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<b>Preauthorization</b>	Yes	<b>Review Dates:</b> 11/16, 11/17	

***Preauthorization is required for testing for Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Myelodysplastic Syndrome and Myeloproliferative Neoplasms.***

*The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient's contract at the time the services are rendered.*

### **Description**

In the treatment of Philadelphia chromosome (Ph)–positive leukemias various nucleic acid-based laboratory methods may be used to detect the BCR-ABL1 fusion gene for confirmation of the diagnosis; for quantifying mRNA BCR-ABL1 transcripts during and after treatment to monitor disease progression or remission; and for identification of ABL kinase domain point mutations related to drug resistance when there is inadequate response or loss of response to tyrosine kinase inhibitors (TKIs), or disease progression.

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

Acute promyelocytic leukemia (APML, APL) is the M3 subtype of acute myelogenous leukemia (AML), a cancer of the white blood cells. APL is due to a translocation between chromosomes 15 and 17, t(15;17).

Mutations in the gene encoding Janus kinase 2 (JAK2) protein and in the myeloproliferative leukemia virus oncogene (MPL) encoding the thrombopoietin receptor have been associated with myeloproliferative neoplasms and with acute lymphoblastic leukemia (ALL) in Down syndrome patients.

Approximately 80% of individuals with Chronic Lymphocytic Leukemia exhibit chromosomal aberrations including in genes TP53 and ATM which have been proposed as instructive in management of the disease. A strong relationship has been observed between specific genetic mutations and the clinical course of the disease.

Hairy-cell leukemia (HCL), a rare leukemia, has been associated with the V600E mutation of the BRAF gene.

Classification and treatment of lymphoma may be guided by the identification of mutations which have been associated with specific lymphomas.

In multiple myeloma, clinical outcomes have been variable among patients in the same risk category who received similar therapy. Thus, more specific methods have been sought to more finely classify multiple myeloma, including microarray-based gene expression profile (GEP) analysis that shows the underlying activity of cellular biological pathways that control, for example, cell division or proliferation, apoptosis, metabolism, or other signaling pathways. Microarray-based GEP analysis has been proposed as a means to risk-stratify patients with multiple myeloma to guide treatment decisions.

Somatic (acquired) genetic variants in JAK2, MPL, and CALR genes have been implicated as the underlying molecular genetic drivers for the pathogenesis of myeloproliferative neoplasms (MPNs). This protocol addresses the use of genetic testing of JAK2 and CALR genes for the diagnosis, prognosis, and treatment selection in patients with MPNs.

## Policy

### *Acute Lymphocytic Leukemia*

*BCR/ABL1* testing for messenger RNA transcript levels prior to initiation of treatment and during therapy may be considered **medically necessary** for monitoring of Philadelphia chromosome-positive acute lymphocytic leukemia.

Genetic testing in Acute Lymphocytic Leukemia may be **medically necessary** for MLL translocations and IKZF1.

Evaluation of *ABL* kinase domain point mutations is considered **investigational** for monitoring in advance of signs of treatment failure or disease progression.

### *Acute Myeloid Leukemia*

The following may be considered **medically necessary** in Acute Myeloid Leukemia (AML) in patients with normal karyotype:

- Genetic testing for FLT3 internal tandem duplication (FLT3/ITD).
- NPM1 and CEBPA variant testing.
- Additional genetic testing of the following genes may be considered **medically necessary** if the leukemia has a normal karyotype and FLT3 and NPM1 gene sequences contain no pathogenic variants:
  - DNMT3A
  - IDH1/2

In patients with CBF AML, i.e., t(8;21), KIT gene testing is considered **medically necessary**. KIT variants are associated with decreased remission duration (e.g., EFS and RFS) and decreased overall survival in patients with t(8;21).

The following genetic testing in AML is considered **investigational**:

- Genetic testing for FLT3 internal tandem duplication (FLT3/ITD) and NPM1 mutations.
- Genetic testing for FLT3 tyrosine kinase domain (FLT3/TKD) mutations.
- Genetic testing for FLT3 or NPM1 mutations to detect minimal residual disease.

### *Acute Myelomonocytic Leukemia*

Genetic testing in Acute Myelomonocytic Leukemia for (AML/ETO t(8;21) translocation) may be considered **medically necessary**.

### *Acute Promyelocytic Leukemia*

Genetic testing for Acute Promyelocytic Leukemia (APML) may be considered **medically necessary**:

- if a diagnosis of APML is suspected but cannot be established by morphological, FISH, or cytogenetic analysis
- for PML/RARalpha, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (e.g., promyelocytic leukemia) translocation analysis.

### *Chronic Myelogenous Leukemia*

BCR/ABL1 qualitative testing may be considered **medically necessary** for diagnosis of chronic myeloid leukemia.

BCR/ABL1 testing for messenger RNA transcript levels may be considered **medically necessary** for monitoring of chronic myeloid leukemia treatment response and remission.

Evaluation of ABL kinase domain point mutations to evaluate patients for tyrosine kinase inhibitor resistance may be considered **medically necessary** if there is inadequate initial response to treatment or any sign of loss of response; and/or when there is progression of the disease to the accelerated or blast phase.

ABL kinase domain mutation testing may be considered **medically necessary** to identify T315I mutation or as a panel (which includes T315I) of the most common and clinically important mutations.

### *Chronic Lymphocytic Leukemia*

Chromosome deletion analysis may be considered **medically necessary** to determine prognosis by:

- Multi-gene FISH analysis to detect deletion of the LSI TP53, LSI ATM, and LSI D13S319 probe targets and gain of the D12Z3 sequence from untreated patients with B-cell CLL to dichotomize CLL (the 13q-, +12, or normal genotype group versus the 11q- or 17p-group).
- Immunoglobulin heavy chain variable region (IGHV or IgVH) gene mutation status.
- ZAP-70 analysis by flow cytometry.

### *Hairy Cell Leukemia*

Genetic testing for BRAF V600E may be considered **medically necessary** in order to confirm the diagnosis of HCL.

### *Lymphoma*

Cytogenetic testing may be considered **medically necessary** in the analysis of lymphoma and is the method of choice to detect the following abnormalities that guide classification and treatment:

- T-cell clonality analysis, IGVH analysis, or targeted FISH analysis
- Follicular lymphoma/t(14;18) IgH/BCL2
- Mantle cell lymphoma/t(11;14) IgH/CCND1
- Marginal zone lymphoma/t(11;18) API/MALT1
- Burkitt lymphoma/t(8;14) IgH/CMYC most commonly or t(2;8) or t(8;22)
- Anaplastic large cell lymphoma/t(2;5) NPM/ALK

### *Multiple Myeloma*

Microarray-based gene expression profile testing is considered **investigational** in Multiple Myeloma.

### *Myelodysplastic Syndrome*

Analysis of TET2 may be **medically necessary** to guide the use of hypomethylating agents.

Analysis of TP53 may be considered **medically necessary** to determine prognosis and need for bone marrow transplant.

Multi-gene panel testing in Myelodysplastic Syndrome is considered **investigational**.

*Myelofibrosis, Essential Thrombocythemia and Polycythemia Vera*

Genetic testing may be **medically necessary** in patients with suspected myelofibrosis, or essential thrombocythemia, when the diagnosis is unclear after bone marrow morphologic and cytogenetic analysis, to establish a diagnosis in the following situations:

- If BCR-ABL testing is negative it may define a myeloproliferative disease with a distinct leukemia subtype.
- Testing for the Janus Kinase 2 (JAK2; JAK2V617F) gene mutation for the initial diagnostic assessment of adults presenting with clinical, laboratory, or pathological findings suggesting classic forms of polycythemia vera (PV).
- Testing for the Janus Kinase 2 (JAK2; JAK2V617F) gene mutation for the initial diagnostic assessment of BCR-ABL negative adults presenting with clinical, laboratory, or pathological findings suggesting classic forms of essential thrombocythemia (ET) or primary myelofibrosis (PMF).
- MPL Gene Mutations in exon 10 and CALR testing in the diagnosis of patients presenting with clinical, laboratory, or pathologic findings suggesting essential thrombocythemia or primary myelofibrosis.

Testing for the Janus Kinase 2 (JAK2; JAK2V617F) gene mutation is **investigational** for:

- Diagnostic assessment of myeloproliferative disorders (MPD)/myeloproliferative neoplasms (MPN) in children.
- The Quantitative assessment of JAK2V617F allele burden subsequent to qualitative detection of JAK2V617F.

**Medicare Advantage**

In addition to or in place of the above policy statements, testing for the following genes may be considered **medically necessary** for Medicare Advantage members when the stated criteria is met:

- Testing for ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase) in patients with acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) to guide therapeutic decision making;
- Testing for BCR/ABL in the evaluation of individuals with chronic myelogenous leukemia or BCR-ABL positive acute lymphoblastic leukemia to evaluate treated individuals who manifest suboptimal response to initial tyrosine kinase inhibitor therapy or loss of response to tyrosine kinase inhibitor therapy;
- BRAF gene analysis for patients who have hairy cell leukemia when needed to determine if a medically necessary therapy is a reasonable option given the individual's specific clinical presentation;
- Testing for CEBA (CCAAT/enhancer binding protein [C/EBP], alpha) in patients with acute myelogenous leukemia (AML) to guide therapeutic decision making;
- Testing for CALR (calreticulin) in the initial diagnostic work-up of BCR-ABL negative, JAK2-negative adults with clinical, laboratory, or pathological findings suggesting polycythemia vera (PV), essential thrombocythemia (ET) or primary myelofibrosis (PMF);
- Testing for FLT3 in acute myeloid leukemia (AML) to guide therapeutic decision making;
- Testing for IGH@ (Immunoglobulin heavy chain locus) for acute myeloid leukemia (AML) and lymphoma, B-cell to guide therapeutic decision making;

- JAK2 V617F genotyping in the initial diagnostic work-up of BCR-ABL negative, JAK2-negative adults with clinical, laboratory, or pathological findings suggesting myeloproliferative neoplasm (MPN) (polycythemia vera (PV), essential thrombocythemia (ET) or primary myelofibrosis (PMF)) or a myelodysplastic syndrome (MDS). **Note:** Also JAK2 testing (exons 12 and 13) in individuals for whom PV is a strong consideration.
- Testing for KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) in patients who have acute myeloid leukemia (AML) to guide therapeutic decision making;
- Testing for NPM1 (nucleophosmin) in patients with acute myeloid leukemia (AML) to guide therapeutic decision making;
- Testing for PDGFRA (platelet-derived growth factor receptor, alpha polypeptide) in PDGFRA-associated chronic eosinophilic leukemia to guide therapeutic decision making;
- Testing for PML/RARALPHA, (t(15;17)) in patients with promyelocytic leukemia;
- Testing for TRB@ (T CELL antigen receptor, BETA) for individuals with acute lymphoid leukemia and T cell prolymphocytic leukemia to guide therapeutic decision-making;
- Testing for TRG@ (T CELL antigen receptor, GAMMA) for individuals with acute lymphoid leukemia and T cell prolymphocytic leukemia to guide therapeutic decision-making;
- Testing for IGH@BCL2 (t(14;18)) for patients who have non-Hodgkin's lymphoma to guide therapeutic decision-making;
- Testing for MPL (myeloproliferative leukemia virus oncogene, thrombopoietin receptor, TPOR) common variants (e.g., W515A, W515K, W515L, W515R) and/or exon 10 sequence in the initial work-up of BCR-ABL negative, JAK2 negative, and CALR negative adults with clinical, laboratory, or pathological findings
- Testing for JAK2 (Janus kinase 2) exon 12 sequence and exon 13 sequence in the initial work-up of BCR-ABL and JAK2 (V617F variant) negative adults with clinical, laboratory, or pathological findings suggesting polycythemia vera.
- Testing for TP53 (tumor protein 53) targeted sequence analysis of 2-5 exons and/or full gene sequence or targeted sequence analysis of > 5 exons in individuals who have Acute Myelogenous Leukemia or Myelodysplastic Disease to guide therapeutic decision-making.

For Medicare Advantage genomic sequencing procedures are considered **not medically necessary**.

A targeted genomic sequential analysis panel, hematolymphoid neoplasm, DNA analysis, 5-50 genes (see policy guidelines\*\*) will be considered **medically necessary** in the evaluation of blood or bone marrow samples in the following clinical circumstances:

- Newly diagnosed patients with acute myelogenous leukemia (AML) who are undergoing induction therapy, and who are suitable candidates for post-induction transplantation or consolidation therapy at the time of testing, and meet one of the following cytogenetic criteria:
  - normal karyotype
  - core binding factor
- Previously diagnosed patients with AML, who have not responded to induction chemotherapy, or who have progressed following induction. The patient must be a candidate for transplantation at the time of the testing.
- Patients with AML, who have responded to treatment, either chemotherapy or transplantation, with evidence of relapse.

### Medicare Advantage Policy Guidelines

\*\*Targeted genomic sequence analysis panel, hematolymphoid neoplasm, DNA analysis, 5-50 genes is a useful representation of the aggregate of these gene tests, and may be used as long as the panel contains, at a minimum, five or more gene tests for molecular biomarkers determined to meet Medicare coverage criteria. (See table 1 and table 2).

The following molecular genetic biomarkers are considered **medically necessary** for diagnosis and management of AML when ordered in reference to the preceding statement.

Table 1 Biomarkers that require a molecular diagnostics method (either via panel or individually):

Gene	Alteration	Clinical Utility	NCCN Biomarkers Category
<i>CEBPA</i>	Mutation	Favorable risk	2A
<i>FLT3</i>	Internal tandem duplication	Poor risk	2A
<i>KIT</i>	Mutation	Intermediate risk	2A
<i>NPM1</i>	Insertion mutation	Favorable risk	2A
<i>TP53</i>	Mutations, deletions	Poor risk	2A
<i>RUNX1</i>	Mutation	Distinct Diagnostic Category; Poor prognosis	*

\* WHO 2016 AML Classification

Table 2 Biomarkers that can be assessed by either a molecular diagnostics method (panel only) or by a cytogenetics method:

Gene	Alteration	Clinical Utility
<i>PML-RARA</i>	Rearrangement	All-trans retinoic acid
<i>BCR-ABL1</i>	Rearrangement	Poor risk
<i>CBFB-MYH11</i>	Rearrangement	Favorable risk
<i>DEK-NUP214</i>	Rearrangement	Poor risk
<i>MLLT3-KMT2A</i>	Rearrangement	Intermediate risk
<i>Other KMT2A</i>	Rearrangements	Poor risk
<i>GATA2, MECOM</i>	Rearrangement	Poor risk
<i>RUNX1-RUNX1T1</i>	Rearrangement	Favorable risk
Deletion 5, 5q	Copy number loss	Poor risk
Deletion 7, 7q	Copy number loss	Poor risk
Trisomy 8	Copy number gain	Intermediate risk

## Background

### *Acute Lymphocytic Leukemia*

ALL is characterized by the proliferation of immature lymphoid cells in the bone marrow, peripheral blood and other organs. ALL is the most common childhood tumor, and represents 75% to 80% of acute leukemias in children. ALL represents only 20% of all leukemias in the adult population. Median age at diagnosis is 14 years; 60% of patients are diagnosed at younger than 20 years of age. Current survival rates for patients with ALL have improved dramatically over the past several decades, primarily in children, largely due to better understanding of the molecular genetics of the disease, the incorporation of risk-adapted therapy, and new targeted agents. Current treatment regimens have a cure rate among children of about 80%. The long-term prognosis among adults is poor, with cure rates of 30% to 40%. Prognosis variation is explained, in part, by different subtypes among age groups, including the BCR-ABL fusion gene, which has a poor prognosis and is much less common in childhood ALL.

In ALL, the Ph chromosome is found in 3% of children and 25% to 30% of adults. Two clinically important variants are p190 and p210; p190 is generally associated with ALL, while p210 is most often seen in CML. The product of BCR-ABL1 is also a functional tyrosine kinase; the kinase domain of the BCR-ABL protein is the same as the kinase domain of the normal ABL protein. However, the abnormal BCR-ABL protein is resistant to normal regulation. Instead, the enzyme is constitutively activated and drives unchecked cellular signal transduction resulting in excess cellular proliferation. Determining the qualitative presence of the BCR-ABL1 fusion gene is not necessary to establish a diagnosis of ALL.

### Treatment and Response and Minimal Residual Disease

Before initiation of therapy for ALL, quantification of the BCR-ABL transcript is necessary to establish baseline levels for subsequent quantitative monitoring of response during treatment.

Quantitative determination of BCR-ABL1 transcript levels during treatment allows for a very sensitive determination of the degree of patient response to treatment. Evaluation of trial samples has consistently shown that the degree of molecular response correlates with risk of progression. In addition, the degree of molecular response at early time points predicts improved rates of progression-free and event-free survival. Conversely, rising BCR-ABL1 transcript levels predict treatment failure and the need to consider a change in management. With the established poor prognosis of Ph-positive ALL, standard ALL chemotherapy alone has long been recognized as a suboptimal therapeutic option, with 60% to 80% of patients achieving complete response (CR), significantly lower than that achieved in Ph-negative ALL.<sup>4</sup> The inclusion of TKIs to frontline induction chemotherapy has improved CR rates, exceeding 90%.<sup>4</sup>

Treatment response is evaluated initially by hematologic response (normalization of peripheral blood counts), then by cytogenetic response (percent of cells with Ph-positive metaphase chromosomes in a bone marrow aspirate). Complete cytogenetic response (CCyR; 0% Ph-positive metaphases) is expected by six to 12 months after initial treatment with the TKI imatinib.<sup>3</sup>

### Resistance

Imatinib treatment does not usually result in complete eradication of malignant cells. Not uncommonly, malignant clones resistant to imatinib may be acquired or selected during treatment (secondary resistance), resulting in disease relapse. In addition, a small fraction of chronic phase malignancies that express the fusion gene do not respond to treatment, indicating intrinsic or primary resistance. When the initial response to treatment is inadequate or there is a loss of response, resistance mutation analysis is recommended to support a diagnosis of resistance (based on hematologic or cytogenetic relapse) and to guide the choice of alternative doses or treatments.<sup>3,6</sup>

For patients with increasing levels of BCR-ABL1 transcripts, there is no strong evidence to recommend specific treatment; possibilities include continuation of therapy with dasatinib or nilotinib at the same dose, imatinib dose escalation from 400 mg to 800 mg daily, as tolerated or therapy change to an alternate second-generation TKI.<sup>3</sup>

### *Acute Myeloid Leukemia*

AML is a group of diverse hematologic malignancies characterized by the clonal expansion of myeloid blasts in the bone marrow, blood, and/or other tissues. It is the most common type of leukemia in adults, and is generally associated with a poor prognosis. It is estimated that, in 2014, 18,860 people would be diagnosed with AML and 10,460 would die of the disease. The median age at diagnosis is 66 years, with approximately one in three patients diagnosed at 75 years of age or older.<sup>28</sup>

### Diagnosis and Prognosis of AML

The most recent World Health Organization (WHO) classification (2016) reflects the increasing number of acute leukemias that can be categorized based on underlying cytogenetic abnormalities (i.e., at the level of the chromosome including chromosomal translocations or deletions) or molecular genetic abnormalities (i.e., at the level of the function of individual genes, including gene variants). These cytogenetic and molecular changes form distinct clinico-pathologic-genetic entities with diagnostic, prognostic, and therapeutic implications.<sup>31</sup> Conventional cytogenetic analysis (karyotyping) is considered to be a mandatory component in the diagnostic evaluation of a patient with suspected acute leukemia, because the cytogenetic profile of the tumor is considered to be the most powerful predictor of prognosis in AML and is used to guide the current risk-adapted treatment strategies.

Molecular variants have been analyzed to subdivide AML with normal cytogenetics into prognostic subsets. In AML, three of the most frequent molecular changes with prognostic impact are mutations of CEBPA encoding a transcription factor, mutations of the FLT3 gene, encoding a receptor of tyrosine kinase involved in hematopoiesis, and mutation of the NPM1 gene, encoding a shuttle protein within the nucleolus. "AML with mutated NPM1 or CEBPA" were included as provisional entities in the 2016 WHO classification of acute leukemias. AML with FLT3 mutations is not considered a distinct entity in the 2016 classification, although WHO recommends determining the presence of FLT3 mutations because of the prognostic significance.<sup>31</sup>

### Treatment

AML has a highly heterogeneous clinical course, and treatment generally depends on the different risk stratification categories.<sup>28</sup> Depending on the risk-stratification category, treatment modalities may include intensive remission induction chemotherapy, hypomethylating agents, clinical trials with innovative compounds, palliative cytotoxic treatment, or supportive care only. For patients who achieve complete remission (CR) after induction treatment, possible postremission treatment options include intensive consolidation therapy, maintenance therapy, or autologous or allogeneic hematopoietic cell transplant.<sup>28</sup>

### FLT3 Variants

FMS-like tyrosine kinase (FLT3) plays a critical role in normal hematopoiesis and cellular growth in hematopoietic stem and progenitor cells. Mutations in FLT3 are one of the most frequently encountered mutations in AML, and approximately 30% of AML patients harbor some form of FLT3 mutation.<sup>32</sup> FLT3 mutations are divided into two categories: (1) internal tandem duplications (FLT3-ITD) mutations, which occur in or near the juxtamembrane domain of the receptor, and (2) point mutations resulting in single amino acid substitutions within the activation loop of the tyrosine kinase domain (FLT3-TKD).

FLT3-ITD mutations are much more common than FLT3-TKD variants, occurring in 25% of newly diagnosed adult cases of AML, versus FLT3-TKD mutations, occurring in about 7% of patients. FLT3-ITD variants are a well-documented adverse prognostic marker, particularly in patients younger than 60 years of age and with normal-



or intermediate-risk cytogenetics, and is associated with an increased risk of relapse and inferior overall survival (OS).<sup>32-34</sup> Patients with FLT3-ITD mutations have a worse prognosis when treated with conventional chemotherapy, compared with patients with wild-type (WT; i.e., nonmutated) FLT3. Once FLT3-ITD AML relapses, the disease is rapidly fatal.

The prognostic impact of FLT3-TKD variants is less certain, and has only been studied in small numbers of patients.<sup>32, 35</sup> FLT3 tyrosine kinase inhibitors are under active clinical investigation.

#### NPM1 Mutations

The most common molecular aberration in AML is a mutation of NPM1, which is found in 46% to 64% of cytogenetically normal AML (CN-AML) and 9% to 18% of cytogenetically abnormal AML.<sup>28</sup> Up to 50% of AML with mutated NPM1 also carry an FLT3-ITD. Mutated NPM1 confers an independent favorable prognosis for patients with CN-AML and either the presence or absence of an FLT3-ITD. Retrospective studies of banked clinical samples suggest that an NPM1 mutation may mitigate the negative prognostic effect of an FLT3-ITD variant, but possibly only if the FLT3-ITD-to-WT allelic ratio is low.<sup>33</sup> The prognostic impact in patients with an abnormal karyotype is unclear.<sup>28</sup>

#### CEBPA Variants

CEBPA (CCAAT/enhancer binding protein) is a transcription-factor gene that plays a role in cell cycle regulation and cell differentiation. Variants to CEBPA are found in approximately 15% of AML patients with a normal karyotype.<sup>42-44</sup> CEBPA mutations can be either biallelic (double mutations) or monoallelic. Monoallelic mutations are prognostically similar to CEBPA WT and do not confer a favorable prognosis in cytogenetically normal AML; double variants of CEBPA have shown a better prognosis with higher rates of CR and OS after standard induction chemotherapy.<sup>45-46</sup>

#### Acute Myelomonocytic Leukemia

Acute myelomonocytic leukemia one of the more common types of acute myelogenous leukemia, characterized by both malignant monocytes and myeloblasts. A characteristic chromosomal abnormality observed in AML-M4 is inv (16). Treatment includes intensive multidrug chemotherapy and in selected cases allogeneic bone marrow transplantation. Nevertheless, outcome of AML remains poor with an overall survival of 35-60%.

#### Acute Promyelocytic Leukemia

APL is a form of AML. In APL, there is an abnormal accumulation of promyelocytes and a deficiency of mature red blood cells in the myeloid line of cells. This disease occurs in approximately one in 250,000 people in the United States and accounts for about 10% of AML diagnosis. APL usually occurs in middle-aged adults and responds favorably to treatments including retinoids, chemotherapy and, most recently, arsenicals.

A somatic mutation may occur as a translocation between chromosomes 15 and 17, t(15;17), fusing part of the PML gene with part of the RARA gene. The protein produced from this fused gene is known as PML-RAR $\alpha$  and produces a different effect, interfering with the normal function of both than the PML and the RAR $\alpha$  proteins. As a result, white blood cells (WBCs) fail to mature appropriately beyond the promyelocyte stage, growing and dividing too rapidly so that they accumulate excessively in the bone marrow and prevent normal WBCs from developing. The PML-RARA gene fusion accounts for up to 98 percent of cases of APL. Translocations involving the RARA gene and other genes have been identified in a few cases of APL.

#### *Chronic Myelogenous Leukemia*

CML is a clonal disorder of myeloid hematopoietic stem cells, accounting for 15% of adult leukemias. The disease occurs in chronic, accelerated, and blast phases, but is most often diagnosed in the chronic phase. If left untreated, chronic phase disease will progress within three to five years to the accelerated phase, characterized by any of several specific criteria such as 10% to 19% blasts in blood or bone marrow, basophils comprising 20% or

more of the white blood cell count, very high or very low platelet counts, etc.<sup>1</sup> From the accelerated phase, the disease progresses into the final phase of blast crisis, in which the disease behaves like an acute leukemia, with rapid progression and short survival. Blast crisis is diagnosed by the presence of either more than 20% myeloblasts or lymphoblasts in the blood or bone marrow, large clusters of blasts in the bone marrow on biopsy, or development of a solid focus of leukemia outside the bone marrow.

Although CML is diagnosed primarily by clinical and cytogenetic methods, qualitative molecular testing is needed to confirm the presence of the BCR-ABL1 fusion gene, particularly if the Ph chromosome was not found and to identify the type of fusion gene, as this information is necessary for subsequent quantitative testing of fusion gene messenger RNA transcripts. If the fusion gene is not confirmed, then the diagnosis of CML is called into question.

Extensive clinical data have led to the development of congruent recommendations and guidelines developed both in North America and in Europe concerning the use of various types of molecular tests relevant to the diagnosis and management of CML. These tests are also useful in the accelerated and blast phases of this malignancy.

Imatinib (Gleevec®) a TKI, was originally developed to specifically target and inactivate the Abl tyrosine kinase portion of the BCR-ABL1 fusion protein to treat patients with CML. In patients with chronic phase CML, early imatinib study data indicated a high response rate to imatinib compared with standard therapy, and long-term follow-up has shown that continuous treatment of chronic phase CML results in “durable responses in [a] large proportion of the patients with a decreasing rate of relapse.”<sup>3</sup> As a result, imatinib became the primary therapy for most patients with newly diagnosed chronic phase CML.

For CML, testing is appropriate at baseline before the start of imatinib treatment and testing is appropriate every three months when the patient is responding to treatment. After a complete cytogenetic response is achieved, testing is recommended every three months for two years and then every three to six months thereafter.

Without complete cytogenetic response, continued monitoring at three-month intervals is recommended. It has been assumed that the same time points for monitoring imatinib are appropriate for dasatinib and nilotinib as well and will likely also be applied to bosutinib and ponatinib.

### *Chronic Lymphocytic Leukemia*

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in the Western world, accounting for approximately 30% of all leukemia diagnosis in the United States, with an incidence of 4.7 cases per 100,000. It has a tremendously variable clinical course so that survival ranges from months to years. The median age at diagnosis of CLL is approximately 70 years, but it may present in younger individuals, often as poor-risk disease with significantly reduced life expectancy.

Chronic lymphocytic leukemia (CLL) is a neoplasm of hematopoietic origin characterized by the accumulation of lymphocytes with a mature, generally well-differentiated morphology. In CLL, these cells accumulate in blood, bone marrow, lymph nodes, and spleen.

CLL tends to present as asymptomatic enlargement of the lymph nodes, and is indolent in nature although it can undergo transformation to a more aggressive form of disease (e.g., Richter transformation). Low- and intermediate-risk CLL demonstrates relatively good prognoses with median survivals of six to 10 years; however, the median survival of high-risk CLL may only be two years. Although typically responsive to initial therapy, CLL is rarely cured by conventional therapy, and nearly all patients ultimately die of their disease.

### *Hairy Cell Leukemia*

Hairy Cell Leukemia (HCL) is classified as a Mature B-Cell Neoplasm, this rare disease is so named for the appearance of B-lymphocytes which have hair like projections when viewed under a microscope. Hairy cell

leukemia is a chronic disease with no generally accepted staging system. It accounts for approximately 2% of leukemia diagnosis annually in the United States, about 600-800 cases. Typically women are less likely to be affected than men, and middle aged or older adults are most at risk.

Remission is often possible and most likely longstanding. Although relapse is likely remission may be achievable more than once with survival frequently for 10 years or longer after diagnosis. The etiology of HCL is unknown, although it has been recognized that BRAF V600E mutation is present in most individuals with this disease. Vemurafenib is a drug which targets the effects of BRAF mutations and has been reported to promote a positive response in HCL, including for those with relapsed or refractory disease.

### *Lymphomas*

In general, non-hodgkin's lymphoma can be divided into two prognostic groups, indolent and aggressive. Indolent NHL has a relatively good prognosis, with a median survival of 10 years; however, it is not curable in advanced clinical stages.<sup>93</sup> Early stage indolent NHL (stage 1 or 2) may be effectively treated with radiation alone.<sup>93</sup> Although indolent NHL is responsive to radiation and chemotherapy, a continuous rate of relapse is seen in advanced stages.<sup>93</sup> These patients can often be retreated if their disease remains of the indolent type. Indolent NHL may transform into a more aggressive form, which is generally treated with regimens that are used for aggressive, recurrent NHL. Histologic transformation to higher grade lymphoma occurs in up to 70% of patients with low-grade lymphoma,<sup>94</sup> and median survival with conventional chemotherapy is one year or less.

Follicular Lymphoma (FL) is the most common indolent NHL (70%-80% of cases), and often the terms indolent lymphoma and FL are used synonymously. Also included in the indolent NHL are SLL/CLL, lymphoplasmacytic lymphoma, marginal zone lymphomas, and cutaneous T-cell lymphoma.

Aggressive NHL has a shorter natural history; however, 30% to 60% of these patients can be cured with intensive combination chemotherapy regimens.<sup>93</sup> Aggressive lymphomas include DLBCL, MCL, PTCL, anaplastic large cell lymphoma, and Burkitt lymphoma.

Mantle Cell Lymphoma (MCL) comprises approximately 65% to 68% of NHL and has been recognized within the past 15 years as a unique lymphoma subtype with a particularly aggressive course. MCL is characterized by a chromosomal translocation t(11;14), and the term mantle cell lymphoma was proposed in 1992 by Banks et al.<sup>95</sup> MCL shows a strong predilection for elderly men, and most cases (70%) present with disseminated (stage 4) disease and extranodal involvement is common. Localized MCL is quite rare. MCL has a median survival of approximately two to four years, and although most patients achieve remission with first-line therapy, relapse inevitably occurs, often within 12 to 18 months. MCL is rarely, if ever, cured with conventional therapy, and no standardized therapeutic approach to MCL is used.

Most peripheral t-cell lymphomas (PTCLs) are aggressive and fall into the category of PTCL, unspecified (PTCL-u) or PTCL-NOS, angioimmunoblastic or anaplastic large cell which, combined make up approximately 60% to 70% of T cell lymphomas. PTCLs are less responsive to standard chemotherapy than diffuse large B-cell lymphomas and carry a worse prognosis than aggressive B cell counterparts. Survival rates at five years with standard chemotherapy regimens range from 20% to 35%.

### *Multiple Myeloma*

Multiple myeloma is a malignant plasma-cell dyscrasia characterized by clonal proliferation of plasma cells derived from B cells in the bone marrow.<sup>102</sup> It accounts for about one in every 100 cancers and 13% of hematologic cancers. The annual age-adjusted incidence is about six cases per 100,000 persons, with median age at diagnosis of about 70 years. Before the advent of current treatment protocols, most patients with multiple myeloma succumbed to their disease within five to 10 years; in the prechemotherapy era, median survival was less than one year. Among patients who present at an age younger than 60 years, 10-year overall survival with current treatment protocols now may exceed 30%.<sup>103</sup>

Criteria for the diagnosis, staging, and response assessment of multiple myeloma have been reported by the International Myeloma Working Group and are in widespread use.<sup>97, 106, 107</sup> The decision to treat is based on criteria set forth in the diagnosis of multiple myeloma, which includes calcium elevation; renal insufficiency; anemia; and bone disease (i.e., CRAB). Patients with monoclonal gammopathy of undetermined significance (MGUS) or smoldering myeloma do not require therapy, irrespective of any associated risk factors, except on specifically targeted protocols.

Asymptomatic (smoldering) multiple myeloma and monoclonal gammopathy of undetermined significance (MGUS) currently require only ongoing clinical observation, because early treatment with conventional chemotherapy has shown no benefit. However, for symptomatic patients diagnosed with multiple myeloma, prompt induction therapy is indicated. Despite achievement of complete remission and apparent eradication of disease, the clinical response is transitory in all cases, and multiple myeloma is considered incurable with current approaches.

#### *Myelodysplastic Syndromes/Myeloproliferative Neoplasms*

Myelodysplastic syndromes (MDS) can occur as a primary (idiopathic) disease or can be secondary to cytotoxic therapy, ionizing radiation, or other environmental insult. Chromosomal abnormalities are seen in 40% to 60% of patients, frequently involving deletions of chromosome 5 or 7, or an extra chromosome as in trisomy 8. Most MDS diagnoses occur in individuals older than age 55 to 60 years, with an age adjusted incidence of approximately 62% among individuals older than age 70 years. Patients either succumb to disease progression to acute myeloid leukemia (AML) or to complications of pancytopenias. Patients with higher blast counts or complex cytogenetic abnormalities have a greater likelihood of progressing to AML than do other patients.

Treatment of nonprogressing MDS has involved best supportive care, including red blood cell (RBC) and platelet transfusions and antibiotics. Active therapy was given only when MDS progressed to AML or resembled AML with severe cytopenias. Given the spectrum of treatments available, the goal of therapy must be decided upfront whether it is to improve anemia; thrombocytopenia; or neutropenia, eliminate the need for RBC transfusion, achieve complete remission, or cure the disease.

Allo-HSCT is the only approach with curative potential, but its use is governed by patient age, performance status, medical comorbidities, the patient's risk preference, and severity of MDS at presentation.

#### *Chronic Myeloproliferative Neoplasms*

Chronic myeloproliferative neoplasms (MPN) are clonal bone marrow stem cell disorders; as a group, approximately 8400 MPN are diagnosed annually in the United States. Like MDS, MPN primarily occur in older individuals, with approximately 67% reported in patients aged 60 years and older.

MPNs are characterized by the slow but relentless expansion of a clone of cells with the potential evolution into a blast crisis similar to AML. MPN share a common stem cell-derived clonal heritage, with phenotypic diversity attributed to abnormal variations in signal transduction as the result of a spectrum of mutations that affect protein tyrosine kinases or related molecules. The unifying characteristic common to all MPN is effective clonal myeloproliferation resulting in peripheral granulocytosis, thrombocytosis, or erythrocytosis that is devoid of dyserythropoiesis, granulocytic dysplasia, or monocytosis.

In indolent, nonprogressing cases, therapeutic approaches are based on relief of symptoms. Supportive therapy may include prevention of thromboembolic events. Hydroxyurea may be used in cases of high-risk essential thrombocytosis and polycythemia vera, and intermediate- and high-risk primary myelofibrosis.

In November 2011, FDA approved the orally administered selective Janus kinase 1 and 2 inhibitor ruxolitinib for the treatment of intermediate- or high-risk myelofibrosis.

Myeloablative allo-HSCT has been considered the only potentially curative therapy, but because most patients are of advanced age with attendant comorbidities, its use is limited to those who can tolerate the often severe

treatment-related adverse effects of this procedure. However, use of reduced-intensity conditioning for allo-HSCT has extended the potential benefits of this procedure to selected individuals with these disorders.

### *Myeloproliferative Neoplasms*

MPNs are uncommon overlapping blood diseases characterized by the production of one or more blood cell lines. The most common forms of MPNs include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML). A common finding in many MPNs is clonality and a central pathogenic feature is the detection of a somatic (acquired) pathogenic variant in disease-associated genes. Pathogenic variants in disease-associated genes result in constitutively activated tyrosine kinase enzyme or cell surface receptor.

The paradigm for use of molecular genetics to revolutionize patient management is CML. A unique chromosomal translocation t(9;22), the Philadelphia chromosome, leads to a unique gene rearrangement (*BCR-ABL*) creating a fusion gene that encodes for a constitutively active Bcr-abl fusion protein. These findings led to the development of targeted tyrosine kinase inhibitor drug therapy (imatinib) that produces long-lasting remissions.

Diagnosis and monitoring of patients with Ph-negative MPNs have been challenging because many of the laboratory and clinical features of the classic forms of these diseases—PV, ET, and PMF—can be mimicked by other conditions such as reactive or secondary erythrocytosis, thrombocytosis, or myeloid fibrosis. Additionally, these entities can be difficult to distinguish on morphologic bone marrow exam, and diagnosis can be complicated by changing disease patterns: PV and ET can evolve into PMF or undergo leukemic transformation. World Health Organization criteria were published as a benchmark for diagnosis in 2001<sup>1</sup> and updated in 2008 and 2016.<sup>28</sup> Applying these criteria have been challenging because they involve complex diagnostic algorithms, rely on morphologic assessment of uncertain consistency, and require tests that are not well-standardized or widely available, such as endogenous erythroid colony formation.

### Molecular Genetics of Ph-Negative MPNs

#### JAK2 GENE

The JAK2 gene, located on chromosome 9, contains the genetic code for making the Janus kinase 2 protein, a nonreceptor tyrosine kinase. The Janus kinase 2 (JAK2) protein is part of the JAK/STAT signal transduction pathway that is important for the controlled production of blood cells from hematopoietic stem cells. Somatic (acquired) variants in the JAK2 gene are found in patients with PV ( $\approx 96\%$ ), ET (50%), and PMF (50%).<sup>35</sup>

#### CALR GENE

The CALR gene, located on chromosome 19, contains the genetic code for making the calreticulin protein, a multifunctional protein located in the endoplasmic reticulum, cytoplasm, and cell surface. The calreticulin protein is thought to play a role in cell growth and division and regulation of gene activity. Somatic variants in the CALR gene are associated with ET and PMF.<sup>36</sup>

#### MPL GENE

The MPL gene, located on chromosome 1, contains the genetic code for making the thrombopoietin receptor, a cell surface protein that stimulates the JAK/STAT signal transduction pathway. The thrombopoietin receptor is critical for the cell growth and division of megakaryocytes, which produce platelets involved in blood clotting. Somatic variants in the MPL gene are associated with ET and PMF.<sup>37</sup>

### **Regulatory Status**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory

Improvement Amendments (CLIA). The BCR/ABL1 qualitative and quantitative genotyping tests, and ABL KD mutation tests, are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for *JAK2* testing and *MPL* mutation testing.

Clinically validated FLT3 mutation testing is performed with a polymerase chain reaction–based assay of genomic DNA isolated from the leukemic cells, either from blood or bone marrow. Testing for FLT3 may involve a duplex assay, which tests for both types of FLT3 mutations (ITD and TKD), however, some laboratories only test for ITD mutations, as the prognostic effect of TKD mutations is uncertain.<sup>6</sup> Several Laboratories offer these tests.

### Related Protocols

Hematopoietic Cell Transplantation for Acute Lymphoblastic Leukemia

Hematopoietic Cell Transplantation for Chronic Myeloid Leukemia

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Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. **Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.**

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