

The JAK2^{V617F} Tyrosine Kinase Mutation in Myeloproliferative Disorders: Status Report and Immediate Implications for Disease Classification and Diagnosis

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Janus kinase 2 (JAK2) is a cytoplasmic protein-tyrosine kinase that catalyzes the transfer of the γ -phosphate group of adenosine triphosphate to the hydroxyl groups of specific tyrosine residues in signal transduction molecules. JAK2 mediates signaling downstream of cytokine receptors after ligand-induced autophosphorylation of both receptor and enzyme. The main downstream effectors of JAK2 are a family of transcription factors known as signal transducers and activators of transcription (STAT) proteins. The myeloproliferative disorders (MPD), a subgroup of myeloid malignancies, are clonal stem cell diseases characterized by an expansion of morphologically mature granulocyte, erythroid, megakaryocyte, or monocyte lineage cells. Among the traditionally classified MPD, the disease-causing mutation has been delineated, thus far, for only chronic myeloid leukemia (ie, *bcr/abl*). In the past 3 months, 7 different studies have independently described a close association between an activating JAK2 mutation (JAK2^{V617F}) and the classic *bcr/abl*-negative MPD (ie, polycythemia vera, essential thrombocythemia, myelofibrosis with myeloid metaplasia) as well as the less frequent occurrence of the same mutation in both atypical MPD and the myelodysplastic syndrome. The particular finding is consistent with previous observations that have implicated the JAK/STAT signal transduction pathway in the pathogenesis of *bcr/abl*-negative MPD, including the phenotype of growth factor independence and/or hypersensitivity. The current article summarizes this new information and discusses its implications for both classification and diagnosis of MPD.

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AML = acute myeloid leukemia; ATP = adenosine triphosphate; CMD = chronic myeloid disorders; CML = chronic myeloid leukemia; CMML = chronic myelomonocytic leukemia; CNL = chronic neutrophilic leukemia; CPTK = cytoplasmic PTK; EEC = endogenous erythroid colony; Epo = erythropoietin; EpoR = Epo receptor; ET = essential thrombocythemia; FISH = fluorescent in situ hybridization; HES = hypereosinophilic syndrome; IL = interleukin; JAK = Janus kinase; JH = JAK homology; LOH = loss of heterozygosity; MDS = myelodysplastic syndromes; MMM = myelofibrosis with myeloid metaplasia; MPD = myeloproliferative disorders; PCR = polymerase chain reaction; PDGFR = platelet-derived growth factor receptor; PK = protein kinases; PP = protein phosphatases; PTK = protein-tyrosine kinases; PTP = protein-tyrosine phosphatase; PV = polycythemia vera; RCM = red cell mass; RPTK = receptor PTK; SM = systemic mastocytosis; STAT = signal transducers and activators of transcription; UMPD = unclassified MPD; WHO = World Health Organization

Myeloproliferative disorders (MPD) are characterized by neoplastic expansion of relatively mature granulocyte, erythroid, megakaryocyte, or monocyte lineage cells. The MPD are traditionally classified into "classic" and "atypical" subcategories.^{1,2} The former includes chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis with myeloid metaplasia (MMM). Examples of atypical MPD include

chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, chronic neutrophilic leukemia (CNL), chronic basophilic leukemia, chronic eosinophilic leukemia, hypereosinophilic syndrome (HES), systemic mastocytosis (SM), and unclassified MPD (UMPD).³⁻⁵ Analysis of X chromosome inactivation patterns in informative female cases as well as other cytogenetic and/or molecular studies has long established both classic and atypical MPD as clonal stem cell disorders.⁶⁻¹⁵ However, the disease-causing mutation in most cases has remained elusive.¹⁶

In 1960, Nowell and Hungerford¹⁷ described the first disease-specific cytogenetic marker in MPD, the Philadelphia chromosome in CML. In the 1980s, molecular genetic techniques became available and revealed a specific oncogenic mutation (ie, *bcr/abl*) harbored by the Philadelphia chromosome.¹⁸⁻²⁷ Since then, additional MPD-specific mutant molecules have been identified, and examples of specific phenotype-genotype pairs in *bcr/abl*-negative MPD include SM and either *FIP1L1-PDGFR* or *c-kit*^{D816V} mutation,²⁸⁻³⁴ chronic eosinophilic leukemia and rearrangements of *PDGFRB*,³⁵⁻⁴⁷ stem cell leukemia/lymphoma syndrome and rearrangements of *FGFR1*,⁴⁸⁻⁶⁶ and juvenile myelomonocytic leukemia and mutations affecting the RAS signaling pathway (eg, *PTPN11*, *NFI*).⁶⁷⁻⁷¹ Furthermore, a new mutation affecting the Janus tyrosine kinase 2 (JAK2^{V617F}) has just been described in a large proportion of patients with PV, MMM, or ET⁷²⁻⁷⁶ and in a smaller proportion with other myeloid disorders.^{77,78} Such advances in the molecular pathogenesis of MPD provide the basis for the new molecular-based disease classification and diagnostic systems as well as identify mutant molecules that might serve as therapeutic targets for rational therapy, including the use of small molecule kinase inhibitors.⁷⁹

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BACKGROUND ON PROTEIN KINASES AND JAK2

Protein kinases (PK) are enzymes that catalyze protein phosphorylation, whereas protein phosphatases (PP) do the opposite: regulate PK activity through protein dephosphorylation.⁸⁰ Such phosphotransfer and hydrolysis reactions are key to cellular signal transduction.⁸⁰ In the human genome, there are more than 520 PK and 130 PP, and both are broadly classified into either tyrosine-specific or serine/threonine-specific categories based on the target protein residues that are either phosphorylated or dephosphorylated.⁸¹ Accordingly, protein-tyrosine kinases (PTK) are PK that catalyze the transfer of the γ -phosphate group of adenosine triphosphate (ATP) to the hydroxyl groups of specific tyrosine residues in signal transduction molecules.

In general, there are 2 categories of PTK: receptor PTK (RPTK) and cytoplasmic PTK (CPTK).^{81,82} In humans, there are approximately 90 known PTK genes; 58 encode for RPTK and 32 for CPTK.⁸³ Examples of MPD-pertinent RPTK include platelet-derived growth factor receptor (PDGFR), stem cell factor receptor (Kit), fibroblast growth factor receptor, vascular endothelial growth factor receptor, and *fms*-related tyrosine kinase 3 (Flt3).^{81,82} Examples of CPTK include the Janus family of kinases (JAK1, JAK2, JAK3, tyrosine kinase 2 [TYK2]),^{84,85} the Src family of kinases (homologues of the Rous sarcoma virus oncoprotein),⁸⁶ and Abl kinase (homologue of the Abelson murine leukemia virus oncoprotein).⁸⁷⁻⁸⁹

Janus kinase 2 (JAK2) is a cytoplasmic (ie, nonreceptor) PTK, and its structure is uniquely characterized by the presence of 2 homologous kinase domains: JAK homology (JH) 1, which is functional, and JH2, which lacks kinase activity (ie, pseudokinase).^{84,88,90} The Janus CPTK (eg, JAK2) mediate signaling downstream of cytokine receptors, via distinct type I and II cytokine receptors, as well as RPTK.⁹¹ Engagement of a cytokine receptor with ligand (eg, interferons, interleukins [ILs], growth factors) results in receptor dimerization as well as both autophosphorylation and transphosphorylation of both the receptor and the receptor-associated JAK.^{92,93} The activated JAK-cytokine receptor complex leads to recruitment and phosphorylation of substrate molecules including signal transducers and activators of transcription (STAT) proteins (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6).^{94,95} This results in activation/dimerization of STAT proteins with subsequent translocation into the nucleus and interaction with specific regulatory elements to induce target gene transcription.^{94,95} JAK/STAT signaling is regulated at multiple levels by distinct mechanisms including, for example, direct dephosphorylation of JAK2 by specific protein-tyrosine phosphatase (PTP) (eg, SHP-1), proteolytic degradation of JAK2 through binding with a family of suppres-

sors of cytokine signaling (eg, SOCS-1), and inhibition of DNA binding of STAT by protein inhibitors of activated STAT.⁹⁶⁻⁹⁹

The JAK/STAT signal transduction pathway plays a major role in both cellular proliferation and cell survival.^{94,95,100} In hematopoiesis, for example, definitive erythropoiesis and cytokine response by myeloid progenitors have been shown to be absent in *JAK2* knockout mice.^{101,102} The kinase domain of JAK2 mediates anti-apoptotic signals in hematopoietic cells by inducing bcl-2 production.¹⁰³ Abnormalities affecting either members of the JAK/STAT signaling pathway or its regulatory elements have been associated with various tumor phenotypes including hematologic malignancies. For example, germline *JAK3* mutations have been associated with certain forms of autosomal recessive severe combined immunodeficiency syndrome.^{85,104} Within the purview of somatic mutations, *JAK2* has been identified as a fusion partner of *ETV6/TEL* in t(9;12)(p24;p13) that is associated with both T-cell and pre-B-cell type acute lymphoid leukemia and atypical CML in transformation.^{105,106} Such fusion results in constitutive activation of JAK2, PI3 kinase, ERK, SAPK/JNK, and P38 signaling pathways and/or promotes cytokine-independent proliferation of IL-3-dependent Ba/F3 cell lines.¹⁰⁶⁻¹⁰⁸ Furthermore, in knockout mouse model experiments, STAT5 was found to be essential for *TEL/JAK2*-induced myeloproliferative disease.¹⁰⁹ *JAK2* was also the fusion partner in *PCMI-JAK2*-associated acute or chronic myeloid disorders (CMDs) associated with eosinophilia.¹¹⁰ The leukemogenic potential of mutant *JAK* has also been demonstrated in *Drosophila*.¹¹¹

Several lines of evidence have previously implicated the JAK/STAT pathway in the pathogenesis and the phenotype of erythropoietin (Epo) independence and/or hypersensitivity in MPD.¹¹²⁻¹¹⁴ In the latter regard, a recent study identified the PI3 kinase, JAK2/STAT5, and Src kinase signaling pathways as being essential for both Epo-dependent and Epo-independent erythroid differentiation.¹¹⁵ Similarly, activating mutations of Epo receptor (EpoR) have been associated with constitutive phosphorylation of JAK2 and STAT5.¹¹⁶ Furthermore, the failure to negatively regulate JAK2, in moth-eaten mice lacking SHP-1 expression, produced myeloid cell Epo hypersensitivity, whereas intact enzyme activity had been shown to down-regulate Epo-induced proliferative signals.^{117,118} These observations have broader implications because the phenomenon of abnormal growth factor response is not specific to PV, and it can also be seen in other MPD and involve other growth factors (insulin-like growth factor 1, IL-3, granulocyte-macrophage colony-stimulating factor, stem cell factor, thrombopoietin).¹¹⁹⁻¹³¹ Other JAK/STAT-implicating observations in MPD include constitutive activation of

TABLE 1. Summary of Published Literature on JAK2^{V617F} Mutational Frequencies in Myeloproliferative Disorders*

Reference	Overall (homozygous) JAK2 ^{V617F} mutational frequencies in myeloproliferative disorders			
	Polycythemia vera	Essential thrombocythemia	Myelofibrosis with myeloid metaplasia	Other myeloid disorders
Baxter et al ⁷²	97% (26%) N=73	57% (0%) N=51	50% (19%) N=16	NA
James et al ⁷³	89% (30%) N=45	43% (NA) N=21	43% (NA) N=7	NA
Kralovics et al ⁷⁴	65% (27%) N=128	23% (3%) N=93	57% (22%) N=23	CML 0% (n=9)
Levine et al ⁷⁵	74% (25%) N=164	32% (3%) N=115	35% (9%) N=46	NA
Zhao et al ⁷⁶	83% (NA) N=24	NA NA	NA NA	NA
Steensma et al ⁷⁷	NA	NA	NA	MDS 5% (n=101) CMML 3% (n=119) HES 0% (n=11) SM 25% (n=8) CNL 17% (n=6)
Jones et al ⁷⁸	81% (33%) N=72	41% (7%) N=59	43% (29%) N=35	CML 0% (n=18) CMML/UMPD 20% (n=152) HES/CEL 2% (n=134) SM 0% (n=28) CNL 33% (n=6) AML 0% (n=17)

*Mutational frequencies in different studies are not comparable because of the use of assays with variable sensitivity. AML = acute myeloid leukemia; CEL = chronic eosinophilic leukemia; CML = chronic myeloid leukemia; CMML = chronic myelomonocytic leukemia; CNL = chronic neutrophilic leukemia; HES = hypereosinophilic syndrome; MDS = myelodysplastic syndrome; NA = not applicable; SM = systemic mastocytosis; UMPD = unclassified MPD. Reproduced with permission from Tefferi A, Gilliland DG. JAK2 in myeloproliferative disorders is not just another kinase. *Cell Cycle*. August 2005;4(8). Copyright 2000-2005. Landes Bioscience. All rights reserved.

STAT3,¹³² up-regulation of negative control elements of the cell cycle (p16/p14),^{133,134} and abundance, in erythroid precursors, of antiapoptotic proteins (eg, Bcl-x_L).^{135,136} The latter molecule is down-regulated by AG490-induced inhibition of JAK2.¹³⁷ Most recently, Lnk, an adaptor protein, was identified as a modulator of signaling involving Epo-R and JAK2.¹³⁸

SUMMARY OF PUBLISHED STUDIES OF JAK2^{V617F} MUTATION AND MPD

Polycythemia vera and other classic *bcr/abl*-negative MPD (ET and MMM) are true clonal stem cell diseases with myeloid and lymphoid lineage involvement.^{9,12,14,15,139-144} However, the primary oncogenic event had remained elusive. Nonspecific cytogenetic abnormalities are found at diagnosis in approximately 50% of patients with MMM, 10% to 20% with PV, and less than 5% with ET.¹⁴⁵⁻¹⁴⁷ The most frequent karyotypic lesions include del(13q), del(20q), trisomies 8 and 9, and abnormalities of chromosome 1.^{145,146,148-150} In addition, both fluorescent in situ hybridization (FISH) and comparative genomic hybridization studies have suggested frequent abnormalities of chromo-

some 9p in both chromosomal gains^{146,151,152} and loss of heterozygosity (LOH).^{141,153} Because chromosome 9p24 houses the *JAK2* gene and because of the previously discussed collection of evidence that implicates the JAK/STAT pathway in the pathogenesis of *bcr/abl*-negative MPD, it was reasonable to pursue *JAK2* mutation studies in PV, ET, and MMM. To date, 5 separate studies on the association of JAK2^{V617F} and *bcr/abl*-negative MPD have either been published or are in press (Table 1).⁷²⁻⁷⁶ Furthermore, 2 additional studies have demonstrated the less frequent occurrence of JAK2^{V617F} in both atypical MPD and myelodysplastic syndromes (MDS) (Table 1).^{77,78} The newly identified somatic point mutation is a G-C to T-A transversion resulting in the substitution of valine by phenylalanine at codon 617 (JAK2^{V617F}). The JAK2^{V617F} occurs within the autoinhibitory JH2 domain (pseudokinase domain; exon 12 of the *JAK2* gene), and therefore the oncogenic mechanism probably involves enhanced kinase activity that resides in the JH1 domain.¹⁵⁴⁻¹⁵⁷

In one report from the United States, high-throughput sequencing of granulocyte DNA of both activation loop and autoinhibitory domains of 85 PTK in 93 patients with PV identified a recurrent *JAK2* point mutation (JAK2^{V617F}).⁷⁵

Subsequently, focused mutation screening detected the specific abnormality in 121 (74%) of 164 patients with PV (homozygous in 25%), 37 (32%) of 115 patients with ET (homozygous in 3%), and 16 (35%) of 46 patients with MMM (homozygous in 9%).⁷⁵ The somatic nature of the mutation was confirmed by buccal smear analysis that displayed wild-type allele in 301 (96%) of 313 patients. The mutation was not detected in 270 control subjects, confirming that the allele is not a common polymorphism in the general population. FISH and quantitative genomic polymerase chain reaction (PCR) analyses indicated that homozygosity for the mutant allele was a result of mitotic recombination rather than LOH. Clinical correlative studies disclosed significant associations between the presence of a mutant allele and female sex in PV (83% vs 64%) and longer duration of disease in homozygous JAK2^{V617F} patients with either PV or ET. Functional assays revealed constitutive phosphorylation of JAK2^{V617F} when expressed in 293 T cells and induction of Epo hypersensitivity in Ba/F3-EpoR cells by JAK2^{V617F}. The study also showed that HEL erythroleukemia cell lines expressed homozygous JAK2^{V617F} that is constitutively phosphorylated, as were its downstream effectors STAT5 and ERK. Both cell growth and proliferation signals were inhibited by small molecule inhibitor of JAK2.

A report from investigators at Cambridge University in the United Kingdom screened for JAK2^{V617F} in granulocytes, T cells, and bone marrow hematopoietic colonies.⁷² Mutation screening by sequence analysis revealed the presence of the mutant allele in granulocytes from 53 (73%) of 73 patients with PV (homozygous in 26%), 6 (12%) of 51 with ET (homozygous in 0%), and 7 (44%) of 16 with MMM (homozygous in 19%). The authors did not find the particular mutation in T cells from 30 patients who had the mutant allele in their granulocytes. Similarly, JAK2^{V617F} was not detected in 90 control samples. Because of issues of mixed clonality, the authors used a more sensitive allele-specific PCR that can detect a heterozygous mutation in 3% of a test sample and were able to demonstrate the presence of JAK2^{V617F} in 71 (77%) of 73 patients with PV, 29 (57%) of 51 with ET, and 8 (50%) of 16 with MMM but not in the control samples. In PV, the incidence of a homozygous defect was not influenced by the time of diagnosis. Metaphase FISH and microsatellite PCR studies showed mitotic recombination rather than LOH in patients with homozygous defects. Hematopoietic colony studies revealed the presence of JAK2^{V617F} in both erythroid and granulocyte-macrophage progenitor cells. There was also a concordance of heterozygosity between granulocytes and individual colonies as well as the uniform presence of the mutant allele in all Epo-independent colonies. Although restricted by small sample size, the presence of the mutant

allele in patients with either ET or MMM did not confer a different clinical course.

Investigators in France used a biological approach to the problem in which they tested for inhibition of endogenous erythroid colony (EEC) growth using short interfering RNA for various candidate genes including JAK2.⁷³ They demonstrated interference with EEC with JAK2-directed short interfering RNA. In subsequent DNA sequence analysis of granulocytes from patients with PV, the authors then sequenced all coding exons and intron-exon junctions of JAK2 and identified the JAK2^{V617F} mutation. Subsequently, exon 12, which houses JAK2^{V617F}, was sequenced in 45 patients with PV, 21 with ET, 7 with MMM, 35 with secondary erythrocytosis, and 15 controls. The respective mutation detection rates were 89%, 43%, 43%, 0%, and 0%, respectively. Furthermore, the mutation was not detected in their granulocytes in T cells from 3 patients who displayed the mutant allele as well as CD34-derived erythroblasts and platelets. Similar to the observation from the 2 aforementioned studies, homozygosity was shown to be secondary to duplication of the mutant allele rather than LOH. Additional functional studies showed Epo-independent constitutive STAT activity in JAK2- and STAT5-deficient γ -2A cells conferred by transfections with JAK2^{V617F}. Cotransfection studies with both mutant and wild-type allele suggested that the latter might function as a negative dominant allele. Similarly, transduction with the wild-type allele neutralized JAK2^{V617F}-mediated growth factor independence by murine Ba/F3 cell lines. Incidentally, the latter and other cell lines attained Epo-hypersensitivity when transfected by JAK2^{V617F}. Finally, the authors were able to induce in vivo erythrocytosis in mice after transplanting them with murine bone marrow transduced with a retrovirus containing JAK2^{V617F}.

Investigators from Switzerland and Italy collaborated to analyze patients using a genetic approach that involved 244 patients with MPD, including 128 with PV, 93 with ET, and 23 with MMM.⁷⁴ This group had observed 9pLOH in MPD patients using genome-wide screening for LOH; 9pLOH was observed in 34% of patients with PV, 22% with MMM, and 3% with ET. The LOH region in all instances included the JAK2^{V617F} locus. DNA sequence analysis subsequently demonstrated that 51 patients with LOH carried the mutant allele in their granulocytes; 8 were heterozygous. Furthermore, all patients with 9pLOH were shown to have 2 copies of 9p, and both maternal and paternal chromosomes were affected equally. On the other hand, granulocyte mutation screening studies in all study patients, regardless of the presence or absence of 9pLOH, disclosed the presence of JAK2^{V617F} in 83 (65%) of 128 patients with PV (homozygous in 27%), 21 (23%) of 93 with ET (homozygous in 3%), 13 (57%) of 23 with MMM

(homozygous in 22%), 0 of 11 with secondary erythrocytosis, 0 of 9 with CML, and 0 of 71 healthy controls. Homozygous abnormality was not seen in patients without 9pLOH. Concomitantly tested "normal" tissue from 89 MPD patients was negative for the specific mutation. Interestingly, hair follicle cells from 2 patients with a heterozygous mutation pattern in their buccal mucosa cells were negative for the mutation, suggesting contamination with blood in the latter instance. In general, the incidence of EEC growth was significantly higher in patients with the JAK2^{V617F} mutation. The authors were also able to demonstrate JAK2^{V617F}-mediated growth factor hypersensitivity and enhanced STAT5 phosphorylation in Ba/F3 cell lines. Clinically, disease duration was the shortest in MPD patients with wild-type allele and longest in those with homozygous mutations. In addition, that particular study suggested a higher incidence of myelofibrosis, hemorrhage, and thrombosis in patients with the mutations vs those without.

Investigators from Vanderbilt University used a candidate gene approach to screen for mutations in PV patients.⁷⁶ Genes encoding a number of PKs and PPs, including JAK, Abl, Src, PDGFR, SHP-1, SHP-2, PTP-MEG-1, and PTP-MEG-2, were sequenced and ultimately resulted in identification of the JAK2^{V617F} mutation. The investigators detected the mutation in complementary DNA prepared from RNA extracted from either peripheral blood mononuclear cells or purified erythroid colony-forming cells from 20 (83%) of 24 patients with PV but in none of 12 controls. The authors also found that complementary DNA as opposed to genomic DNA mutation analysis was more sensitive in detecting the mutant allele, a phenomenon attributed to a growth advantage by cells carrying the mutant allele. Using HeLa cell lines, the study demonstrated a higher degree of substrate phosphorylation and JAK2 autophosphorylation associated with JAK2^{V617F} compared to the wild-type enzyme.

Two additional studies have analyzed the occurrence of JAK2^{V617F} in both atypical MPD and MDS.^{77,78} In one of these studies,⁷⁷ mutation screening for JAK2^{V617F} was performed in 245 patients with either atypical MPD or MDS. The study revealed the occurrence of the specific mutation in 3 (3%) of 119 patients with CMML, 5 (5%) of 101 with MDS, 2 (25%) of 8 with SM, 1 (17%) of 6 with CNL, and 0 of 11 patients with HES. Among the positive cases, 1 patient with MDS and 1 with CNL were homozygous for JAK2^{V617F}. In the second study involving a total of 679 subjects,⁷⁸ JAK2^{V617F} was detected in 13 (25%) of 53 patients with UMPD, 17 (17%) of 99 with either CMML or CML-like UMPD (2 of 6 patients with CNL expressed the mutation), 2 (2%) of 127 with HES, 0 of 7 with *FIP1L1*-PDGFRA⁺ eosinophilic/mast cell disorder, 0 of 28 with

SM, 0 of 17 with acute myeloid leukemia (AML), 0 of 18 with CML, 0 of 4 with secondary erythrocytosis, and 0 of 160 healthy controls. The latter study also included 72 patients with PV, 59 with ET, and 35 with MMM with the JAK2^{V617F} mutational frequencies of 81%, 41%, and 43%, respectively.⁷⁸ In both studies, the JAK2 mutation did not occur in otherwise molecularly defined cases, including *c-kit* D816V-associated SM and MPD associated with tyrosine kinase fusions.^{77,78}

DISCUSSION AND CLINICAL IMPLICATIONS

The data from the aforementioned 7 studies clearly establish a close link between JAK2^{V617F} and classic *bcr/abl*-negative MPD. However, the association has been neither invariable (the mutation is reported to be absent in 3%-35% of patients with PV and in approximately 50% of those with either ET or MMM)⁷²⁻⁷⁶ nor specific (JAK2^{V617F} also occurs in a spectrum of atypical MPD and MDS).^{77,78} Furthermore, the absence of the mutant allele in T cells^{72,73} suggests that JAK2^{V617F} might be a myeloid lineage-specific event that contrasts with the perception that MPD are clonal stem cell diseases with myeloid and lymphoid lineage involvement.¹⁵ Such issues present a pathogenetically challenging scenario that is further compounded by the possibility that wild-type JAK2 might⁷³ or might not⁷⁵ function as a negative dominant allele. Nevertheless, there is clear evidence that JAK2^{V617F} results in constitutive JAK2 activity and enhanced JAK2-STAT signaling.⁷³⁻⁷⁶ Furthermore, some of the aforementioned studies have demonstrated JAK2^{V617F}-induced cytokine hypersensitivity in cell lines as well as erythrocytosis in mice, both of which are reminiscent of the PV phenotype in humans.⁷³⁻⁷⁵ Therefore, it is plausible that JAK2^{V617F} has definite biological relevance regardless of whether or not it represents a disease-causing mutation, and its potential as a therapeutic target is suggested by the in vitro demonstration of inhibition of JAK2^{V617F}-induced cell growth and proliferation by small molecule JAK2 inhibitors.⁷⁵

JAK2 contains JH1 through JH7 domains. The JAK2^{V617F} occurs within the ATP-binding region of the autoinhibitory JH2 domain (pseudokinase domain), and it is believed to produce conformational changes that interfere with the normal regulatory interaction of the JH2 domain with the functionally active JH1 domain.^{76,154-157} On the other hand, construction of a JAK2 JH2 homology domain suggested mutation topology that is solvent exposed, and the corresponding amino acid substitution might not affect the fold of the domain.⁷⁵ Regardless, more work is needed to elucidate the mechanism of JAK2^{V617F}-induced cellular transformation in MPD. Similarly, additional laboratory and clinical investigations are needed to explain the imperfect

TABLE 2. Semimolecular Classification of Chronic Myeloid Disorders*

1. Myelodysplastic syndrome	
2. Myeloproliferative disorders	
Classic	
I. Molecularly defined	
1. Chronic myeloid leukemia (<i>bcr/abl</i> ⁺)	
II. Clinicopathologically assigned (<i>bcr/abl</i> ⁻ and frequently associated with JAK2 ^{V617F} mutation)	
1. Essential thrombocythemia	
2. Polycythemia vera	
3. Myelofibrosis with myeloid metaplasia	
Atypical	
I. Molecularly defined	
1. <i>PDGFRA</i> -rearranged eosinophilic/mast cell disorders (eg, <i>FIP1L1-PDGFR</i>)	
2. <i>PDGFRB</i> -rearranged eosinophilic disorders (eg, <i>TEL/ETV6-PDGFRB</i>)	
3. Systemic mastocytosis associated with <i>c-kit</i> mutation (eg, <i>c-kit</i> ^{D816V})	
4. 8p11 myeloproliferative syndrome (eg, <i>ZNF198/FIM/RAMP-FGFR1</i>)	
II. Clinicopathologically assigned (infrequently associated with JAK2 ^{V617F} mutation)	
1. Chronic neutrophilic leukemia	
2. Chronic eosinophilic leukemia, molecularly not defined	
3. Hypereosinophilic syndrome	
4. Chronic basophilic leukemia	
5. Chronic myelomonocytic leukemia	
6. Juvenile myelomonocytic leukemia (associated with recurrent mutations of RAS signaling pathway molecules including <i>PTPN11</i> and <i>NF1</i>)	
7. Systemic mastocytosis, molecularly not defined	
8. Unclassified myeloproliferative disorder	

*JAK2 = Janus kinase 2; PDGFR = platelet-derived growth factor receptor. Reprinted with permission from Tefferi and Gilliland.⁵

JAK2^{V617F} genotype-phenotype association: the mutant allele has been detected in diverse clinicopathologic entities, but not every patient with PV carries the mutation. It is possible that JAK2^{V617F} is responsible for some portions of but not the complete phenotype in MPD, and in this capacity, it is not clear at the present time if JAK2^{V617F} represents a subclone or is part of the disease-initiating event. It is expected that future studies of the pathogenesis in JAK2^{V617F}-negative MPD will focus on possible abnormalities of other JAK/STAT pathway molecules or their regulatory elements. From a therapeutic standpoint, one can envision targeting JAK2 directly or its downstream effectors. In this regard, several lead compounds, such as AG-490, have already been identified, and it is only a matter of time before clinical trials involving small molecule JAK2 inhibitors are developed.¹⁵⁸⁻¹⁶⁰

The current classification of myeloid disorders is based on a constellation of clinical, bone marrow histological, cytochemical, chromosomal, and immunophenotypic features.¹⁶¹ The World Health Organization (WHO) system for classification of myeloid neoplasms separates AML from CMD on the basis of the presence or absence, respectively, of AML-defining morphologic or cytogenetic features.³

According to WHO, the CMD are further classified into 3 broad categories—MDS, MPD, and MDS/MPD—as well as SM.³ Again, distinction among these disorders is based primarily on bone marrow histological parameters. With the recent discovery of the association between JAK2^{V617F} and *bcr/abl*-negative MPD as well as between a subset of eosinophilic disorders and rearrangement *PDGFRA*,²⁸ it is time to start the process of a transition from a largely pathologic classification of CMD into a semimolecular system that recognizes molecularly defined categories (Table 2).⁵

From a clinical diagnostics point of view, the demonstrated absence of JAK2^{V617F} in either normal controls or patients with secondary erythrocytosis^{73,74,78} offers an opportunity to incorporate mutation screening for JAK2^{V617F} into current diagnostic algorithms for both PV and ET (Figures 1 and 2).^{162,163} In routine clinical practice, the diagnosis of PV is suspected in the presence of either an “increased” venous hematocrit or a characteristic clinical feature of PV (eg, a thrombotic event, aquagenic pruritus, splenomegaly, erythromelalgia or other symptoms of acral ischemia, leukocytosis, thrombocytosis, or microcytosis). The diagnosis of ET is entertained after ruling out the possibility of reactive thrombocytosis.

When PV is clinically suspected, the first-line set of tests should include serum Epo level and leukocyte alkaline phosphatase score and not red cell mass (RCM) measurement (Figure 1). This is because RCM measurement does not distinguish between PV and secondary polycythemia, whereas relative polycythemia is readily appreciated from patient history and review of previous laboratory records. The suboptimal value of RCM measurement for the diagnosis of PV was recently demonstrated in a systematic study,¹⁶⁴ and the test has fallen out of favor in many countries including Sweden where more than 80% of hematologists seldom or never use RCM measurement for the diagnosis of PV.¹⁶⁵

Once the results of the serum Epo level are available, further evaluation for PV is warranted only if the serum Epo level is low or normal because the specific diagnosis is highly unlikely in the presence of an increased serum Epo level (Figure 1).^{166,167} In the presence of a low serum Epo level, we recommend proceeding directly to bone marrow examination because of the high likelihood of the diagnosis (of note, however, there are rare cases of congenital polycythemia that are associated with a low serum Epo level).¹⁶⁸ In contrast, in the presence of a normal serum Epo level, bone marrow examination is recommended only if either the leukocyte alkaline phosphatase score is elevated or the patient manifests one of the aforementioned characteristic features of PV. Similarly, in evaluating thrombocytosis that is not believed to be reactive, we recommend bone marrow examination not only to confirm the diagnosis

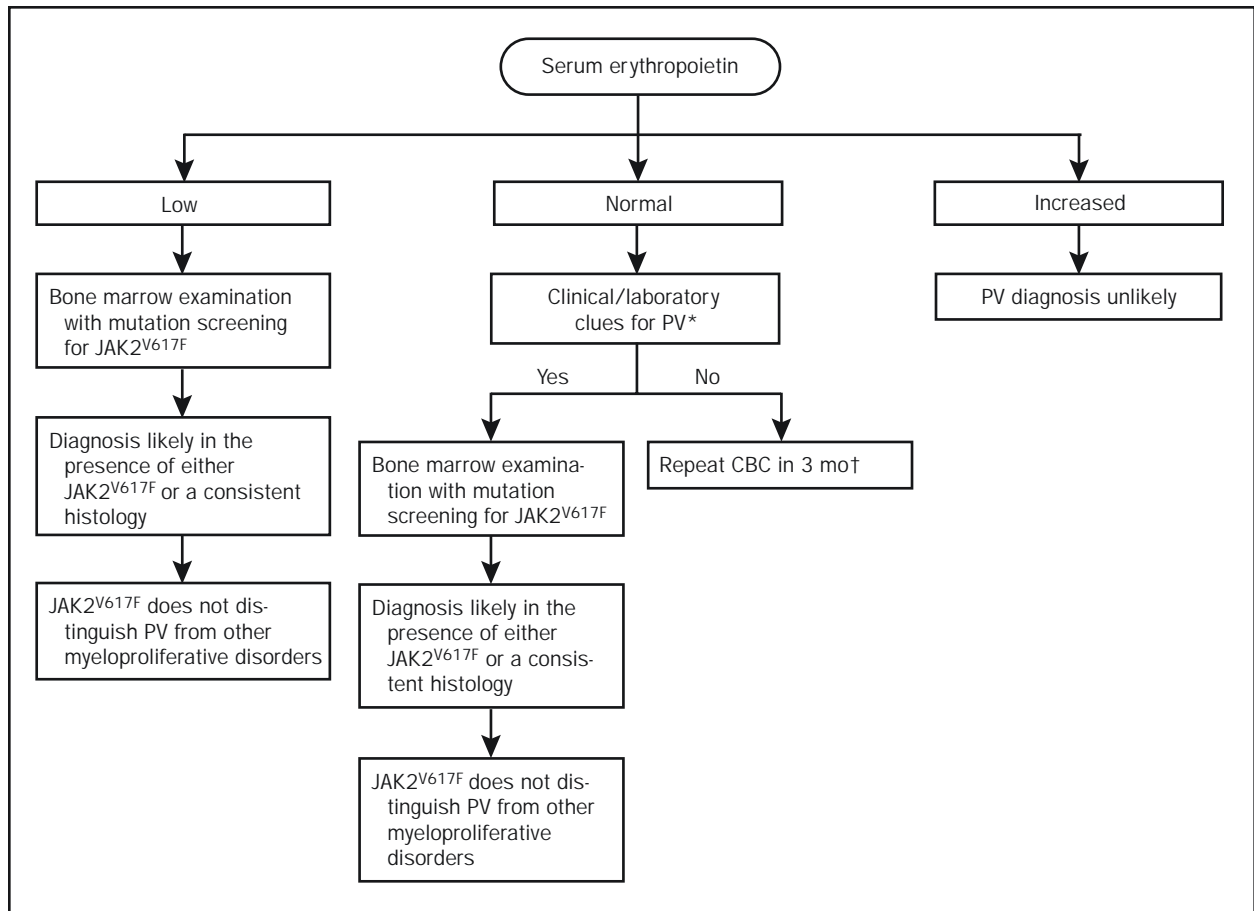


FIGURE 1. Diagnostic algorithm for polycythemia vera (PV).

*Clinical clues for PV include splenomegaly, thrombosis, aquagenic pruritus, and erythromelalgia. Laboratory clues for PV include thrombocytosis, leukocytosis, and increased leukocyte alkaline phosphatase score. Janus kinase 2 (JAK2) screening is to detect the V617F mutation that occurs in most patients with PV. BM = bone marrow; CBC = complete blood cell count; MPD = myeloproliferative disorders.

†Alternatively, one can consider mutation screening for JAK2^{V617F} to help decide necessity of BM examination.

(clonal as opposed to reactive thrombocythemia is associated with abnormal megakaryocyte morphology and clustering) but also to differentiate ET from other causes of clonal thrombocythemia.

In the hands of an experienced clinical hematopathologist, the histological changes in the bone marrow should help confirm the diagnosis of an MPD, that includes both PV and ET, as opposed to reactive myeloproliferation that includes secondary polycythemia.¹⁶⁹ Characteristic bone marrow histological features of MPD include both numerical and morphologic abnormalities of megakaryocytes including cluster formation, increased reticulin fibrosis, and bone marrow hypercellularity.¹⁷⁰ However, the utility of bone marrow histology in the diagnosis of PV is not universally appreciated; thus, we believe that the concomitant information from JAK2 mutation screening is complemen-

tary in that regard (Figures 1 and 2). On the other hand, peripheral blood mutation screening cannot currently substitute for bone marrow histology because JAK2^{V617F} is not always detected in patients with PV and is absent in almost half of patients with ET.^{74,75,78} However, in the asymptomatic patient with a normal serum Epo level who displays no evidence of a characteristic feature of PV, one can consider using the information from peripheral blood JAK2^{V617F} screening in deciding whether to pursue bone marrow biopsy.

One can argue about the possibility of bypassing bone marrow examination if the peripheral blood mutation screening reveals the presence of JAK2^{V617F}. However, such an action is currently considered premature and restricts one from obtaining useful baseline information from bone marrow histology and cytogenetic analysis. First, the

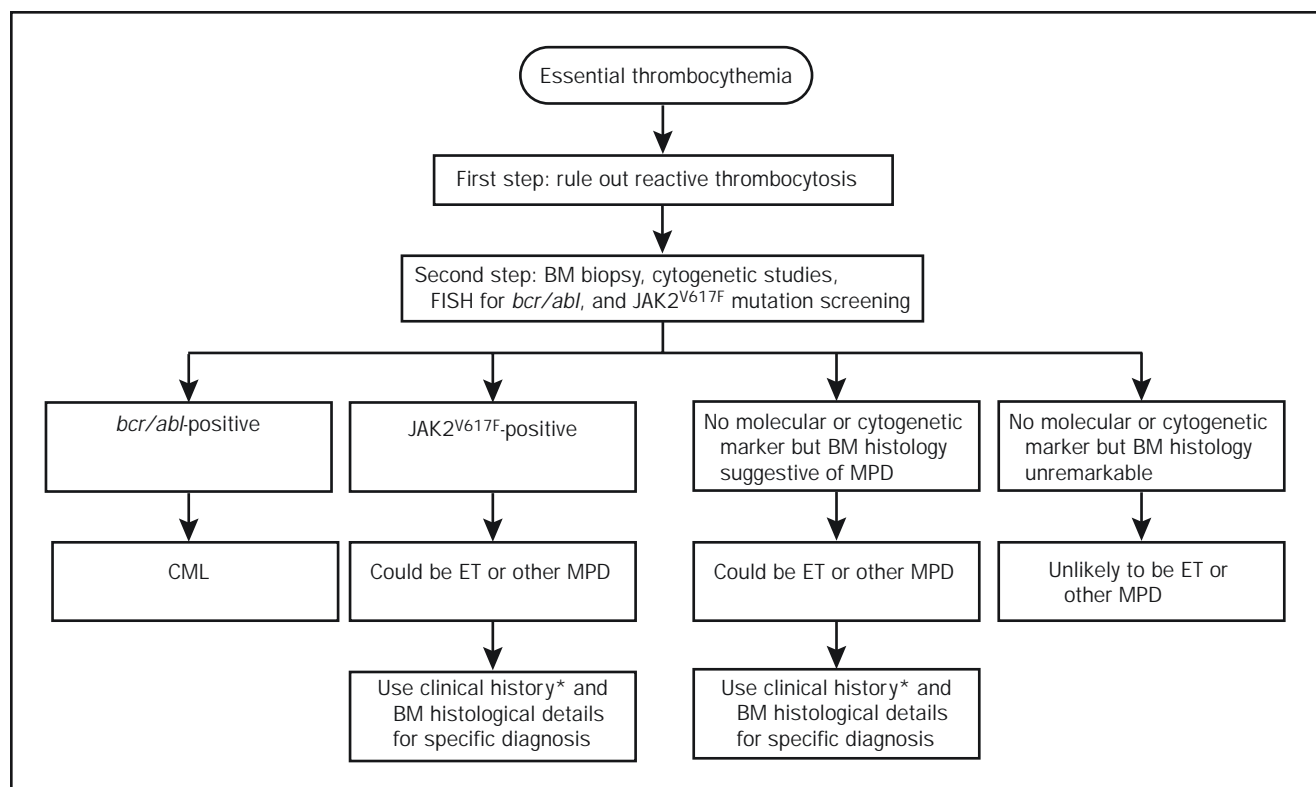


FIGURE 2. Diagnostic algorithm for essential thrombocythemia (ET) that incorporates JAK2^{V617F} mutation screening.

*In addition to clinical history, laboratory tests that are helpful in distinguishing reactive thrombocytosis from ET include serum ferritin, peripheral blood smear, and C-reactive protein. BM = bone marrow; CML = chronic myeloid leukemia; FISH = fluorescent in situ hybridization; JAK2 = Janus kinase 2; MDS = myelodysplastic syndrome; MMM = myelofibrosis with myeloid metaplasia; MPD = myeloproliferative disorder; PV = polycythemia vera.

currently presumed 100% specificity of JAK2^{V617F} for clonal myeloid disorders vs a reactive process should be validated in a larger group of patients with either secondary polycythemia or reactive thrombocytosis. Second, bone marrow histological examination and cytogenetic analysis at diagnosis are performed not only to confirm the presence of a clonal disorder but also to provide baseline information that is used during assessment of clonal evolution at a future date.¹⁷¹ Furthermore, cytogenetic analysis might identify molecularly relevant markers that could provide additional pathogenetic insight and facilitate the differential diagnosis among the subcategories of CMD.

Finally, the remarkably close association between PV and JAK2^{V617F} has further undermined the already limited value of RCM measurement¹⁶⁴ and specialized biological assays for the diagnosis of PV.¹⁶² The latter include EEC formation,¹⁷² megakaryocyte thrombopoietin receptor (Mpl) expression,^{173,174} neutrophil *PRV-1* transcription,¹⁷⁵ and platelet-rich plasma serotonin level.¹⁷⁶ This is welcome news because each of these assays entails a certain level of

expertise that is not widely available for routine clinical use. Obviously, for routine clinical practice, feasibility of such a process requires availability of laboratory screening tests for JAK2^{V617F}. At present, both our institutions offer the particular test on a research basis.

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