BCR-ABL1 Testing in Chronic Myelogenous Leukemia and Acute Lymphoblastic Leukemia

Policy

Blue Cross and Blue Shield of Kansas City (Blue KC) will provide coverage for BCR-ABL1 Testing when it is determined to be medically necessary because the criteria shown below are met.

Note: Genetic testing may be excluded in some contracts. Verify benefits prior to review of Medical Necessity.

When Policy Topic is covered

Chronic Myelogenous Leukemia

BCR/ABL1 qualitative testing for the presence of the fusion gene is considered medically necessary for diagnosis of chronic myeloid leukemia (see Considerations).

BCR/ABL1 testing for messenger RNA transcript levels by quantitative real-time reverse transcription-polymerase chain reaction at baseline prior to initiation of treatment and at appropriate intervals during therapy (see Considerations) is 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 is considered medically necessary when there is inadequate initial response to treatment or any sign of loss of response (see Considerations); and/or when there is progression of the disease to the accelerated or blast phase.

Acute Lymphoblastic Leukemia

BCR/ABL1 testing for messenger RNA transcript levels by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) at baseline prior to
initiation of treatment and at appropriate intervals during therapy (see Considerations) is considered medically necessary for monitoring of Philadelphia chromosome-positive acute lymphoblastic leukemia treatment response and remission.

Evaluation of ABL kinase domain point mutations to evaluate patients for tyrosine kinase inhibitor resistance is considered medically necessary when there is inadequate initial response to treatment or any sign of loss of response.

**When Policy Topic is not covered**

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

**Considerations**

**Diagnosis of CML and ALL**

Qualitative molecular confirmation of the cytogenetic diagnosis (ie, detection of the Philadelphia chromosome) is necessary information for the accurate diagnosis of CML. Identification of the Philadelphia chromosome is not necessary for the diagnosis of ALL, however, molecular phenotyping is generally performed at the time of initial assessment (See Determining baseline RNA transcript levels and subsequent monitoring subsection).

Distinction between molecular variants (ie, p190 vs. p210) is necessary information for accurate results in subsequent monitoring assays.

**Determining baseline RNA transcript levels and subsequent monitoring**

Determination of BCR-ABL1 messenger RNA transcript levels should be done by quantitative real-time RT-PCR-based assays, and reported results should be standardized according to the International Scale.

For CML, testing is appropriate at baseline before the start of imatinib treatment and testing is appropriate every 3 months when the patient is responding to treatment. After a complete cytogenetic response is achieved, testing is recommended every 3 months for 3 years, then every 3 to 6 months thereafter.

Without attainment of a complete cytogenetic response, continued monitoring at 3-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 (see Rationale section for more information).

For ALL, the optimal timing remains unclear and depends upon the chemotherapy regimen used.
**TKI resistance**

For CML, inadequate initial response to TKIs is defined as failure to achieve complete hematologic response at 3 months, only minor cytologic response at 6 months or major (rather than complete) cytogenetic response at 12 months.

Unlike in CML, resistance in ALL to TKIs is less well studied. In patients with ALL who are receiving a TKI, a rise in the BCR-ABL level while in hematologic CR or clinical relapse warrants mutational analysis.

Loss of response to tyrosine kinase inhibitors is defined as hematologic relapse, cytogenetic relapse or 1 log increase in *BCR-ABL1* transcript ratio and therefore loss of major molecular response.

Kinase domain mutation testing is usually offered either as a single test to identify T315I mutation or as a panel (which includes T315I) of the most common and clinically important mutations.

### Description of Procedure or Service

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
</tr>
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</table>
| Individuals:  
- With suspected chronic myelogenous leukemia for diagnosis  
  
Interventions of interest are:  
  - *BCR/ABL1* fusion gene qualitative testing to confirm diagnosis and establish baseline for monitoring treatment | Comparators of interest are:  
  - Diagnosis only by clinical and cytogenetic methods | Relevant outcomes include:  
  - Test accuracy  
  - Test validity | |
| Individuals:  
- With a diagnosis of chronic myelogenous leukemia  
  
Interventions of interest are:  
  - *BCR/ABL1* quantitative testing at appropriate intervals during therapy for monitoring treatment response and remission | Comparators of interest are:  
  - Cytogenetics | Relevant outcomes include:  
  - Disease-specific survival  
  - Test accuracy  
  - Test validity  
  - Change in disease status | |
| Individuals:  
- With a diagnosis of chronic myelogenous leukemia, inadequate initial response, loss of response, and/or disease progression  
  
Interventions of interest are:  
  - Evaluation for ABL kinase domain point mutations to assess for tyrosine kinase inhibitor resistance | Comparators of interest are:  
  - Standard workup without genetic testing | Relevant outcomes include:  
  - Test accuracy  
  - Test validity  
  - Medication use | |
| Individuals:  
- With a diagnosis of Philadelphia chromosome‒positive acute lymphoblastic leukemia  
  
Interventions of interest are:  
  - *BCR/ABL1* quantitative testing at baseline prior to and during treatment to monitor treatment response and remission | Comparators of interest are:  
  - Cytogenetics | Relevant outcomes include:  
  - Disease-specific survival  
  - Test accuracy  
  - Test validity  
  - Change in disease status | |
| Individuals:  
- With Philadelphia  
  
Interventions of interest are: | Comparators of interest are: | Relevant outcomes include: |
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.

The evidence for BCR/ABL1 fusion gene qualitative testing to confirm diagnosis and establish baseline for monitoring treatment in individuals who have suspected chronic myelogenous leukemia (CML) includes validation studies. Relevant outcomes are test accuracy and test validity. The sensitivity of testing with reverse transcription-polymerase chain reaction is high compared with conventional cytogenetics. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

The evidence for quantitative BCR/ABL1 quantitative testing at appropriate intervals during therapy for monitoring treatment response and remission in individuals who have a diagnosis of CML or at baseline prior to and during treatment to monitor treatment response and remission in individuals who have acute lymphoblastic leukemia (ALL) includes a randomized trial and case series. Relevant outcomes are disease-specific survival, test accuracy and validity, and change in disease status. Studies have shown a high sensitivity of this type of testing and a strong correlation with outcomes, including risk of disease progression and survival, and may stratify patients to different treatment options. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

The evidence for evaluation of ABL kinase domain (KD) point mutations to assess for TKI resistance in individuals who have a diagnosis of CML or who have Ph chromosome-positive ALL and signs of treatment failure or disease progression includes a systematic review of pharmacogenetic testing for TKIs and case series reporting the presence of KD mutations detected at imatinib failure. Relevant outcomes are test accuracy and validity and medication use. Studies have shown a correlation between certain types of mutations, treatment response, and the selection of subsequent treatment options. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.
Background

Disease

Chronic Myelogenous Leukemia
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 3 to 5 years to the accelerated phase, characterized by any of several specific criteria such as 10-19% 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. (1) 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.

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

Acute Lymphoblastic Leukemia
Acute lymphoblastic 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. The 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 advances in the 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 ~80%. The long-term prognosis among adults is poor, with cure rates of 30% to 40%, 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, as compared with adult ALL.

Disease Genetics.
Ph-positive leukemias are characterized by the expression of the oncogenic fusion protein product BCR-ABL1, resulting from reciprocal translocation between chromosomes 9 and 22. This abnormal fusion gene characterizes CML. In ALL, with increasing age, the frequency of genetic alterations associated with favorable outcomes declines and alterations associated with poor outcomes, such as BCR-ABL1, are more common.(2) In ALL, the Ph is found in 3% of children and 25% to 30% of adults. Depending on the exact location of the fusion, the molecular weight
of the protein can range from 185 to 210 kDa. Two clinically important variants are p190 and p210; p190 is generally associated with acute lymphoblastic leukemia, while p210 is most often seen in CML. The product of \textit{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.

**Diagnosis**
Although CML is diagnosed primarily by clinical and cytogenetic methods, qualitative molecular testing is needed to confirm the presence of the \textit{BCR-ABL1} fusion gene, particularly if the Ph chromosome was not found, and to identify the type of fusion gene, because 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.

Determining the qualitative presence of the \textit{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 CML or ALL, quantification of the \textit{BCR-ABL} transcript is necessary to establish baseline levels for subsequent quantitative monitoring of response during treatment.

Quantitative determination of \textit{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 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 \textit{BCR-ABL1} transcript levels predicts treatment failure and the need to consider a change in management. Quantitative polymerase chain reaction (PCR)–based methods and international standards for reporting have been recommended and adopted for treatment monitoring.

Imatinib (Gleevec), a tyrosine kinase inhibitor (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."(3) As a result, imatinib became the primary therapy for most patients with newly diagnosed chronic phase CML.

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 a complete response, significantly lower
than that achieved in Ph-negative ALL. The inclusion of TKIs to frontline induction chemotherapy has improved complete response rates, exceeding 90%.

Treatment response is evaluated initially by hematologic response (normalization of peripheral blood counts), then by cytogenetic response (percentage of cells with Ph-positive metaphase chromosomes in a bone marrow aspirate). Complete cytogenetic response (CCyR; 0% Ph-positive metaphases) is expected by 6 to 12 months after initial treatment with the TKI imatinib. It is well established that most “good responders” who are considered to be in morphologic remission but relapse may still have considerable levels of leukemia cells, referred to as minimal residual disease (MRD.) Among children with ALL who achieve a complete response by morphologic evaluation after induction therapy, 25% to 50% may still have detectable MRD based on sensitive assays. Current methods used for MRD detection include flow cytometry (sensitivity of MRD detection, 0.01%), or PCR-based analyses (Ig and T-cell receptor gene rearrangements or analysis of BCR-ABL transcripts), which are the most sensitive methods of monitoring treatment response (sensitivity, 0.001%). Most ALL patients can be tested with Ig and T-cell receptor gene arrangement analysis, whereas only Ph-positive patients can be tested with PCR analysis of BCR-ABL transcripts is.

**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. The molecular basis for resistance is explained in the following section. 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.

Structural studies of the ABL-imatinib complex have resulted in the design of second-generation ABL inhibitors, including dasatinib (Sprycel®) and nilotinib (Tasigna®), which were initially approved by the U.S. Food and Drug Administration (FDA) for treatment of patients resistant or intolerant to prior imatinib therapy. More recently, trials of both agents in newly diagnosed chronic phase patients showed that both are superior to imatinib for all outcomes measured after 1 year of treatment, including CCyR (primary outcome), time to remission, and rates of progression to accelerated phase or blast crisis. Although initial follow-up was short, early and sustained complete cytogenetic response was considered a validated marker for survival in CML. On June 17, 2010, FDA approved nilotinib for the treatment of patients with newly-diagnosed chronic phase CML. Dasatinib was approved on October 28, 2010, for the same indication.

For patients with increasing levels of BCR-ABL 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 are all options.(3)

**Molecular Resistance**
Molecular resistance is most often explained as genomic instability associated with the creation of the abnormal BCR-ABL1 gene, usually resulting in point mutations within the ABL1 gene kinase domain (KD) that affects protein kinase-TKI binding. BCR-ABL1 KD point mutations account for 30% to 50% of secondary resistance.(6) At least 58 different KD mutations have been identified in CML patients.(9) The degree of resistance depends on the position of the mutation within the KD (ie, active site) of the protein. Some mutations are associated with moderate resistance, and are responsive to higher doses of TKIs, while other mutations may not be clinically significant. Two mutations, designated T315I and E255K (nomenclature indicates the amino acid change and position within the protein), are consistently associated with resistance. The T315I mutation is relatively common at frequencies ranging from 4% to 19%, depending on the patient population; it is more common in patients with advanced phase CML than in patients with early chronic phase CML.(10-12)

The presence of ABL KD point mutations is associated with treatment failure. A large number of mutations have been detected, but extensive analysis of trial data with low-sensitivity mutation detection methods has identified a small number of mutations consistently associated with treatment failure with specific TKIs; guidelines recommend testing for, and use of information on these specific mutations in subsequent treatment decisions. The recommended method is sequencing with or without denaturing high-performance liquid chromatography screening to reduce the number of samples to be sequenced. Targeted methods that detect the mutations of interest for management decisions are also acceptable if designed for low sensitivity. High-sensitivity assays are not recommended.

Unlike imatinib, fewer mutations are associated with resistance to dasatinib or nilotinib.(13,14) For example, Guilhot et al(15) and Cortes et al(16) studied the use of dasatinib in imatinib-resistant CML patients in the accelerated phase and in blast crisis, respectively, and found that dasatinib response rates did not vary by the presence or absence of baseline tumor cell BCR-ABL1 mutations. However, neither dasatinib nor nilotinib are effective against resistant clones with the T315I mutation,15,9 and new agents and treatment strategies are in development for patients with T315I resistance.

In a recent follow-up study of nilotinib by le Coutre et al.(17) 137 patients with accelerated phase CML were evaluated after 24 months. Sixty-six percent maintained major cytogenetic responses at 24 months. The estimates of overall and progression-free survival rates at 24 months were 70% and 33%, respectively. Grade 3/4 neutropenia and thrombocytopenia were each observed in 42% of patients.
Rarely, other acquired cytogenetic abnormalities such as BCR-ABL gene amplification and protein overexpression have also been reported. Resistance unrelated to kinase activity may result from additional oncogenic activation or loss of tumor suppressor function, and may be accompanied by additional karyotypic changes.

Unlike in CML, resistance in ALL to TKIs is less well studied. In patients with ALL receiving a TKI, a rise in the BCR-ABL level while in hematologic complete response or clinical relapse warrants mutational analysis.

**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

* Note that new BCR-ABL KD mutations also occur in about 80-90% of cases of acute lymphoblastic leukemia in relapse after TKI treatment, and in CML blast transformation.

Improvement Amendments (CLIA). The BCR/ABL1 qualitative and quantitative genotyping tests and ABL kinase domain 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.

**Rationale**
This evidence review was created in February 2013 and has been updated with a search of the MEDLINE database through February 2, 2016 (see Appendix Table 1 for genetic testing categories).

Various types of laboratory tests for *BCR-ABL1* detection are associated with chronic myelogenous leukemia (CML) and have different clinical uses. Briefly, they are:

1. **Diagnosis:** patients who do not have the *BCR-ABL1* fusion gene by definition do not have CML. In contrast, identification of the *BCR-ABL1* fusion gene is necessary, although not sufficient, for diagnosis. Relevant test technologies are cytogenetics (karyotyping; recommended) or fluorescence in situ hybridization (FISH; acceptable in the absence of sufficient sample for karyotyping).

2. **Monitoring** *BCR-ABL1* RNA transcripts for residual disease during treatment/disease remission; relevant, standardized test technology is quantitative reverse-transcription polymerase chain reaction (RT-PCR). Note that a baseline measurement after confirmation of CML diagnosis, and before treatment begins is strongly recommended.

3. **Identification and monitoring of mutations for drug resistance at response failure or disease progression;** various test technologies are in use (not standardized).
Diagnosis/Pretreatment Workup

Chronic Myelogenous Leukemia
While the diagnosis of CML is based on the presence of characteristic cellular abnormalities in bone marrow, the presence of the Philadelphia (Ph) chromosome and/or confirmation of the BCR-ABL1 fusion gene is essential. The initial evaluation of chronic phase CML should include bone marrow cytogenetics, not only to detect the Ph chromosome, but also to detect other possible chromosomal abnormalities.(19) If bone marrow is not available, FISH analysis with dual probes for BCR and ABL genes or qualitative RT-PCR can provide qualitative confirmation of the fusion gene and its type.(19) Baseline measurement of BCR-ABL transcript levels are recommended as part of the initial evaluation, providing confirmation of the fusion gene, ensuring that it is detectable (rare variants requiring nonstandard probes may occur), and providing baseline for monitoring response to treatment.(19)

Acute Lymphoblastic Leukemia
The diagnosis of acute lymphoblastic leukemia (ALL) is made by demonstrating 20% or greater bone marrow lymphoblasts; demonstration of the BCR-ABL fusion gene is not essential. However, identification of specific molecular subtypes is recommended at the time of diagnosis for optimal risk evaluation and treatment planning. The initial evaluation of ALL patients should include bone marrow sample for RT-PCR for BCR-ABL to establish the presence or absence of BCR-ABL, as well as baseline transcript quantification.(4)

Monitoring for Residual Disease During Treatment and Disease Remission

Chronic Myelogenous Leukemia
Quantitative RT-PCR (qRT-PCR) measurement of BCR-ABL1 RNA transcript levels is the method of choice for assessing response to treatment because of the high sensitivity of the method and strong correlation with outcomes.(3) Compared with conventional cytogenetics, qRT-PCR is more than 3 logs more sensitive(20) and can detect 1 CML cell in the background of 100,000 or more normal cells. qRT-PCR testing can be conducted on peripheral blood, eliminating the need for bone marrow sampling. The goal of treatment is complete molecular response (CMR; no detectable BCR-ABL transcript levels by qRT-PCR). However, only a small minority of patients achieve CMR on imatinib.(21) More often, patients achieve a major molecular response (MMR; a 3-log reduction from the standardized baseline of the International Scale (IS; not from the actual baseline level of the individual patient). Results from the IRIS trial showed that patients who had a CMR or MMR had a negligible risk of disease progression at 1 year, and a significantly lower risk of disease progression at 5 years than patients who had neither.(22) At 8-year follow-up, none of the patients who achieved an MMR at 1 year progressed to the accelerated phase of disease or to a blast crisis. Similar near absence of progression in patients who achieved an MMR has been reported in registration studies of nilotinib and dasatinib.(7,8,21)
The degree of molecular response has been reported to correlate with risk of progression in patients treated with imatinib. Timing of the molecular response is also important; the degree of molecular response at early time points predicts the likelihood of achieving CMR or MMR and predicts improved rates of progression-free and event-free survival. While early and strong molecular response predicts durable long-term remission rates and progression-free survival, studies have not been conclusive that molecular response is predictive of overall survival.

Based on imatinib follow-up data, it is recommended that, for patients with a complete cytogenetic response (CCyR), molecular response to treatment be measured every 3 months for 2 years, then every 3 to 6 months thereafter. Without CCyR, continued monitoring at 3-month intervals is recommended. It has been assumed that the same time points for monitoring imatinib are appropriate for dasatinib and nilotinib, and will likely also be applied to bosutinib and ponatinib.

Rising BCR-ABL1 transcript levels are associated with increased risk of mutations and of treatment failure. However, what constitutes a clinically significant rise to warrant mutation testing is not known. Factors affecting the clinically significant change include the variability of the specific assay used by the laboratory and the level of molecular response achieved by the patient. Thresholds used include 2- to 10-fold increases, and increases of 0.5 to 1 log, respectively. Because of potential variability in results and lack of agreement across studies for an acceptable threshold, rising transcript levels alone are not viewed as sufficient to trigger mutation testing or changes in treatment.

**Standardization of BCR-ABL1 Quantitative Transcript Testing**

A substantial effort has been made to standardize the BCR-ABL1 qRT-PCR testing and reporting across academic and private laboratories. In 2006, the National Institute of Health Consensus Group proposed an IS for BCR-ABL1 measurement. The IS defines 100% as the median pretreatment baseline level of BCR-ABL1 RNA in early chronic phase CML; as determined in the pivotal IRIS trial, MMR is defined as a 3-log reduction relative to the standardized baseline, or 0.1% BCR-ABL1 on the IS. In the assay, BCR-ABL1 transcripts are quantified relative to 1 of 3 recommended reference genes (e.g., ABL) to control for the quality and quantity of RNA and to normalize for potential differences between tests. Percent ratios on the IS are determined at local labs by a test-specific conversion factor: IS percent ratio = local percent ratio \( \times \) conversion factor. Until reference standards become broadly available, patient specimens must be exchanged between the local laboratory and an IS reference laboratory to establish a laboratory-specific conversion factor (available online at http://www.whereareyouontheis.com/Default.aspx). In the United States, many laboratories offer BCR-ABL quantitative testing (e.g., Quest, ARUP, LabCorp, Mayo), and most specify on their websites that results are standardized to the IS.
Acute Lymphoblastic Leukemia
Despite significantly higher complete response (CR) rates with tyrosine kinase inhibitors (TKIs) in Ph-positive ALL, the response is typically short-lived and relapses are common.(4) The principal aim of after remission therapy is to eradicate minimal residual disease (MRD), which is the prime cause for relapse.(4)

Studies in children and adults with ALL have demonstrated a strong correlation between MRD and risk for relapse, as well as the prognostic significance of measuring MRD during and after initial induction therapy. A commonly used cutoff to define MRD positivity is 0.01%, with patients who attain an MRD less than 0.01% early during therapy having high odds of remaining in continuous CR with contemporary postremission therapy.(44)

A study of 3184 B-cell ALL children enrolled in the AIEOP-BFM ALL 2000 treatment protocol demonstrated that a risk classification algorithm based on MRD measurements using PCR on days 33 and 78 of treatment was superior to that of other risk stratification criteria based on white blood cell count, age, early response to prednisone, and genetic subtype.(44,45) Patients with an MRD less than 0.01% on day 33 (42%) had a 5-year event-free survival of 92.3%.

NCCN recommendations state that the timing of testing for MRD depends on the ALL chemotherapy regimen used and may occur during or after completion of induction therapy or at additional time points.

MRD is also a strong prognostic factor for children and adolescents with first-relapse ALL who achieve a second remission.(44) Patients with an MRD of 0.01% or more are eligible for allogeneic hematopoietic cell transplantation, whereas achievement of MRD negativity may be an indication for chemotherapy.(44)

Identification of ABL Kinase Domain Mutations (Mutations Associated With TKI Resistance)

Chronic Myelogenous Leukemia
Screening for BCR-ABL1 kinase domain (KD) point mutations (ie, single-nucleotide polymorphisms) in chronic phase CML is recommended for patients with (1) inadequate initial response to TKI treatment, (2) with evidence of loss of response, or (3) who have progressed to accelerated or blast phase CML.(3) Testing for KD point mutations, in the setting of potential treatment failure, helps to select from among other possible TKI treatments or allogeneic cell transplantation. The following discussion focuses only on KD point mutations.

In 2010, the Agency for Healthcare Research and Quality published a systematic review on BCR-ABL1 pharmacogenetic testing for TKIs in CML.(46) The report concluded that the presence of any BCR-ABL1 mutation does not predict differential response to TKI therapy, although the presence of the T315I mutation uniformly predicts TKI failure. The review was strongly criticized by respected pathology organizations for insufficient attention to several issues. Importantly, the report grouped studies that used KD mutation screening methods with those
that used targeted methods, and grouped studies that used mutation detection technologies with very different sensitivities. The report discounted issues related to analytic validity. However, in this clinical scenario, assays used for different reasons (screening vs targeted) and assays with very different sensitivities may lead to different clinical conclusions.

**Point Mutation Detection Methods**
Currently, methods for detecting drug resistance mutations are not standardized; clinical laboratories may choose among different methods. Some can detect specific, known mutations (eg, targeted mutation analysis) or screen for all possible mutations (eg, direct sequencing); sensitivity also varies by method.

Particular methods to detect BCR-ABL KD mutations will greatly influence the detection frequency, analytic sensitivity and the clinical impact of testing. The various mutation detection methods available have widely differing analytic sensitivities, from the least sensitive direct Sanger sequencing to the highly sensitive mutation-specific quantitative PCR methods.

Direct Sanger sequencing screens for all possible mutations but has low sensitivity, detecting a mutation present in approximately 1 in 5 BCR-ABL transcripts. Denaturing high-performance liquid chromatography (DHPLC) is a screening method with initially higher sensitivity to detect the presence or absence of mutations. Follow-up Sanger sequencing of positive samples is required to identify the mutations present; final sensitivity of this method is the sensitivity of sequencing. Targeted methods, used either to screen for only the most common, clinically relevant mutations or to monitor already identified mutations after a therapy change, can offer either limited sensitivity (eg, pyrosequencing) or very high sensitivity (eg, allele-specific PCR).

**KD Point Mutations and Treatment Outcomes**
Branford et al summarized available evidence in 2009 regarding KD mutations detected at imatinib treatment failure, and subsequent treatment success or failure with dasatinib or nilotinib.(47) Studies referenced used direct Sanger sequencing, with or without DHPLC screening, to identify mutations at low sensitivity. The authors conducted a survey of mutations detected in patients at imatinib failure at their own institution and compared results with a collation of mutations derived from the literature. For both, the T315I mutation was most common; although about 100 mutations have been reported, the 7 most common (at residues T315, Y253, E255, M351, G250, F359, and H396) accounted for 60% to 66% of all mutations in both surveys. Detection of the T315I mutation at imatinib failure is associated with lack of subsequent response to high-dose imatinib or to dasatinib or nilotinib. For these patients, allogeneic cell transplantation was the only available treatment until the approval of new agents (eg, ponatinib).(48) Most common, however, does not necessarily correspond to clinically significant. Based on the available clinical studies, most imatinib-resistant mutations remain sensitive to dasatinib and nilotinib. However, preexisting or emerging mutations T315A, F317L/I/V/C, and V299L are associated with decreased clinical efficacy with dasatinib treatment following imatinib failure.
Similarly, preexisting or emerging mutations Y253H, E255K/V, and F359V/C have been reported to have decreased clinical efficacy with nilotinib treatment following imatinib failure. In the Branford survey, 42% of patients tested had T315I or one of the dasatinib- or nilotinib-resistant mutations.(47) As a result, guidelines recommend mutation analysis only at treatment failure, and use of the T315I mutation and the identified dasatinib- and nilotinib-resistant mutations to select subsequent treatment.(3,39) Absent any of these actionable mutations, various treatment options are available. Note that these data were obtained from studies of patients all initially treated with imatinib. No data are available on mutations developing during first-line therapy with dasatinib or nilotinib.(49)

ABL KD mutational analysis is recommended if there is inadequate initial response (failure to achieve complete hematologic response at 3 months, only minor cytologic response at 6 months or major [rather than complete] cytogenetic response at 12 months) or any sign of loss of response (defined as hematologic relapse, cytogenetic relapse, or 1-log increase in BCR-ABL1 transcript ratio and therefore loss of major molecular response). Mutation testing is also recommended for progression to accelerated or blast phase CML. Treatment recommendations based on mutation(s) are shown in Table 1.

### Table 1. Treatment Options Based on BCR-ABL1 KD Point Mutation Status at Imatinib Treatment Failure

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Treatment Recommendation</th>
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<tbody>
<tr>
<td>T315I</td>
<td>Ponatinib,a HCT, or clinical trial</td>
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<tr>
<td>V299L, T315A, F317L/V/I/C</td>
<td>Consider nilotinib or bosutiniba rather than dasatinib</td>
</tr>
<tr>
<td>Y253H, E255K/V, F359V/C/I</td>
<td>Consider dasatinib or bosutiniba rather than nilotinib</td>
</tr>
<tr>
<td>Any other mutation</td>
<td>Consider high-dose imatinib, or dasatinib, nilotinib, or bosutiniba</td>
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</tbody>
</table>

a Recently approved; added in advance of National Comprehensive Cancer Network update, from which guidelines in this table have been modified. Ponatinib active in treatment-resistant patients with T315I mutation.(48,50) Bosutinib is active across BCR-ABL1 mutations including dasatinib- and nilotinib-resistant mutations, except T315I, and after treatment failure with imatinib, dasatinib, or nilotinib.(51,52)

Because only a small number of mutations have been recommended as clinically actionable, targeted assays may also be used to screen for the presence of actionable mutations at treatment failure. Quantitative, targeted assays may also be used to monitor levels of already identified clinically significant mutations after starting a new therapy because of initial treatment failure. Targeted assays use different technologies that can be very sensitive and pick up mutated cell clones at very low frequencies in the overall malignant population. Banked samples from completed trials have been studied with high-sensitivity assays to determine if monitoring treatment can detect low-level mutations that predict treatment failure well in advance of clinical indications. Some results have been positive, but not all mutations detected in advance predict treatment failure; more study is recommended before these assays are used for monitoring in advance of treatment failure.(39,47) A direct correlation between low-sensitivity and high-sensitivity assays and a limited correlation with clinical outcomes support
recommendations of sequencing, with or without DHPLC screening, for identification of mutations. (53) Although high-sensitivity assays identified more mutations than did sequencing, the clinical impact of identifying additional mutations is uncertain.

Mutations other than point mutations can be detected in the *BCR-ABL1* gene, including alternate splicing, insertions, deletions, and/or duplications. The clinical significance of such altered transcripts is unclear, and reporting such mutations is not recommended. (6,49)

**Acute Lymphoblastic Leukemia**
Unlike in CML, resistance in ALL to TKIs is less well studied. Resistance does not necessarily arise from dominant tumor clone(s), but possibly in response to TKI-driven selective pressure and/or competition of other coexisting subclones. (4) In patients with ALL receiving a TKI, a rise in the BCR-ABL protein level while in hematologic CR or clinical relapse warrants mutational analysis. (4)

**Ongoing and Unpublished Clinical Trials**
Some currently unpublished trials that might influence this review are listed in Table 2.

**Table 2. Summary of Key Trials**

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
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<tbody>
<tr>
<td>Ongoing</td>
<td></td>
<td></td>
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<tr>
<td>NCT01343173</td>
<td>Multicenter Trial Estimating the Persistence of Molecular Remission in Chronic Myeloid Leukaemia in Long Term After Stopping Imatinib</td>
<td>220</td>
<td>Jul 2017</td>
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<tr>
<td>NCT01578213</td>
<td>Validation of Digital-PCR Analysis Through Programmed Imatinib Interruption in PCR Negative CML Patients (ISAV)</td>
<td>100</td>
<td>Nov 2018</td>
</tr>
<tr>
<td>Unpublished</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT00760877a</td>
<td>An Open Label, Randomized Study of Nilotinib vs. Standard Imatinib (400/600 mg QD) Comparing the Kinetics of Complete Molecular Response for CML-CP Patients With Evidence of Persistent Leukemia by RQ-PCR</td>
<td>206</td>
<td>Jul 2015 (completed)</td>
</tr>
<tr>
<td>NCT01580059a</td>
<td>Extending Molecular Responses With Nilotinib in Newly Diagnosed Chronic Myeloid Leukemia (CML) Patients in Chronic Phase</td>
<td>419</td>
<td>Nov 2014 (completed)</td>
</tr>
<tr>
<td>NCT01342679</td>
<td>A Study of Complete Molecular Response for Chronic Myeloid Leukemia in Chronic Phase Patients, Treated With Dasatinib</td>
<td>21</td>
<td>Sep 2014 (completed)</td>
</tr>
</tbody>
</table>

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

**Summary of Evidence**
The evidence for BCR/ABL1 fusion gene qualitative testing to confirm diagnosis and establish baseline for monitoring treatment in individuals who have suspected
chronic myelogenous leukemia (CML) includes validation studies. Relevant outcomes are test accuracy and test validity. The sensitivity of testing with reverse transcription-polymerase chain reaction is high compared with conventional cytogenetics. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

The evidence for quantitative BCR/ABL1 quantitative testing at appropriate intervals during therapy for monitoring treatment response and remission in individuals who have a diagnosis of CML or at baseline prior to and during treatment to monitor treatment response and remission in individuals who have acute lymphoblastic leukemia (ALL) includes a randomized trial and case series. Relevant outcomes are disease-specific survival, test accuracy and validity, and change in disease status. Studies have shown a high sensitivity of this type of testing and a strong correlation with outcomes, including risk of disease progression and survival, and may stratify patients to different treatment options. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

The evidence for evaluation of ABL kinase domain (KD) point mutations to assess for tyrosine kinase inhibitor resistance in individuals who have a diagnosis of CML or who have Philadelphia chromosome–positive ALL and signs of treatment failure or disease progression includes a systematic review of pharmacogenetic testing for TKIs and case series reporting the presence of KD mutations detected at imatinib failure. Relevant outcomes are test accuracy and validity and medication use. Studies have shown a correlation between certain types of mutations, treatment response, and the selection of subsequent treatment options. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

Practice Guidelines and Position Statements
National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN) practice guidelines (v.1.2016) on chronic myelogenous leukemia outline recommended methods for diagnosis and treatment management of CML, including BCR-ABL1 tests for diagnosis, monitoring, and ABL KD mutations, and were referred to extensively in this document.(3)

The NCCN practice guidelines (v.2.2015) on ALL state that, if minimal residual disease (MRD) is being evaluated, the initial measurement should be performed on completion of induction therapy; additional time points for MRD evaluation may be useful, depending on the specific treatment protocol or regimen used. MRD is an essential component of patient evaluation during sequential therapy.(54)

European LeukemiaNet
The European LeukemiaNet management recommendations for CML are similar to those of NCCN.(31,38) The U.S. Association for Molecular Pathology(6) and
European LeukemiaNet recommendations for KD mutation analysis(39) both provide very similar guidelines.

Other
In 2010, technical recommendations for MRD assessment and definitions for response based on MRD results were made in an effort to standardize MRD measurements and MRD data reporting in European ALL trials.(55)

U.S. Preventive Services Task Force Recommendations
Not applicable.

Medicare National Coverage
There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

References


33. Branford S, Rudzki Z, Parkinson I, et al. Real-time quantitative PCR analysis can be used as a primary screen to identify patients with CML treated with imatinib who have BCR-ABL kinase domain mutations. Blood. Nov 1 2004;104(9):2926-2932. PMID 15256429


**Billing Coding/Physician Documentation Information**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81170</td>
<td>ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase) (eg, acquired imatinib tyrosine kinase inhibitor resistance), gene analysis, variants in the kinase domain</td>
</tr>
<tr>
<td>81206</td>
<td>BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; major breakpoint, qualitative or quantitative</td>
</tr>
<tr>
<td>81207</td>
<td>BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; minor breakpoint, qualitative or quantitative</td>
</tr>
<tr>
<td>81208</td>
<td>BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; other breakpoint, qualitative or quantitative</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (eg, 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)</td>
</tr>
<tr>
<td>81403</td>
<td>Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of &gt;10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)</td>
</tr>
<tr>
<td>0040U</td>
<td>BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis, major breakpoint, quantitative. MRDx BCR-ABL Test</td>
</tr>
</tbody>
</table>

**ICD-10 Codes**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C91.00-</td>
<td>Acute lymphoblastic leukemia [ALL], code range</td>
</tr>
<tr>
<td>C91.02</td>
<td>Chronic myeloid leukemia, BCR/ABL-positive code range</td>
</tr>
<tr>
<td>C92.10</td>
<td>Atypical chronic myeloid leukemia, BCR/ABL-negative code range</td>
</tr>
</tbody>
</table>

**Additional Policy Key Words**

N/A

**Policy Implementation/Update Information**

6/1/2013 New policy; may be considered medically necessary.

6/1/14 Policy statements added for ALL, medically necessary prior to initiation
of treatment for disease monitoring and to evaluate for TKI resistance.

Updated Description. Title also changed to add ALL. Updated
Considerations to include ALL and TKI. Added CPT codes 81401, 81403

6/1/15  No policy statement changes.
6/1/16  Added CPT 81170. No policy statement changes.
6/1/17  No policy statement changes.

Appendix

Appendix Table 1. Categories of Genetic Testing Addressed in 2.04.85

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>1a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>1b. Prognostic</td>
<td></td>
</tr>
<tr>
<td>1c. Therapeutic</td>
<td></td>
</tr>
<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>2a. Diagnostic</td>
<td>X</td>
</tr>
<tr>
<td>2b. Prognostic</td>
<td>X</td>
</tr>
<tr>
<td>2c. Therapeutic</td>
<td>X</td>
</tr>
<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
<td></td>
</tr>
<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
<td></td>
</tr>
<tr>
<td>5. Reproductive testing</td>
<td></td>
</tr>
<tr>
<td>5a. Carrier testing: preconception</td>
<td></td>
</tr>
<tr>
<td>5b. Carrier testing: prenatal</td>
<td></td>
</tr>
<tr>
<td>5c. In utero testing: aneuploidy</td>
<td></td>
</tr>
<tr>
<td>5d. In utero testing: mutations</td>
<td></td>
</tr>
<tr>
<td>5e. In utero testing: other</td>
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</tr>
<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
<td></td>
</tr>
</tbody>
</table>

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provisions/exclusions, take precedence over Medical Policy and must be considered first in
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