropathy or myelosuppression was infrequent but possible. A Phase-1 study did not uncover better activity at higher doses (>2 mg/day), which were instead associated with increased myelosuppression [72].

JAK2 inhibitor ATP mimetics. JAK2 inhibitor ATP mimetics that are currently in clinical trials include ruxolitinib (INCB018424), SAR302503 (TG101348), CYT387, lestaurtinib (CEP-701), SB1518, AZD1480, BMS911543, LY2784544, and XL019 (clinicalTrials.gov). Results of these studies so far suggest substantial differences among these drugs in their toxicity and efficacy profiles, some of which

might be linked to their variable in vitro activity against other JAK and non-JAK kinase targets. For the purposes of this review, I will focus on three of these drugs whose results have now been formally published as full papers: INCB018424, TG101348, and CEP701.

Ruxolitinib is a JAK1/JAK2 inhibitor. The drug was evaluated in 153 patients with PMF or post-PV/ET MF, in a Phase-1/2 study [73]. Dose limiting toxicity (DLT) was thrombocytopenia and the maximum tolerated dose (MTD) was either 25 mg twice-daily or 100 mg once-daily. Adverse events included thrombocytopenia, anemia, and a "cytokine rebound reaction" upon drug discontinuation, characterized by acute relapse of symptoms and splenomegaly [74]. Non-hematologic adverse events were infrequent. Grade 3/4 thrombocytopenia or anemia (in transfusion-independent patients at baseline) respectively occurred in 39% and 43% of patients receiving the drug at 25 or 10 mg twice daily. Among all evaluable patients, 44% experienced $\geq$50% decrease in palpable spleen size. Improvement in constitutional symptoms (fatigue, pruritus, abdominal discomfort, early satiety, night sweats, and exercise tolerance) and weight gain were seen in the majority of patients. Four (14%) of 28 transfusion-dependent patients became transfusion-independent. The drug's effect on *JAK2*V617F allele burden or bone marrow pathology was negligible but a major reduction in proinflammatory cytokines (e.g., IL-1RA, IL-6, TNF-a, MIP-1b) was documented and coincided with improvement in constitutional symptoms.

Two randomized studies comparing ruxolitinib with either placebo or best supportive care have now been published [75,76]. In the COMFORT-1 trial that compared the drug with placebo (n=309), the spleen response rate was approximately 42% for ruxolitinib versus $<$1% for placebo. In addition, about 46% of patients experienced substantial improvement in their constitutional symptoms. However, the benefit of the drug was antagonized by ruxolitinib-associated anemia (31% vs. 13.9%) and thrombocytopenia (34.2% vs. 9.3%). In the COMFORT-2 trial that compared the drug with "best available therapy" (n=219), the spleen response was 28.5% with ruxolitinib vs. 0% otherwise but the drug was detrimental in terms of thrombocytopenia (44.5% vs. 9.6%), anemia (40.4% vs. 12.3%), and diarrhea (24.0% vs. 11.0%). The long-term outcome of ruxolitinib therapy in MF was recently reported and disclosed a very high treatment discontinuation rate (92% after a median time of 9.2 months) and the occurrence of severe withdrawal symptoms during ruxolitinib treatment discontinuation ("ruxolitinib withdrawal syndrome") characterized by acute relapse of disease symptoms, accelerated splenomegaly, worsening of cytopenias, and occasional hemodynamic decompensation, including a septic shock-like syndrome [74].

TG101348, a selective JAK2 inhibitor, was evaluated in 59 patients with PMF or post-PV/ET MF, in a Phase-1/2 study [77]. The DLT was a reversible and asymptomatic increase in serum amylase/lipase and the MTD was 680 mg/day. Grade 3 or 4 adverse events were all reversible and dose-dependent and included nausea (3%), vomiting (3%), diarrhea (10%), asymptomatic mild increases in serum lipase (27%), transaminases (27%) or creatinine (24%), thrombocytopenia (24%), and anemia (35%). By 6 or 12 months of treatment, 39% and 47% of patients, respectively, experienced a $\geq$50% decrease in palpable spleen size. In addition, the majority of patients with early satiety, fatigue, night sweats, cough, or pruritus reported a durable resolution of their symptoms. Almost all patients with thrombocytosis and the majority with leukocytosis had normalization of their counts. Among 23 patients with a baseline *JAK2*V617F allele burden of $>$20%, 9 (39%) had $\geq$50% decrease in allele burden. Effect on bone marrow pathology was limited. In general, response was not affected by the presence of *JAK2*V617F.

CEP-701 is a JAK2 and FLT-3 inhibitor [78]. Twenty-two *JAK2*V617F-positive MF patients received the drug orally at 80 mg twice-daily and 6 (27%) experienced clinical improvement including reduction in spleen size in three patients and red blood cell transfusion independency in two patients. No improvement was seen in bone marrow fibrosis or *JAK2*V617F allele burden. Side effects included Grades 3–4 anemia in 14% of the patients, thrombocytopenia 23%, and diarrhea (9%). Grade 1 or 2 nausea and vomiting were seen in 50% and 27% of patients, respectively.

The above observations demonstrate major differences in toxicity and activity profile among several JAK inhibitor small molecules and underscore the need to evaluate more such drugs before making any conclusions regarding the value of anti-JAK2 therapy in MF or related MPN. It is also becoming evident that some of the salutary effects of these drugs might be the result of a potent anti-cytokine activity.

mTOR inhibitors. JAK-STAT activation leads to Akt/mTOR activation as well and it is therefore reasonable to evaluate the therapeutic activity of Akt and mTOR inhibitors. In a Phase 1/2 study involving the mTOR inhibitor everolimus including 39 MF patients [79], the commonest toxicity was Grades 1–2 stomatitis. A $>$50% reduction in splenomegaly occurred in 20% of the patients evaluated and the constitutional symptoms response was 69%; 80% experienced complete resolution of pruritus. Drug effect on cytosis or anemia was modest and on *JAK2*V617F burden negligible. Overall IWG-MRT response rate was 23%.

Recommendations: Considering the lack of effective drug therapy in PMF, the risk of transplant-related complications might be justified in those patients in whom median survival is expected to be $<$5 years and leukemic transformation risk $>$20%. These include DIPSS-plus high or intermediate-2 risk patients as well as those with either unfavorable karyotype or a platelet count of $<100 \times 10^9$/L. Non-transplant candidates are best managed with experimental drug therapy. I have yet to be satisfied by the value of any currently available JAK inhibitor and strongly advise patients to continue participating in clinical trials.

Management of refractory disease and specific disease complications

Hydroxyurea-refractory splenomegaly is often managed by splenectomy [80]. Other indications for splenectomy include symptomatic portal hypertension, thrombocytopenia, and frequent red blood cell transfusions. In a recent report of 314 splenectomized patients with MF [81], more than 75% benefited from the procedure and the benefit lasted for a median of one year; specific benefits included becoming transfusion-independent and resolution of severe thrombocytopenia. Perioperative complications occurred in 28% of the patients and included infections, abdominal vein thrombosis, and bleeding. Overall perioperative mortality rate was 9%. Approximately 10% of patients experienced progressive hepatomegaly and 29% thrombocytosis after splenectomy. Median survival after splenectomy was 19 months. Leukemic transformation was documented in 14% of patients whose survival was not different than that of patients without "leukemic transformation" [82].

Splenic irradiation (100 cGy in 5–10 fractions) induces transient reduction in spleen size but can be associated with severe pancytopenia [80]. Non-hepatosplenic EMH might involve the vertebral column, lymph nodes, pleura,

and peritoneum (ascites) and is effectively treated with low-dose radiotherapy (100–1,000 cGy in 5–10 fractions) [80]. Diagnosis of MF-associated pulmonary hypertension is confirmed by a technetium 99 m sulfur colloid scintigraphy and treatment with single-fraction (100 cGy) whole-lung irradiation has been shown to be effective [80]. Single fraction of 100–400 cGy involved field radiotherapy has also been shown to benefit patients with MF-associated extremity pain [80].

Transjugular intrahepatic portosystemic shunt might be considered to alleviate symptoms of portal hypertension [80]. Recent technical advances in the procedure and the introduction of specially coated stents have greatly improved shunt patency and clinical efficacy of transjugular intrahepatic portosystemic shunt (TIPS) in general. Current TIPS indications include recurrent variceal bleeding and refractory ascites, both of which could accompany advanced MF. The therapeutic value of TIPS has not been systematically studied in MF but relevant information is available from several case reports that confirm feasibility and efficacy [83–88].

Recommendations: At present, my first-line choice for the management of drug-refractory splenomegaly is participation in experimental drug therapy. Both splenectomy and low-dose radiotherapy are reasonable alternative treatment options. Prophylactic therapy with hydroxyurea is advised to prevent post-splenectomy thrombocytosis [89]. Post-splenectomy thrombosis might be prevented by instituting short-term systemic anticoagulation. Laparoscopic splenectomy is not advised in the setting of MF [90] and data on the value of splenic artery embolization are limited [91–93]. I do not believe that splenectomy increases the risk of leukemic transformation [82].

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