JAK2, MPL, and CALR Testing for Myeloproliferative Neoplasms

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Last Review: 06/2018
Next Review: 06/2019

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 testing may be considered medically necessary in the diagnosis of patients presenting with clinical, laboratory, or pathologic findings suggesting polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF). Based on criteria from the World Health Organization, documentation of a serum erythropoietin level below the reference range for normal is recommended before JAK2 testing (See Considerations).

MPL and CALR testing may be considered medically necessary in the diagnosis of patients presenting with clinical, laboratory, or pathologic findings suggesting essential thrombocythemia or primary myelofibrosis.

When Policy Topic is not covered
JAK2, MPL and CALR 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

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 and CALR variants is warranted.
CRITERIA FOR POLYCYTHEMIA TESTING
Based on the World Health Organization (WHO) major and minor criteria (see Table PG1), documentation of serum erythropoietin level below the reference range for normal meets a minor criterion for polycythemia vera. Therefore, serum erythropoietin testing is recommended before JAK2 testing.

Table PG1. WHO Diagnostic Criteria for Polycythemia Vera

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Criteria</td>
<td></td>
</tr>
<tr>
<td>- Increased hemoglobin level (&gt;16.5 g/dL in men or &gt;16.0 g/dL in women)</td>
<td></td>
</tr>
<tr>
<td>- Increased hematocrit (&gt;49% in men or &gt;48% in women)</td>
<td></td>
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<tr>
<td>- Other evidence of increased red cell volume</td>
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<tr>
<td>- Bone marrow biopsy showing hypercellularity for age with trilineage maturation, including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)</td>
<td></td>
</tr>
<tr>
<td>- JAK2 V617F or JAK2 exon 12 variant detected</td>
<td></td>
</tr>
<tr>
<td>Minor Criterion</td>
<td>Serum erythropoietin level below the reference range for normal</td>
</tr>
</tbody>
</table>

WHO: World Health Organization.

Genetics Nomenclature Update
The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG2). The Society’s nomenclature is recommended by the Human Variome Project, the HUman Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG3 shows the recommended standard terminology—“pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” and “benign”—to describe variants identified that cause Mendelian disorders.

Table PG2. Nomenclature to Report on Variants Found in DNA

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Variant</td>
<td>Change in the DNA sequence</td>
<td></td>
</tr>
<tr>
<td>Familial variant</td>
<td>Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
<td></td>
</tr>
</tbody>
</table>
Table PG3. ACMG-AMP Standards and Guidelines for Variant Classification

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

Genetic Counseling
Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual’s family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Description of Procedure or Service

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals: With a suspected myeloproliferative neoplasm</td>
<td>Interventions of interest are: Genetic testing for JAK2</td>
<td>Comparators of interest are: Standard clinical management without genetic testing</td>
<td>Relevant outcomes include: Overall survival, Disease-specific survival, Test accuracy, Test validity, Resource utilization</td>
</tr>
<tr>
<td>Individuals: With a suspected myeloproliferative neoplasm</td>
<td>Interventions of interest are: Genetic testing for MPL</td>
<td>Comparators of interest are: Standard clinical management without genetic testing</td>
<td>Relevant outcomes include: Overall survival, Disease-specific survival, Test accuracy, Test validity, Resource utilization</td>
</tr>
<tr>
<td>Individuals: With a suspected myeloproliferative neoplasm</td>
<td>Interventions of interest are: Genetic testing for CALR</td>
<td>Comparators of interest are: Standard clinical management without genetic testing</td>
<td>Relevant outcomes include: Overall survival, Disease-specific survival, Test accuracy, Test validity, Resource utilization</td>
</tr>
</tbody>
</table>

Somatic (acquired) genetic variants in JAK2, MPL, and CALR genes have been implicated as the underlying molecular genetic drivers for the pathogenesis of myeloproliferative neoplasms (MPNs). This policy addresses the use of genetic testing of JAK2 and CALR genes for the diagnosis, prognosis, and treatment selection in patients with MPNs.

For individuals with a suspected MPN who receive genetic testing for JAK2, the evidence includes case series, retrospective studies, meta-analyses, and randomized control trials. Relevant outcomes include overall survival, disease-
specific survival, test accuracy and validity, and resource utilization. For patients with suspected Philadelphia chromosome-negative (Ph-negative) MPN, JAK2 variants are found in nearly 100% of those with polycythemia vera, 60% to 65% of those with essential thrombocythemia, and 60% to 65% of those with primary myelofibrosis. In individuals with suspected MPN, a positive genetic test for JAK2 satisfies a major criterion for the 2016 World Health Organization classification for Ph-negative MPNs and eliminates secondary or reactive causes of erythrocytosis and thrombocythemia from the differential diagnosis. The presence of a documented JAK2 variant may aid in the selection of ruxolitinib, a JAK2 inhibitor; ruxolitinib, however, is classified as a second-line therapy. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with a suspected MPN who receive genetic testing for MPL, the evidence includes case series and retrospective studies. Relevant outcomes include overall survival, disease-specific survival, test accuracy and validity, and resource utilization. For patients with suspected Ph-negative MPN, MPL variants are found in approximately 5% of those with essential thrombocythemia (ET) and primary myelofibrosis cases (PMF). In individuals with suspected MPN, a positive genetic test for MPL satisfies a major criterion for the 2016 World Health Organization classification for ET and PMF and eliminates secondary or reactive causes of thrombocythemia from the differential diagnosis. The goal of ET treatment is to alleviate symptoms and minimize thrombotic events and bleeding irrespective of MPL variant status. For PMF, hematopoietic cell transplantation is the only treatment with curative potential while most other treatment options focus on alleviation of symptoms. However, in both ET and PMF, establishing the diagnosis through MPL genetic testing does not in and of itself result in changes in management that would be expected to improve net health outcome. Thus clinical utility has not been established. The evidence is insufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with a suspected MPN who receive genetic testing for CALR, the evidence includes case series and retrospective studies. Relevant outcomes include overall survival, disease-specific survival, test accuracy and validity, and resource utilization. For patients with suspected Ph-negative MPN, CALR variants are found in approximately 20% to 25% of those with ET and PMF. For individuals with suspected MPN, a positive genetic test for CALR satisfies a major criterion for the WHO classification for ET and PMF and eliminates secondary or reactive causes of thrombocythemia from the differential diagnosis. The goal of ET treatment is to alleviate symptoms and minimize thrombotic events and bleeding irrespective of CALR variant status. For PMF, hematopoietic cell transplantation is the only treatment with curative potential while most other treatment options focus on alleviation of symptoms. However, in both ET and PMF, establishing the diagnosis through CALR genetic testing does not result in changes in management that would be expected to improve net health outcome. Thus clinical utility has not been established. The evidence is insufficient to determine that the technology results in a meaningful improvement in the net health outcome.
Background
Myeloproliferative Neoplasms
Myeloproliferative neoplasms (MPNs) are rare overlapping blood diseases characterized by the production of one or more blood cell lines. The most common forms of MPNs include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML). A common finding in many MPNs is clonality and a central pathogenic feature the detection of a somatic (acquired) pathogenic variant in disease-associated genes. Pathogenic variants in disease-associated genes result in constitutively activated tyrosine kinase enzyme or cell surface receptor.

CML and Philadelphia Chromosome
The paradigm for the use of molecular genetics to revolutionize patient management is CML. A unique chromosomal translocation t(9;22), the Philadelphia chromosome (Ph), leads to a unique gene rearrangement (BCR-ABL) creating a fusion gene that encodes for a constitutively active Bcr-abl fusion protein. These findings led to the development of targeted tyrosine kinase inhibitor drug therapy (imatinib) that produces long-lasting remissions. The remainder of this evidence review focuses only on the non-Ph or Ph-negative MPNs with a focus on genetic testing for JAK2, CALR, and MPL.

Ph-Negative MPNs
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, or 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 and updated in 2008 and 2016. Applying these criteria have been challenging 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.

Molecular Genetics of Ph-Negative MPNs

JAK2 Gene
The JAK2 gene, located on chromosome 9, contains the genetic code for making the Janus kinase 2 protein, a nonreceptor tyrosine kinase. The Janus kinase 2 (JAK2) protein is part of the JAK/STAT signal transduction pathway that is important for the controlled production of blood cells from hematopoietic stem cells. Somatic (acquired) variants in the JAK2 gene are found in patients with PV (≈96%), ET (50%), and PMF (50%).

CALR Gene
The CALR gene, located on chromosome 19, contains the genetic code for making the calreticulin protein, a multifunctional protein located in the endoplasmic
Reticulum, cytoplasm, and cell surface. The calreticulin protein is thought to play a role in cell growth and division and regulation of gene activity. Somatic variants in the \textit{CALR} gene are associated with ET and PMF.\textsuperscript{5}

\textbf{MPL Gene}

The \textit{MPL} gene, located on chromosome 1, contains the genetic code for making the thrombopoietin receptor, a cell surface protein that stimulates the JAK/STAT signal transduction pathway. The thrombopoietin receptor is critical for the cell growth and division of megakaryocytes, which produce platelets involved in blood clotting. Somatic variants in the \textit{MPL} gene are associated with ET and PMF.\textsuperscript{6}

\textbf{Frequency of JAK2, CALR, and MPL Somatic Variants in Ph-Negative MPNs}

Ph-negative MPNs are characterized by their molecular genetic alterations. Table 1 summarizes the driver genes and somatic variants associated with specific Ph-negative MPNs.\textsuperscript{7}

\begin{table}[h!]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Ph-Negative MPNs & \textbf{JAK2 Somatic Variant Detected,} & \textbf{CALR Somatic Variant Detected,} & \textbf{MPL Somatic Variant Detected,} \\
 & \% of Patients & \% of Patients & \% of Patients \\
\hline
Polycythemia vera & \textit{JAK2} V617F, 95\% & & \\
 & \textit{JAK2} exon12 variants, 5\% & & \\
\hline
Essential thrombocythemia & \textit{JAK2} V617F, 60\%-65\% & \textit{CALR} exon 9 indels, 20\%-25\% & \textit{MPL} exon 10 variants, 5\% \\
\hline
Primary myelofibrosis & \textit{JAK2} V617F, 60\%-65\% & \textit{CALR} exon 9 indels, 20\%-25\% & \textit{MPL} exon 10 variants, 5\% \\
\hline
\end{tabular}
\caption{Frequency of JAK2, CALR, and MPL Somatic Variants in Ph-Negative MPNs\textsuperscript{2}}
\end{table}

\textsuperscript{7}MPN: myeloproliferative neoplasm; Ph: Philadelphia chromosome.

\textbf{Regulatory Status}

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for \textit{JAK2}, \textit{CALR}, and \textit{MPL} testing under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

\textbf{Rationale}

This evidence review was created in January 2010 and has been updated regularly searches of the MEDLINE database. The most recent literature review was performed through April 25, 2017 (see Appendix Table 1 for genetic testing categories).

Validation of the clinical use of any genetic test focuses on 3 main principles: (1) analytic validity, which refers to the technical accuracy of the test in detecting a
variant that is present or in excluding a variant that is absent; (2) clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and (3) clinical utility (ie, 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). The following is a summary of the key literature.

**Diagnosis**

**Classic Myeloproliferative Neoplasms**
Diagnosis of the various classic forms of myeloproliferative neoplasms (MPNs) has been based most recently on a complex set of clinical, pathologic, and biologic criteria first introduced by the Polycythemia Vera Study Group in 1996 or by the World Health Organization (WHO) in 2001 (updated 2008 and 2016). Both classifications use a combination of clinical, pathologic, and/or biologic 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 Background section (discussed above), some diagnostic methods (eg, bone marrow microscopy) are not well-standardized, and others (eg, endogenous erythroid colony formation) are neither standardized nor widely available.

**Nonclassic Forms of MPNs**
Although the most common Ph-negative MPNs include what is 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 variants in some of these cases.

**JAK2 Testing in Individuals with a suspected myeloproliferative neoplasm**

**Clinical Context and Test Purpose**
The purpose of JAK2 testing of individuals with a suspected MPN is to establish a molecular genetic diagnosis of MPN to inform management decisions.

The question addressed in this evidence review is: In individuals with a suspected MPN, does the use of JAK2 testing result in improvement in health outcomes?

The following PICOTS were used to select literature to inform this review.

**Patients**
The relevant population of interest includes individuals with a suspected MPN.
**Interventions**
The relevant intervention of interest is genetic testing for JAK2.

**Comparators**
The relevant comparator of interest is standard clinical management without genetic testing.

**Outcomes**
The potential beneficial outcomes of primary interest include establishing a molecular genetic diagnosis of PV, ET, or PMF to inform management decisions when test results are provided.

**Timing**
The time frame for outcomes measures varies from several months for the improvement of symptoms to long-term survival as a result of disease-related complications.

**Setting**
Patients with a suspected MPN are actively managed by hematologists and oncologists.

**Analytic Validity**
McClure et al (2006) described 2 assays: allele-specific polymerase chain reaction (AS-PCR) and a melting curve assay to detect JAK2 V617F. The AS-PCR test has a maximum sensitivity of 0.01% to 0.1% while the melting curve analysis assay has a moderate sensitivity of 1% to 10%.

Rapado et al (2009) reported on high-resolution melting (HRM) analysis for JAK2 exon 12 and 14 variants. The analytic sensitivity was reported at near 1% for the detection of both JAK2 exon 12 and 14 variants.

In 2013, the European LeukemiaNet and MPN&MPNr–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 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 wild-type and mutated cell line DNA and plasmid standards. DNA detection was verified using pyrosequencing. Sensitivity and specificity of the 2 best-performing assays were further assessed in serial samples from 28 patients who underwent allogeneic hematopoietic cell transplantation for JAK2 V617F–positive disease and in 100 peripheral blood samples from healthy controls.
**Section Summary: Analytic Validity**

Evidence on analytic validity includes multiple validation studies published by test developers and systematic evaluation of published and unpublished laboratory assays. The analytic validity is expected to be high when testing is performed according to optimal laboratory standards.

**Clinical Validity**

**JAK2 V617F Variant**

In March and April 2005, 4 separate groups using different modes of discovery and different measurement techniques reported on the presence of a novel somatic (acquired) single-nucleotide variant (SNV) in the conserved autoinhibitory pseudokinase domain of the gene encoding JAK2 protein in patients with classic MPNs. The SNV caused a valine-to-phenylalanine substitution at amino acid position 617 (JAK2 V617F) leading to a novel somatic gain-of-function SNV that resulted in loss of autoinhibition of the JAK2 tyrosine kinase. JAK2 V617F is a constitutively activated kinase that recruits and phosphorylates 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.

The JAK2 V617F variant was present in blood and bone marrow from a variable portion of patients with classic BCR-ABL-negative (ie, Ph-negative) MPNs including 65% to 97% of patients with PV, 23% to 57% with ET, and 35% to 56% with PMF (see Table 2). The variant was initially reported to be absent in all normal subjects and patients with secondary erythrocytosis, although very low levels of cells carrying the variant have been reported in a small subset of healthy individuals.

<table>
<thead>
<tr>
<th>Study</th>
<th>Variant Detection Method</th>
<th>PV</th>
<th>ET</th>
<th>PMF</th>
<th>Normals</th>
<th>Secondary Erythrocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baxter et al (2005)</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>
</tr>
<tr>
<td>Levine et al (2005)</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>
</tr>
<tr>
<td>James et al (2005)</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>
</tr>
<tr>
<td>Kralovics et al (2005)</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>
</tr>
<tr>
<td>Jones et al (2005)</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>
</tr>
<tr>
<td>Tefferi et al (2006)</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>
</tr>
<tr>
<td>Zhao et al (2005)</td>
<td>DNA sequencing</td>
<td>20/24 (83)</td>
<td>NR</td>
<td>NR</td>
<td>0/12 (0)</td>
<td>NR</td>
</tr>
</tbody>
</table>
In almost a dozen reports (all case series), it has been suggested that the JAK2 mutated protein potentially caused the disease because it was demonstrated 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 given transplanted bone marrow cells infected with a retrovirus containing the variant developed a myeloproliferative syndrome.\(^{20}\)

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

Early reports have 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 variant, that \textit{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 \textit{JAK2} V617F-negative classic MPNs.\(^{30}\) Two important caveats should be noted about this test: (1) a negative result cannot be used to rule out classic MPN and (2) a positive result is credible evidence that a classic MPN is present but alone is insufficient to subclassify the disease category.

In recognition of the value of this marker for refining the diagnostic workup of patients suspected of having Ph-negative MPNs, several reports recommended new diagnostic algorithms.\(^{31,32}\) The 2001 WHO criteria were revised in 2008 and 2016 to incorporate genetic testing in patient workup.\(^{2,3,33}\)

In 2006, James et al compared PV diagnosed using either WHO or the Polycythemia Vera Study Group criteria with a streamlined diagnostic approach for PV using a 2-step algorithm (elevated hematocrit levels and the presence of the \textit{JAK2} V617F variant).\(^{31}\) Although the groups studied were small (45 patients with a
Polycythemia Vera Study Group diagnosis of PV, 61 patients meeting WHO criteria), use of the 2-step algorithm resulted in a correct diagnosis in 96% (the Polycythemia Vera Study Group criteria) or 93% (WHO criteria) of patients with PV.

Studies of new drugs targeted to treat the mutated tyrosine kinase in patients with MPNs are expected to cast additional light on the functionality of the observed JAK2 V617F variant and are likely to contribute not only to refined treatment choices but also to better understanding of the diagnostic role of this important marker.

**JAK2 Exon 12 Variants**
In 2007, Scott et al identified 4 somatic gain-of-function variants in JAK2 exon 12 in 10 of 11 PV patients without the JAK2 V617F variant. Patients with a JAK2 exon 12 variant differed from those with the JAK2 V617F variant, 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 variants with similar functional consequences in patients with PV and patients with idiopathic erythrocytosis. Based on these findings, it was concluded that the identification of JAK2 exon 12 variants provides a diagnostic test for JAK2 V617F–negative patients who present with erythrocytosis. Of note, different variants in the same gene appear to have different effects on signaling, resulting in distinct clinical phenotypes.

The 2016 WHO criteria specifically recommended testing for JAK2 exon 12 variants in patients with suspected PV (presumably in patients who are JAK2 V617F–negative). The criteria suggested testing for JAK2 V617F or other clonal markers in patients with ET.

**Section Summary: Clinical Validity**
Evidence of the clinical validity of JAK2 V617F and exon 12 variant testing includes prospective studies and case series. In PV patients, the JAK2 V617F variants were found in approximately 95% of cases while JAK2 exon 12 variants were found in the 5%. In ET and PMF patients, JAK2 V617F variants were detected in more than 50% of cases. Additionally, the 2016 WHO diagnostic criteria incorporated the JAK2 V617F variants for PV, ET, and PMF and JAK2 exon 12 variants for PV.

**Clinical Utility**
Testing for JAK2 V617F or JAK2 exon 12 variants have potential clinical utility in several different clinical scenarios:

1. Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic MPNs (PV, ET, or PMF);
2. Phenotyping of disease subtypes in patients with MPNs to establish disease prognosis;

**Molecular Genetic Diagnosis for Ph-negative MPNs**
Diagnosis of Ph-negative MPNs is challenging due to the overlapping clinical features across MPNs, reactive processes, and complex diagnostic algorithms. The presence of JAK2 V617F or JAK2 exon 12 variants is considered a major criterion for the PV. The presence of a JAK2 variant is also a major criterion for ET and PMF.

**Molecular Profiling: Phenotype and Genotype Associations and Impact on Prognosis**
The use of JAK2 V617F testing has been used as a front-line diagnostic test in the evaluation of Ph-negative MPN patients. Efforts have been made to link the presence of JAK2 variants and the quantitative measurement of its allele burden with clinical features and biologic behavior. Unfortunately, due to differences in disease definitions, differences in methods of testing, differences in sample type (bone marrow vs circulating blood cells), and differences in study designs, the literature in this area is conflicting and inconclusive.

Because most patients with PV exhibit the JAK2 V617F variant, attention has focused on differences in the disease’s presence in the homozygous vs heterozygous state and on whether allele burden correlates with clinical or laboratory features.

Studies have reported on a range of findings, including the association between homozygous states and 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 lack of an association with thrombosis, fibrotic transformation, and need for chemotherapy.

The impact of the presence of the JAK2 V617F variants in patients with ET is also controversial. In several studies, the presence of this variant 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 2009 meta-analysis by Dahabreh et al surveyed 394 studies on outcomes in ET. Reviewers concluded that thrombosis but not myelofibrosis or leukemia appeared to be influenced by the presence of JAK2 variants. Reviewers 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 the use of testing in patients with more general thrombosis or primary thrombocytosis. Results for splanchnic events have been contradictory.
In a 2010 retrospective study that assessed JAK2 V617F variants in patients treated for thrombosis in ET and in unselected patients with splanchic vein thrombosis, JAK2 V617F variants were more common in patients with splanchic vein thrombosis; further the study 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 variants appeared at risk for arterial thrombosis but not for venous thrombosis.

**Disease Monitoring**
A 2009 report by Hussein et al 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 variant 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 variants required fewer blood transfusions but exhibited poorer overall survival than those without the variant.
- Patients with JAK2 V617F variants did not show differences in the incidence of thrombosis, overall survival, or leukemia-free survival.
- Patients with homozygous JAK2 V617F variants showed 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.

The most sensitive assay performed consistently across various quantitative PCR platforms and detected mutant allele (ie, minimal residual disease) in transplant recipients at 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 hematopoietic cell transplantation. Although the study supported the analytic validity of the assay, given the inconsistency of outcomes when JAK2 V617F testing is used for treatment monitoring (described earlier), the utility of this assay or any JAK2 V617F test for treatment monitoring is uncertain. Other investigators have studied methods to improve JAK2 and MPL variant testing using quantitative PCR and novel approaches (eg, an electrochemical DNA biosensor).

**Treatment**

*Treatment With Hydroxyurea*
Several reports have suggested that JAK2 V617F–positive patients are more sensitive to treatment with hydroxyurea than JAK2 V617F–negative patients. In a 2010 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.54

Treatment With JAK2 Inhibitors
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 are useful in symptom relief.55 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 the 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 MPNs have been reported.56-58 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.59

In November 2011, ruxolitinib (a JAK kinase inhibitor) was approved by the U.S. Food and Drug Administration for the treatment of intermediate- and high-risk myelofibrosis (including primary myelofibrosis, post-polycythemia vera myelofibrosis, and postessential thrombocythemia myelofibrosis) based on results from 2 randomized controlled trials (RCTs). One, a double-blind RCT (2012) in patients with intermediate- to high-risk myelofibrosis, randomized participants to twice-daily oral ruxolitinib (n=155) or to placebo (n=154) and followed patients for 76 weeks (Controlled Myelofibrosis Study with oral JAK Inhibitor Treatment [COMFORT-I]).60 The primary outcome (a ≥35% 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, there were 10 (6.5%) deaths in the ruxolitinib group and 14 (9.1%) deaths in the placebo group (Kaplan-Meier method, p=0.33). With 4 additional months of follow-up (median, 51 weeks total follow-up), there were 13 (8.4%) total deaths in the ruxolitinib group and 24 (15.6%) total deaths in the placebo group (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 (11%) and placebo (10.6%) groups. In post hoc subgroup analysis of patients with the JAK2 V617F variant, 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 variant, 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 variant were -52.6% in the ruxolitinib group and +42.8% in the placebo group (higher scores indicate more severe symptoms); in patients without the variant, changes in total symptom score were -28.1% and +37.2%, respectively.
A second trial by Harrison et al (2012) reached similar conclusions (COMFORT-II).61 Patients with intermediate- or high-risk primary myelofibrosis, postpolycythemia vera myelofibrosis, or postessential thrombocytopenia myelofibrosis received oral ruxolitinib (n=146) or best available therapy (n=73). No differences in overall survival were 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, the incidence of spleen reduction was 33% in the ruxolitinib group and 0% in the control group; in the JAK2 V617F-negative subgroup, the 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.

A follow-up to COMORT-I, published in 2015, provided data on a median 3-year follow-up.62 At a median of 149 weeks (range, 19-175 weeks), 77 (49.7%) of the 155 patients originally randomized to ruxolitinib were still receiving therapy. One hundred eleven of 154 patients whom originally received placebo crossed over to receive ruxolitinib, and, of these, 57 (51.4%) were still receiving the drug. Of the patients originally randomized to receive therapy, discontinuation rates were 21% at 1 year, 35% at 2 years, and 51% at year 3. Reasons for discontinuing ruxolitinib were disease progress (23.1%), adverse events (19.2%), death (19.2%), and withdrawal of consent (15.4%). The initial primary outcome measure of this study was a reduction in spleen volume, and, in this follow-up study, reductions in spleen size were durable with longer term treatment. Mean percentage change from baseline was -31.6% at week 24 and -34.1% at week 144. Of patients initially randomized to ruxolitinib, 91 (59%) of 155 of patients achieved a 35% or more reduction in spleen volume at any time during study follow-up. The probability of maintaining this same reduction for at least 132 weeks was 0.53, and more than 80% of patients maintained a reduction of at least 10%. Regarding overall survival, 42 patients randomized to ruxolitinib died while 54 in the placebo group died. With a median follow-up of 149.1 and 149.3 weeks for ruxolitinib and placebo, respectively, the hazard ratio for overall survival favored patients in the ruxolitinib arm (hazard ratio, 0.69; 95% confidence interval, 0.46 to 1.03; p=0.067). Anemia and thrombocytopenia were the most common adverse hematologic events and were highest during the first 6 months of therapy, both of which subsequently increased to a new steady state. The most common nonhematologic adverse events, which occurred more commonly in the ruxolitinib group, were ecchymosis (18.7%), dizziness (14.8%), and headache (14.8%). Additionally, more patients treated with study drug developed urinary tract infections and herpes zoster, although the incidence of these infections did not increase with length of therapy. All herpes zoster infections were grade 1 or 2, and no other opportunistic infections were identified during follow-up. Four new cases of acute myeloid leukemia were reported since the first analysis published in
2012, two in patients originally randomized to ruxolitinib and two in the placebo arm, for a total of 8 cases since the study began. The rate of leukemic transformation per person-year of ruxolitinib exposure was 0.0121 per person-year and 0.0233 per person-year in patients originally randomized to ruxolitinib or placebo, respectively.

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 the JAK2 V617F variant in selecting or monitoring patients for new treatments or residual neoplasia remains undefined.

Treatment With Imetelstat
Other drugs to treat MPNs are being evaluated. Two studies in 2015 described imetelstat, which inhibits telomerase enzymatic activity. One was a pilot study looking at its use in patient myelofibrosis, the other a phase 2 study looking at imetelstat in patients with ET. Both studies demonstrated hematologic and molecular responses in patients with JAK2 variants, although clinically significant myelosuppression may be an obstacle to its use.

In 2015, results of a phase 2 study for pacritinib were published. This drug is a Janus kinase 2, JAK2 V617F, and Fms-like tyrosine kinase 3 inhibitor, which has demonstrated a favorable safety profile with promising efficacy in phase 1 studies in patients with primary and secondary myelofibrosis.

Section Summary: Clinical Utility
Direct evidence for the clinical utility of JAK2 testing includes meta-analyses, retrospective studies, and RCTs. Evidence for JAK2 testing for phenotyping and monitoring provides conflicting results. However, the presence of JAK2 V617F or JAK2 exon 12 variants is considered a major criterion for the diagnosis of PV, ET, and PMF. JAK2 V617F and JAK2 exon 12 testing allow secondary or reactive erythrocytosis or thrombocytosis to be differentiated from PV, ET, and PMF.

MPL Testing in Individuals with a suspected myeloproliferative neoplasm

Clinical Context and Test Purpose
The purpose of MPL testing of individuals with a suspected MPN is to establish a molecular genetic diagnosis of MPN to inform management decisions.

The question addressed in this evidence review is: In individuals with a suspected MPN, does the use of MPL testing result in improvement in health outcomes?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest includes individuals with a suspected MPN.
**Interventions**
The relevant intervention of interest is genetic testing for *MPL*.

**Comparators**
The relevant comparator of interest is standard clinical management without genetic testing.

**Outcomes**
The potential beneficial outcomes of primary interest include establishing a molecular genetic diagnosis of ET or PMF to inform management decisions when test results are positive.

**Timing**
The time frame for outcomes measures varies from several months for the improvement of symptoms to long-term survival as a result of disease-related complications.

**Setting**
Patients with a suspected MPN are actively managed by hematologists and oncologists.

**Analytic Validity**
Ivanova et al (2011) published on a novel multiplex bead-based assay with locked nucleic acids (LNA)-modified probes for detection of *MPL* exon 10 variants. The assay was designed to detect specific *MPL* variants, W515L, W515K, W515A, and W515R. The sensitivity of the assay was reported at 1% for detection of variants.

Furtado et al (2013) reported on a novel AS-PCR-based assay for the detection of *MPL* exon 10 variants. The assay was designed to detect most recurrent *MPL* exon 10 variants associated with ET and PMF and included W515L, W515K, W515A, and S505N. The sensitivity was reported at 2.5% variant allele frequency. In the study, Sanger sequencing did not detect 7 positive cases when the variant allele frequency was less than 5%. Sanger sequencing could not reliably detect 10 cases with variant allele frequencies between 5% and 15%.

**Section Summary: Analytic Validity**
Evidence on the analytic validity of detecting *MPL* exon 10 variants is limited. However, it is expected that the analytic validity will be high when testing is performed according to optimal laboratory standards.

**Clinical Validity**
In 2006, Pikman et al surveyed *JAK2* variant–negative patients with suspected ET and PMF to determine whether variants in pathways complementary to *JAK2* signaling could be identified. A genetic variant of the thrombopoietin receptor gene (*MPL*) at codon 515 (exon 10) causing a change from tryptophan to leucine (*MPL* W515L) was discovered.
Subsequent studies have identified additional variants including \textit{MPL} S505N, \textit{MPL} W515Ki, and \textit{MPL} W515Kii in a small but growing number of patients with ET and PMF (see Table 3).\cite{68-71} Although these variants can be found in both \textit{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>
<thead>
<tr>
<th>Study</th>
<th>Variant 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)\cite{67}</td>
<td>DNA sequencing</td>
<td>0/10 (0)</td>
<td>0/50 (0)</td>
<td>4/45 (8.8)</td>
<td>0/270 (0)</td>
<td>JAK2 negative</td>
<td></td>
</tr>
<tr>
<td>Pardanani et al (2006)\cite{68}</td>
<td>Site 1: PCR with DNA sequencing</td>
<td>0/38 (0)</td>
<td>2/167 (1)</td>
<td>8/198 (4)</td>
<td>0/64 (0)</td>
<td>3/118 (2.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Site 2: DNA sequencing</td>
<td>0/20 (0)</td>
<td>2/151 (1)</td>
<td>5/92 (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer et al (2008)\cite{69}</td>
<td>PCR testing</td>
<td>–</td>
<td>Prel: 3/88 (3.4)</td>
<td>Pros: 32/776 (4.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancrazi et al (2008)\cite{70}</td>
<td>PCR testing</td>
<td>0/50 (0)</td>
<td>–</td>
<td>19/217 (8.7)</td>
<td>0/60 (0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Ruan et al (2009)\cite{71}</td>
<td>PCR testing</td>
<td>0/32 (0)</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>
</tr>
<tr>
<td>Schnittger et al (2009)\cite{72}</td>
<td>PCR testing</td>
<td>–</td>
<td>19/356 (5.3)</td>
<td>10/193 (5.2)</td>
<td>–</td>
<td>2/269 (0.8)</td>
<td></td>
</tr>
</tbody>
</table>

Values are n/N (%).
ET: essential thrombocythemia; MPN: myeloproliferative neoplasm; PCR: polymerase chain reaction; Ph: Philadelphia chromosome; PMF: primary myelofibrosis; Prel: preliminary; Pros: prospective; PV: polycythemia vera.

Similar to observations about \textit{JAK2} V617F–negative variants in exon 12, \textit{MPL} exon 10 variants appear to demonstrate an autoinhibitory role leading to receptor activation in the absence of thrombopoietin binding. Expression of the \textit{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.\cite{68}

Although data sets are small, \textit{MPL} exon 10 variants are unique and have been demonstrated to be associated with MPNs, and exhibit in vitro and murine model behavior consistent with a causative role in MPNs. The 2016 WHO criteria specifically cited testing \textit{MPL} exon 10 variants in patients with ET and PMF. The criteria included testing for \textit{MPL} exon 10 variants in patients with ET and PMF.

\textbf{Section Summary: Clinical Validity}
Evidence of the clinical validity \textit{MPL} exon 10 variants includes case series. In patients with ET and PMF, the \textit{MPL} exon 10 variants were found in approximately
5% of cases. In ET and PMF patients, the 2016 WHO incorporated \textit{MPL} exon 10 variants as a major criterion for the diagnosis of ET and PMF.

**Clinical Utility**
Testing for \textit{MPL} exon 10 variants has potential clinical utility in several different clinical scenarios:

1. Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic ET or PMF;

\textit{MPL} exon 10 variants are detected in approximately 5% of patients with ET and PMF. No RCTs were identified that used the results of \textit{MPL} exon 10 variant testing to guide treatment and management decisions. The goals of treatment and management for ET are to alleviate symptoms and minimize complications of disease such as thrombotic events and bleeding, though establishing the diagnosis does not lead to preventive management. For PMF, hematopoietic cell transplantation is the only treatment with curative potential while most other treatment options focus on alleviation of symptoms. However, in both ET and PMF, establishing the diagnosis through \textit{MPL} genetic testing does not result in changes in management that would be expected to improve net health outcome.

**Section Summary: Clinical Utility**
Direct evidence for the clinical utility of \textit{MPL} testing is lacking. While \textit{MPL} exon 10 testing has potential utility in diagnosing ET and PMF using the 2016 WHO major criteria for MPNs and excluding reactive or secondary causes of thrombocytosis, there is no change in management that would be expected to improve net health outcome. Thus clinical utility has not been established.

**\textit{CALR2} Testing in Individuals with a suspected myeloproliferative neoplasm**

**Clinical Context and Test Purpose**
The purpose of \textit{CALR} testing of individuals with a suspected MPN is to establish a molecular genetic diagnosis of MPN to inform management decisions.

The question addressed in this evidence review is: In individuals with a suspected MPN, does the use of \textit{CALR} testing result in improvement in health outcomes?

The following PICOTS were used to select literature to inform this review.

**Patients**
The relevant population of interest includes individuals with a suspected MPN.

**Interventions**
The relevant intervention of interest is genetic testing for \textit{CALR}.
**Comparators**
The relevant comparator of interest is standard clinical management without genetic testing.

**Outcomes**
The potential beneficial outcomes of primary interest include establishing a molecular genetic diagnosis of PV, ET or PMF to inform management decision when test results are positive.

**Timing**
The time frame for outcomes measures varies from several months for the improvement of symptoms to long-term survival as a result of disease-related complications.

**Setting**
Patients with a suspected MPN are actively managed by hematologists and oncologists.

**Analytic Validity**
Zinke et al (2015) have published on the development of a real-time quantitative PCR test for the rapid and sensitive detection of CALR type 1 and type 2 variants in exon 9, comprising 85% of all variants detected in CALR. The type 1 variant is a 52 base pair deletion and type 2 variant is a 5 base pair insertion. Both type 1 and type 2 variants lead to a frameshift with a subsequent modification to the CALR protein C-terminus. The quantitative PCR test has a reported sensitivity of less than 0.1% for CALR type 1 variant and less than 0.01% for CALR type 2 variant.

Murugesan et al (2016) has published on the analytic validation of a molecular diagnostic assay for CALR exon 9 indels (insertions/deletions). Forty specimens were analyzed using fragment length analysis to detect indels in CALR exon 9 and reported a sensitivity of 5% for detection of indels.

**Section Summary: Analytic Validity**
Evidence on the analytic validity of tests for detection of CALR variants is limited. However, it is expected that analytic validity will be high when testing is performed according to optimal laboratory standards.

**Clinical Validity**
Klampfl et al (2013) performed whole exome sequencing in patients with PMF who were previously identified as negative for JAK2 and MPL variants to search for a novel molecular marker for MPNs. Somatic insertions or deletions (indels) in exon 9 of CALR were initially detected in small cohort of 6 patients using whole exome sequencing. Subsequent resequencing of 1107 samples from patients with JAK2-negative and MPL-negative MPNs found that CALR exon 9 indels were absent in all cases of PV in this population. For patients with ET and PMF, CALR variants were detected in 67% and 88%, respectively. In total, 36 unique indels were identified resulting in a frameshift that led to mutated calreticulin proteins with
novel C-terminal peptides. Patients with CALR exon 9 indels were also found to have longer overall survival and lower risk of thrombosis.

Nangalia et al (2013) performed whole exome sequencing on 1345 hematologic cancers, 1517 other cancers, and 550 controls to assess the presence or absence of variants in CALR. Nineteen unique variants located in exon 9 resulting +1 base pair frameshift were detected from a total of 148 CALR variants identified. CALR variants were detected in most patients with JAK2-negative MPNs.

Rumi et al (2014) reported on CALR exon 9 variants somatically acquired in familial cases of ET and PMF. CALR exon 9 indels were found in 20% to 25% of sporadic patients with ET and PMF. In the small cohort of patients with ET with CALR variants, a lower cumulative incidence of thrombosis and disease progression was noted compared with ET patients with JAK2 V617F variants.

Section Summary: Clinical Validity
Evidence of the clinical validity CALR variants includes retrospective studies and case series. In patients with ET and PMF, the CALR exon 9 insertions and deletions (indels) were found in approximately 20% to 25% of cases, respectively. In ET and PMF patients, the 2016 WHO incorporated CALR exon 9 variants as a major criterion for the diagnosis of ET and PMF.

Clinical Utility
Testing for CALR exon 9 variants has potential clinical utility in several different clinical scenarios:

1. Diagnosis of patients with clinical, laboratory, or pathologic findings suggesting classic ET or PMF;

CALR exon 9 variants are detected in approximately 5% of patients with ET and PMF. No RCTs were identified that used the results of CALR exon 9 variant testing to guide treatment and management decisions. The goals of treatment and management for ET are to alleviate symptoms and minimize complications of disease such as thrombotic events and bleeding, though establishing the diagnosis does not lead to preventive management. For PMF, hematopoietic cell transplantation is the only treatment with curative potential while most other treatment options focus on alleviation of symptoms.

Rumi et al (2014) described JAK2 and CALR variant status in defining subtypes of ET with substantially different clinical course and outcomes. The presence of a CALR variant was associated with lower risk for thrombotic events in ET cases compared with JAK2 V617F ET cases. No significant differences in myelofibrotic transformations were noted. However, establishing the diagnosis through CALR genetic testing does not result in changes in management that would be expected to improve net health outcome.
Section Summary: Clinical Utility
Direct evidence for the clinical utility of \textit{CALR} testing is lacking. While \textit{CALR} exon 9 testing has potential clinical utility in diagnosing ET and PMF using the 2016 WHO major criteria for MPNs and excluding reactive or secondary causes of thrombocytosis, there is no change in management that would be expected to improve net health outcome. Thus clinical utility has not been established.

Summary of Evidence
For individuals with a suspected myeloproliferative neoplasms (MPN) who receive genetic testing for \textit{JAK2}, the evidence includes case series, retrospective studies, meta-analyses, and randomized control trials. Relevant outcomes include overall survival, disease-specific survival, test accuracy and validity, and resource utilization. For patients with suspected Philadelphia chromosome-negative (Ph-negative) MPN, \textit{JAK2} variants are found in nearly 100\% of those with polycythemia vera, 60\% to 65\% of those with essential thrombocythemia, and 60\% to 65\% of those with primary myelofibrosis. In individuals with suspected MPN, a positive genetic test for \textit{JAK2} satisfies a major criterion for the 2016 World Health Organization classification for Ph-negative MPNs and eliminates secondary or reactive causes of erythrocytosis and thrombocythemia from the differential diagnosis. The presence of a documented \textit{JAK2} variant may aid in the selection of ruxolitinib, a \textit{JAK2} inhibitor; ruxolitinib, however, is classified as a second-line therapy. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with a suspected MPN who receive genetic testing for \textit{MPL}, the evidence includes case series and retrospective studies. Relevant outcomes include overall survival, disease-specific survival, test accuracy and validity, and resource utilization. For patients with suspected Ph-negative MPN, \textit{MPL} variants are found in approximately 5\% of those with essential thrombocythemia (ET) and primary myelofibrosis cases (PMF). In individuals with suspected MPN, a positive genetic test for \textit{MPL} satisfies a major criterion for the 2016 World Health Organization classification for ET and PMF and eliminates secondary or reactive causes of thrombocythemia from the differential diagnosis. The goal of ET treatment is to alleviate symptoms and minimize thrombotic events and bleeding irrespective of \textit{MPL} variant status. For PMF, hematopoietic cell transplantation is the only treatment with curative potential while most other treatment options focus on alleviation of symptoms. However, in both ET and PMF, establishing the diagnosis through \textit{MPL} genetic testing does not in and of itself result in changes in management that would be expected to improve net health outcome. Thus clinical utility has not been established. The evidence is insufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with a suspected MPN who receive genetic testing for \textit{CALR}, the evidence includes case series and retrospective studies. Relevant outcomes include overall survival, disease-specific survival, test accuracy and validity, and resource utilization. For patients with suspected Ph-negative MPN, \textit{CALR} variants are found in approximately 20\% to 25\% of those with ET and PMF. For individuals with suspected MPN, a positive genetic test for \textit{CALR} satisfies a major criterion for the
WHO classification for ET and PMF and eliminates secondary or reactive causes of thrombocythemia from the differential diagnosis. The goal of ET treatment is to alleviate symptoms and minimize thrombotic events and bleeding irrespective of CALR variant status. For PMF, hematopoietic cell transplantation is the only treatment with curative potential while most other treatment options focus on alleviation of symptoms. However, in both ET and PMF, establishing the diagnosis through CALR genetic testing does not result in changes in management that would be expected to improve net health outcome. Thus clinical utility has not been established. The evidence is insufficient to determine that the technology results in a meaningful improvement in the net health outcome.

Supplemental Information

Practice Guidelines and Position Statements
World Health Organization major criteria for myeloproliferative neoplasms (2016) are as follows:

Polycythemia vera: “Presence of JAK2 V617F or other functionally similar mutation such as JAK2 exon 12 mutation”
Essential thrombocythemia: “Demonstration of JAK2 V617F or other clonal marker, or in the absence of a clonal marker, no evidence for reactive thrombocytosis”
Primary myelofibrosis: “Demonstration of JAK2 V617F or other clonal marker (eg, MPL W515K/L), 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. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials
Some currently unpublished trials that might influence this review are listed in Table 4.

Table 4. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ongoing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01259856a</td>
<td>Randomized Trial of Pegylated Interferon Alfa-2a Versus Hydroxyurea in Polycythemia Vera (PV) and Essential Thrombocythemia (ET)</td>
<td>168</td>
<td>Jun 2017</td>
</tr>
<tr>
<td>NCT02292446a</td>
<td>Expanded Treatment Protocol (ETP) of Ruxolitinib in Patients with Polycythemia Vera who are hydroxyurea resistant or intolerant and for whom no</td>
<td>500</td>
<td>Dec 2017</td>
</tr>
<tr>
<td>NCT Number</td>
<td>Description</td>
<td>Patients</td>
<td>Completed Date</td>
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<td>NCT01969838</td>
<td>Momelotinib Versus Ruxolitinib in Subjects with Myelofibrosis (Simplify 1)</td>
<td>432</td>
<td>Jun 2018</td>
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<tr>
<td>NCT02101268</td>
<td>Efficacy of Momelotinib Versus Best Available Therapy in Anemic or Thrombocytopenic Subjects with Primary Myelofibrosis (MF), Post-polycythemia Vera MF or Post-essential Thrombocythemias MF (Simplify 2)</td>
<td>156</td>
<td>Jun 2018</td>
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<td>NCT02577926</td>
<td>Ruxolitinib Versus Best Available Therapy in Patients with High-risk Polycythemia Vera or High-risk Essential Thrombocytemia – The Ruxo-BEAT Trial</td>
<td>380</td>
<td>Dec 2020</td>
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<td><strong>Unpublished</strong></td>
<td>Pegylated Interferon Alpha-2b Versus Hydroxyurea in Polycythemia Vera (PROUD-PV)</td>
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<td>NCT02055781</td>
<td>Oral Pacritinib Versus Best Available Therapy to Treat Myelofibrosis with Thrombocytopenia (PAC326)</td>
<td>300</td>
<td>Dec 2016 (terminated)</td>
</tr>
</tbody>
</table>

NCT: national clinical trial.

Denotes industry-sponsored or cosponsored trial.

References


48. 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
77. Rumi E, Harutyunyan AS, Pietra D, et al. CALR exon 9 mutations are somatically acquired events in familial cases of essential thrombocythemia or primary myelofibrosis. Blood. Apr 10 2014;123(15):2416-2419. PMID 24553179

**Billing Coding/Physician Documentation Information**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>81219</td>
<td>CALR (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9</td>
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<tr>
<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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<tr>
<td>81403</td>
<td>Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of &gt;10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)</td>
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<tr>
<td>0017U</td>
<td>Oncology (hematolymphoid neoplasia), JAK2 mutation, DNA, PCR amplification of exons 12-14 and sequence analysis, blood or bone marrow, report of JAK2 mutation not detected or detected. JAK2 Mutation</td>
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<td>0027U</td>
<td>JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, targeted sequence analysis exons 12-15. JAK2 Exons 12 to 15 Sequencing</td>
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**ICD-10 Codes**

- C96.2 Malignant mast cell tumors
- C92.10-C92.12 Chronic myeloid leukemia code range
- D45 Polycythemia vera
- D47.3 Essential thrombocytemia
- D47.4 Osteomyelofibrosis

**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.
- 9/1/17 CALR testing added to the policy. Policy revised with updated genetics nomenclature. Policy statements updated to clarify that JAK2 testing is medically necessary for PV, ET and PMF and added recommendation for documentation of serum erythropoietin levels prior to JAK2 testing, MPL testing is medically necessary for ET and PMF, and new medical
necessity statement added for CALR testing in ET and PMF. Title changed to “JAK2, MPL, and CALR Testing for Myeloproliferative Neoplasms”.

6/1/18  No policy statement changes.

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**APPENDIX**

**Appendix Table 1. Categories of Genetic Testing Addressed in 2.04.60**

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
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</thead>
<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>1a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>1b. Prognostic</td>
<td></td>
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<tr>
<td>1c. Therapeutic</td>
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<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
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</tr>
<tr>
<td>2a. Diagnostic</td>
<td>X</td>
</tr>
<tr>
<td>2b. Prognostic</td>
<td>X</td>
</tr>
<tr>
<td>2c. Therapeutic</td>
<td>X</td>
</tr>
<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
<td></td>
</tr>
<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
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</tr>
<tr>
<td>5. Reproductive testing</td>
<td></td>
</tr>
<tr>
<td>5a. Carrier testing: preconception</td>
<td></td>
</tr>
<tr>
<td>5b. Carrier testing: prenatal</td>
<td></td>
</tr>
<tr>
<td>5c. In utero testing: aneuploidy</td>
<td></td>
</tr>
<tr>
<td>5d. In utero testing: familial variants</td>
<td></td>
</tr>
<tr>
<td>5e. In utero testing: other</td>
<td></td>
</tr>
<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
<td></td>
</tr>
</tbody>
</table>

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