The Journal of Molecular Diagnostics, Vol. 13, No. 5, September 2011

DOI: 10.1016/j.jmoldx.2011.05.007

Review

Uses and Abuses of *JAK2* and *MPL* Mutation Tests in Myeloproliferative Neoplasms

A Paper from the 2010 William Beaumont Hospital Symposium on Molecular Pathology

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JAK2*V617F is sufficiently prevalent in *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs) to be useful as a clonal marker. *JAK2*V617F mutation screening is indicated for the evaluation of erythrocytosis, thrombocytosis, splanchnic vein thrombosis, and otherwise unexplained *BCR-ABL1*-negative granulocytosis. However, the mutation does not provide additional value in the presence of unequivocal morphologic diagnosis, and its presence does not necessarily distinguish one MPN from another or provide useful prognostic information. In general, quantitative cell-based *JAK2*V617F mutation assays are preferred because the additional information obtained on mutant allele burden enhances diagnostic certainty and facilitates monitoring of response to treatment. *JAK2* exon 12 mutation screening is indicated only in the presence of *JAK2*V617F-negative erythrocytosis that is associated with a subnormal serum erythropoietin level. *MPL* mutations are neither frequent nor specific enough to warrant their routine use for MPN diagnosis, but they may be useful in resolving specific diagnostic problems. The practice of *en bloc* screening for *JAK2*V617F, *JAK2* exon 12, and *MPL* mutations is scientifically irrational and economically irresponsible. (*J Mol Diagn 2011, 13:461–466; DOI: 10.1016/j.jmoldx.2011.05.007*)

Morphology is the cornerstone of current diagnosis and classification in myeloid malignancies.[1] Cytochemical, immunophenotypic, cytogenetic, and molecular data enhance diagnostic accuracy and form the basis for the World Health Organization classification of myeloid malignancies into five main categories[2]: acute myeloid leukemia, myelodysplastic syndromes (MDSs), myeloproliferative neoplasms (MPNs), MDS/MPN overlap, and platelet-derived growth factor receptor gene or fibroblast growth factor receptor 1 gene rearranged myeloid/lymphoid neoplasms associated with eosinophilia. The World Health Organization MPN category includes eight subcategories: chronic myelogenous leukemia, polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), mastocytosis, chronic eosinophilic leukemia not otherwise specified, chronic neutrophilic leukemia, and MPN unclassifiable.[1] Among these subcategories, the first four (ie, chronic myelogenous leukemia, PV, ET, and PMF) are currently referred to as "classic" MPNs, because they were included in the original description of myeloproliferative disorders by William Dameshek.[3] In general, current evidence supports consideration of all myeloid malignancies, including MPN, as clonal stem cell diseases.

JAK2 and *MPL* mutations occur across the spectrum of myeloid malignancies, including MPN, MDS, MDS/MPN, and acute myeloid leukemia (Table 1).[4–7] These mutations are most prevalent in the *BCR-ABL1*-negative classic MPN (ie, PV, ET, and PMF), which are morphologically characterized by the absence of both cellular dysplasia

Accepted for publication May 23, 2011.

This article is partly based on material presented by the authors at the William Beaumont Hospital 19th Annual Symposium on Molecular Pathology: Clinical Applications of Genomic Medicine, which took place September 22-23, 2010, in Troy, MI.

CME Disclosure: None of the authors disclosed any relevant financial relationships.

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Table 1. Currently Known Mutations in *BCR-ABL1*-Negative Myeloproliferative Neoplasms

Mutations	Chromosome location	Mutational frequency, %
JAK2	9p24	
PV		~96[4]
ET		~55[4]
PMF		~65[4]
BP-MPN		~50[4]
JAK2 exon 12 mutation [4]	9p24	
PV		~3[4]
MPL	1p34	
ET		~3[4]
PMF		~10[4]
BP-MPN		~5[4]
LNK	12q24.12	
PV		Rare[20,21]
ET		Rare[19,20]
PMF		Rare[19,20]
BP-MPN		~10[20]
TET2	4q24	
PV		~16[4]
ET		~5[4]
PMF		~17[4]
BP-MPN		~17[4]
ASXL1	20q11.1	
ET		~3[32]
PMF		~13
BP-MPN		~18
IDH1/IDH2	2q33.3/15q26.1	
PV		~2[24]
ET		~1[24]
PMF		~4[24]
BP-MPN		~20[24]
EZH2	7q36.1	
PV		~3[34]
PMF		~7
DNMT3A	2p23	
PV		~7[35]
PMF		~7[35,36]
BP-MPN		~14[35,36]
CBL	11q23.3	
PV		Rare[22]
ET		Rare[22]
MF		~6[22]
IKZF1	7p12	
CP-MPN		Rare[27]
BP-MPN		~19[27]

BP-MPN, blast-phase MPN; CP-MPN, chronic phase MPN; MF, both PMF and post-ET/PV myelofibrosis.

and monocytosis and the presence of abnormal megakaryocytes that are increased in number and often found in clusters. Megakaryocyte morphology and degree of trilineage proliferation differ among the three *BCR-ABL1*-negative classic MPNs. Megakaryocytes are large, hyperlobulated, and mature appearing in ET[8]; immature appearing with hyperchromatic and irregularly folded bulky nuclei in PMF[8]; and pleomorphic without maturation defects in PV.[9,10] Megakaryocyte changes in *BCR-ABL1*-negative MPN are accompanied by left-shifted granulocyte proliferation in PMF, trilineage proliferation in PV, and otherwise normal-appearing bone marrow in ET. Overt bone marrow fibrosis is absent in prefibrotic PMF, which is otherwise characterized by the aforementioned PMF-associated changes in megakaryocyte morphology and increased granulocyte proliferation.[11] Controversy is ongoing about the utility of morphology alone to distinguish ET from early PMF[12]; however, this is irrelevant because clinical pathologists never base their diagnostic impressions on morphology alone, and they also consider clinical, cytogenetic, and molecular information.[1,11]

Clinically, PV and ET are characterized by erythrocytosis and thrombocytosis, respectively, and leukocytosis, splenomegaly, thrombohemorrhagic complications, vasomotor disturbances, pruritus, and a small risk of disease progression into acute leukemia or myelofibrosis.[13] PMF is characterized by anemia, splenomegaly, extramedullary hematopoiesis, constitutional symptoms, and a higher risk of leukemic progression.[14] Median survival exceeds 15 years in both ET and PV, but it is significantly shorter in PMF. The goal of therapy in PV and ET is to prevent thrombotic complications. Low-dose aspirin is the cornerstone of therapy in both PV and ET.[15] In addition, phlebotomy is required in PV and hydroxyurea therapy in high-risk disease (history of thrombosis or age >60 years).[13] PMF is managed according to risk category from the Dynamic International Prognostic Scoring System.[16] Patients with low-risk or intermediate-1 PMF are managed by observation alone or conventional drug therapy, whereas allogeneic stem cell transplantation or experimental drug therapy might be necessary for intermediate-2 or high-risk disease.

Overview of Mutations Associated with BCR-ABL1-*Negative MPNs*

The disease-initiating mutation(s) in *BCR-ABL1*-negative MPN is unknown. However, *JAK2*V617F is present in most patients with PV, ET, or PMF, and a minority of patients with these diseases also harbor *JAK2* exon 12, *MPL*, *LNK*, *CBL*, *TET2*, *ASXL1*, *IDH*, *IKZF1*, *EZH2*, or *DNMT3A* mutations (Table 1).[4] These mutations are currently thought to represent secondary events and to lack both disease specificity and mutual exclusivity. Some patients carry more than one mutation, and clonal hierarchy in such instances appears to be unpredictable.[17]

JAK2 (Janus kinase 2) maps to chromosome 9p24. *JAK2*V617F is located on exon 14 and occurs in ~96% of patients with PV, 55% with ET, and 65% with PMF.[4] *JAK2*V617F contributes to abnormal myeloproliferation in MPN, whereas such effect is erythroid lineage weighted with *JAK2* exon 12 mutation.[4] *MPL* (myeloproliferative leukemia virus oncogene) maps to chromosome 1p34, and *MPL* mutations usually involve exon 10 and contribute to primarily megakaryocytic myeloproliferation.[4] *MPL* mutational frequencies are estimated at 3% in ET and 10% in PMF.[4] *LNK* (as in Links) maps to chromosome 12q24.12 and encodes for a membrane-bound adaptor protein that negatively regulates JAK2 signaling.[18] *LNK* mutations usually involve exon 2, are inactivating, and occur in ~10% of patients with blast-phase MPN, whereas they are infrequent in chronic-phase disease.[19–21]

CBL (Casitas B-lineage lymphoma proto-oncogene) maps to chromosome 11q23.3, and its mutations involve exons 8 and 9.[22] CBL is an E3-ubiquitin ligase that marks mutant kinases for degradation.[23] *CBL* mutations are rare

Table 2. Clinical Indications for Screening *JAK2* and *MPL* Mutations

Mutation	Screening appropriate	Not indicated
*JAK2*V617F	Erythrocytosis	For purposes of MPN prognostication
	Thrombocytosis	To differentiate one MPN from another
	Bone marrow fibrosis	Testing both blood and bone marrow
	BCR-ABL1-negative granulocytosis	
	Unexplained monocytosis	
	Unexplained splenomegaly	
	Aquagenic pruritus	
	Splanchnic vein thrombosis	
	Testing either blood or bone marrow	
JAK2 exon 12 mutation	*JAK2*V617F-negative erythrocytosis and low Epo	Before *JAK2*V617F screening
	Suspected *JAK2*V617F-negative post-PV MF	In the presence of *JAK2*V617F
		For diagnosis of ET or PMF
MPL mutation	Thrombocytosis and morphologically equivocal for ET	For diagnosis of PV Morphologically confirmed ET or PMF
	Marrow fibrosis and morphologically equivocal for PMF	*JAK2*V617F positive

Epo, serum erythropoietin level; MF, myelofibrosis.

in PV and ET but are reported to occur in ~6% of patients in myelofibrosis.[22] *IDH1* and *IDH2* (isocitrate dehydrogenase) map to chromosomes 2q33.3 and 15q26.1, respectively, and their mutations involve exon 4.[24] *IDH* mutations induce formation of 2-hydroxyglutarate, which is thought to be oncogenic.[25] *IDH* mutational frequencies are estimated at 2% in PV, 1% in ET, 4% in PMF, and 20% in blast-phase MPN.[24] IKAROS family zinc finger 1 (*IKZF1*) maps to chromosome 7p12 and is thought to function as a transcription regulator and tumor suppressor.[26] *IKZF1* mutations are rare in chronic-phase MPN but might be detected in ~19% of patients with blast-phase MPN.[27]

TET oncogene family member 2 (*TET2*) maps to chromosome 4q24. *TET2* mutations occur across several of the gene's 12 exons.[4] TET proteins catalyze conversion of 5-methylcytosine to 5-hydroxymethylcytosine,[28,29] and, accordingly, *TET2* mutations are thought to contribute to epigenetic dysregulation of transcription. *TET2* mutational frequencies are estimated at 16% in PV, 5% in ET, 17% in PMF, and 17% in blast-phase MPN.[4] Additional sex combs-like 1 (*ASXL1*) maps to chromosome 20q11.1, and *ASXL1* mutations involve exon 12. Wild-type ASXL1 is needed for normal hematopoiesis[30] and might be involved in transcriptional repression.[31] *ASXL1* mutations

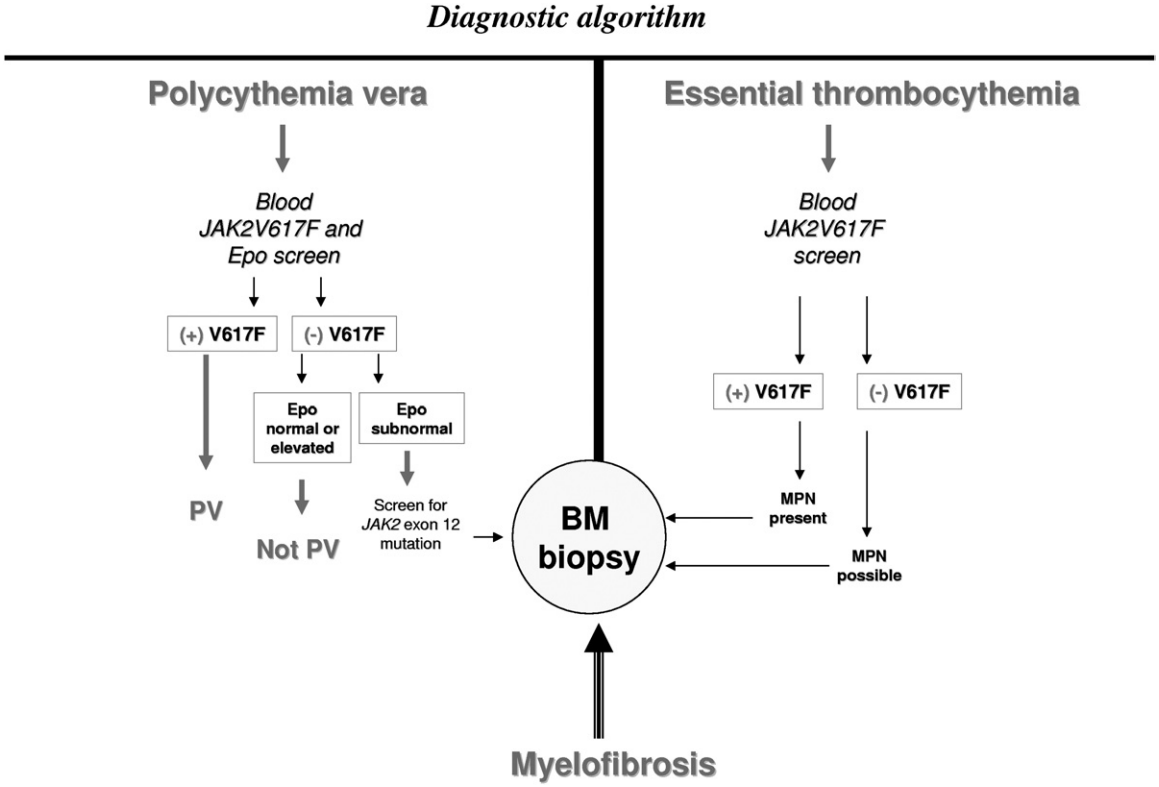

Figure 1. A contemporary diagnostic algorithm for MPNs, including PV, ET, and PMF. Epo, erythropoietin; V617F, *JAK2V617F*; BM, bone marrow.

are rare in PV or ET[32] but were reported at ~13% in PMF and 18% in blast-phase MPN.[33] Enhancer of zeste homolog 2 (*EZH2*) maps to chromosome 7q36.1, and *EZH2* mutations involve exons 10, 18, and 20.[34] Wild-type EZH2 is part of a histone methyltransferase and might function both as a tumor suppressor and an oncogene.[34] *EZH2* mutational frequencies are reported at ~3% in PV[34] and 7% in PMF.[33]

Most recently, DNA methyltransferase 3a (*DNMT3A*) mutations were reported in patients with acute myeloid leukemia, MDS, and MPN; mutational frequencies in the latter were reported at ~7% for PV, 6% for PMF, and 14% for blast-phase MPN.[35] Another study of 46 patients with PMF, 22 with post-ET/PV myelofibrosis, and 11 with blast-phase MPN reported corresponding *DNMT3A* mutational frequencies of 7%, 0%, and 0%, respectively.[36] All 13 *DNMT3A* mutations reported in those two studies were heterozygous, and the most frequent mutations affected amino acid R882. *DNMT3A* mutations in those two MPN studies were documented to occur in the presence or absence of *JAK2*, *IDH*, *ASXL1*, or *TET2* mutations.

In summary, activating *JAK2* and *MPL* mutations and *LNK* loss-of-function result in constitutive JAK-STAT activation and induce MPN-like disease in mice.[19,37–39] *TET2*, *ASXL1*, and *EZH2* mutations might contribute to epigenetic dysregulation of transcription.[28,29,31]

JAK2 *and* MPL *Mutation Screening in Routine Clinical Practice*

The decision to screen for *JAK2* or *MPL* mutations, in the context of routine clinical practice, is based on a number of facts and possibilities:

- *JAK2*V617F is present in most patients with PV, ET, or PMF but lacks disease specificity or prognostic value.[40]
- *JAK2*V617F also occurs in other myeloid malignancies and is therefore useful as a clonal marker in the evaluation of otherwise unexplained *BCR-ABL1*-negative granulocytosis or monocytosis.[41]
- *JAK2*V617F, but not *JAK2* exon 12 or *MPL* mutations,[42] has been shown to identify occult MPN in patients with splanchnic vein thrombosis,[43] but the yield of mutation screening in the evaluation of non-splanchnic thrombosis is very low.[44,45]
- *JAK2* mutations are present in virtually all patients with PV; therefore their screening is reasonable in the presence of characteristic symptoms of PV such as aquagenic pruritus or unexplained splenomegaly, even if the complete blood count picture is not suggestive of PV.[46]
- *JAK2*V617F studies are concordant between peripheral blood and bone marrow; therefore, patients may be screened with either specimen type, but analyzing both blood and marrow is unnecessary.[47,48]
- *JAK2* exon 12 mutations are rare in ET or PMF, and their occurrence in PV is almost always associated with the absence of *JAK2*V617F and the presence of a subnormal serum erythropoietin level.[46,49]
- The incidence of *MPL* mutations in MPN are too low (see above) to warrant their routine use in MPN diagnosis, except for clarification of equivocal morphology in the diagnosis of ET or PMF.[7]
- *JAK2* and *MPL* mutations do not occur in healthy subjects or in those with non-clonal causes of myeloproliferation.[7,50,51]

Taking the above-mentioned factors into consideration, we have outlined the clinical scenarios in which *JAK2* or *MPL* mutations are indicated (Table 2) and also provided a diagnostic algorithm for PV, ET, and PMF (Figure 1). At present, it is not essential to use quantitative assays for mutation screening, although we prefer to use cell-based quantitative assays because diagnosis certainty is enhanced in the presence of >1% mutant allele burden.[50] In addition, a ≥50% *JAK2*V617F allele burden suggests the presence of homozygous mutations, which are atypical for ET,[40,52] but a lower mutant allele burden does not necessarily exclude the presence of homozygously mutated cells and is, therefore, of limited value in distinguishing ET from PV or PMF. Cell-based quantitative assays are also useful to monitor treatment response, including assessment of minimal residual disease after allogenic stem cell transplantation.[53–56]

Recent studies in PV have suggested the association of higher mutant allele burden with a higher risk of fibrotic transformation[57] and in PMF a lower mutant allele burden with inferior survival.[58,59] However, the lack of assay standardization across different laboratories undermines the practical translation of such observations, at present. In general, mutation screening outcome does not appear to be influenced by or whether peripheral blood or bone marrow is used as the source of test samples.[47] Finally, it is important to note that most commercial assays use ≥1% sensitivity level to minimize false-positive test results; however, positive signals in the 0.01% to 1% range should not be discarded, especially in the context of the appropriate clinical scenario.

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