

## Genomic Testing for Hematopoietic Neoplasms

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### 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.<sup>1</sup> Myeloid neoplasms occur when there is a proliferation of myeloid cells that have originated from a primitive stem cell.<sup>2</sup> In contrast, lymphoid neoplasms are composed of a lymphocytic cell population which is usually malignant (clonal) by molecular genetic and/or immunophenotypic analysis.<sup>3</sup>

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. Related Policies

Policy Number	Policy Title
AHS-F2019	Flow Cytometry
AHS-M2101	Mutation Analysis in Myeloproliferative Neoplasms
AHS-M2175	Minimal Residual Disease

### III. 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 the differential diagnosis of chronic myeloid leukemia (CML) or acute lymphoblastic leukemia (ALL) or for optimal risk stratification and treatment planning for individuals diagnosed with B-cell ALL

(B-ALL), one time qualitative or quantitative RT-PCR testing on blood or bone marrow for identification of the *BCR-ABL1* fusion gene transcript type, including determination of transcript size (e.g., p190 vs. p210), **MEETS COVERAGE CRITERIA**.

- 3) 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.
- 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 optimal risk stratification and treatment planning for individuals diagnosed with chronic lymphocytic leukemia (CLL) or small lymphocytic leukemia (SLL), one time testing with any of the following tests (when not already performed during initial diagnosis) **MEETS COVERAGE CRITERIA**:
  - a) FISH to detect +12; del(11q); del(13q); del(17p).
  - b) *TP53* mutation testing.
  - c) CpG-stimulated metaphase karyotype for complex karyotype.
  - d) Immunoglobulin heavy chain variable region gene (IGHV) mutation testing.
- 8) 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.
- 9) 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%.
- 10) 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.
- 11) 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 CRITERIA** 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.
- 12) For individuals with CML, the use of FISH to monitor response to TKI therapy **DOES NOT MEET COVERAGE CRITERIA**.
- 13) 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.*

14) 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.**

15) For diagnosis or prognosis of myeloid or lymphoid neoplasms, all other testing not addressed above **DOES NOT MEET COVERAGE CRITERIA.**

**NOTES:**

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

**IV. Table of Terminology**

Term	Definition
<i>ABL1</i>	<i>Abelson murine leukemia viral oncogene homolog 1</i>
ACAs	Additional chromosomal abnormalities
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
<i>ANKRD26</i>	<i>Ankyrin repeat domain containing 26</i>
AP	Accelerated phase
AP-CML	Accelerated phase-chronic myeloid leukemia
APL	Acute promyelocytic leukemia
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
<i>ASXL1</i>	<i>ASXL transcriptional regulator 1</i>
<i>ASXL2</i>	<i>ASXL transcriptional regulator 2</i>
BAALC	Brain and acute leukemia cytoplasmic
B-ALL	B-cell acute lymphoblastic leukemia
<i>BCR-ABL1</i>	<i>BCR activator of RhoGEF and GTPase- ABL proto-oncogene 1, non-receptor tyrosine kinase</i>
BCSH	British Committee for Standards in Haematology
<i>BLNK</i>	<i>B-cell linker</i>
BM	Bone marrow
BP	Blast phase
BPDCN	Blastic plasmacytoid dendritic cell neoplasm
<i>c-KIT</i>	<i>Commonly used alias for KIT proto-oncogene, receptor tyrosine kinase</i>
CAP	College of American Pathologists
CBC	Complete blood count
CBF	Core-binding factor
<i>CALR</i>	<i>Calreticulin</i>
<i>CBFA2</i>	<i>CBFA2/RUNX1 partner transcriptional co-repressor 2</i>
CCyR	Complete cytogenetic response

CEBPA	<i>CCAAT/enhancer binding protein alpha</i>
cGH	Comparative genomic hybridization
CK	Complex karyotype
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLL	Chronic lymphocytic leukemia
CMA	Chromosomal microarray
CMS	Centers for Medicare and Medicaid
CML	Chronic myeloid leukemia
CMP	Comprehensive metabolic panel
CN	Normal karyotypes
CP-CML	Chronic phase-chronic myeloid leukemia
CR	Complete hematologic remission
CT	Computed tomography
<i>CRLF2</i>	<i>Cytokine receptor-like factor 2</i>
<i>CSF1R</i>	<i>Colony stimulating factor 1 receptor</i>
CV	Coefficient of variation
<i>CXCR4</i>	<i>C-X-C chemokine receptor type 4</i>
<i>DDX41</i>	<i>DEAD-box helicase 41</i>
<i>d17p</i>	<i>Deletion of chromosome 17p</i>
DNA	Deoxyribonucleic acid
<i>DNMT3A</i>	<i>Deoxyribonucleic acid methyltransferase 3A</i>
dPCR	Digital polymerase chain reaction
<i>EBF1</i>	<i>Early B cell factor-1</i>
ELN	European Leukemia Net
EMSO	European Society For Medical Oncology
EPOR	Erythropoietin receptor
ERG	ETS transcription factor ERG
EVI1	Ecotropic virus integration site 1 protein homolog
<i>EZH2</i>	<i>Enhancer of zeste 2 polycomb repressive complex 2 subunit</i>
FCI	Flow cytometric immunophenotyping
FDA	Food and Drug Administration
FDG-PET	Fluorodeoxyglucose positron emission tomography
FISH	Fluorescence in situ hybridization
<i>FLT3</i>	<i>FMS-like tyrosine kinase 3</i>
FNA	First-needle aspiration
GATA2	GATA binding protein 2
HCT	Hematopoietic cell transplantation
<i>IDH1</i>	<i>Isocitrate dehydrogenase 1</i>
<i>IDH2</i>	<i>Isocitrate dehydrogenase 2</i>
IGHV	Immunoglobulin heavy chain variable region gene
IHC	Immunohistochemistry

<i>IKZF1</i>	<i>Ikaros zinc finger 1</i>
<i>IL7R</i>	<i>Interleukin-7 receptor</i>
ITD	Internal tandem duplications
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
<i>JAK2</i>	<i>Janus kinase 2</i>
<i>JNK</i>	<i>c-Jun N-terminal kinase</i>
<i>KIT</i>	<i>KIT proto-oncogene, receptor tyrosine kinase</i>
<i>KRAS</i>	<i>KRAS proto-oncogene, GTPase</i>
LAIP	Leukemia-associated immunophenotypes
LCDs	Local Coverage Determinations
LDH	Lactate dehydrogenase
LOD	Limit of detection
LTD	Laboratory-developed test
MBL	Monoclonal B lymphocytosis
<i>MBNL1</i>	<i>Muscleblind like splicing regulator 1</i>
MCL	Mantle cell lymphoma
MDNs	Myelodysplastic neoplasms
MECOM	MDS1 and EVI1 complex locus
MEIS1	Muscleblind like splicing regulator 1
MFC	Multiparameter flow cytometry
miRNA	Micro ribonucleic acid
Mkneg	Non-chromosomal karyotype
<i>MLL-PTD</i>	<i>MLL -Partial Tandem Duplication</i>
MMR	Major molecular response
<i>MPL</i>	<i>MPL proto-oncogene, thrombopoietin receptor</i>
MRD	Minimal residual disease
<i>MYD88</i>	<i>Myeloid differentiation primary response 88</i>
<i>MYH11</i>	<i>Myosin heavy chain 11</i>
EMZL	Extranodal marginal zone lymphoma
NCCN	National Comprehensive Cancer Network
<i>NF1</i>	<i>Neurofibromin 1</i>
NGS	Next generation sequencing
<i>NOTCH1</i>	<i>Neurogenic locus notch homolog protein 1</i>
<i>NPM1</i>	<i>Nucleophosmin 1</i>
<i>NPM1mut</i>	<i>NPM1 mutations</i>
<i>NRAS</i>	<i>NRAS proto-oncogene, GTPase</i>
<i>NTRK3</i>	<i>Neurotrophic tyrosine receptor kinase 3</i>
OS	Overall survival
PB	Peripheral blood
PCR	Polymerase chain reaction
<i>PDGFRB</i>	<i>Platelet-derived growth factor receptor beta</i>

PEDALL	Pediatric Acute Lymphoblastic Leukemia
Ph	Philadelphia
<i>PI3K</i>	<i>Phosphatidylinositol 3-kinase</i>
PMF	Polyamine modulated factor
<i>PML-RAR</i>	<i>Promyelocytic leukemia-retinoic acid receptor</i>
PT	Prothrombin time
PTT	Partial thromboplastin time
<i>PTK2B</i>	<i>Protein tyrosine kinase 2 beta</i>
<i>PTPN11</i>	<i>Protein phosphatase non-receptor type 11</i>
RNA	Ribonucleic acid
RT-PCR	Reverse transcription- polymerase chain reaction
<i>RUNX1</i>	<i>RUNX family transcription factor 1</i>
<i>RUNX1T1</i>	<i>RUNX1 partner transcriptional co-repressor 1</i>
sDMR	Sustained deep molecular response
<i>SF3B1</i>	<i>Splicing factor 3b subunit 1</i>
<i>SH2B3</i>	<i>SH2B adapter protein 3</i>
<i>SH3</i>	<i>Src homology 3</i>
<i>SPI1</i>	<i>Spi-1 proto-oncogene</i>
<i>SRSF2</i>	<i>Serine and arginine rich splicing factor 2</i>
SLL	Small lymphocytic leukemia
<i>STAT5</i>	<i>Signal transducer and activator of transcription 5</i>
<i>TET2</i>	<i>Tet methylcytosine dioxygenase 2</i>
TFR	Treatment-free remission
<i>TKD</i>	<i>Tyrosine kinase domain</i>
TKI	Tyrosine Kinase Inhibitor
T-LGLL	T-cell large granular lymphocyte leukemia
<i>TP53</i>	<i>Tumor protein 53</i>
<i>TYK2</i>	<i>Tyrosine kinase 2</i>
VAF	Variant allele frequency
WGS	Whole-genome sequencing
WHO	World Health Organization
<i>WT1</i>	<i>Wilms' tumor 1</i>

## V. Scientific Background

### *Acute Lymphoblastic Leukemia (ALL)*

Acute lymphocytic leukemia occurs when a bone marrow cell develops a mutation in its genetic material. ALL can be of B-cell precursor (BCP) lineage (BCP-ALL) or, less commonly, T-cell precursor lineage (T-ALL). However, “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*)."<sup>4</sup>

### *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.<sup>5-7</sup>

A clinical presentation of AML includes symptoms related to complications of pancytopenia, including weakness and fatigability, infections of variable severity, and hemorrhagic findings.<sup>7</sup> Analysis of gene sequencing of AML cases generally reveal more than ten significant gene mutations; many of which are thought to participate in leukemogenesis.<sup>8</sup> 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*.<sup>9</sup>

The second most common mutation in AML (27%) is of nucleophosmin (*NPM1*), a ubiquitously expressed phosphoprotein that normally shuttles between the nucleus and cytoplasm.<sup>9,10</sup> *NPM1* mutations are associated with improved outcomes although the mechanism is not known. Concurrent mutations (such as an *FLT3* mutation) may influence prognosis, but generally *NPM1* patients without concurrent mutations have better prognoses.<sup>11</sup>

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.<sup>11</sup>

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.<sup>11</sup>

The *KIT* mutations also comprise about six percent of AML cases. *KIT* encodes the receptor for a stem cell factor, and prognoses are varied.<sup>11,12</sup> Some researchers suggest that of all *KIT* mutations, the D816 mutation is the most unfavorable prognostic factor in AML patients.<sup>13</sup>

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,<sup>11</sup> although some researchers strongly support the theory that *WT1* mutations are associated with poor AML prognoses.<sup>14</sup> The *WT1* mutation status of AML patients may also change during disease progression. New research has suggested that after allogeneic stem cell transplantation, AML relapse could be due to a gain in *WT1* gene alterations and a “high mutation load.”<sup>15</sup>

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*).<sup>11</sup>

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.<sup>11</sup>

*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 constitutively active growth stimulus whereas *TP53* encodes a transcriptional activator of growth inhibitory genes.<sup>16,17</sup>

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.<sup>11</sup>

### *Chronic Lymphoblastic Leukemia (CLL)*

In Western countries, CLL is the most common form of leukemia; in the United States, it accounts for 25 to 30 percent of all leukemias.<sup>18</sup> CLL and SLL (small lymphocytic leukemia) are mature B cell neoplasms characterized by a progressive accumulation of monoclonal B lymphocytes. It should be noted that “CLL is considered to be identical (ie, one disease with different manifestations) to the non-Hodgkin lymphoma SLL. The malignant cells seen in CLL and SLL have identical pathologic and immunophenotypic features. The term CLL is used when the disease manifests primarily in the blood, whereas the term SLL is used when involvement is primarily nodal.”<sup>18</sup> It is also worth mentioning that “In less than 10 percent of patients, CLL/SLL presents primarily in the lymph nodes as a non-Hodgkin lymphoma (ie, SLL)” and that “this presentation accounts for less than 5 percent of all non-Hodgkin lymphomas.”<sup>18</sup>

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.”<sup>19</sup>

### *Chronic myeloid leukemia (CML)*

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.<sup>20</sup> 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.<sup>21</sup> 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<sup>22</sup> that activates a number of downstream signaling molecules, including PI3K, AKT, JNK, RAS and STAT5.<sup>23</sup> This then disrupts cellular signal transduction pathways, leading to issues in the regulation of both apoptosis and cell proliferation,<sup>24</sup> ultimately leading to factor-independent and leukemogenic cell growth.<sup>25</sup>

Detection of the Ph chromosome is the hallmark of CML and is found in up to 95 percent of patients.<sup>26</sup> 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.<sup>21</sup> 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,”<sup>25</sup> as the 210-KDa fusion protein BCR-ABL is essential for initiation, maintenance, and progression of CML.<sup>23</sup> 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 *FLT3* point mutations in the activation loop of the kinase domain. Higher ratios of mutated alleles compared to wild-type alleles confer worse prognoses.<sup>11</sup>

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.<sup>25,27</sup> This reciprocal translocation also generates a shortened derivative chromosome 22, known as the Philadelphia (Ph) chromosome.<sup>21</sup> 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).<sup>26</sup> 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.<sup>28</sup> Predominately a childhood disease, approximately 60% of cases were diagnosed in patients younger than 20 years of age.<sup>29</sup>

### **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.<sup>30</sup> Other tools, such as flow cytometry and next generation sequencing (NGS) have also been used for AML prognostic and diagnostic purposes.

Amipasavate, 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 ( $r_2 > 0.99$ )”; further, “when compared with Western blotting results, FLT3 protein expression levels in leukemia patient's bone marrow samples were demonstrated in the same trend.”<sup>31</sup> 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.”<sup>31</sup>

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  $>2000\times$ , 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.<sup>32</sup>

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).<sup>25</sup> 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.<sup>33</sup> 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 (CML) patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2 as a class II device with special controls.<sup>34</sup> 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 follows 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.<sup>36-</sup>

<sup>38</sup>

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.<sup>39</sup>

Bolouri, et al. (2018) examined 993 children’s genetic data from the Children’s Oncology Group (COG) 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.”<sup>40</sup>

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.”<sup>41</sup>

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.<sup>42</sup>

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.”<sup>43</sup>

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 hydroxymethylation 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).<sup>44</sup>

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 (*NPM1mut*) and karyotype were the only independent predictors of survival, also noting that *NPM1mut* showed a prognostic impact on both normal (CN) and non-chromosomal (Mkneg) karyotypes. The authors concluded that “elderly patients with CN/Mkneg-*NPM1mut* 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.”<sup>45</sup>

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.<sup>46</sup>

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.<sup>48</sup> 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%).<sup>47</sup>

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.”<sup>49</sup> Patients with *WT1* mutations also had significantly worse event-free and overall survival rates ( $P=0.007$  and  $P<0.001$ , respectively).<sup>49</sup>

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 five percent. “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."<sup>50</sup>

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.<sup>51</sup>

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."<sup>27</sup>

## VI. 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.<sup>52</sup>

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.”<sup>53</sup> Their list of acute myeloid leukemias is presented below.<sup>53</sup>

Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with <i>PML::RARA</i> fusion
Acute myeloid leukaemia with <i>RUNX1::RUNX1T1</i> fusion
Acute myeloid leukaemia with <i>CBFB::MYH11</i> fusion
Acute myeloid leukaemia with <i>DEK::NUP214</i> fusion
Acute myeloid leukaemia with <i>RBM15::MRTFA</i> fusion
Acute myeloid leukaemia with <i>BCR::ABL1</i> fusion
Acute myeloid leukaemia with <i>KMT2A</i> rearrangement
Acute myeloid leukaemia with <i>MECOM</i> rearrangement
Acute myeloid leukaemia with <i>NUP98</i> rearrangement
Acute myeloid leukaemia with <i>NPM1</i> mutation
Acute myeloid leukaemia with <i>CEBPA</i> 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

Moreover, in defining acute myeloid leukemia, *myelodysplasia-related* (AML-MR), WHO offers the following abnormalities and mutations of interest:<sup>53</sup>

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

**Defining cytogenetic abnormalities**

- Complex karyotype (≥3 abnormalities)
- 5q deletion or loss of 5q due to unbalanced translocation
- Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation
- 11q deletion
- 12p deletion or loss of 12p due to unbalanced translocation
- Monosomy 13 or 13q deletion
- 17p deletion or loss of 17p due to unbalanced translocation
- Isochromosome 17q
- idic(X)(q13)

**Defining somatic mutations**

- ASXL1*
- BCOR*
- EZH2*
- SF3B1*
- SRSF2*
- STAG2*
- U2AF1*
- ZRSR2*

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.<sup>52</sup>

They state that: “With regard to chronic myeloid leukemia, BCR-ABL1<sup>+</sup>, most cases of CML in chronic phase can be diagnosed from peripheral blood (PB) 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.”<sup>52</sup>

They also introduced a provisional classification of ALL: B-ALL with translocations involving tyrosine kinases or cytokine receptors (“BCR-ABL1-like ALL”). They note that “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.”<sup>52</sup>

In the 5th Edition of the World Health Organization Classification of Hematolymphoid Tumors, the WHO updated their cytogenetic and molecular genetic information regarding AML. They highlight the following in their update:

- “Newly discovered recurrent cytogenetic abnormalities listed in *AML with other defined genetic abnormalities* include *RUNX1T3 (CBFA2T3)::GLIS*, *KAT6A::CREBBP*, *FUS::ERG*, *MNX1::ETV6*, and *NPM1::MLF1*. Those listed in “B-LBL/L with other defined genetic abnormalities” include *DUX4* rearrangement, *MEF2D* rearrangement, *ZNF384* rearrangement, *NUTM1* rearrangements, *MYC* rearrangement, *PAX5alt* or *PAX5 p.P80R*. With more data accumulated, neoplasms with these new genetic abnormalities will very likely become separated subtypes in the next edition, and there will be more newly discovered genetic alterations listed here. Biallelic *TP53* alterations have been frequently found in MDNs, acute erythroid leukemia, and myeloid neoplasms post cytotoxic therapy, and they usually predict a worse prognosis”
- “Gene mutations in the MAPK pathway are commonly seen in histiocytic neoplasms. *CXCR4* mutations are detected in a significant proportion of lymphoplasmacytic lymphoma cases, mostly concurrent with *MYD88* mutations, and the presence of them is associated with resistance to ibrutinib therapy (32). The mutational profiles of extranodal MZL (EMZL) and nodal MZL differ, and there are significant genetic differences among EMZLs arising in different anatomic sites”
- “*STAT3* mutation is commonly seen in CD8+ T-LGLL and gamma/delta T-LGLL, and it is associated with neutropenia and unfavorable overall survival; while *STAT5b* mutation is associated with a

poor prognosis only in CD8+ T-LGLL but has no prognostic impact in CD4+ T-LGLL and gamma/delta T-LGLL.”<sup>54</sup>

Though the WHO did not take any specific stance with these in their new version, these mutations and pathways may be worth keeping abreast of for the future.

### **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.

There, the societies recommend that “the pathologist should review recent or concurrent CBC counts and leukocyte differentials and evaluate a peripheral blood (PB) smear (*Strong recommendation*).” Moreover, they note that “in addition to performing morphologic assessment (blood and BM), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (ie, karyotype), appropriate molecular genetic and/or FISH testing, and FCI. The flow cytometry panel should be sufficient to distinguish AML (including APL), including early T-ALL, B-ALL, and AL of ambiguous lineage in all patients diagnosed with AL. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis (*Strong recommendation*).”<sup>55</sup>

In those patients with suspected or confirmed AL, “the pathologist may request and evaluate cytochemical studies to assist in the diagnosis and classification of AML (*Expert consensus opinion*).” Furthermore, “for patients with suspected or confirmed AL, the pathologist may use flow cytometry in the evaluation of CSF (*Recommendation*)” and that “the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of MRD (*Strong recommendation*).” It is also mentioned that “for patients with AL other than those with ALL who are receiving intrathecal therapy, the treating clinician may, under certain circumstances, obtain a CSF sample when there is no clinical contraindication. The treating clinician or pathologist should ensure that a cell count is performed and that examination and enumeration of blasts on a cytocentrifuge preparation are performed and are reviewed by the pathologist (*Expert consensus opinion*).”<sup>55</sup>

Many genes should be tested for patients with suspected or confirmed B-ALL. In pediatric patients, “the pathologist or treating clinician should ensure that testing for t(12;21)(p13.2;q22.1); *ETV6-RUNX1*, t(9;22)(q34.1;q11.2); *BCR-ABL1*, *KMT2A (MLL)* translocation, *iAMP21*, and trisomy 4 and 10 is performed (*Strong recommendation*),” while for adult patients, they assert “the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2); *BCR-ABL1* is performed. In addition, testing for *KMT2A (MLL)* translocations may be performed. (*Strong recommendation* for testing for t(9;22)(q34.1;q11.2) and *BCR-ABL1*; *Recommendation* for testing for *KMT2A (MLL)* translocations).”<sup>55</sup>

The CAP and the ASH also propound that “for patients with suspected or confirmed ALL, the pathologist or treating clinician may order appropriate mutational analysis for selected genes that influence diagnosis, prognosis, and/or therapeutic management, which include, but are not limited to, *PAX5*, *JAK1*,

*JAK2*, and/or *IKZF1* for B-ALL and *NOTCH1* and/or *FBXW7* for T-ALL. Testing for overexpression of *CRLF2* may also be performed for B-ALL (*Recommendation*).”

The appropriate molecular genetic testing for AML is discussed starting from 16<sup>th</sup> 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<sup>th</sup> 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); CBFβ-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); CBFβ-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 20<sup>th</sup> 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.<sup>56</sup>

### **American Society of Clinical Oncology (ASCO)**

The ASCO fully endorses the 2017 CAP and ASH guideline regarding the initial diagnostic work-up of acute leukemia.<sup>55</sup> 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 evaluation of AML in individuals 18 years of age or older, the NCCN guidelines recommend a history and a physical, complete blood count (CBC), platelets, differential, comprehensive metabolic panel (CMP), uric acid, lactate dehydrogenase (LDH), B12 and folic acid evaluation, prothrombin time (PT), partial thromboplastin time (PTT), and fibrinogen as part of the workup. Moreover, they recommend 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.

In terms of molecular analyses, they list “*ASXL1*, *BCOR*, *KIT*, *EZH2*, *FLT3* [ITD (internal tandem duplication) and TKD (tyrosine kinase domain)], *NPM1*, *CEBPA*, *IDH1*, *IDH2*, *RUNX1*, *TP53*, and other mutations” as targets. A “comprehensive pathology report, including diagnosis of AML (acute myeloid leukemia) within

the context of the diagnostic system used, blast count, cellularity, morphologic dysplasia, and mutation status if available” is also mentioned, and the NCCN adds to “consider additional molecular and genetic testing for heritable hematologic malignancy predisposition in a subset of patients, particularly in patients <50 years and those with a family history.” For patients with potential hematopoietic cell transplantation (HCT) in the future, HLA typing is also noted, “(except for patients with a major contraindication to HCT).”<sup>57</sup> The NCCN also highlights that “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.”<sup>57</sup>

The NCCN cautions that 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 r favorable-, intermediate-, or poor/adverse-risk groups, additional tests are needed to provide a full picture of the genetic factors that contribute to risk.”<sup>57</sup>

As a part of monitoring during therapy for individuals with AML, the NCCN recommends a bone marrow aspirate/biopsy 14 to 21 days after the “start of therapy to document hypoplasia. If hypoplasia is not documented or indeterminate, repeat biopsy within 7 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.”<sup>57</sup>

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: *TET2*, *ASXL1*, *ZRSR2*, *SRSF2*, *TP53*, *NRAS*, *IDH2*, and *ETV6*).”<sup>57</sup>

#### *Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Leukemia (SLL)*

For the diagnosis of chronic lymphocytic leukemia and small lymphocytic leukemia, the NCCN notes the following components as essential:

- “Hematopathology review of peripheral blood smear and all slides with at least one paraffin block representative of the tumor, if the diagnosis was made on a lymph node or bone marrow biopsy.
- Flow cytometry of blood is adequate for the diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL).
  - CLL diagnosis requires presence of monoclonal B lymphocytes  $\geq 5 \times 10^9/L$  in peripheral blood based on flow cytometry<sup>9</sup>

- Clonality of B cells should be confirmed by flow cytometry
- Adequate immunophenotyping to establish diagnosis by flow cytometry<sup>b</sup>: kappa/lambda, CD19, CD20, CD5, CD23, CD10, CD200; if flow cytometry is used to establish diagnosis, also include cytosin for cyclin D1 or fluorescence in situ hybridization (FISH) for t(11;14); t(11q;v) to exclude mantle cell lymphoma (MCL)
- SLL diagnosis requires presence of lymphadenopathy and/or splenomegaly with monoclonal B lymphocytes  $\leq 5 \times 10^9/L$  in peripheral blood
- SLL diagnosis should be confirmed by histopathology evaluation of lymph node biopsy
- Biopsy is generally not required. If diagnosis is not established by flow cytometry, then proceed with lymph node biopsy. Bone marrow aspirate with biopsy can be pursued if peripheral blood and lymph node biopsy material are nondiagnostic. Fine-needle aspiration (FNA) or core needle biopsy alone is not generally suitable for the initial diagnosis of CLL/SLL. In certain circumstances, when a lymph node is not easily accessible for excisional or incisional biopsy, a combination of core needle biopsy and FNA biopsy in conjunction with appropriate ancillary techniques for the differential diagnosis (ie, immunohistochemistry [IHC], flow cytometry) may be sufficient for diagnosis.
  - Adequate immunophenotyping to establish diagnosis by IHC<sup>b</sup>: CD3, CD5, CD10, CD20, CD23, cyclin D1, LEF1, SOX11<sup>c</sup>
- Absolute monoclonal B lymphocyte count<sup>a</sup>.<sup>58</sup>

In superscript a, the NCCN cautions that “absolute monoclonal B lymphocyte count  $< 5000/mm^3$  that persists more than 3 months in the absence of palpable adenopathy or other clinical features of lymphoproliferative disorder is MBL. Cells of the same phenotype may be seen in reactive lymph nodes; therefore, diagnosis of SLL should only be made when effacement of lymph node architecture is seen. Bone marrow examination is not helpful for the diagnosis of MBL.” Moreover, they note in superscript b that “Typical immunophenotype: CD5+, CD23+, CD43+/-, CD10-, CD19+, CD20 dim, slg dim+, and cyclin D1-. Note: Some cases may be slg bright+ or CD23- or dim; some MCL may be CD23+; cyclin D1 immunohistochemistry or FISH for t(11;14) should be considered in all cases, especially for those with an atypical immunophenotype (ie, CD23 dim or negative, CD20 bright, slg bright). CD200 positivity may distinguish CLL from mantle cell lymphoma (MCL), which is usually CD200.”<sup>58</sup>

Other analyses that may be “informative for prognostic and/or therapy determination” include

- “FISH to detect: +12; del(11q); del(13q); del(17p)
- *TP53* sequencing
- CpG-stimulated metaphase karyotype for complex karyotype (CK)
- Molecular analysis to detect: Immunoglobulin heavy chain variable region gene (IGHV) mutation status
- Beta-2-microglobulin.”<sup>58</sup>

During workup, history and physical exam including measurement of size of liver and spleen and palpable lymph nodes, performance status, B symptoms, complete blood count (CBC) with differential, comprehensive metabolic panel, and beta-2-microglobulin are essential according to the NCCN. They also note that the following tests may be “useful under certain circumstances”: quantitative

immunoglobulins; reticulocyte count, haptoglobin, and direct antiglobulin test (Coombs); chest/abdominal/pelvic CT with contrast of diagnostic quality, if clinically indicated; uric acid; lactate dehydrogenase (LDH); unilateral bone marrow aspirate and biopsy (may be informative for the diagnosis of immune-mediated or disease-related cytopenias); hepatitis B and C testing if treatment is contemplated; pregnancy testing in patients of childbearing age if systemic therapy or RT is planned; discussion of fertility preservation; and FDG-PET/CT scan to direct nodal biopsy, if histologic transformation is suspected.<sup>58</sup>

### Chronic Myeloid Leukemia

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

Monitoring Response to TKI Therapy and Mutational Analysis	
Test	Recommendation
<b>Hematologic</b>	CBC every 1–2 weeks for the first 1–2 months (or until stable normalization of blood counts) and thereafter as indicated based on the persistence of cytopenias
<b>Bone marrow cytogenetics<sup>1</sup></b>	At diagnosis Response milestones not reached Any signs of loss of hematologic response Any sign of loss of CCyR or its molecular response correlate (MR2.0: BCR::ABL1 [IS] ≤1%) - defined as an increase in <i>BCR::ABL1</i> transcript to >1%
<b>qPCR using IS [International Scale]</b>	At diagnosis Every 3 months after initiating treatment. After <i>BCR::ABL1</i> (IS) ≤1% <sup>2</sup> (MR2.0) <sup>2</sup> has been achieved, every 3 months for 2 years and every 3-6 months thereafter If there is 1-log increase in <i>BCR::ABL1</i> transcript levels with MMR, qPCR should be repeated in 1-3 months
<b>BCR::ABL kinase domain mutation analysis<sup>3</sup></b>	CP-CML Response milestones not reached <ul style="list-style-type: none"> <li>○ Any sign of loss of hematologic response</li> <li>○ Any sign of loss of CCyR or its molecular response correlate (MR2.0: BCR::ABL1 [IS] ≤1%) – defined as an increase in <i>BCR::ABL1</i> transcript to &gt;1%</li> <li>○ 1-log increase in <i>BCR::ABL1</i> transcript levels and loss of MMR</li> </ul> Disease progression to AP-CML or BP-CML
<p><sup>1</sup>FISH has been inadequately studied for monitoring response to treatment.</p> <p><sup>2</sup>CCyR correlates with <i>BCR-ABL1</i> (IS) ≤1% (MR2.0).</p> <p><sup>3</sup>Consider myeloid mutation panel to identify <i>BCR::ABL1</i>–independent resistance mutations in patients with no <i>BCR::ABL1</i> kinase domain mutations</p>	

The NCCN (2025) 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 with a minimum of 20 metaphases is useful to detect additional chromosomal abnormalities (ACAs) in Ph-positive cells, also known as clonal cytogenetic evolution. If bone marrow evaluation is not feasible, fluorescence in situ hybridization (FISH) on the bone marrow or a peripheral blood specimen with dual probes for BCR and ABL1 genes can be used 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.”
- “Qualitative RT-PCR or D-FISH should be considered for detecting atypical BCR::ABL1 transcripts if there is discordance between FISH and qPCR results.”<sup>59</sup>

The NCCN provides the following early treatment response milestones for those with CML:<sup>59</sup>

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

BCR::ABL1 (IS)	3 months	6 months	12 months <sup>9</sup>
>10% <sup>r</sup>	YELLOW	RED	
>1%–10% <sup>s</sup>	GREEN		ORANGE
>0.1%–1%	GREEN		LIGHT GREEN
≤0.1%	GREEN		

COLOR	CONCERN	CLINICAL CONSIDERATIONS <sup>u</sup>	RECOMMENDATIONS <sup>l,m,u</sup>
RED	TKI-resistant disease <sup>t</sup>	<ul style="list-style-type: none"> <li>• Evaluate patient adherence and <a href="#">drug interactions</a></li> <li>• Consider <a href="#">BCR::ABL1 kinase domain mutational analysis</a><sup>v</sup></li> <li>• Consider bone marrow cytogenetic analysis to assess additional chromosomal abnormalities (ACAs)</li> </ul>	Switch to alternate TKI (CML-5) (other than imatinib) and evaluate for allogeneic HCT
YELLOW	Possible TKI resistance <sup>t</sup>	<ul style="list-style-type: none"> <li>• Evaluate patient adherence and <a href="#">drug interactions</a></li> <li>• Consider <a href="#">BCR::ABL1 kinase domain mutational analysis</a><sup>v</sup></li> </ul>	Switch to alternate TKI (CML-5) or Continue same TKI <sup>r</sup>
ORANGE	Possible TKI resistance <sup>t</sup>	<ul style="list-style-type: none"> <li>• Evaluate patient adherence and <a href="#">drug interactions</a></li> <li>• Consider <a href="#">BCR::ABL1 kinase domain mutational analysis</a><sup>v</sup></li> <li>• Consider bone marrow cytogenetic analysis to assess for complete cytogenetic response (CCyR) at 12 mo</li> </ul>	Consider switch to alternate TKI <sup>s</sup> (CML-5) or Continue the same TKI if CCyR is achieved
LIGHT GREEN	TKI-sensitive disease	<ul style="list-style-type: none"> <li>• Evaluate patient adherence and <a href="#">drug interactions</a></li> <li>• If treatment goal is long-term survival: ≤1% optimal</li> <li>• If treatment goal is treatment-free remission: ≤0.1% optimal</li> </ul>	<ul style="list-style-type: none"> <li>• If optimal: continue same TKI</li> <li>• If not optimal: shared decision-making with patient<sup>t,w</sup></li> </ul>
GREEN	TKI-sensitive disease	<ul style="list-style-type: none"> <li>• Evaluate patient adherence and <a href="#">drug interactions</a></li> <li>• Monitor response (CML-G)</li> </ul>	Continue same TKI <sup>x</sup>

For those discontinuing TKI therapy, the NCCN recommends molecular monitoring every one to two months in the first six months following discontinuation, bimonthly monitoring during months seven to twelve, and quarterly monitoring thereafter (indefinitely) in individuals who remain in MMR (MR3; BCR::ABL1 ≤0.1% IS).<sup>59</sup>

### Acute Lymphoblastic Leukemia (ALL)

Regarding ALL, the NCCN 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” predicated “on hematopathology review of bone marrow aspirate and biopsy materials, which includes:

- Morphologic assessment of Wright-Giemsa-stained bone marrow aspirate smears, and hematoxylin and eosin (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 (MRD) analysis
- Karyotyping of G-banded metaphase chromosomes.”<sup>60</sup>

Regarding molecular characterization of ALL, it is stated that

- “Cytogenetic and molecular prognostic risk stratification for B-cell ALL (B-ALL)
- Optimal risk stratification and treatment planning require 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 (ie, p190 vs. p210)
  - Comprehensive testing by next-generation sequencing (NGS) for gene fusions and pathogenic mutations is recommended.
- Additional optional tests include:
  - Assessment with chromosomal microarray (CMA)/array comparative genomic hybridization (cGH) in cases of aneuploidy or inadequate karyotype.”<sup>60</sup>

According to the NCCN, “BCR::ABL1-like or Ph-like ALL is a subgroup of B-cell lineage ALL associated with unfavorable prognosis,” and it should be noted that “although this subgroup is Ph-negative, there is an otherwise similar genetic profile to the Ph-positive ALL subgroup including mutation of the *IKZF1* gene.” Nevertheless, “this subtype is typically associated with gene fusions and mutations that activate tyrosine kinase pathways as the common mechanism of transformation,” and 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.”<sup>60</sup>

As a part of surveillance for individuals with ALL, the NCCN highlights that “an assessment of bone marrow aspirate can be considered as clinically indicated at a frequency of 3 to 6 months for the first 5 years; if a bone marrow aspirate is performed, flow cytometry with additional studies that may include comprehensive cytogenetics, FISH, molecular tests, and MRD assessments should be carried out. While there is insufficient evidence to guide MRD monitoring for patients with Ph-negative disease following completion of maintenance therapy, the approval of blinatumomab, and potentially future therapies for MRD-positive relapse, may warrant testing in this regard.” The NCCN also states that “sequential MRD assessments should be considered for patients who have achieved a complete molecular remission (undetectable levels). The frequency may be increased if MRD levels are detectable or for those discontinuing TKI.”<sup>59,60</sup>

#### Pediatric Acute Lymphoblastic Leukemia (PEDALL)

The NCCN also produces guidelines focusing on Pediatric Acute Lymphoblastic Leukemia (PEDALL). 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
- Additional optional tests include:
  - Additional assessment (eg, microarray comparative genomic hybridization [CGH] and/or NGS) in cases of aneuploidy or inadequate karyotype
  - Whole transcriptome sequencing to identify B-lymphoblastic leukemia/lymphoma (B-ALL/LL) subtypes defined by gene expression profile (ie, *ETV6::RUNX1*-like, *PAX5alt*, *MYC*).<sup>61</sup>

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.” They note that “if bone marrow aspirate is done: Flow cytometry with additional studies that may include comprehensive cytogenetics, FISH, molecular testing, and MRD testing” and suggest that one can “consider periodic *BCR::ABL1* transcript-specific quantification (*BCR::ABL1*+ ALL).”<sup>61</sup>

The guidelines also state that “the *BCR-ALB1*-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.”<sup>61</sup>

### 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.

The 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  $\geq$  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  $\leq 40$  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.<sup>62</sup> It is also noted that “certain gene alleles (eg, *CHEK2* I200T and truncating *DDX41* variants) are overwhelmingly likely to be germline and should prompt consideration of germline testing when identified even once.”<sup>62</sup>

Furthermore, ELN lists tests and procedures recommended to be performed at diagnosis for a patient with AML in the Table 4, captured below.<sup>62</sup>

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

Tests and procedures	
<p><b>Tests to establish the diagnosis</b></p> <ul style="list-style-type: none"> <li>Complete blood count and differential count*</li> <li>Bone marrow aspirate†</li> <li>Bone marrow trephine biopsy‡</li> <li>Immunophenotyping by flow cytometry (see Table 5)</li> </ul>	<p><b>Additional tests and procedures</b></p> <ul style="list-style-type: none"> <li>Complete physical examination<sup>c</sup></li> <li>Performance status (ECOG/WHO score)</li> <li>Geriatric assessment<sup>d</sup> (optional)</li> <li>Biochemistry, coagulation tests<sup>e</sup></li> <li>Hepatitis A, B, C; HIV-1 testing; CMV, EBV, HSV, VZV</li> <li>Serum pregnancy test<sup>f</sup></li> <li>Eligibility assessment for allogeneic HCT (incl. HLA-typing)<sup>g</sup></li> <li>Chest x-ray, 12-lead electrocardiogram, echocardiography or MUGA (on indication)</li> <li>Computed tomography of the chest (on indication)<sup>h</sup></li> <li>Lumbar puncture (on indication)<sup>i</sup></li> <li>Information on oocyte and sperm cryopreservation<sup>j</sup></li> <li>Biobanking<sup>k</sup></li> </ul>
<p><b>Genetic analyses</b></p> <p>Cytogenetics§</p> <p>Screening for gene mutations required for establishing the diagnosis and to identify actionable therapeutic targets#</p> <ul style="list-style-type: none"> <li>• <i>FLT3</i>,# <i>IDH1</i>, <i>IDH2</i></li> <li>• <i>NPM1</i></li> <li>• <i>CEBPA</i>,# <i>DDX41</i>, <i>TP53</i>; <i>ASXL1</i>, <i>BCOR</i>, <i>EZH2</i>, <i>RUNX1</i>, <i>SF3B1</i>, <i>SRSF2</i>, <i>STAG2</i>, <i>U2AF1</i>, <i>ZRSR2</i></li> </ul> <p>Screening for gene rearrangements**</p> <ul style="list-style-type: none"> <li>• <i>PML::RARA</i>, <i>CBFB::MYH11</i>, <i>RUNX1::RUNX1T1</i>, <i>KMT2A</i> rearrangements, <i>BCR::ABL1</i>, other fusion genes (if available)</li> </ul>	<p>Results preferably available within</p> <ul style="list-style-type: none"> <li>• 5-7 d</li> <li>• 3-5 d</li> <li>• 3-5 d</li> <li>• 1st cycle</li> <li>• 3-5 d</li> </ul>
<p>Additional genes recommended to test at diagnosis††</p> <ul style="list-style-type: none"> <li>• <i>ANKRD26</i>, <i>BCORL1</i>, <i>BRAF</i>, <i>CBL</i>, <i>CSF3R</i>, <i>DNMT3A</i>, <i>ETV6</i>, <i>GATA2</i>, <i>JAK2</i>, <i>KIT</i>, <i>KRAS</i>, <i>NRAS</i>, <i>NF1</i>, <i>PHF6</i>, <i>PPM1D</i>, <i>PTPN11</i>, <i>RAD21</i>, <i>SETBP1</i>, <i>TET2</i>, <i>WT1</i></li> </ul>	
<p><b>Medical history</b></p> <ul style="list-style-type: none"> <li>Demographics and medical history‡‡</li> <li>Detailed family history<sup>a</sup></li> <li>Patient bleeding history<sup>b</sup></li> <li>Analysis of comorbidities</li> </ul>	

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, CBFβ::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.”<sup>62</sup> 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.”<sup>62</sup> 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 <sup>9</sup>	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	<ul style="list-style-type: none"> <li>t(8;21)(q22;q22.1)/RUNX1::RUNX1T1†,‡</li> <li>inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11†,‡</li> <li>Mutated NPM1†,§ without FLT3-ITD</li> <li>bZIP in-frame mutated CEBPA  </li> </ul>
Intermediate	<ul style="list-style-type: none"> <li>Mutated NPM1†,§ with FLT3-ITD</li> <li>Wild-type NPM1 with FLT3-ITD (without adverse-risk genetic lesions)</li> <li>t(9;11)(p21.3;q23.3)/MLL3::KMT2A†,¶</li> <li>Cytogenetic and/or molecular abnormalities not classified as favorable or adverse</li> </ul>
Adverse	<ul style="list-style-type: none"> <li>t(6;9)(p23.3;q34.1)/DEK::NUP214</li> <li>t(v;11q23.3)/KMT2A-rearranged#</li> <li>t(9;22)(q34.1;q11.2)/BCR::ABL1</li> <li>t(8;16)(p11.2;p13.3)/KAT6A::CREBBP</li> <li>inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM(EVI1)</li> <li>t(3q26.2;v)/MECOM(EVI1)-rearranged</li> <li>-5 or del(5q); -7; -17/abn(17p)</li> <li>Complex karyotype,** monosomal karyotype††</li> <li>Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2‡‡</li> <li>Mutated TP53<sup>9</sup></li> </ul>

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 include:

- “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).”<sup>63</sup>

In 2023, ELN published recommendations for the diagnosis and management of CML. In the guidelines they recommend:

- “Cytogenetics along with FISH and/or RT-PCR should be used in all cases to confirm a diagnosis of CML. The limitations of each approach as standalone tests need to be understood and, where appropriate, included in clinical reports.
- Cytogenetic testing should include a screen for ACAs at diagnosis.
- BCR::ABL1 mRNA transcript type should be determined for all cases prior to treatment to enable appropriate follow up.
- The possibility of a rare BCR::ABL1 variant should be excluded. If testing for rare variants is not available, the diagnostic report should clearly state that the presence of a BCR::ABL1 remains a possibility and that further testing in an appropriate reference laboratory should be performed.”<sup>64</sup>

The ELN also added the following tables with recommended tests for diagnostic workup of CML patients and for monitoring or investigating CML patients on treatment.<sup>64</sup>

	ROUTINE DIAGNOSTIC WORKUP	EXPERIMENTAL/CLINICAL TRIALS <sup>c</sup>
Interphase FISH	<ul style="list-style-type: none"> <li>• <b>Recommended for initial screening<sup>a</sup></b></li> </ul> <p>Pros: Picks up all <i>BCR::ABL1</i> rearrangements irrespective of breakpoint. Usually performed on PB. May be used as primary screen for <i>BCR::ABL1</i> or to investigate cases that show discrepant results between cytogenetics and RT-PCR.</p> <p>Cons: does not detect ACAs or identify transcript type therefore positive cases need to be followed up by both cytogenetics and RT-PCR.</p>	<ul style="list-style-type: none"> <li>• <b>Acceptable for initial screening</b></li> </ul>
Qualitative RT-PCR	<ul style="list-style-type: none"> <li>• <b>Recommended for initial screening<sup>a</sup></b></li> <li>• <b>Strongly recommended for determining <i>BCR::ABL1</i> transcript type in all confirmed CML patients</b></li> <li>• <b>Mandatory to detect atypical <i>BCR::ABL1</i> variants<sup>b</sup></b></li> </ul> <p>Pros: Only routine technique to determine exact <i>BCR::ABL1</i> transcript type<sup>b</sup>. Usually performed on PB.</p> <p>Cons: No commercial test available to detect most atypical <i>BCR::ABL1</i> variants, but essential to cover atypical variants if used as a primary screen. Sequence confirmation may be required. Cannot identify ACAs therefore positive cases need to be followed up by cytogenetics. Nested RT-PCR should not generally be used due to the risk of artefacts and contamination.</p>	<ul style="list-style-type: none"> <li>• <b>Mandatory</b></li> </ul>
Cytogenetics	<ul style="list-style-type: none"> <li>• <b>Mandatory for all CML cases</b></li> </ul> <p>Pros: Only routine technique that can detect prognostically significant ACAs. Can usually be performed on PB but may require BM. May be performed after initial screening by FISH or RT-PCR. Metaphase FISH may be useful to investigate variant translocations.</p> <p>Cons: Up to 5% of CML patients have a normal karyotype therefore not recommended in isolation as an initial screening tool. Positive cases need to be followed up by RT-PCR to determine <i>BCR::ABL1</i> transcript type.</p>	<ul style="list-style-type: none"> <li>• <b>Mandatory</b></li> </ul>
Quantitative RT-qPCR	<ul style="list-style-type: none"> <li>• <b>Not generally recommended</b></li> </ul> <p>Pros: may provide additional prognostic information by providing baseline to determine early response kinetics. May be used as an initial screen for CML cases identified by FISH or cytogenetics to identify (by exclusion) those who need investigation for atypical <i>BCR::ABL1</i> variants.</p> <p>Cons: approach to determine early response kinetics not standardized.</p>	<ul style="list-style-type: none"> <li>• <b>Strongly recommended</b></li> </ul> <p><i>GUSB</i> or <i>BCR</i> recommended as reference genes in preference to <i>ABL1</i> to determine early response kinetics within the first 1-3 months</p>
NGS panel for myeloid and lymphoid genes	<ul style="list-style-type: none"> <li>• <b>Not recommended for CP</b></li> <li>• <b>Suggested for <i>de novo</i> BP</b></li> </ul> <p>Pros: May provide additional prognostic information and identify targets for therapy</p> <p>Cons: Very limited clinical actionability, even in BC</p>	<ul style="list-style-type: none"> <li>• <b>Strongly recommended for CP and <i>de novo</i> BP</b></li> </ul> <p><i>ASXL1</i> mutations associated with adverse prognosis in some contexts but further studies required to define actionability</p>
<i>BCR::ABL1</i> TKD mutations	<ul style="list-style-type: none"> <li>• <b>Not recommended for either CP or <i>de novo</i> BP</b></li> </ul> <p>Mutations very unlikely to be detected prior to TKI therapy</p>	<ul style="list-style-type: none"> <li>• <b>Not generally recommended (but should be considered)</b></li> </ul>

<sup>a</sup>FISH and qualitative RT-PCR may be considered as alternative approaches for initial screening, i.e. initial identification of CML patients.

<sup>b</sup>RNAseq may be a better alternative to qualitative RT-PCR but is not widely available.

<sup>c</sup>Depending on trial design and objectives.

	ROUTINE INVESTIGATIONS ON THERAPY	EXPERIMENTAL/CLINICAL TRIALS
<b>Interphase FISH</b>	<ul style="list-style-type: none"> <li>• <b>Not recommended</b></li> </ul> May be useful for monitoring response if quality-controlled RT-qPCR not available, including patients with atypical <i>BCR::ABL1</i> fusions. Cannot be used to define MMR or DMR.	<ul style="list-style-type: none"> <li>• <b>Not recommended</b></li> </ul>
<b>Qualitative RT-PCR</b>	<ul style="list-style-type: none"> <li>• <b>Not recommended</b></li> </ul> Very limited value for monitoring response to treatment.	<ul style="list-style-type: none"> <li>• <b>Not recommended</b></li> </ul>
<b>Cytogenetics</b>	<ul style="list-style-type: none"> <li>• <b>Suggested at overt hematological relapse, failure according to ELN, or suspected/overt disease progression</b></li> <li>• <b>Considered in cases in remission but abnormal blood counts</b></li> </ul> Only technique that can detect prognostically-significant ACAs acquired during the course of disease, and chromosome abnormalities in Ph-negative cells.	<ul style="list-style-type: none"> <li>• <b>Should be considered</b></li> </ul>
<b>Quantitative RT-qPCR or RT-dPCR</b>	<ul style="list-style-type: none"> <li>• <b>Mandatory for routine molecular monitoring</b></li> </ul> Only technique(s) that can quantify disease levels over full response range specific by ELN and other clinical recommendations, including DMR	<ul style="list-style-type: none"> <li>• <b>Mandatory</b></li> </ul>
<b>NGS panel for myeloid and lymphoid genes</b>	<ul style="list-style-type: none"> <li>• <b>Suggested at overt hematological relapse and suspected or overt disease progression</b></li> </ul> May be helpful to confirm progression and occasionally identify potential therapeutic targets	<ul style="list-style-type: none"> <li>• <b>Recommended</b></li> </ul> Panel analysis during remission useful to determine if variants detected pre-treatment are somatic/germline/clonal hematopoiesis
<b><i>BCR::ABL1</i> TKD mutations</b>	<ul style="list-style-type: none"> <li>• <b>Strongly recommended in cases who fail to reach defined ELN milestones or loss of MMR on TKI therapy</b></li> </ul> Informs subsequent treatment in many cases	<ul style="list-style-type: none"> <li>• <b>Strongly recommended</b></li> </ul>

### International Workshop on Chronic Lymphocytic Leukemia (iwCLL)

In the iwCLL guidelines for the diagnosis, indications for treatment, response assessment, and supportive management of CLL, the iwCLL reference the World Health Organization in describing “CLL as leukemic, lymphocytic lymphoma, being only distinguishable from small lymphocytic lymphoma (SLL) by its leukemic manifestation” and adduce that “CLL, by definition, is always a disease of neoplastic B cells, whereas the entity formerly described as T-CLL is now called T-cell prolymphocytic leukemia.”<sup>65</sup> The iwCLL states that “The diagnosis of CLL requires the presence of  $\geq 5 \times 10^9/L$  B lymphocytes in the peripheral blood, sustained for at least 3 months. The clonality of these B lymphocytes needs to be confirmed by demonstrating immunoglobulin light chain restriction using flow cytometry.” They also assert that “CLL or SLL might be suspected in otherwise healthy adults who have an absolute increase in clonal B lymphocytes, but who have  $< 5 \times 10^9/L$  B lymphocytes in the blood. However, in the absence of lymphadenopathy or organomegaly (as detected by physical examination or imaging studies), or of disease-related cytopenias or symptoms, the presence of  $< 5 \times 10^9/L$  B lymphocytes is defined as monoclonal B lymphocytosis (MBL),” explaining that “The presence of a cytopenia caused by a typical marrow infiltrate establishes the diagnosis of CLL regardless of the number of peripheral blood B lymphocytes or of the lymph node involvement.” Meanwhile, “The definition of SLL requires the presence of lymphadenopathy and the absence of cytopenias caused by a clonal marrow infiltrate. Additionally, the number of B lymphocytes in the peripheral blood should be  $< 5 \times 10^9/L$ . In SLL, the diagnosis should be confirmed by histopathological evaluation of a lymph node biopsy or biopsy of other tissues.”<sup>65</sup>

They also mention that “it is important to verify that the patient has CLL and not some other lymphoproliferative disease that can masquerade as CLL, such as hairy cell leukemia or leukemic manifestations of mantle cell lymphoma, marginal zone lymphoma, splenic marginal zone lymphoma with circulating villous lymphocytes, or follicular lymphoma. To achieve this, it is necessary to evaluate the blood smear, the immunophenotype, and, in some cases, the genetic features of the circulating lymphoid cells.”<sup>65</sup>

### 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.”<sup>66</sup>

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).”<sup>67</sup>

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.”<sup>67</sup>

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 *IDH1/2* 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.”<sup>67</sup>

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.<sup>67</sup>

Table 1, produced by BSH and referenced earlier, is captured below:<sup>67</sup>

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)	√	√ <sup>a</sup>
(within 5 working days)		
Rapid <i>FLT3</i> ITD and TKD mutation screen (within 72 hours)	√	√
Rapid <i>NPM1</i> mutation screen (within 72 hours)	√	
Multi-target NGS panel including minimum of:	√	
<i>IDH1/2</i> screen	√ <sup>b,c</sup>	
<i>TP53</i> screen	√ <sup>b,c</sup>	
<i>ASXL1</i> screen	√	
<i>RUNX1</i> screen	√	

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

<sup>a</sup>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.

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

<sup>c</sup>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*.

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 panel an 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.”<sup>68</sup>

## 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.”<sup>69</sup>

### 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 ( $\geq 5$  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.”<sup>70</sup>

### Chronic Lymphocytic Leukemia

According to ESMO, “the diagnosis of CLL is established by the following criteria:

- Presence of  $\geq 5 \times 10^9/l$  monoclonal B lymphocytes in the peripheral blood. The clonality of the circulating B lymphocytes needs to be confirmed by demonstrating light chain restriction using flow cytometry.
- The leukaemia cells found in the blood smear are characteristically small, mature-appearing lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin. Larger, atypical lymphocytes or prolymphocytes may be seen but must not exceed 55%.”<sup>71</sup>

Also worth noting is that “CLL cells co-express the B-cell surface antigens CD19 and CD20 together with CD5, CD23, CD43 and CD200. The levels of surface CD20, surface immunoglobulin (Ig) and CD79b are characteristically low compared with those found on normal B cells” and that “each clone of leukaemia cells is restricted to expression of either kappa or lambda Ig light chains, or has no apparent expression of either of the two.” ESMO also states that “Other lymphoma entities to be differentiated from CLL are mantle cell lymphoma (MCL), leukaemic marginal zone lymphoma (MZL) (in particular the splenic variant) and lymphoplasmacytic lymphoma.” This differentiation can be made as “tumour cells may express B-cell surface antigens and CD5, but in most cases, they do not express CD23, in particular MZL. For cases that express CD23, reverse transcription polymerase chain reaction (RT-PCR) for determination of cyclin D1 overexpression, and FISH for detecting a translocation (11;14), but also CD200 expression, are useful for establishing the diagnosis of MCL.” Moreover, “SOX11 staining may be used on tumour

biopsies. A diagnosis of MZL is supported by negative or low CD43 expression and high expression of CD180.”<sup>71</sup>

Ultimately, the ESMO proposes two recommendations regarding the diagnosis of CLL:

- “Diagnosis is usually possible by immunophenotyping of peripheral blood only [III, A].
- LN biopsy and/or bone marrow biopsy may be helpful if immunophenotyping is not conclusive for the diagnosis of CLL [IV, A].”<sup>71</sup>

During diagnostic and staging work-up, the ESMO recommends also that

- “Binet and Rai staging systems with clinical symptoms are relevant for treatment indication [III, A].
- del(17p), TP53 mutations and IGHV status are relevant for choice of therapy and should be assessed before treatment [III, A].
- Routine evaluation of del(17p), TP53 mutation and IGHV status in early and asymptomatic stage is not recommended [V, D].
- Routine imaging during a watch-and-wait period is not recommended unless there are clinical symptoms [V, E].”<sup>71</sup>

The ESMO also recommends that “after the first year, when all patients should be seen at 3-monthly intervals, patients can be followed every 3-12 months depending on burden and dynamics of the disease” using history and physical examinations including a careful palpation of all LN areas, spleen and liver and complete blood cell count and differential count.<sup>71</sup>

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<sup>72</sup>

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.”<sup>72</sup> 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 three months.<sup>72</sup> 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.<sup>72</sup>

## 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)

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.

On April 28, 2017 the FDA approved the LeukoStrat<sup>®</sup> 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<sup>®</sup> CDx *FLT3* Mutation Assay is used as an aid in the selection of patients with AML for whom RYDAPT<sup>®</sup> (midostaurin) treatment is being considered. The LeukoStrat<sup>®</sup> 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.”<sup>73</sup>

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*<sup>®</sup> (enasidenib).”<sup>74</sup>

### VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81120	IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (e.g., glioma), common variants (e.g., R132H, R132C)
81121	IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (e.g., glioma), common variants (e.g., R140W, R172M)
81170	ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase) (e.g., acquired imatinib tyrosine kinase inhibitor resistance), gene analysis, variants in the kinase domain
81175	ASXL1 (additional sex combs like 1, transcriptional regulator) (e.g., myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; full gene sequence
81176	ASXL1 (additional sex combs like 1, transcriptional regulator) (e.g., myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; targeted sequence analysis (e.g., exon 12)
81195	Cytogenomic (genome-wide) analysis, hematologic malignancy, structural variants and copy number variants, optical genome mapping (OGM)
81206	BCR/ABL1 (t(9:22)) (e.g., chronic myelogenous leukemia) translocation analysis; major breakpoint, qualitative or quantitative
81207	BCR/ABL1 (t(9:22)) (e.g., chronic myelogenous leukemia) translocation analysis; minor breakpoint, qualitative or quantitative
81208	BCR/ABL1 (t(9:22)) (e.g., chronic myelogenous leukemia) translocation analysis; other breakpoint, qualitative or quantitative
81218	CEBPA (CCAAT/enhancer binding protein [C/EBP], alpha) (e.g., acute myeloid leukemia), gene analysis, full gene sequence
81245	FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia), gene analysis; internal tandem duplication (ITD) variants (i.e., exons 14, 15)
81246	FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia), gene analysis; tyrosine kinase domain (TKD) variants (e.g., D835, I836)
81261	IGH@ (Immunoglobulin heavy chain locus) (e.g., leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (e.g., polymerase chain reaction)
81262	IGH@ (Immunoglobulin heavy chain locus) (e.g., leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); direct probe methodology (e.g., Southern blot)

CPT	Code Description
81263	IGH@ (Immunoglobulin heavy chain locus) (e.g., leukemia and lymphoma, B-cell), variable region somatic mutation analysis
81272	KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (e.g., gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (e.g., exons 8, 11, 13, 17, 18)
81273	KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (e.g., mastocytosis), gene analysis, D816 variant(s)
81310	NPM1 (nucleophosmin) (e.g., acute myeloid leukemia) gene analysis, exon 12 variants
81334	RUNX1 (runt related transcription factor 1) (e.g., acute myeloid leukemia, familial platelet disorder with associated myeloid malignancy) gene analysis, targeted sequence analysis (e.g., exons 3-8)
81351	TP53 (tumor protein 53) (e.g., Li-Fraumeni syndrome) gene analysis; full gene sequence
81352	TP53 (tumor protein 53) (e.g., Li-Fraumeni syndrome) gene analysis; targeted sequence analysis (e.g., 4 oncology)
81401	Molecular pathology procedure, Level 2 (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81403	Molecular pathology procedure, Level 4 (e.g., analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons) Gene: <i>DNMT3A</i>
81405	Molecular pathology procedure, Level 6 (e.g., analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis) Genes: <i>WT1</i> , <i>TP53</i>
81450	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81451	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81455	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81479	Unlisted molecular pathology procedure
88237	Tissue culture for neoplastic disorders; bone marrow, blood cells
88271	Molecular cytogenetics; DNA probe, each (e.g., FISH)
88272	Molecular cytogenetics; chromosomal in situ hybridization, analyze 3-5 cells (e.g., for derivatives and markers)
88273	Molecular cytogenetics; chromosomal in situ hybridization, analyze 10-30 cells (e.g., for microdeletions)
88274	Molecular cytogenetics; interphase in situ hybridization, analyze 25-99 cells

CPT	Code Description
88275	Molecular cytogenetics; interphase in situ hybridization, analyze 100-300 cells
88291	Cytogenetics and molecular cytogenetics, interpretation and report
0016U	Oncology (hematolymphoid neoplasia), RNA, BCR/ABL1 major and minor breakpoint fusion transcripts, quantitative PCR amplification, blood or bone marrow, report of fusion not detected or detected with quantitation Proprietary test: QuantideX® Lab/Manufacturer: University of Iowa, Department of Pathology, Asuragen
0023U	Oncology (acute myelogenous leukemia), DNA, genotyping of internal tandem duplication, p.D835, p.I836, using mononuclear cells, reported as detection or non-detection of FLT3 mutation and indication for or against the use of midostaurin Proprietary test: LeukoStrat® CDx FLT3 Mutation Assay Lab/Manufacturer: LabPMM LLC/Invivoscribe Technologies, Inc.
0040U	BCR/ABL1 (t(9;22)) (e.g., chronic myelogenous leukemia) translocation analysis, major breakpoint, quantitative Proprietary test: MRDx® Lab/Manufacturer: MolecularMD
0046U	FLT3 (fms-related tyrosine kinase 3) (e.g., acute myeloid leukemia) internal tandem duplication (ITD) variants, quantitative Proprietary test: FLT3 ITD MRD by NGS Lab/Manufacturer: LabPMM LLC/Invivoscribe Technologies, Inc.
0049U	NPM1 (nucleophosmin) (e.g., acute myeloid leukemia) gene analysis, quantitative Proprietary test: NPM1 MRD by NGS Lab/Manufacturer: LabPMM LLC/Invivoscribe Technologies, Inc.
0413U	Oncology (hematolymphoid neoplasm), optical genome mapping for copy number alterations, aneuploidy, and balanced/complex structural rearrangements, DNA from blood or bone marrow, report of clinically significant alterations Proprietary test: DH Optical Genome Mapping/Digital Karyotyping Assay Lab/Manufacturer: Bionano Genomics

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## IX. Evidence-based Scientific References

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**X. Review/Revision History**

Effective Date	Summary
02/01/2026	<p>Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity and consistency:</p> <p>Note edited to change “2” to “two”: “Note: For two or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.”</p> <p>Off-cycle coding modification: Added CPT code 81195 (effective date 1/1/2025)</p> <p>Fixed number transposition in CPT table, 81352 was recorded in table as 83152</p>
01/01/2025	<p>Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria:</p> <p>Former CC3 was edited to include the use of BCR-ABL1 fusion transcript identification in the differential diagnosis between CML and ALL. Because it is now broader than a single hematopoietic neoplasm type, it was moved to be CC2. Now reads: “2) For the differential diagnosis of chronic myeloid leukemia (CML) or acute lymphoblastic leukemia (ALL) or for optimal risk stratification and treatment planning for individuals diagnosed with B-cell ALL (B-ALL), one time qualitative or quantitative RT-PCR testing on blood or bone marrow for identification of the BCR-ABL1 fusion gene transcript type, including determination of transcript size (e.g., p190 vs. p210), MEETS COVERAGE CRITERIA.”</p> <p>New CC7: “7) For optimal risk stratification and treatment planning for individuals diagnosed with chronic lymphocytic leukemia (CLL) or small lymphocytic leukemia (SLL), one time testing with any of the following tests (when not already performed during initial diagnosis) MEETS COVERAGE CRITERIA:</p> <ul style="list-style-type: none"> <li>a) FISH to detect +12; del(11q); del(13q); del(17p).</li> <li>b) TP53 mutation testing.</li> <li>c) CpG-stimulated metaphase karyotype for complex karyotype.</li> <li>d) Immunoglobulin heavy chain variable region gene (IGHV) mutation testing.”</li> </ul> <p>Note was updated to reflect changes to Avalon’s definition of a genetic panel within R2162. Now reads: “Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.”</p> <p>Added CPT code 88237, 81450, 81451, 81455, 81261, 81262, 81263, 81351, 81352, 88291</p>
12/01/2024	Initial Policy Implementation

