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# Genomic Testing for Hematopoietic Neoplasms

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| Policy Number:<br>AHS – M2182 | Prior Policy Name and Number:<br>Not applicable |
| Original Effective Date:<br>October 01, 2024 | Current Effective Date:<br>February 1, 2025 |
| Line(s) of Business:<br>HMO; PPO; QUEST Integration; Medicare; FEP | Precertification:<br>Required. Refer to GTM Utilization Review Matrix |

## I. Policy Description

Hematologic cancers impact the normal production and function of blood cells. These cancers often begin in bone marrow, where stem cells develop into white blood cells, red blood cells, or platelets. Hematologic cancers such as myeloid and lymphoid neoplasms occur when there is an uncontrolled growth of abnormal cells which overtake the development of normal blood cells. An overabundance of abnormal cells interferes with the regular functions of normal cells (ACCC, 2022). Myeloid neoplasms occur when there is a proliferation of myeloid cells that have originated from a primitive stem cell (NCBI, 2023b). In contrast, lymphoid neoplasms are composed of a lymphocytic cell population which is usually malignant (clonal) by molecular genetic and/or immunophenotypic analysis (NCBI, 2023a). For guidance on flow cytometry in hematopoietic neoplasms, please refer to AHS-F2019-Flow Cytometry.

For guidance on minimal/measurable residual disease (MRD) in hematopoietic neoplasms, please refer to AHS-M2175-Minimal Residual Disease.

## II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the initial diagnosis and profiling of hematopoietic neoplasms, the following testing **MEETS COVERAGE CRITERIA**:
   a) Bone marrow cytogenetics **and/or** fluorescent in situ hybridization (FISH) of bone marrow samples.
   b) Multigene panel testing.
2) For individuals diagnosed with acute lymphoblastic leukemia (ALL) or pediatric ALL (PEDALL) who are under surveillance, the following tests **MEET COVERAGE CRITERIA** once every 3 to 6 months for at least 5 years:
   a) Bone marrow cytogenetics.
   b) Bone marrow FISH.
   c) Multigene panel testing.
3) For optimal risk stratification and treatment planning for individuals diagnosed with B-cell ALL (B-ALL), one time qualitative or quantitative reverse transcription polymerase chain reaction (RT-PCR) testing on blood or bone marrow for identification of the *BCR-ABL1* fusion gene transcript type, including determination of transcript size (i.e., p190 vs. p210), **MEETS COVERAGE CRITERIA**.

4) For all individuals with Philadelphia chromosome positive ALL (Ph+ ALL), *BCR-ABL1* transcript-specific quantification **MEETS COVERAGE CRITERIA** at the following frequency:
   a) Once per month for individuals who have detectable levels of *BCR-ABL1* transcript.
   b) Once every three months for individuals who have undetectable levels of *BCR-ABL1* transcript.
5) For individuals diagnosed with acute myeloid leukemia (AML) who had cytogenetic abnormalities at diagnosis, cytogenetic or FISH testing **MEETS COVERAGE CRITERIA** at the following time points:
   a) Fourteen to twenty-one days after the start of therapy to document hypoplasia.
      i) When hypoplasia is documented, repeat analysis seven to fourteen days later to clarify persistence of leukemia.
      ii) When hypoplasia is not documented, repeat analysis at the time of hematologic recovery to document remission.
   b) By 42 days post-treatment with either standard-dose or high-dose cytarabine induction for poor-risk AML, regardless of the degree of hematologic recovery.
6) For individuals with AML who have experienced relapsed/refractory disease after the completion of post-remission therapy, multigene panel testing **MEETS COVERAGE CRITERIA**.
7) For individuals diagnosed with chronic myeloid leukemia (CML), quantitative reverse transcription polymerase chain reaction (qPCR) testing for the *BCR-ABL1* fusion gene transcript **MEETS COVERAGE CRITERIA** at the following frequency:
   a) To establish baseline levels at diagnosis.
   b) For individuals undergoing TKI therapy:
      i) Every 3 months after initiation of therapy until major molecular response (MMR) (*BCR-ABL1* (IS) < 1% (>0.1%-1%)) has been achieved.
      ii) Every 3 months for 2 years and every 3-6 months thereafter.
      iii) If there is a 1-log increase in *BCR-ABL1* transcript levels with MMR, repeat in 1-3 months.
8) For individuals diagnosed with CML who are pursuing or who are actively undergoing TKI therapy, bone marrow cytogenetics **MEETS COVERAGE CRITERIA** at the following time points:
   a) To establish cytogenetic changes.
   b) When there is a failure to reach response milestones.
   c) When there is any sign of loss of hematologic response.
   d) When there is any sign of loss of complete cytogenetic response (CCyR) or its molecular response correlate, defined as an increase in *BCR-ABL1* transcript to >1%.
9) Evaluation of BCR-ABL kinase domain point mutations in patients with CML **MEETS COVERAGE CRITERIA** at the following time points:
   a) When the individual has chronic phase CML.
   b) When there is failure to reach response milestones.
   c) When there is any sign of loss of hematologic response.
   d) When there is any sign of loss of CCyR or its molecular response correlate, defined as an increase in *BCR-ABL1* transcript to 1%.
   e) When there is a 1-log increase in *BCR-ABL1* transcript levels and a loss of MMR.
   f) When the disease progresses to accelerated or blast phase.
10) For individuals with CML who are undergoing treatment discontinuation with TKI therapy and who remain in MMR after discontinuation of therapy, qPCR on blood or bone marrow for the *BCR-ABL1* fusion gene transcription **MEETS COVERAGE CRITERA** at the following frequency:
   a) Every month for the first 6 months following discontinuation.

b) Once every two months in months 7-12 following discontinuation.
c) Once every three months beginning 12 months following discontinuation of therapy, as long as the individual remains in MMR.

11) For individuals with CML, the use of FISH to monitor response to TKI therapy **DOES NOT MEET COVERAGE CRITERIA.**
12) Simultaneous testing of both bone marrow and blood for monitoring purposes **DOES NOT MEET COVERAGE CRITERIA**.

*The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.*

13) For the diagnosis or prognosis of individuals with confirmed acute leukemia, global/gene specific methylation, microRNA (miRNA) expression, or gene expression analysis **DOES NOT MEET COVERAGE CRITERIA**.
14) For diagnosis or prognosis of myeloid or lymphoid neoplasms, all other testing not addressed above **DOES NOT MEET COVERAGE CRITERIA.**

**NOTES:**

**Note:** For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

## III. Table of Terminology

| Term | Definition |
|---|---|
| *ABL1* | *Abelson murine leukemia viral oncogene homolog 1* |
| AML | Acute myeloid leukemia |
| *ANKRD26* | *Ankyrin repeat domain containing 26* |
| APL | Acute promyelocytic leukemia |
| ASCO | American Society of Clinical Oncology |
| ASH | American Society of Hematology |
| *ASXL1* | *ASXL transcriptional regulator 1* |
| *ASXL2* | *ASXL transcriptional regulator 2* |
| BAALC | Brain and acute leukemia cytoplasmic |
| *BCR-ABL1* | *BCR activator of RhoGEF and GTPase- ABL proto-oncogene 1, non-receptor tyrosine kinase* |
| BCSH | British Committee for Standards in Haematology |
| *c-KIT* | Commonly used alias for *KIT proto-oncogene, receptor tyrosine kinase* |
| CAP | College of American Pathologists |
| CBF | Core-binding factor |
| *CALR* | *Calreticulin* |
| *CBFA2* | *CBFA2/RUNX1 partner transcriptional co-repressor 2* |
| *CEBPA* | *CCAAT/enhancer binding protein alpha* |
| CLIA '88 | Clinical Laboratory Improvement Amendments of 1988 |
| CMS | Centers for Medicare and Medicaid |
| CML | Chronic myeloid leukemia |
| CN | Normal karyotypes |
| CR | Complete hematologic remission |
| *DDX41* | *DEAD-box helicase 41* |

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|---|---|
| DNA | Deoxyribonucleic acid |
| *DNMT3A* | *Deoxyribonucleic acid methyltransferase 3A* |
| ELN | European LeukemiaNet |
| EMSO | European Society For Medical Oncology |
| ERG | ETS transcription factor ERG |
| EVI1 | Ecotropic virus integration site 1 protein homolog |
| *EZH2* | *Enhancer of zeste 2 polycomb repressive complex 2 subunit* |
| FDA | Food and Drug Administration |
| FISH | Fluorescence in situ hybridization |
| *FLT3* | *FMS-like tyrosine kinase 3* |
| GATA2 | GATA binding protein 2 |
| *IDH1* | *Isocitrate dehydrogenase 1* |
| *IDH2* | *Isocitrate dehydrogenase 2* |
| ITD | Internal tandem duplications |
| *JAK2* | *Janus kinase 2* |
| *KIT* | *KIT proto-oncogene, receptor tyrosine kinase* |
| *KRAS* | *KRAS proto-oncogene, GTPase* |
| LTD | Laboratory-developed test |
| *MBNL1* | *Muscleblind like splicing regulator 1* |
| MECOM | MDS1 and EVI1 complex locus |
| MEIS1 | Muscleblind like splicing regulator 1 |
| miRNA | Micro ribonucleic acid |
| Mkneg | Non-chromosomal karyotype |
| *MLL-PTD* | *Mll -Partial Tandem Duplication* |
| *MPL* | *MPL proto-oncogene, thrombopoietin receptor* |
| MRD | Minimal residual disease |
| *MYH11* | *Myosin heavy chain 11* |
| NCCN | National Comprehensive Cancer Network |
| NGS | Next generation sequencing |
| *NPM1* | *Nucleophosmin 1* |
| *NPM1*mut | *NPM1* mutations |
| *NRAS* | *NRAS proto-oncogene, GTPase* |
| PCR | Polymerase chain reaction |
| PMF | Polyamine modulated factor 1 |
| *PML-RAR* | *Promyelocytic leukemia-retinoic acid receptor* |
| RNA | Ribonucleic acid |
| RT-PCR | Reverse transcription- polymerase chain reaction |
| *RUNX1* | *RUNX family transcription factor 1* |
| *RUNX1T1* | *RUNX1 partner transcriptional co-repressor 1* |
| SPI1 | Spi-1 proto-oncogene |
| *SF3B1* | *Splicing factor 3b subunit 1* |
| *SRSF2* | *Serine and arginine rich splicing factor 2* |
| *TET2* | *Tet methylcytosine dioxygenase 2* |
| *TKD* | *Tyrosine kinase domain* |
| *TP53* | *Tumor protein 53* |
| WHO | World Health Organization |
| *WT1* | *Wilms' tumor 1* |

## IV. Scientific Background

*Acute myeloid leukemia (AML)*

Acute myeloid leukemia is the most common acute leukemia in adults (80%), with a median age at diagnosis of 65 years. An AML diagnosis in adults is generally associated with a poor prognosis. This disease is much less common in children younger than ten years of age, as less than ten percent of acute leukemias are diagnosed as AML in this age group (Schiffer & Gurbaxani, 2022; Siegel et al., 2017; Yamamoto & Goodman, 2008).

A clinical presentation of AML includes symptoms related to complications of pancytopenia, including weakness and fatigability, infections of variable severity, and hemorrhagic findings (Schiffer & Gurbaxani, 2022). Analysis of gene sequencing of AML cases generally reveal more than ten significant gene mutations; many of which are thought to participate in leukemogenesis (CGARN, 2013). The most common gene mutations are as follows: *FLT3* (28%), *NPM1* (27%), *DNMT3A* (26%), *IDH1* or *IDH2* (20%), *NRAS* or *KRAS* (12%), *RUNX1* (10%), *TET2* (8%), *TP53* (8%), *CEBPA* (6%), and *WT1* (6%). Mutations impacting signal activation are frequent (60% of cases); the most common of which are mutations in *FLT3* (Stock, 2022).

The second most common mutation in AML (27%) is of nucleophosmin (NPM1), a ubiquitously expressed phosphoprotein that normally shuttles between the nucleus and cytoplasm (Falini et al., 2005; Stock, 2022). *NPM1* mutations are associated with improved outcomes although the mechanism is not known. Concurrent mutations (such as an *FTL3* mutation) may influence prognosis, but generally *NPM1* patients without concurrent mutations have better prognoses (Schiffer, 2023).

The *CCAAT/enhancer binding protein alpha* (*CEBPA*) gene mutation is also common in AML. *CEBPA* encodes a transcription factor necessary for myeloid differentiation. This mutation is one of the two mutations associated with familial leukemia and consists of about ten percent of AML cases. Familial AML with a *CEBPA* mutation has a phenotype similar to those of "sporadic AML with biallelic *CEBPA* mutations," but most of the current data revolves around the assessment of *CEBPA* double mutations. *CEBPA* single mutations and hypermethylated *CEBPA* requires further study (Schiffer, 2023).

Isocitrate dehydrogenase (IDH) 1 and 2 mutations comprise approximately 15% of AML cases. These mutations are mutually exclusive with *Tet Methylcytosine Dioxygenase 2* (*TET2*) and *Wilms' tumor 1* (*WT1*) mutations but are commonly seen with *NPM1* and *DNA methyltransferase 3A* (*DNMT3A*) mutations. Data on the prognoses of these mutations is varied (Schiffer, 2023).

The *KIT* mutations also comprise about six percent of AML cases. *KIT* encodes the receptor for a stem cell factor, and prognoses are varied (Castells, 2021; Schiffer, 2023). Some researchers suggest that of all *KIT* mutations, the D816 mutation is the most unfavorable prognostic factor in AML patients (Yui et al., 2017).

Approximately eight percent of AML cases consist of *WT1* gene mutations. *WT1* encodes a transcriptional regulator for genes involved in maturation and growth. Again, the prognosis of this mutation is mixed (Schiffer, 2023), although some researchers strongly support the theory that *WT1* mutations are associated with poor AML prognoses (Hou et al., 2010). The *WT1* mutation status of AML patients may also change during disease progression. New research has suggested that after

allogenic stem cell transplantation, AML relapse could be due to a gain in *WT1* gene alterations and a "high mutation load" (Vosberg et al., 2018).

The *ASXL Transcriptional Regulator 1 (ASXL1)* and *ASXL Transcriptional Regulator 2* (*ASXL2)* may also be mutated in AML cases. *ASXL1* has an unclear function, but it is speculated to be related to histone post-translational modifications. The frequency of *ASXL1* is varied, as estimates range from six percent to 30%. Furthermore, *ASXL1* mutations are mutually exclusive with *NPM1* mutations, and *ASXL2* mutations are associated with *RUNX1* mutations (also known as *AML1* or *CBFA2*) (Schiffer, 2023).

The *DNMT3A* gene amounts to 20%-22% of AML cases. This gene plays a role in epigenetic modifications for development and differentiation. Mutations in this gene affect hematopoietic stem cell differentiation. Prognoses of this gene mutation have been mixed (Schiffer, 2023).

*Tumor protein 53* (*TP53*) and RAS and may also be present in AML cases and may be accompanied by other genetic abnormalities. RAS regulates cell signal transduction, and its mutation leads to a constitutionally active growth stimulus whereas *TP53* encodes a transcriptional activator of growth inhibitory genes (Frucht & Lucas, 2023; Rai & Stilgenbauer, 2023).

Gene expression profiling and microRNA expression profiling may also contribute to assessment and management of AML. Gene expression profiling has been used to differentiate between risk groups based on cytogenetic evaluation whereas microRNA profiling evaluates the regulation of gene expression. However, neither technique is used regularly in clinical practice as these techniques have yet to be widely validated (Schiffer, 2023).

*Chronic myeloid leukemia*

The first human malignancy in which a specific chromosomal defect, known as the minute or Philadelphia (Ph) chromosome, could be linked to pathogenetic events of leukemogenesis (Nowell & Hungerford, 1960). The Ph chromosome translocation (t(9;22)(q34;q11.2)) fuses the breakpoint cluster region protein (*BCR*) gene from chromosome 22 with the Abelson murine leukemia viral oncogene homolog 1 (*ABL1*) proto-oncogene from chromosome nine in a head-to-tail manner to form the transcriptionally active *BCR-ABL* fusion gene (Schrijver & Zehnder, 2022). The fusion of *BCR* at the 5' side in *ABL* alters the tightly regulated function of the Src homology 3 (SH3) domain, disabling control over the tyrosine kinase enzyme. The resulting chimeric BCR-ABL protein has constitutively elevated tyrosine phosphokinase activity (Kurzrock et al., 2003) that activates a number of downstream signaling molecules, including PI3K, AKT, JNK, RAS and STAT5 (Ren, 2005). This then disrupts cellular signal transduction pathways, leading to issues in the regulation of both apoptosis and cell proliferation (Warmuth et al., 1999), ultimately leading to factor-independent and leukemogenic cell growth (Van Etten, 2023).

Detection of the Ph chromosome is the hallmark of CML and is found in up to 95 percent of patients (Leoni & Biondi, 2015). In approximately five percent of CML cases, the Ph chromosome cannot be detected, and *BCR-ABL1* formation is attributed to microscopically undetectable translocations or variant complex translocations involving a third chromosome (Schrijver & Zehnder, 2022). Independent of which other chromosomes are involved in variant translocations, the generation of the

*BCR-ABL* fusion gene is the "fundamental cause of Ph-positive leukemias" (Van Etten, 2023), as the 210-KDa fusion protein BCR-ABL is essential for initiation, maintenance, and progression of CML (Ren, 2005). Testing for BCR-ABL1 detects both the Ph chromosome and fusion gene or its transcripts. The BCR-ABL1 transcript is the RNA copy made by the cell.

The FMS-like tyrosine kinase 3 (FLT3) is a transmembrane tyrosine kinase receptor that stimulates cell proliferation upon activation. Both internal tandem duplications (ITDs) of different lengths and point mutations in the activating loop of the kinase domain result in ligand-independent activation of the FLT3 receptor and a proliferative signal. A *FLT3-ITD* mutation has been shown to have a poor prognosis in contrast to *FTL3* point mutations in the activation loop of the kinase domain. Higher ratios of mutated alleles compared to wild-type alleles confer worse prognoses (Schiffer, 2023).

The *BCR-ABL1*, refers to the fusion gene resulting from a reciprocal translocation that joins the *ABL1* gene from chromosome 9 to the *BCR* gene on chromosome 22 and is necessary for the development of chronic myeloid leukemia (Tachibana et al., 2022) (Van Etten, 2023). This reciprocal translocation also generates a shortened derivative chromosome 22, known as the Philadelphia (Ph) chromosome (Schrijver & Zehnder, 2022). The Ph chromosome is a diagnostic hallmark, present in 95% of people with CML and approximately three to five percent children and 25%–40% adults with acute lymphoblastic leukemia (ALL) (Leoni & Biondi, 2015). An aggressive form of cancer resulting from the neoplastic transformation of lymphoid precursors characterized by the presence of too many lymphoblasts or lymphocytes in the bone marrow and peripheral blood (PDQ, 2023). Predominately a childhood disease, approximately 60% of cases were diagnosed in patients younger than 20 years of age (Pui, 2011).

*Acute Lymphoblastic Leukemia (ALL)*
Acute lymphocytic leukemia occurs when a bone marrow cell develops a mutation in its genetic material. "(ALL) is of B-cell precursor (BCP) lineage (BCP-ALL) or, less commonly, T-cell precursor lineage (T-ALL). Both comprise multiple subtypes commonly defined by structural chromosomal alterations that are initiating lesions, with secondary somatic (tumor-acquired) DNA copy number alterations and sequence mutations that contribute to leukemogenesis. Chromosomal alterations include aneuploidy and chromosomal rearrangements that result in oncogene deregulation or expression of chimeric fusion genes. The prevalence of these alterations varies according to age and identification is important for diagnosis, risk classification, and, for some lesions, targeted therapy. Common genomic features of *BCR-ABL1*–like ALL are alterations of B-lymphoid transcription factor genes (particularly *IKZF1* deletions) and genetic alterations deregulating cytokine receptor and tyrosine kinase signaling. These include rearrangements and mutation of *CRLF2* (approximately 50%), rearrangements of *ABL*-class tyrosine kinase genes (12%), rearrangements of *JAK2* (7%) and the erythropoietin receptor gene (*EPOR*; three to ten percent), mutations activating JAK-STAT signaling (11%) and Ras signaling (*NRAS*, *KRAS*, *PTPN11*, and *NF1*; six percent), and less common kinase alterations (*FLT3*, *NTRK3*, *BLNK, TYK2*, and *PTK2B*)" (Iacobucci & Mullighan, 2017).

*Chronic Lymphoblastic Leukemia (CLL)*
The CLL exomes carry approximately "5–20 somatically acquired mutated genes per individual case, much fewer than many other solid cancers. Importantly, the somatically acquired mutation status of

sequence variants in genes, like other studies of genomic changes in cancer, necessitates confirmation of the absence of these candidate mutations in paired non-tumorous DNA (T-cell–, buccal cell– or skin-derived DNA). Mutations in *TP53* are of major clinical relevance, are often associated with del17p and gain in frequency over time. *TP53* mutated and associated del17p states substantially lower response rates, remission duration, and survival in CLL. Mutations in *NOTCH1* and *SF3B1* are recurrent, often associated with progressive CLL" (Amin A. Nisar, 2016).

***Analytic Validity***

There is very limited published literature on the analytic validity and clinical validity of genetic testing for *FLT3* and *NPM1* mutations in AML. However, the analytic validity of PCR in general is extremely high (Leonard, 2016). Other tools, such as flow cytometry and next generation sequencing (NGS) have also been used for AML prognostic and diagnostic purposes.

Ampasavate et al. (2019) have developed a quantitative protocol and flow cytometry-based method for monitoring an anti-FLT3 interaction. The FLT3 biomarker has been previously identified as a poor prognostic marker for AML patients. This method can rapidly identify intact FLT3 on the leukemic cell surface. "The results demonstrated good linearity (r2 > 0.99)"; further, "when compared with Western blotting results, FLT3 protein expression levels in leukemia patient's bone marrow samples were demonstrated in the same trend" (Ampasavate et al., 2019). The researchers state that this technique is reliable, rapid, effective and "provided a practical analysis of FLT-3 biomarker levels which is valuable for physician decision in acute leukemia treatment" (Ampasavate et al., 2019).

Alonso et al. (2019) have researched the utility of a 19-gene NGS panel for AML diagnostic purposes. This targeted NGS panel was studied in a cohort of 162 patients with AML. The authors note that "The assay yielded a median read depth >2000×, with 88% of on-target reads and a mean uniformity >93% without significant global strand bias. The method was sensitive and specific, with a valid performance at the clinical variant allele frequency cutoff of 3% for point mutations and five percent for insertions or deletions." The researchers conclude that this is a "reliable and reproducible method" for AML diagnoses (Alonso et al., 2019).

Molecular testing for the diagnosis of CML confirms typical findings in the blood and bone marrow by the demonstration of the Ph chromosome, the *BCR-ABL1* fusion gene or the BCR-ABL1 fusion mRNA. Molecular testing techniques include conventional cytogenetics, fluorescence in situ hybridization (FISH) analysis and reverse transcription polymerase chain reaction (RT-PCR) (Van Etten, 2022). Conventional cytogenetic karyotyping is no longer the diagnostic modality of choice due to its requirements for a highly skilled staff, culturing of cells, long turnaround time, and lower sensitivity (5-10%). Despite this, conventional cytogenetics are still the gold standard, and "should be performed" especially at diagnosis to detect additional clonal abnormalities (Yeung et al., 2016). FISH is more sensitive (0.1-5%) than karyotyping and can be performed on peripheral blood in addition to bone marrow and tissue. FISH can detect certain very rare translocations not usually detectable by the vast majority of commercial and laboratory-developed RT-PCR assays, but FISH is highly specific to the targeted region and may miss other chromosomal changes. Quantitative RT-PCR is the most sensitive technique currently available (0.001-0.01% sensitivity).

On July 22, 2016 the FDA approved the QuantideX qPCR BCR-ABL IS Kit as an *in vitro* nucleic acid amplification test for the quantitation of BCR-ABL1 and ABL1 transcripts in total RNA from whole blood of diagnosed t(9;22) positive Chronic Myeloid Leukemia (Tachibana et al.) patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2 as a class II device with special controls (FDA, 2016). Brown et al. (2019) performed a study to describe the analytical validation of this kit. They were able to find that "the test has a limit of detection of MR molecular response] 4.7 (0.002% IS) and a linear range from MR0.3 (50%IS) to MR4.7 (0.002%IS) for both Major transcripts. Single-site and multisite precision studies demonstrated a maximum SD of 0.13 MR (30% CV within the assay range between MR0.7 and MR3.7)."

***Clinical Utility and Validity***

The clinical utility of testing fallows for further risk stratification, prognostication, and guide management decisions in patients with AML. Several studies have concluded that *FLT3* and *NPM1* mutation testing in cytogenetically normal AML is useful for prognosis and treatment decision making (DeZern et al., 2011; Pastore et al., 2014; Willemze et al., 2014).

Devillier et al. (2015) sought to identify biological and prognostic subgroups based on genetic mutations in AML patients. A total of 125 AML patients with myelodysplasia-related changes ("MRC") were evaluated. The authors focused on the 26 patients with *ASXL1* mutations and 28 with *TP53* mutations. The *ASXL1* mutation cohort was found to have a higher proportion of marrow dysgranulopoiesis and an overall survival (OS) rate that was below average for wild-types (14% for *ASXL1* mutants, 37% for wild-types). The *TP53* cohort was found to have a "complex karyotype" and predicted a poor outcome with unfavorable cytogenetic risk AML. Both mutations were found to be an independent factor associated with shorter OS (Devillier et al., 2015).

Bolouri et al. (2018) examined 993 children's genetic data from the Children's Oncology Group (Khoury et al.) AML trials to characterize the molecular landscape of AML. The authors found that certain somatic variants, such as *MBNL1,* were "disproportionately prevalent" in children compared to adults. However, certain variants common in adults such as *TP53*, were not found in children. Other mutations such as *NRAS* and *KRAS* were "frequent" in pediatric AML. The authors concluded that their results "highlight the need for and facilitate the development of age-tailored targeted therapies for the treatment of pediatric AML" (Bolouri et al., 2018).

Jongen-Lavrencic et al. (2018) conducted a study of 482 patients 18 to 65 years with newly diagnosed AML. Targeted next-generation sequencing (NGS) was carried out at diagnosis and after induction therapy (during complete remission). At least one mutation was detected in 430 (89.2%) patients, and mutations persisted in 51.4% of those patients during complete remission. The detection of minimal residual disease was associated with a significantly increased relapse rate than no detection. Persistent DTA mutations (mutations in *DNMT3A*, *TET2*, and *ASXL1*) were not correlated with an increased relapse rate. Overall, the authors concluded, "A comparison of sequencing with flow cytometry for the detection of residual disease showed that sequencing had significant additive prognostic value. Among patients with AML, the detection of molecular minimal residual disease during complete remission had significant independent prognostic value with respect to relapse and survival rates, but the detection

of persistent mutations that are associated with clonal hematopoiesis did not have such prognostic value within a 4-year time frame" (Jongen-Lavrencic et al., 2018).

Kuwatsuka et al. (2018) evaluated the genetic background of 103 young adults and their subsequent clinical outcomes. The 103 cases included mutations in *FLT3-ITD, KIT, CEBPA, NRAS, KRAS, WT1, MLL-PTD*, and *NPM1*. Overall, *FLT3-ITD* and *NPM1* mutations were associated with a greater mortality risk. *NPM1* mutations conferred a 100% survival rate in the absence of *FLT3-ITD* mutations, but *FLT3-ITD* conferred only a 35% survival without *NPM1* mutations (Kuwatsuka et al., 2018).

Zhu et al. (2017) assessed the effect of gene mutations on the subsequent cytogenetic aberrations. A total of 560 patients were enrolled, and the authors examined the following alterations: "*CEBPA*, *NPM1, FLT3, C-KIT, NRAS, WT1, DNMT3A, MLL-PTD* and *IDH1/2*, as well as expression levels of MECOM, ERG, GATA2, WT1, BAALC, MEIS1 and SPI1." The investigators found that the expression levels of MECOM, MEIS1, and BAALC influenced cytogenetic aberration. Further, *FLT3*, *C-KIT*, and *NRAS* mutations all contained a "conversed" expression profile of MEIS1, WT1, GATA2, and BAALC expression. The investigators also noted "*FLT3, DNMT3A, NPM1* and biallelic *CEBPA* represented the mutations associated with the prognosis of AML in our group" (Zhu et al., 2017).

Papaemmanuil et al. (2016) examined the relationship between genotype and pathophysiology in AML. A total of 1540 patients with 5234 driver mutations across 76 genes were studied. The authors found three genomic subcategories in addition to the currently defined AML subgroups: mutations in genes encoding chromatin, RNA splicing regulators or both (such as *ASXL1* or *RUNX1*), *TP53* mutations, chromosomal aneuploidies or both (unusual karyotypes and *TP53*), and *IDH2* mutations. The authors noted that "patients with chromatin–spliceosome and *TP53*–aneuploidy AML had poor outcomes." The *NPM1* cohort was the largest of the sample (27%, 436 patients) and 319 of those patients also carried a DNA methylation or hydroxylmethylation gene, such as *IDH1/2* or *TET2*. The authors also noted that *NPM1-DNMT3A–NRASG12/13* had an "unexpectedly benign" prognosis, and the *NPM1* subgroup's prognoses were largely determined by the context in which the NPM1 mutation occurred (such as in *NRAS, IDH*, and so on) (Papaemmanuil et al., 2016).

Sperr et al. (2016) evaluated the effect of a genetic mutation and karyotype on the efficacy of treatment for elderly patients. A total of 192 patients over 60 years old were enrolled, and 115 of these patients achieved "complete hematologic remission (CR)." The authors stated that *NPM1* mutations (*NPM1*mut) and karyotype were the only independent predictors of survival, also noting that *NPM1*mut showed a prognostic impact on both normal (CN) and non-chromosomal (Mkneg) karyotypes. The authors concluded that "elderly patients with CN/Mkneg-*NPM1*mut or core binding factor AML can achieve long term median continuous CR when treated with intensive induction and consolidation therapy whereas most elderly patients with CN/Mkneg-NPM1wild-type or CN/Mkpos AML may not benefit from intensive chemotherapy" (Sperr et al., 2016).

Heiblig et al. (2019) assessed the impact of *NPM1* subtypes on treatment outcomes. One hundred seventy-five patients were examined. The authors found that out of the *NPM1* AML cases, 73% (128) were "Type A" mutations (TCTG at exon 12) and 27% (47) were "Non Type-A mutations" (Type B: CATG and Type D: CGTG). The Type-A mutations were found to achieve minimal residual disease (MRD)

earlier than non Type-A mutations. However, non-type A mutations achieved better rates of medial survival (Heiblig et al., 2019).

D'Adda et al. (2019) evaluated the effect of the BCR-ABL transcript on efficacy of TKIs. The *BCR*-ABL1 fusion gene may cause CML pathogenesis due to several breakpoints; the most common occur around exon 13 and 14 of the *BCR* gene and cause the formation of e13a2 and e14a2 transcripts (Greenfield et al., 2019). Out of 173 sampled patients, 67 had the e13a2 transcript, and 106 had the e14a2 version. The patients with the e14a2 version were more likely to achieve a deep molecular response to TKIs (sustained or otherwise). After 68 months, the sustained deep molecular response (sDMR) rate was 39.6% for e14a2 patients compared to 19.6% for e13a2 patients. Overall, the maximum rate of sDMR for e13a2 patients was 37%, after 60 months. Furthermore, only two patients (three percent) with the e13a2 transcript achieved treatment-free remission (TFR) whereas 25 of e14a2 patients achieved TFR (23%) (D'Adda et al., 2019).

Xu et al. (2020) have analyzed data from 220 normal karyotype AML pediatric patients. Participants were selected from the Cancer Genome Atlas database. It was found that 12.7% of these patients had *WT1* mutations, and that "the *WT1*-mutated group suffered lower rates of complete remission (CR) ($P < 0.001$ and $P < 0.001$, respectively) but higher rates of minimal residual disease (MRD) ($P = 0.003$ and $P = 0.021$, respectively) after both one and two courses of induction chemotherapy" (Xu et al., 2020). Patients with *WT1* mutations also had significantly worse event-free and overall survival rates ($P=0.007$ and $P<0.001$, respectively) (Xu et al., 2020).

Sasaki et al. (2020) studied the impact of *ASXL1, DNMT3A, JAK2, TET2*, and *TP53* mutations on survival in newly diagnosed acute myeloid leukemia (AML) patients. The 421 bone marrow aspirates were studied using NGS for these mutations with a minimum variant allele frequency (VAF) of 5%. "A total of 71 patients (17%) had *ASXL1* mutations, 104 patients (25%) had *DNMT3A* mutations, 16 patients (4%) had *JAK2* mutations, 82 patients (20%) had *TET2* mutations, and 86 patients (20%) had *TP53* mutations." The median VAF of *ASXL1* was 34.31% (range, 1.17%-58.62%), *DNMT3A* was 41.76% (range, 1.02%-91.66%), *JAK2* was 46.70% (range, 10.4%-71.7%), *TET2* was 42.78% (range, 2.26%-95.32%), and *TP53* was 45.47% (range, 1.15%-93.74%). In patients with these mutations, the median overall survival was 11 months. In patients without these mutations, the overall survival was 27 months. The authors conclude that "The VAF of *ASXL1, DNMT3A, JAK2, TET2, TP53*, and *NPM1* mutations is associated with worse prognosis in patients with newly diagnosed AML" (Sasaki et al., 2020).

Duncavage et al. (2021) investigated the clinical utility and accuracy of whole-genome sequencing (WGS) for the purpose of risk stratification in patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). The results from 263 patients were compared with findings from cytogenetic analysis and targeted sequencing. When conducting the WGS, they found that all 40 recurrent translocations and 91 copy-number alterations found on cytogenetic analysis were identified on WGS. There were also new clinically reportable genomic events among 17.0% of the patient sample. The standard AML risk groups, "as defined by sequencing results instead of cytogenetic analysis, correlated with clinical outcomes." WGS was also able to classify patients with inconclusive

cytogenetic analysis results into risk groups. This demonstrates that WGS could increase the diagnostic yield of AML and MDS and supplement the accuracy of cytogenetic analysis (Duncavage et al., 2021).

In a retrospective study, Yang reported the clinical significance of *BCR-ABL1* mutations in patients with Philadelphia chromosome-positive CML who underwent allogeneic hematopoietic cell transplantation. Of 315 patients in this study, point mutations were detected in 152 patients. One hundred and one of these 152 patients (66%) had T315I mutations and 51 had mutations other than T315I. The patients were followed 38 months later and overall survival at three years was worse in patients with the mutations than the non-mutation group, especially in the chronic phase of CML. Overall survival in the non-T315I group was significantly worse than that in the no-mutation group. The authors conclude that "mortality risk was significantly higher in patients with the BCR-ABL1 mutation than in patients without the mutation" (Tachibana et al., 2022).

## V. Guidelines and Recommendations

**World Health Organization (WHO)**

In a revision of the fourth edition on Classification of Tumors of Hematopoietic and Lymphoid Tissues, World Health Organization (WHO) incorporated new molecular genetic findings and clinical data into its classification of acute leukemias. WHO expanded on the prognostic significance of various gene mutations for each AML subtype. For example, for the AML with recurrent genetic abnormalities, inv(3) (q21.3; q26.2) does not represent a fusion gene, but rather a repositioning of a distal GATA2 enhancer leading to activation of MECOM (EVI1) expression and GATA2 haploinsufficiency. The AML with *CEBPA* mutation is defined based on biallelic mutation instead of single mutations because of prognostic significance. The provisional two categories are also added such as AML with *RUNX1* for de novo AML without preexisting cytogenetic abnormalities associated with MDS and AML with *BCR-ABL1* fusion gene. AML with *NPM1* or biallelic *CEBPA* mutations and multilineage dysplasia are now considered separately instead of being a part of AML with myelodysplasia-related changes because of a lack of prognostic significance. The complete list for acute myeloid neoplasms 2016 WHO classification is shown in Figure 1 (Arber et al., 2016).

In the absence of *JAK2, CALR,* and *MPL* mutations, the presence of another clonal marker is included as one of the major diagnostic criteria for PMF. Additional mutation in *ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2* and *SF3B1* genes are noted to be of use in determining the clonal nature of the disease.

In the fifth edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms, WHO places greater emphasis in "the separation of AML with defining genetic abnormalities from AML defined by differentiation". The latter eschews the previously ambiguous categorical term AML NOS, and other notable changes include the "elimination of the 20% blast requirement for AML types with defining genetic abnormalities (with the exception of AML with BCR::ABL1 fusion and AML with CEBPA mutation)" and "the introduction of a section on AML with other defined genetic alterations, a landing spot for new and/or uncommon AML subtypes that may (or may not) become defined types in future editions of the classification" (Khoury et al., 2022). Their list of acute myeloid leukemias is presented below.

| Acute myeloid leukaemia with defining genetic abnormalities |
|---|
| Acute promyelocytic leukaemia with *PML*::*RARA* fusion |
| Acute myeloid leukaemia with *RUNX1*::*RUNX1T1* fusion |
| Acute myeloid leukaemia with *CBFB*::*MYH11* fusion |
| Acute myeloid leukaemia with *DEK*::*NUP214* fusion |
| Acute myeloid leukaemia with *RBM15*::*MRTFA* fusion |
| Acute myeloid leukaemia with *BCR*::*ABL1* fusion |
| Acute myeloid leukaemia with *KMT2A* rearrangement |
| Acute myeloid leukaemia with *MECOM* rearrangement |
| Acute myeloid leukaemia with *NUP98* rearrangement |
| Acute myeloid leukaemia with *NPM1* mutation |
| Acute myeloid leukaemia with *CEBPA* mutation |
| Acute myeloid leukaemia, myelodysplasia-related |
| Acute myeloid leukaemia with other defined genetic alterations |

| Acute myeloid leukaemia, defined by differentiation |
|---|
| Acute myeloid leukaemia with minimal differentiation |
| Acute myeloid leukaemia without maturation |
| Acute myeloid leukaemia with maturation |
| Acute basophilic leukaemia |
| Acute myelomonocytic leukaemia |
| Acute monocytic leukaemia |
| Acute erythroid leukaemia |
| Acute megakaryoblastic leukaemia |

(Khoury et al., 2022)

Moreover, in defining acute myeloid leukemia, *myelodysplasia-related* (AML-MR), WHO offers the following abnormalities and mutations of interest:

Cytogenetic and molecular abnormalities defining acute myeloid leukaemia, myelodysplasia-related.

| Defining cytogenetic abnormalities | Defining somatic mutations |
|---|---|
| Complex karyotype (≥3 abnormalities) | *ASXL1* |
| 5q deletion or loss of 5q due to unbalanced translocation | *BCOR* |
| Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation | *EZH2* |
| 11q deletion | *SF3B1* |
| 12p deletion or loss of 12p due to unbalanced translocation | *SRSF2* |
| Monosomy 13 or 13q deletion | *STAG2* |
| 17p deletion or loss of 17p due to unbalanced translocation | *U2AF1* |
| Isochromosome 17q | *ZRSR2* |
| idic(X)(q13) | |

(Khoury et al., 2022).

The WHO published guidelines on the classification of myeloid neoplasms and acute leukemia. In the recent revision of the fourth edition on Classification of Tumors of Hematopoietic and Lymphoid issues, World Health Organization (WHO) incorporated new molecular genetic findings and clinical data into its classification of acute leukemias (Arber et al., 2016).

They state that: “With regard to chronic myeloid leukemia , BCR-ABL1$^{+}$, most cases of CML in chronic phase can be diagnosed from peripheral blood (Papaemmanuil et al.) findings combined with detection of t(9;22)(q34.1;q11.2) or, more specifically, *BCR-ABL1* by molecular genetic techniques. However, a bone marrow (BM) aspirate is essential to ensure sufficient material for a complete karyotype and for morphologic evaluation to confirm the phase of disease. In the era of tyrosine-kinase inhibitor (TKI) therapy, newly diagnosed patients may have a nearly normal lifespan, but regular

monitoring for *BCR-ABL1* burden and for evidence of genetic evolution and development of resistance to TKI therapy is essential to detect disease progression" (Arber et al., 2016).

They also introduced a provisional classification of ALL: B-ALL with translocations involving tyrosine kinases or cytokine receptors ("BCR-ABL1–like ALL").

"This newly recognized entity is assuming increasing importance because of its association with an adverse prognosis and responses of some cases to TKI therapies; however, it has been difficult to define in the clinical setting. It was originally described separately by different groups who demonstrated a series of cases of poor-prognosis childhood ALL with gene expression profiles similar to those seen in cases of ALL with BCR-ABL1, though different algorithms applied to the same sets of cases did not classify all cases the same way.

The cases with translocations involving tyrosine kinase genes involve many different genes including ABL1 (with partners other than BCR), as well as other kinases including ABL2, PDGFRB, NTRK3, TYK2, CSF1R, and JAK2. Over 30 different partner genes have been described. Some patients, especially those with EBF1-PDGFRB translocations, have shown remarkable responses to TKI therapy, even after failing conventional therapy" (Arber et al., 2016).

**College of American Pathologists (CAP) and American Society of Hematology (ASH)**
Following recent progress in molecular genetic findings and 2016 WHO classification of acute leukemias, the College of American Pathologists (CAP) and the American Society of Hematology (ASH) have formed an expert panel to review and establish guidelines for appropriate laboratory testing. The published guideline provides twenty-seven guideline statements ranging from recommendations on what testing is appropriate for the diagnostic and prognostic evaluation of leukemias to where the testing should be performed and how results should be reported. The appropriate molecular genetic testing for AML is discussed starting from 16$^{th}$ guideline statement.

The expert panel strongly recommends testing for *FLT3-ITD* in adult and pediatric patients with suspected or confirmed AML of any type. They also recommend testing for other mutational analysis that could include, but not limited to, *IDH1, IDH2, TET2, WT1, DNMT3A*, and/or *TP53* for prognostic and/or therapeutic purposes (Statement 16).

In the 17$^{th}$ guideline statement, expert panel strongly recommends testing for *KIT* mutation in adult patients with confirmed core-binding factor (CBF) AML (AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22) /t(16;16)(p13.1;q22); CBFB-MYH11). It is only an expert consensus opinion for testing *KIT* mutation in pediatric patients with confirmed core binding factor AML (AML with t(8;21)(q22;q22.1); RUNX1- RUNX1T1 or inv(16)(p13.1q22) /t(16;16)(p13.1;q22); CBFB-MYH11) which is not a strong recommendation (Statement 17).

The strong recommendation is also given for patients other than those with confirmed core binding factor AML, APL, or AML with myelodysplasia-related cytogenetic abnormalities that testing is needed for mutational analysis for *NPM1*, *CEBPA*, and *RUNX1* (Statement 19).

In the 20th guideline statement, expert panel is providing no recommendation on either for or against the use of global/gene specific methylation, microRNA (miRNA) expression, or gene expression analysis for diagnosis or prognosis in patients with confirmed acute leukemia.

Finally, in their last statement, expert panel strongly recommends the use of current WHO terminology for the final diagnosis and classification of acute leukemias (Arber et al., 2017).

**American Society of Clinical Oncology (ASCO)**
The ASCO has announced an endorsement of the 2017 CAP and ASH guideline regarding the initial diagnostic work-up of acute leukemia (de Haas et al., 2019). The ASCO supports all twenty-seven guideline statements. The statements relevant to this policy are noted in the 2017 CAP and ASH guidelines above.

**National Comprehensive Cancer Network (NCCN)**
*Acute myeloid leukemia*
For the initial evaluation of AML in individuals 18 years of age or older, the NCCN guidelines recommends bone marrow core biopsy and aspirate analyses (including immunophenotyping by immunohistochemistry stains plus flow cytometry) and analysis for chromosomal structural variations using cytogenetics, FISH, or whole genome sequencing. During the initial evaluation for AML, the NCCN also recommends "molecular analyses (*ASXL1*, *c-KIT*, *FLT3* [ITD (internal tandem duplication) and TKD (tyrosine kinase domain)], *NPM1*, *CEBPA* [biallelic], *IDH1, IDH2*, *RUNX1*, *TP53*, and other mutations. . . A variety of gene mutations are associated with specific prognoses (category 2A) and may guide medical decision-making (category 2B). Other genetic lesions may have therapeutic significance. The field of genomics in myeloid malignancies and related implications in AML are evolving rapidly. Mutations should be tested in all patients. Multiplex gene panels and targeted next-generation sequencing (NGS) analysis are recommended for the ongoing management of AML and various phases of treatment" (NCCN, 2023c).

Per the NCCN, the "importance of obtaining adequate samples of marrow or peripheral blood at diagnosis for full karyotyping and FISH cytogenetic analysis for the most common abnormalities cannot be overemphasized. Although FISH studies for common cytogenetic abnormalities may allow for rapid screening to identify either favorable- or unfavorable-risk groups, additional tests are needed to provide a full picture of the genetic factors that contribute to risk" (NCCN, 2023c).

As a part of monitoring during therapy for individuals with AML, the NCCN recommends a bone marrow aspirate/biopsy 14-21 days after the "start of therapy to document hypoplasia. If hypoplasia is not documented or indeterminate, repeat biopsy in 7–14 days to clarify persistence of leukemia. If hypoplasia, then repeat biopsy at time of hematologic recovery to document remission. If cytogenetics were initially abnormal, include cytogenetics as part of the remission documentation." Furthermore, as a follow up to both standard-dose cytarabine induction therapy or high-dose cytarabine induction for poor-risk AML, the NCCN recommends that all patients should have bone marrow aspirate and biopsy by day 42 post-treatment, "regardless of the degree of hematologic recovery. When performed, BM aspirate and biopsy should include cytogenetic and molecular studies, as appropriate" (NCCN, 2023c).

As a part of surveillance and therapy for individuals with relapsed/refractory disease after the completion of consolidation, the NCCN recommends comprehensive genomic profiling to determine mutation status of actionable genes.

For the evaluation or workup for blastic plasmacytoid dendritic cell neoplasm in individuals 18 years of age or older, the NCCN recommends cytogenetic analysis (karyotype and/or FISH) and molecular analysis "(most common aberrations include: *ASXL1, IDH1-2, IKZF1-3, NPM1, NRAS, TET1-2, TP53, U2AF1, ZEB2*)".

*Chronic Myeloid Leukemia*

The NCCN's recommendations for CML include the following table:

| **Monitoring Response to TKI Therapy and Mutational Analysis** | |
|---|---|
| **Test** | **Recommendation** |
| **Bone marrow cytogenetics[1]** | At diagnosis<br>Failure to reach response milestones<br>Any signs of loss of hematologic response<br>Any sign of loss of CCyR or its molecular response correlate defined as an increase in *BCR-ABL1* transcript to >1%. |
| **qPCR using IS [International Scale]** | At diagnosis<br>Every 3 months after initiating treatment. After *BCR-ABL1* (IS) ≤1%[2] has been achieved, every 3 months for 2 years and every 3 – 6 months thereafter<br>If there is 1-log increase in *BCR-ABL1* transcript levels with MMR, qPCR should be repeated in 1 – 3 months |
| **BCR-ABL kinase domain mutation analysis** | Chronic phase<br>Failure to reach response milestones<br>Any sign of loss of hematologic response<br>Any sign of loss of CCyR or its molecular response correlate defined as an increase in *BCR-ABL1* transcript to >1%.<br>1-log increase in *BCR-ABL1* transcript levels and loss of MMR<br>Disease progression to accelerated or blast phase[3] |
| **[1]FISH has been inadequately studied for monitoring response to treatment.**<br>**[2]CCyR correlates with *BCR-ABL1* (IS) ≤1%.**<br>**[3]Consider myeloid mutation panel to identify BCR-ABL1–independent resistance mutations in patients with no BCR-ABL1 kinase domain mutations** | |

The NCCN (2023b) also states that for the diagnosis and workup of CML-1:

- "Initial evaluation should consist of a history and physical exam, including palpation of spleen, complete blood count (CBC) with differential, chemistry profile, and hepatitis B panel. Bone marrow aspirate and biopsy for morphologic and cytogenetic evaluation and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to establish the presence of quantifiable *BCR-ABL1* mRNA transcripts at baseline are recommended to confirm the diagnosis of CML.
-

- Bone marrow cytogenetics should be done at initial workup to detect additional chromosomal abnormalities in Ph-positive cells (ACA/Ph$^+$), also known as clonal cytogenetic evolution. If bone marrow evaluation is not feasible, fluorescence *in situ* hybridization (FISH) on a peripheral blood specimen with dual probes for *BCR* and *ABL1* genes is an acceptable method to confirm the diagnosis of CML.
- 
- Quantitative RT-PCR (qPCR) should be done at initial workup to establish the presence of quantifiable *BCR-ABL1* mRNA transcripts. qPCR for the detection of atypical *BCR-ABL1* transcripts should be considered if there is discordance between FISH and qPCR results" (NCCN, 2023b).
- 

The NCCN provides the following early treatment response milestones for those with CML:

**EARLY TREATMENT RESPONSE MILESTONES**
**CRITERIA FOR RESPONSE AND RELAPSE**

| *BCR::ABL1* (IS) | 3 months | 6 months | 12 months[m] |
|---|---|---|---|
| >10%[n] | YELLOW | RED | |
| >1%–10% | GREEN | | YELLOW |
| >0.1%–1% | GREEN | | LIGHT GREEN |
| ≤0.1% | GREEN | | |

| COLOR | CONCERN | CLINICAL CONSIDERATIONS[p] | RECOMMENDATIONS[p] |
|---|---|---|---|
| RED | TKI-resistant disease[o] | • Evaluate patient adherence and drug interactions<br>• Consider *BCR::ABL1* kinase domain mutational analysis[q]<br>• Consider bone marrow cytogenetic analysis to assess additional chromosomal abnormalities (ACAs) | Switch to alternate TKI (CML-5) (other than imatinib) and evaluate for allogeneic HCT |
| YELLOW | Possible TKI resistance[o] | • Evaluate patient adherence and drug interactions<br>• Consider *BCR::ABL1* kinase domain mutational analysis[q]<br>• Consider bone marrow cytogenetic analysis to assess for MCyR at 3 mo or CCyR at 12 mo | Switch to alternate TKI (CML-5) or<br>Continue same TKI (CML-G)[r]<br>and Consider evaluation for allogeneic HCT |
| LIGHT GREEN | TKI-sensitive disease | • Evaluate patient adherence and drug interactions<br>• If treatment goal is long-term survival: ≤1% optimal<br>• If treatment goal is treatment-free remission: ≤0.1% optimal | • If optimal: continue same TKI (CML-G)<br>• If not optimal: shared decision-making with patient[o,s] |
| GREEN | TKI-sensitive disease | • Monitor response (CML-E)<br>• Evaluate patient adherence and drug interactions | Continue same TKI (CML-G)[t] |

(NCCN, 2023b)

For those discontinuing TKI therapy, the NCCN recommends molecular monitoring every one to two months in the first six months following discontinuation, bimonthly monitoring in months seven to twelve, and quarterly monitoring thereafter (indefinitely) in individuals who remain in MMR (NCCN, 2023b).

*Acute Lymphoblastic Leukemia (ALL)*

Regarding ALL, the NCCN (NCCN, 2023a) recommends testing of marrow or peripheral blood lymphoblasts using various techniques. The guidelines state the following:

"The diagnosis of ALL generally requires demonstration of ≥20% bone marrow lymphoblasts, upon hematopathology review of bone marrow aspirate and biopsy materials, which includes:

- Morphologic assessment of Wright-Giemsa-stained bone marrow aspirate smears, and H&E-stained core biopsy and clot sections
- Comprehensive flow cytometric immunophenotyping

- Baseline flow cytometric and/or molecular characterization of leukemic clone to facilitate subsequent minimal/measurable residual disease analysis
- Karyotyping of G-banded metaphase chromosomes"

"Optimal risk stratification and treatment planning requires testing marrow or peripheral blood lymphoblasts for specific recurrent genetic abnormalities using:

- Interphase fluorescence in situ hybridization (FISH) testing, including probes capable of detecting the major recurrent genetic abnormalities;
- Reverse transcriptase polymerase chain reaction (RT-PCR) testing *BCR-ABL1* in B-ALL (quantitative or qualitative) including determination of transcript size (i.e., p190 vs p210).
- Comprehensive testing by next-generation sequencing (NGS) for gene fusions and pathogenic mutations is recommended.
- Assessment with chromosomal microarray (CMA)/array cGH in cases of aneuploidy or failed karyotype" (NCCN, 2023a).

Per the NCCN, these gene fusions and mutations include *ABL1, ABL2, CRLF2, CSF1R, EPOR, JAK1, JAK2, JAK3, TYK2, PDGFRβ, PDGFRα, FGFR, EBF1, FLT3, IL7R, NTRK3, PTL2B*, and *SH2B3* genes".

As a part of surveillance under their "Other General Measures" for individuals with ALL, the NCCN recommends, that bone marrow aspirate "can be considered as clinically indicated at a frequency of up to 3 to 6 months for at least 5 years. . . If bone marrow aspirate is done: Flow cytometry with additional studies that may include comprehensive cytogenetics, FISH, molecular testing, and MRD assessment. . . Periodic *BCR::ABL1* transcript-specific quantification (Ph+ ALL)" (NCCN, 2023b). When discussing periodic as a frequency, the NCCN notes "no more than every 3 months" as a more specific time frame for individuals with complete molecular remission (undetectable levels). However, "increased frequency may be indicated for detectable levels" (NCCN, 2023b).

*Pediatric Acute Lymphoblastic Leukemia (PEDALL)*

In 2019, the NCCN first issued a new set of guidelines titled *Pediatric Acute Lymphoblastic Leukemia*. These guidelines have been updated since the original issue date, with the most recent revision having been on March 10, 2023. For PEDALL, the NCCN provides the following recommendation for genetic characterization:

"GENETIC CHARACTERIZATION
Optimal risk stratification and treatment planning require testing marrow or peripheral blood lymphoblasts for specific recurrent genetic abnormalities using:

- Karyotyping of G-banded metaphase chromosomes
- Interphase fluorescence in situ hybridization (FISH) testing, including probes capable of detecting the major recurrent genetic abnormalities
- Reverse transcriptase-polymerase chain reaction (RT-PCR) testing for BCR::ABL1 in B-ALL (quantitative or qualitative) including determination of transcript size (i.e., p190 vs. p210) If BCR::ABL1 negative: encourage testing for gene fusions and mutations associated with BCR::ABL1–like (Ph-like) ALL

• Assessment of various potentially actionable or prognostic mutations and gene fusions via next-generation sequencing (NGS) or alternative methods" (NCCN, 2024).

As a part of surveillance under their "Procedures and Molecular Testing" of individuals with PEDALL, the NCCN recommends testing of "Bone marrow aspirate and cerebrospinal fluid (CSF) for suspected relapse. . . If bone marrow aspirate is done: Flow cytometry with additional studies that may include comprehensive cytogenetics, FISH, molecular testing, and MRD testing. . . Consider periodic BCR::ABL1 transcript-specific quantification (Ph+ ALL)" (NCCN, 2024).

The guidelines also state that "The *BCR-ALB1*-like (Ph-like) phenotype is associated with recurrent gene fusions and mutations that activate tyrosine kinase pathways and includes gene fusions involving *ABL1, ABL2, CRLF2, CSF1R, EPOR, JAK2 or PDGFRB* and mutations involving *CRLF2, FLT3, IL7R, SH2B3, JAK1, JAK3, and JAK2* (in combination with *CRLF2* gene functions). Testing of these abnormalities at diagnosis may aid in risk stratification" (NCCN, 2024).

**European LeukemiaNet (ELN) Working Party**

In 2022, an international expert panel on behalf of the ELN published recommendations for the diagnosis and management of AML.

ELN remarks that "Germline predisposition risk should be considered for all patients diagnosed with a hematopoietic malignancy regardless of age, because some germline predisposition alleles, like those in DDX41, can drive hematopoietic malignancies in older age" and notes key features of clinical presentation warranting consideration of clinical testing for a germline predisposition allele(s), including

- "Personal history of ≥ two cancers, one of which is a hematopoietic malignancy (order does not matter)
- Personal history of a hematopoietic malignancy plus:
    - Another relative within two generations with another hematopoietic malignancy, or
    - Another relative within two generations with a solid tumor diagnosed at age 50 or younger, or
    - Another relative within two generations with other hematopoietic abnormalities
- Presence of a deleterious gene variant in tumor profiling that could be a germline allele, especially if that variant is present during remission*
- Age of diagnosis of hematopoietic malignancy at an earlier age than average (eg, MDS diagnosed ≤ 40 y)
- Germline status of a variant is confirmed by:
- Its presence in DNA derived from a tissue source not likely to undergo somatic mutation frequently (eg, cultured skin fibroblasts or hair follicles) AND at a variant allele frequency consistent with the germline (generally considered between 30-60%), or
- Its presence in at least two relatives at a variant allele frequency consistent with the germline" (Döhner et al., 2022).

Furthermore, ELN lists tests and procedures recommended to be performed at diagnosis for a patient with AML in the below table:

**Table 4. Tests and procedures at diagnosis for a patient with AML**

| Tests and procedures | | |
|---|---|---|
| **Tests to establish the diagnosis**<br>Complete blood count and differential count*<br>Bone marrow aspirate†<br>Bone marrow trephine biopsy‡<br>Immunophenotyping by flow cytometry (see Table 5) | | **Additional tests and procedures**<br>Complete physical examination[c]<br>Performance status (ECOG/WHO score)<br>Geriatric assessment[d] (optional)<br>Biochemistry, coagulation tests[e]<br>Hepatitis A, B, C; HIV-1 testing; CMV, EBV, HSV, VZV<br>Serum pregnancy test[f]<br>Eligibility assessment for allogeneic HCT (incl. HLA-typing)[g]<br>Chest x-ray, 12-lead electrocardiogram, echocardiography or MUGA (on indication)<br>Computed tomography of the chest (on indication)[h]<br>Lumbar puncture (on indication)[i]<br>Information on oocyte and sperm cryopreservation[j]<br>Biobanking[k] |
| **Genetic analyses** | Results preferably available within | |
| Cytogenetics§ | • 5-7 d | |
| Screening for gene mutations required for establishing the diagnosis and to identify actionable therapeutic targets# | | |
| • *FLT3*,¶ *IDH1*, *IDH2* | • 3-5 d | |
| • *NPM1* | • 3-5 d | |
| • *CEBPA*,# *DDX41*, *TP53*; *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2* | • 1st cycle | |
| Screening for gene rearrangements** | | |
| • *PML::RARA*, *CBFB::MYH11*, *RUNX1::RUNX1T1*, *KMT2A* rearrangements, *BCR::ABL1*, other fusion genes (if available) | • 3-5 d | |
| Additional genes recommended to test at diagnosis†† | | |
| • *ANKRD26*, *BCORL1*, *BRAF*, *CBL*, *CSF3R*, *DNMT3A*, *ETV6*, *GATA2*, *JAK2*, *KIT*, *KRAS*, *NRAS*, *NF1*, *PHF6*, *PPM1D*, *PTPN11*, *RAD21*, *SETBP1*, *TET2*, *WT1* | | |
| **Medical history** | | |
| Demographics and medical history‡‡ | | |
| Detailed family history[a] | | |
| Patient bleeding history[b] | | |
| Analysis of comorbidities | | |

(Döhner et al., 2022).

According to ELN, "Conventional cytogenetic analysis is mandatory in the evaluation of AML. If conventional cytogenetics fails, fluorescence in situ hybridization is an alternative to detect specific abnormalities like RUNX1::RUNX1T1, CBFB::MYH11, KMT2A (MLL), and MECOM (EVI1) gene fusions, or myelodysplasia-related chromosome abnormalities, eg, loss of chromosome 5q, 7q, or 17p material". ELN also asserts that "Molecular genetic testing should screen for all the genetic abnormalities that define disease and risk categories or that are needed for targeted treatment modalities" listed above in their Table 4, and that for "patients with mutant NPM1 and core-binding factor (CBF)- AML, it is recommended to perform baseline molecular assessment by quantitative polymerase chain reaction (qPCR) or droplet digital PCR (dPCR) to facilitate MRD monitoring after treatment" (Döhner et al., 2022). ELN recognizes that "Immunophenotyping by multiparameter flow cytometry (MFC) is required to diagnose AML accurately by identifying cell surface and intracellular markers" and that "It is also important to identify leukemia-associated immunophenotypes (LAIP) for subsequent MRD monitoring by MFC. In cases where an aspirate is unobtainable and circulating blasts are absent, myeloid phenotype may be confirmed on a core biopsy using immunohistochemistry" (Döhner et al., 2022).

Cell-surface and cytoplasmic markers of interest for the diagnosis of AML and MPAL as reported by ELN is shown below (Table 5). Lastly, this ELN special report included an updated table of risk classifications at initial diagnosis based on new data (Table 6).

**Table 5. Expression of cell-surface and cytoplasmic markers for the diagnosis of AML and MPAL**

| Diagnosis of AML | |
|---|---|
| Diagnosis of AML | |
| Precursor marker | CD34, CD117, HLA-DR |
| Myeloid markers | Cytoplasmic MPO, CD33, CD13 |
| Myeloid maturation markers | CD11b, CD15, CD64, CD65 |
| Monocytic markers | CD14, CD36, CD64, CD4, CD38, CD11c |
| Megakaryocytic markers | CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa), CD36 |
| Erythroid markers | CD235a (glycophorin A), CD71, CD36 |
| Diagnosis of MPAL | |
| Myeloid lineage | MPO (flow cytometry, immunohistochemistry or cytochemistry), or monocytic differentiation (at least 2 of the following: non-specific esterase cytochemistry, CD11c, CD14, CD64, lysozyme), or at least two myeloid markers, ie, CD177, CD33, CD13 |
| T-lineage | Strong cytoplasmic CD3 (with antibodies to CD3 ε chain) or surface CD3 |
| B-lineage[9] | Strong CD19 with at least one of the following strongly expressed: cytoplasmic CD79a, cCD22 or CD10, or weak CD19 with at least two of the following strongly expressed: CD79a, cCD22 or CD10 |
| Core MRD markers | CD34, CD117, CD45, CD33, CD13, CD56, CD7, HLA-DR If monocytic: CD64, CD11b, CD4 (in addition) |

**Table 6. 2022 ELN risk classification by genetics at initial diagnosis***

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

An ELN expert panel has released guidelines for the assessment of measurable residual disease (MRD) of AML that were updated in 2021. First, for molecular MRD recommendations, "techniques for molecular MRD assessment should reach an LOD [limit of detection] of $10^{-3}$ or lower. To achieve this LOD, qPCR, dPCR, or error-corrected NGS using UMIs [unique molecular identifiers] is recommended (Grade of Recommendation: B)."

Other recommendations for molecular MRD testing included:

- "For patients with mutant *NPM1*, CBF AML (*RUNX1-RUNX1T1* or *CBFB-MYH11*), or APL (*PML-RARA*), we recommend molecular MRD assessment by qPCR or dPCR. (Grade of Recommendation: A)"

- "Leukemia-specific PCR assays (eg, for *NPM1, PML-RARA,* or *CBF* AML) are preferred over fewer specific markers, such as *WT1* or *EV11* expression (Grade of Recommendation: B)"
- "Targeted NGS-MRD using specific mutations identified at diagnosis vs agnostic panel approaches has different strengths and limitations, but both approaches can be considered, depending on sensitivity, turnaround time, resource use, setting (research, clinical trial, or clinical routine), and ability to standardize methodology and reporting. (Grade of Recommendation: B)"
- "If a panel approach is used for NGS-MRD, emerging variants not found at diagnosis should be reported only if confidently detected above background noise. (Grade of Recommendation: B)"
- "For NGS-MRD, we recommend considering all detected mutations as potential MRD markers, with the limitations detailed in recommendations B9 to B11 (listed below) (Grade of Recommendation: B)"
    - "Germline mutations (VAF of ~50 in genes *ANKRD26*, *CEBPA*, *DDX41*, *ETV6*, *GATA2*, *RUNX1*, and *TP53*) should be excluded as NGS-MRD markers, as they are noninformative for MRD. (Grade of Recommendation: A)"
    - "Mutations in *DNMT3A*, *TET2*, and *ASXL1* (DTA) can be found in age-related clonal hematopoiesis and should be excluded from MRD analysis. (Grade of Recommendation: A)"
    - "Mutations in signaling pathway genes (*FLT3-ITD*, *FLT3-TKD*, *KIT*, and *RAS*, among others) most likely represent residual AML when detected, but are often subclonal and have a low negative predictive value. These mutations are best used in combination with additional MRD markers. (Grade of Recommendation: B)" (Heuser et al., 2021).

**British Society for Haematology (BSH)**

The Haemato-oncology Task Force of BCSH (British Committee for Standards in Haematology) had produced a draft outline of guidelines for the diagnosis and management of AML in pregnancy, which was subsequently reviewed by the British Society of Haematology due to a consultation process resulting in the name change from BCSH to BSH in 2016. These guidelines state that "As for non-pregnant patients, acute myeloid leukaemia (AML) should be diagnosed using the World Health Organization (WHO) classification (Grade 1A)" and [w]here a diagnosis of leukemia is suspected, care must be taken to ensure that marrow samples are directed for immunophenotypic, cytogenetic and molecular analysis to allow accurate sub-typing and understanding of prognostic features" (Ali et al., 2015).

On the management of older patients with frailty and acute myeloid leukemia, BSH asserts that "Older adults who are suspected of having AML and are deemed to be fit to receive anti-leukaemia therapy should undergo the same diagnostic workup as any other patient (Table 1)" (Dennis et al., 2022).

That is, at diagnosis, "Patients should have a bone marrow examination (aspirate and trephine biopsy) for blast enumeration [with a 300 (or 500 where indicated) differential cell count]" and "flow cytometry to characterise the leukaemic clone immunophenotype to confirm lineage, and because this provides a means of assessing measurable residual disease (MRD) in the absence of a molecular marker". Moreover, "For patients with a high white blood count at presentation, diagnostic workup may be performed on the peripheral blood in lieu of a bone marrow examination, and this may also be a suitable approach for frail patients, for whom best supportive care is the most appropriate treatment

option. Consideration should also be given (with appropriate patient consent) to taking trial samples at the time of diagnostic marrow sampling" (Dennis et al., 2022).

The BSH notes that "Risk stratification remains informed by cytogenetic analysis, and, given that some treatments (e.g., CPX-351) are currently only licensed in the UK for therapy-related AML or AML with myelodysplasia-related changes (AML-MRC), knowledge of cytogenetic abnormalities is a prerequisite to offer the most appropriate treatment." But while "This is likely to be most efficiently achieved using a multi-target next-generation sequencing (NGS) panel approach with a maximum turnaround time of 21 days", "we [BSH] recommended rapid screening (turnaround time within 72 hours) for mutations of *NPM1*, *FLT3*, *IDH1/2* and *TP53*, given that the presence of *NPM1* and I*DH1/2* mutations may inform treatment decisions, as these mutations have been shown to confer a superior response to venetoclax/azacitidine), whilst *TP53* mutations confer resistance to chemotherapy and an overall poor outcome (Dennis et al., 2022).

The BSH also recommends that "At relapse, patients who remain fit to receive anti-leukaemia therapy, should be re-screened for actionable/targetable mutations. At the least, this should include re-screening for mutations in FLT3 (both internal tandem duplication; ITD and tyrosine kinase domain; TKD), given the availability of gilteritinib", along with "*IDH1/2* mutations (if trial recruitment is an option). It should be noted that *FLT3* mutations may be acquired or lost at relapse". As such,

- All patients should have their disease assessed by morphology, immunophenotyping, cytogenetics and molecular studies at presentation.
- At relapse, patients should be re-screened for *FLT3* mutations as a minimum.
- Peripheral blood is a suitable alternative to bone marrow for the diagnostic evaluations if there are circulating blast cells.
- All cases should be discussed at an appropriate local or regional haemato-oncology MDT (Dennis et al., 2022).

Table 1, produced by BSH and referenced earlier, is captured below:

**TABLE 1.** Diagnostic investigations for AML in the older adult

| Diagnostic | Diagnosis | Relapse |
|---|---|---|
| Bone marrow aspirate | √ | √ |
| Bone marrow trephine biopsy | √ | √ |
| Flow cytometric immunophenotyping | √ | √ |
| Cytogenetic analysis (G-banding and FISH panels) (within 5 working days) | √ | √[a] |
| Rapid *FLT3* ITD and TKD mutation screen (within 72 hours) | √ | √ |
| Rapid *NPM1* mutation screen (within 72 hours) | √ | |
| Multi-target NGS panel including minimum of: | √ | |
| *IDH1/2* screen | √[b,c] | |
| *TP53* screen | √[b,c] | |
| *ASXL1* screen | √ | |
| *RUNX1* screen | √ | |

Abbreviations: AML, acute myeloid leukaemia; FISH, fluorescence in situ hybridisation.

[a]If no unfavourable risk cytogenetic abnormalities were found at diagnosis, or if the patient has experienced a prolonged remission (>12 months), then cytogenetics should be rechecked at relapse.

[b]Rapid mutation screening assays for *IDH1/2* and *TP53* are recommended as these mutations may impact early treatment decisions.

[c]National Genomic Test directory for Cancer (https://www.england.nhs.uk/publication/national-genomic-test-directories/) currently recommends screening for mutations in following genes at diagnosis; *NPM1*, *CEBPA*, *RUNX1*, *FLT3*, *IDH1*, *IDH2*, *KIT*, *WT1*, *ASXL1*, *SRSF2*, *STAG2*, *RAD21*, *TP53*, *KRAS*, *NRAS*, *MLL* (*KMT2A*)-PTD, *PPM1D*.

(Dennis et al., 2022).

In a paper on best practices for laboratory testing of UK patients with AML, BSH recommends that "For a suspected acute leukaemia, most multiparametric flow cytometry laboratories apply a two-stage diagnostic panelan acute leukaemia screen (such as the Euroflow A LOT combination)" in order to confirm leukaemic blasts and allow lineage assessment, "followed immediately by an AML-specific panel if appropriate". Furthermore, "extended secondary testing may be required to diagnose blastic plasmacytoid dendritic cell neoplasm (BPDCN) or ambiguous leukaemias or acute megakaryoblastic leukaemia" (Mehta et al., 2023).

**European Society for Medical Oncology (ESMO)**

The ESMO released guidelines for the diagnosis of acute myeloid leukemia in adult patients. They recommend molecular testing for *c-KIT, FLT3-ITD, FLT3-TKD, NPM1, CEBPA, IDH1, IDH2,* and *TP53* mutations. They state that "next-generation sequencing (NGS) of a panel of genes commonly mutated in AML provides important additional prognostic and therapeutic information" (Heuser et al., 2020).

*Chronic Myeloid Leukemia*

The ESMO has published guidelines for diagnosis, treatment, and follow-up of CML. These guidelines include a table which is shown below:

Table 2: Recommendations for diagnostic work-up, assessment of response and monitoring (Hochhaus et al., 2017)

| Test | Baseline (diagnostic work-up) | To assess the response | To monitor the response and the treatment |
|---|---|---|---|
| **Blood counts and differential** | Yes | Every 15 days until a CHR [complete hematological response] without significant cytopaenias has been achieved | Every 3 months |
| **BM [bone marrow], cytology** | Yes | No | No |
| **BM, karyotype** | Yes | At 3 and 6 months | Then every 6 months until CCyR [complete cytogenetic response] has been achieved |
| **Blood, iFISH** | No | No | Only if cytogenetics of BM metaphases cannot be analyzed or is normal and molecular response cannot be assessed |
| **Blood, RT-PCR (qualitative)** | Yes | No | No |
| **Blood, qRT-PCR (quantitative, BCR–ABL %)** | No | Every 3 months | Every 4–6 weeks in first year after treatment discontinuation |
| **Mutational Analysis** | Only in AP [accelerated phase] or BP [blast phase] | No | Only in the case of failure |

The ESMO has also stated that a "diagnosis must be confirmed by cytogenetics showing t(9;22)(q34;q11), and by multiplex RT-PCR showing BCR–ABL1 transcripts" (Hochhaus et al., 2017). Other warning signs include "Major route cytogenetic aberrations (+8, iso(17q), +19, +22q-), chromosome 3 aberrations and BM fibrosis at diagnosis signs"; further, a quantification of BCR-ABL mRNA is required every 3 months (Hochhaus et al., 2017). Finally, the ESMO acknowledges that

mutation analysis is "due" in case of failure of first-line therapy or if BCR-ABL transcript levels increase. However, ESMO recommends against baseline mutational analysis in patients with newly diagnosed CML-CP (Hochhaus et al., 2017).

*Acute Lymphoblastic Leukemia (ALL)*

The ESMO also published clinical practice guidelines for ALL in 2016 and note that "standard cytogenetics/FISH and especially RT-PCR are routinely performed to obtain a rapid diagnosis of Ph+ ALL and identify certain intermediate/high- and high-risk karyotypes or gene, mainly:

a. t(4;11)(q21;q23)/MLL-AFA4, abn11q23/MLL, t(1;19)(q23; p13)/PBX-E2A, t(8;14) or other abn14q32 in non-Burkitt ALL
b. del(6q), del(7p), del(17p), −7, +8, low hypodiploidy, i.e., with 30–39 chromosomes/near triploidy with 60–78 chromosomes
c. complex (≥5 unrelated clonal abnormalities), and
d. T-ALL lacking NOTCH1/FBXW7 mutations and/or with RAS/PTEN abnormalities

The more prognostically favorable cytogenetic/genetic subsets are t(12;21)( p13;q22)/TEL-AML1 + ALL (rare in adults) and hyperdiploid ALL, and NOTCH-1/FBXW7-mutated T-ALL (Hoelzer et al., 2016)."

## VI. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status.

HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

## VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-

database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

**Food and Drug Administration (FDA)**

On April 28, 2017 the FDA approved the LeukoStrat® CDx *FLT3* Mutation Assay as a "PCR-based, in vitro diagnostic test designed to detect internal tandem duplication (ITD) mutations and the tyrosine kinase domain mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML). The LeukoStrat® CDx FLT3 Mutation Assay is used as an aid in the selection of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered. The Leukostrat® CDx FLT3 Mutation Assay is to be performed only at Laboratory for Personalized Molecular Medicine (LabPMM) LLC, a single site laboratory located at 6330 Nancy Ridge Dr., San Diego, CA 92121."

On August 1, 2017 the FDA approved the Abbott RealTime *IDH2* as an "in vitro polymerase chain reaction (PCR) assay for the qualitative detection of single nucleotide variants (SNVs) coding nine *IDH2* mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) in DNA extracted from human blood (EDTA) or bone marrow (EDTA). Abbott RealTime IDH2 is for use with the Abbott m2000rt System. Abbott RealTime IDH2 is indicated as an aid in identifying acute myeloid leukemia (AML) patients with an isocitrate dehydrogenase-2 (IDH2) mutation for treatment with IDHIFA® (enasidenib)."

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

## VIII. Evidence-based Scientific References


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## IX. Policy History

| Action Date | Action |
|---|---|
| 2/01/2025 | Policy created |