IMPORTANT REMINDER

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

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

DESCRIPTION

Genetic mutation testing for ABL1, CALR, CEBPA, FLT3, JAK2, KIT, MPL, NPM1, and/or TP53 genes may inform the diagnostic, prognostic, and treatment selection processes for select myeloid neoplasms and leukemias (acute or chronic).

MEDICAL POLICY CRITERIA

Notes:

- This policy does not address testing for BCR/ABL1 (t(9;22)) translocation analysis (i.e., CPT codes 81206-8 and 0016U) which may be considered medically necessary.
- Please refer to the Cross References section below for genetic testing not addressed in this policy, including but not limited to single-gene testing.

I. BCR-ABL kinase domain (ABL1)

   A. Genetic mutation testing for ABL1 may be considered medically necessary to evaluate patients when either of the following (1. or 2.) are met:

   1. In patients with chronic myelogenous (myeloid) leukemia (CML), to monitor response to tyrosine kinase inhibitor therapy, when either of the following (a. or b.) are met: (See Policy Guidelines)
a. In chronic phase, when there is failure to reach response milestones; or when there is any sign of loss of response (defined as hematologic or cytogenetic relapse); or when there is 1-log increase in \( BCR-ABL1 \) transcript levels and loss of major molecular response (MMR); OR

b. When there is progression of the disease to the accelerated or blast phase.

2. In patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL), to evaluate for tryosine kinase inhibitor resistance when there is an inadequate initial response to treatment or any sign of loss of response. (See Policy Guidelines)

B. Genetic mutation testing for \( ABL1 \) is considered investigational for all other circumstances, including but not limited to monitoring, management, or selecting treatment for patients with any condition.

II. \( CALR, JAK2, \) and/or \( MPL \)

A. Genetic mutation testing may be considered medically necessary to confirm a diagnosis for myeloid neoplasms when any of the following are met:

1. \( CALR, JAK2, \) and/or \( MPL \) when clinical, laboratory, or pathological findings suggest the following:
   a. Essential thrombocythemia (ET)
   b. Prefibrotic/early stage primary myelofibrosis (pre-PMF)
   c. Primary myelofibrosis (PMF) (overt PMF)

2. \( CALR, JAK2 \) (V617F), and/or \( MPL \) when clinical, laboratory, or pathological findings suggest myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T).

3. \( JAK2 \) (V617F) and/or \( JAK2 \) exon 12 when clinical, laboratory, or pathological findings suggest polycythemia vera (PV).

B. \( CALR, JAK2, \) and/or \( MPL \) genetic mutation testing is considered investigational for all other circumstances, including but not limited to monitoring, management, or selecting treatment for patients with any condition.

III. \( CEBPA, FLT3, KIT, NPM1, \) and/or \( TP53 \) for Acute Myeloid Leukemia

A. Genetic mutation testing for \( CEBPA, FLT3 \) internal tandem duplication (\( FLT3-ITD \)), \( KIT \), and/or \( NPM1 \) may be considered medically necessary when either of the following (1. or 2.) are met:

1. Evaluation for acute leukemia, or


B. Genetic mutation testing for \( TP53 \) may be considered medically necessary for risk stratification in patients with acute myeloid leukemia.

C. Genetic mutation testing of \( FLT3-ITD \) and \( FLT3 \) tryosine kinase domain (\( FLT3-TKD \)) mutations may be considered medically necessary for patients with newly diagnosed acute myeloid leukemia for treatment selection with FDA-approved kinase inhibitors (See Policy Guidelines).
D. Genetic mutation testing for CEBPA, FLT3-ITD, FLT3-TKD, KIT, NPM1, and/or TP53 is considered investigative for all other circumstances.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutation(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

Strategies for testing may include testing for individual genes or in combination, such as in a panel.

BCR-ABL KINASE DOMAIN MUTATION ANALYSIS

Chronic Myelogenous (Myeloid) Leukemia

In chronic phase CML, following primary treatment, National Comprehensive Cancer Network Guidelines for Chronic Myeloid Leukemia (v2.2017) response milestones are defined at 3-, 6-, 12-, and >12-months for BCR-ABL1 measurements according to the International Scale (IS). Measurements are expressed as ratios, which correspond to the percent ratio between BCR-ABL1 and the endogenous control transcript.

Accelerated or blast phase CML are defined by hematologic, cytogenetic, and/or response-to-tryosine kinase inhibitor (TKI) criteria.[1,2]

Philadelphia Chromosome-positive Acute Lymphoblastic Leukemia

Unlike in CML, resistance in ALL to TKIs is less well studied. In patients with ALL that are receiving a TKI, a rise in the BCR-ABL level while in hematologic complete response or clinical relapse warrants mutational analysis.

FLT3 TESTING FOR TREATMENT WITH RYDAPT® (MIDOSTAURIN)

For patients who have newly diagnosed acute myeloid leukemia, eligibility for Rydapt® (midostaurin) may be guided by testing with the LeukoStrat® CDx FLT3 Mutation Assay offered by Invivoscribe.
BACKGROUND

DIAGNOSING MYELOID NEOPLASMS AND ACUTE LEUKEMIA

Myeloid neoplasms may be acute or chronic, are a type of hematologic malignancy, and usually derive from bone marrow progenitor cells that normally develop into erythrocytes, granulocytes (neutrophils, basophils, and eosinophils), monocytes, or megakaryocytes. Classification of myeloid neoplasms and acute leukemias has evolved over the past decade, based in part on the advancement of available technologies and results from repeat validation studies.

In recent history, diagnosis of the various forms of myeloid neoplasms has been based on a complex set of clinical, pathological, and biological criteria first introduced by the Polycythemia Vera Study Group (PVSG) in 1996\(^3,\text{4}\) and the World Health Organization (WHO) in 2001.\(^5\) Both of these classifications use a combination of clinical, pathological, and/or biological criteria to arrive at a definitive diagnosis, predominantly reliant on status of Philadelphia chromosome presence. An important component of the diagnostic process is a clinical and laboratory assessment to rule out reactive or secondary causes of disease. Some diagnostic methods (e.g., bone marrow microscopy) are not well standardized and others (e.g., endogenous erythroid colony formation) are neither standardized nor widely available.\(^6-\text{8}\)

Diagnosis and monitoring of patients with Philadelphia chromosome negative myeloid neoplasms poses a challenge because many of the laboratory and clinical features of these diseases can be mimicked by other conditions such as reactive or secondary erythrocytosis, thrombocytosis or myeloid fibrosis. In addition, these entities can be difficult to distinguish on morphological bone marrow exam and diagnosis can be complicated by changing disease patterns.

The most up-to-date classification and benchmark for diagnosis is a result of collaboration between the Society for Hematopathology and the European Association for Haematopathology, and is published by the World Health Organization (WHO).\(^2,\text{9}\) The 2016 version is the fourth edition published by the WHO for classification of tumors of the hematopoietic and lymphoid tissues. This edition varies from the previous WHO versions predominantly due to advances in available technologies to identify unique biomarkers associated with myeloid neoplasms and acute leukemias. The current classification of myeloid neoplasm and acute leukemia subgroups are delineated in Table 1

<table>
<thead>
<tr>
<th>Myeloproliferative neoplasms (MPN)</th>
<th>Chronic myeloid leukemia (CML), BCR-ABL1$^+$</th>
</tr>
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</table>

\(^*)\text{For\ full criteria, see Table 1. WHO myeloid neoplasm and acute leukemia classification.}\n
WHO myeloid neoplasm and acute leukemia classification

Chronic neutrophilic leukemia (CNL)
Polycythemia vera (PV)
Primary myelofibrosis (PMF)
PMF, prefibrotic/early stage
PMF, overt fibrotic stage
Essential thrombocythemia (ET)
Chronic eosinophilic leukemia, not otherwise specified (NOS)
MPN, unclassifiable
Mastocytosis
Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRα, PDGFRβ, or FGFR1, or with PCM1-JAK2
Myeloid/lymphoid neoplasms with PDGFRα rearrangement
Myeloid/lymphoid neoplasms with PDGFRβ rearrangement
Myeloid/lymphoid neoplasms with FGFR1 rearrangement
Provisional entity: Myeloid/lymphoid neoplasms with PCM1-JAK2
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia (aCML), BCR-ABL1−
Juvenile myelomonocytic leukemia (JMMML)
MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
MDS/MPN, unclassifiable
Myelodysplastic syndromes (MDS)
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood
Myeloid neoplasms with germ line predisposition
Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11
APL with PML-RARA
AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
AML with t(6;9)(p23;q34.1);DEK-NUP214
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2);
GATA2, MECOM
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1
Provisional entity: AML with BCR-ABL1
AML with mutated NPM1

WHO myeloid neoplasm and acute leukemia classification

AML with biallelic mutations of CEBPA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2);
BCR-ABL1
MPAL with t(v;11q23.3); KMT2A rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS

B-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);BCR-ABL1
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);KMT2A rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy

T-lymphoblastic leukemia/lymphoma
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma
It is important to note that the presence of any one or more of the mutations included in this policy in itself may not be sufficient to confirm a diagnosis, rather, testing may help support other clinical, laboratory, or pathological findings.

**TREATMENT MONITORING**

The paradigm for use of molecular information to revolutionize patient management is CML. A unique chromosomal change (the Philadelphia chromosome) and an accompanying unique gene rearrangement (BCR-ABL) resulting in a continuously activated tyrosine kinase enzyme were identified. These led to the development of a targeted tyrosine kinase inhibitor drug therapy (imatinib) that produces long-lasting remissions.

**REGULATORY STATUS**

More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for gene mutation testing related to myeloid neoplasms and acute lymphoblastic leukemia. 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; LDTs must meet the general regulatory standards of 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.

The FDA Centers for Devices and Radiological Health (CDRH), for Biologics Evaluation and Research (CBER), and for Drug Evaluation and Research (CDER) developed a draft guidance on in vitro companion diagnostic devices, which was released on July 14, 2011,[10] to address the “emergence of new technologies that can distinguish subsets of populations that respond differently to treatment.” As stated, the FDA encourages the development of treatments that depend on the use of companion diagnostic devices “when an appropriate scientific rationale supports such an approach.” In such cases, the FDA intends to review the safety and effectiveness of the companion diagnostic test as used with the therapeutic treatment that depends on its use. The rationale for co-review and approval is the desire to avoid exposing patients to preventable treatment risk.

LeukoStrat® CDx FLT3 Mutation Assay (Invivoscribe) is the first FDA-approved companion diagnostic test for fms related tyrosine kinase 3 (FLT3) mutation testing for patients with newly diagnosed acute myeloid leukemia. The test is used with Rydapt® (midostaurin), an FDA-approved kinase inhibitor, indicated for adult patients, in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation. The LeukoStrat® CDx FLT3 Mutation Assay (Invivoscribe) received FDA premarket approval in April 2017.

**EVIDENCE SUMMARY**

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

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

The focus of this review is on evidence related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

**BCR-ABL1 (ABL1) KINASE DOMAIN MUTATION ANALYSIS IN CML AND ALL**

**ABL1 Mutations for CML**

Screening for BCR-ABL1 kinase domain point mutations (i.e. single nucleotide polymorphisms) in chronic phase CML is recommended for patients with inadequate initial response to TKI treatment, those with evidence of loss of response, and for patients who have progressed to accelerated or blast phase CML.[3] The focus of the following discussion is on kinase domain point mutations and treatment outcomes in systematic reviews.

**Systematic Reviews**

In 2010, the Agency for Healthcare Research and Quality published a systematic review on BCR-ABL1 pharmacogenetic testing for tyrosine kinase inhibitors in CML.[11] Thirty-one publications of BCR-ABL1 testing met the eligibility criteria and were included in the review (20 of dasatinib, 7 of imatinib, 3 of nilotinib, and 1 with various TKIs). The report concluded that the presence of any BCR-ABL1 mutation does not predict differential response to TKI therapy, although the presence of the T315I mutation uniformly predicts TKI failure. However, during the public comment period the review was strongly criticized by respected pathology organizations for lack of attention to several issues that were subsequently insufficiently addressed in the final report. Importantly, the review grouped together studies that used kinase domain mutation screening methods with those that used targeted methods, and grouped together studies that used mutation detection technologies with very different sensitivities. The authors dismissed the issues as related to analytic validity and beyond the scope of the report. However, in this clinical scenario assays with different intent (screening vs. targeted) and assays of very different sensitivities may lead to different clinical conclusions, so an understanding of these points is critical.

Branford et al. summarized much of the available evidence regarding kinase domain mutations detected at imatinib failure, and subsequent treatment success or failure with dasatinib or nilotinib.[12] The T315I mutation was most common; although about 100 mutations have been reported, the 7 most common (at residues T315, Y253, E255, M351, G250, F359, and H396) accounted for 60-66% of all mutations. However, preexisting or emerging mutations T315A, F317L/I/V/C, and V299L are associated with decreased clinical efficacy with dasatinib treatment following imatinib failure. Detection of the T315I mutation at imatinib failure is associated with lack of subsequent response to high-dose imatinib, or to dasatinib or nilotinib. For these patients, allogeneic stem-cell transplantation remained the only available treatment until the advent of new agents such as ponatinib.[13] However these mutations do not correspond to clinical significance, and based on clinical studies, the majority of imatinib-resistant mutations remain sensitive to dasatinib and nilotinib.
Preexisting or emerging mutations T315A, F317L/I/V/C, and V299L are associated with decreased clinical efficacy with dasatinib treatment following imatinib failure. Similarly, preexisting or emerging mutations Y253H, E255K/V, and F359V/C have been reported for decreased clinical efficacy with nilotinib treatment following imatinib failure. In the survey reported by Branford et al., a total of 42% of patients tested had T315I or one of these dasatinib- or nilotinib-resistant mutations. In the absence of any of these actionable mutations, various treatment options are available. Note that these data have been obtained from studies in which patients were all initially treated with imatinib; no data are available regarding mutations developing during first-line therapy with dasatinib or nilotinib.[14]

**ABL1** Mutations for ALL

Unlike in CML, resistance in ALL to TKIs is less well studied. Resistance does not necessarily arise from dominant tumor clone(s), but possibly in response to TKI-driven selective pressure and/or by competition of other coexisting subclones.[15] In patients with ALL that are receiving a TKI, a rise in the BCR-ABL level while in hematologic CR or clinical relapse warrants mutational analysis.

**CALRETICULIN (CALR), JANUS KINASE 2 (JAK2), AND MPL PROTO-ONCOGENE, THROMBOPOIETIN RECEPTOR (MPL) MUTATION ANALYSIS**

**Diagnosing Myeloid Neoplasms**

Testing for the following markers is required to meet WHO diagnostic criteria for patients with all of the most common Philadelphia chromosome-negative MPNs. 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. However, the most recent revisions to the WHO criteria are heavily based on repeat validation studies.[2]

**JAK2**

In 2005, a novel somatic gain-of-function point mutation was discovered in the conserved autoinhibitory pseudokinase domain of the Janus kinase 2 (**JAK2**) protein in patients with MPNs. The mutation was present in blood and bone marrow from a variable portion of patients with BCR-ABL negative (i.e., Philadelphia chromosome-negative) MPNs including 65% to 97% of patients with PV, 23% to 57% with ET, and 35% to 56% with IMF.[8,10,16-23] It was initially reported to be absent in all normal subjects and in patients studied with secondary erythrocytosis,[8,10,13,16-20,22-24] although recently very low levels of mutated cells have been reported to be found in a small subset of the healthy population.[25,26] Reports have appeared identifying **JAK2**V617F mutations in some of these cases.[10,27]

In 2007 Scott et al.[28] identified four somatic gain-of-function mutations in the **JAK2** exon 12 section of 10 of 11 PV patients without the **JAK2**V617F mutation. Patients with a **JAK2** exon 12 mutation differed from those with the **JAK2**V617F mutations, presenting at a younger age with higher hemoglobin levels and lower platelet and white cell counts. Erythroid colonies could be grown from their blood samples in the absence of exogenous erythropoietin, and mice treated with transfected bone marrow transplants developed a myeloproliferative syndrome.

Findings were subsequently confirmed by a number of investigators who identified additional mutations with similar functional consequences in patients with PV and in patients with idiopathic erythrocytosis.[29,30] 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.[28]

**MPL**

In 2006 Pikman et al.[31] surveyed JAK2 mutation-negative patients with suspected ET and PMF to determine if mutations in pathways complementary to Janus kinase two 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[32-36]. While this mutation can be found in both JAK2V617-positive and negative patients, it is obviously of value in the latter in helping to establish a clonal basis of the observed disease process.

**CALR**

Evidence for CALR demonstrates that a significant proportion of patients with myeloproliferative neoplasms and normal JAK2V617F status.[37-39] Mutations in exon 9 of CALR are found in 20-35% of all patients with ET and myelofibrosis (MF). Fifty-two base pair deletions (Type 1) and five base pair insertions (Type 2) are the most common.

**Phenotype/Genotype Associations and Impact on Prognosis**

While there has been great interest in the use of the JAK2V617F test as a front-line diagnostic test in the evaluation of myeloproliferative patients, there has also been a growing effort to link the presence of this mutation and the quantitative measurement of its allele burden with clinical features and biological behavior. Unfortunately, due to differences in disease definitions testing methods, sample type (bone marrow versus circulating blood cells) and study design, the literature in this area is conflicting and inconclusive.

Since most patients with PV do exhibit the mutation, attention has been focused in this disease on differences in its presence in the homozygous versus heterozygous state and on whether allele burden correlates with clinical or laboratory features. Studies have suggested a range of findings including association of homozygous states with older age, higher hemoglobin level at diagnosis, leukocytosis, more frequent pruritus, increased incidence of fibrotic transformation, and larger spleen volumes.[21,40] Studies comparing quantitative measurements of allele burden with disease manifestations have demonstrated both a positive and a lack of association with thrombosis, fibrotic transformation, and need for chemotherapy.[41,42]

The impact of the presence of JAK2V617F 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.[16,22] Discrepant results have been reported for thrombotic events and for fibrotic transformation.[43] A recent meta-analysis by Dahabreh et al.[44] surveyed some 394 studies on the subject of outcomes in ET. Dahabreh concluded thrombosis but not myelofibrosis or leukemia appeared to be influenced by the presence of JAK2 mutations. Dahabreh cautioned that there was a need for prospective studies to determine how this information might be used in treatment choices.
Thrombotic effects have been reported to be most pronounced for splanchnic vascular events\cite{45} and there has been little support for use of testing in patients with more general thrombosis or primary thrombocytosis.\cite{46} 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,\cite{47} 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.\cite{47} However, the outcome of routine testing in both settings remained unclear. In a recent international collaborative study of patients with ET, patients with JAK2 $^{V617F}$ mutations appeared at risk for arterial thrombosis but not for venous thrombosis.\cite{48}

A 2009 report by Hussein et al\cite{49} demonstrated that although there was significant overlap in JAK2$^{V617F}$ F allele burden among various MPN entities, quantitative measurements suggested discriminatory differences between patients with ET and the prefibrotic-stage of PMF.

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

- Patients with JAK2$^{V617F}$ mutations required fewer blood transfusions but exhibited poorer overall survival than those without the mutation.\cite{13}
- Patients with JAK2$^{V617F}$ mutations did not show differences in the incidence of thrombosis, overall survival, or leukemia-free survival.\cite{50}
- Patients with homozygous JAK2$^{V617F}$ mutations show an increased evolution toward large splenomegaly, need of splenectomy and leukemic transformation.\cite{51}
- Patients with low allele burdens appeared to exhibit shortened survival, perhaps because they represented a myelodepleted subset of affected patients.\cite{50,52}

In 2013, a joint systematic evaluation of JAK2 $^{V617F}$ quantitative polymerase chain reaction (qPCR) assays was conducted to identify “an assay that, beyond being robust enough for routine diagnostic purposes, also showed the best performance profile when used for predicting outcome following an allogeneic transplant.” Effective assays can detect an allele burden as low as 1%.\cite{54} Investigators assessed three unpublished laboratory-developed tests and six published assays in 12 laboratories. 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 JAK2 $^{V617F}$ -positive disease and in 100 peripheral blood samples from healthy controls, respectively. The most sensitive assay performed consistently across various qPCR platforms and detected mutant allele (ie, minimal residual disease) in transplant recipients a median of 22 weeks (range, 6-85 weeks) before relapse. The authors suggested that the assay could be used to guide management of patients undergoing allogeneic HSCT. Although the study supports the analytic validity of the assay, given the inconsistency of outcomes when JAK2 $^{V617F}$ testing is used for treatment monitoring (described above), utility of this assay or any JAK2 $^{V617F}$ test for treatment monitoring is uncertain. Other investigators have studied methods to improve JAK2 and MPL mutation testing using qPCR\cite{55,56} and novel approaches (eg, an electrochemical DNA biosensor).\cite{57}
Limited studies suggest distinct clinical outcomes associated with MPL and CALR mutation status; however, the findings should be confirmed by additional research.

It is suggested that ET patients with CALR mutations have lower polycythemic transformation rates, but not lower myelofibrotic transformation rate, compared with ET patients harboring a JAK2 mutation. Chen et al. (2014) reported a higher platelet count, younger age of diagnosis, lower leukocyte count, and decreased risk for thrombosis, compared with a JAK2 positive ET population. In 2014, Tefferi et al. reported survival and blast transformation in PMF were significantly affected by mutational status, though not in ET. The outcome was best in CALR-mutated patients and worst in JAK2/CALR/MPL negative PMF patients. CALR-mutated ET has also been associated with better thrombosis-free survival and lower leukocyte counts. However, overall survival has been reported as not different among CALR mutated and non-mutated ET.

**Treatment**

Due to the strong epidemiologic and biologic literature linking JAK2 pathway mutations to occurrence of MPNs, there has been considerable recent attention on using JAK2 as a molecular target for drug discovery. In preclinical and early clinical studies, a number of promising JAK2 inhibitors have been identified and reports have suggested some of these are useful in symptom relief. 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. A considerable amount of study will be required to sort through issues of safety and efficacy of these new treatments before they enter routine clinical use. Several early phase and preliminary treatment trials evaluating the safety and efficacy of tyrosine kinase inhibitors in patients with JAK2V617F-positive myeloproliferative neoplasms have been reported. It has recently been noted that benefits from tyrosine kinase therapy may not be specific for JAK2V617F-positive myeloproliferative neoplasms but may be observed in wild type disease as well.

**CEBPA, FLT3-ITD, FLT3-TKD, KIT, NPM1, AND TP53 MUTATION ANALYSIS FOR ACUTE MYELOID LEUKEMIA**

**Acute Myeloid Leukemia**

Acute myeloid leukemia (AML) is a group of diverse hematologic malignancies characterized by the clonal expansion of myeloid blasts in the bone marrow, blood, and/or other tissues. It is the most common type of leukemia in adults, and is generally associated with a poor prognosis. It was estimated that, in 2014, 18,860 people would be diagnosed with AML and 10,460 would die of the disease. Median age at diagnosis is 66 years, with approximately 1 in 3 patients diagnosed at 75 years of age or older.

**Diagnosis and Prognosis of AML**

Conventional cytogenetic analysis (karyotyping) is considered to be a mandatory component in the diagnostic evaluation of a patient with suspected acute leukemia, because the cytogenetic profile of the tumor is considered to be the most powerful predictor of prognosis in AML and is used to guide the current risk-adapted treatment strategies. Molecular variants including those in CCAAT/enhancer-binding protein alpha (CEBPA); FMS-like tyrosine kinase 3 (FLT3); the tyrosine kinase receptor, KIT; nucleophosmin (NPM1); and tumor protein p53 (TP53) genes have been analyzed to subdivide AML into prognostic subsets. Patients with better-prognosis
disease (for example, core-binding factor AML) based on cytogenetics, and a mutation in the c-KIT gene of leukemic blast cells, do just as poorly with postremission standard chemotherapy as patients with cytogenetically poor-risk AML.[68] Similarly, individuals with cytogenetically normal AML (intermediate-prognosis disease) can be subcategorized into groups with better or worse prognosis based on the mutational status of the NPM1 and the FLT3 gene. Thus, patients with mutations in NPM1 but without FLT3-ITD have postremission outcomes with standard chemotherapy that are similar to those with better-prognosis cytogenetics; in contrast, patients with any other combination of mutations in those genes have outcomes similar to those with poor-prognosis cytogenetics.[69]

The World Health Organization (WHO) classification of AML was adapted by the National Comprehensive Cancer Network (NCCN) to estimate individual patient prognosis to guide management, as shown in Table 2:[70]

<table>
<thead>
<tr>
<th>Risk Status</th>
<th>Cytogenetic Factors</th>
<th>Molecular Abnormalities</th>
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<tbody>
<tr>
<td>Favorable-risk</td>
<td>Core binding factor: inv(16) or t(16;16) or t(15;17)</td>
<td>Normal cytogenetics: NPM1 mutation in the absence of FLT3-ITD or isolated biallelic CEBPA mutation</td>
</tr>
</tbody>
</table>
| Intermediate-risk | Normal cytogenetics  
+8 alone  
t(9;11)  
Other non-defined | Normal cytogenetics: with FLT3-ITD mutation  
TP53 mutation |
| Poor-risk     | Complex (≥3 clonal chromosomal abnormalities)  
-5, 5q-, -7, 7q-, 11q23 - non t(9;11)  
Inv3, t(3;3)  
t(6;9)  
t(9;22) | Normal cytogenetics: with FLT3-ITD mutation  
TP53 mutation |

Systematic Reviews for CEBPA, FLT3-ITD, KIT, NPM1, and TP53

Recent systematic reviews with meta-analyses have highlighted the evolving classification of AML into distinct molecular subtypes based on for CEBPA, FLT3-ITD, KIT, NPM1, and TP53, particularly in patients with normal karyotype.[71-76] These studies support the WHO and NCCN risk status classifications, and additionally highlight the importance of KIT testing in the initial evaluation and for prognosis.

FLT3 tryosine kinase domain

The FLT3 gene encodes a receptor tryosine kinase involved in hematopoiesis, of which two major activating mutations have been identified in patients with AML: the internal tandem duplications (ITD) and tryosine kinase domain (TKD) point mutations. While a significant body of literature demonstrates the diagnostic and prognostic value of FLT3-ITD testing, FLT3-TKD research is controversial. Studies have found presence of FLT3-TKD mutations to be associated with shorter disease free survival time and decreased overall survival,[77-80] though other studies have reported no impact on prognosis,[69,81-83] and even favorable outcomes with overall survival when compared to those with FLT3-wild type[84,85].
**FLT3-ITD and FLT3-TKD in AML Treatment**

The LeukoStrat® CDx FLT3 Mutation Assay offered by Invivoscribe. According to Invivoscribe, the test is indicated at initial diagnosis of AML to determine eligibility for Rydapt® (midostaurin), and may also be used for risk stratification.[86] The assay includes internal tandem duplication mutation testing for FLT3 as well as mutations in the tyrosine kinase domain. Rydapt® (midostaurin) is an FDA-approved kinase inhibitor, indicated for adult patients, in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation.[87] The assay is an FDA-approved companion diagnostic test for use with Rydapt® (midostaurin) and therefore may be standard of care in screening patients for use with this specific kinase inhibitor. However, due to the studies outlined above, FLT3-TKD testing (which is included in this assay) for risk stratification warrants additional research in well-designed studies.

**PRACTICE GUIDELINE SUMMARY**

**WORLD HEALTH ORGANIZATION (WHO)**

Policy criteria are consistent with WHO (2016) diagnostic criteria for myeloid neoplasms and acute leukemia for JAK2, MPL, and CALR.

**NATIONAL COMPREHENSIVE CANCER NETWORK (NCCN)**

Policy criteria are mostly consistent with NCCN guidelines for Chronic Myeloid Leukemia (v2.2017)[1], Acute Lymphoblastic Leukemia (v2.2016)[88], Acute Myeloid Leukemia (v1.2017)[70], and Myeloproliferative Neoplasms (v2.2017)[89].

**SUMMARY**

**BCR-ABL kinase domain (ABL1)**

In chronic myeloid leukemia, there is enough research to show clinical utility for evaluation of ABL1 mutations for tyrosine kinase inhibitor (TKI) resistance. TKI resistance in acute lymphoblastic leukemia (ALL) has not been studied as well as in CML. However, there is enough research to show ABL1 mutation testing for evaluation of TKI resistance may lead to an improvement in health outcomes for patients with ALL who are receiving a TKI. Practice guidelines based on research recommend ABL1 testing for ALL and CML in specific clinical scenarios. Therefore, ABL1 mutation testing for evaluation of TKI resistance may be considered medically necessary when policy criteria are met. Due to insufficient evidence, evaluation of ABL1 mutations is considered investigational when policy criteria are not met.

**CALR, JAK2, and/or MPL**

There is enough research on the clinical validation of JAK2 (in some scenarios for select mutations only), MPL, and CALR as a distinctive marker of patients with select myeloid neoplasms. Testing for these markers is recommended in practice guidelines based on research for patients with the most common Philadelphia chromosome-negative neoplasms. Therefore, JAK2, MPL, and/or CALR genetic testing may be considered medically necessary as a diagnostic test when policy criteria are met. There is not enough research to know if JAK2, MPL, and CALR mutation testing to establish disease prognosis or to select or monitor therapy leads to changes that improve overall health outcomes. This remains an active area of investigation. Therefore, JAK2, MPL, and/or CALR mutation testing is
considered investigational for prognostic testing or to direct therapeutic treatments for any condition due to insufficient evidence.

**CEBPA, FLT3, KIT, NPM1, and/or TP53 for Acute Myeloid Leukemia**

There is enough research to know that evaluation of CEBPA, FLT3 internal tandem duplication (FLT3-ITD), KIT, and/or NPM1 gene mutations inform the diagnostic process for acute leukemia. These genes, in addition to TP53 are also important molecular markers for risk stratification for patients with acute myeloid leukemia (AML). Policy criteria are in alignment with practice guidelines based on research which recommend testing for these markers. There is less research to support mutation testing for FLT3 tryosine kinase domain (FLT3-TKD), and it is unclear if FLT3-TKD testing impacts overall health outcomes. However, both FLT3-ITD and FLT3-TKD are included in at least one FDA-approved companion diagnostic test. Therefore, genetic testing for CEBPA, FLT3, KIT, NPM1, and/or TP53 mutations may be considered medically necessary for select patients when policy criteria are met. Due to insufficient evidence and no recommendations from practice guidelines, testing for these genes is considered investigational when policy criteria are not met.

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**REFERENCES**

9. Barbui, T, Thiele, J, Vannucchi, AM, Tefferi, A. Rationale for revision and proposed changes of the WHO diagnostic criteria for polycythemia vera, essential


50. 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*. 2008 Apr;22(4):756-61. PMID: 18216871


91. BlueCross BlueShield Association Medical Policy Reference Manual "JAK2 and MPL Mutation Analysis in Myeloproliferative Neoplasms." Policy No. 2.04.60

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<tr>
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<td>0017U</td>
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<td>NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, exon 12 variants</td>
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*Date of Origin: August 2010*