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REVIEW ARTICLE

Myeloproliferative Neoplasms: Molecular Pathophysiology, Essential Clinical Understanding, and Treatment Strategies

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ABSTRACT

To update oncologists on pathogenesis, contemporary diagnosis, risk stratification, and treatment strategies in *BCR-ABL1*–negative myeloproliferative neoplasms, including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Recent literature was reviewed and interpreted in the context of the authors' own experience and expertise. Pathogenetic mechanisms in PV, ET, and PMF include stem cell–derived clonal myeloproliferation and secondary stromal changes in the bone marrow and spleen. Most patients carry an activating *JAK2* or *MPL* mutation and a smaller subset also harbors *LNK*, *CBL*, *TET2*, *ASXL1*, *IDH, IKZF1,* or *EZH2* mutations; the precise pathogenetic contribution of these mutations is under investigation. *JAK2* mutation analysis is now a formal component of diagnostic criteria for PV, ET, and PMF, but its prognostic utility is limited. Life expectancy in the majority of patients with PV or ET is near-normal and disease complications are effectively (and safely) managed by treatment with low-dose aspirin, phlebotomy, or hydroxyurea. In PMF, survival and quality of life are significantly worse and current therapy is inadequate. In ET and PV, controlled studies are needed to show added value and justify the risk of unknown long-term health effects associated with nonconventional therapeutic approaches (eg, interferon-alfa). The unmet need for treatment in PMF dictates a different approach for assessing the therapeutic value of new drugs (eg, JAK inhibitors, pomalidomide) or allogeneic stem-cell transplantation.

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INTRODUCTION

William Dameshek was the first to call attention to the clinical and bone marrow morphologic similarities between chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[1] He recognized their common trait of unregulated trilineage myeloproliferation and accordingly assigned the term myeloproliferative disorders (MPD) to describe them in a seminal 1951 commentary.[2] Dameshek's almost 60-year-old insight regarding the pathogenesis of MPD proved to be accurate in that all four MPD originate from a common ancestral clone (or oligoclones) that arises from a polyclonal, but disease susceptible, stem cell pool. Considering this current understanding of the clonal structure in MPD and their propensity to transform into acute myeloid leukemia (AML), it was appropriate for the 2008 WHO classification system subcommittee to recommend change in terminology from MPD to myeloproliferative neoplasms (MPN).[3] The WHO MPN category includes not only CML, PV, ET, and PMF, but also chronic neutrophilic leukemia, chronic eosinophilic leukemia-not otherwise specified, mastocytosis, and MPN unclassifiable. The current review focuses on PV, ET, and PMF, which are operationally subcategorized as *BCR-ABL1*–negative MPN (Fig 1).

CLONES AND MUTATIONS

PV, ET, and PMF are stem cell–derived clonal (ie, monoclonal or oligoclonal) diseases.[4] However, clonal architecture and hierarchy in these diseases is complex and not always predictable.[4] Currently known MPN-associated mutations involve *JAK2* (exon 14[5-8] and exon 12),[9] *MPL* (exon 10),[10,11] *TET2,*[12] *ASXL1,*[13] *IDH1,*[14,15] *IDH2*[14,16] *CBL,*[17] *IKZF1,*[18] *LNK,*[19] and *EZH2.*[20] Most of these mutations originate at the progenitor cell level but they do not necessarily represent the primary clonogenic event and are not mutually exclusive (Fig 2).[4]

JAK2V617F

*JAK2*V617F (Janus kinase 2; 9p24) is the most prevalent mutation in *BCR-ABL1*–negative MPN: mutational frequency of approximately 96% in PV, 55% in ET, and 65% in PMF. The mutation affects the noncatalytic (pseudokinase) domain of JAK2 and disrupts its kinase-regulatory activity. *JAK2*V617F induces PV-, ET-,

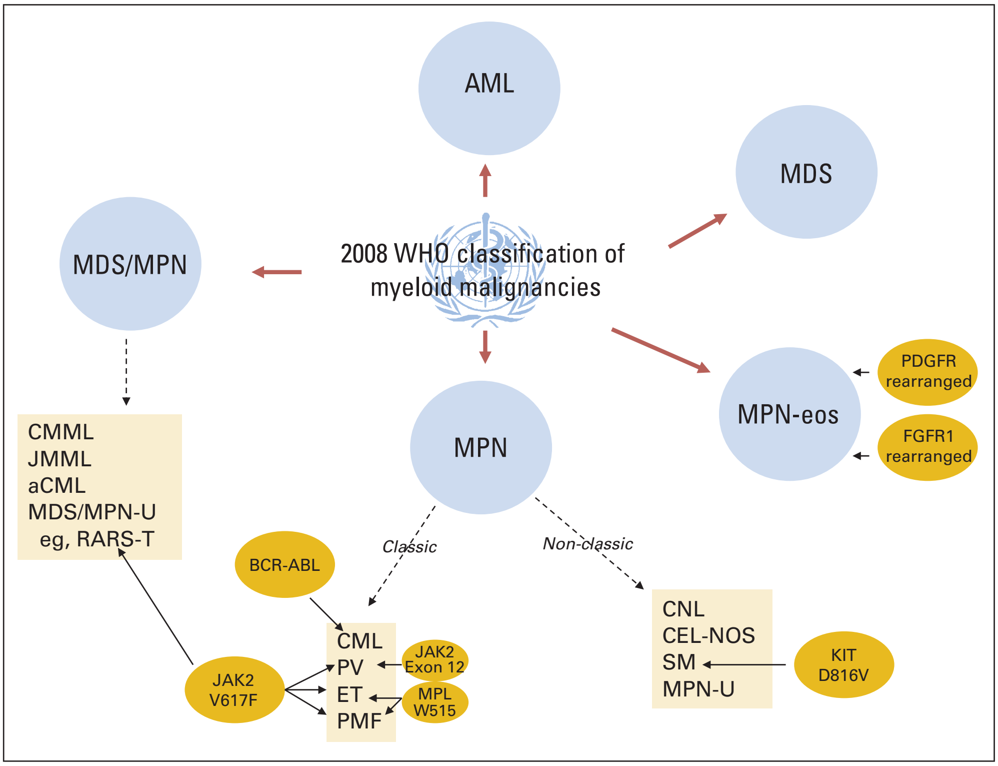

Fig 1. The 2008 WHO classification system for myeloid neoplasms: acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), MDS/MPN, and MPN eosinophilia (eos), platelet-derived growth factor receptor (PDGFR), or fibroblast growth factor receptor (FGFR1)–rearranged myeloid/lymphoid malignancies associated with eos. The MDS/MPN category includes chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia (aCML, *BCR-ABL1* negative), and MDS/MPN, unclassifiable (MDS/MPN-U) including refractory anemia with ring sideroblasts and thrombocytosis (RARS-T). The MPN category includes chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia–not otherwise specified (CEL-NOS), systemic mastocytosis (SM), and MPN unclassifiable (MPN-U). JAK2, Janus kinase 2.

or PMF-like disease in mice by experimental manipulation of its allele burden.[21] *JAK2*V617F homozygosity is ascribed to mitotic recombination and is prevalent in PV and PMF but infrequent in ET.[22] These observations suggest a cause-effect relationship with clonal erythrocytosis.

The presence of *JAK2*V617F in MPN has been associated with older age, higher hemoglobin level, leukocytosis, and lower platelet count.[23] In PV, a higher mutant allele burden has been associated with pruritus and fibrotic transformation.[24] *JAK2*V617F presence or increased allele burden does not appear to affect thrombosis risk, survival or leukemic transformation in PV, ET, or PMF.[23,24] *JAK2*V617F can become undetectable during leukemic transformation and a lower mutant allele burden has been associated with inferior survival in PMF.[25,26]

JAK2 *Exon 12 Mutations*

JAK2 exon 12 mutations are relatively specific to *JAK2*V617F-negative PV and mutational frequency among all patients with PV is estimated at 3%.[9] *JAK2* N542-E543del is the most frequent among the many *JAK2* exon 12 mutations so far described.[9,27-29] One of these mutations (ie, *JAK2*K539L) has been shown to cause erythrocytosis in mice.[9] *JAK2* exon 12 mutation–positive patients are often heterozygous for the mutation and are usually characterized by predominantly erythroid myelopoiesis, subnormal serum erythropoietin level, and younger age at diagnosis.[9,27-29]

Myeloproliferative Leukemia Virus Mutations

Myeloproliferative leukemia virus (*MPL* oncogene; 1p34) W515L mutation was first described in *JAK2*V617F-negative PMF and induces a PMF-like disease with thrombocytosis in mice.[10] Subsequently, *MPL*W515K and other exon 10 *MPL* mutations were described in approximately 3% of patients with ET and 10% of those with PMF.[11,30-32] *MPL* mutations in MPN have been associated with older age, female sex, lower hemoglobin level, and higher platelet count.[30-32]

TET2 *Mutations*

TET2 (TET oncogene family member 2; 4q24) mutations are seen in both *JAK2*V617F positive and negative MPN with mutational frequencies of approximately 16% in PV, 5% in ET, 17% in PMF, 14% in post-PV MF, 14% in post-ET MF, and 17% in blast phase MPN.[12,33] *TET2* mutations in MPN can either antedate or follow the acquisition of a *JAK2* mutation, or occur independently leading to a biclonal pattern.[34] *TET2* and *ASXL1* may contribute to epigenetic regulation of hematopoeisis.[15,33]

Additional Sex Combs-Like 1 Mutations

Additional sex combs-like 1 (*ASXL1*; 20q11.1) mutations are seen in approximately 8% of patients with MPN, 11% with MDS, 43% of with chronic myelomonocytic leukemia (CMML), 7% with primary and 47% with secondary AML.[35,36] Among 64 patients with MPN, heterozygous mutations of *ASXL1* were identified in five patients who were all *JAK2*V617F negative (three PMF, one ET, and one blast phase ET).[13]

Isocitrate Dehydrogenase Mutations

Isocitrate dehydrogenase (*IDH1* and *IDH2;* 2q33.3 and 15q26.1, respectively) mutations were studied in 1,473 patients with MPN; mutational frequencies were 0.8% for ET, 1.9% for PV, 4.2% for PMF, 1% for post-PV/ET MF, and 21.6% for blast-phase MPN.[37,38] Mutant *IDH* was documented in the presence or absence of *JAK2*, *MPL,* and *TET2* mutations. *IDH* mutations are

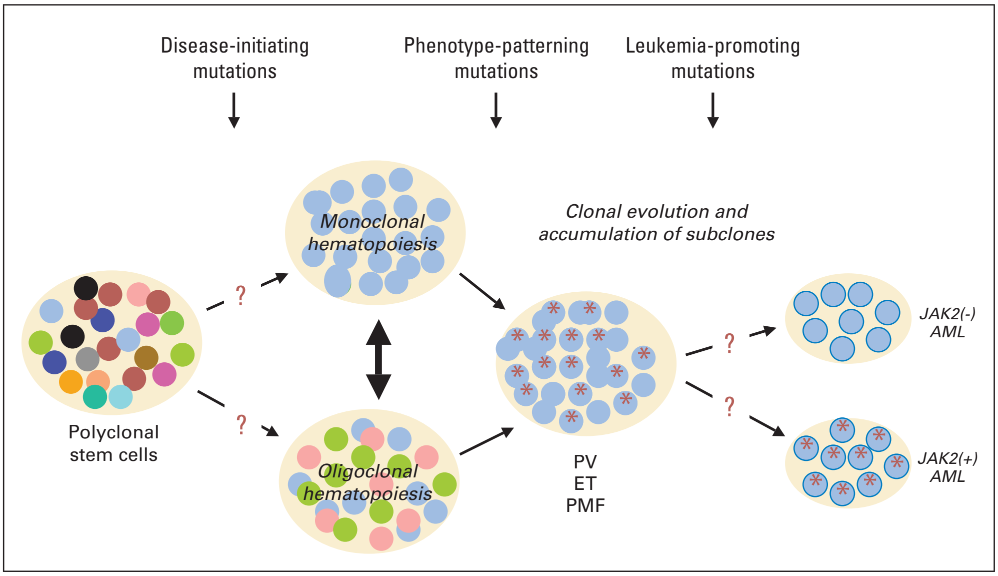

Fig 2. Current concept regarding clonal origination and evolution in *BCR-ABL1*–negative myeloproliferative neoplasms: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). *JAK2*, Janus kinase 2; AML, acute myeloid leukemia.

heterozygous and affect three specific arginine residues: R132 (*IDH1*), R172 (the *IDH1* R132 analogous residue on *IDH2*), and R140 (*IDH2*). The specific mutation variants so far seen in MPN include *IDH1*R132C, *IDH1*R132S, and *IDH2*R140Q. Functional characterization of *IDH* mutations suggests neoenzymatic activity in converting α-ketoglutarate to the putatively oncogenic 2-hydroxyglutarate.

Casitas B-Lineage Lymphoma Mutations

Casitas B-lineage lymphoma (*CBL* proto-oncogene; 11q23.3) mutations in myeloid malignancies are usually associated with 11q acquired uniparental disomy and are seen in approximately 17% of patients with juvenile myelomonocytic leukemia and 11% of those with CMML.[39] Most *CBL* mutations in juvenile myelomonocytic leukemia are homozygous, which suggests a tumor suppressor function for the normal protein. In a recent study that included 74 patients with PV, 24 with ET and 53 with PMF, *CBL* mutations in either exon 8 or 9 were identified in three patients (6%) with PMF.[17]

IKAROS Family Zinc Finger 1 Mutations

IKAROS family zinc finger 1 (*IKZF1;* 7p12) mutations are prevalent in blast phase CML or *BCR-ABL1*–positive ALL, suggesting a pathogenetic contribution to leukemic transformation.[40] A recent study in *BCR-ABL1*–negative MPN revealed a 19% and less than 0.5% *IKZF1* mutational frequency in blast and chronic phase disease, respectively.[18]

LNK Mutations

LNK (12q24.12) encodes for LNK, which is a plasma membrane-bound adaptor protein whose function includes inhibition of wild type and mutant JAK2 signaling.[41] *LNK* exon 2 loss-of-function mutations were recently described in *JAK2*V617F-negative ET or PMF.[19] Both mutations involved the *LNK* pleckstrin homology domain.[19] In a more recent study of 61 patients with blast-phase MPN,[42] nine novel heterozygous *LNK* mutations were identified in eight patients (13%); eight affected the pleckstrin homology domain. *LNK* mutations were not detected in 78 additional patients with chronic phase MPN, but were reported in otherwise unexplained erythrocytosis with subnormal serum erythropoietin level.[43]

EZH2 Mutations

EZH2 (7q36.1) encodes the catalytic subunit of the polycomb repressive complex 2, a histone H3 lysine 27 methyltransferase with putative epigenetic effect. A recent study described homozygous *EZH2* mutations in nine of 12 individuals with 7q acquired uniparental disomy.[20] Among 614 patients with myeloid disorders, 42 harbored 49 monoallelic or biallelic *EZH2* mutations. Mutational frequency was highest in MDS/MPN (12%) and in MF (13%).

CONTEMPORARY DIAGNOSIS

Diagnosis of PV, ET, or PMF is based on a composite assessment of clinical and laboratory features (Table 1).[44] Figure 3 provides a practical diagnostic algorithm that begins with peripheral blood mutation screening for *JAK2*V617F.

The laboratory detection of *JAK2*V617F is highly sensitive (97% sensitivity) and virtually 100% specific for distinguishing PV from other causes of increased hematocrit[45,46]; the possibility of false-positive or false-negative mutation test result is effectively addressed by the concomitant measurement of serum erythropoietin level, which is expected to be subnormal in more than 85% of patients with PV.[47] A subnormal serum erythropoietin level in the absence of *JAK2*V617F mandates additional mutational analysis for *JAK2* exon 12 mutation in order to capture some of the approximately 3% of patients with PV who are *JAK2*V617F negative.[27] Bone marrow examination is not essential for the diagnosis of PV because the WHO diagnostic criteria for PV does not require the absence of bone marrow fibrosis (Table 1).

When evaluating thrombocytosis, the detection of *JAK2*V617F confirms the presence of an underlying MPN, but its absence does not rule out the possibility because 50% of patients with ET are *JAK2*V617F negative.[48] Furthermore, other *JAK2*V617F-positive MPN can mimic ET in their presentation. Therefore, bone

Table 1. WHO Diagnostic Criteria for PV, ET, and PMF

Criteria		PV*		ET*		PMF*
	2008 WHO Diagnostic Criteria					
Major	1	Hgb > 18.5 g/dL (men) > 16.5 g/dL (women) or†	1	Platelet count ≥ 450 × 10^9/L	1	Megakaryocyte proliferation and atypia‡ accompanied by either reticulin and/or collagen fibrosis, or§
	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	1	BM trilineage myeloproliferation			1	Leukoerythroblastosis
	2	Subnormal serum Epo level			2	Increased serum LDH level
	3	EEC growth			3	Anemia
					4	Palpable splenomegaly

Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; Hgb, hemoglobin; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndromes; BM, bone marrow; Epo, erythropoietin; LDH, lactate dehydrogenase; EEC, endogenous erythroid colony; Hct, hematocrit.
*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.
†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.
‡Small to large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.
§Or in the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis (ie, prefibrotic PMF).

marrow examination is often necessary to make an accurate morphologic diagnosis of ET and distinguish it from other myeloid neoplasms including prefibrotic PMF.[49]

Bone marrow fibrosis associated with *JAK2*V617F, trisomy 9, or 13q- is consistent with the diagnosis of PMF. The presence of dwarf megakaryocytes raises the possibility of CML that should be pursued with *BCR-ABL1* fluorescent in situ hybridization or polymerase chain reaction analysis. PMF is not always easy to distinguish from acute myelofibrosis, which is an AML-related myeloid neoplasm, or fibrotic MDS or CMML. Such distinction, however, is not always critical from the standpoint of management. 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.[50]

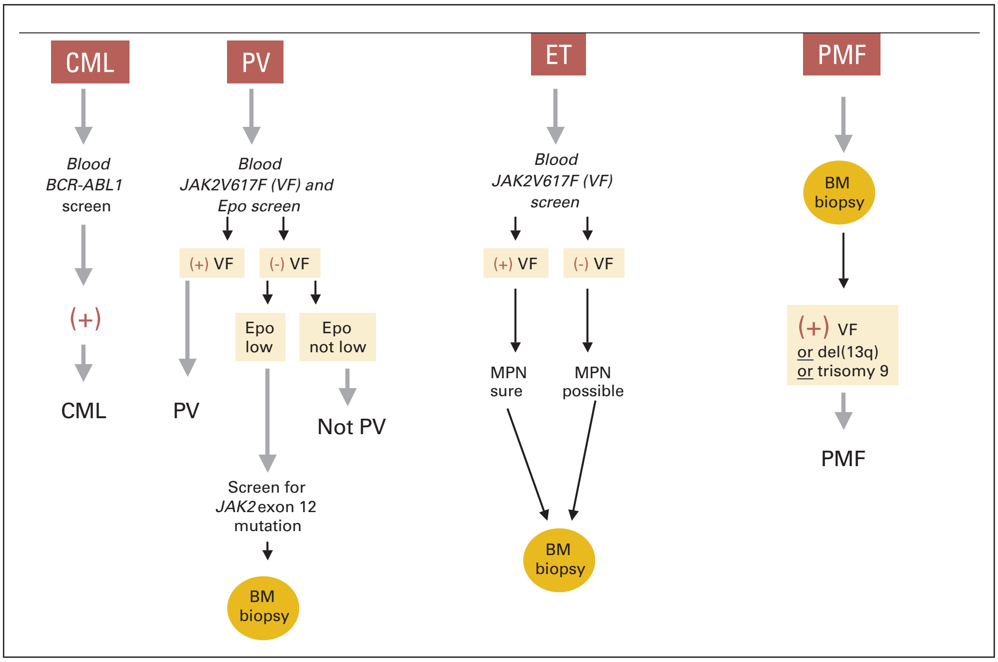

Fig 3. Diagnostic algorithm for chronic myelogenous leukemic (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). JAK2, Janus kinase 2; Epo, erythropoietin; MPN, myeloproliferative neoplasm; BM, bone marrow.

RISK STRATIFICATION

Current risk stratification in PV and ET is designed to estimate the likelihood of thrombotic complications.[51] Age ≥ 60 years and history of thrombosis are the two risk factors used to classify patients with PV or ET into low (zero risk factors) and high (one or two risk factors) risk groups (Table 2).[52-56] In addition, because of the potential risk for bleeding, low-risk patients with extreme thrombocytosis (platelet count > 1,000 × 10^9/L) are considered separately (Table 2).[57] The presence of cardiovascular risk factors is currently not taken under consideration during formal risk categorization.

Risk factors for shortened survival in both PV and ET include history of thrombosis, leukocytosis, advanced age, and anemia.[53-55,58] Leukocytosis has also been associated with leukemic or fibrotic transformation in PV. The relationship between thrombosis and leukocytosis,[59,60] thrombosis and *JAK2*V617F,[23] or pregnancy-associated complications and *JAK2*V617F[61] have been examined by both Mayo Clinic and Italian investigators with findings that were conflicting and inconclusive.

The International Prognostic Scoring System for PMF uses five independent predictors of inferior survival: age older than 65 years, hemoglobin lower than 10 g/dL, leukocyte count higher than 25 × 10^9/L, circulating blasts ≥ 1%, and presence of constitutional symptoms.[62] The International Working Group for MPN Research and Treatment subsequently developed a dynamic prognostic model (Dynamic International Prognostic Scoring System [DIPSS]) that utilizes the same prognostic variables but can be applied at any time during the disease course.[63] DIPSS was recently modified into DIPSS-plus by incorporating three additional DIPSS-independent risk factors: platelet count lower than 100 × 10^9/L, red cell transfusion need, and unfavorable karyotype (Table 2).[52] The latter includes complex karyotype or single or two abnormalities including +8, −7/7q-, i(17q), −5/5q-, 12p-, inv(3), or 11q23 rearrangement.[64] The four DIPSS-plus risk categories are low, intermediate-1, intermediate-2, and high with respective median survivals of 15.4, 6.5, 2.9, and 1.3 years.

More recent data suggest inferior survival in PMF associated with nullizygosity for *JAK2* 46/1 haplotype,[65] low *JAK2*V617F allele burden,[25] and increased plasma levels of interleukin (IL) -8, IL-2R, or IL-15.[52] In addition, the concept of an accelerated phase disease was introduced and a survival of shorter than 1 year and leukemic transformation were predicted by the presence of ≥ 10% circulating blasts in blood or bone marrow, platelet count lower than 50 × 10^9/L, or chromosome 17 abnormalities.[66] In an earlier study, leukemic transformation in PMF was associated with platelet count lower than 100 × 10^9/L and circulating blasts ≥ 3%.[67]

It is not clear how well the aforementioned prognostic models apply to patients with post-PV/ET MF. However, the presence of an abnormal karyotype, hemoglobin lower than 10 g/dL, platelet count lower than 100 × 10^9/L, leukocyte count higher than 30 × 10^9/L, and older age have all been associated with inferior survival in such patients.[68,69] Therefore, it is currently reasonable to manage patients with post-PV/ET MF in a similar fashion to that of PMF. This might change in the future considering the fact that patients with post-PV MF are always *JAK2* mutation positive and carry a larger mutant allele burden, and therefore might respond differently to novel drugs, such as JAK inhibitors.

TREATMENT

Current drug therapy for PV, ET, or PMF is not curative and there is little evidence to suggest a favorable effect on survival. Allogeneic stem-cell transplantation (alloSCT) is potentially curative in PMF (or post-ET/PV MF), but its utility is limited by the relatively high incidence of treatment-related mortality and morbidity. The goal of current therapy in PV and ET is to prevent thrombohemorrhagic complications and in PMF (or post-PV/ET MF) to alleviate anemia, symptomatic splenomegaly, or constitutional symptoms. To that end, conventional, investigational and transplant-based therapies are employed and further elaborated below.

Table 2. Risk Stratification and Risk-Adapted Therapy in ET, PV, and PMF

Risk Groups PV and ET	Management ET	Management PV	Management PMF	DIPSS Plus[52] Risk Groups PMF
Low risk (age < 60 years and no thrombosis history)	Low-dose aspirin	Low-dose aspirin + phlebotomy*	Observation or conventional drugs†	Low risk (no risk factors‡)
Low risk with extreme thrombocytosis§	Low-dose aspirin‖	Low-dose aspirin‖ + phlebotomy	Observation or conventional drugs† or experimental drugs	Intermediate-1 risk (1 risk factor‡)
High risk (age ≥ 60 years or thrombosis history)	Low-dose aspirin + hydroxyurea¶	Low-dose aspirin + phlebotomy + hydroxyurea¶	AlloSCT or experimental drugs	Intermediate-2 risk (2 or 3 risk factors‡)
			AlloSCT or experimental drugs	High risk (≥ 4 risk factors‡)

Abbreviations: ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; DIPSS, Dynamic International Prognostic Scoring System; alloSCT, allogeneic stem-cell transplantation.
*In the presence of aspirin therapy, the hematocrit target can range between 38% and 50% and is set at a level that maintains best performance status.
†Androgen preparations or thalidomide with prednisone for anemia; hydroxyurea for symptomatic splenomegaly.
‡DIPSS plus[52] uses eight 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 two risk factors because of transfusion need (1 risk point) and hemoglobin < 10 g/dL (1 risk point).
§Extreme thrombocytosis is defined as a platelet count of > 1,000 × 10^9/L.
‖Clinically significant acquired von Willebrand syndrome (ristocetin cofactor activity < 30%) should be excluded before the use of aspirin in patients with extreme thrombocytosis.
¶In hydroxyurea-intolerant or -resistant patients, interferon-alfa (age < 60 years) or busulfan or pipobroman (age > 60 years) might be used.

CONVENTIONAL THERAPY

PV and ET

Controlled studies have confirmed the antithrombotic value of low-dose aspirin in PV (all risk categories)[70] and hydroxyurea in ET (high-risk disease).[71,72] In addition, there is uncontrolled evidence to support the need to phlebotomize all patients with PV and a recent study suggested a hematocrit target of lower than 55% as being acceptable in patients receiving aspirin therapy.[56] The best available evidence also supports the use of hydroxyurea in high-risk PV and low-dose aspirin in ET; the latter, especially in the presence of *JAK2*V617F or cardiovascular risk factors.[73,74] In patients with extreme thrombocytosis, the use of aspirin can lead to bleeding complications because of acquired von Willebrand syndrome[75]; therefore, in the presence of platelets higher than $1{,}000 \times 10^9$/L, screening for ristocetin cofactor activity is advised and consideration be given to withhold aspirin therapy if the result shows fewer than 30% activity.

Based on the above, it is reasonable to use low-dose aspirin (81 mg/d; range, 40 to 100 mg/d) in all patients with PV or ET provided there are no major contraindications, including clinically significant acquired von Willebrand syndrome. In addition, phlebotomy is indicated in all patients with PV and a hematocrit target of 45% is advised, but not mandated. High-risk patients with PV or ET should also receive hydroxyurea in order to minimize their risk of thrombosis (starting dose 500 mg twice per day). The dose of hydroxyurea is titrated to keep platelets lower than 400×10^9/L and WBC higher than 2×10^9/L. However, it is to be noted that the recommended platelet target is not based on controlled evidence. Women of child-bearing potential and those who are pregnant are managed in the same general manner other than the preferred use of interferon (INF) -α in high-risk disease.[61]

Patients with PV or ET who are either intolerant or resistant to hydroxyurea are effectively managed by INF-alpha[76,77] or busulfan.[78,79] Among these two second-line drugs, we prefer the use of INF-α for patients younger than age 65 years and busulfan in the older age group, although there is no controlled evidence to support or refute such a strategy. Two recent studies of pegylated INF-α (approximately 90-μg subcutaneously weekly) in PV and ET reported hematologic remissions of approximately 80% accompanied by decreases in *JAK2*V617F allele burden (complete molecular remission rate of 5% to 10%).[76,77] In one of the two studies,[76] 77 patients were evaluable after a median follow-up of 21 months and 76% and 70% of patients with ET or PV, respectively, achieved a complete hematologic remission, mostly in the first 3 months; adverse effects were recorded in 96% of the patients and 22% had discontinued treatment (10% drop-out rate for INF-α–related events). In our experience, the adverse effect profile of INF-α is worse than that of hydroxyurea and the reported hematologic response rates are not necessarily superior. Furthermore, long-term health effects of INF-α and impact on survival and disease complications are unknown. Therefore, a controlled study is needed before INF-α is recommended for first-line therapy in either PV or ET. Busulfan is started at 4 mg/d, withheld in the presence of platelets lower than 100×10^9/L or WBC lower than 3×10^9/L, and the dose is reduced to 2 mg/d if the corresponding levels are lower than 150×10^9/L and 5×10^9/L.

There is unsubstantiated fear among primary care givers regarding drug leukemogenicity with use of hydroxyurea or busulfan. The fact of the matter is that there is not a single controlled study in either PV or ET that shows these drugs to be more leukemogenic than any other drug or treatment approach.[55,58,80] The most recent randomized study in this regard found no difference in leukemia risk among patients receiving either hydroxyurea or anagrelide.[72] In a much earlier study, the European Organization for Research on Treatment of Cancer randomly assigned 293 patients to treatment with either ^{32}P or oral busulfan and the results favored busulfan in terms of both first remission duration and overall survival and a leukemia conversion rate of only 1.4%.[79] Another randomized study in PV found no difference in leukemia risk between hydroxyurea and pipobroman.[81] Similarly, the two largest noncontrolled studies in ET[82] and PV[80] do not support the concern that leukemia might arise from the use of hydroxyurea, and there is additional evidence to that effect from long-term studies of patients receiving hydroxyurea for sickle cell disease.[83]

The evidence for busulfan leukemogenicity in the context of treatment for PV or ET is equally weak and inappropriately extrapolated from older patients with advanced phase disease and exposed to multiple cytoreductive drugs. In 65 busulfan-treated patients with PV followed between 1962 and 1983, overall median survival was 11.1 years and 19 years in patients whose disease was diagnosed before age 60 years.[84] Only two patients (3.5%) treated with busulfan alone developed acute leukemia. The safety and efficacy of busulfan treatment in ET was recently underlined by a long-term study of 36 patients older than 60 years[78]; no instances of AML or other malignancies were documented after a median follow-up of 72 months (range, 30 to 300). In comparison, the baseline risk of leukemic transformation among 605 patients with ET[58] and 459 patients with PV,[55] treated at a single institution mostly without cytoreductive therapy or hydroxyurea alone, was 3.3% and 7.4%, respectively.

Other treatment options in PV and ET include pipobroman (not available in the United States), anagrelide, and radiophosphorus. The latter has been associated with a delayed risk of leukemic transformation in patients with PV and its use is currently limited to patients older than 65 years.[73] In regard to anagrelide use, a large randomized study compared the drug with hydroxyurea, both in combination with aspirin, in high-risk patients with ET and demonstrated an overall superiority of hydroxyurea over anagrelide.[72] Hydroxyurea was better tolerated and associated with significantly less risk of arterial thrombosis, major hemorrhage, and fibrotic transformation. In contrast, anagrelide displayed better activity against venous thrombosis. A more recent smaller randomized study found no difference between hydroxyurea and anagrelide in the incidence of ET-related events, but treatment discontinuation rate was higher in the anagrelide arm.[85]

Myelofibrosis

Low-risk[52] patients with PMF can be observed without any therapeutic intervention (Table 2). High or intermediate-2 risk patients should be considered for investigational drug therapy or alloSCT. Management of intermediate-1 risk patients should be individualized and might include observation, conventional drug therapy, or participation in investigational drug trials.

Anemia and symptomatic splenomegaly are the main indications for treatment in PMF. Anemia is treated with androgens (eg, testosterone enanthate 400- to 600-mg intramuscularly weekly, oral fluoxymesterone 10 mg three times per day), prednisone (0.5 to 1.0

mg/kg/d), danazol (600 mg/d), thalidomide (50 mg/d), or lenalidomide (10 mg/d).[86] We currently do not recommend the use of erythropoiesis stimulating agents because they exacerbate splenomegaly and are ineffective in transfusion-dependent patients.[87] Prostate cancer screening in men is necessary when considering treatment with androgen preparations. Response rates to prednisone, androgen preparations, or danazol are in the vicinity of 20% and response durations average about 1 to 2 years.

Thalidomide and lenalidomide are relatively new drugs in the context of MF therapy. Anemia response rate is approximately 20% with single-agent thalidomide therapy (50 to 200 mg/d)[88,89] whereas the addition of prednisone to low-dose thalidomide (50 mg/d) appeared to attenuate thalidomide-associated adverse effects and increase the response rate.[90] However, the usual adverse effect of peripheral neuropathy remains unaltered. Single-agent lenalidomide therapy was associated with a 22% anemia response rate, but grade 3 or 4 thrombocytopenia or neutropenia was seen in one third of the patients.[91] Severe myelosuppression was also the main issue with combined lenalidomide and prednisone therapy, and the anemia response rates in two recent studies were 19%[92] and 30%.[93] Lenalidomide works best in the presence of del(5q31).[94] Both thalidomide and lenalidomide improve thrombocytopenia and splenomegaly in approximately 10% of patients.[88-90,93]

The drug of choice for symptomatic splenomegaly in PMF is hydroxyurea (starting dose 500 mg three times per day). Hydroxyurea-refractory patients are often managed by splenectomy since the value of other conventional drugs in this regard is limited.[95] Other indications for splenectomy include symptomatic portal hypertension and frequent RBC transfusions. The perioperative mortality of splenectomy in PMF is between 5% and 10%. Postsplenectomy complications occur in approximately 50% of the patients and include bleeding, thrombosis, hepatomegaly, extreme thrombocytosis, leukocytosis, and an increase in circulating blasts.[95]

Splenic irradiation (1.0 to 5.0 Gy in 5 to 10 fractions) induces transient reduction in spleen size but could be associated with life-threatening pancytopenia.[95] Nonhepatosplenic extramedullary hematopoiesis might involve the vertebral column, lymph nodes, pleura, and peritoneum (ascites) and is effectively treated with low-dose radiation therapy (1.0 to 10.0 Gy in 5 to 10 fractions).[95] Diagnosis of MF-associated pulmonary hypertension is confirmed by a technetium 99m sulfur colloid scintigraphy and treatment with single-fraction (1.0 Gy) whole-lung irradiation has been shown to be effective.[95] Single fraction 1.0 to 4.0 Gy involved-field therapy has also been shown to benefit patients with MF-associated extremity pain.[95] Transjugular intrahepatic portosystemic shunt might be considered to alleviate symptoms of portal hypertension.[95]

INVESTIGATIONAL DRUG THERAPY

Although many drugs are currently being evaluated in MF, PV, and ET, the current discussion is limited to three drugs that have shown the most promising activity in MF, so far: pomalidomide and two JAK inhibitor ATP mimetics (TG101348 and INCB018424).

Pomalidomide

Pomalidomide is a second-generation immunomodulatory drug and in a phase II randomized study, 25% of patients with anemia responded to the drug used alone (0.5 or 2 mg/d) or in combination with prednisone (median response duration > 1 year).[96] At the dose level of 0.5 mg/d, the drug did not cause either neuropathy or myelosuppression. However, pomalidomide had limited activity in reducing spleen size. In a most recent study involving 58 patients with MF receiving single-agent pomalidomide (0.5 mg/d), anemia response rates ranged from 38% in *JAK2*V617F-positive patients with palpable spleen size of smaller than 10 cm to 0% in *JAK2*V617F-negative patients.[97]

TG101348

TG101348, a selective JAK2 inhibitor, was evaluated in 59 patients with PMF or post-PV/ET MF, in a phase I/II study.[98] The dose-limiting toxicity was a reversible and asymptomatic increase in serum amylase/lipase and the maximum-tolerated dose was 680 mg/d. Adverse events were all reversible and dose dependent and included nausea/vomiting, diarrhea, thrombocytopenia, and anemia. The gastrointestinal adverse effects that occurred in up to 69% of the patients were mostly grade 1 or 2 (only 3% were grade 3), dose dependent, and transient in almost all instances. Asymptomatic mild increases in serum lipase, transaminases, or creatinine were seen in 27%, 27%, and 24%, respectively. Among 37 anemic but nontransfusion-dependent patients, 35% experienced worsening of anemia that was recorded as grade 3 or 4. The corresponding figures for thrombocytopenia and neutropenia were 24% and 10%. Among all patients completing at least one or six cycles of treatment, 42% and 59%, respectively, experienced a ≥ 50% decrease in palpable spleen size during the first 6 months of therapy. 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. Furthermore, among 23 patients with a baseline *JAK2*V617F allele burden of higher than 20%, nine (39%) had ≥ 50% decrease in allele burden. In general, response was not affected by the presence of *JAK2*V617F.

INCB018424

INCB018424, a JAK1 and JAK2 inhibitor, was evaluated in 153 patients with PMF or post-PV/ET MF, in a phase I/II study.[99] The dose-limiting toxicity was reversible thrombocytopenia and the maximum-tolerated dose was either 25-mg twice daily or 100-mg once daily mg/d. Adverse events were all reversible and dose-dependent and included thrombocytopenia, anemia, and a cytokine rebound reaction on drug discontinuation, characterized by acute and intense relapse of symptoms and splenomegaly. Nonhematologic adverse events were remarkably infrequent. Grade 3 or 4 thrombocytopenia occurred in 29% and 10% of patients receiving the drug at 25- or 10-mg twice daily. The corresponding figures for anemia, in transfusion-independent patients at baseline, were 27% and 16%. Among all evaluable patients, 44% experienced ≥ 50% decrease in palpable spleen size. Improvement in constitutional symptoms (eg, fatigue, pruritus, abdominal discomfort, early satiety, night sweats, and exercise tolerance) and weight gain were seen in the majority of patients, even at lower doses (10-mg twice daily). Four (14%) of 28 transfusion-dependent patients became transfusion independent. Ten of 17 patients with thrombocytosis normalized their count at 3 months and mean leukocyte count decreased from 29.8 to 16×10^9/L. The drug's effect on *JAK2*V617F allele burden was negligible, but a major reduction in proinflammatory cytokines (eg, IL-1RA, IL-6, TNF-a, MIP-1b) was documented and coincided with improvement in constitutional symptoms.

INCB018424 has also been studied in patients with hydroxyurea-refractory/intolerant PV and ET.[100] Not surprisingly, the spleen and constitutional symptoms benefits seen in patients with MF were also seen in patients with PV and ET. The drug was effective in controlling erythrocytosis in PV, but less so in normalizing platelet count in ET. Regardless, it is currently not clear what the drug could potentially offer over and above what can be readily obtained from the use of INF-α or busulfan in hydroxyurea failures.

Other Investigational Drugs Currently in Clinical Trials

Other drugs that are currently in clinical trials for MF, PV, or ET include other kinase inhibitors (eg, CYT387, CEP-701, AZD1480, SB1518) and histone deacetylase inhibitors (eg, ITF2357, MK-0683, panobinostat; http://ClinicalTrials.gov). Among these, CYT387 appears to be the most promising because preliminary results suggest significant response rates in anemia, splenomegaly, and constitutional symptoms.[101]

alloSCT

The largest study of alloSCT in PMF (n = 289) comes from the Center for International Bone Marrow Transplant Research and included a variety of donor types and conditioning regimens.[102] Five-year disease-free survival and treatment-related mortality were 33% and 35% for matched related and 27% and 50% for unrelated transplants, respectively. Outcome was not favorably affected by reduced intensity conditioning.[102] In another reduced intensity conditioning transplant study from the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation, 103 patients (median age, 55 years) with PMF or post-PV/ET MF were prospectively studied and 5-year disease-free survival was estimated at 51%.[103] Chronic graft-versus-host disease occurred in 49% of the patients and relapse (29%) was predicted by high-risk disease and prior splenectomy.[103] The respective chronic graft-versus-host disease and relapse rates for matched related transplants in the Center for International Bone Marrow Transplant Research study were 40% and 32% and history of splenectomy did not affect outcome.[102] Considering all these observations, the risk of transplant-related complications might be justified in patients with expected median survival of shorter than 5 years (ie, DIPSS-plus high and intermediate-2 risk categories; Table 2). Post-transplant outcome is poor in the presence of high-risk disease, advanced age, unrelated donor, or HLA mismatch.[102,103]

CONCLUDING REMARKS

Pathogenetic mechanisms in *BCR-ABL1*-negative MPN are not as straight forward as they are in CML. Therefore, we should curb our expectations from anti-JAK2 treatment strategies and instead pay attention to additional pathogenetic insight from correlative laboratory studies. Furthermore, it has become apparent that JAK inhibitor ATP mimetics are far from being similar in their toxicity and activity profiles and one must avoid making premature conclusions about their ultimate therapeutic value.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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