JAK2 and MPL Mutation Analysis in Myeloproliferative Neoplasms

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Policy
Blue Cross and Blue Shield of Kansas City (Blue KC) will provide coverage for JAK2 and MPL Mutation Analysis in Myeloproliferative Neoplasms when it is determined to be medically necessary because the criteria shown below are met.

When Policy Topic is covered
JAK2 tyrosine kinase and MPL mutation testing may be considered medically necessary in the diagnosis of patients presenting with clinical, laboratory, or pathologic findings suggesting classic forms of myeloproliferative neoplasms (MPN), that is, polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF).

When Policy Topic is not covered
JAK2 tyrosine kinase and myeloproliferative leukemia (MPL) mutation testing may be considered investigational in all other circumstances including, but not limited to, the following situations:
- Diagnosis of nonclassic forms of MPNs
- Molecular phenotyping of patients with MPNs
- Monitoring, management, or selecting treatment in patients with MPNs
- Diagnosis or selection of treatment in patients with Down syndrome and acute lymphoblastic leukemia

Considerations
Testing strategy
Patients suspected to have 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, eg, in exon 12, is warranted.

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

Mutations in the gene encoding Janus kinase 2 (JAK2) protein and in the myeloproliferative leukemia virus oncogene (MPL) encoding the thrombopoietin receptor have been associated with myeloproliferative neoplasms and with acute lymphoblastic leukemia (ALL) 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 will address the potential use of JAK2 mutations in the diagnosis or selection of treatment in patients with Down syndrome and ALL.

There is an extensive body of literature on the clinical validation of JAK2 V617F as a distinct marker of patients with Philadelphia chromosome (Ph)–negative classic myeloproliferative neoplasms (MPNs). In almost a dozen reports (all case series), JAK2 V617F has emerged as a unique clonal finding in patients with polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF). Although the association between defined disease and presence of the marker has been variable depending on detection methods used and study design, test specificity is virtually 100%. In patients with PV tested using polymerase chain reaction (PCR) methodology, sensitivity also may approach 100% (reports up to 97%). In the subset of patients with suspected PV who are JAK2 V617F–negative classic myeloproliferative neoplasms (MPNs). In almost a dozen reports (all case series), JAK negative, there is compelling evidence from several case series suggesting that other JAK2 mutations (involving exon 12) may be identified.

Given the difficulty of using classic criteria (morphology and complex tests such as erythropoietin measurements or measurements of endogenous erythroid colony formation), JAK2 testing will facilitate the diagnostic workup. The presence of this marker biologically and clinically is a convincing substitute for the need to rule out reactive causes of erythrocytosis. Testing for this marker is recommended in clinical practice guidelines for patients with all of the most common Ph-negative MPNs. Therefore, JAK2 testing may be considered medically necessary as a diagnostic test for patients with signs and symptoms of MPN.

Mutation testing to establish disease phenotype (eg, disease prognosis) or to select or monitor therapy remains an area of intense interest with a growing number of studies, particularly drug trials. Patients with MPNs who received JAK inhibitor therapy experienced improvements in splenomegaly and symptoms regardless of JAK2 mutation status. Recently, multiple additional mutations have been identified in patients with various MPN disorders. These appear to have less specificity than the JAK2 and MPL mutations, and their use in understanding, diagnosing, and treating disease remains a matter requiring further study. It is currently unclear whether these mutations carry broad, albeit nonspecific pathogenic relevance to MPNs or whether they are simply passenger mutations with little or no functional relevance. JAK2 testing for prognosis or to direct therapy is considered investigational.
Background

MPNs are uncommon overlapping blood diseases characterized by the production of 1 or more blood cell lines and include chronic myeloid leukemia (CML), PV, ET, PMF, systemic mastocytosis, chronic eosinophilic leukemia, and others. A common finding in many MPNs is clonality, and a central pathogenic feature is a mutated version of a 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 (Ph) and an accompanying unique gene rearrangement (BCR-ABL) resulting in a continuously activated tyrosine kinase enzyme were identified. These findings led to the development of targeted tyrosine kinase inhibitor drug therapy (imatinib) that produces long-lasting remissions.

Diagnosis and monitoring of patients with Ph-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. Additionally, these entities can be difficult to distinguish on morphologic bone marrow exam, and diagnosis can be complicated by changing disease patterns: PV and ET can evolve into PMF or undergo leukemic transformation. World Health Organization criteria were published as a benchmark for diagnosis in 2001(1) and updated in 2008.(2) These have been challenging to use because they involve complex diagnostic algorithms, rely on morphologic assessment of uncertain consistency, and require tests that are not well-standardized or widely available, such as endogenous erythroid colony formation.

In March and April 2005, 4 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 encoding JAK2 protein in patients with classic MPNs. The mutation caused 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 (so-called JAK-Stat signaling). The result is cell proliferation independent of normal growth factor control. These findings were subsequently confirmed, and additional mutations affecting the JAK2 gene—mutations in exon 12 or in complementary pathways such as thrombopoietinreceptor- pathway mutations in MPL exon 10—were identified. These mutations were seen with varying but reliable frequency in patients with classic MPNs, and with uncommon and erratic frequency in other MPNs. Additionally, unique cases of JAK2 mutations were reported in a subset of patients with Down syndrome–associated ALL.

Although these mutations were of importance in better understanding the biology of MPNs, they also were of immediate interest as laboratory tools to aid in diagnosis and management of disease. To that end, at least 4 potential intended uses for mutation testing have been considered, including:
a. Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic MPNs (PV, ET, or PMF);
b. Diagnosis or selection of treatment for patients with Down syndrome ALL;
c. Phenotyping of disease subtypes in patients with MPNs to establish disease prognosis;
d. Identification, selection, and monitoring of treatment.

Many diagnostic procedures are available for JAK2 testing and MPL mutation testing. Variable analytic and clinical performance has been reported, suggesting that nucleic acid amplification methodologies are more sensitive than mutation sequence analysis. It appears that there can be considerable interassay and interlaboratory variability in testing results.

**Rationale**
The original policy was based on a literature search using MEDLINE for the period of March 2005 through November 2009. The search identified 1313 publications including 150 reviews, 41 clinical trials, 17 editorials, 2 meta-analyses, and 1 observational prospective study. The literature review for the most recent update was performed on January 17, 2015.

**Tyrosine Kinase Mutation Analysis and Diagnosis of Philadelphia Chromosome–Negative Myeloproliferative Neoplasms**

**Diagnosis of Classic Myeloproliferative Neoplasms**
Diagnosis of the various classic forms of myeloproliferative neoplasms (MPNs) has been based most recently on a complex set of clinical, pathological, and biological criteria first introduced by the Polycythemia Vera Study Group (PVSG) in 1996(3,4) or by the World Health Organization (WHO) in 2001(1) (updated 2008(2)). Both classifications use a combination of clinical, pathological, and/or biological criteria to reach definitive diagnoses. Varying combinations of these criteria are used to determine whether a patient has polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF), ie, MPNs that are Philadelphia chromosome (Ph)–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 section, some diagnostic methods (eg, bone marrow microscopy) are not wellstandardized, (5-7) and others (eg, endogenous erythroid colony formation) are neither standardized nor widely available.

In March 2005, a novel somatic gain-of-function point mutation was discovered in the conserved autoinhibitory pseudokinase domain of the JAK2 gene in patients with MPNs. The mutation was present in blood and bone marrow from a variable portion of patients with classic BCR-ABL–negative (ie, Phnegative) MPNs including 65% to 97% of patients with PV, 23% to 57% with ET, and 35% to 56% with PMF (see Table 1). The mutation was initially reported to be absent in all normal subjects and in patients with secondary erythrocytosis,(7-17) although recently
very low levels of mutated cells have been reported in a small subset of healthy individuals.(18,19)

That the \textit{JAK2} V617F–negative classic myeloproliferative neoplasms (MPNs). In almost a dozen reports (all case series), \textit{JAK} mutated protein potentially caused the disease was suggested by the demonstration that cell lines transfected with \textit{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 \textit{JAK2}. In vivo, mice irradiated and then transplanted with bone marrow cells infected with retrovirus containing the mutation developed a myeloproliferative syndrome.(9)

Table 1: Frequency of \textit{JAK2} V617F Mutations in Patients With Classic Philadelphia Chromosome–Negative Myeloproliferative Neoplasm

<table>
<thead>
<tr>
<th>Study</th>
<th>Mutation Detection Method</th>
<th>PV</th>
<th>ET</th>
<th>PMF</th>
<th>Normal</th>
<th>Secondary Erythrocytosis</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baxter et al (2005)7</td>
<td>DNA sequencing, PCR</td>
<td>71/73 (97%)</td>
<td>29/51 (57%)</td>
<td>8/16 (50%)</td>
<td>0/90 (0%)</td>
<td>NR</td>
<td>Case series</td>
</tr>
<tr>
<td>Levine et al (2005)8</td>
<td>DNA sequencing</td>
<td>121/164 (74%)</td>
<td>37/115 (32%)</td>
<td>16/46 (35%)</td>
<td>0/269 (0%)</td>
<td>NR</td>
<td>Case series</td>
</tr>
<tr>
<td>James et al (2005)9</td>
<td>DNA sequencing</td>
<td>40/45 (88%)</td>
<td>9/21 (43%)</td>
<td>3/7 (43%)</td>
<td>0/15 (0%)</td>
<td>0/35 (0%)</td>
<td>Case series</td>
</tr>
<tr>
<td>Kralovics et al (2005)10</td>
<td>DNA sequencing</td>
<td>83/128 (65%)</td>
<td>21/94 (23%)</td>
<td>13/23 (56%)</td>
<td>0/142 (0%)</td>
<td>0/11 (0%)</td>
<td>Case series</td>
</tr>
<tr>
<td>Jones et al (2005)11</td>
<td>PCR testing</td>
<td>58/72 (81%)</td>
<td>24/59 (41%)</td>
<td>15/35 (43%)</td>
<td>0/160 (0%)</td>
<td>0/4 (0%)</td>
<td>Case series</td>
</tr>
<tr>
<td>Tefferi et al 2006 12</td>
<td>PCR testing</td>
<td>36/38 (95%)</td>
<td>12/46 (55%)</td>
<td>3/10 (30%)</td>
<td>NR</td>
<td>0/19 (0%)</td>
<td>Case series</td>
</tr>
<tr>
<td>Zhao et al 2005 13</td>
<td>DNA sequencing</td>
<td>20/24 (83%)</td>
<td>NR</td>
<td>NR</td>
<td>0/12 (0%)</td>
<td>NR</td>
<td>Case series</td>
</tr>
<tr>
<td>Campbell et al (2005)14</td>
<td>PCR testing</td>
<td>NR</td>
<td>414/776 (53%)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Prospective, case series</td>
</tr>
<tr>
<td>Wolanskyj et al (2005)15</td>
<td>PCR testing</td>
<td>NR</td>
<td>73/150 (49%)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Case series</td>
</tr>
<tr>
<td>Campbell et al (2006)16</td>
<td>PCR testing</td>
<td>NR</td>
<td>NR</td>
<td>83/152 (55%)</td>
<td>NR</td>
<td>NR</td>
<td>Case series</td>
</tr>
<tr>
<td>Tefferi et al (2005)17</td>
<td>PCR testing</td>
<td>NR</td>
<td>NR</td>
<td>80/157 (51%)</td>
<td>NR</td>
<td>NR</td>
<td>Case series</td>
</tr>
</tbody>
</table>

ET: essential thrombocythemia; NR: not reported; PCR: polymerase chain reaction; PMF: primary myelofibrosis; PV: polycythemia vera.

Although almost all studies were retrospective case series and/or cross-sectional studies, and although both analytic 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 \textit{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 Ph-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 JAK2 V617F–negative classic myeloproliferative neoplasms (MPNs). In almost a dozen reports (all case series), JAK positive patients.(20) Two important caveats should be noted in use of this test: (1) A negative result cannot be used to rule out classic MPN; and (2) a positive result is excellent evidence that a classic MPN is present but alone is insufficient to subclassify the disease category.

In recognition of the value of this new marker in refining the diagnostic workup of patients suspected of having Ph-negative MPNs, several reports recommended new diagnostic algorithms.(21,22) The 2001 WHO criteria were revised in 2008 to incorporate mutation testing in patient workup.(2,23)

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 with earlier diagnostic criteria or on clinical outcomes. Because 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 evaluated JAK2 V617F testing in comparison with previously established PVSG or WHO criteria.

In 2005, James et al(21) compared PV diagnosed using WHO or PVSG criteria with a streamlined diagnostic approach for PV using a 2-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, 61 patients meeting WHO criteria), use of the 2-step algorithm resulted in a correct diagnosis in 96% (PVSG criteria) or 93% (WHO criteria) of patients with PV.

In 2008, Kondo et al(24) 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, 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 also to better understanding of the diagnostic role of this important marker.
Diagnosis of Nonclassic Forms of MPNs
Although the most common Ph-negative MPNs include what are commonly referred to as classic forms of this disorder (PV, ET, PMF), rare patients may show unusual manifestations of nonclassic forms of MPNs, such as chronic myelomonocytic leukemia, hypereosinophilic syndrome, systemic mastocytosis, chronic neutrophilic leukemia, or others. Reports have identified JAK2 V617F mutations in some of these cases.(11,25) Due to the paucity of data about the significance of JAK2 V617F or MPL mutations in these disease settings, testing in patients with these diseases should be considered investigational.

Other Tyrosine Kinase or Related Mutations
In 2007, Scott et al(26) identified 4 somatic gain-of-function mutations in JAK2 exon 12 in 10 of 11 PV patients without the JAK2 V617F mutation. Patients with a JAK2 exon 12 mutation differed from those with the JAK2 V617F mutation, 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.(27,28) 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 section). Of note, different mutations in the same gene appear to have different effects on signaling, resulting in distinct clinical phenotypes.(26) This perhaps explains the unexpected finding of various JAK2 mutations in patients with Down syndrome–associated acute lymphoblastic lymphoma (ALL).

In 2006, Pikman et al(29) surveyed JAK2 mutation–negative patients with suspected ET and PMF to determine whether mutations in pathways complementary to JAK2 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 (see Table 2).(30-33) Although these mutations can be found in both JAK2 V617F–positive and –negative patients, they are of particular value in the latter group for establishing a clonal basis of the observed disease process.

Table 2: Frequency of MPL 515 Mutations in Patients With Philadelphia Chromosome–Negative Myeloproliferative Neoplasm

<table>
<thead>
<tr>
<th>Study</th>
<th>Mutation Detection Method</th>
<th>PV</th>
<th>ET</th>
<th>PMF</th>
<th>Normals</th>
<th>Other MPNs</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pikman et al (2006)29</td>
<td>DNA sequencing</td>
<td>0/10</td>
<td>0/50</td>
<td>4/45</td>
<td>0/270</td>
<td></td>
<td>JAK2 negative</td>
</tr>
</tbody>
</table>
### JAK2 and MPL Mutation Analysis in Myeloproliferative Neoplasms

<table>
<thead>
<tr>
<th>Study</th>
<th>Site 1: PCR with DNA sequencing</th>
<th>Site 2: DNA sequencing</th>
<th>0/38 (0%)</th>
<th>2/167 (1%)</th>
<th>8/198 (4%)</th>
<th>0/64 (0%)</th>
<th>3/118 (2.5%)</th>
<th>10/193 (5.2%)</th>
<th>2/269 (0.8%)</th>
<th>167 (1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pardanani et al (2006)</td>
<td>0/204 (0%)</td>
<td>Preliminary 3/88 (3.4%)</td>
<td>Prospective 32/776 (4.1%)</td>
<td>2/151 (1%)</td>
<td>5/92 (5%)</td>
<td>0/64 (0%)</td>
<td>3/118 (2.5%)</td>
<td>167 (1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer et al (2008)</td>
<td>Preliminary 8/112 (7.1%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pancrazzi et al (2008)</td>
<td>0/50 (0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruan et al (2009)</td>
<td>7/199 (3.5%)</td>
<td>3/24 (12.5%)</td>
<td>0/52 (0%)</td>
<td>0/29 (0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schnitter et al (2009)</td>
<td>19/356 (5.3%)</td>
<td>10/193 (5.2%)</td>
<td></td>
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<td></td>
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<td></td>
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</tr>
</tbody>
</table>

ET: essential thrombocythemia; MPN: myeloproliferative neoplasm; PCR: polymerase chain reaction; PMF: primary myelofibrosis; PV: polycythemia vera.

Similar to observations about *JAK2* V617F–negative mutations in exon 12, *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 3 independent cell lines, and transplantation of mice with bone marrow expressing this allele resulted in a distinct myeloproliferative disorder.(30)

Although 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) and for *MPL* W515L/K in patients with PMF (presumably in patients who are *JAK2* V617F–negative). The criteria suggest testing for *JAK2* V617F or other clonal markers, such as *MPL*, in patients with ET (see Policy Guidelines section).

**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; the disease process appears to be exclusively B cell in origin. In 2007, Malinge et al published a case report(35) describing a novel JAK2 mutation in a patient with Down syndrome and B-cell precursor ALL. 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(36) studied 88 patients with Down syndrome-associated ALL for JAK2 mutations and compared these to 216 patients with sporadic ALL. Five mutant alleles were identified in 16 (18%) of the patients with Down syndrome, 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 (loss of the short [p] arm of chromosome 21 and duplication of the long [q] arm). This finding was subsequently confirmed by Gaikwad et al (37) who found that 20% of patients with Down syndrome 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 subject to future study.

**Molecular Profiling: Phenotype/Genotype Associations and Impact on Prognosis**

Although 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 also has 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.

Because most patients with PV 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 reported 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.(38,39) Studies that compared quantitative measurements of allele burden with disease manifestations have demonstrated both a positive association and lack of an association with thrombosis, fibrotic transformation, and need for chemotherapy.(40,41)

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.(14,15) Discrepant results have been reported for thrombotic events and for fibrotic transformation.(42) A 2009 meta-analysis by Dahabreh et al (43) surveyed 394 studies on the subject of outcomes in ET. Dahabreh et al concluded that thrombosis but not myelofibrosis or leukemia appeared to be influenced by the presence of JAK2 mutations. Dahabreh et al 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,(44) and there has been little support for use of testing in patients with more general thrombosis or primary thrombocytosis.(45) Results for splanchnic events have been contradictory. In 1 retrospective study that assessed JAK2 V617F in patients treated for thrombosis in ET and in unselected patients with splanchnic vein thrombosis,(46) JAK2 V617F mutations were more common in patients with splanchnic vein thrombosis and appeared to identify a subset of
patients who might benefit from antiplatelet therapy. However, the outcome of routine testing in both settings remained unclear. In a 2011 international collaborative study of patients with ET, patients with JAK2 V617F mutations appeared at risk for arterial thrombosis but not for venous thrombosis.(47)

A 2009 report by Hussein et al(48) demonstrated that although there was significant overlap in JAK2 V617F 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. In a series of confusing and noncongruent articles, it has been concluded that:

- Patients with JAK2 V617F mutations required fewer blood transfusions but exhibited poorer overall survival than those without the mutation.(16)
- Patients with JAK2 V617F mutations did not show differences in the incidence of thrombosis, overall survival, or leukemia-free survival.(49)
- Patients with homozygous JAK2 V617F mutations showed an increased evolution toward large splenomegaly, need of splenectomy, and leukemic transformation.(50)
- Patients with low allele burdens appeared to exhibit shortened survival, perhaps because they represented a myelodepleted subset of affected patients.(49,51)

In 2013, European LeukemiaNet and MPN&MPNr (related diseases)–EuroNet undertook a joint systematic evaluation of JAK2 V617F quantitative polymerase chain reaction (qPCR) assays 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.”(52) Effective assays can detect an allele burden as low as 1%. (53) Investigators assessed 3 unpublished laboratory developed tests and 6 published assays in 12 laboratories in 7 countries. The detection limit of each assay was determined in 7 quality control rounds comprising serial dilutions of centrally distributed wildtype 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. 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 earlier), 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(54,55) and novel approaches (eg, an electrochemical DNA biosensor).(56)
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 that some of these are useful in symptom relief.\(^{(57)}\) Many patients with these diseases have good responses to 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.\(^{(58-60)}\) It also has been noted that benefits from tyrosine kinase therapy may not be specific for JAK2 V617F–positive MPNs but may be observed in wild-type disease as well.\(^{(61)}\)

Although identification of a drug producing long-term remission (like imatinib in chronic myeloid leukemia [CML]) is the ultimate goal, discovery likely will 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.

Several reports suggest that JAK2 V617F–positive patients are more sensitive to treatment with hydroxyurea than JAK2 V617F–negative patients.\(^{(42)}\) In 1 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 that serial testing in patients taking hydroxyurea should be confined to clinical studies.\(^{(62)}\)

On November 16, 2011, FDA-approved ruxolitinib (a JAK kinase inhibitor) for the treatment of intermediate- and high-risk myelofibrosis (including primary myelofibrosis, postpolycythemia vera myelofibrosis, and postessential thrombocytopenia myelofibrosis) based on results from 2 randomized controlled trials (RCTs). One, a double-blind RCT in patients with intermediate- to high-risk myelofibrosis,\(^{(63)}\) randomized participants to twice-daily oral ruxolitinib (n=155) or placebo (n=154) and followed patients for 76 weeks. The primary outcome, a 35% or greater reduction in spleen volume at or after 24 weeks, was observed in 41.9% of patients treated with ruxolitinib compared with 0.7% in the placebo group (p<0.001). At the prospectively defined data cutoff of 32 weeks median follow-up, there were 10 deaths in the ruxolitinib group (6.5%) versus 14 deaths in the placebo group (9.1%) (Kaplan-Meier method, p=0.33). With 4 additional months of follow-up (median, 51 weeks total follow-up), there were 13 total deaths in the ruxolitinib group (8.4%) versus 24 total deaths in the placebo group (15.6%) (Kaplan-Meier method, p=0.04). Myelofibrosis symptom score at 24 weeks improved 45.9% from baseline in patients who received ruxolitinib and
5.3% in placebo patients. Discontinuations due to adverse events were similar in the ruxolitinib and placebo groups (11% and 10.6%, respectively). In post hoc subgroup analysis of patients with the JAK2 V617F mutation, mean changes in spleen volume at 24 weeks were -34.6% in the ruxolitinib group and +8.1% in the placebo group; in patients without the mutation, mean changes in spleen volume were -23.8% and +8.4%, respectively. Changes in total symptom score at 24 weeks in patients with the JAK2 V617F mutation were -52.6% in the ruxolitinib group and +42.8% in the placebo group (higher scores indicate more severe symptoms); in patients without the mutation, changes in total symptom score were -28.1% and +37.2%, respectively.

A second trial by Harrison et al reached similar conclusions.(64) Patients with intermediate- or high-risk primary myelofibrosis, postpolycythemia vera myelofibrosis, or postessential thrombocythemia myelofibrosis received oral ruxolitinib (n=146) or best available therapy (n=73). No difference in overall survival was observed between the 2 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 with 0% in the control group (p<0.001). In the JAK2 V617F–positive subgroup, incidence of spleen reduction was 33% in the ruxolitinib group and 0% in the control group; in the JAK2 V617F–negative subgroup, incidence of spleen reduction was 14% in the ruxolitinib group and 0% in controls. In the ruxolitinib group, patients had an improved overall quality of life and a reduction in myelofibrosis symptoms compared with no benefit in the control group. Serious adverse events were similar between groups: anemia occurred in 5% of patients in the ruxolitinib group and 4% of the control group, pneumonia occurred in 1% of the ruxolitinib group and 5% of the control group, and 8% of patients in the ruxolitinib group and 5% in the control group discontinued treatment.

**Ongoing and Unpublished Clinical Trials**
An online search of ClinicalTrials.gov found several ongoing studies using the search terms “myeloproliferative,” “JAK2,” and “MPL.” Most studies do not require presence of a mutation. However, 2 phase 3 trials of treatments for PV do require presence of the JAK2 V617F mutation. These are summarized in Table 3.

**Table 3. Phase 3 Trials of Treatments for JAK2 V617F–Positive Polycythemia Vera**

<table>
<thead>
<tr>
<th>NCT Number</th>
<th>Title</th>
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<th>Completion Date&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>NCT01259856</td>
<td>Randomized Trial of Pegylated Interferon Alfa-2a Versus Hydroxyurea in Polycythemia Vera (PV) and Essential Thrombocythemia (ET)</td>
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<td>NCT01949805</td>
<td>Pegylated Interferon Alpha-2b Versus Hydroxyurea in Polycythemia Vera (PROUD-PV)</td>
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<sup>a</sup> Estimated.  
<sup>b</sup> Expected.

**Summary of Evidence**
There is an extensive body of literature on the clinical validation of JAK2 V617F as a distinct marker of patients with Philadelphia chromosome (Ph)–negative classic...
myeloproliferative neoplasms (MPNs). In almost a dozen reports (all case series), JAK2 V617F has emerged as a unique clonal finding in patients with polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF). Although the association between defined disease and presence of the marker has been variable depending on detection methods used and study design, test specificity is virtually 100%. In patients with PV tested using polymerase chain reaction methodology, sensitivity also may approach 100% (reports up to 97%).

In the subset of patients with suspected PV who are JAK2 V617F−negative, there is compelling evidence from several case series suggesting that other JAK2 mutations (involving exon 12) may be identified.

Given the difficulty of using classic criteria (morphology and complex tests such as erythropoietin measurements or measurements of endogenous erythroid colony formation), JAK2 testing will facilitate the diagnostic workup. The presence of this marker biologically and clinically is a convincing substitute for the need to rule out reactive causes of erythrocytosis. Testing for this marker is recommended in clinical practice guidelines for patients with all of the most common Ph-negative MPNs. Therefore, JAK2 testing may be considered medically necessary as a diagnostic test for patients with signs and symptoms of MPN.

Mutation testing to establish disease phenotype (eg, disease prognosis) or to select or monitor therapy remains an area of intense interest with a growing number of studies, particularly drug trials. Patients with myeloproliferative neoplasms who received JAK inhibitor therapy experienced improvements in splenomegaly and symptoms regardless of JAK2 mutation status. Recently, multiple additional mutations have been identified in patients with various MPN disorders. These appear to have less specificity than the JAK2 and MPL mutations, and their use in understanding, diagnosing, and treating disease remains a matter requiring further study. It is currently unclear whether these mutations carry broad, albeit nonspecific pathogenic relevance to MPNs or whether they are simply passenger mutations with little or no functional relevance. JAK2 testing for prognosis or to direct therapy is considered investigational.

Practice Guidelines and Position Statements

**WHO Criteria for MPN (2008)**

**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 (eg, 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.

**U.S. Preventive Services Task Force Recommendations**

Not applicable.
Medicare National Coverage

There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

References:
49. Tefferi A, Lasho TL, Huang J, et al. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. Leukemia. Apr 2008;22(4):756-761. PMID 18216871

Billing Coding/Physician Documentation Information

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<td>81270</td>
<td>JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant</td>
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<td>81402</td>
<td>Molecular pathology procedure, Level 3 (eg, &gt;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 of 1 exon, loss of heterozygosity [LOH], uniparental disomy [UPD])</td>
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<td>81403</td>
<td>Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of &gt;10 ampicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)</td>
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ICD-10 Codes

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<td>D47.4</td>
<td>Osteomyelofibrosis</td>
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Additional Policy Key Words

N/A

Policy Implementation/Update Information

6/1/16  New Policy; considered medically necessary when criteria is met.
6/1/17  No policy statement changes.

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