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

**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.

**Background**

**Disease:**

**CML**

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% 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. (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.

**ALL**

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 about 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 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.

**Treatment and response and minimal residual disease:** Imatinib (Gleevec®) was originally developed to specifically target and inactivate the ABL tyrosine kinase portion of the BCR-ABL1 fusion protein in order 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.

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 6-12 months after initial treatment with the TKI imatinib. (3) It has been well established that most “good responders” that 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 (CR) by morphologic evaluation after induction therapy, approximately 25% to 50% may still have detectable MRD based on sensitive assays. Current methods used for MRD detection include flow cytometer (which affords a sensitivity of MRD detection of 0.01%), or polymerase chain reaction (PCR)-based analyses (Ig and T-cell receptor gene rearrangements or analysis of BCR-ABL transcripts), which are the most sensitive method of monitoring treatment response, with a sensitivity of 0.001%. (4) Ig and T-cell receptor gene arrangement analysis is applicable for most ALL patients, whereas PCR analysis of BCR-ABL transcripts is applicable only in Ph-positive patients.
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 CR, significantly lower than that achieved in PH-negative ALL (5) The inclusion of TKIs to frontline induction chemotherapy has improved CR rates, exceeding 90% (5)

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. (3, 6)

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 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. (7, 8) 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-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 400mg to 800mg daily as tolerated or therapy change to an alternate second-generation TKI are all options. (3)

Molecular resistance: Resistance is most often explained at the molecular level by genomic instability associated with the creation of the abnormal BCR-ABL1 gene, usually resulting in point mutations within the ABL1 kinase domain that affects protein kinase-TKI binding. BCR-ABL1 kinase domain (KD) point mutations account for 30-50% of secondary resistance. (6, 9) 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 kinase domain (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)

\(^{a}\) Note that new BCR-ABL KD mutations also occur in about 80% to 90% of cases of acute lymphoblastic leukemia in relapse after TKI treatment, and in CML blast transformation.
Compared to 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 (9, 15) 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 (17), 137 patients with accelerated phase CML were evaluated after 24 months. Sixty-six percent of patients maintained major cytogenic 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. (18) 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. (6)

**Regulatory Status**

The BCR-ABL1 qualitative and quantitative genotyping tests and ABL KD mutation tests are not manufactured test kits and have not been reviewed by the FDA. Rather, they are laboratory-developed tests (LDT), offered by clinical laboratories licensed under Clinical Laboratory Improvement Amendments (CLIA) for high-complexity testing.

**Related Policies**

None

**Policy**

*This policy statement applies to clinical review performed for pre-service (Prior Approval, Precertification, Advanced Benefit Determination, etc.) and/or post-service claims.*

**Chronic Myelogenous Leukemia**

BCR/ABL 1 qualitative testing for the presence of the fusion gene may be considered medically necessary for diagnosis of chronic myeloid leukemia (see Policy Guidelines section).

BCR/ABL 1 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 Policy Guidelines section) 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 when there is inadequate initial response to
treatment or any sign of loss of response (see Policy Guidelines section); and/or when there is progression of the disease to the accelerated or blast phase.

Evaluation of \textit{ABL} kinase domain point mutations may be considered \textit{investigational} for monitoring in advance of signs of treatment failure or disease progression.

**Acute Lymphoblastic Leukemia**

\textit{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 Policy Guidelines section) may be considered \textit{medically necessary} for monitoring of Philadelphia chromosome-positive acute lymphoblastic leukemia treatment response and remission.

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

Evaluation of \textit{ABL} kinase domain point mutations may be considered \textit{investigational} for monitoring in advance of signs of treatment failure or disease progression.

**Policy Guidelines**

**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).

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

**Determining Baseline RNA Transcript Levels and Subsequent Monitoring**

Determination of \textit{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; every 3 months for 3 years, then every 3-6 months thereafter. After a complete cytogenetic response is achieved, testing is recommended every 3 months for 3 years and 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 posatinib. (See Rationale 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 TKIs is defined as hematologic relapse, cytogenetic relapse or 1 log increase in BCR/ABL1 transcript ratio and therefore loss of major molecular response (MMR).

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.

Rationale

Various types of laboratory tests involving BCR-ABL1 detection are associated with CML and have different clinical uses. Briefly, these 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 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/disease progression; various test technologies are in use (not standardized).

Diagnosis/Pretreatment Work-Up

Chronic Myelogenous Leukemia (CML)

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 are essential to diagnosis. The initial evaluation of chronic phase CML should include bone marrow cytogenetics, not only to detect the Ph chromosome, but 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 reverse transcriptase 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 non-standard probes may occur) as well as a baseline for monitoring response to treatment. (19)

**Acute Lymphoblastic Leukemia (ALL)**

The diagnosis of acute lymphoblastic leukemia (ALL) is made by demonstrating 20% or greater bone marrow lymphoblasts and demonstration that the BCR-ABL fusion gene is not essential to diagnosis; 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. (5)

**Monitoring for Residual Disease During Treatment/Disease Remission**

Quantitative RT-PCR measurement of BCR-ABL RNA transcript levels is the method of choice for measuring response to treatment because of the high sensitivity of the method and strong correlation with outcomes. (3) Compared to conventional cytogenetics, quantitative RT-PCR (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. Quantitative RT-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 (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 compared to patients who had neither. (22) At 8 year follow-up, none of the patients who achieved a 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. (23) 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. (24-27) 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. (28-30)

Based on imatinib follow-up data, it is recommended that for patients with a complete cytogenetic response, molecular response to treatment be measured every 3 months for 3 years, then every 3-6 months thereafter. (3, 31) Without complete cytogenic response (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 as well (3), and will likely also be applied to bosutinib and posatinib.

Rising BCR-ABL1 transcript levels are associated with increased risk of mutations and of treatment failure. (32-37) However, the amount of rise that is considered clinically significant for considering mutation testing is not known. Factors affecting the clinically significant change include the variability of the specific assay used by the laboratory, as well as the level of molecular response achieved by the patient. Thresholds used include 2- to 10-fold increases, and increases of 0.5-1 log. (31, 33, 38) 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. (39)

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 International Scale (IS) for BCR-ABL1 measurement. (40) 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. (41) In the assay, BCR-ABL1 transcripts are quantified relative to one of 3 recommended reference genes (eg, ABL) to control for the quality and quantity of RNA and to normalize for potential differences between tests. (42, 43) Percent ratios on the IS are determined at local labs by a test-specific conversion factor (IS % ratio = local % ratio x 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 (http://www.whereareyouontheis.com/Default.aspx). In the United States, many laboratories offer BCR-ABL quantitative testing (eg, Quest, ARUP, LabCorp and 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 the use of tyrosine kinase inhibitors (TKIs) in Ph-positive ALL, the response is typically short-lived and relapses are common. (5) The principal aim of post remission therapy is eradicating minimal residual disease (MRD), which is the prime cause for relapse. (5)

Studies in both 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 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 by 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 MRD less than 0.01% on day 33 (42%) had a 5-year event free survival of 92.3% (±0.9%).

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

MRD is also a strong prognostic factor for children and adolescents with first-relapse ALL who achieve a second remission. (44) Patients with MRD of 0.01% or more are eligible for allogeneic hematopoietic stem 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 (CML)
Screening for BCR-ABL1 kinase domain (KD) point mutations (ie single nucleotide polymorphisms) in chronic phase CML is recommended for patients with inadequate initial response to TKI treatment, those with evidence of loss of response, and for patients who have progressed to accelerated or blast phase CML. (3) The purpose of testing for KD point mutations is, in the setting of potential treatment failure, to help select among other possible TKI treatments or allogeneic stem 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. However, during the public comment period the review was strongly criticized by respected pathology organizations for lack of attention to several issues that were subsequently insufficiently addressed in the final report. Importantly, the review grouped together studies that used KD mutation screening methods with those that used targeted methods, and grouped together studies that used mutation detection technologies with very different sensitivities. The authors dismissed the issues as related to analytic validity and beyond the scope of the report. However, in this clinical scenario assay with different intent (screening vs. targeted) and assays of very different sensitivities may lead to different clinical conclusions and an understanding of these points is critical.

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

The particular methods to detect BCR-ABL KD mutations will have great influence on the detection frequency, analytical sensitivity and the clinical impact of testing. The various mutation detection methods available have widely different 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-ABL1 transcripts. Denaturing high performance liquid chromatography (DHPLC) is also a screening method with initially higher sensitivity to detect the presence or absence of any 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).

Kinase Domain Point Mutations and Treatment Outcomes: Branford et al. (47) have summarized much of the available evidence regarding KD mutations detected at imatinib failure, and subsequent treatment success or failure with dasatinib or nilotinib. The 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 it 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 stem cell transplantation remained the only available treatment until the advent of new agents such as ponatinib. (48) 'Most common,' however, does not necessarily correspond to clinically significant. Based on the available clinical studies, the majority of 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 for decreased clinical efficacy with nilotinib treatment following imatinib failure. In the survey reported by Branford et al., a total of 42% of patients tested had T315I or one of these 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 the subsequent treatment. (3, 39) In the absence of any of these actionable mutations, various treatment options are available. Note that these data have been obtained from studies in which patients were all initially treated with imatinib. No data are available regarding 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 cytological 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-ABL1KD 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), HSCT or clinical trial</td>
</tr>
<tr>
<td>V299L, T315A, F317L/V/I/C</td>
<td>Consider nilotinib or bosutinib(^a) rather than dasatinib</td>
</tr>
<tr>
<td>Y253H, E255K/V, F359V/V/I</td>
<td>Consider dasatinib or bosutinib(^a) rather than nilotinib</td>
</tr>
<tr>
<td>Any other mutation</td>
<td>Consider high dose imatinib, or dasatinib, nilotinib, or bosutinib(^a)</td>
</tr>
</tbody>
</table>

\(^a\) HSCT: hematopoietic stem cell transplantation.

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

Since 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 following initial treatment failure. Targeted assays use different technologies, which can be made very sensitive to 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. While some results have been positive, not all mutations detected in advance predict treatment failure and more study is recommended before these assays are used for monitoring in advance of treatment failure. (39, 47) A direct correlation of 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 the additional mutations was viewed as uncertain.

Other types of mutations in addition to 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 by competition of other coexisting subclones. (5) 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. (5)

**Ongoing and Unpublished Clinical Trials**

Over 100 ongoing clinical trials resulted from a search of clinical trials.gov for 'BCR-ABL1 and CML'; many of these trials are treatment regimen-related. Available online at: (http://www.clinicaltrials.gov/ct2/results?term=BCR%2FABL+CML&recr=Open&no_unk=Y).
Five ongoing trials were found that have direct genetic testing/molecular testing involvement:

1. (NCT01343173) The Multicenter Trial Estimating the Persistence of Molecular Remission in Chronic Myeloid Leukemia in Long Term After Stopping Imatinib (STIM 2) will measure the rate of molecular relapse defined by the rate of patients having a significant increasing of BCR-ABL transcript for two years after stopping treatment. Secondary outcomes include overall survival, molecular profile of patient, treatment costs, and event free survival. The study has an estimated enrollment of 220 and an anticipated completion date of 2017. [http://www.clinicaltrials.gov/ct2/show/NCT01343173].

2. (NCT01342679) A Study of Complete Molecular Response for Chronic Myeloid Leukemia in Chronic Phase Patients, Treated with Dasatinib (CMR-CML) will measure the rate of complete molecular response (CMR) after treatment with dasatinib. Secondary outcomes include progression free survival, and number of participants with adverse events. The status of this study is unknown; last verified in April 2011. [http://www.clinicaltrials.gov/ct2/show/NCT01342679].

3. (NCT00760877) Nilotinib Versus Standard Imatinib (400/600 mg QD) Comparing the Kinetics of Complete Molecular Response (CMR) for CML-CP (chronic phase) Patients With Evidence of Persistent Leukemia by RQ-PCR will measure the rate of confirmed best cumulative Complete Molecular Response within the first year of study therapy with imatinib or nilotinib. Secondary outcomes include kinetics of CMR achieved in both treatment arms, progression free survival, EFS and OS between the two arms, and kinetics of CMR achieved after crossover. This study has an estimated enrollment of 207 and an anticipated completion date of July 2015. [http://www.clinicaltrials.gov/ct2/show/NCT00760877].

4. (NCT01578213) Validation of Digital-PCR Analysis Through Programmed Imatinib Interruption in PCR Negative CML Patients will measure the negative predictive value ratio (rNPV) of dPCR over Q-RT-PCR. Secondary outcomes include rate of molecular and cytogenetic relapse, rate of dPCR positive patients, rate of dPCR negative patients, rate of patients who are maintaining dPCR negativity for 36 month, time to molecular relapse, overall survival, quality of life, and rate of patients progressing or developing resistance. This study has an estimated enrollment of 100 and an anticipated completion date of November 2018. [http://www.clinicaltrials.gov/ct2/show/NCT01578213].

5. (NCT01580059) Extending Molecular Responses With Nilotinib in Newly Diagnosed Chronic Myeloid Leukemia (CML) Patients in Chronic Phase will evaluate efficacy, using molecular response, of nilotinib 300 mg BID in the treatment of newly diagnosed CML-CP patients. No secondary outcomes were defined. This study has an estimated enrollment of 418 and an anticipated completion date of November 2018. [http://www.clinicaltrials.gov/ct2/show/NCT01580059].

Policy Guidelines and Position Statements

The National Comprehensive Cancer Network (NCCN) Practice Guidelines v.1.2015 Chronic Myelogenous Leukemia recommended methods for diagnosis and treatment management of CML, including BCR-ABL1 tests for diagnosis, monitoring, and ABL kinase domain mutations, and were referred to extensively in this document. (3) The European LeukemiaNet management
recommendations for CML are very similar to those of NCCN and have also been cited in this document. (31) The U.S. Association for Molecular Pathology (6) and European LeukemiaNet recommendations for KD mutation analysis (39) have been referenced; both provide very similar guidelines.

The NCCN Practice Guidelines v.2.2014 Acute Lymphoblastic Leukemia 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 over the course of sequential therapy. If a patient is not treated in an academic center, there are commercially available tests available for MRD assessment. (54)

In 2010, recommendations were published on the technical requirements for MRD assessment and definitions for response based on MRD results, as a result of a consensus development meeting. (55) The recommendations were made in an effort to standardize MRD measurements and MRD data reporting in European ALL trials.

**U.S. Preventive Services Task Force Recommendations**

Not applicable.

**Summary**

CML

Extensive clinical data has 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 chronic myelogenous leukemia (CML). These tests are also useful in the accelerated and blast phases of this malignancy. Appropriate uses are summarized as follows:

**Diagnosis:** 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 Philadelphia chromosome (Ph) 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.

**Monitoring During Treatment with Tyrosine Kinase Inhibitors (TKIs):** 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. Quantitative polymerase-chain reaction (PCR)-based methods and international standards for reporting have been recommended and adopted for treatment monitoring.
Treatment Failure: The presence of ABL KD point mutations are associated with treatment failure; a large number of mutations have been detected, but extensive analysis of trial data with low sensitivity mutation detection methods have identified a small number of mutations that are consistently associated with treatment failure with specific TKIs; guidelines recommend testing for, and using information regarding these specific mutations in subsequent treatment decisions. The recommended method is sequencing with or without denaturing high performance liquid chromatography (DHPLC) screening to reduce the number of samples that need 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.

While the existing evidence is associational in nature, the body of evidence that has been accumulated and the consequences of the management decisions involved, along with international agreement on recommendations of the use of molecular assays, support the medical necessity of the use of the assays as described. Other uses and other types of assays are considered investigational.

ALL

Diagnosis: the presence of the BCR-ABL1 fusion gene is not necessary to establish a diagnosis of ALL. However, before initiation of therapy, identification of the BCR-ABL transcript is necessary for risk stratification and quantification to establish baseline levels for subsequent monitoring of response during treatment.

Monitoring during treatment with TKIs: 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. For ALL, the optimal timing remains unclear and depends upon the chemotherapy regimen used.

Treatment Failure: 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.

Medicare National Coverage

There is no national coverage determination.

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


48. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A Pivotal Phase 2 Trial of Ponatinib in Patients with Chronic Myeloid Leukemia (CML) and Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia (Ph+ALL) Resistant or Intolerant to Dasatinib orNilotinib, or with the T315I BCR-ABL Mutation: 12-Month Follow-up of the PACE Trial. American Society of Hematology 54th Annual Meeting, December 2012 2012: Abstract 163. PMID


Policy History

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<tr>
<th>Date</th>
<th>Action</th>
<th>Reason</th>
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<tbody>
<tr>
<td>June 2013</td>
<td>New Policy</td>
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<tr>
<td>June 2014</td>
<td>Update Policy</td>
<td>Policy updated with literature review. References 3, 5, 6, 46-47, 56-57 added. Policy statements added for ALL, medically necessary prior to initiation of treatment, for disease monitoring and to evaluate for TKI resistance. Title also changed to add ALL.</td>
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Keywords

BCR/ABL1 genotyping
Genetic testing, chronic myeloid leukemia
BCR/ABL1 mRNA quantification
ABL kinase domain mutation testing

This policy was approved by the FEP® Pharmacy and Medical Policy Committee on June 19, 2015 and is effective July 15, 2015.

Signature on File

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