BCR-ABL1—Negative Myeloproliferative Neoplasms: A Review of Molecular Biology, Diagnosis, and Treatment

Erik Vakil,[1] Ayalew Tefferi[2]

Abstract

In 2008, the World Health Organization expanded the classification of myeloproliferative disorders based on increasing amounts of molecular and cytogenetic data. Myeloproliferative neoplasms (MPN) that do not contain the *BCR-ABL1* mutation include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). *JAK2V617F* is the best characterized mutation in *BCR-ABL1*–negative neoplasms, with an estimated prevalence of more than 95% in PV, 50% in ET, and 50% in PMF. Current diagnostic strategies are increasingly reliant on molecular markers, and their prognostic value continues to be investigated. The use of aspirin, hydroxyurea, and phlebotomy for PV and ET, and the use of androgens, steroids, chemotherapy, and radiation therapy for PMF continues to be the mainstay of therapy. The only potentially curative therapy is allogeneic hematopoietic stem cell transplantation, but treatment-related mortality remains high. There have been promising results from clinical trials that involve the JAK tyrosine kinase inhibitors TG101384 and INCB018424, but their role in future therapy is yet to be established. Despite the optimism, it is increasingly apparent that pathogenicity in BCR-ABL1-negative MPN is more complex than for chronic myeloid leukemia, and a pathognomonic mutation may not be forthcoming.

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Introduction

In 1951, Dameshek[1] organized chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) into an inclusive disease category that he termed "myeloproliferative disorders" (MPD). His rationale was based on similarities in the trilineage proliferation of hematopoietic stem cells and disease phenotypes. The World Health Organization (WHO), first in 2001 and again in 2008, began reclassifying these disorders in light of evolving histologic, cytogenetic, and molecular information.[2,3] The latest iteration replaced the term myeloproliferative disorders with "myeloproliferative neoplasms" (MPN) and incorporated systemic mastocytosis, chronic eosinophilic leukemia-not otherwise specified, chronic neutrophilic leukemia, and MPN-unclassifiable into the existing MPD category. The value of cellular markers as a complement to histologic classification was first validated in CML with the discovery of the Philadelphia chromosome[4] and the characterization of the oncogenic *BCR-ABL1* fusion protein.[5-7] In 2005, it was observed that many patients with "classic *BCR-ABL1*–negative" MPDs (PV, ET, and PMF; Figure 1) carried the somatic mutation *JAK2*V617F,[8-10] which had important implications for classification, diagnosis, and potential targeted therapy. Additional cytogenic and molecular markers have since been characterized, and their role continues to evolve.

Data from the North American Association of Central Cancer Registries and the Surveillance, Epidemiology, and End Results program have estimated the annual age-adjusted incidence of *BCR-ABL1*–negative MPD to be 2.1 per 100,000, with an overall 3-year survival rate of 80%.[11] This review will focus on the latest developments in the molecular biology, diagnosis, and treatment of classic *BCR-ABL1*–negative MPNs.

Molecular Biology

Cytogenetics

The role of cytogenetics in the classification of MPN is less defined than for the pathognomonic t(9;22) karyotype of CML. Research

[1]Biochemistry and Molecular Biology, Royal College of Surgeons in Ireland, Dublin, Republic of Ireland
[2]Department of Hematology, Mayo Clinic, SW, Rochester, MN

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Address for correspondence: Ayalew Tefferi, MD, Department of Hematology, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905; e-mail contact: tefferi.ayalew@mayo.edu

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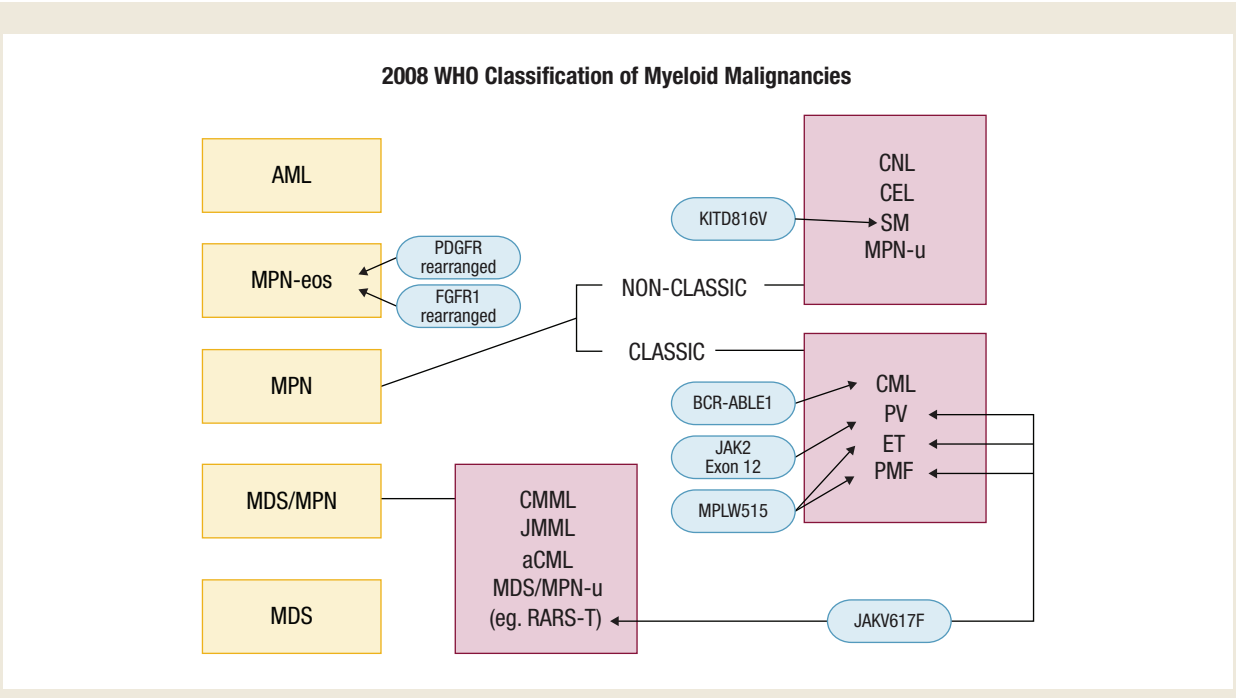
Figure 1 The World Health Organization (WHO) 2008 Classification of Myeloid Malignancies and Associated Genotypic Abnormalities: Acute Myeloid Leukemia (AML); Myelopreliferative Neoplasm Associated Esosinophilia (MPN-eos); Myelodysplatic Syndrome (MDS). MDS/MPN Includes Subtypes Chronic Myelomonocytic Leukemia (CMML), Juvenile Myelomonocytic Leukemia (JMML), Atypical Chronic Myeloid Leukemia–BCR-ABL1 Negative (aCML), MDS/MPN Unspecified (MDS/MPN-u), Which Includes the Refractory Anemia With Ring Sideblasts and Thrombocytosis (RARS-T). MPN Includes Nonclassic Subtypes Chronic Neutrophilic Leukemia (CNL), Chronic Eosinophilic Leukemia (CEL), Systemic Mastocytosis (SM), and MPN Unspecified (MPN-u). Classic Subtypes Include Chronic Myeloid Leukemia–BCR-ABL1 positive (CML), Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primary Myelofibrosis (PMF)



has primarily focused on the prognostic implication of cytogenetics in *BCR-ABL1*-negative MPNs. In a study of 137 patients with PV, 11% displayed abnormal genotypes, with Y- (7% of male patients), +8, +9, 20q-, and abnormalities of chromosome 1, 13q-, 11q-, 3p-, and dup13 were observed with the highest frequency.[12] Seven percent of 402 patients with ET displayed a Y- (6% of male patients) or +8, +9, 5q-, 6-, 20q-, and abnormalities of chromosome 1 and 11.[13] The strongest correlation was found in PMF, where 33% of 109 patients had cytogenetic abnormalities, including +8, 13q-, 20q-, and abnormalities of chromosomes 1, 7, and 9.[14]

Clinical phenotypes that correspond to specific cytogenetic patterns were variable among the disease groups. The majority of abnormalities in PV were associated with age ≥60 years. Similar cytogenetic profiles in ET did not predominate in the older age group but were associated with palpable splenomegaly; concurrent tobacco use; venous thrombosis; and anemia, with a hemoglobin <10 g/dL. [12,13] The prognostic value was only demonstrated for PMF when an "unfavorable" cytogenetic profile (complex karyotype, +8 or abnormalities other than isolated +9, 13q-, or 20q-) predicted inferior survival not already accounted for by the International Prognostic Scoring System for PMF.[14,15]

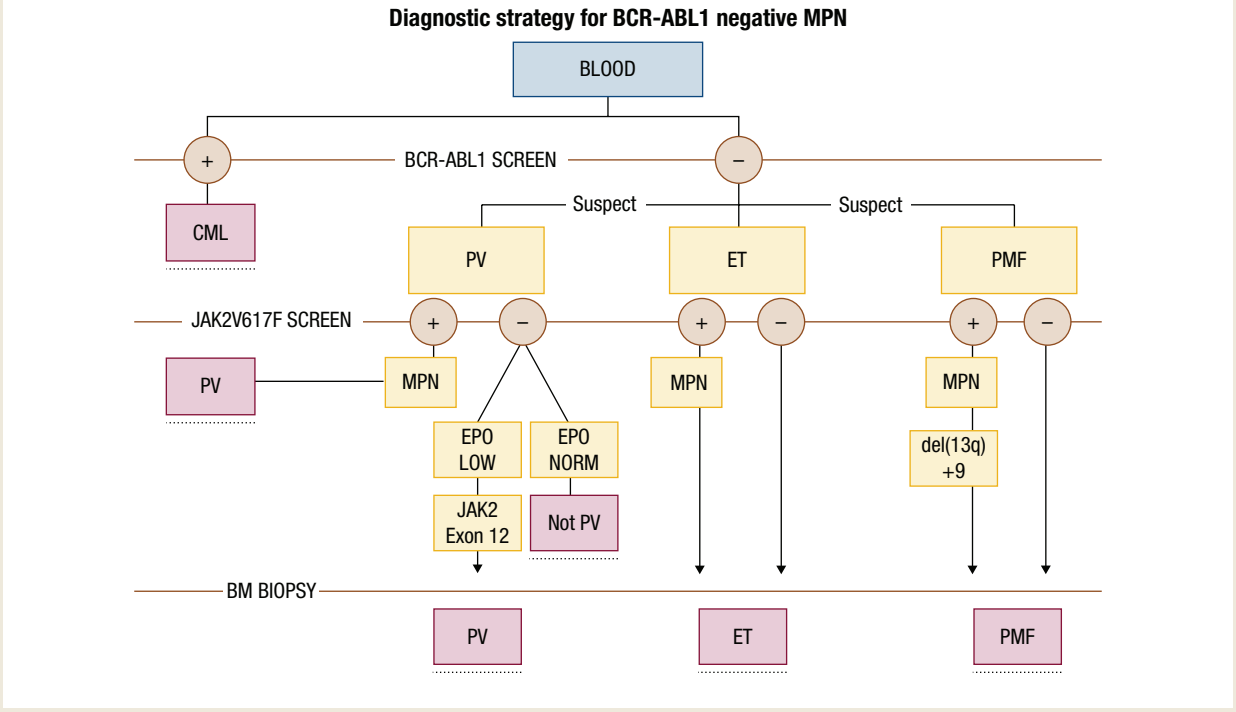
Oncogenes

JAK2. JAK2V617F is the best characterized mutation in *BCR-ABL1*–negative MPN. Its estimated prevalence is more than 95% in PV,[16-19] 50% in ET,[20-23] and 50% in PMF,[24] and its value as a diagnostic marker is improved by a low prevalence in non-MPN myeloproliferative diseases. It is found in only 3% of patients with de novo acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS)[25-27] and is not seen in nonmyeloid malignancies[28,29] or reactive myeloproliferation.[30] The exception is an MDS subtype, refractory anemia with ring sideroblasts and thrombocytosis, in which the frequency of *JAK2V617F* mutation is estimated to be 50%.[31]

JAK2V617F is a tyrosine kinase gain-of-function mutation in exon 14 that results from a guanine-to-thymine transversion at nucleotide 1849 with substitution of valine to phenylalanine at codon 617.[8-10,32] The in vitro expression of *JAK2V617F* results in the constitutive activation of the JAK-STAT pathway and resultant cytokine-independent growth.[33] In vivo studies demonstrated the induction of PV-, ET-, and PMF-like diseases in *JAK2V617F*-positive transgenic mice and the relative disease phenotypes could be partially anticipated by allelic burden.[34,35] Allele heterozygosity is more common in ET compared with homozygosity in PV and PMF, which is thought to result from mitotic recombination.[10,36] In humans, however, recent evidence indicates that acquisition of *JAK2V617F* may not be the primary oncogenic event[37,38] and the relative predisposition to *JAK2V617F* mutation may be more contributory to disease phenotype.[39-42]

Disease phenotypes associated with *JAK2V617F* mutations include older age, higher hemoglobin levels, leukocytosis, and lower

Figure 2 Proposed Diagnostic Strategy for Myeloproliferative Neoplasms (MPN). Initial Screening Should Be for BCR-ABL1. Positive Test is Diagnostic for Chronic Myeloid Leukemia (CML). If Clinical Suspicion Exists for BCR-ABL1 Negative MPN, Then Screen for JAK2V617F Mutation. JAK2 is Diagnostic for MPN. Bone Marrow (BM) Biopsy is Required for Diagnosis of Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF), and Can Be Helpful in JAK2 Negative Polycythemia Vera (PV)



platelet count.[43] A higher allelic burden in PV is associated with pruritus and increased splenomegaly, and has been implicated in myelofibrotic transformation.[43-45] A lower allelic burden in PMF is associated with decreased leukemic-free survival and an inferior prognosis.[46,47] The prognostic value of *JAK2V617F* in PV and ET is yet to be established.

Additional *JAK2* mutations have now been described on exon 12, with N542-E543 being the most common.[48] These mutations are specific for PV and are observed in predominantly patients who are *JAK2V617F* negative, with an overall frequency of 3%.[49,50] Disease phenotypes associated with *JAK2* exon 12 mutations include younger age at diagnosis, subnormal erythropoietin (EPO) levels, and isolated erythrocytosis at clinical onset.[49,51]

MPL. *MPL* was identified as a candidate for MPN pathogenesis when it was described in the downstream signaling pathway required for *JAK2V617F*-mediated transformation of myeloprogenitor cells.[52] Subsequent analysis identified the *MPLW515L* mutation that resulted in a MPN-like disorder in a murine bone marrow transplant assay.[53] *MPLW515L* results from a guanine-to-thymine transversion at nucleotide 1544, with substitution of tryptophan to leucine at codon 515.[53] Additional *MPL* mutations have since been described at the same locus, with an overall *MPL515* mutational frequency estimated at 4% for ET and 11% for PMF. As with *JAK2* exon 12 mutations, these mutations occur predominantly in *JAK2V617F*-negative clones.[54-56] Disease phenotypes associated with *MPL* mutations include older age, female gender, lower hemoglobin level, and higher platelet count.[57-59]

TET2. The role of *TET2* in MPN was established while investigating a chromosomal rearrangement at locus 4q24 previously described in patients with AML and MDS.[60,61] *TET2* is a putative tumor suppressor gene and mutational frequency is estimated at 16% for PV, 5% for ET, and 17% for PMF.[62] The role of *TET2* in the classification of MPN is limited because *TET2* mutations have been identified in most other myeloid malignancies.[61,63,64] The only described disease phenotype associated with *TET2* in MPN is older age.

Other. Mutations in *ASXL1*, *IDH1*, *IDH2*, *CBL*, *IKAROS,* and *LNK* genes have all been described with variable frequencies in MPN.[65-70] Their role in the classification and diagnosis is yet to be established.

Diagnosis

The initial step in an accurate diagnosis must be the exclusion of the *BCR-ABL1* translocation in patients who present with signs, symptoms, or laboratory values consistent with PV, ET, or PMF. Screening for *JAK2V617F* may help increase clinical suspicion of MPN and may be diagnostic in PV, and eliminate the need for bone marrow biopsy (Figure 2). The specific diagnostic criteria are presented below.

Table 1 2008 World Health Organization Diagnostic Criteria for Polycythemia Vera

Diagnosis Requires Meeting Both Major Criteria OR **the First Major Criteria and 2 Minor Criteria**	
Major Criteria	1. Hemoglobin >18.5 g/dL (men), >16.5 g/dL (women) **OR** hemoglobin or hematocrit >99th percentile of reference range for age, sex, or altitude of residence **OR** red cell mass >25% above mean normal predicted **OR** hemoglobin >17 g/dL (men), >15 g/dL if representing a sustained increase of ≥2 g/dL from baseline that is not attributed to correction of iron deficiency 2. Presence of *JAK2V617F* or *JAK2* exon 12 mutation
Minor Criteria	1. Bone marrow trilineage myeloproliferation 2. Sub-normal serum erythropoietin levels 3. Endogenous erythroid colony growth

(Adapted from Tefferi et al.)[76]

Table 2 2008 World Health Organization Diagnostic Criteria for Essential Thrombocythemia

Diagnosis Requires Meeting all 4 Major Criteria	
Major Criteria	1. Sustained platelet count ≥450 × 10^9/L before treatment 2. Bone marrow proliferation of enlarged, mature megakaryocytes 3. Not meeting the World Health Organization criteria for chronic myelogenous leukemia, polycythemia vera, primary myelofibrosis, myelodysplatic syndrome, or other myeloid malignancies 4. Presence of *JAK2V617F* or other clonal markers **OR** no evidence of reactive thrombocytosis*

* The presence of reactive thrombosis does not exclude a diagnosis of essential thrombocytosis in the presence of 3 other major criteria.
(Adapted from Tefferi et al.)[76]

Table 3 2008 World Health Organization Diagnostic Criteria for Primary Myelofibrosis

Diagnosis Requires Meeting All 3 Major Criteria AND **2 Minor Criteria**	
Major Criteria	1. Megakaryocyte proliferation with atypia* in association with reticulin and/or collagen fibrosis **OR** in the absence of reticulin fibrosis, an increase in bone marrow cellularity associated with granulocyte proliferation and often decreased erythrocyte proliferation 2. Not meeting the World Health Organization criteria for chronic myelogenous leukemia, polycythemia vera, primary myelofibrosis, myelodysplatic syndrome, or other myeloid malignancies 3. Presence of *JAK2V617F* or other clonal makers **OR** no evidence of reactive marrow fibrosis*
Minor Criteria	1. Leukoerythroblastosis 2. Increased serum lactate dehydrogenase level 3. Anemia 4. Palpable splenomegaly

* The presence of reactive marrow fibrosis does not exclude a diagnosis of primary myelofribrosis in the presence of numerous other criteria.
(Adapted from Tefferi et al.)[76]

PV

Clinical suspicion of PV is most often raised in the context of elevated peripheral blood (PB) counts. Erythrocytosis with a hemoglobin level >18.5 g/dL in men and >16.5 g/dL in women or a hemoglobin level >17 g/dL in men and >15 g/dL in women if representing a sustained increase of ≥2 g/dL from baseline are consistent but not diagnostic for PV (Table 1).[71] Further analysis involves a *JAK2V617* PB mutation screen and measurement of a serum EPO level. *JAK2V617* is present in more than 95% of patients with PV, and serum EPO levels are appropriately subnormal in more than 90% of patients.[72,73] JAK2 exon-12 mutations are present in the majority of patients who were *JAK2V617* negative and 3% of patients with PV overall.[50] A *JAK2* exon-12 mutation screen may be warranted in patients who were *JAK2V617F* negative, particularly in those with subnormal EPO levels. Additional investigations may include bone marrow biopsy that demonstrate trilineage myeloproliferation and/or the presence of endogenous erythroid colonies on peripheral smear. Bone marrow proliferation of pleomorphic, loosely clustered megakaryocytes with unique stromal changes and without maturation defects is commonly seen in PV but is not diagnostic.[74,75]

ET

ET should be suspected in patients who present with a platelet count ≥450 × 10^9/L and when clinical evidence of reactive thrombocytosis is not obvious (Table 2).[76] Demonstration of a *JAK2V617* PB mutation confirms the presence of MPN and is highly suggestive of ET in the absence of erythrocytosis. Approximately 45% of patients will be *JAK2V617* negative and alternative diagnoses that may mimic ET should be considered, including CML, prefibrotic PMF, and PV.[77] The presence of large hyperlobulated and mature-appearing megakaryocytes without erythroid or granulocyte proliferation is diagnostic for ET according to the WHO guidelines, but reliability may depend on skilled hematopathologists.[78-80]

PMF

Clinical suspicion of PMF may be raised in the context of nonspecific derangements in PB counts and evidence of extramedullary hematopoiesis (ie, palpable splenomegaly). PMF can be diagnosed by bone marrow biopsy that shows proliferation of hyperchromic megakaryocytes with large irregular nuclei, in association with either reticulin or collagen fibrosis, and in the absence of reactive fibrosis (Table 3).[80] An accurate diagnosis is more likely when one or more of a *JAK2V617F* mutation, cytogenetic abnormalities (+9, del[13q]), anemia, elevated serum LDH, and leukoerythroblastosis are present. Bone marrow fibrosis secondary to PV or ET can be differentiated from PMF by bone marrow histology.[81]

Table 4 Risk Stratification and Treatment in Polycythemia Vera

Risk Designation for PV	Criteria	Treatment
Low Risk	Age < 60 **AND** no history of thrombosis	Low dose aspirin
Low Risk with Extreme Thrombocytosis	Platelets > 1000 × 10^9/L	Low dose aspirin **IF NO** AVWS + phlebotomy to Hct < 50%
High Risk	Age ≥ 60 **OR** history of thrombosis	Low dose aspirin + phlebotomy + hydroxyurea (1st line) **OR** INFα (age < 60) **OR** busulfan **OR** pipobrom, an (age > 60)

Abbreviations: AVWS = acute von Willebrand syndrome; Hct = hematocrit; PV = polycythemia vera.
(Adapted from Tefferi et al.)[76]

Table 5 Risk Stratification and Treatment in Essential Thrombocythemia

Risk Designation for ET	Criteria	Treatment
Low Risk	Age < 60 **AND** no history of thrombosis	Low dose aspirin
Low Risk with Extreme Thrombocytosis	Platelets > 1000 × 10^9/L	Low dose aspirin **IF NO** AVWS
High Risk	Age ≥ 60 **OR** history of thrombosis	Low dose aspirin + phlebotomy to Hct < 50% + hydroxyurea (1st line) **OR** INFα (age < 60) **OR** busulfan **OR** pipobroman (age > 60)

Abbreviations: AVWS = acute von Willebrand syndrome; ET = essential thrombocythemia; Hct = hematocrit.
(Adapted from Tefferi et al.)[76]

Treatment

Current Paradigms

Current treatments for *BCR-ABL1*-negative MPN are considered noncurative and focus primarily on reducing the risk of complications. Morbidity and mortality in PV and ET is related to thrombophilia and, along with PMF, the progression to acute leukemia. Risk stratification in PV and ET is based on thrombotic risk, and survival in PMF is estimated by the International Prognostic Scoring System.[82,83] Treatments are based on the risk profile of individual patients.

PV. Patients with PV are considered low risk if they are <60 years old and have no history of thrombosis or are high risk if they are ≥60 years old or have a history of thrombosis. Low-risk patients with platelet counts >1000 × 10^9/L are considered separately (Table 4).[84-87]

Aspirin for thromboprophylaxis in PV was previously limited because of a documented risk of gastrointestinal bleeding with high doses (900 mg/day).[88] Equivalent antithrombotic efficacy with low-dose aspirin (100 mg/day) has now been demonstrated and is currently recommended for all patients diagnosed with PV regardless of risk profile and in the absence of contraindications.[89] The risk of hemorrhage secondary to acquired von Willebrand syndrome may preclude the use of aspirin in patients with a ristocetin cofactor activity <30%. A screening assay may be warranted in patients with thrombocytosis >1000 × 10^9/L because the risk of acquired von Willebrand syndrome increases with increasing platelets levels.[90]

Clinical trials conducted by the Polycythemia Vera Study Group support the use of hydroxyurea in conjunction with aspirin in patients with high-risk PV.[91] Dosing should aim to decrease platelets below 400 × 10^9/L and maintain leukocytes above 2 × 10^9/L. Hydroxyurea also has been shown to effectively reduce the *JAK2V617* allelic burden (molecular remission), although the long-term implications of this remain unclear.[92-94]

There is longstanding, yet debatable evidence that a reduction in hematocrit (Hct) level to <45% reduces the risk of vaso-occlusive events.[95,96] However, phlebotomy is still recommended with current guidelines that suggest an Hct target of <50%.[87] Women who are pregnant or who are likely to become pregnant can be managed with aspirin and venesection but should use interferon (INF)-α in place of hydroxyurea when the later is indicated.[97]

ET. Patients with ET are risk stratified in the same manner as those with PV (Table 5). The use of aspirin in reducing thrombotic complications in ET is less defined than for PV, because no formal clinical trials have been conducted. A retrospective study of 68 patients with ET found that the risk of thrombotic events in patients who receive aspirin (500 mg/day) was 3.6 per 100 person-years compared with 32.3 per 100 person-years in patients under observation only.[98] Extrapolation from studies conducted in patients with PV may suggest commensurate efficacy of aspirin in patients with ET. Current recommendations support the use of aspirin as first-line therapy in all risk classes unless contraindicated.[99] Antithrombotic efficacy of hydroxyurea in ET is well established and is recommended for all high-risk patients.[100,101]

INF-α. The most-effective alternative treatments to hydroxyurea in PV and ET are INF-α and busulfan. There is increasing interest in INF-α due to its nonleukemogenic potential; however, the risk of leukemic transformation with hydroxyurea and busulfan therapies has likely been overstated. In 2 large observational studies, therapy with hydroxyurea was not shown to contribute to an increased rate of transformation to acute myeloid leukemia or myelodysplastic syndrome in patients with PV[102] or ET.[103] Studies that compared hydroxyurea with anagrelide[101] and with pipobroman[104] also showed no difference in leukemogenic potential between these drugs. Similar conclusions were reached in studies of patients with PV and ET treated with busulfan.[105,106]

Two principle studies have helped to establish INF-α as an effective therapy. The first reported pegylated INF-α (90 μg/week) induced hematologic remission in approximately 80% of patients with PV and 81% of patients with ET (complete remission in 70% with PV and 76% with ET) and molecular remission in 54% of patients with PV and 38% with ET.[107] Adverse effects were reported in 96%

Table 6 Risk Stratification and Treatment in Primary Myelofibrosis

Risk Designation for PMF	Criteria	Treatment
Low Risk	No IPSS risk factors*	Observation
Intermediate-1 Risk	One risk factor	Androgens **OR** thalidomide + prednisone for anemia **AND/OR** hydroxyurea for symptomatic splenomegaly
Intermediate-1 Risk with Transfusions OR **Unfavorable Karyotype**	Complex karyotype, +8 or abnormalities other than isolated +9, 13q- or 20q-	Allogeneic HCT (age < 65) **OR** experimental therapy
Intermediate-2 Risk	Two risk factors	Allogeneic HCT (age < 65) **OR** experimental therapy
High Risk	≥ Three risk factors	Allogeneic HCT (age < 65) **OR** experimental therapy

Abbreviations: HCT = hematopoietic stem cell transplant; IPSS = international prognostic scoring system; PMF = primary myelofibrosis.
* age > 65, hemoglobin <10 g/dL, leukocytosis > 25×10^9/L, circulating blasts > 1%, and presence of constitutional symptoms.
(Adapted from Tefferi et al.)[76]

of patients, and treatment was stopped in 10% of these due to toxicity.[107] The second study was a phase II clinical trial that reported complete hematologic remission in 94.6% of patients who received pegylated INF-α (90-135 μ/week) after 31.4 months of median follow-up and 21.4% of patients in complete molecular remission.[108] Adverse effects were reported in 89% of patients, and treatment was stopped in 24% due to toxicity.[108] Overall, the response rates for INF-α are promising but remain comparable with those already achieved by hydroxyurea. Given the high-toxicity profile of INF-α and the questionable benefit of its nonleukemogenic properties, further research is needed to establish INF-α as a first-line therapy.

PMF. Age >65 years old, hemoglobin level <10 g/dL, leukocyte count >25×10^9/L, circulating blasts ≥1%, and the presence of constitutional symptoms are considered negative predictive risk factors by the International Prognostic Scoring System. Patients with PMF are considered low risk if they have no risk factors, intermediate-1 risk if they have 1 risk factor, intermediate-2 if they have 2 risk factors, and high risk if they have 3 or more risk factors. Patient-designated intermediate-1 risk but who are transfusion dependent or have an unfavorable karyotype are managed as high risk (Table 6).[83] Inferior predictors of survival in PMF secondary to PV and ET are similar for de novo disease, and patients may be risk stratified in a comparable manner.[109,110] Treatment for PMF is dependent on both the absolute risk category and the presence of anemia, splenomegaly, or both. No treatment is indicated for low-risk patients.

Intermediate-1–risk patients who have anemia should be treated with either erythropoietin stimulating agents, testosterone enanthate (400-600 mg intramuscularly/week), fluoxymesterone (10 mg orally, 3 times a day [t.id.]), prednisone (0.5-1.0 mg/kg/day), danazol (600 mg/day), thalidomide (50-200 mg/day), or lenalidomide (10 mg/day).[111] Single-agent use of any of these drugs have comparable response rates of approximately 20% that last 1-2 years.[112-114] In patients with both anemia and splenomegaly, erythropoietin stimulating agents are not recommended because increased extramedullary hematopoesis can lead to worsening splenomegaly.[115]

In patients who have symptomatic anemia, thalidomide or lenalidomide should be considered, although therapy may be limited by toxicity. Combination therapy with thalidomide and prednisone or lenalidomide and prednisone is associated with higher response rates, 40% and 30%, respectively, and decreased toxicity.[98] Increased response rates have also been reported when lenalidomide is used in patients with del(5)(q31).[116]

In patients who have symptomatic splenomegaly, hydroxyurea (500 mg t.i.d.) should be used. Splenectomy is considered in refractory patients, patients who require frequent transfusions or who have significant portal hypertension.[117] Adverse effects of splenectomy in patients with PMF include bleeding, thrombosis, thrombocytopenia, leukocytosis, increased circulating blasts, and hepatomegaly.[117] Adverse effects may be attenuated with prophylactic cytoreduction when using hydroxyurea. Mortality for splenectomy in PMF is 5%-10%. Splenic irradiation when using either 100-500 cGy in 5-10 fractions or 100 cGy in 4 fractions may help transiently reduce spleen size. The latter has been shown to reduce the incidence of life-threatening pancytopenia.[117]

Low-dose radiation therapy (100-1000 cGy in 5-10 fractions) also is effective at temporarily resolving symptoms associated with nonsplenic sites of extramedullary hematopoiesis.[117] Single-fraction irradiation (100 cGy) of the lungs may be effective for pulmonary hypertension and 400 cGy for extremity pain associated with PMF.[117] Intermediate-1–risk patients who are transfusion dependent or with unfavorable cytogenetics, intermediate-2–risk patients, and high-risk patients are candidates for investigational drug therapy or allogeneic stem cell transplantation.

Investigation Drug Therapy

The oncogenic role of mutant *JAK2V617F* tyrosine kinase in patients with *BCR-ABL1*–negative MPN has not been fully established. However, the demonstrated oncogenecity in transgenic mice provides a reasonable rationale for JAK2 inhibition as a targeted treatment strategy. The most promising JAK2 inhibitors are TG101348 and INCB018424. A phase I/II study that investigated TG101348 in 59 patients with PMF or post-PV/ET MF reported that 67% of patients achieved resolution of leukocytosis, thrombocytosis, and constitutional symptoms, and decreased splenomegaly, whereas 44% achieved >50% reduction in *JAK2V617F* allelic burden.[118] Adverse effects were mild and included nausea, vomiting, diarrhea, anemia, and asymptomatic increases in serum amylase, lipase, and transaminases.

INCB018424 is a JAK1/ JAK2 inhibitor, and phase I/II studies involved 153 patients with PMF or post-PV/ET MF, 34 patients with PV, and 39 with ET.[119-121] Ninety-three percent of patients with PMF showed a decrease in spleen size of >20 cm. Thirty-five percent of patients who received a low dose and 59% who received a high dose showed >50% reduction in splenomegaly overall. Consti-

tutional symptoms improved significantly in 40%-60%, and dose-dependent weight gain was reported in patients with low body mass index. All the patients with PV no longer required phlebotomy after treatment, but only 13% of patients with ET had a reduction of platelets to $<400 \times 10^9$/L. *JAK2FV617F* allelic burden was only decreased 13% in bone marrow and 9% in peripheral blood from baseline. Adverse effects included thrombocytopenia, anemia, and a rapid return of symptoms after discontinuation of therapy. A complete review of other JAK2 inhibitors is discussed elsewhere.[122,123]

Allogeneic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplant (HCT) is currently the only therapy with curative potential for PMF; however, treatment-related mortality remains high. HCT may be warranted in intermediate-2–risk or high-risk patients in which median survival is expected to be <5 years. Allogeneic HCT is also potentially curative in PV and ET, but the already high rates of long-term survival with these diseases preclude the use of aggressive therapy with a high risk of mortality.

A series of small studies that involved 55 patients with PMF,[124] 25 patients with PMF, [125] and 104 patients with PMF, PV, or ET[126] reported a 47% 5-year probability of survival, 41% projected 2-year overall survival, and 58% 3-year survival, respectively. A recent large retrospective study involved 289 patients with PMF receiving HCT from either HLA-matched identical siblings (162 patients), HLA-matched nonidentical family members (26 patients) or HLA-matched unrelated individuals (101 patients).[127] The probability of 5-year disease-free survival was 33%, 22%, and 27%, respectively. Treatment-related mortality at 100 days was 18%, 19%, and 35% respectively. Conditioning regimes and graft-versus-host disease (GVHD) prophylaxis was variable, and significant differences were not found between protocols. A prospective phase II study evaluated the efficacy of reduced intensity conditioning followed by HCT in 103 patients with PMF or post-PV/ET MF. Disease-free survival was 51% for related HLA-matched donors and 67% for unrelated donors.[128] Twenty-seven patients died during follow-up, with a cumulative nonrelapse mortality at 1 year of 16%. The incidence of acute GVHD all grades was 38% and chronic GVHD was 49%.

Conclusion

Karyotyping and cytogenic profiling is increasingly important in evaluating patients with *BCR-ABL1*-negative MPN. JAK2V617F remains the best clinical marker of disease despite the identification of numerous other mutations prevalent in subsets of MPN. The reclassification of MPN by the WHO reflects the increasingly heterogenic character of MPDs. Objective prognostic markers have yet to be established for PV and ET, and the risk of thrombosis continues to be the main criteria for risk stratification. Because long-term survival is high with PV and ET, survival prediction may only be valuable if aggressive therapy is to be considered. Traditional therapies for PV and ET remain effective, but the treatments and prognosis for PMF are comparatively poor. The optimism for targeted JAK2 tyrosine kinase inhibitors has been tempered with increasing data to suggest that the oncogenic role of JAK2 mutations may be more complex than for *BCR-ABL1* in CML. Perhaps additional emphasis should be placed on understanding the pathogenicity of these diseases to gain insight in other potential therapies.

Disclosures

The authors have stated that they have no conflicts of interest.

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