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Medical Policy Manual

Topic: JAK2 and MPL Mutation Analysis in Myeloproliferative Neoplasms

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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION[1]

Myeloproliferative neoplasms (MPNs)

Myeloproliferative neoplasms (MPNs) are a category of uncommon overlapping blood diseases characterized by the production of one or more blood cells --chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), systemic mastocytosis, chronic eosinophilic leukemia, and others. A common finding in many of the MPNs is clonality, and a central pathogenic feature is the presence of a mutated version of the tyrosine kinase enzyme, such that it is abnormally constitutively activated. The paradigm for use of this information to revolutionize patient management is CML. A unique chromosomal change (the Philadelphia chromosome) and an accompanying unique gene rearrangement (BCR-ABL) resulting in a continuously activated tyrosine kinase enzyme were identified. These led to discovery of a targeted tyrosine kinase inhibitor drug treatment (imatinib) that produces long-lasting remissions.

Diagnosis and monitoring of patients with Philadelphia chromosome negative MPNs have been challenging because many of the laboratory and clinical features of the classic forms of these diseases – PV, ET, and PMF -- can be mimicked by other conditions such as reactive or secondary erythrocytosis, thrombocytosis or myeloid fibrosis. In addition, these entities can be difficult to distinguish on morphological bone marrow exam and diagnosis can be complicated by changing disease patterns: PV and ET can evolve into PMF or undergo leukemic transformations. World Health Organization (WHO) criteria were published as a benchmark for diagnosis in 2001. These have been challenging to use

because they involve complex diagnostic algorithms, rely on morphological assessment of uncertain consistency, and require tests such as endogenous erythroid colony formation that are not well standardized or widely available.

Mutations in the gene coding for the Janus kinase 2 (*JAK2*) protein and in the gene myeloproliferative leukemia virus oncogene (*MPL*) coding for the thrombopoietin receptor have been associated with myeloproliferative neoplasms (MPNs) and with acute lymphoblastic leukemia in Down syndrome patients. This policy addresses the use of *JAK2* and *MPL* gene mutation testing for diagnosis, prognosis, and treatment selection in patients with myeloproliferative neoplasms. This policy also addresses the potential use of *JAK2* mutations in the diagnosis or selection of treatment in patients with Down syndrome acute lymphoblastic leukemia.

Janus kinase 2 (JAK2) protein

JAK2 is one of the four Janus family nonreceptor protein tyrosine kinases; *JAK1*, *JAK2* and *TYK2* are ubiquitously expressed in mammalian cells.[2] *JAK2* is located on chromosome 9p24 and includes 25 exons and its protein is made up of 1132 amino acids. Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling is important for a wide spectrum of cellular processes, including proliferation, survival or normal functioning of hematopoietic, immune, cardiac and other cells.

Oncogenic *JAK1*, *JAK2* and *JAK3* mutations have been associated with both lymphoid and myeloid neoplasms. Of particular relevance to MPN, *JAK2*V617F was discovered in 2004, is located on exon 14, and occurs in 96% of patients with PV, 55% with ET, and 65% with PMF[3]. In March and April of 2005 four separate groups using different modes of discovery and different measurement techniques reported the presence of a novel somatic point mutation in the conserved autoinhibitory pseudokinase domain of the gene coding for the *JAK2* protein in patients with classic MPNs. The mutation was noted to cause a valine-to-phenylalanine substitution at amino acid position 617 ($JAK2^{V617F}$). Loss of *JAK2* autoinhibition caused by $JAK2^{V617F}$ results in constitutive activation of the kinase and in recruitment and phosphorylation of substrate molecules including signal transducers and activators of transcript (STAT) proteins (JAK-Stat signaling). The result is cell proliferation independent of normal growth factor control.

JAK2 gene exon 12 mutations are specific to JAK2V617-negative PV and were first described in 2007[4]. *JAK2* exon 12 mutations include in-frame deletions, point mutations and duplications, mostly affecting seven highly conserved amino-acid residues (F537–E543). The clinical course of these patients seems to be similar to that of patients with *JAK2*V617F-positive PV.[2]

Myeloproliferative leukemia virus oncogene (MPL)

MPL, located on chromosome 1p34, includes 12 exons and encodes for the thrombopoietin receptor with mutational frequencies at 3% in ET and 10% in PMF.[2,5] *MPL* is the key growth and survival factor for megakaryocytes. Gain-of-function germline *MPL* mutations have been associated with familial thrombocytosis, *MPL*W515L results from a G to T transition at nucleotide 1544 (exon 10), resulting in a tryptophan to leucine substitution at codon 515. *MPL*W515L was first described in 2006 among patients with *JAK2*V617F-negative PMF. *MPL* mutant-induced oncogenesis also results in constitutive JAK-STAT activation. The incidence of *MPL* mutations in MPN is low and suggested to be limited to the need for clarification on the diagnosis of ET or PMF.[5]

While these mutations were of importance in better understanding the biology of the MPNs, they were also of immediate interest as laboratory tools to aid in diagnosis and management of disease. To that end, at least four potential intended uses for mutation testing have been considered, including:

1. Diagnosis of patients with clinical, laboratory or pathological findings suggesting classic MPNs (PV, ET, or PMF);
2. Diagnosis or selection of treatment for patients with Down syndrome-associated acute lymphoblastic leukemia (ALL);
3. Phenotyping of disease subtypes in patients with MPNs to establish disease prognosis;
4. Identification, selection and monitoring of treatment.

More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for *JAK2* gene mutation testing and *MPL* testing. These tests are available as laboratory developed procedures under the U.S. Food and Drug Administration enforcement discretion policy for laboratory developed tests. Variable analytical and clinical performance has been reported, suggesting that the nucleic acid amplification methodologies are more sensitive than mutation sequence analysis. It appears that there can be considerable inter-assay and inter-laboratory variability in the generation of testing results.

MEDICAL POLICY CRITERIA

I. *JAK2* tyrosine kinase and *MPL* mutation testing may be considered **medically necessary** when all of the following criteria are met:

   A. Clinical, laboratory, or pathological findings suggest classic forms of myeloproliferative neoplasms (MPN), which include any of the following:

      1. Polycythemia vera (PV)

      2. Essential thrombocythemia (ET)

      3. Primary myelofibrosis (PMF)

   B. Patients suspected of having polycythemia vera (PV) should first be tested for the most common finding $JAK2^{V617F}$. If testing is negative, further testing to detect other *JAK2* tyrosine kinase mutations, e.g., in exon 12, is warranted.

   C. Patients suspected to have essential thrombocythemia (ET) or primary myelofibrosis (PMF) should first be tested for *JAK2* mutations. If testing is negative, further testing to detect *MPL* mutations is warranted.

II. *JAK2* tyrosine kinase and MPL mutation testing is considered **investigational** in all other circumstances, including but not limited to the following:

   A. Diagnosis of nonclassic forms of MPNs

   B. Molecular phenotyping of patients with MPNs

   C. Monitoring, management, or selecting treatment in patients with MPNs

D. Diagnosis or selection of treatment in patients with Down syndrome and acute lymphoblastic leukemia

SCIENTIFIC EVIDENCE[1]

Validation of the clinical use of any genetic test focuses on 3 main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

Diagnosis of Classic Myeloproliferative Neoplasms

Diagnosis of the various classic forms of myeloproliferative neoplasms (MPNs) has been most recently based on a complex set of clinical, pathological, and biological criteria first introduced by the Polycythemia Vera Study Group (PVSG) in 1996[6,7] and the World Health Organization (WHO) in 2001.[8] Both of these classifications use a combination of clinical, pathological, and/or biological criteria to arrive at a definitive diagnosis. Varying combinations of these criteria are used to determine if a patient has polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF), MPNs that are Philadelphia chromosome negative. (An important component of the diagnostic process is a clinical and laboratory assessment to rule out reactive or secondary causes of disease.)

As noted in the Description, some diagnostic methods (i.e., bone marrow microscopy) are not well standardized[9-11] and others (i.e., endogenous erythroid colony formation) are neither standardized nor widely available.

As noted, in March of 2005, a novel somatic gain-of-function point mutation was discovered in the conserved autoinhibitory pseudokinase domain of the Janus kinase 2 (*JAK2*) protein in patients with MPNs. The mutation was present in blood and bone marrow from a variable portion of patients with classic BCR-ABL negative (i.e., Philadelphia chromosome-negative) MPNs including 65% to 97% of patients with PV, 23% to 57% with ET, and 35% to 56% with IMF (see Table 1). It was initially reported to be absent in all normal subjects and in patients studied with secondary erythrocytosis,[3,11-20] although recently very low levels of mutated cells have been reported to be found in a small subset of the healthy population.[21,22]

That the $JAK2^{V617F}$ -mutated protein was potentially causal for the disease was suggested by the demonstration that cell lines transfected with $JAK2^{V617F}$ could be maintained in culture for several weeks in the absence of growth factor and that dependency was restored by transduction of wild-type *JAK2*. In vivo, mice irradiated and then transplanted with bone marrow cells infected with retrovirus containing the mutation developed a myeloproliferative syndrome.[3]

Table 1: Frequency of *JAK2*^{V617F} Mutations in Patients with Classic Philadelphia Chromosome-Negative MPN							
Study	**Mutation Detection Method**	**PV**	**ET**	**PMF**	**Normals**	**Secondary Erythro-cytosis**	**Comment**
Baxter 2005[11]	DNA sequencing, PCR	71/73 (97%)	29/51 (57%)	8/16 (50%)	90/90 (0%)		Case series
Levine 2005[12]	DNA sequencing	121/164 (74%)	37/115 (32%)	16/46 (35%)	0/269 (0%)		Case series
James 2005[3]	DNA sequencing	40/45 (88%)	9/21 (43%)	3/7 (43%)	0/15 (0%)	0/35 (0%)	Case series
Kravolics 2005[13]	DNA sequencing	83/128 (65%)	21/94 (23%)	13/23 (56%)	0/142 (0%)	0/11 (9%)	Case series
Jones 2005[14]	*Polymerase chain reaction (PCR)* testing	58/72 (81%)	24/59 (41%)	15/35 (43%)	0//160 (0%)	0/4 (0%)	Case series
Tefferi 2006[15]	PCR testing	36/38 (95%)	12/46 (55%)	3/10 (30%)		0/19 (0%)	Case series
Zhoa 2005[16]	DNA sequencing	20/24 (83%)			0/12 (0%)		Case series
Campbell 2005[17]	PCR testing		414/776 (53%)				Prospective, case series
Wolanski 2005[18]	PCR testing		73/150 (49%)				Case series
Campbell 2005[19]	PCR testing			83/152 (55%)			Case series
Tefferi 2005[20]	PCR testing			80/157 (51%)			Case series

Although almost all studies reported were retrospective and/or cross-sectional case series and although both analytical and clinical performances appear dependent on the laboratory method used to detect the mutation, there has been impressive consistency across studies in demonstrating that the *JAK2*^{V617F} mutation is a highly specific marker for clonal evidence of an MPN.

Early reports suggested that specificity was 100%, although sensitivity was variable (as high as 97% in patients with PV but only 30% to 50% in patients with ET or PMF). A result of the extraordinary specificity observed was that in the setting of evaluating a patient with a suspected Philadelphia chromosome- negative MPN, the predictive value of a positive test also approached 100%. It was recognized within months of the discovery of this mutation, that *JAK2*^{V617F} testing could dramatically expedite diagnosis by reducing the need for complex workups of secondary or reactive causes of the observed proliferative process in the *JAK2*^{V617F} - positive patients.[23] Two important caveats should be noted in use of this test. A negative result cannot be used to rule out a classic MPN. A positive result is excellent evidence that a classic MPN is present but alone is insufficient to subclassify the disease category present.

In recognition of the value of use of this new marker in refining the diagnostic workup of patients suspected of having Philadelphia chromosome-negative MPNs, several reports recommending new algorithms for diagnosis were published.[24,25] The 2001 World Health Organization (WHO) criteria were revised in 2008 to reflect incorporation of the test in patient workup.[26,27]

It is important to note that the 2008 WHO revision represents expert consensus and is not based on independent validation of the 2008 criteria compared to earlier diagnostic criteria or on clinical outcomes. Since these previous criteria were themselves based on expert consensus alone, the importance of such comparative studies may be a moot point. However, 2 small cross-sectional comparative studies have been performed evaluating *JAK2*^{V617F} testing directly against previously established PVSG or WHO criteria.

In 2005, James et al[24] compared PV diagnosed using WHO or PVSG criteria with a streamlined diagnostic approach for PV using a two-step algorithm (elevated hematocrit and the presence of the *JAK2*^{V617F} mutation). Although the study group was small (45 patients with a PVSG diagnosis of PV and 61 patients meeting WHO criteria), use of the two-step algorithm resulted in a correct diagnosis in 96% (PVSG criteria) or 93% (WHO criteria) of patients with PV.

In 2008 Kondo et al[28] compared the 2001 WHO classification and the 2008 classification in a small study of 75 patients undergoing evaluation for MPN. Using the 2001 criteria, 57 patients were diagnosed with MPNs including 16 with PV, 37 with ET, and 4 with PMF. Using the 2008 criteria 59 patients were diagnosed with MPNs. The PV and PMF categories were in complete agreement. The 2008 criteria caused reclassification of 2 patients (1 with erythrocytosis and 1 with thrombocytosis) into the ET category.

Ongoing studies of new drugs targeted to treat the mutated tyrosine kinase in patients with MPN are expected to cast additional light on the functionality of the observed *JAK2*^{V617F} mutation and are likely to contribute not only to refined treatment choices but to improved insight into the diagnostic role of this important marker.

Diagnosis of Non-Classical Forms of MPNs

While the most common Philadelphia negative MPNs include what are commonly referred to as classic forms of this disorder (PV, ET, and PMF), patients may rarely show unusual manifestations of this proliferative hematopoietic disorder including non-classical forms of MPNs such as chronic myelomonocytic leukemia, hypereosinophilic syndrome, systemic mastocytosis, chronic neutrophilic leukemia, or others. Reports have appeared identifying *JAK2*^{V617F} mutations in some of these cases.[14,29]

Other Tyrosine Kinase or Related Mutations

In 2007 Scott et al[4] identified four somatic gain-of-function mutations in the *JAK2* exon 12 section of 10 of 11 PV patients without the *JAK2*^{V617F} mutation. Patients with a *JAK2* exon 12 mutation differed from those with the *JAK2*^{V617F} mutations, presenting at a younger age with higher hemoglobin levels and lower platelet and white cell counts. Erythroid colonies could be grown from their blood samples in the absence of exogenous erythropoietin, and mice treated with transfected bone marrow transplants developed a myeloproliferative syndrome.

Findings were subsequently confirmed by a number of investigators who identified additional mutations with similar functional consequences in patients with PV and in patients with idiopathic erythrocytosis.[30,31] Based on these findings it was concluded that the identification of *JAK2* exon 12

mutations provides a diagnostic test for *JAK2*^{V617F} -negative patients who present with erythrocytosis (see Policy Guidelines). Of note, different mutations in the same gene appear to have different effects on signaling, resulting in distinct clinical phenotypes.[4] This perhaps explains the surprise findings of a series of *JAK2* mutations in patients with Down syndrome acute lymphoblastic lymphoma (ALL).

In 2006 Pikman et al[32] surveyed *JAK2* mutation-negative patients with suspected ET and PMF to determine if mutations in pathways complementary to Janus kinase 2 signaling could be identified. A mutation of the thrombopoietin receptor gene (MPL) at codon 515 (exon 10) causing a change from tryptophan to leucine (*MPL*^{W515L}) was discovered.

Subsequent studies identified additional mutations including *MPL*^{S505N}, *MPL*^{W515Ki}, and *MPL*^{W515Kii} in a small but growing number of patients with ET and PMF[33-37] (see Table 2). While this mutation can be found in both *JAK2*^{V617} - positive and negative patients. It is obviously of particular value in the latter in helping to establish a clonal basis of the observed disease process.

TABLE 2: Frequency of MPL 515 Mutations in Patients with Philadelphia Chromosome-Negative MPN

Study	Mutation Detection Method	PV	ET	PMF	Normals	Other MPNs	Comment
Pikman[32] 2006	DNA sequencing	0/10 (0%)	0/50 (0%)	4/45 (8.8%)	0/270(0%)		*JAK2* Negative
Pardanani[33] 2006	Site 1: PCR with DNA sequencing; Site 2: DNA sequencing	0/38 (0%) 0/204 (0%)	2/167 (1%) 2/151 (1%)	8/198 (4%) 5/92 (5%)	0/64 (0%)	3/118 (2.5%)	
Beer[34] (2008)	*PCR testing*		Preliminary 3/88 (3.4%) Prospective 32/776 (4.1%)	Preliminary 8/112 (7.1%)			
Pancrazzi[35] (2008)	PCR *testing*	0/50 (0%)		19/217 (8.7%)	0/60 (0%)		
Ruan[36] (2009)	PCR *testing*	0/32 (0%)	7/199 (3.5%)	3/24 (12.5%)	0/52 (0%)	0/29 (0%)	
Schnittger[37] (2009)	PCR *testing*		19/356 (5.3%)	10/193 (5.2%)		2/269 (.8%)	

Similar to the observations made on the *JAK2*^{V617F} -negative mutations involving exon 12, the MPL exon 10 mutations appeared to demonstrate an autoinhibitory role leading to receptor activation in the absence of thrombopoietin binding. Expression of the MPL allele resulted in cytokine-independent growth of three independent cell lines and transplantation of mice with bone marrow expressing this allele results in a distinctive myeloproliferative disorder.[33]

Although the data sets are small, the *JAK2* exon 12 and MPL exon 10 mutations are unique, appear to be associated with MPNs, and exhibit in vitro and murine model behavior consistent with a causative role in MPNs. The 2008 WHO criteria specifically cite testing for *JAK2* exon 12 mutations in patients with suspected PV (presumably in patients who are *JAK2*^{V617F} negative), specifically cite testing for *MPL*$^{W515L/K}$ in patients with PMF (presumably in patients who are *JAK2*^{V617F} negative), and suggest patients with ET be subject to testing for *JAK2*^{V617F} or other clonal markers such as MPL testing in patients with ET.

Mutations of JAK2 in Acute Lymphoblastic Leukemias Associated with Down Syndrome

Children with Down syndrome have a 10- to 20-fold increased risk of developing acute leukemia. The mechanisms for this are unknown; interestingly, the disease process appears to be exclusively B cell in origin. In 2007 Malinge et. al[38] published a case report describing a novel *JAK2* mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic lymphoma. Speculating that this finding might relate to the role the JAK/signal transducer and activator of transcription (STAT) signaling pathway played in early B-cell development, Bercovich et al[39] studied 88 patients with Down syndrome-acquired acute lymphoblastic leukemia (ALL) for *JAK2* mutations and compared these to 216 patients with sporadic ALL. Five mutant alleles were identified in 16 (18%) of the Down syndrome patients, all at a highly conserved arginine residue (R683) on exon 16. These mutations immortalized primary mouse hematopoietic progenitor cells in vitro. Only a single non-Down syndrome patient exhibited this mutation, and this patient was found to have an isochromosome 21Q. This finding was subsequently confirmed by Gaikwad et al[40] who found 20% of Down syndrome patients with ALL exhibited a point mutation at this location. The role of this abnormality and efforts to consider treatment modifications based on its finding remain subjects for future study.

Molecular Profiling –Phenotype/Genotype Associations and Impact on Prognosis

While there has been great interest in the use of the *JAK2*^{V617F} test as a front line diagnostic test in the evaluation of myeloproliferative patients, there has also been a growing effort to link the presence of this mutation and the quantitative measurement of its allele burden with clinical features and biological behavior. Unfortunately, due to differences in disease definitions, differences in methods of testing, differences in sample type (bone marrow versus circulating blood cells) and differences in study design, the literature in this area is conflicting and inconclusive.

Since the vast majority of patients with PV do exhibit the mutation, attention has been focused in this disease on differences in its presence in the homozygous versus heterozygous state and on whether allele burden correlates with clinical or laboratory features, Studies have suggested a range of findings including association of homozygous states with older age, higher hemoglobin level at diagnosis, leukocytosis, more frequent pruritus, increased incidence of fibrotic transformation, and larger spleen volumes.[41,42] Studies comparing quantitative measurements of allele burden with disease manifestations have demonstrated both a positive and a lack of association with thrombosis, fibrotic transformation, and need for chemotherapy.[43,44]

The impact of the presence of *JAK2*^{V617F} in patients with ET is also controversial. In several studies, the presence of this mutation has been associated with advanced age, higher hemoglobin levels, increased leukocyte count, lower platelet count, and a higher rate of transformation to PV.[17,18] Discrepant results have been reported for thrombotic events and for fibrotic transformation.[45] A recent meta-analysis by Dahabreh et al[46] surveyed some 394 studies on the subject of outcomes in ET. Dahabreh concluded thrombosis but not myelofibrosis or leukemia appeared to be influenced by the presence of *JAK2* mutations. Dahabreh cautioned that there was a need for prospective studies to determine how this information might be used in treatment choices.

Thrombotic effects have been reported to be most pronounced for splanchnic vascular events[47] and there has been little support for use of testing in patients with more general thrombosis or primary thrombocytosis.[48] Results for splanchnic events have been contradictory. In one retrospective study performed looking at *JAK2*^{V617F} in patients treated for thrombosis in ET and in unselected patients with splanchnic vein thrombosis,[49] *JAK2* ^{V617F} mutations did occur with increased frequency in patients with splanchnic vein thrombosis and appeared to identify a subset of patients who might benefit from antiplatelet therapy.[49] However, the outcome of routine testing in both settings remained unclear. In a recent international collaborative studies of patients with ET, patients with *JAK2* ^{V617F} mutations appeared at risk for arterial thrombosis but not for venous thrombosis.[50]

A recent report by Hussein et al[51] demonstrated that although there was significant overlap in *JAK2*^{V617F} F allele burden among various MPN entities, quantitative measurements suggested discriminatory differences between patients with ET and the prefibrotic-stage of PMF.

JAK2^{V617F} mutational status and allele burden appear particularly poorly defined in patients with PMF. A series of confusing and non-congruent articles has resulted in the following conclusions:

- Patients with *JAK2*^{V617F} mutations required fewer blood transfusions but exhibited poorer overall survival than those without the mutation.[19]
- Patients with *JAK2*^{V617F} mutations did not show differences in the incidence of thrombosis, overall survival, or leukemia-free survival.[52]
- Patients with homozygous *JAK2*^{V617F} mutations show an increased evolution toward large splenomegaly, need of splenectomy and leukemic transformation.[53]
- Patients with low allele burdens appeared to exhibit shortened survival, perhaps because they represented a myelodepleted subset of affected patients.[52,54]

In 2013, a joint systematic evaluation of *JAK2* ^{V617F}[55] quantitative polymerase chain reaction (qPCR) assays was conducted to identify “an assay that, beyond being robust enough for routine diagnostic purposes, also showed the best performance profile when used for predicting outcome following an allogeneic transplant.” Effective assays can detect an allele burden as low as 1%.[56] Investigators assessed 3 unpublished laboratory-developed tests and 6 published assays in 12 laboratories. The detection limit of each assay was determined in 7 quality control rounds comprising serial dilutions of centrally-distributed wild-type and mutated cell line DNA and plasmid standards. DNA detection was verified by pyrosequencing. Sensitivity and specificity of the 2 best-performing assays were further assessed in serial samples from 28 patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) for *JAK2* ^{V617F}-positive disease and in 100 peripheral blood samples from healthy controls, respectively. The most sensitive assay performed consistently across various qPCR platforms and detected mutant allele (ie, minimal residual disease) in transplant recipients a median of 22 weeks

(range, 6-85 weeks) before relapse. The authors suggested that the assay could be used to guide management of patients undergoing allogeneic HSCT. Although the study supports the analytic validity of the assay, given the inconsistency of outcomes when *JAK2*^{V617F} testing is used for treatment monitoring (described above), utility of this assay or any *JAK2*^{V617F} test for treatment monitoring is uncertain. Other investigators have studied methods to improve *JAK2* and *MPL* mutation testing using qPCR[57,58] and novel approaches (eg, an electrochemical DNA biosensor).[59]

Treatment

Due to the strong epidemiologic and biologic literature linking *JAK2* pathway mutations to occurrence of MPNs, there has been considerable recent attention on using *JAK2* as a molecular target for drug discovery. In preclinical and early clinical studies, a number of promising *JAK2* inhibitors have been identified and reports have suggested some of these are useful in symptom relief.[60] Many patients with these diseases have a good response to other therapies with cytotoxic drugs, and the natural course of disease, particularly for PV and ET, can be quite indolent. Considerable study will be required to sort through issues of safety and efficacy of these new treatments before they enter routine clinical use. Several early phase and preliminary treatment trials evaluating the safety and efficacy of tyrosine kinase inhibitors in patients with *JAK2*^{V617F} -positive myeloproliferative neoplasms have been reported.[61-63] It has recently been noted that benefits from tyrosine kinase therapy may not be specific for *JAK2*^{V617F} -positive myeloproliferative neoplasms but may be observed in wild type disease as well.[64]

While the identification of a drug producing long-term remissions such as imatinib in chronic myeloid leukemia (CML) is the ultimate goal, it will likely be complicated by the complexity of molecular processes occurring in patients with these other MPNs and the fact that *JAK2*^{V617F} alone does not appear to be a unique or absolutely necessary event in many patients with these diseases. The role of *JAK2*^{V617F} in selecting or monitoring patients for new treatments or residual neoplasia remains undefined.

There are several reports suggesting *JAK2*^{V617F} -positive patients are more sensitive to treatment with hydroxyurea than negative patients.[45] In one study of hydroxyurea treatment in patients with PV or ET harboring the *JAK2*^{V617F} gene, serial changes in allele burden were observed. However, the value of these findings was unclear, and the authors concluded serial testing in patients on this drug should be confined to clinical studies.[65]

On November 16, 2011, the U.S. FDA approved ruxolitinib (a JAK kinase inhibitor) for the treatment of intermediate- and high-risk myelofibrosis (including primary myelofibrosis, post-polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis) due to results from 2 randomized controlled trials (RCTs). One, a double-blind RCT in patients with intermediate to high-risk myelofibrosis, randomized participants to twice-daily oral ruxolitinib (n=155) or placebo (n=154) and followed patients for 76 weeks).[66] The primary outcome, reduction in spleen volume of 35% or more at 24 or more weeks, was observed in 41.9% of patients treated with ruxolitinib compared with 0.7% in the placebo group ($p<0.001$). Survival analysis by Kaplan Meier curves estimated thirteen deaths in the ruxolitinib group (8.4%) and 24 in the placebo group (15.6%) over a median follow-up of 51 weeks ($p=0.04$). This significant association was not observed at the prospectively defined data cutoff of median 32 weeks follow-up ($p=0.33$). A myelofibrosis symptom score at 24 weeks showed an improvement of 45.9% in patients who received ruxolitinib compared with 5.3% in placebo patients. Discontinuation in the ruxolitinib and placebo groups due to adverse events was similar (11% to 10.6% respectively). *Ad hoc s*ubgroup analyses on patients with JAK2V617F mutations indicated reduction in spleen volume of 34.6% compared to an increase of 8.1% in placebo. Patients with JAK2V617F

mutation improved total symptom score by 52.6% in ruxolitinib compared to a worsening of 42.8% at 24 weeks in the placebo arm.

A second trial by Harrison et al. (NCT00934544) reached similar conclusions. Patients with intermediate- or high-risk primary myelofibrosis, post-polycythemia vera myelofibrosis, or post-essential thrombocythemia myelofibrosis patients received oral ruxolitinib (n=146) or best available therapy (n=73).[67] No difference in overall survival was observed between the two groups at 48 weeks. Twenty-eight percent of patients in the ruxolitinib group had at least a 35% reduction in spleen volume at 48 weeks compared to 0% in the best available treatment group ($p<0.001$). Among the subgroup of patients who were JAK^{V617F-} positive, spleen reduction was 33% in the ruxolitinib group and 0% in the best available therapy group. In the ruxolitinib group, patients had an improved overall quality-of-life and a reduction in myelofibrosis symptoms, compared to no benefit that was observed in the control group. Serious adverse events were similar between groups: anemia occurred in 5% of patients in the ruxolitinib group and 4% in the best-available-therapy group, pneumonia occurred in 1% of ruxolitinib group and 5% in the best-available-therapy group, and discontinuation of treatment in the ruxolitinib group occurred in 8% of patients and 5% in the best-available-therapy group.

Clinical Practice Guidelines

World Health Organization (WHO)

In 2007 an ad hoc group of experts in the area of MPNs formed a working group to reformulate the 2001 WHO diagnostic criteria for classic cases of MPN. This group recommended the use of *JAK2* testing for diagnosis of all three common Philadelphia chromosome-negative MPN variants – PV, ET, and PMF. Revised criteria were published by WHO in 2008[27].

- PV – Major criteria: presence of *JAK2*^{V617F} or other functionally similar mutation such as *JAK2* exon 12 mutation.

- ET- Major criteria: demonstration of *JAK2*^{V617F} or other clonal marker, or in the absence of a clonal marker, no evidence for reactive thrombocytosis.

- PMF- Major criteria: demonstration of *JAK2*^{V617F} or other clonal marker (e.g., *MPL*^{W515K} or *MPL*^{W515L}) or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic disease.

Summary

There is an extensive body of literature providing information on the clinical validation of *JAK2*^{V617F} as a distinctive marker of patients with Philadelphia chromosome-negative classic myeloproliferative neoplasms (MPNs). The evidence on the clinical validation of *MPL* as a marker of patients with Philadelphia chromosome-negative MPNs is growing. Therefore, *JAK2* and *MPL* genetic testing may be considered medically necessary as a diagnostic test for patients with signs and symptoms of classic MPN. There is insufficient data on the additional mutations that have been identified in patients with various non-classical MPN disorders. It is unclear whether these additional mutations have functional relevance. *JAK2* and *MPL* mutation testing is considered investigational for the diagnosis of non-classic forms of MPN, prognostic testing or to direct therapeutic treatments of MPN.

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CROSS REFERENCES

Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

CODES	NUMBER	DESCRIPTION
CPT	81270	*JAK2* (*Janus kinase 2*) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant
	81402	Molecular pathology procedure, Level 3 (eg, >10 SNPs, 2-10 methylated variants, or 2-10 somatic variants [typically using non-sequencing target variant analysis], immunoglobulin and T-cell receptor gene rearrangements, duplication/deletion variants 1 exon) – which includes *MPL (myeloproliferative leukemia virus oncogene, thrombopoietin receptor TPOR)* (eg, myeloproliferative disorder), common variants (eg, W515A, W515K, W515L, W515R)
	81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons – which includes *JAK2 (Janus kinase 2)* (eg, myeloproliferative disorder), exon 12 sequence and exon 13 sequence, if performed
HCPCS	None	