Medical Policy

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Title: Genetic Testing for *FLT3, NPM1, CEBPA, IDH1 and IDH2* Variants in Acute Myeloid Leukemia

Description/Background

Acute Myeloid Leukemia (AML)

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 20,380 new cases of AML and 11,310 deaths from AML in the United States in 2023.[1,]

Diagnosis and Prognosis of AML

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, three 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 classification. The 2008 World Health Organization classification recommended determining the presence of *FLT3* variants because of the prognostic significance.[3,]

Treatment

AML has a highly heterogeneous clinical course, and treatment generally depends on the different risk stratification categories.[4,] 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 post-remission treatment options include intensive consolidation therapy, maintenance therapy, or autologous or allogeneic hematopoietic cell transplant.[4,]

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.[8,] 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. MRD 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 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 limited based on several factors. First, some patients may have relapse despite having no MRD, while others do not relapse despite being MRD positive. Standardization have recently been introduced for identifying certain individual markers for MRD assessment as well as threshold values to define MRD positive and MRD negative samples.

***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.[9,] *FLT3* variants are divided into two 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 a 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 reduced remission rates and/or worse survival in some studies.[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 vs benign cells in the sample tested and by the percentage of cells with zero, one, or two 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. For this reason, and the overall lack of sensitivity of the assay (see the Clinically Valid section), the assay is considered to be unsuitable for use as a marker of minimal residual disease. 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 LFS and overall survival, while other studies have found no prognostic value, or potentially a benefit if a NPMI mutation is also present. Next generation FLT3 tyrosine kinase inhibitors, with greater specificity for FLT3, have been under clinical investigation including gilteritinib, which was approved by the Food and Drug Administration (FDA) in 2018.[9,13,14]

NPM1 **Variants**

The most common molecular aberration in AML is a variant of *NPM1*, which is found 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. 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 to *CEBPA* are found in approximately 15% of AML patients with a normal karyotype.[9,16,17] *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; double variants of *CEBPA* have shown a better prognosis with higher rates of complete remission and overall survival after standard induction chemotherapy.[18-21]

***IDH 1* and *2* Variants**
Isocitrate dehydrogenase 1 and 2 (*IDH* 1 and 2) are the most frequently mutated metabolic genes in human cancer. The presence of and *IDH1* or *IDH2* variation has both diagnostic and prognostic significance in central nervous system tumors and prognostic value in hematologic disorders, such as myelodysplastic syndrome or acute myeloid leukemia.

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, Lab PMM, and ARUP Laboratories, 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 Food and Drug Administration 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 (AML) with a *FLT3* mutation as detected by an FDA-approved test.

Medical Policy Statement

Genetic testing for *FLT3* internal tandem duplication (*FLT3*-ITD), *FLT3* tyrosine kinase domain (*FLT3-TKD*), *NPM1*, *CEBPA, IDH1 and IDH2* variants may be considered established in acute myeloid leukemia (if testing for all variants, panel testing [code 81450] may be appropriate).

Genetic testing for *FLT3*, *NPM1*, and *CEBPA* variants to detect minimal residual disease is considered experimental/investigational in AML.

Inclusionary and Exclusionary Guidelines

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

Genetic testing for *IDH1* and *IDH2* variants is intended for use as diagnostic and prognostic value in hematologic disorders, such as acute myeloid leukemia.

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

81120 81121 81218 81245 81246 81310
81450*

*Code is appropriate when testing for all (*FLT3, NPM1, CEBPA, IDH1 and IDH2*) variants.

Other codes (investigational, not medically necessary, etc.):

N/A

Note: Individual policy criteria determine the coverage status of the CPT/HCPCS code(s) on this policy. Codes listed in this policy may have different coverage positions (such as established or experimental/investigational) in other medical policies.

Rationale

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.

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 vs allogeneic transplantation remain unclear in cytogenetically normal (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 AML;
- whether to do allogeneic or autologous transplantation vs chemotherapy as consolidation therapy for an AML patient in the first remission;
- whether to use investigational therapies such as FLT3 inhibitors.

Genetic testing can be used during the initial evaluation of leukemia to provide prognostic information and guide treatment decisions. It also has an evolving role in the assessment of measurable residual disease (MRD) to assess the risk of relapse.

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, giltertinib) 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 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 following **PICOs** were used to select literature to inform this review.

Populations
The populations of interest are individuals with newly diagnosed CN-AML, those in the first remission, and those who have relapsed.

Interventions
The intervention of interest is testing *for FLT3*, *NMP1*,or *CEBPA* variants. During initial assessment of AML, genetic testing provides prognostic risk assessment and helps guide treatment decisions. MRD evaluation is intended to assess risk for relapse and guide potential preemptive therapy. Decisions about management of AML are generally made by patients and hematologists or oncologists in the secondary or tertiary care setting.

Comparators
The comparator of interest is risk stratification without *FLT3*, *NMP1*,or *CEBPA* genetic testing, either for initial evaluation or MRD.

Outcomes
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), *NMP1*,or *CEBPA* variants compared with patients without *FLT3*-ITD, *NMP1*,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, NMP1*, or *CEBPA* Variants

Study	Design	Participants	Outcomes
Port et al (2014)	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)	Systematic review of 10 studies published before Aug 2014	6219 patients with AML	Any AML:
			• *CEBPA* monoallelic vs. WT
			  ○ OS HR=1.1 (95% CI, 0.9 to 1.5)
			  ○ EFS HR=1.1 (95% CI, 0.8 to 1.5)
			• *CEBPA* biallelic vs. WT:
			  ○ OS HR=0.4 (95% CI, 0.3 to 0.5)
			  ○ EFS HR=0.4 (95% CI, 0.3 to 0.5)
			CN-AML:
			• *CEBPA* monoallelic vs. WT:
			  ○ OS HR=1.1 (95% CI, 0.9 to 1.5)
			  ○ EFS HR=0.9 (95% CI, 0.7 to 1.2)
			• *CEBPA* biallelic vs. WT:
			  ○ OS HR=0.3 (95% CI, 0.2 to 0.4)
			  ○ EFS HR=0.4 (95% CI, 0.3 to 0.5)

Dickson et al (2016)[4]	Retrospective analysis of patients enrolled in an	662 AML patients >60 y	1-y OS: • *CEBPA*, biallelic: 75%

Study	Design	Participants	Outcomes
	RCT between 1990 and 1998		• *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)[4]	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)	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)[5]	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)	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)
Issa et al (2022)	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 (an FLT3 inhibitor) or a placebo following each of 4 cycles of induction and consolidation chemotherapy.[27] Patients with ITD subtype (74%), tyrosine kinase domain subtype (23%), and both subtypes (2%) were included. There were no significant differences in remission or survival estimates between treatment groups.

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 Table 3 and 4).[28] Patients with ITD (77%), and tyrosine kinase domain (23%) subtypes were included. The addition of midostaurin did not affect complete remission rates or time to complete remission; 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 tyrosine kinase domain subtype.[29.] 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=0.044), 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=0.244).

Perl et al (2019) published results from an RCT evaluating patients with relapsed/refractory *FLT3*-mutated AML who were randomized to gilteritinib (an FLT3 inhibitor) or salvage chemotherapy (see Tables 3 and 4).[30.] Patients with the ITD subtype (88.4%), tyrosine kinase domain subtype (8.4%), and both subtypes (1.9%) were included. 60.6% of patients had relapsed disease, with 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 (an FLT3 inhibitor)

or salvage chemotherapy.[31] 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)	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)	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)	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)	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)				
	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)				
	CR rate (95% CI)	59 (54 to 64)	54 (48 to 59)	NS

	Time to complete remission (range), median days	35 (20-60)	35 (20-60)	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=0.002
Perl et al (2019)				
	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)				
	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; NR: not reported; *NS*: not significant; RCT: randomized controlled trial; SCT: stem cell transplantation.

Retrospective Studies

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)	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)	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 vschemo: • RFS HR=0.2 (1 to 0.5) • OS HR=0.5 (0.2 to 1.2) Auto-HCT vschemo: • RFS HR=0.4 (0.2 to 0.8) • OS HR=0.6 (0.2 to 1.4)
Willemze et al (2014)	Retrospective analysis of EORTC-GIMEMA AML-12 RCT conducted	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:

	between 1999 and 2008		• HR=0.70 (0.47 to 1.04)
Chou et al (2014)	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 ✓ OS HR=0.5 (0.3 to 0.8) • *NPM1* variant with *FLT3*-ITD WT: ✓ OS HR=0.4 (0.2 to 0.7) Allo-HCT: • *CEBPA* biallelic vs other: ✓ OS HR=0.3 (0.1 to 1.2) • *NPM1* variant with *FLT3*-ITD WT: ✓ OS HR=NR
Ma et al (2015)	Systematic review of 9 studies of chemo vs. HCT published between 1989 and 2013	Patients with *AML, FLT3-ITD* variant	Allo-HCT vschemo: • 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)	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 ✓ Relapse rate, 37% vs. 59% (p=0.02) ✓ DFS=47% vs. 41% (p=0.45) ✓ TRM=16% vs. 0% (p=0.008) • Patients with high *FLT3*-ITD allelic ratio ✓ Relapse rate, 15% vs. 53% (p=0.007) ✓ DFS 65% vs. 40% (p=0.08) ✓ TRM=19% vs. 7% (p=0.08)
Ahn et al (2016)	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=0.001) 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)	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)	Retrospective analysis of patients from 4 trials who achieved CR after 1	Intermediate risk patients receiving the following post-remission treatment: chemo (n=148); auto-HCT	Auto-HCT vs. chemo: no difference in OS, RFS, relapse, or NRM Allo-HCT with MAC vs. chemo: no difference OS

	or 2 induction chemo cycles	(n=168); allo-HCT with MAC (n=137); and allo-HCT with RIC (n=68)	• 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)	Retrospective analysis of patients enrolled in 4 clinical trials or the Study Alliance Leukemia registry and biorepository	4708 patients who received intensive chemotherapy followed by risk-stratified consolidation, with the option of HCT for eligible patients (5.1% with CEBPA mutations)	Biallelic CEBPA vs. unselected single CEBPA mutation vs. CEBPA-WT: 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)	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: OS HR=0.63 (0.41 to 0.98) RFS HR=0.55 (0.35 to 0.84) Patients with NPM1-WT: Median OS 19.6 months vs. 14.6 months (p=.023) Median RFS 7.7 months vs. 4.6 months (p=.003) Patients with FLT3 mutations: Median OS 28.2 months vs. 9.7 months (p=.114) Median RFS 23.1 months vs. 4.6 months (p=.032)

			Patients with FLT3-WT: Median OS 24.7 months vs. 15.2 months (p=.013) Median RFS 10.2 months vs. 4.9 months (p=.001) Patients with NPM1 mutations vs. NPM1-WT: Placebo arm: OS HR=0.69 (0.49 to 0.97) RFS HR=0.65 (0.47 to 0.91) Oral azacitidine arm: OS HR=0.52 (0.36 to 0.75) 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: non-relapse 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[43-49] 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. OS 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. 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). 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=0.003) and *CEBPA* double variant (HR=0.468; 95% CI, 0.265 to 0.828; p=0.009) 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=0.008 and p=0.001, 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* tyrosine kinase domain variants is uncertain. *NPM1* variants are found in approximately half of the 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). 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 tested 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 double-mutation *CEBPA*, 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 individuals 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*, *NPM1NMP1*, 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*-mutated AML; results of studies evaluating the use of MRD with this variant is summarized in Table 5.

Table 5. Prognostic Value of *NPM1* MRD Assessment

Study	Design	Participants	MRD Assessment	Outcomes
Ivey et al (2016)	Retrospective evaluation of samples obtained from patients who had undergone intensive treatment in the National Cancer Research Institute AML17 trial	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,	Positive MRD status vs. negative MRD status in peripheral blood following the second chemotherapy cycle (retrospective cohort):

	(April 2009 to May 2012), with a prospective evaluation period (June 2012 to December 2014) to make up a validation cohort		using a threshold setting of 0.1	• 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)[,]	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)	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% For those with low MRD levels, *FLT3*-ITD variant vs. *FLT3*-ITD wild-type: • 2-year OS: 25% vs. 77%
Grob et al (2022)	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; MRD: measurable residual disease; OS: overall survival; RT-qPCR: reverse-transcriptase quantitative polymerase chain reaction.

Outcomes Based on MRD Assessment of Genetic Variants

Results from retrospective analyses 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)	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.

Testing for *IDH1* and *IDH2* in Myelodysplastic Syndrome or Acute Myeloid Leukemia

Clinical Context and Test Purpose

The question addressed in this evidence review is: Does *IDH1 and IDH2* genetic testing in individuals with AML improve outcomes?

The following **PICOs** were used to select literature to inform this review.

Populations

The populations of interest are individuals with newly diagnosed AML, those in the first remission, and those who have relapsed.

Interventions
The intervention of interest is testing *for IDH1 and IDH2* variants.

Comparators
The comparator of interest is risk stratification without *IDH1 and IDH2* genetic testing.

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

Desia et al (2018) studied the premalignant mutational landscape of AML and its impact on risk and time to diagnosis.[58] They identified 212 women from the Women's Health Initiative who were healthy at study baseline, but eventually developed AML during follow-up (median time: 9.6 years). Deep sequencing was performed on peripheral blood DNA of these cases and compared to age-matched controls that did not develop AML. It was discovered that mutations in IDH1, IDH2, TP53, DNMT3A, TET2 and spliceosome genes significantly increased the odds of developing AML. All subjects with TP53 mutations (n = 21 out of 21 patients) and IDH1 and IDH2 (n = 15 out of 15 patients) mutations eventually developed AML in the study. The presence of detectable mutations years before diagnosis suggests that there is a period of latency that precedes AML during which early detection, monitoring and interventional studies should be considered.

According to Petrova et al (2018), molecular screening plays a major role in prognostic categorization and subsequent definition of treatment strategies for AML.[59] In a retrospective study, ninety patients with de novo AML using Sanger sequencing (exon 4, IDH1 and IDH2) were evaluated. Twenty-two patients (24%) were identified who harbored mutations in IDH1 and IDH2 genes. Fourteen (64%) of them had other commonly used minimal residual disease markers. Eight of the 22 patients had IDH1 mutations, 13 had IDH2 mutations and 1 had both IDH1 and IDH2 mutations. In this cohort, IDH1 and 2 marker responded to the treatment in all of the patients and reflected the onset of the relapse very well. Given this data, the authors concluded that IDH1 and IDH2 mutations can be used as a reliable and cost-effective marker for monitoring.

Brunner et al (2019) prospectively evaluated IDH1 and IDH2 mutational status and outcomes of patients receiving standard chemotherapy for newly diagnosed AML.[60] Serial samples of serum, urine, and bone marrow aspirates were collected from patients newly diagnosed with AML. IDH1 and IDH2 mutations and estimated variant allele frequencies were identified. Disease free survival and overall survival were evaluated with log-rank tests and Cox regression. Two hundred and two patients were treated for AML; 51 harbored IDH1/2 mutations. IDH1/2-mutated patients had significantly higher 2-hydroxyglutarate (2HG) levels in serum, urine, bone marrow aspirates, and aspirate cell pellets than wild-type patients. A serum 2HG level greater than 534.5 ng/mL was 98.8% specific for the presence of an IDH1/2 mutation. Patients with IDH1/2-mutated AML treated with 7+3-based induction had a 2-year event-free survival (EFS) rate of 44% and a 2-year OS rate of 57%. There was no difference in complete remission rates, EFS, or OS between IDH1/2-mutated and wild-type patients. Decreased serum 2HG levels on day 14 as a proportion of the baseline were significantly associated with improvements in EFS (P = .047) and OS (P = .019) in a multivariate

analysis. The authors concluded that among patients with IDH1/2-mutated AML, 2HG levels are highly specific for the mutational status at diagnosis, and they have prognostic relevance in patients receiving standard chemotherapy.

Recently, Ok et al (2019) evaluated 80 AML patients with know IDH1 or IDH2 mutations and assessed their bone marrow at the time of remission to determine the potential impact of persistent IDH1 or IDH2 mutations.[61] Approximately 40% of AML patients given standard treatment in this cohort had persistent mutations in IDH 1 and IDH2. Patients with an IDH1 or IDH2 mutation had an increased risk of relapse after 1 year of follow-up compared to patients without a detectable IDH 1/IDH2 mutation (59% vs. 24%; p<0.01). However, a persistent mutation was not associated with a shorter time to relapse. High *IDH1/2* mutation burden (mutant allelic frequency ≥10%) did not correlate with relapse rate (77% *vs.* 86% for patients with a low burden, i.e., mutant allelic frequency <10%; *P*=0.66). Persistent mutations were also observed in *NPM1*, *DNMT3A* and *FLT3* during remission, but *IDH1/2* mutations remained significant in predicting relapse by multivariate analysis. Flow cytometry was comparable and complementary to next-generation sequencing-based assay for predicting relapse. Monitoring for persistent *IDH1/2* mutations in patients with acute myeloid leukemia in remission can provide information that could be used to justify early interventions, with the hope of facilitating longer remissions and better outcomes in these patients.

Section Summary
The *IDH1* and *IDH2* variant is quite common in AML, particularly in patients with normal karyotypes, and has been associated with disease relapse. Approximately half of patient who have standard treatment have persistent *IDH1/IDH2* mutations. Patients with persistent *IDH1/IDH2* mutations may have an increased risk of relapse after 1 year of follow-up compared to patients without a detectable *IDH1/IDH2* mutation; therefore, monitoring for persistent IDH1/IDH2 mutations in patients with AML in remission may provide information that could be used for early interventions.

SUMMARY OF EVIDENCE
For individuals who have AML who receive genetic testing for variants in *FLT3*, *NPM1*, and *CEBPA* to risk-stratify AML, the evidence includes RCTs, retrospective observational studies, and systematic reviews of these studies. The relevant outcomes are OS, disease-specific survival, test validity, and treatment-related mortality and morbidity. *FLT3*-ITD variants confer a poor prognosis, whereas *NPM1* (without the *FLT3*-ITD variant) and biallelic *CEBPA* variants confer a favorable prognosis. The prognostic effect of *FLT3* TKD variants is uncertain. Data have suggested an OS benefit with transplantation for patients with *FLT3*-ITD, but do not clearly demonstrate an OS benefit of transplantation for patients with *NPM1* and *CEBPA* variants. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have AML with a genetic variant in *FLT3*, *NPM1*, and *CEBPA,* the evidence for measurable residual disease (MRD) monitoring of these genetic variants is limited to retrospective observational studies. Relevant outcomes are overall survival, disease-specific survival, test validity, and treatment-related mortality and morbidity. Detection of MRD based on *NPM1* variant presence is associated with higher risks for relapse and lower overall survival; prospective evaluations using MRD results to direct prognostic evaluation and treatment decisions are needed. For the use of genetic variants to detect MRD, the evidence is

insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have AML who receive genetic testing for variants in IDH1 and IDH2, the evidence includes retrospective and observational studies. Data have suggested that patients with persistent *IDH1/IDH2* mutations have an increased risk of disease relapse. Monitoring for these persistent mutations may provide an opportunity for early interventions. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

PRACTICE GUIDELINES AND POSITION STATEMENTS

National Comprehensive Cancer Network

Current National Comprehensive Cancer Network guidelines for acute myeloid leukemia (AML) (v.2.2024) provide the following recommendations[9]:

For the evaluation for acute leukemia, "bone marrow core biopsy and aspirate analysis, including immunophenotyping and cytochemistry."

"Several gene mutations are associated with specific prognoses (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 and 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 Stratification by Biological Disease Factors For Patients

Risk Category	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/RUNX1::RUNX1T1b,c • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11[b,c] • Mutated NPM1[b,d] without FLT3-ITD • bZIP in-frame mutated CEBPA[e]
Intermediate	• Mutated NPM1[b,d] with FLT3-ITD • Wild-type NPM1 with FLT3-ITD (without adverse-risk genetic lesions • t(9;11)(p21.3;q23.3)/MLLT3::KMT2A[b,f] • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Poor/Adverse	• t(6;9)(p23.3;q34.1)/DEK::NUP214 • t(v;11q23.3)/KMT2A-rearranged[g] • 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,[h] monosomal karyotype[i] • Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2j Mutated TP53[k,l]

Adapted from NCCN guidelines for AML (v.2.2021).

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, 20-50 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 Dohner et al (2017).[49]
ITD: internal tandem duplication.

Table 9. 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 alone >35% to 40%*: allo-HCT	Oral azacitidine	• Intermediate-dose cytarabine with or without anthracycline • FLAG-IDA chemotherapy • MEC chemotherapy
CD33-positive AML with favorable- or intermediate-risk disease	"7 + 3" with ("other" option) or without	• Intermediate-dose cytarabine with ("other" option) or without	--	

	gemtuzumab ozogamicin	gemtuzumab ozogamicin, and/or • If relapse probability with chemotherapy alone >35% to 40%*: allo-HCT		• 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
Without *FLT3* mutation				• *IDH1* mutation: ivosidenib • *IDH2* mutation: enasidenib

Adapted from Dohner et al (2017).[49]
HCT: hematopoietic cell transplant.

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			
NCT05383014	FLT3-ITD Gene Mutation and CD135 Expression in Acute Myeloid Leukemia.	82	Jan 2024
NCT05023707	Anti-FLT3 CAR T-cell Therapy in FLT3 Positive Relapsed/ Refractory Acute Myeloid Leukemia	5	Jun 2025
NCT05432401	TAA05 Injection in the Treatment of Adult Patients With FLT3-positive Relapsed/Refractory Acute Myeloid Leukemia	18	Jun 2025
Unpublished			
NCT01296178	First line treatment adapted to risk of acute myeloblastic leukemia in patients less than or equal to 65 years	200	Dec 2021
NCT02156297	Sorafenib to treat AML patients with FLT3-ITD mutation, a non-interventional cohort study	100	Aug 2022
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	Sept 2016 (completed)
NCT01237808	Study of low-dose cytarabine and etoposide with or without all trans retinoic acid in older patients not eligible for intensive	144	Aug 2022

	chemotherapy with acute myeloid leukemia and NPMI1 mutation		

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

Government Regulations

National:

No national coverage determination available.

Local:

MolDX: Abbott RealTime *IDH1* and *IDH2* testing for Acute Myeloid Leukemia Coding and Billing Guidelines. (A55738), effective on or after 06/01/2023.

The **Abbott RealTime *IDH1*** by Abbott Molecular is the only test that has received FDA approval to be used as an aid in identifying acute myeloid leukemia (AML) patients with an isocitrate dehydrogenase-1(IDH1) mutation for treatment with TIBSOVO® (ivosidenib). TIBSOVO® (ivosidenib) is an isocitrate dehydrogenase-1 (*IDH1*) inhibitor indicated for the treatment of adult patients with relapsed or refractory acute myeloid leukemia (AML) with a susceptible *IDH1* mutation as detected by an FDA-approved test.

Abbott RealTime *IDH1* by Abbott Molecular meets the reasonable and necessary criteria for Medicare reimbursement, effective 7/20/2018.

The **Abbott RealTime *IDH2*** by Abbott Molecular is the only test that has received FDA approval to be used as an aid in identifying acute myeloid leukemia (AML) patients with an isocitrate dehydrogenase-2 mutation for treatment with enasidenib (IDHIFA®).

Abbott RealTime *IDH2* by Abbott Molecular meets the reasonable and necessary criteria for Medicare reimbursement, effective 8/1/2017.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

- Genetic Testing BCR/ABL1 Testing in Chronic Myelogenous Leukemia and Acute Lymphoblastic Leukemia
- Genetic Testing-JAK2, MPL and CALR Testing for Myeloproliferative Neoplasms

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through May 2024, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
9/1/19	7/24/19		Joint policy established
9/1/20	6/16/20		Routine policy maintenance, no change in policy status.
9/1/21	6/15/21		Routine policy maintenance, updated rationale section, added references 1, 8, 12-14, 23-26, 29-31. No change in policy status.
9/1/22	6/21/22		Reorganized rationale section, no change in policy status.
9/1/23	6/13/23		Rationale updated, references 49-54 added. No change in policy status. Vendor managed: N/A (ds)
9/1/24	6/11/24		Routine policy maintenance. Elements in MPS removed. No change in policy status. Vendor managed: N/A (ds)

Next Review Date: 2nd Qtr. 2025

Pre-Consolidation Medical Policy History

Original Policy Date	Comments
BCN:	Revised:
BCBSM:	Revised:

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GENETIC TESTING FOR *FLT3, NPM1*, *CEBPA, IDH1 AND IDH2* VARIANTS IN ACUTE MYELOID LEUKEMIA

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered per policy
BCNA (Medicare Advantage)	See government section
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member's certificate and is not guaranteed. Please consult the individual member's certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.