Name of Policy:
JAK2 and MPL Mutation Analysis in Myeloproliferative Neoplasms

Policy #: 405       Latest Review Date:  February 2015  
Category:  Laboratory       Policy Grade: A

Background/Definitions:
As a general rule, benefits are payable under Blue Cross and Blue Shield of Alabama health plans only in cases of medical necessity and only if services or supplies are not investigational, provided the customer group contracts have such coverage.

The following Association Technology Evaluation Criteria must be met for a service/supply to be considered for coverage:

1. The technology must have final approval from the appropriate government regulatory bodies;
2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes;
3. The technology must improve the net health outcome;
4. The technology must be as beneficial as any established alternatives;
5. The improvement must be attainable outside the investigational setting.

Medical Necessity means that health care services (e.g., procedures, treatments, supplies, devices, equipment, facilities or drugs) that a physician, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury or disease or its symptoms, and that are:

1. In accordance with generally accepted standards of medical practice; and
2. Clinically appropriate in terms of type, frequency, extent, site and duration and considered effective for the patient’s illness, injury or disease; and
3. Not primarily for the convenience of the patient, physician or other health care provider; and
4. Not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient’s illness, injury or disease.
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 acute lymphoblastic leukemia.

Myeloproliferative neoplasms (MPNs) are uncommon overlapping blood diseases characterized by the production of one or more blood cells and includes chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocytosis (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 findings led to the development of targeted tyrosine kinase inhibitor drug therapy (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 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 (WHO) criteria were published as a benchmark for diagnosis in 2001 and updated in 2008. 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 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 encoding JAK2 protein in patients with classic MPNs. The mutation was noted to cause a valine-to-phenylalanine substitution at amino acid position 617 (JAK2V617F). Loss of JAK2 autoinhibition, caused by JAK2V617F, 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 thrombopoietin-receptor-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. In addition, unique cases of \textit{JAK2} mutations were reported in a subset of patients with Down syndrome-associated acute lymphoblastic leukemia (ALL).

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:

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

More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for \textit{JAK2} testing and \textit{MPL} mutation testing. These tests are available as laboratory developed procedures under the U.S. Food and Drug Administration (FDA) enforcement discretion policy for laboratory developed tests. 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 the generation of testing results.

**Policy:**

\textit{JAK2 tyrosine kinase and MPL mutation testing meets} Blue Cross and Blue Shield of Alabama’s medical criteria for coverage when used for diagnosis of patients presenting with clinical, laboratory, or pathological finding suggesting myeloproliferative neoplasms (MPN), that is, polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF).

\textit{JAK2 tyrosine kinase and MPL mutation does not meet} Blue Cross and Blue Shield of Alabama’s medical criteria for coverage and is 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 (ALL)

\textit{Blue Cross and Blue Shield of Alabama does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Cross and Blue Shield of Alabama administers benefits based on the member’s contract and corporate medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.}
Key Points:
A literature search identified 1313 publications including 150 reviews, 41 clinical trials, 17 editorials, two meta-analyses, and one observational prospective study. The literature review for the most recent update was performed through January 17, 2015.

Tyrosine Kinase Mutation Analysis and the Diagnosis of Philadelphia Chromosome Negative MPNs
Diagnosis of classic myeloproliferative neoplasms
Diagnosis of the various classic forms of myeloproliferative neoplasms 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 or the World Health Organization (WHO) in 2001. 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 PV, ET or PMF. 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 and others (i.e., endogenous erythroid colony formation) are neither standardized nor widely available.

In March of 2005, a novel somatic gain-of-function point mutation was discovered in the conserved auto-inhibitory 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 (i.e., Philadelphia chromosome-negative) 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, although recently very low levels of mutated cells have been reported in a small subset of healthy individuals.

That the JAK2$^{V617F}$ mutated protein potentially caused 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...
Table 1: Frequency of *JAK2*<sup>V617F</sup> Mutations in Patients with Classic Philadelphia Chromosome Negative MPN

<table>
<thead>
<tr>
<th>Study</th>
<th>Mutation Detection Method</th>
<th>PV</th>
<th>ET</th>
<th>PMF</th>
<th>Normals</th>
<th>Secondary Erythro-Cytosis</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baxter 2005</td>
<td>DNA sequencing, PCR</td>
<td>71/73</td>
<td>29/51</td>
<td>8/16</td>
<td>0/90</td>
<td>0%</td>
<td>Case series</td>
</tr>
<tr>
<td>Levine 2005</td>
<td>DNA sequencing</td>
<td>121/164</td>
<td>37/115</td>
<td>16/46</td>
<td>0/269</td>
<td>0%</td>
<td>Case series</td>
</tr>
<tr>
<td>James 2005</td>
<td>DNA sequencing</td>
<td>40/45</td>
<td>9/21</td>
<td>3/7</td>
<td>0/15</td>
<td>0%</td>
<td>Case series</td>
</tr>
<tr>
<td>Kravolics 2005</td>
<td>DNA sequencing</td>
<td>83/128</td>
<td>21/94</td>
<td>13/23</td>
<td>0/142</td>
<td>0%</td>
<td>Case series</td>
</tr>
<tr>
<td>Jones 2005</td>
<td>PCR Testing</td>
<td>58/72</td>
<td>24/59</td>
<td>15/35</td>
<td>0/160</td>
<td>0%</td>
<td>Case series</td>
</tr>
<tr>
<td>Tefferi 2006</td>
<td>PCR Testing</td>
<td>36/38</td>
<td>12/46</td>
<td>3/10</td>
<td>--</td>
<td>0%</td>
<td>Case series</td>
</tr>
<tr>
<td>Zhoa 2005</td>
<td>DNA sequencing</td>
<td>20/24</td>
<td>NR</td>
<td>NR</td>
<td>0/12</td>
<td>0%</td>
<td>Case series</td>
</tr>
<tr>
<td>Campbell 2005</td>
<td>PCR Testing</td>
<td>NR</td>
<td>414/776</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Prospective, case series</td>
</tr>
<tr>
<td>Wolanski 2005</td>
<td>PCR Testing</td>
<td>NR</td>
<td>73/150</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Case series</td>
</tr>
<tr>
<td>Campbell 2006</td>
<td>PCR Testing</td>
<td>NR</td>
<td>NR</td>
<td>83/152</td>
<td>NR</td>
<td>NR</td>
<td>Case series</td>
</tr>
<tr>
<td>Tefferi 2005</td>
<td>PCR Testing</td>
<td>NR</td>
<td>NR</td>
<td>80/157</td>
<td>NR</td>
<td>NR</td>
<td>Case series</td>
</tr>
</tbody>
</table>

NR, not reported; PCR, polymerase chain reaction

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*<sup>V617F</sup> mutation is a highly specific marker for clonal evidence of a 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*<sup>V617F</sup> testing could dramatically expedite diagnosis by reducing the need for complex work-ups of secondary or reactive causes of the observed proliferative process in the *JAK2*<sup>V617F</sup> positive patients. 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 work-up of patients suspected to have Philadelphia-negative MPNs, several reports recommending new algorithms for diagnosis were published. The 2001 WHO criteria were revised in 2008 to reflect incorporation of the test in patient work-up.

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, two 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 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 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 four 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 two patients (one with erythrocytosis and one 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 nonclassical 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 nonclassical forms of MPNs such as chronic myelomonocytic leukemia, hypereosinophilic syndrome, systemic mastocytosis, chronic neutrophilic leukemia, or others. Reports have appeared that identify $JAK2^{V617F}$ mutations in some of these cases. Due to the paucity of data about the significance or use of $JAK2^{V617F}$ or $MPL$ mutations in these disease settings, use of the test in patients with these diseases should be considered investigational.

**Other Tyrosine Kinase or Related Mutations**

In 2007, Scott et al. identified four somatic gain-of-function mutations in the $JAK2$ exon 12 section of ten out of eleven 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. Based on these findings it was concluded that the identification of JAK2 exon 12 mutations provides a diagnostic test for JAK2V617F negative patients who present with erythrocytosis. Of note, different mutations in the same gene appear to have different effects on signaling resulting in distinct clinical phenotypes. 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 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 (MPLW515L) was discovered.

Subsequent studies identified additional mutations including MPLS505N, MPLW515Ki, and MPLW515Kii in a small but growing number of patients with ET and PMF. While this mutation can be found in both JAK2V617F-positive and negative patients, it is of particular value in the latter in helping to establish a clonal basis of the observed disease process.
Similar to the observations made on the \( JAK2^{V617F} \)-negative mutations involving exon 12, the \( MPL \) exon 10 mutations appeared to demonstrate an auto-inhibitory 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.

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 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) signalling pathway played in early B-cell development, Bercovich et al studied 88 patients with Down syndrome-acquired 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 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**

Although there has been great interest in the use of the \( JAK2^{V617F} \) test as a front line diagnostic 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.

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 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. Studies that compared quantitative measurements of allele burden with disease
manifestations have demonstrated both a positive association and a lack of association with thrombosis, fibrotic transformation and need for chemotherapy.

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. Discrepant results have been reported for thrombotic events and for fibrotic transformation. A recent meta-analysis by Dahabreh et al surveyed some 394 studies on the subject of outcomes in ET. Dahabreh concluded thrombosis but not myelofibrosis or leukemia did appear 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 and there has been little support for use of testing in patients with more general thrombosis or primary thrombocytosis. 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 $JAK2^{V617F}$ 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. However, the outcome of routine testing in both settings remained unclear. In recent international collaborative studies of patients with ET, patients with $JAK2^{V617F}$ mutations appeared at risk for arterial thrombosis but not for venous thrombosis.

A 2009 report by Hussein et al demonstrated that although there was significant overlap in $JAK2^{V617F}$ allele burden among various MPN entities, that quantitative measurements did suggest 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 non-congruent 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.
- Patients with $JAK2^{V617F}$ mutations did not show differences in the incidence of thrombosis, overall survival or leukemia free survival.
- Patients with homozygous $JAK2^{V617F}$ mutations show an increased evolution toward large splenomegaly, need of splenectomy and leukemic transformation.
- Patients with low allele burdens appeared to exhibit shortened survival perhaps because they represented a myelodepleted subset of affected patients.

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.” Effective assays can detect an allele burden as low as 1%. Investigators assessed three unpublished laboratory-developed tests and six published assays in 12 laboratories in seven countries. The detection limit of each assay was determined in seven 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 two best-performing assays were further assessed in serial samples from 28 patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) for \( \text{JAK2}^{V617F} \)-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 (i.e., 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 \( \text{JAK2}^{V617F} \) testing is used for treatment monitoring (described earlier), utility of this assay or any \( \text{JAK2}^{V617F} \) test for treatment monitoring is uncertain. Other investigators have studied methods to improve \( \text{JAK2} \) and \( \text{MPL} \) mutation testing using qPCR and novel approaches (e.g., an electrochemical DNA biosensor).

**Treatment**

Due to the strong epidemiologic and biologic literature linking \( \text{JAK2} \) pathway mutations to occurrence of MPNs, there has been considerable recent attention on using \( \text{JAK2} \) as a molecular target for drug discovery. In preclinical and early clinical studies, a number of promising \( \text{JAK2} \) inhibitors have been identified and reports have suggested some of these are useful in symptom relief. 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. It has recently been noted that benefits from tyrosine kinase therapy may not be specific for \( \text{JAK2}^{V617F} \)-positive myeloproliferative neoplasms but may be observed in wild-type disease as well.

Although identification of a drug producing long-term remissions such as imatinib in 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 \( \text{JAK2}^{V617F} \) alone does not appear to be a unique or absolutely necessary event in many patients with these diseases. The role of \( \text{JAK2}^{V617F} \) in selecting or monitoring patients for new treatments or residual neoplasia remains undefined.

Several reports suggest that \( \text{JAK2}^{V617F} \)-positive patients are more sensitive to treatment with hydroxyurea than \( \text{JAK2}^{V617F} \)-negative patients. In one study of hydroxyurea treatment in patients with PV or ET harboring the \( \text{JAK2}^{V617F} \) 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.

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 two 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. 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. 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. Patients with intermediate- or high-risk primary myelofibrosis, postpolycythemia vera myelofibrosis, or post-essential thrombocytopenia 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.

**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). In almost a dozen reports (all case series), $JAK2^{V617F}$ has been found as a unique clonal finding in patients with polycythemia vera (PV), essential thrombocytopenia primary myelofibrosis. While the association between defined diseases and the presence of the marker has been rather variable depending on the detection methods used and the study designs applied (see Table 1 in Rationale section above) test specificity is virtually 100%. Patients with PV tested using PCR methodology appear to have a test sensitivity that may approach 100% (reports up to 97%) and in the subset of patients with suspected PV who are $JAK2^{V617F}$-negative, there is compelling evidence in several case series to suggest other $JAK2$ mutations (involving exon 12) may be identified.

Given the difficulty in 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 MPNs that are Philadelphia chromosome negative. Therefore, JAK2 testing may be considered medically necessary as a diagnostic test for patients with signs and symptoms of MPN.

Mutations testing to establish disease phenotype (e.g., disease prognosis) or to select or monitor therapy remains an area of intense interest with a growing number of studies, in particular 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 if these carry a broad, albeit nonspecific pathogenetic 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 (e.g. $MPL^{W515K}$ or $MPL^{W515C}$) 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.

**Key Words:**
JAK2, tyrosine kinase, myeloproliferative neoplasms, MPN, polycythemia vera, PV, essential thrombocytopenia, ET, primary myelofibrosis, PMF, myeloproliferative leukemia, MPL, MPL mutation

**Approved by Governing Bodies:**
More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for JAK2 testing and MPL mutation testing. These tests are available as laboratory developed procedures under the U.S. Food and Drug Administration (FDA) enforcement discretion policy for laboratory-developed tests (LDTs). Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA), and laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, FDA does not require regulatory review of LDTs.
**Benefit Application:**
Coverage is subject to member’s specific benefits. Group specific policy will supersede this policy when applicable.
ITS: Home Policy provisions apply
FEP: Special benefit consideration may apply. Refer to member’s benefit plan. FEP does not consider investigational if FDA approved and will be reviewed for medical necessity.

**Current Coding:**
CPT codes:

- **81219**  
  *CALR* (calreticulin) (e.g. myeloproliferative disorders), gene analysis, common variants in exon 9 *(effective 01/01/2016)*

- **81270**  
  Jak2 (Janus Kinase 2) (e.g., myeloproliferative disorder) gene analysis, p.val617phe (*v617f*) variant *(effective 01/01/2012)*

- **81402**  
  Molecular pathology procedure, Level 3 (e.g., >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]) includes – *CYP21A2* (cytochrome *P450, family 21, subfamily A, polypeptide 2*) (e.g., congenital adrenal hyperplasia, 21-hydroxylase deficiency), common variants (e.g., IVS2-13G, P30L, I172N, exon 6 mutation cluster [I235N, V236E, M238K], V281L, L307FfsX6, Q318X, R356W, P453S, G110VfsX21, 30-kb deletion variant)

- **81403**  
  Molecular pathology procedure, Level 4 (NEW TESTING)

**Previous Coding:**
CPT codes:

There are no specific CPT codes for JAK2 or MPL mutation analysis. Multiple codes describing genetic analysis would likely be used (e.g., codes from **83890-83914**) *(deleted effective 01/01/13)*

**References:**


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


Policy History:
Medical Policy Group, January 2010 (1)
Medical Policy Administration Committee, February 2010
Available for comment February 23-April 8, 2010
Medical Policy Group, January 2011: Key Points
Medical Policy Group, December 2011 (1): Added Code 81270 for 2012 Updates
Medical Policy Group, January 2012 (1): Policy title changed to reflect that MPL is not a tyrosine kinase; Update to Key Points and References related to MPP update; no change in policy statement; Added new codes 81402 and 81403 related to JAK2 and MPL testing
Medical Policy Group, December 2012 (3): 2013 Coding Update, verbiage change on 81402 & 81403 and code range 83890-83914 deleted effective 01/01/2013.
Medical Policy Panel, February 2013
Medical Policy Group, February 2013 (1): Update to Key Points and References; no change to policy statement.
Medical Policy Panel, February 2014
Medical Policy Group, February 2014 (1): Update to Key Points and References; no change to policy statement.
Medical Policy Group, November 2014: 2015 Coding Update to wording in codes 81402 & 81403
Medical Policy Group, January 2015: Update to description for CPT code 81402
Medical Policy Panel, February 2015  
Medical Policy Group, February 2015 (3): 2015 Updates to Description, Key Points, Approved by Governing Bodies and References  

This medical policy is not an authorization, certification, explanation of benefits, or a contract. Eligibility and benefits are determined on a case-by-case basis according to the terms of the member’s plan in effect as of the date services are rendered. All medical policies are based on (i) research of current medical literature and (ii) review of common medical practices in the treatment and diagnosis of disease as of the date hereof. Physicians and other providers are solely responsible for all aspects of medical care and treatment, including the type, quality, and levels of care and treatment.  

This policy is intended to be used for adjudication of claims (including pre-admission certification, pre-determinations, and pre-procedure review) in Blue Cross and Blue Shield’s administration of plan contracts.