Genetic Testing for FLT3 and NPM1, and CEBPA Mutations in Acute Myeloid Leukemia

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Policy
Blue Cross and Blue Shield of Kansas City (Blue KC) will provide coverage for Genetic Testing for FLT3, NPM1, and CEBPA Mutations in Acute Myeloid Leukemia when it is determined to be medically necessary because the criteria shown below are met.

Note: This is a type of genetic testing that may be excluded in some contracts. Verify benefits prior to review for Medical Necessity.

When Policy Topic is covered
Genetic testing for FLT3 internal tandem duplication (FLT3/ITD), NPM1, and CEBPA mutations may be considered medically necessary in cytogenetically normal AML. (see Considerations)

When Policy Topic is not covered
Genetic testing for FLT3 internal tandem duplication (FLT3/ITD), NPM1, and CEBPA mutations is considered investigational in all other situations.

Genetic testing for FLT3 tyrosine kinase domain (FLT3/TKD) mutations is considered investigational.

Genetic testing for FLT3, NPM1, and CEBPA mutations to detect minimal residual disease is considered investigational.

Considerations
This testing is intended to guide management decisions in patients who would receive treatment other than low-dose chemotherapy or best supportive care.

Description of Procedure or Service

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| • Cytogenetically normal AML | • Genetic testing for mutations in FLT3, NPM1, CEBPA to risk stratify AML | • Treatment based on conventional cytogenetics and patient characteristics | • Overall survival  
• Other test performance measures |

AML: acute myeloid leukemia.

Treatment of acute myeloid leukemia (AML) is based upon risk stratification, mainly patient age and tumor cytogenetics. The identification of mutations in several genes, including FLT3, NPM1, and CEBPA, have been proposed to allow for further segregation in the management of this heterogeneous disease.

*FLT3* internal tandem duplication (*FLT3*-ITD) mutations are known to confer a very poor prognosis, whereas *NPM1* and biallelic *CEBPA* mutations have been shown to confer an independently favorable prognosis. Limited data suggest that a coexistent *NPM1* mutation may mitigate the negative prognostic effect of an *FLT3*-ITD mutation, if both mutations are present. The prognostic effect of *FLT3* tyrosine kinase domain (*FLT3*-TKD) mutations is uncertain.

Data on the analytic and the clinical validity of *FLT3*, *NPM1*, and *CEBPA* mutation testing are lacking. Data on the clinical utility of testing for these mutations is limited to retrospective analyses, and consist predominantly of studies of the effect of the presence of an *FLT3*-ITD mutation in patients who underwent hematopoietic stem cell transplant (HSCT) versus those who did not. Although some controversy exists as to the survival benefit in transplanting a patient with an *FLT3*-ITD mutation, retrospective studies, in general, have suggested a survival benefit for these poor-risk patients, and major professional societies and guidelines recommend testing for these mutations to risk stratify and to inform treatment management decisions, including possible HSCT.

Therefore, evidence is sufficient to determine that genetic testing for *FLT3*-ITD, *NPM1*, and *CEBPA* mutations improves the net health outcome for patients with cytogenetically normal AML. Evidence is insufficient to determine that genetic testing for *FLT3*-TKD mutations improves the net health outcome for cytogenetically normal AML.

**Background**
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 is estimated that, in 2014, 18,860 people will be diagnosed with AML and 10,460 will die of the disease. The median age at diagnosis is 66 years, with approximately 1/3 of patients diagnosed at 75 years of age or older.(1)

**Diagnosis and Prognosis of AML**
The most recent World Health Organization (WHO) classification (2008) reflects the increasing number of acute leukemias that can be categorized based on underlying cytogenetic abnormalities (ie, at the level of the chromosome including chromosomal translocations or deletions) or molecular genetic abnormalities (ie, at
the level of the function of individual genes, including gene mutations. These cytogenetic and molecular changes form distinct clinico-pathologic-genetic entities with diagnostic, prognostic, and therapeutic implications. 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. Younger adult patients are usually categorized into 3 different risk groups based on cytogenetics (good, intermediate, poor risk).

Molecular mutations have been analyzed to subdivide AML with normal cytogenetics into prognostic subsets. In AML, 3 of the most frequent molecular changes with prognostic impact are mutations of CEBPA encoding a transcription factor, mutations of the FLT3 gene, encoding a receptor of tyrosine kinase involved in hematopoiesis, and mutation of the NPM1 gene, encoding a shuttle protein within the nucleolus. “AML with mutated NPM1 or CEBPA” were included as provisional entities in the 2008 WHO classification of acute leukemias. AML with FLT3 mutations is not considered a distinct entity in the 2008 classification, although WHO recommends determining the presence of FLT3 mutations because of the prognostic significance.

Recent reviews highlight the evolving classification of AML into distinct molecular subtypes. (1, 3-5)

Treatment
AML has a highly heterogeneous clinical course, and treatment generally depends on the different risk-stratification categories. (1) Depending on the risk-stratification category, treatment modalities may include intensive remission induction chemotherapy, hypomethylating agents, clinical trials with innovative compounds, palliative cytotoxic treatment or supportive care only. For patients who achieve a complete remission (CR) after induction treatment, possible postremission treatment options include intensive consolidation therapy, maintenance therapy or autologous or allogeneic hematopoietic stem-cell transplantation (HSCT). (1)

FLT3 mutations
FMS-like tyrosine kinase (FLT3) plays a critical role in normal hematopoiesis and cellular growth in hematopoietic stem and progenitor cells. Mutations in FLT3 are one of the most frequently encountered mutations in AML, and approximately 30% of AML patients harbor some form of FLT3 mutation. (6) FLT3 mutations are divided into 2 categories: 1) internal tandem duplications (FLT3/ITD) mutations, which occur in or near the juxtamembrane domain of the receptor, and 2) point mutations resulting in single amino acid substitutions within the activation loop of the tyrosine kinase domain (FLT3/TKD).

FLT3/ITD mutations are much more common than FLT3/TKD mutations, occurring in 25% of newly diagnosed adult cases of AML, versus FLT3/TKD mutations, occurring in ~7% of patients. FLT3/ITD are a well-documented adverse prognostic
marker, particularly in patients younger than 60 years of age and with normal or intermediate risk cytogenetics, and is associated with an increased risk of relapse and inferior overall survival (OS). (6-8). Patients with FLT3/ITD mutations have a worse prognosis when treated with conventional chemotherapy, compared with patients with wild type (ie, nonmutated) FLT3. Although remission can be achieved in patients with FLT3/ITD mutations using conventional induction chemotherapy at a frequency similar to other AML patients, the remission durations are shorter and relapse rates are higher. The median time to relapse in patients with a FLT3/ITD mutation is 6 to 7 months compared with 9 to 11 months in patients with other AML subtypes. (6) Once FLT3/ITD AML relapses, the disease is rapidly fatal.

Because of the high risk of relapse, hematopoietic stem-cell transplantation (HSCT) as consolidation of a first remission for a FLT3/ITD AML patient is often a consideration. However, this must be weighed against the treatment-related mortality associated with a transplant. (6)

The clinical significance of an FLT3 mutation varies according to the nature of the mutation and the context in which it occurs. Longer FLT3/ITD mutations have been associated with reduced remission rates and/or worse survival in some studies. (6)

For FLT3/ITD mutations, allelic ratio refers to the number of ITD-mutated alleles compared with the number of WT (nonmutated) alleles. This ratio is influenced by the number of malignant versus benign cells in the sample tested and by the percentage of cells with 0, 1 or 2 mutated alleles. In most cases, the mutation detected at diagnosis is also present at relapse. However, in some cases, as FLT3/ITD-positive AML evolves from diagnosis to relapse, the mutation present at diagnosis may be absent (or undetectable) at relapse. This is most commonly seen in cases in which the mutant allele burden is low (5% to 15%) at diagnosis. (6) For this reason, and the overall lack of sensitivity of the assay (see Clinical Validity), the assay is considered to be unsuitable for use as a marker of minimal residual disease. (6) Higher mutant to WT allelic ratios have been associated with worse outcomes. (6)

The prognostic impact of FLT3-TKD mutations is less certain, and has only been studied in small numbers of patients.6,9 FLT3 tyrosine kinase inhibitors are under active clinical investigation.

NPM1
The most common molecular aberration in AML is a mutation of NPM1, which is found in 46% to 64% of cytogenetically normal AML (CN-AML) and 9% to 18% of cytogenetically abnormal AML. (1) Up to 50% of AML with mutated NPM1 also carry a FLT3/ITD. Mutated NPM1 confers an independent favorable prognosis for patients with CN-AML and either the presence or absence of a FLT3/ITD. Retrospective studies of banked clinical samples suggest that a NPM1 mutation may mitigate the negative prognostic effect of an FLT3/ITD, but possibly only if the FLT3/ITD to WT allelic ratio is low. (6) The prognostic impact in patients with an abnormal karyotype is unclear. (1)
**CEBPA Mutations**

*CEBPA* (CCAAT/enhancer binding protein) is a transcription-factor gene that plays a role in cell cycle regulation and cell differentiation. Mutations to *CEBPA* are found in approximately 15% of AML patients with a normal karyotype.\(^10\)-\(^12\) *CEBPA* mutations can be either biallelic (double mutations) or monoallelic. Monoallelic mutations are prognostically similar to *CEBPA WT* and do not confer a favorable prognosis in cytogenetically normal AML; double mutations of *CEBPA* have shown a better prognosis with higher rates of CR and OS after standard induction chemotherapy.\(^13\),\(^14\)

**Regulatory Status**

No U.S. food and Drug Administration (FDA) cleared genetic tests for *FLT3* or *NPM*, or *CEBPA* were found. Thus, these genetic tests are offered as laboratory-developed tests. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA).

Clinically validated *FLT3* mutation testing is performed with a polymerase chain reaction (PCR)-based assay of genomic DNA isolated from the leukemic cells, either from the blood or bone marrow. Testing for *FLT3* may involve a duplex assay which tests for both types of *FLT3* mutations (*ITD* and *TKD*), however, some laboratories only test for *ITD* mutations, as the prognostic effect of *TKD* mutations is uncertain.\(^6\)

Several Laboratories offer these tests including Quest Diagnostics, Medical Genetic Laboratories of Baylor College, Geneva Labs of Wisconsin, LabPMM and ARUP Laboratories.

**Rationale**

This policy was created in July 2014 and the most recent update is based on a search of the MEDLINE database through May 28, 2015 (see Appendix Table 1 for genetic testing categories). Literature that describes the analytic validity, clinical validity, and clinical utility of genetic testing for *FLT3*, *NPM1*, and *CEBPA* mutations was sought.

**Analytic Validity**

Analytic validity is the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent.

No published data on the analytic validity of *FLT3*, *NPM1*, or *CEBPA* mutation testing are identified.

**Clinical Validity**

Clinical validity is the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease.
Published data on the clinical validity of FLT3 testing is lacking, however, a review article highlights that a major limitation of most polymerase chain reaction (PCR) assays for FLT3 internal tandem duplication (FLT3-ITD) mutations is lack of sensitivity compared with PCR assays for other acute myeloid leukemia (AML)–associated genetic alterations. The sensitivity of the PCR assays is a function of the amount of sample DNA and the number of PCR cycles. However, for the FLT3-ITD assay, increasing the number of cycles does not increase the sensitivity because the PCR primers used to amplify the mutant allele also amplify the wild-type (WT) allele, and the shorter WT allele has a competitive advantage over the mutant allele, because it takes more time to complete a PCR cycle for the longer length mutant allele. The longer the mutation (insertion), the greater the PCR bias.

This bias can be minimized using fewer PCR cycles, but this could affect sensitivity if there is a low burden of leukemia cells in the sample.

Published data on the clinical validity of testing for NPM1 or CEBPA mutations are not identified.

**Clinical Utility**
Clinical utility is 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 literature on the use of these markers consists of retrospective analyses, and no prospective studies have been published to date.

Most of the literature consists of analyses of FLT3-ITD mutations and survival outcomes with the use of allogeneic hematopoietic stem cell transplantations (HSCT) in patients depending on the presence of this type of mutation. In general, the data support use of HSCT in patients with FLT3-ITD mutations, however, not all studies have shown consistent results.

Gale et al first reported the results of a retrospective analysis of FLT3 status in patients enrolled in 2 trials in the United Kingdom. The trials included 1135 adult patients with AML, of whom 141 received autologous HSCT and 170 an allogeneic HSCT in first complete remission (CR), based on donor availability. An FLT3-ITD was detected in 283 of the total study population of 1135. Of the patients who underwent autologous HSCT (n=141), 37 (26%) were FLT3-ITD-positive, and among those who received an allogeneic HSCT (n=170), 35 (21%) were FLT3-ITD-positive. The clinical investigators were not aware of FLT3-ITD status and did not direct treatment based on FLT3 mutation status. There was no difference in effect on relapse rate with the use of autologous versus allogeneic HSCT (odds ratio [OR], 2.39; confidence interval [CI], 1.24 to 4.62 for autologous; OR=1.31; 95% CI, 0.56 to 3.06 for allogeneic; p=0.3), nor between patients who did or did not receive a transplant (p=0.4).
They did additional analysis of the effect of allogeneic HSCT in \textit{FLT3}-ITD-positive patients by performing a donor versus no donor comparison of 683 patients in whom \textit{FLT3}-ITD status was available. No difference in relapse rate was noted in \textit{FLT3}-ITD-positive versus -negative patients (OR=0.70; 95% CI, 0.53 to 0.92 vs OR=0.59; 95% CI, 0.40 to 0.87; respectively; \(p=.5\)). The authors concluded that there is no strong evidence that \textit{FLT3} status should influence the decision whether to proceed to transplant.

In 2012, Brunet et al retrospectively compared outcomes for \textit{FLT3}-ITD AML patients registered in the European Group for Blood and Marrow Transplantation (EBMT) who underwent a myeloablative allogeneic HSCT in first remission with patients without the mutation.\textsuperscript{16} Of 1467 patients who met inclusion criteria (age \(\geq 18\) years, de novo AML, normal cytogenetics at diagnosis, myeloablative allogeneic HSCT performed between 2000 and 2008), 206 (14\%) had \textit{FLT3}-ITD data. \textit{FLT3}-ITD was present in 120 patients, absent in 86. At 2 years, the relapse incidence was 30\%±5\% versus 16\%±5\% (\(p=0.006\)) in \textit{FLT3}-ITD-positive versus \textit{FLT3}-ITD-negative patients, and leukemia-free survival (LFS) was 58\%±5\% versus 71\%±6\% (\(p=0.04\)) in \textit{FLT3}-positive patients versus -negative patients, respectively. Although the presence of \textit{FLT3}-ITD led to a higher relapse risk and inferior LFS in this study when compared with the \textit{FLT3}-negative patients, the observed 2-year LFS of 58\% and the relapse risk of 30\% in the patients with the \textit{FLT3}-ITD mutation compares favorably with outcomes reported in patients with \textit{FLT3}-ITD mutations after post remission chemotherapy (ie, who did not undergo transplant), who have been reported to have a median survival of 2.5 months.

Bornhäuser et al reported the results of the AML 96 study of the DSIL (German Study Initiative Leukemia) in which 999 patients 60 years of age or younger were prospectively included between 1996 and 2003 and stratified according to cytogenetic risk category.\textsuperscript{17} Of patients with intermediate-risk cytogenetics, 555 were available for evaluation of \textit{FLT3} mutation status; 175 (31.5\%) were \textit{FLT3}-ITD-positive. The rate of remission after 2 cycles of induction chemotherapy, including high-dose Ara-C, did not differ among patients with and without \textit{FLT3}-ITD (68\% vs 63\%). The investigators decided to determine the impact of different consolidation therapies on overall survival (OS) and the probability of relapse with respect to \textit{FLT3}-ITD mutation status. Patients underwent allogeneic HSCT (n=103), autologous HSCT (n=141) if no donor was available, or conventional consolidation chemotherapy consisting of high-dose Ara-c (n=132) if the patient could not mobilize autologous cells. After a median follow-up of 53 months, OS did not differ significantly between \textit{FLT3}-ITD-positive and -negative patients having undergone autologous or allogeneic HSCT. In the group that received conventional consolidation chemotherapy, \textit{FLT3}-ITD-positive patients had an inferior probability of survival (21\% vs 46\%; hazard ratio [HR], 2.2; 95\% CI, 1.4 to 3.5; \(p=0.001\)), and the relapse probability was significantly higher in \textit{FLT3}-ITD-positive than in -negative patients (94\% vs 59\%; \(HR=4.0\); 95\% CI, 2.5 to 6.6; \(p<0.001\)).

DeZern et al reviewed the clinical data from November 2004 to October 2008 of 133 consecutive patients with previously untreated AML.\textsuperscript{18} Patients were between the ages of 20 and 59 years and received induction and consolidation therapy at
Johns Hopkins, and were followed through August 2010. Thirty-one patients (23%) harbored an FLT3-ITD mutation. Induction success was similar between the 2 groups, with 20 of 31 (65%) of FLT3-ITD mutation patients and 52 of 85 (61%) of WT patients. Of the 20 FLT3-ITD patients in CR (CR1), 11 (55%) underwent allogeneic HSCT, 9 myeloablative, and 2 nonmyeloablative. The FLT3-ITD patients who did not undergo HSCT either did not have a suitable donor or had precluding comorbidities. Seventeen (33%) of the WT patients underwent HSCT in CR1; 14 myeloablative, 1 syngeneic, 1 autologous, and 1 nonmyeloablative allogeneic. Median relapse-free survival (RFS) was 8.6 months (range, 5.3-43.3 months) versus 54.1 months (range, 6.4-69.9 months) in the FLT3-ITD transplant group (p=0.03). Median OS in the WT nontransplant group versus the WT transplant group was 57.3 months (range, 3.9-64.4) versus 60 months, respectively (p=0.02). The authors concluded that their study suggests an advantage of HSCT in patients with FLT3-ITD in early CR1. However, the number of patients transplanted was small.

Willemze et al conducted a randomized trial in 1942 newly diagnosed patients with AML, ages 15 to 60 years, to compare remission induction treatment containing either standard or high-dose cytarabine. In both arms, patients who achieved CR received consolidation therapy with either an autologous or an allogeneic HSCT. Patients were subclassified as good risk, intermediate risk, bad risk, very bad risk, or unknown risk, according to cytogenetics and FLT3-ITD mutation. Testing for FLT3-ITD mutation showed that, in the standard dose cytarabine group, 50% were negative, 13% were positive, and 37% were unknown. In the high-dose cytarabine group, 48% were negative, 14% were positive, and 38% were unknown. All patients with an FLT3-ITD mutation were categorized as very bad risk. OS at 6 years in the patients categorized as very bad risk was 20% in the standard cytarabine group and 31% in the high-dose group (HR=0.70; 95% CI, 0.47 to 1.04; p=0.02). The authors concluded that patients with very bad risk cytogenetics and/or FLT3-ITD mutation benefitted from high-dose cytarabine induction treatment.

Pratcorona et al reported on the outcomes of 303 patients with intermediate-risk cytogenetics AML who were treated with intensive chemotherapy. They analyzed the effect of the ratio of FLT3-ITD to FLT3 WT, depending on the presence of an NPM1 mutation. FLT3-ITD mutations were identified in 94 (31%) of patients and NPM1 mutations in 161 (53%) of patients (65 patients harbored both mutations). To further confirm the prognostic value of the FLT3-ITD mutations to WT ratio, patients were subdivided into FLT3wt, FLT3-ITD/wt with an allelic ratio less than 0.5 (low ratio), and FLT3-ITD/wt with an allelic ratio of 0.5 or higher (high ratio). The 0.5 cutoff value was chosen based on maximum clinical prognostic impact derived at that threshold because, in this series, this cutoff showed the greatest difference in relapse rate in patients with FLT3-ITD. Among the patients with NPM1 mutations, FLT3wt and low-ratio groups showed similar OS, relapse risk, and LFS. High-ratio patients had a worse outcome. In patients without NPM1 mutations, FLT3-ITD subgroups showed comparable outcomes, with a higher risk of relapse and shortened OS than WT FLT3 patients.
Pastore et al reported on 349 patients with CN-AML and an NPM1 mutation who were treated with induction chemotherapy as part of the AMLGC99 trial (NCT00266136). The aim of the study was to assess if different NPM1 mutations are prognostic for RFS and OS. A minority of patients (16%) underwent allogeneic stem cell transplant in the first CR. NPM1 mutation combined with FLT3-ITD was associated with a reduced OS (HR=2.04; 95% CI, 1.40 to 2.96; p<0.001) and RFS (HR=2.45; 95% CI, 1.60 to 3.75; p<0.001). In the authors’ assessment of co-occurring FLT3-ITD and NPM1 mutations, the type of mutation did not result in a statistically significant change in OS or RFS between NPM1 mutation types. The authors concluded that NPM1 mutations can be prognostic for OS and RFS in CN-AML patients but further stratification by type of mutation does not appear to be useful.

Chou et al conducted a retrospective analysis of 325 adult AML patients to determine the prognostic significance of 8 mutations, including CEBPA, FLT3-ITD, and NPM1, on OS between patients who received allogeneic HSCT (n=100) and those who did not (n=255). Karyotype included favorable (n=51), intermediate (n=225), and unfavorable (n=40). Patients were selected from a single Taiwanese hospital between 1995 and 2007. Pediatric patients and those receiving only supportive care were excluded from the study. Patients received induction chemotherapy followed by allogeneic stem cell transplant, or consolidation chemotherapy for those patients who did not achieve CR. In the nonallogeneic HSCT patients, NPM1/FLT3-ITDwt (HR=0.363; 95% CI, 0.188 to 0.702; p=0.003) and CEBPA double mutation (HR=0.468; 95% CI, 0.265 to 0.828; p=0.009) were significant good prognostic factors of OS in a multivariate analysis. All other gene mutations failed to have a significant impact on OS in the HSCT and non-HSCT groups in the multivariate analysis. The authors presented survival curves stratified by CEBPA and FLT3-ITD mutations and found that, in the non-HSCT group, CEBPA and FLT3-ITDwt mutations were prognostic of improved OS (p=0.008 and p=0.001, respectively), but, in the allogeneic HSCT group, neither mutation had a prognostic effect. The inability to detect mutations of prognostic significance in the HSCT group could be due to the small number of patients with the studied mutations (CEBPA=9, NPM1=13, FLT3-ITD=25).

Li et al conducted a meta-analysis of 10 studies to evaluate the prognostic significance of CEBPA mutations in patients with AML. A total of 6219 subjects were analyzed, with the percentage of patients with CN-AML ranging from 56% to 100%. Three studies examined the CN-AML population exclusively. Seven studies examined CEBPA monoallelic mutation patients and showed no significant differences in event-free survival (EFS; HR=1.11; 95% CI, 0.85 to 1.46; p=0.42) or OS (HR=1.11; 95% CI, 0.84 to 1.45; p=0.43). Three studies examined CEBPA monoallelic mutation patients who had CN-AML and also found no differences in EFS (HR=0.88; 95% CI, 0.65 to 1.19; p=0.407) or OS (HR=1.11; 95% CI, 0.85 to 1.46; p=0.085). However, the pooled analysis of 8 studies with biallelic mutation CEBPA patients found a favorable prognosis for EFS (HR=0.41; 95% CI, 0.32 to 0.52; p<0.001) and OS (HR=0.37; 95% CI, 0.27 to 0.50; p<0.001). This positive prognostic effect on EFS (HR=0.38; 95% CI, 0.29 to 0.49; p<0.001) and OS (HR=0.32; 95% CI, 0.23 to 0.43; p<0.001) remained when restricting the
analysis to 4 studies of CN-AML. The authors concluded that biallelic CEBPA mutations are associated with an improved prognosis in CN-AML patients and could help stratify patient risk for clinical treatment.

Pastore et al examined 88 CN-AML patients with CEBPA mutations treated with induction chemotherapy who were enrolled in either the AMLCG99, AMLCG2008, or the HD98-A clinical trials.23 Forty-five (51%) patients had CEBPA biallelic mutations; NPM1 or FLT3-ITD mutations were present in 19% and 6% of patients, respectively. Patients with biallelic CEBPA mutations had a significantly longer median survival time of 9.6 years compared with 1.7 years in patients with monoallelic CEBPA mutations (p=0.008). Results with biallelic CEBPA mutations showed a significantly longer median RFS of 9.4 years compared with 1.5 years in monoallelic CEBPA (p=0.021). The authors adjusted for the potential confounding effects of NPM1 and FLT3-ITD mutations, which occurred more commonly in monoallelic CEBPA patients, and found that the positive impact of biallelic CEBPA mutations on OS and RFS remained.

Ongoing and Unpublished Clinical Trials
A search of ClinicalTrials.gov in June 2015 did not identify any ongoing or unpublished trials that would likely influence this policy.

Summary of Evidence
Acute myeloid leukemia (AML) is a heterogeneous disease and treatment is based on risk stratification, mainly by patient age and tumor cytogenetics (karyotyping), which allow for patients to be divided into good, intermediate, and poor risk categories. The identification of mutations in several genes, including FLT3, NPM, and CEBPA, have been proposed to allow for further segregation of prognostic categories in the cytogenetically normal group.

FLT3 internal tandem duplication (FLT3-ITD) mutations are known to confer a very poor prognosis, whereas NPM1 and biallelic CEBPA mutations have been shown to confer an independently favorable prognosis. Limited data suggest that a coexistent NPM1 mutation may mitigate the negative prognostic effect of an FLT3-ITD mutation, if both mutations are present. The prognostic effect of FLT3 tyrosine kinase domain (FLT3-TKD) mutations is uncertain.

Data on the analytic and the clinical validity of FLT3, NPM1, and CEBPA mutation testing are lacking. Data on the clinical utility of testing for these mutations is limited to retrospective analyses, and consist predominantly of studies of the effect of the presence of an FLT3-ITD mutation in patients who underwent hematopoietic stem cell transplant (HSCT) versus those who did not. Although some controversy exists as to the survival benefit in transplanting a patient with an FLT3-ITD mutation, retrospective studies, in general, have suggested a survival benefit for these poor-risk patients, and major professional societies and guidelines recommend testing for these mutations to risk stratify and to inform treatment management decisions, including possible HSCT.
Therefore, evidence is sufficient to determine that genetic testing for \textit{FLT3-ITD, NPM1, and CEBPA} mutations improves the net health outcome for patients with cytogenetically normal AML. Evidence is insufficient to determine that genetic testing for \textit{FLT3-TKD} mutations improves the net health outcome for cytogenetically normal AML.

**Practice Guidelines and Position Statements**

**National Comprehensive Cancer Network**
The National Comprehensive Cancer Network guidelines for Acute Myeloid Leukemia\(^\text{24}\) (v.1.2015) provide the following recommendations:

For the evaluation and initial workup for suspected acute leukemias, bone marrow analysis with cytogenetics (karyotype) with or without fluorescence in situ hybridization (FISH) is necessary to establish the diagnosis of AML; cryopreservation of samples for evaluation of other markers, including FLT3-ITD and NPM1 mutations.

Evaluation of several molecular markers (e.g., FLT3, NPM1, CEBPA, and c-KIT) may be important for risk assessment and prognostication, and may also guide treatment decisions.

**Alberta Provincial Hematology Tumour Team**
The Alberta Provincial Hematology Tumour Team\(^\text{25}\) issued a 2009 guideline on AML that includes the recommendation for molecular analysis in cases with normal karyotypes, including FMS-like tyrosine kinase 3 (FLT3).

**U.S. Preventive Services Task Force Recommendations**
Not applicable.

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

**References**


Billing Coding/Physician Documentation Information

81218  CEBPA (CCAAT/enhancer binding protein [C/EBP], alpha) (eg, acute myeloid leukemia), gene analysis, full gene sequence

81245  FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis, internal tandem duplication (ITD) variants (ie, exons 14, 15)

81246  FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis; tyrosine kinase domain (TKD) variants (eg, D835, I836)

81310  NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, exon 12 variants

81403  Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)

ICD-10 Codes

C92.00-C92.02  Acute myeloblastic leukemia code range

C92.60-C92.62  Acute myeloid leukemia with 11q23-abnormality code range

C92.A0-C92.A2  Acute myeloid leukemia with multilineage dysplasia code range

Additional Policy Key Words

N/A

Policy Implementation/Update Information

9/1/14  New policy; considered investigational.

9/1/15  Title revised and medically necessary statement added for CEBPA mutation testing. Added CPT codes 81246, 81403

9/1/16  Added CPT 81218. No policy statement changes.

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