2.04.124	Genetic Testing for FLT3, NPM1, and CEBPA Variants in Cytogenetically Normal Acute Myeloid Leukemia		
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Section:	2.0 Medicine	**Page:**	Page 1 of 28

Policy Statement

I. Genetic testing for *FLT3* internal tandem duplication (*FLT3*-ITD), *NPM1*, and *CEBPA* variants may be considered **medically necessary** in cytogenetically normal acute myeloid leukemia (see Policy Guidelines section).

II. Genetic testing for *FLT3-ITD*, *NPM1*, and *CEBPA* variants is considered **investigational** in all other situations.

III. Genetic testing for *FLT3* tyrosine kinase domain (FLT3-TKD) variants is considered **investigational**.

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

NOTE: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

Policy Guidelines

Genetic testing for cytogenetically normal acute myeloid leukemia is intended to guide management decisions in individuals who would receive treatment other than low-dose chemotherapy or best supportive care.

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.

Coding

There is specific CPT coding for the following testing:

- **0023U**: Oncology (acute myelogenous leukemia), DNA, genotyping of internal tandem duplication, p.D835, p.I836, using mononuclear cells, reported as detection or non-detection of FLT3 mutation and indication for or against the use of midostaurin
- **0046U**: FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia) internal tandem duplication (ITD) variants, quantitative.
- **0049U**: NPM1 (nucleophosmin) (e.g., acute myeloid leukemia) gene analysis, quantitative. This code is for NPM1 MRD by NGS by LabPMM LLC
- **0050U**: Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements. This code is for MyAML NGS Panel by LabPMM LLC
- **81218**: CEBPA (CCAAT/enhancer binding protein [C/EBP], alpha) (e.g., acute myeloid leukemia), gene analysis, full gene sequence

- **81245**: FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia), gene analysis; internal tandem duplication (ITD) variants (i.e., exons 14, 15)
- **81246**: FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia), gene analysis; tyrosine kinase domain (TKD) variants (e.g., D835, I836)
- **81310**: NPM1 (nucleophosmin) (e.g., acute myeloid leukemia) gene analysis, exon 12 variants

Effective October 1, 2022, the following CPT code has been **deleted**:

- **0056U**: Hematology (acute myelogenous leukemia), DNA, whole genome next-generation sequencing to detect gene rearrangement(s), blood or bone marrow, report of specific gene rearrangement(s)

Description

Treatment of acute myeloid leukemia (AML) is based on risk stratification, primarily related to patient age and tumor cytogenetics. In patients with cytogenetically normal AML, the identification of variants in several genes, including FLT3, NPM1, and CEBPA, has been proposed to allow for further segregation in the management of this heterogeneous disease.

Related Policies

- Hematopoietic Cell Transplantation for Acute Myeloid Leukemia

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

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. Several laboratories offer these tests, including Quest Diagnostics, Medical Genetic Laboratories of Baylor College, Geneva Labs of Wisconsin, LabPMM, and ARUP Laboratories, and they are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed under 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.

In May 2017, the FDA granted approval for midostaurin (Rydapt®, Novartis Pharmaceuticals). Rydapt is a targeted therapy to be used in combination with chemotherapy when an *FLT3* variant is detected by the LeukoStrat® CDx *FLT3* Mutation Assay (Invivoscribe). In 2018, gilteritinib (Xospata®, Astellas Pharma US) was approved by the FDA for the treatment of relapsed or refractory acute myeloid leukemia with a *FLT3* mutation as detected by an FDA-approved test.

Rationale

Background

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. The American Cancer Society has estimated there will be 20,050 new cases of AML and 11,540 deaths from AML in the United States in 2022.[1,]

Diagnosis and Prognosis of Acute Myeloid Leukemia

The most recent World Health Organization classification (2022) reflects the increasing number of acute leukemias that can be categorized based on underlying cytogenetic abnormalities (i.e., at the level of the chromosome including chromosomal translocations or deletions) or molecular genetic abnormalities (i.e., at the level of the function of individual genes, including gene variants) and those distinguished by differentiation without defining genetic abnormalities. These cytogenetic and molecular changes form distinct clinicopathologic-genetic entities with diagnostic, prognostic, and therapeutic implications.[2,] 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 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 variants of *CEBPA*, encoding a transcription factor, variants of the *FLT3* gene, encoding a receptor of tyrosine kinase involved in hematopoiesis, and a variant of the *NPM1* gene, encoding a shuttle protein within the nucleolus. "AML with *NPM1* mutation" and "AML with *CEBPA* mutation" were included as categories in the 2022 World Health Organization classification of acute leukemias. AML with *FLT3* variants is not considered a distinct entity in the 2022 or prior 2016 classifications.[2,3,] The 2008 World Health Organization classification recommended determining the presence of *FLT3* variants because of the prognostic significance.[4,]

Treatment

AML has a highly heterogeneous clinical course, and treatment generally depends on the different risk stratification categories.[5,] Depending on the risk stratification category, treatment modalities may include intensive remission induction chemotherapy, hypomethylating agents, enrollment in clinical trials with innovative compounds, palliative cytotoxic treatment, or supportive care only. For patients who achieve complete remission after induction treatment, possible postremission treatment options include intensive consolidation therapy, maintenance therapy, or autologous or allogeneic hematopoietic cell transplant.

Measurable (Minimal) Residual Disease Monitoring

Relapse in AML is believed to be due to residual clonal cells that remain following "complete response" after induction therapy but are below the limits of detection using conventional morphologic assessment.[6,] Residual clonal cells that can be detected in the bone marrow or blood are referred to as measurable residual disease (MRD), also known as minimal residual disease. Measurable residual disease assessment is typically performed by multiparameter flow cytometry or polymerase chain reaction with primers for common variants. It is proposed that finding MRD at different time points in the course of the disease (e.g., after initial induction, prior to allogenic transplantation) may be able to identify patients at a higher risk for relapse. In those with a high risk of relapse during the first remission, stem cell transplantation may be a more appropriate treatment approach. Studies in both children and adults with AML have demonstrated the correlation between MRD and risk for relapse. The role of MRD monitoring in AML is evolving, and important limitations

remain. Some patients may have relapse despite having no MRD, while others do not relapse despite being MRD positive. Standards have recently been introduced for identifying certain individual markers for MRD assessment, and threshold values delineating MRD positivity and negativity have recently been defined for multiparameter flow cytometry and some variants detected by polymerase chain reaction or other methods.[7,]

FLT3 Variants

FMS-like tyrosine kinase (*FLT3*) plays a critical role in normal hematopoiesis and cellular growth in hematopoietic stem and progenitor cells. Variants in *FLT3* are among the most frequently encountered in AML.[8,] *FLT3* variants are divided into 2 categories: (1) internal tandem duplications (*FLT3*-ITD) variants, 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 variants are much more common than *FLT3*-TKD variants, occurring in 30% of newly diagnosed adult cases of AML, versus *FLT3*-TKD variants, occurring in about 10% of patients.[9,] *FLT3*-ITD variants are a well-documented adverse prognostic marker, particularly in patients younger than 60 years of age with normal- or intermediate-risk cytogenetics, and are associated with an increased risk of relapse and inferior overall survival.[8,10,11,] Patients with *FLT3*-ITD variants have a worse prognosis when treated with conventional chemotherapy, compared with patients with wild-type (WT; i.e., nonmutated) *FLT3*. Although remission can be achieved in patients with *FLT3*-ITD variants 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 an *FLT3*-ITD variant is 6 to 7 months compared with 9 to 11 months in patients with other AML subtypes.[8,]

Because of the high-risk of relapse, hematopoietic cell transplantations as consolidation therapy of the first remission for an *FLT3*-ITD AML patient is often considered. However, this treatment must be weighed against the treatment-related mortality associated with a transplant.[8,]
The clinical significance of an *FLT3* variant varies by the nature of the variant and the context in which it occurs. Longer *FLT3*-ITD variants have been associated with worse overall survival.[12,]
For *FLT3*-ITD variants, the *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 variant detected at diagnosis is also present at relapse. However, in some cases, as *FLT3* -ITD positive AML evolves from diagnosis to relapse, the variant present at diagnosis may be absent (or undetectable) at relapse. This is most commonly seen where the mutant allele burden is low (5%-15%) at diagnosis.[8,] The assays for detecting *FLT3*-ITD , was previously considered to be unsuitable for use as a marker of minimal residual disease.[8,] Higher mutant-to-WT allelic ratios have been associated with worse outcomes.[8,]

The prognostic impact of *FLT3*-TKD variants is less certain and conflicting. Some studies have suggested a negative impact of tyrosine kinase domain variants on event-free survival and overall survival, while other studies have found no prognostic value, or potentially a benefit if a NPM1 mutation is also present.[13,14,9,] Next generation FLT3 tyrosine kinase inhibitors with greater specificity for FLT3 have been under clinical investigation, including gilteritinib, which was approved by the U.S. Food and Drug Administration (FDA) in 2018.[13,]

NPM1 Variants

A common molecular aberration in AML is a variant of *NPM1*, which is found in 28% to 35% of AML cases and is more common in cytogenetically normal AML.9, Up to 50% of AML with mutated NPM1 also carry an FLT3-ITD.[15,] Mutated *NPM1* confers an independent favorable prognosis for patients with cytogenetically normal AML and either the presence or absence of an *FLT3*-ITD variant. Retrospective studies of banked clinical samples have suggested that an *NPM1* variant may mitigate

the negative prognostic effect of an *FLT3*-ITD variant, but possibly only if the *FLT3*-ITD-to-WT allelic ratio is low.[8,] The prognostic impact in patients with an abnormal karyotype is unclear.[15,]

CEBPA Variants

CEBPA (CCAAT/enhancer-binding protein) is a transcription factor gene that plays a role in cell cycle regulation and cell differentiation. Variants of *CEBPA* are found in approximately 7% to 11% of AML patients.[16,17,9,] *CEBPA* variants can be either biallelic (double variants) or monoallelic. Monoallelic variants are prognostically similar to *CEBPA* WT variant and do not confer a favorable prognosis in cytogenetically normal AML, with the exception of mutations in the basic leucine zipper region; double variants of *CEBPA* and variants with single mutations in the basic leucine zipper region have shown a better prognosis with higher rates of complete remission and overall survival after standard induction chemotherapy.[18,19,20,21,]

Literature Review

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Promotion of greater diversity and inclusion in clinical research of historically marginalized groups (e.g., People of Color [African-American, Asian, Black, Latino and Native American]; LGBTQIA (Lesbian, Gay, Bisexual, Transgender, Queer, Intersex, Asexual); Women; and People with Disabilities [Physical and Invisible]) allows policy populations to be more reflective of and findings more applicable to our diverse members. While we also strive to use inclusive language related to these groups in our policies, use of gender-specific nouns (e.g., women, men, sisters, etc.) will continue when reflective of language used in publications describing study populations.

Testing for *FLT3*, *NPM1*, and *CEBPA* Variants to Risk-Stratify Acute Myeloid Leukemia

Clinical Context and Test Purpose

Optimal decisions regarding treatment intensity and chemotherapy-based consolidation therapy versus allogeneic transplantation remain unclear in cytogenetically normal acute myeloid leukemia (CN-AML). The purpose of genetic testing in patients who have CN-AML is to provide prognostic risk stratification information that may inform decisions regarding:

- whether to use standard or increased treatment intensity in induction therapy, consolidation therapy, or in relapsed/refractory acute myeloid leukemia (AML);
- whether to do allogeneic or autologous transplantation versus chemotherapy as consolidation therapy for an AML patient in the first remission;
- whether to use therapies such as FLT3 inhibitors.

Genetic testing can be used during the initial evaluation of leukemia to provide prognostic information and guide treatment decisions.

Induction therapy usually consists of 7 days of continuous-infusion cytarabine at 100 to 200 mg/m^2 with 3 days of anthracycline. Studies have shown greater efficacy at higher doses but also increased toxicity.

Transplantation reduces the risk of recurrence but is typically associated with at least a 20% treatment-related mortality risk.

Side effects of FLT3 inhibitors (e.g., sorafenib, sunitinib, midostaurin, quizartinib, gilteritinib) include QT prolongation, nausea, vomiting, diarrhea, anemia, abnormal liver function tests, increased bilirubin, fever, and fatigue. Currently, the FLT3 inhibitor midostaurin has been approved by the U.S. Food and Drug Administration to be used in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation. Sorafenib and sunitinib are approved for treatment of other malignancies. Gilteritinib is only approved for treatment of relapsed or refractory AML.

The question addressed in this evidence review is: Does *FLT3, NPM1,* or *CEBPA* genetic testing improve the net health outcome in individuals with AML?

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is patients with CN-AML, including newly diagnosed, those in the first remission, and those who have relapsed.

Interventions
The intervention of interest is testing *for FLT3*, *NPM1*, or *CEBPA* variants. During initial assessment of AML, genetic testing provides prognostic risk assessment and helps guide treatment decisions.

Comparators
The comparator of interest is risk stratification without *FLT3*, *NPM1*, or *CEBPA* genetic testing.

Outcomes
The general outcomes of interest are overall survival, disease-free survival, test validity, treatment-related mortality, and treatment-related morbidity.

Outcomes are focused on overall- and cancer-specific mortality, although treatment-related morbidity in the short- and long-term is also a focus.

The assays can be conducted during diagnostic evaluation, to aid in the treatment decision process.

Study Selection Criteria
For the evaluation of clinical validity of the genetic tests for *FLT3*, *NPM1*, and *CEBPA*, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Prognosis of patients with *FLT3* internal tandem duplication (ITD), *NPM1*, or *CEBPA* variants compared with patients without *FLT3*-ITD, *NPM1*, or *CEBPA* variants are described in Table 1. Results from systematic reviews are presented when available and individual studies are included if they described a population not represented in the systematic reviews.

Table 1. Survival Outcomes of Patients With *FLT3*-ITD, *NPM1*, or *CEBPA* Variants

Study	Design	Participants	Outcomes
Port et al (2014)[22,]	Systematic review of 19 studies published between 2000 and 2012, with 4 studies included in the meta-analysis	1942 patients with CN-AML <60 y in meta-analysis	*FLT3*-ITD WT vs. *FLT3*-ITD variant: • OS HR=1.9 (95% CI, 1.6 to 22) • RFS HR=1.8 (95% CI, 1.5 to 2.2) *NPM1* WT vs. *NPM1* variant: • OS HR=0.6 (95% CI, 0.5 to 0.7) • RFS HR=0.6 (95% CI, 0.5 to 0.6) *CEBPA* WT vs. *CEBPA* variant: • OS HR=0.4 (95% CI, 0.3 to 0.5) • RFS HR=0.4 (95% CI, 0.3 to 0.6)
Li et al (2015)[19,]	Systematic review of 10 studies published before Aug 2014	6219 patients with AML	Any AML: • *CEBPA* monoallelic vs. WT o OS HR=1.1 (95% CI, 0.9 to 1.5) o EFS HR=1.1 (95% CI, 0.8 to 1.5) • *CEBPA* biallelic vs. WT: o OS HR=0.4 (95% CI, 0.3 to 0.5) o EFS HR=0.4 (95% CI, 0.3 to 0.5) CN-AML: • *CEBPA* monoallelic vs. WT: o OS HR=1.1 (95% CI, 0.9 to 1.5) o EFS HR=0.9 (95% CI, 0.7 to 1.2) • *CEBPA* biallelic vs. WT: o OS HR=0.3 (95% CI, 0.2 to 0.4) o EFS HR=0.4 (95% CI, 0.3 to 0.5)
Dickson et al (2016)[23,]	Retrospective analysis of patients enrolled in an RCT between 1990 and 1998	662 AML patients >60 y	1-y OS: • *CEBPA*, biallelic: 75% • *NPM1* variant, *FLT3*-ITD WT: 54% • All others: 33% 3-y OS: • *CEBPA*, biallelic: 17% • *NPM1* variant, *FLT3-ITD* WT: 29% • All others: 12%
Wu et al (2016)[24,]	Systematic review of 10 cohort studies published between 1995 and 2015	1661 pediatric patients with AML	*FLT3*-ITD WT vs. *FLT3*-ITD variant: • OS HR=2.2 (95% CI, 1.6 to 3.0) • EFS HR=1.7 (95% CI, 1.4 to 2.1)
Kuwatsuka et al (2017)[25,]	Retrospective analysis of patients enrolled in 2 clinical trials between 2001 and 2010	103 adolescent and young adults (age range, 15-39 y) with AML	*FLT3*-ITD WT vs. *FLT3*-ITD variant: • OS HR=2.1 (95% CI, 1.1 to 4.1) • EFS HR=2.4 (95% CI, 1.3 to 4.2) *NPM1* WT vs. *NPM1* variant: • OS HR=0.2 (95% CI, 0.06 to 1.0) • RFS HR=0.2 (95% CI, 0.09 to 0.7)
Rinaldi et al (2020)[26,]	Systematic review of 10 studies published between 1999 to 2020	1513 adult, non-transplant patients with AML	*FLT3*-ITD WT vs. *FLT3*-ITD variant: • OS HR=1.91 (95% CI, 1.59 to 2.30) • EFS HR=1.64 (95% CI, 1.26 to 2.14)
Tarlock et al (2021)[20,]	Retrospective analysis of patients enrolled in 4 clinical trials between 1996 and 2016	2958 children and young adults with AML (5.4% with *CEBPA* mutations in the basic leucine zipper region)	*CEBPA* WT vs. *CEBPA* biallelic vs. *CEBPA* single mutation in basic leucine zipper region: • 5-year OS 61% vs. 81% vs. 89% (p<.001 for WT vs. others; p=.259 for single vs. biallelic mutations) • 5-year EFS 46% vs. 64% vs. 64% (p<.001 for WT vs. others, p=.777 for single vs. biallelic mutations)

Study	Design	Participants	Outcomes
Issa et al (2022)[27,]	Retrospective analysis of patients treated at a single center between 2012 and 2020	1722 adults with relapsed or refractory AML (12% with *NPM1* mutations)	*NPM1* WT vs. *NPM1* variant: • OS 5.5 months vs. 6.1 months (p=.07) • RFS 5.6 months vs. 5.5 months (p=.4)

AML: acute myeloid leukemia; CI: confidence interval; CN; cytogenetically normal; EFS: event-free survival; HR: hazard ratio; ITD: internal tandem duplication; OS, overall survival; RCT: randomized controlled trial; RFS: recurrence-free survival; WT; wild-type.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

The literature on the use of genetic markers for initial evaluation consists mostly of retrospective analyses and RCTs evaluating FLT3 inhibitors in patients with confirmed *FLT3* variants.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Randomized Controlled Trials

Knapper et al (2017) published results from 2 RCTs in which patients with previously untreated AML and confirmed *FLT3* variants were randomized to lestaurtinib (a FLT3 inhibitor) or placebo following each of 4 cycles of induction and consolidation chemotherapy (see Tables 2 and 3).[28,] Patients with ITD subtype (74%), tyrosine kinase domain subtype (TKD, 23%), and both subtypes (2%) were included. There were no significant differences in remission or survival estimates between treatment groups (see Table 3).

Stone et al (2017) published results from an RCT in which patients with previously untreated AML and confirmed *FLT3* variants were randomized to standard chemotherapy with or without midostaurin (see Tables 2 and 3).[29,] Patients with ITD (77%) and TKD (23%) subtypes were included. The addition of midostaurin did not affect complete remission rates or time to complete remission in the overall cohort; however, overall and event-free survival was significantly better in the midostaurin group than in the placebo group (see Table 3). Voso et al (2020) published a subgroup analysis of the trial evaluating outcomes in patients with the TKD subtype.[30,] In this subgroup, 5-year event-free survival was significantly better in the midostaurin group than in the placebo group (45.2% vs. 30.1%; hazard ratio [HR], 0.66; 95% confidence interval [CI], 0.45 to 0.99; p=.044), but 5-year overall survival was similar between the 2 treatment groups (65.9% vs. 58.0%; HR, 0.74; 95% CI, 0.44 to 1.23; p=.244).
Perl et al (2019) published results from an RCT evaluating patients with relapsed/refractory *FLT3*-mutated AML who were randomized to gilteritinib (a FLT3 inhibitor) or salvage chemotherapy (see Tables 2 and 3).[31,] Patients with the ITD subtype (88.4%), TKD subtype (8.4%), and both subtypes (1.9%) were included. Overall, 60.6% of patients had relapsed disease, and 39.4% had primary refractory disease. Median overall survival and percent of patients achieving complete remission was significantly better with gilteritinib.

Cortes et al (2019) published results from an RCT evaluating patients with relapsed/refractory *FLT3*-mutated AML who were randomized to quizartinib (a FLT3 inhibitor) or salvage chemotherapy (see Tables 2 and 3).[32,] Only patients with the *FLT3* ITD subtype were included. One third of patients had

refractory disease, while the rest had relapsed disease. Overall survival was improved with quizartinib compared to salvage chemotherapy.

Table 2. Summary of RCT Characteristics

					Treatment	
Study	**Countries**	**Sites**	**Dates**	**Participants**	**Active**	**Comparator**
Knapper et al (2017)[28,]	England,Denmark, New Zealand	>130	May 2002 to Dec 2014	Patients with previously untreated AML and confirmed *FLT3* variants, mostly <60 y	• n=300 • 4 cycles of induction and consolidation chemotherapy, followed by lestaurtinib (FLT3 inhibitor)	• n=200 • 4 cycles of induction and consolidation chemotherapy, followed by placebo
Stone et al (2017)[29,]	17 in North America, Europe, Australia	225	May 2008 to Oct 2011	Patients with previously untreated AML and confirmed *FLT3* variants, 18-59 y	• n=360 • Standard chemotherapy plus midostaurin (kinase inhibitor)	• n=357 • Standard chemotherapy plus placebo
Perl et al (2019)[31,]	14 in North America, Europe, Asia	107	Oct 2015 to Sept 2018	Patients with refractory or relapsed AML and confirmed *FLT3* variants, 19-85 y	• n=247 • Gilteritinib	• n=124 • Salvage chemotherapy
Cortes et al (2019)[32,]	19 in North America, Europe, Asia	152	May 2014 to Sept 2017	Patients with refractory or relapsed AML and confirmed *FLT3* variants (with or without allo-HCT), median age 56 y	• n=245 • Quizartinib	• n=122 • Salvage chemotherapy

allo-HCT: allogenic hemopoietic stem cell transplant; AML: acute myeloid leukemia; RCT: randomized controlled trial.

Table 3. Summary of RCT Outcomes

Study	Outcomes	Active	Control	HR (95% CI)
Knapper et al (2017)[28,]				
	CR + CRi			1.4 (0.7 to 2.8)
	5-y overall survival	NR	NR	0.9 (0.7 to 1.1)
	5-y overall survival, censored at SCT	NR	NR	0.9 (0.7 to 1.3)
	5-y cumulative incidence, relapse	NR	NR	0.9 (0.7 to 1.1)
	5-y cumulative incidence, death in remission	NR	NR	1.1 (0.6 to 2.0)
	5-y relapse-free survival	NR	NR	0.9 (0.7 to 1.1)
Stone et al (2017)[29,]				
	CR rate (95% CI)	59 (54 to 64)	54 (48 to 59)	*NS*
	Time to complete remission (range), median days	35 (20-60)	35 (20 to 60)	*NS*
	Overall survival (95% CI), median months	75 (31 to NR)	26 (19 to 43)	0.8 (0.6 to 1.0)
	Event-free survival (95% CI), median months	8.2 (5 to 11)	3 (2 to 6)	p=.002
Perl et al (2019)[31,]				
	Overall survival (95% CI), median months	9.3 (7.7 to 10.7)	5.6 (4.7 to 7.3)	0.64 (0.49 to 0.83)
	Event-free survival (95% CI), median months	2.8 (1.4 to 3.7)	0.7 (0.2 to NE)	0.79 (0.58 to 1.09)
	CR rate (95% CI)	21.2 (NR)	10.5 (NR)	10.6 (2.8 to 18.4)
Cortes et al (2019)[32,]				
	Overall survival (95% CI), median months	6.2 (5.3 to 7.2)	4.7 (4.0 to 5.5)	0.76 (0.58 to 0.98)
	Event-free survival (95% CI), median months	1.4 (0 to 1.9)	0.9 (0.1 to 1.3)	0.90 (0.70 to 1.16)

CI: confidence interval; CR: complete remission; CRi: complete remission with incomplete peripheral blood count recovery; HR: hazard ratio; NE: not evaluable; NR: not reported; NS: not significant; RCT: randomized controlled trial; SCT: stem cell transplantation.

Retrospective Studies

Outcomes Based on Genetic Variant Status

Literature from retrospective analyses describing outcomes by type of treatment for patients with and without *FLT3*-ITD, *CEBPA*, and *NPM1* variants are shown in Table 4. Results from systematic reviews are presented when available and individual studies are shown if the populations were not included in the scope of the systematic reviews. Narrative summaries of select studies are presented following the table.

Most of the literature consists of analyses of *FLT3*-ITD variants and survival outcomes with the use of allogeneic hematopoietic cell transplantations (allo-HCT) in patients depending on the presence of this type of variant. In general, the data support use of HCT in patients with *FLT3*-ITD variants, however, not all studies have shown consistent results.[8,]

Table 4. Retrospective Analyses of Results by Treatment of Patients With and Without Genetic Variants

Study	Design	Participants	Outcomes Estimate (95% CI)
Schlenk et al (2008)[33,]	Retrospective analysis of patients in 4 AML therapy RCTs conducted between 1993 and 2004	872 adults <60 y with CN-AML, 53% *NPM1* variant, 31% *FLT3*-ITD variant, 11% *FLT3*-TKD variant, 13% *CEBPA* variant	Allo-HCT vs. other consolidation therapy: • *NPM1* without *FLT3*-ITD • Relapse rate HR=0.9 (0.5 to 1.8) Other genotypes (excluding *CEBPA*, *NPM1* without *FLT3*-ITD): • Relapse rate HR=0.6 (0.4 to 0.9)
Schlenk et al (2013)[34,]	Retrospective analysis of patients in 7 AML therapy RCTs conducted between 1987 and 2009	124 adults <60 y with CN-AML who were *CEBPA* biallelic and had CR after induction therapy	Allo-HCT vs. chemo: • RFS HR=0.2 (0.1 to 0.5) • OS HR=0.5 (0.2 to 1.2) Auto-HCT vs. chemo: • RFS HR=0.4 (0.2 to 0.8) • OS HR=0.6 (0.2 to 1.4)
Willemze et al (2014)[35,]	Retrospective analysis of EORTC-GIMEMA AML-12 RCT conducted between 1999 and 2008	613 patients with AML, ages 15-60 y; 126 (21%) *FLT3*-ITD variant	Patients with *FLT3*-ITD variant categorized as very bad risk: • OS at 6 y in patients at very bad risk 20% in standard cytarabine group vs. 31% in high-dose group: • HR=0.70 (0.47 to 1.04)
Chou et al (2014)[36,]	Retrospective analysis of patients from Taiwanese university hospital between 1995 and 2007	325 adults with AML who received conventional induction chemo; 81 (25%) *FLT3*-ITD, 69 (21%) *NPM1*, 33 (10%) *NPM1* with *FLT*-ITD WT, 42 (13%) *CEBPA* biallelic	Non-allo-HCT: • *CEBPA* biallelic vs. other o OS HR=0.5 (0.3 to 0.8) • *NPM1* variant with *FLT3*-ITD WT: o OS HR=0.4 (0.2 to 0.7) Allo-HCT: • *CEBPA* biallelic vs. other: o OS HR=0.3 (0.1 to 1.2) • *NPM1* variant with *FLT3*-ITD WT: o OS HR=NR
Ma et al (2015)[37,]	Systematic review of 9 studies of chemo vs. HCT published between 1989 and 2013	Patients with AML, *FLT3*-ITD variant	Allo-HCT vs. chemo: • OS OR=2.9 (2.0 to 4.1) • DFS OR=2.8 (1.9 to 4.3) • Relapse rate OR=0.1 (0.05 to 0.2)
Tarlock et al (2016)[38,]	Retrospective analysis of 2 AML RCTs conducted between 2003 and 2005	183 children with AML, *FLT3*-ITD variant who received standard chemo and HCT	Standard chemo with vs. without gemtuzumab ozogamicin: • Overall o Relapse rate, 37% vs. 59% (p=.02)

Study	Design	Participants	Outcomes Estimate (95% CI)
			○ DFS=47% vs. 41% (p=.45) ○ TRM=16% vs. 0% (p=.008) • Patients with high *FLT3*-ITD allelic ratio ○ Relapse rate, 15% vs. 53% (p=.007) ○ DFS 65% vs. 40% (p=.08) ○ TRM=19% vs. 7% (p=.08)
Ahn et al (2016)[39],	Retrospective analysis of patients from 7 institutions in South Korea from 1998 to 2012	404 CN-AML patients ages ≥15 y treated with conventional induction chemo; 51 (13%) *CEBPA* biallelic	Overall, by *CEBPA:* • 5-y OS biallelic, 62% (43% to 82%) • 5-y OS monoallelic, 44% (19% to 69%) • 5-y OS WT=26% (19% to 32%) Biallelic vs. others: • HR=0.4 (p=.001) Among *CEBPA* biallelic: • Chemo: ○ 5-y OS=60% (40% to 81%) ○ 5-y EFS=39% (15% to 64%) ○ 5-y relapse incidence, 38% (17% to 59%) • Allo-HCT: ○ 5-y OS=72% (54% to 90%) ○ 5-y EFS=73% (55% to 90%) ○ 5-y relapse incidence, 8% (1% to 23%)
Brunner et al (2016)[40],	Retrospective analysis of patients at 2 U.S. institutions between 2008 and 2014	81 consecutive AML patients who underwent *FLT3*-ITD testing who achieved CR with induction chemo followed by allo-HCT	Sorafenib maintenance therapy vs. no sorafenib • 2-y OS=81% vs. 62%; HR=0.3 (0.1 to 0.8) • 2-y PFS=82% vs. 53%; HR=0.3 (0.1 to 0.8)
Versluis et al (2017)[41],	Retrospective analysis of patients from 4 trials who achieved CR after 1 or 2 induction chemo cycles	Intermediate risk patients receiving the following postremission treatment: chemo (n=148); auto-HCT (n=168); allo-HCT with MAC (n=137); and allo-HCT with RIC (n=68)	Auto-HCT vs. chemo: no difference in OS, RFS, relapse, or NRMAllo-HCT with MAC vs. chemo: no difference OS • RFS: HR=0.7 (0.5 to 1.0) • Relapse: HR=0.2 (0.1 to 0.3) • NRM: HR=9.1 (2.7 to 30.4) Allo-HCT with RIC vs. chemo: no difference in NRM • OS HR=0.5 (0.3 to 0.9) • RFS HR=0.5 (0.3 to 0.8) • Relapse HR=0.3 (0.2 to 0.6) Allo-HCT with MAC vs. auto-HCT: no difference in OS or RFS • Relapse HR=0.3 (0.2 to 0.5) • NRM HR=5.7 (2.3 to 13.9) Allo-HCT with RIC vs. auto-HCT: no difference in NRM: • OS HR=0.6 (0.4 to 1.0) • RFS HR=0.6 (0.4 to 1.0) • Relapse HR=0.5 (0.3 to 0.9)
Taube et al (2022)[21],	Retrospective analysis of patients enrolled in 4 clinical trials or the	4708 patients who received intensive chemotherapy followed	Biallelic *CEBPA* vs. unselected single *CEBPA* mutation vs. *CEBPA*-WT:

Study	Design	Participants	Outcomes Estimate (95% CI)
	Study Alliance Leukemia registry and biorepository	by risk-stratified consolidation, with the option of HCT for eligible patients (5.1% with *CEBPA* mutations)	• Median OS 103.2 months vs. 21.9 months vs. 19.3 months, p<.001 • Median EFS 20.7 months vs. 9.4 months vs. 7.0 months, p<.001 Biallelic *CEBPA* vs. single mutation in basic leucine zipper region of *CEBPA* vs. single mutation in transcription activation domain of *CEBPA* vs. *CEBPA*-WT: • Median OS 103.2 months vs. 63.3 months vs. 12.7 months vs. 17.9 months • Median EFS 20.7 months vs. 17.1 months vs. 5.7 months vs. 7.0 months Multivariate analysis indicated *CEBPA* variants with a single mutation in the basic leucine zipper region were independently associated with prolonged OS (HR, 0.62; 95% CI, 0.42 to 0.92) and EFS (HR, 0.537; 95% CI, 0.37 to 0.77) after controlling for cytogenetic risk group, age, white blood cell count, diagnosis of treatment-related AML, *FLT3* mutations, *NPM1* mutations, and receipt of allogeneic HCT in first CR.
Döhner et al (2022)[42,]	Retrospective analysis of patients enrolled in the QUAZAR AML-001 trial	469 patients age 55 years or older with AML with intermediate- or poor-risk cytogenetics who achieved CR following intensive chemotherapy and were not considered candidates for HCT, and were then randomized to receive maintenance therapy with oral azacitidine or placebo	Oral azacitidine vs. placebo: • Patients with *NPM1* mutations: o OS HR=0.63 (0.41 to 0.98) o RFS HR=0.55 (0.35 to 0.84) • Patients with *NPM1*-WT: o Median OS 19.6 months vs. 14.6 months (p=.023) o Median RFS 7.7 months vs. 4.6 months (p=.003) • Patients with *FLT3* mutations: o Median OS 28.2 months vs. 9.7 months (p=.114) o Median RFS 23.1 months vs. 4.6 months (p=.032) • Patients with *FLT3*-WT: o Median OS 24.7 months vs. 15.2 months (p=.013) o Median RFS 10.2 months vs. 4.9 months (p=.001) Patients with *NPM1* mutations vs. *NPM1*-WT: • Placebo arm: o OS HR=0.69 (0.49 to 0.97) o RFS HR=0.65 (0.47 to 0.91) • Oral azacitidine arm: o OS HR=0.52 (0.36 to 0.75) o RFS HR=0.46 (0.31 to 0.66) Patients with *FLT3* mutations vs. *FLT3*-WT: • Placebo arm: OS HR=1.25 (0.83 to 1.89) • Oral azacitidine arm: OS HR=0.96 (0.60 to 1.54)

allo: allogeneic; AML: acute myeloid leukemia; auto: autologous; chemo: chemotherapy; CI: confidence interval; CN; cytogenetically normal; CR: complete remission; DFS: disease-free survival; EFS: event-free survival; HCT:

hematopoietic cell transplantation; HR: hazard ratio; ITD: internal tandem duplication; MAC: myeloablative conditioning; NR: not reported; NRM: nonrelapse mortality; OR: odds ratio; OS: overall survival; PFS: progression-free survival; RCT: randomized controlled trial; RFS: recurrence-free survival; RIC: reduced-intensity conditioning; TKD: tyrosine kinase domain; TRM: treatment-related mortality; WT: wild-type.

Ma et al (2015)[37,] performed a systematic review including 7 studies[43,44,45,46,47,48,49,] published up to December 2012 that described the use of HCT or chemotherapy in patients with AML in the first complete remission who had *FLT3*-ITD variants. All studies were retrospective or nonrandomized controlled analyses. Allo-HCT was associated with a longer OS (OR , 2.9; 95% CI, 2.0 to 4.1), longer DFS (OR , 2.8; 95% CI, 1.9 to 4.3), and reduction in relapse rate (OR , 0.1; 95% CI, 0.05 to 0.2) compared with chemotherapy. Overall survival and DFS rates favored allo-HCT but did not differ significantly between allo-HCT and autologous HCT (OS OR , 1.4; 95% CI, 0.8 to 2.4; DFS OR , 1.6; 95% CI, 0.8 to 3.3); however, relapse rates were lower for allo-HCT (OR , 0.4, 95% CI, 0.2 to 0.7).

Willemze et al (2014) conducted a randomized trial in 1942 patients newly diagnosed with AML, ages 15 to 60 years, to compare remission induction treatment containing standard or high-dose cytarabine.[35,] In both arms, patients who achieved complete remission received consolidation therapy with either autologous HCT or allo-HCT. Patients were subclassified as a good risk, intermediate risk, bad risk, very bad risk, or unknown risk, according to cytogenetics and *FLT3*-ITD variant. Testing for *FLT3*-ITD variants showed that, in the standard-dose cytarabine group, 50% were negative, 13% were positive, and 37% were indeterminate. In the high-dose cytarabine group, 48% were negative, 14% were positive, and 38% were indeterminate. All patients with an *FLT3*-ITD variant were categorized as a very bad risk. Overall survival 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=.02). Trialists concluded that patients with very bad risk cytogenetics and/or *FLT3*-ITD variants benefited from high-dose cytarabine induction treatment.

Chou et al (2014) retrospectively analyzed 325 adults with AML to determine the prognostic significance of 8 variants, including *CEBPA*, *FLT3*-ITD, and *NPM1*, on OS between patients who received allo-HCT (n=100) and those who did not (n=255).[36,] Karyotype included favorable (i.e., variant *CEBPA* or *NPM1* but without *FLT3*-ITD; 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 allo-HCT or consolidation chemotherapy for those patients who did not achieve complete remission. In the non-allo-HCT patients, *NPM1* variant/*FLT3*-ITD WT (HR , 0.363; 95% CI, 0.188 to 0.702; p=.003) and *CEBPA* double variant (HR , 0.468; 95% CI, 0.265 to 0.828; p=.009) were significant good prognostic factors of OS in a multivariate analysis. None of the other gene variants had a significant impact on OS in the HCT and non-HCT groups in the multivariate analysis. Authors presented survival curves stratified by *CEBPA* and *FLT3*-ITD variants and found that, in the non-HCT group, *CEBPA* and *FLT3*-ITD WT variants were prognostic of improved OS (p=.008 and p=.001, respectively), but, in the allo-HCT group, neither variant had a prognostic effect. The inability to detect variants of prognostic significance in the HCT group could have been due to the small number of patients with the studied variants (*CEBPA*=9, *NPM1*=13, *FLT3*-ITD=25).

Section Summary: Testing for *FLT3*, *NPM1*, and *CEBPA* Variants to Risk-Stratify Acute Myeloid Leukemia

The *FLT3*-ITD variant is quite common in AML, particularly in patients with normal karyotypes, and has been associated with poorer survival (overall, event-free, and recurrence-free) in children, younger adults, and older adults. The prognostic effect of FLT3 TKD variants is uncertain. NPM1 variants are found in approximately half of patients with CN-AML. *NPM1* variants are associated with improved outcomes; however, the superior prognosis is limited to those with *NPM1* variants who do not have an *FLT3*-ITD variant. *CEBPA* variants are found in approximately 15% of patients with CN-AML. Patients with *CEBPA* variants have a favorable prognosis, although the effect may be limited to patients who carry 2 copies of the mutant allele (biallelic) and those with single mutations

in the basic leucine zipper region. There are RCTs providing direct evidence of clinical utility, randomizing patients with AML and confirmed *FLT3* variants to different treatments. One RCT evaluated the addition of a FLT3 inhibitor, and 1 evaluated the addition of midostaurin to the chemotherapy regimen in patients with previously untreated AML. No significant difference between treatment groups was found with the addition of the FLT3 inhibitor, while the addition of midostaurin significantly improved OS and event-free survival compared with placebo. Another 2 RCTs evaluated comparative outcomes of treatment with a FLT3 inhibitor versus salvage chemotherapy in relapsed/refractory AML. Both gilteritinib and quizartinib prolonged survival compared to salvage chemotherapy in this population. Additionally, a chain of evidence for clinical utility can be constructed from retrospective analyses suggesting that risk stratification (favorable, intermediate, and poor) based on the presence of *NPM1*, *FLT3*-ITD, or *CEBPA* variants can help guide therapy decisions that are associated with improved outcomes. Patients with a favorable prognosis, including those who have *NPM*1 variants without *FLT3*-ITD variant or those with *CEBPA* biallelic or single basic leucine zipper region-mutant variants, may not derive an OS benefit with allo-HCT. Treatment of patients with intermediate or poor prognosis, including *FLT3*-ITD variant, depends on several risk factors, but HCT may improve outcomes.

Testing for *FLT3*, *NPM1*, or *CEBPA* Variants for Measurable Residual Disease Monitoring

Clinical Context and Test Purpose

The purpose of testing for *FLT3*, *NPM1*, or *CEBPA* variants in patients who have AML is to monitor for measurable residual disease (MRD) that may inform treatment decisions.

The question addressed in this evidence review is: Does *FLT3, NPM1,* or *CEBPA* genetic testing improve the net health outcome in individuals with AML who may have MRD?

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is patients with AML and a variant in *FLT3*, *NPM1*, or *CEBPA*.

Interventions

The intervention of interest is testing for *FLT3*, *NPM1*, or *CEBPA* variants. MRD evaluation is intended to assess risk for relapse and guide potential preemptive therapy.

Comparators

The comparator of interest is MRD surveillance based on morphologic relapse or other MRD methods without *FLT3*, *NPM1*, or *CEBPA* genetic testing.

Outcomes

The general outcomes of interest are overall survival, disease-free survival, test validity, treatment-related mortality, and treatment-related morbidity.

Study Selection Criteria

For the evaluation of clinical validity of the genetic tests for *FLT3*, *NPM1*, and *CEBPA*, studies that meet the following eligibility criteria were considered:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence

Monitoring for MRD can provide prognostic information on the risk of relapse in patients with *NPM1*- or *FLT3*-ITD-mutated AML; results of studies evaluating the use of MRD with these variants are summarized in Table 5.

Table 5. Prognostic Value of *NPM1*or *FLT3*-ITD MRD Assessment

Study	Design	Participants	MRD Assessment	Outcomes
Ivey et al (2016)[50,]	Retrospective evaluation of samples obtained from patients who had undergone intensive treatment in the National Cancer Research Institute AML17 trial (April 2009 to May 2012), with a prospective evaluation period (June 2012 to December 2014) to make up a validation cohort	346 patients with *NPM1*-mutated AML	RT-qPCR using a *NPM1*-specific primer; MRD positivity defined as amplification in at least 2 of 3 replicates with cycle-threshold values of 40 or less, using a threshold setting of 0.1	Positive MRD status vs. negative MRD status in peripheral blood following the second chemotherapy cycle (retrospective cohort): • Risk of relapse at 3 years: 82% vs. 30% (HR=4.80 [95% CI, 2.95 to 7.80]) • OS at 3 years: 24% vs. 75% (HR=4.38 [95% CI, 2.57 to 7.47]) Positive MRD status vs. negative MRD status in peripheral blood following the second chemotherapy cycle (validation cohort): • Risk of relapse at 2 years: 70% vs. 31% (p=.001) • OS at 2 years: 40% vs. 87% (p=.001)
Balsat el al (2017)[51,]	Retrospective evaluation of samples obtained from patients who were enrolled in the ALFA-0702 trial (April 2009 to August 2013)	152 patients with *NPM1*-mutated AML who achieved CR/CRp after induction	RT-qPCR using a *NPM1*-specific primer; a negative MRD was defined as *NPM1* transcript levels below the quantitative detection limit of the assay (0.01%)	Patients with <4-log reduction in *NPM1* from baseline vs. those with >5-log reduction in *NPM1* from baseline: • 3-year CIR: 65.8% vs. 20.5% • 3-year OS: 40.8% vs. 93.1%
Dillon et al (2020)[52,]	Retrospective evaluation of samples obtained from patients who had undergone intensive treatment in the National Cancer Research Institute AML17 trial (2009 to 2014)	107 patients with *NPM1*-mutated AML who underwent an allogenic stem cell transplantation	RT-qPCR using a *NPM1*-specific primer; MRD positivity defined as amplification in at least 2 of 3 replicates with cycle-threshold values of 40 or less, using a threshold setting of 0.1	Any detectable MRD vs. MRD-negative in pre-transplant samples: • 2-year OS: 45% vs. 83% (median OS: 10.5 months vs. not reached [HR=3.60; 95% CI, 1.92 to 6.77]) High MRD levels vs. low MRD levels (<200 copies in peripheral blood and <1000 copies in bone marrow) vs. MRD-negative in pre-transplant samples: • 2-year OS: 13% vs. 63% vs. 83%

Study	Design	Participants	MRD Assessment	Outcomes
				For those with low MRD levels, *FLT3*-ITD variant vs. *FLT3*-ITD wild-type: • 2-year OS: 25% vs. 77%
Grob et al (2022)[53,]	Retrospective analysis of patients enrolled in 3 clinical trials between 2006 and 2017	161 patients with de novo *FLT3*-ITD AML who achieved CR after induction	Capillary fragment length analysis and confirmation by targeted NGS for *FLT3*-ITD at diagnosis and targeted NGS for *FLT3*-ITD MRD assessment in CR; the lower limit of detection of the *FLT3*-ITD MRD assay ranged from allele frequencies of 0.01% to 0.001%	Patients with *FLT3*-ITD MRD detected in CR vs. not: • 4-year cumulative incidence of relapse 75% vs. 33% (HR=3.70 [95% CI, 2.31 to 5.94]) • 4-year OS 31% vs. 57% (HR=2.47 [95% CI, 1.59 to 3.84]) Multivariate analysis indicated *FLT3*-ITD MRD detected in CR was independently associated with risk of relapse (HR=3.55 [95% CI, 1.92 to 6.56]) and reduced overall survival (HR=2.51 [95% CI, 1.42 to 4.43]) when controlling for age, white blood cell count at diagnosis, *NPM1* mutation status at diagnosis, and *FLT3*-ITD allelic ratio at diagnosis.

AML: acute myeloid leukemia; CI: confidence interval; CIR: cumulative incidence of relapse; CR: complete remission; CRp: complete remission with incomplete platelet recovery; HR: hazard ratio; MFC: multiparameter flow cytometry; MRD: measurable residual disease; NGS: next-generation sequencing; OS: overall survival; RT-qPCR: reverse-transcriptase quantitative polymerase chain reaction.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

The literature on the use of genetic markers for MRD evaluation is limited to 1 retrospective analysis.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Outcomes Based on Measurable Residual Disease Assessment of Genetic Variants

Results from a retrospective analysis describing outcomes after preemptive interventions based on MRD are shown in Table 6. Bataller et al (2020) evaluated the use of protocol in *NPM1*-mutated AML that prospectively evaluated MRD status and allowed use of allogenic stem cell transplant in patients with identified molecular failure based on the presence of MRD, instead of waiting for patients to present with morphologic hematologic recurrence.[54,]

Table 6. Retrospective Analyses of Results by Treatment of Patients Based on MRD Assessment of Genetic Variants

Study	Design	Participants	Outcomes Estimate (95% CI)
Bataller et al (2020)[54,]	Retrospective analysis of patients with AML with a *NPM1* mutation without unfavorable cytogenetics who were treated based on the CETLAM-12 protocol MRD was evaluated after each chemotherapy cycle and at 3-month intervals for at least 3 years after CR. Patients with MRD after consolidation or confirmed MRD reappearance after molecular response were defined as molecular failures. After confirmation of molecular failure or an overt morphologic relapse (HemR), allo-HCT was recommended but treatment was at the discretion of the attending physician, which could include salvage chemotherapy	157 adults with *NPM1* mutation AML were included in the CETLAM-12 protocol; 91% achieved CR after 1 or 2 courses of chemotherapy	Outcomes after allo-HCT, patients who developed molecular failure (n=33) vs. HemR without prior molecular failure (n=13): • 2-year OS: 85.7% vs. 42%

allo: allogeneic; AML: acute myeloid leukemia; CR: complete remission; HCT: hematopoietic cell transplantation; MRD: measurable residual disease; OS: overall survival.

Section Summary: Testing for *FLT3*, *NPM1*, and *CEBPA* Variants to Risk-Stratify Acute Myeloid Leukemia

The prognostic value of *NPM1* MRD evaluation has been evaluated retrospectively and found to be associated with higher risks for relapse and lower overall survival. Literature on the use of MRD assessment of genetic variants to direct treatment decisions is limited to 1 retrospective analysis, which found survival benefit in implementing pre-emptive treatment intensification based on *NPM1* variant MRD monitoring.

Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

National Comprehensive Cancer Network

Current National Comprehensive Cancer Network guidelines for acute myeloid leukemia (AML) (v 2.2022) provide the following recommendations[9,]:

For the evaluation for acute leukemia, bone marrow core biopsy and aspirate analysis, including immunophenotyping, cytogenetic analyses, and molecular analyses for *FLT3, NPM1, CEBPA*, and other mutations , are needed to risk stratify patients.

> "Several gene mutations are associated with specific prognoses in a subset of patients (category 2A) and may guide treatment decisions (category 2B). Presently, *c-KIT, FLT3-ITD, FLT3-TKD, NPM1, CEBPA* (biallelic), *IDH1/IDH2, RUNX1, ASXL1, TP53, BCR-ABL,* and *PML-RAR* alpha are

included in this group. All patients should be tested for mutations in these genes, and multiplex gene panels and comprehensive next-generation sequencing (NGS) analysis are recommended for the ongoing management of AML and various phases of treatment. To appropriately stratify therapy options, test results of molecular and cytogenetic analyses of immediately actionable genes or chromosomal abnormalities (e.g., *CBF, FLT3* [ITD or TKD], *NPM1, IDH1,* or *IDH2*) should be expedited."

The guideline defined the following risk status based on molecular abnormalities:

Table 7. Risk Factors Based on Genetic Abnormalities

Risk Category	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1); *RUNX 1-RUNX1T1* • inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* • Bialletic mutated *CEBPA* • Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}
Intermediate	• Mutated *NPM1* and *FLT3*-ITD^{high} • Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3); *MLLT3-KMT2A* • Cytogenetic abnormalities not classified as favorable or adverse
Poor/Adverse	• t(6;9)(p23;q34.1); *DEK-NUP214* • t(v;11q23.3); *KMT2A* rearranged • t(9;22)(q34.1;q11.2); *BCR-ABL*1 • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* • -5 or del(5q); -7; -17/abn(17p) • Complex karyotype, monosomal karyotype • Wild-type *NPM1*and *FLT3*-ITD^{high} • Mutated *RUNX1* • Mutated *ASXL1* • Mutated *TP53*

Adapted from NCCN guidelines for AML (v 2.2022).
ITD: internal tandem duplication

The role of measurable (minimal) residual disease (MRD) assessment for prognosis and treatment is evolving and the use of MRD is still under investigation. Currently available evidence has "demonstrated the correlation between MRD and risks for relapse, as well as the prognostic significance of MRD measurements after initial induction therapy." Limitations of incorporating MRD into routine practice include "a lack of standardization and established cutoff values." The guideline notes that "the most frequently employed methods for MRD assessment include real-time quantitative polymerase chain reactions (RQ-PCR) assays (i.e., *NPM1, CBFB-MYH11, RUNX1-RUNX1T1*) and multicolor flow cytometry (MFC) assays specifically designed to detect abnormal MRD immunophenotypes. The threshold to define MRD+ and MRD- samples depends on the technique and subgroup of AML. Next-generation sequencing (NGS)-based assays to detect mutated genes (targeted sequencing, 20 to 50 genes per panel) is not routinely used, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS."

European LeukemiaNet

The European LeukemiaNet international expert panel recommendations for the diagnosis and management of adults with AML were updated in 2017 and again in 2022.[55,56,]The most recent update reflects the 2022 changes to the World Health Organization classification of AML. The panel recommended that screening for *NPM1*, *CEBPA*, and *FLT3* variants should be part of the diagnostic workup in patients with cytogenetically normal AML because they define disease categories that can inform treatment decisions. Table 8 outlines the risk stratification by genetic variants, and Table 9

summarizes recommended conventional care regimens based on patient fitness and risk characteristics, including mutations and other considerations.

The European LeukemiaNet MRD Working Party is an international expert panel convened with the objective of providing guidelines for technical assessment and clinical use of immunophenotypic and molecular MRD testing in AML; the panel's first consensus recommendations were published in 2018, and updated recommendations were published in 2021.[57,7,] In the 2021 update, the panel recommended that molecular MRD be assessed by real-time quantitative or digital polymerase chain reaction in patients with *NPM1*, *CBFB-MYH11*, or *RUNX1-RUNX1T1* mutations, and by MFC in all other patients. NGS-based MRD monitoring is considered by the panel to be "useful to refine prognosis in addition to MFC but, to date, there are insufficient data to recommend NGS-MRD as a stand-alone technique." The panel also defined MRD positivity thresholds according to whether <FC or polymerase chain reaction techniques were used, and provisional MRD positivity thresholds for NGS techniques.

Table 8. Risk Stratification by Genetic Variant

Risk Category	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1* • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/ *CBFB*::*MYH11* • Mutated *NPM1* without *FLT3*-ITD • Basic leucine zipper in-frame mutated *CEBPA*
Intermediate	• Mutated *NPM1* with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A* • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/ *GATA2*, *MECOM(EVI1)* • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype, monosomal karyotype • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1,* and/or *ZRSR2* • Mutated *TP53*

Adapted from Döhner et al (2022).[56,]
ITD: internal tandem duplication.

Table 9. Selected Conventional Care Regimens by Fitness and Risk Characteristics

Patient Characteristics	Induction Therapy	Consolidation Therapy	Maintenance Therapy	Salvage therapy
Considered fit for intensive therapy				
With *FLT3* mutation	Anthracycline plus cytarabine ("7 + 3") plus midostaurin	• Intermediate-dose cytarabine plus midostaurin and/or • If relapse probability with chemotherapy alone >35% to 40%*: allo-HCT	Midostaurin	Gilteritinib or options for other fit patients listed below
Without *FLT3* mutation	"7 + 3"	• Intermediate-dose cytarabine and/or • If relapse probability with chemotherapy	Oral azacitidine	• Intermediate-dose cytarabine

Patient Characteristics	Induction Therapy	Consolidation Therapy	Maintenance Therapy	Salvage therapy
		alone >35% to 40%*: allo-HCT		with or without anthracycline
CD33-positive AML with favorable- or intermediate-risk disease	"7 + 3" with ("other" option) or without gemtuzumab ozogamicin	• Intermediate-dose cytarabine with ("other" option) or without gemtuzumab ozogamicin, and/or • If relapse probability with chemotherapy alone >35% to 40%*: allo-HCT	--	• FLAG-IDA chemotherapy • MEC chemotherapy • CLAG-M chemotherapy • allo-HCT
AML with myelodysplasia-related changes or therapy-related AML	"7 + 3" or liposomal-coformulated daunorubicin and cytarabine ("other" option)	• Intermediate-dose cytarabine or liposomal-coformulated daunorubicin and cytarabine ("other" option), and/or • If relapse probability with chemotherapy alone >35% to 40%*: allo-HCT	--	
Not considered fit for intensive therapy				
With *FLT3* mutation	• Venetoclax plus either azacitidine or decitabine • Venetoclax plus low-dose cytarabine • *IDH1* mutation: ivosidenib with or without azacitidine • Best supportive care			Gilteritinib • *IDH1* mutation: ivosidenib • *IDH2* mutation: enasidenib
Without *FLT3* mutation				

Adapted from Döhner et al (2022).[56,]
*Examples include intermediate- or adverse-risk disease and/or inadequate clearance of measurable residual disease.
allo: allogeneic, AML: acute myeloid leukemia, HCT: hematopoietic cell transplant.

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
Select currently ongoing and unpublished trials that might influence this review are listed in Table 10.

Table 10. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT01296178	PROTOCOL FOR First Line TREATMENT ADAPTED TO RISK of Acute Myeloblastic Leukemia in Patients LESS THAN OR EQUAL TO 65 YEARS	200	Dec 2021 (last update posted Mar 2021)
NCT02156297	Sorafenib to Treat AML Patients with FLT3-ITD Mutation, a Non-interventional Cohort Study	100	Aug 2022 (last update posted Feb 2020)
NCT02668653[a]	Phase 3, Double-Blind, Placebo-controlled Study of Quizartinib Administered in Combination With Induction and Consolidation	539	Aug 2023

NCT No.	Trial Name	Planned Enrollment	Completion Date
	Chemotherapy, and Administered as Maintenance Therapy in Subjects 18 to 75 Years Old With Newly Diagnosed FLT3-ITD (+) Acute Myeloid Leukemia (QuANTUM-First)		
NCT03031249	Efficacy and Safety of ATO Plus ATRA in Nucleophosmin-1 Mutated Acute Myeloid Leukemia	80	Dec 2022
NCT02927262[a]	A Phase 2 Multicenter, Randomized, Double-Blind, Placebo-controlled Trial of the FLT3 Inhibitor Gilteritinib (ASP2215) Administered as Maintenance Therapy Following Induction/Consolidation Therapy for Subjects with FLT3/ITD AML in First Complete Remission	98	Feb 2024
NCT02997202[a]	A Trial of the FMS-like Tyrosine Kinase 3 (FLT3) Inhibitor Gilteritinib Administered as Maintenance Therapy Following Allogeneic Transplant for Patients With FLT3/Internal Tandem Duplication (ITD) Acute Myeloid Leukemia (AML)	356	Jul 2025
Unpublished			
NCT01237808	Study of Low-Dose Cytarabine and Etoposide With or Without All-Trans Retinoic Acid in Older Patients Not Eligible for Intensive Chemotherapy With Acute Myeloid Leukemia and NPM1 Mutation	144	Jul 2018 (completed; last update posted 8/01/2018)
NCT00860639	Randomized Open Phase III Trial Testing Efficacy of Gemtuzumab Ozogamycin Associated to Intensive Chemotherapy for Patients Aged Between 18-60 Years and Presenting an AML With Intermediate Risk	327	Sep 2016 (completed; last update posted 01/27/2017)

NCT: national clinical trial.
[a] Denotes industry-sponsored or cosponsored trial.

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Documentation for Clinical Review

Please provide the following documentation:

- History and physical, including:
  - Diagnosis and reason for testing
  - Lab reports, demonstrating:
    - Cytogenetic analysis

- Any know genetic testing results

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy.

The following codes are included below for informational purposes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy. Policy Statements are intended to provide member coverage information and may include the use of some codes for clarity. The Policy Guidelines section may also provide additional information for how to interpret the Policy Statements and to provide coding guidance in some cases.

Type	Code	Description
CPT®	0023U	Oncology (acute myelogenous leukemia), DNA, genotyping of internal tandem duplication, p.D835, p.I836, using mononuclear cells, reported as detection or non-detection of FLT3 mutation and indication for or against the use of midostaurin
	0046U	FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia) internal tandem duplication (ITD) variants, quantitative
	0049U	NPM1 (nucleophosmin) (e.g., acute myeloid leukemia) gene analysis, quantitative
	0050U	Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements
	0056U	Hematology (acute myelogenous leukemia), DNA, whole genome next-generation sequencing to detect gene rearrangement(s), blood or bone marrow, report of specific gene rearrangement(s) ***(Deleted code effective 10/1/2022)***
	81218	CEBPA (CCAAT/enhancer binding protein [C/EBP], alpha) (e.g., acute myeloid leukemia), gene analysis, full gene sequence
	81245	FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia), gene analysis; internal tandem duplication (ITD) variants (i.e., exons 14, 15)
	81246	FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia), gene analysis; tyrosine kinase domain (TKD) variants (e.g., D835, I836)
	81310	NPM1 (nucleophosmin) (e.g., acute myeloid leukemia) gene analysis, exon 12 variants
HCPCS	None	

Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

Effective Date	Action
09/30/2014	BCBSA Policy Adoption
01/01/2015	Coding update
01/01/2017	Policy revision without position change
03/01/2017	Policy title change from Genetic Testing for FLT3, NPM1, and CEBPA Mutations in Acute Myeloid Leukemia Policy revision without position change

Effective Date	Action
03/01/2018	Policy title change from Genetic Testing for FLT3, NPM1, and CEBPA Mutations in Cytogenetically Normal Acute Myeloid Leukemia Policy revision without position change
08/01/2018	Coding update
04/01/2019	Policy revision without position change
04/01/2020	Annual review. No change to policy statement. Literature review updated.
03/01/2021	Annual review. No change to policy statement. Policy guidelines and literature updated.
03/01/2022	Annual review. No change to policy statement. Literature review updated.
11/01/2022	Coding update
03/01/2023	Annual review. No change to policy statement. Literature review updated.

Definitions of Decision Determinations

Medically Necessary: Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield, are: (a) consistent with Blue Shield medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the patient; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the Member's illness, injury, or disease.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements and Feedback (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

We are interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration.

For utilization and medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.

Appendix A

POLICY STATEMENT (No changes)	
BEFORE	**AFTER**
Genetic Testing for FLT3, NPM1, and CEBPA Variants in Cytogenetically Normal Acute Myeloid Leukemia 2.04.124 **Policy Statement:** I. Genetic testing for *FLT3* internal tandem duplication (*FLT3-ITD), NPM1, and CEBPA* variants may be considered **medically necessary** in cytogenetically normal acute myeloid leukemia (see Policy Guidelines section). II. Genetic testing for *FLT3-ITD*, *NPM1*, and *CEBPA* variants is considered **investigational** in all other situations. III. Genetic testing for *FLT3* tyrosine kinase domain (*FLT3*-TKD) variants is considered **investigational**. IV. Genetic testing for *FLT3*, *NPM1*, and *CEBPA* variants to detect minimal residual disease is considered **investigational**.	**Genetic Testing for FLT3, NPM1, and CEBPA Variants in Cytogenetically Normal Acute Myeloid Leukemia 2.04.124** **Policy Statement:** I. Genetic testing for *FLT3* internal tandem duplication (*FLT3*-ITD), *NPM1*, and *CEBPA* variants may be considered **medically necessary** in cytogenetically normal acute myeloid leukemia (see Policy Guidelines section). II. Genetic testing for *FLT3-ITD*, *NPM1*, and *CEBPA* variants is considered **investigational** in all other situations. III. Genetic testing for *FLT3* tyrosine kinase domain (*FLT3*-TKD) variants is considered **investigational**. IV. Genetic testing for *FLT3*, *NPM1*, and *CEBPA* variants to detect minimal residual disease is considered **investigational**.