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BCR-ABL 1 Testing

Policy Number: AHS – M2027 – BCR-ABL 1 Testing	Prior Policy Name and Number, as applicable: • M2027 - BCR-ABL 1 Testing for Chronic Myeloid Leukemia
Initial Presentation Date: 11/16/2015 Revision Date: 06/02/2021	

I. Policy Description

Oncogenesis can result when specific genes or regions of chromosomes are translocated elsewhere within the genome. *BCR-ABL1*, for example, refers to the fusion gene resulting from a reciprocal translocation that joins the *ABL1* gene from chromosome 9 to the *BCR* gene on chromosome 22 and is necessary for the development of chronic myeloid leukemia (CML) (Van Etten, 2019). This reciprocal translocation also generates a shortened derivative chromosome 22, known as the Philadelphia (Ph) chromosome (Schrijver & Zehnder, 2020). The Ph chromosome is a diagnostic hallmark, present in 95% of people with CML and approximately 3%–5% children and 25%–40% adults with acute lymphoblastic leukemia (ALL) (Leoni & Biondi, 2015), an aggressive form of cancer resulting from the neoplastic transformation of lymphoid precursors characterized by the presence of too many lymphoblasts or lymphocytes in the bone marrow and peripheral blood (PDQ, 2019). Predominately a childhood disease, approximately 60% of cases were diagnosed in patients younger than 20 years of age (Pui, 2011).

II. Related Policies

Policy Number	Policy Title
AHS-G2124	Serum Tumor Markers For Malignancies
AHS-M2062	Genetic Testing for Acute Myeloid Leukemia
AHS-M2066	Genetic Cancer Susceptibility Using Next Generation Sequencing
AHS-M2101	Mutation Analysis In Myeloproliferative Neoplasms
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare] for

a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx or the manual website

1. Qualitative or quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) testing for identification of the breakpoint cluster region protein- Abelson murine leukemia viral oncogene homolog 1 (*BCR-ABL1)* fusion gene transcript type **MEETS COVERAGE CRITERIA** for the differential diagnosis of chronic myeloid leukemia (CML) or acute lymphoblastic leukemia (ALL).

2. Quantitative testing on blood or bone marrow for the *BCR-ABL1* fusion gene transcript in individuals with CML, using the International Scale reporting convention, for patients prior to initiation or undergoing treatment with tyrosine-kinase inhibitor (TKI) therapy, **MEETS COVERAGE CRITERIA** for any of the following:

    a. As a baseline measurement prior to initiation of TKI therapy

    b. Every 3 months after initiation of therapy after major molecular response (MMR) (*BCR-ABL1* (International Scale (IS)) $\leq$ 1% (>0.1%-1%)) has been achieved

    c. Every 3 months for 2 years and every 3-6 months thereafter

    d. If there is a 1-log increase in *BCR-ABL1* transcript levels with MMR, repeat in 1-3 months.

3. Quantitative testing on blood or bone marrow for the *BCR-ABL1* fusion gene transcript in individuals with CML, using the International Scale reporting convention, for patients undergoing treatment discontinuation with TKI therapy and who remain in MMR after discontinuation of therapy, **MEETS COVERAGE CRITERIA**.

4. Evaluation of *BCR-ABL* kinase domain point mutations in patients with CML **MEETS COVERAGE CRITERIA** when:

    a. There is failure to reach response milestones, OR

    b. There is any sign of loss of response (defined as hematologic or cytogenic relapse), OR

    c. 1-log increase in *BCR-ABL1* transcript levels and loss of MMR, OR

    d. The disease progresses to accelerated or blast phase.

5. Quantitative or qualitative testing on blood or bone marrow for the *BCR-ABL1* fusion gene transcript, including determination of transcript size (ie, p190 vs. p210), in individuals diagnosed with B-cell acute lymphoblastic leukemia (B-ALL), using the International Scale reporting convention, **MEETS COVERAGE CRITERIA** for optimal risk stratification, treatment planning, surveillance, and MRD assessment.

6. Evaluation of *BCR-ABL* kinase domain point mutations in patients with ALL **MEETS COVERAGE CRITERIA** when there is relapsed or refractory disease in Philadelphia (Ph) chromosome positive ALL patients.

7. Testing of both bone marrow and blood for monitoring purposes **DOES NOT MEET COVERAGE CRITERIA.**

IV. Scientific Background

A translocation occurs when specific genes or regions of a chromosome are moved from one chromosome to another. Several human diseases are caused by translocations. In particular, a few types of leukemia (such as acute myeloid leukemia and chronic myeloid leukemia) are known to occur due to spontaneous translocations.

Chronic myeloid leukemia (CML) was the first human malignancy in which a specific chromosomal defect, known as the minute or Philadelphia (Ph) chromosome, could be linked to pathogenetic events of leukemogenesis (Nowell & Hungerford, 1960). The Ph chromosome translocation (t(9;22)(q34;q11.2)) fuses the breakpoint cluster region protein (*BCR*) gene from chromosome 22 with the Abelson murine leukemia viral oncogene homolog 1 (*ABL1*) proto-oncogene from chromosome 9 in a head-to-tail manner to form the transcriptionally active *BCR-ABL* fusion gene (Schrijver & Zehnder, 2020). The fusion of *BCR* at the 5' side in *ABL* alters the tightly regulated function of the Src homology 3 (SH3) domain, disabling control over the tyrosine kinase enzyme. The resulting chimeric BCR-ABL protein has constitutively elevated tyrosine phosphokinase activity (Kurzrock, Kantarjian, Druker, & Talpaz, 2003) that activates a number of downstream signaling molecules, including PI3K, AKT, JNK, RAS and STAT5 (Ren, 2005). This then disrupts cellular signal transduction pathways, leading to issues in the regulation of both apoptosis and cell proliferation (Warmuth, Danhauser-Riedl, & Hallek, 1999), ultimately leading to factor-independent and leukemogenic cell growth (Van Etten, 2019).

Detection of the Ph chromosome is the hallmark of CML and is found in up to 95 percent of patients (Leoni & Biondi, 2015). In approximately 5% of CML cases, the Ph chromosome cannot be detected, and *BCR-ABL1* formation is attributed to microscopically undetectable translocations or variant complex translocations involving a third chromosome (Schrijver & Zehnder, 2020). Independent of which other chromosomes are involved in variant translocations, the generation of the *BCR-ABL* fusion gene is the "fundamental cause of Ph-positive leukemias" (Van Etten, 2019), as the 210-KDa fusion protein BCR-ABL is essential for initiation, maintenance, and progression of CML (Ren, 2005). Testing for BCR-ABL1 detects both the Ph chromosome and fusion gene or its transcripts. The BCR-ABL1 transcript is the RNA copy made by the cell.

Depending on the precise breakpoints in the translocation and RNA splicing, different isoforms of BCR-ABL protein with different molecular weights (p185 BCR-ABL, p210 BCR-ABL and p230 BCR-ABL) can be generated (Ren, 2005). The p210 BCR-ABL isoform, which is the hallmark of CML and also found in one-third of those with Ph+ B-cell acute lymphoblastic leukemia (ALL), is generated from breakpoints in the major breakpoint cluster region (M-bcr) and results in the fusion of exons 13 or 14 from *BCR* with exon 2 of *ABL1* (Van Etten, 2020). A second isoform, p190 BCR-ABL, is generated from breakpoints 5' of the M-bcr within a segment called the minor breakpoint cluster region (m-bcr), and the resulting fusion of exon 1 of *BCR* gene with exon 2 of *ABL1* gene is associated with two-thirds of patients with Ph+ B-cell ALL and a minority of patients with CML (Van Etten, 2019; Verma et al., 2009). A third isoform from a breakpoint 3' from the M-bcr region (u-bcr) resulting in the fusion of exon 19

of the *BCR* gene and exon 2 of the *ABL1* gene is associated with the chronic neutrophilic leukemia variant and with thrombocytosis. These three isoforms display differential increased tyrosine kinase activity that may in part account for the distinct leukemias associated with the different fusions; moreover, they may predict responsiveness to therapy with tyrosine kinase inhibitors (Van Etten, 2019).

The discovery of BCR-ABL-mediated pathogenesis of CML provided the rationale for the design of an inhibitory agent that targets BCR-ABL kinase activity, which eventually led to the creation of tyrosine kinase inhibitors (TKIs) (Negrin & Schiffer, 2019). Protein kinases had been thought to be poor therapeutic targets because of their ubiquitous nature and crucial role in many normal physiologic processes. The development of imatinib mesylate (IM) by Novartis demonstrated that designer kinase inhibitors could be specific (Kurzrock et al., 2003). IM binds to the inactive configuration domain of BCR-ABL kinase, competitively inhibiting the adenosine triphosphate-binding site of the BCR-ABL oncoprotein (Negrin & Schiffer, 2019). IM has shown striking activity in chronic myelogenous leukemia (Kurzrock et al., 2003). By directly targeting the BCR-ABL kinase, IM leads to inhibition of cell proliferation and tumor formation without induction of apoptosis. IM has also been estimated to lead to a 92%-98% reduction of CML colonies without inhibiting normal colony growth (Negrin & Schiffer, 2019). Following the success of IM, other TKIs were developed. Commercially available TKIs for CML treatment are imatinib, dasatinib, nilotinib, bosutinib, and ponatinib. Other newer TKIs with higher potency and activity are also being produced to inhibit additional signaling pathways or overcome resistance (Negrin & Schiffer, 2019).

Molecular monitoring allows the detection of low levels of residual leukemia cells and provides important prognostic information for CML patients (Latremouille-Viau et al., 2017) as molecular responses are predictive of patient outcomes (Bauer & Romvari, 2012). Molecular monitoring during treatment with TKI helps determine whether a patient is responding optimally to treatment, helps identify those at risk of progression, and provides evidence regarding the need to reassess treatment or initiate second-line therapy (Hughes et al., 2006).

Treatment Failure

Despite the excellent efficacy and improved clinical responses, development of resistance in a significant proportion (30-35%) of CML patients on IM therapy has emerged (Ankathil, Azlan, Dzarr, & Baba, 2018). Mutations in the BCR-ABL kinase domain have been identified as the major contributory factor in resistance (O'Hare, Eide, & Deininger, 2007). Hence, *BCR-ABL* mutation analysis is an important component of disease monitoring in patients with clinical signs of resistance (Soverini et al., 2011). Mutation analysis is routinely performed using Sanger sequencing (SS) which is a method of DNA sequencing. However, next-generation sequencing (NGS), a high-throughput approach to DNA sequencing, has recently been utilized as a more sensitive and effective testing method. Compared to SS, NGS is able to detect a lower frequency and earlier existence of mutations, which allows for more effective therapeutic tailoring (Ankathil et al., 2018). *BCR-ABL* mutation screening is clinically relevant to identify CML patients who are more likely to have a poor outcome due to the disease. This is because mutations in different regions of the BCR-ABL tyrosine kinase domain lead to different levels of resistance (Smith et al., 2006) and the type of mutation can potentially indicate whether second- or third-generation TKIs or alternative therapeutic strategies should be given to IM-resistant patients (Milojkovic & Apperley, 2009). However, *BCR-ABL* tyrosine kinase domain (TKD) mutations cannot always explain IM resistance; therefore, additional resistance mechanisms should be addressed. Reduced bio-availability of IM in leukemic cells clonal chromosomal evolution, BCR-ABL amplification,

pharmacogenomic variations, as well as activation of signaling shortcuts, have all been implicated in drug resistance (Ankathil et al., 2018).

Analytical Validity

Molecular testing for the diagnosis of CML confirms typical findings in the blood and bone marrow by the demonstration of the Ph chromosome, the *BCR-ABL1* fusion gene or the BCR-ABL1 fusion mRNA. Molecular testing techniques include conventional cytogenetics, fluorescence in situ hybridization (FISH) analysis and reverse transcription polymerase chain reaction (RT-PCR) (Van Etten, 2020). Conventional cytogenetic karyotyping is no longer the diagnostic modality of choice due to its requirements for a highly skilled staff, culturing of cells, long turnaround time, and lower sensitivity (5-10%). Despite this, conventional cytogenetics are still the gold standard, and "should be performed" especially at diagnosis to detect additional clonal abnormalities (Yeung, Egan, & Radich, 2016). FISH is more sensitive (0.1-5%) than karyotyping and can be performed on peripheral blood in addition to bone marrow and tissue. FISH can detect certain very rare translocations not usually detectable by the vast majority of commercial and laboratory-developed RT-PCR assays, but FISH is highly specific to the targeted region and may miss other chromosomal changes. Quantitative RT-PCR is the most sensitive technique currently available (0.001-0.01% sensitivity).

The quantity of the BCR-ABL1 mRNA transcript is determined in relation to an endogenous control gene (such as *BCR*, *ABL1*, or *GUSB*) to control for specimen quality of the RNA and to obtain semi-quantitative results (Yeung et al., 2016). As differences in laboratory technique and control genes can make it difficult to compare PCR values among laboratories, an international effort to standardize quantitative RT-PCR (qRT-PCR) results led to the development of the International Scale (IS) (Hughes et al., 2006) to provide a common approach for reporting the results of qRT-PCR. The IS is anchored to two values: (1) a standardized baseline value of 100% and (2) a standardized major molecular response (MMR) value set at 0.1%, that is, a 3-log reduction from the standardized baseline (Bauer & Romvari, 2012). However, quantitative BCR-ABL1 transcript levels on the IS vary widely at diagnosis as the ABL1 standard produces unreliable results in samples with high BCR-ABL1 transcript levels, such as samples taken at or near diagnosis (Schiffer & Atallah, 2020).

Clinical trials of discontinuing TKI therapy after previously sustained undetectable BCR-ABL1 transcripts have shown that more than half of patients show evidence of molecular relapse within six months, indicating a population of expandable leukemic cells below the limit of detection of current methods (Mahon et al., 2010; Ross et al., 2013). Thus, there is continued research into more sensitive methods (Yeung et al., 2016).

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

Clinical Validity and Utility

Lima et al. (2011) "compared simultaneously obtained bone marrow (BM) cytogenetics (CTG), peripheral blood (PB) and BM fluorescence in situ hybridization (FISH), and quantitative real-time polymerase chain reaction (Q-PCR) for BCR-ABL1 in monitoring response to treatment with tyrosine kinase inhibitors and homoharringtonine (HHT) in patients with chronic myeloid leukemia." A total of 112 PB and BM FISH samples were obtained along with 132 qPCR samples. The authors found that "excellent correlations (r) were observed between PB and BM FISH (r = 0.95) and PB and BM Q-PCR (r = 0.87), as well as BM CTG and PB FISH (r = 0.89) and PB Q-PCR (r = 0.82)." Conclusions stated that "This correlation was not affected by the presence of the Ph+ variant or additional chromosomal abnormalities, the presence of ABL1 kinase domain mutations, phase of the disease, or treatment (Lima et al., 2011)."

Kantarjian et al. (2003) evaluated the response and minimal residual disease (MRD) of 180 patients with Ph+ chronic-phase, treated with imatinib mesylate by quantitative competitive PCR (QC-PCR). The MRD describes the low number of cancer cells still present in the body after treatment. The authors reported that "the median QC-PCR values for cytogenetic response categories were: no response (Ph, >90%), 36%; minor response (Ph, 35-90%), 22%; partial response (Ph, 1-34%), 7.3%; complete response (Ph, 0%), 0.89%. There was good correlation between cytogenetic and QC-PCR studies ($P < 0.001$; $r = 0.92$) and good concordance between QC-PCR values (>10%, 2-10%, and <2%) and cytogenetic response categories (none, minor, partial, complete) with a concordance rate of 66%, and major discordance of only 10%. Of 170 samples in complete cytogenetic response, 21% still had QC-PCR values of >10%, and 53% had QC-PCR values of <1%. There was excellent concordance between blood and marrow QC-PCR values ($r = 0.965$; $P < 0.01$; concordance rate, 88%; major discordance, 0%)." The authors concluded that "QC-PCR studies provide a useful tool to monitor patients with CML on imatinib mesylate therapy (Kantarjian et al., 2003)."

Goldberg et al. (2013) performed a retrospective chart review of 402 chronic myelogenous leukemia in chronic phase (CML-CP) patients on first-line imatinib therapy analyzing the impact of molecular monitoring frequency on the risk of progression and progression-free survival. The researchers found that "Compared to patients with no qPCR monitoring, those with 3-4 qPCR tests per year had a lower risk of progression (HR = 0.085; p = 0.001) and longer PFS [progression free survival] (HR = 0.088; p = 0.001) after adjusting for potential confounders, as did those patients with 1-2 qPCR tests per year (both $p < 0.02$) (Goldberg et al., 2013)."

Guerin, Chen, Dea, Wu, and Goldberg (2014a) performed a retrospective cohort study of 1205 diagnosed CML-CP patients obtained from two large US administrative claims databases. The researchers aimed to analyze the frequency of molecular monitoring via qPCR and medication adherence. Approximately 41% of patients had no qPCR tests, 31.9% had 1-2 tests, and 27.1% had 3-4 tests. Adherence to therapy was calculated by "medication possession ratio" (MPR) and "proportion of days covered" (PDC). The 3-4 test cohort was found to have higher adherence to therapy (higher MPR and PDC) than the 0 and 1-2 test cohort. The authors concluded that "frequent molecular monitoring (3-4 times per year as recommended in current guidelines) is associated with greater TKI treatment adherence for patients diagnosed with CML" (Guerin et al., 2014a).

Guerin, Chen, Dea, Wu, and Goldberg (2014b) also used a retrospective US claims administrative database to analyze the economic impact of qPCR testing in CML patients on first-line TKIs during the initial 12-months of treatment. A total of 41% of patients had no qPCR tests, 31.9% had 1-2 tests, and 27.1% had 3-4 tests. However, patients that had 3-4 tests also had 44% fewer inpatient admissions than patients with 0 tests, leading to $5663 in all-cause savings for the 3-4 test group. Overall, the

medical service cost savings was calculated to be $5997 for the 3-4 test group. The investigators concluded that "Among CML patients in two large claims databases, nearly three-quarters did not receive adequate molecular monitoring per published guidelines. Those who were more frequently monitored incurred lower medical service costs, with the majority of the difference in costs being related to disease progression. These findings underscore the clinical and economic values of molecular monitoring in CML (Guerin et al., 2014b)."

More recently, Latremouille-Viau et al. (2017) studied direct and indirect effects of qPCR test frequency using multivariate regression models. The authors created an economic model to evaluate the effect of qPCR test frequency on CML treatment in various clinical scenarios and reported their results as the increase from one qPCR test to two. The researchers found that increasing qPCR tests by one led to fewer inpatient days, fewer ER visits, more outpatient service days, and increased TKI adherence. The authors concluded that increasing the qPCR tests from 1 to 2 was associated with a cost savings of $2918 per patient per year (Latremouille-Viau et al., 2017).

Yu, Cui, He, Jing, and Wang (2017) conducted a review on the challenges for standardizing the measurement of *BCR-ABL1*. Though using the IS and conversion factors (CFs) were critical in facilitating "interlaboratory comparison and the process of standardization," the lack of more comprehensive reference materials, including other DNA "atypical subtypes relevant to minor patients with CML and other hematological disorders remain additional attention." This could lead to delays in diagnosis "when a screening test for the common e13a2 and e14a2 transcripts is negative. In other cases, the lack of precise response data that can be aligned with molecular response targets on the *BCR*-ABL1 International Scale (*BCR-ABL1*^{IS}) might limit the clinician's capacity to detect sub-optimal response or impending resistance and alter treatment accordingly" (Pagani et al., 2020).

In understanding the clinical application of *BCR-ABL1* measurement, Yu et al. (2017) also noted that the IRIS (International Randomized Study of Interferon and STI571) trial made successful use of *BCR-ABL1* (IS) to observe patient response to imatinib: "At 6 months, patients who obtained a *BCR-ABL1* (IS) <10% had an EFS [event-free survival] rate >85% at 84 months, much higher than those who did not… Patients who had loss of CCyR [complete cytogenetic response], MMR [major molecular response], or increasing levels of *BCR-ABL1* had increased rate of progression to AP/BC [accelerated phase/blast crisis]." Reviews of the IRIS study recognized how *BCR-ABL1* became critical for quantifying CCyR and MMR during treatment (Yu et al., 2017).

D'Adda et al. (2019) evaluated the effect of the BCR-ABL transcript on efficacy of TKIs. The *BCR*-ABL1 fusion gene may cause CML pathogenesis due to several breakpoints; the most common occur around exon 13 and 14 of the *BCR* gene and cause the formation of e13a2 and e14a2 transcripts (Greenfield et al., 2019). Out of 173 sampled patients, 67 had the e13a2 transcript, and 106 had the e14a2 version. The patients with the e14a2 version were more likely to achieve a deep molecular response to TKIs (sustained or otherwise). After 68 months, the sustained deep molecular response (sDMR) rate was 39.6% for e14a2 patients compared to 19.6% for e13a2 patients. Overall, the maximum rate of sDMR for e13a2 patients was 37%, after 60 months. Furthermore, only 2 patients (3%) with the e13a2 transcript achieved treatment-free remission (TFR) whereas 25 of e14a2 patients achieved TFR (23%) (D'Adda et al., 2019).

Dulucq et al. (2019) researched the impact of a second *BCR-ABL1* transcript decline rate on CML-CP patients treated with imatinib. The researchers analyzed the *BCR-ABL1* transcript decline rate from the three to six-month period after beginning treatment; *ABL1* was used as an internal control gene. A total of 216 CML-CP patients treated with 400 mg of imatinib participated in this study (Dulucq et

al., 2019). Data was sorted into the following categories: event-free (EFS), failure-free (FFS), progression-free (PFS), and overall survivals (OS). The researchers found that the "percentage of BCR-ABL1 decline from month 3 to month 6 was significantly linked to the EFS and the FFS ($p < 0.001$)"; further, the data also showed that "Patients with a decrease below 67% have worse EFS and FFS as compared to those having a higher decrease ($p < 0.001$) (Dulucq et al., 2019)."

Pagani et al. (2020) comments on how genomic DNA Q-PCR, a highly sensitive assay, was able to detect "a case of e19a2 CML with imatinib in association with a T315I kinase domain mutation" that required asciminib to elicit a "deep molecular response determined by patient-specific genomic DNA PCR." This demonstrates the continued, seemingly ubiquitous clinical utility of DNA Q-PCR in testing *BCR-ABL1* transcripts for treatment response patients with CML despite this case being of an unconventional transcript (Pagani et al., 2020).

V. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN) (NCCN, 2020, 2021a, 2021b)

Chronic Myeloid Leukemia (CML)

The NCCN's recommendations for CML include the following table (NCCN, 2021b):

Monitoring Response to TKI Therapy and Mutational Analysis	
Test	**Recommendation**
Bone marrow cytogenetics[1]	• At diagnosis • Failure to reach response milestones • Any signs of loss of response (defined as hematologic or cytogenetic relapse)
qPCR using IS	• At diagnosis • Every 3 months after initiating treatment. After *BCR-ABL1* (IS) ≤1%[2] has been achieved, every 3 months for 2 years and every 3 – 6 months thereafter • If there is 1-log increase in *BCR-ABL1* transcript levels with MMR, qPCR should be repeated in 1 – 3 months

BCR-ABL kinase domain mutation analysis	• Chronic phase
	  ○ Failure to reach response milestones
	  ○ Any sign of loss of response (defined as hematologic or cytogenetic relapse)
	  ○ 1-log increase in *BCR-ABL1* transcript levels and loss of MMR
	• Disease progression to accelerated or blast phase[3]

[1]FISH has been inadequately studied for monitoring response to treatment. [2]CCyR correlates with *BCR-ABL1* (IS) ≤1%. [3]Consider myeloid mutation panel to identify BCR-ABL1–independent resistance mutations in patients with no BCR-ABL1 kinase domain mutations	

The NCCN (2021b) also states that for the diagnosis and workup of CML-1:

- "Initial evaluation should consist of a history and physical exam, including palpation of spleen, complete blood count (CBC) with differential, chemistry profile, and hepatitis B panel. Bone

marrow aspirate and biopsy for morphologic and cytogenetic evaluation and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to establish the presence of quantifiable *BCR-ABL1* mRNA transcripts at baseline are recommended to confirm the diagnosis of CML.

- Bone marrow cytogenetics should be done at initial workup to detect additional chromosomal abnormalities in Ph-positive cells (ACA/Ph^+), also known as clonal cytogenetic evolution. If bone marrow evaluation is not feasible, fluorescence *in situ* hybridization (FISH) on a peripheral blood specimen with dual probes for *BCR* and *ABL1* genes is an acceptable method to confirm the diagnosis of CML."

Acute Lymphoblastic Leukemia (ALL)

Regarding ALL, the NCCN (NCCN, 2021a) recommends testing of marrow or peripheral blood lymphoblasts using various techniques. The guidelines state the following:

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

- Morphologic assessment of Wright-Giemsa-stained bone marrow aspirate smears, and H&E-stained core biopsy and clot sections
- Comprehensive flow cytometric immunophenotyping
- Baseline flow cytometric and/or molecular characterization of leukemic clone to facilitate subsequent minimal/measurable residual disease analysis
- Karyotyping of G-banded metaphase chromosomes

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

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

Pediatric Acute Lymphoblastic Leukemia (PEDALL)

In 2019, the NCCN first issued a new set of guidelines titled *Pediatric Acute Lymphoblastic Leukemia*. These guidelines have been updated since the original issue date, with the most recent revision having been on October 22, 2020. For PEDALL, the NCCN encourages "testing for gene fusions and mutations associated with *BCR*-ABL1-like (Ph-like) ALL (NCCN, 2020)." The guidelines also state that "The *BCR-ALB1*-like (Ph-like) phenotype is associated with recurrent gene fusions and mutations that activate tyrosine kinase pathways and includes gene fusions involving *ABL1, ABL2, CRLF2, CSF1R, EPOR, JAK2 or PDGFRB* and mutations involving *CRLF2, FLT3, IL7R, SH2B3, JAK1, JAK3, and JAK2* (in combination

with *CRLF2* gene functions). Testing of these abnormalities at diagnosis may aid in risk stratification (NCCN, 2020)."

The NCCN table of potentially actionable or prognostic mutations for PEDALL is given below (NCCN, 2020):

Risk Groups	Genetics
Favorable risk features	High hyperdiploidy (51 – 67 chromosomes) • Trisomy of chromosomes 4, 10, and 17 are among trisomies that have the most favorable outcome
	Cryptic t(12;21)(p13;q22): • *ETV6-RUNX1* fusion
Unfavorable risk features	Hypodiploidy (<44 chromosomes)
	KMT2Ar (t[4;11] or others)
	t(9;22)(q34;q11.2) • *BCR-ABL1*
	BCR-ABL1-like (Ph-like) ALL • JAK-STAT ○ *CRLF2r* ○ *EPORr* ○ *JAK1/2/3r* ○ *TYK2r* ○ *SH2B3* mutations ○ *IL7R* mutations ○ *JAK1/2/3* mutations • ABL class rearrangements of: ○ *ABL1* ○ *ABL2* ○ *PDGFRA* ○ *PDGFRB* ○ *FGFR* • Other ○ *NTRKr* ○ *FLT3r* ○ *LYNr* ○ *PTK2Br*
	t(17;19) • *TCF3-HLF* fusion
	Intrachromosomal amplification of chromosome 21 (*iAMP21*)
	Alterations of *IZKF1*‡

‡ NOTE: The NCCN states, "Emerging evidence suggests *DUX4r* ALL is favorable. Additionally in cases of *DUX4r, IZKF1* alterations do not confer poor prognosis (NCCN, 2020)."

European LeukemiaNet (Baccarani et al., 2013; Hochhaus et al., 2020):

The European LeukemiaNet (a publicly funded research network) convened an expert panel to evaluate recommendations for treating CML. These guidelines were published in 2013 and were recently updated in 2020.

In 2013, Baccarani et al. (2013) published the following guidelines on behalf of the European LeukemiaNet:

- "Molecular testing must be performed by RQ-PCR on buffy-coat of more than 10 mL of blood, to measure BCR-ABL1 transcripts level, which is expressed as BCR-ABL1% on the IS. RQ-PCR should be performed every 3 months until a major molecular response (MMR, $MR^{3.0}$ or better) is achieved, then every 3 to 6 months. It is not possible to assess achievement of MMR if the International Scale (IS) is not available. However, if transcripts are not detectable with a threshold sensitivity of 10^{-4}, this is likely in the range of MMR or below. It is important to realize that it is not unusual for PCR results to fluctuate up and down over time, in part because of laboratory technical reasons. If transcript levels have increased >5 times in a single follow-up sample and MMR was lost, the test should be repeated in a shorter time interval, and patients should be questioned carefully about compliance."
- "If cytogenetics is used, it must be performed by chromosome banding analysis (CBA) of marrow cell metaphases, counting at least 20 metaphases, at 3, 6, and 12 months until a complete cytogenetic response (CCyR) is achieved, and then every 12 months. CBA can be substituted by FISH on blood cells only when a CCyR has been achieved."
- "In case of warning, it is recommended to repeat all tests, cytogenetic and molecular, more frequently, even monthly."
- "In case of treatment failure or of progression to AP or BP, cytogenetics of marrow cell metaphases, PCR, and mutational analysis should be performed."

In 2020, Hochhaus et al. (2020) published the following guidelines on behalf of the European LeukemiaNet:

- "Good quality molecular testing is now available worldwide replacing cytogenetic monitoring in most situations and obviating the need for bone marrow aspirations. If a change in therapy is under consideration because of inadequate response or disease progression (drug resistance), a bone marrow aspiration is recommended to assess for cytogenetic clonal evolution. *BCR-ABL1* KD-mutation analysis should also be done in such circumstances.
- Treatment should be managed in cooperation with a specialized referral center where there is rapid access to quality-controlled, reliable tests including chromosome banding analysis (CBA), fluorescence in situ hybridization (FISH) for specific cases, and quantitative reverse transcriptase polymerase chain reaction (qPCR) with mutation analysis (Sanger or next generation sequencing, NGS).

- Cytogenetics should be performed by CBA of Giemsa-stained metaphases from bone marrow cells. A qualitative reverse transcriptase PCR on peripheral blood cells is mandatory to identify the type of BCR-ABL1 transcripts that can be appropriately followed when assessing response to TKI therapy. About 2–4% of patients harbor atypical BCR-ABL1 transcripts lacking ABL1 exon2 (e13a3 or e14a3) or resulting from atypical BCR breakpoints (e.g., e1a2, e6a2, e8a2, or e19a2) that may yield a false negative PCR using routine primer/probe sets in qualitative or quantitative reverse transcriptase PCR protocols. If not tested at diagnosis, a false impression may be given that a patient is in complete molecular response after TKI treatment. A quantitative PCR is not mandatory at diagnosis. If a molecular assay demonstrates BCR-ABL1, but the Ph-chromosome cannot be identified by cytogenetics, a FISH test is required.
- Of importance, more than 80% of recurrences occur within the first 6–8 months after stopping emphasizing the need for frequent monitoring and structured follow-up during this early period. Stopping treatment is a safe procedure at centers with access to high-quality molecular monitoring and with careful patient selection.
- Progression to advanced phase is rare (<2 per thousand patient-years with MMR). Continued molecular monitoring is nevertheless recommended indefinitely."

World Health Organization (WHO) (Arber et al., 2016)

The WHO published guidelines on the classification of myeloid neoplasms and acute leukemia. In the recent revision of the 4th edition on Classification of Tumors of Hematopoietic and Lymphoid issues, World Health Organization (WHO) incorporated new molecular genetic findings and clinical data into its classification of acute leukemias (Arber et al., 2016).

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

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

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

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

College of American Pathologists (CAP) and American Society of Hematology (ASH) (Arber et al., 2017)

Following recent progress in molecular genetic findings and 2016 WHO classification of acute leukemias, the College of American Pathologists (CAP) and the American Society of Hematology (ASH) have formed an expert panel to review and establish guidelines for appropriate laboratory testing (Arber et al., 2017). The published guideline provides twenty-seven guideline statements ranging from recommendations on what testing is appropriate for the diagnostic and prognostic evaluation of leukemias to where the testing should be performed and how results should be reported.

13. For pediatric patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(12;21)(p13.2;q22.1); ETV6- RUNX1, t(9;22)(q34.1;q11.2); BCR-ABL1, KMT2A(MLL) translocation, iAMP 21, and trisomy 4 and 10 is performed. Strong Recommendation

14. For adult patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2) ; BCR-ABL1 is performed. In addition, testing for KMT2A (MLL) translocations may be performed. Strong Recommendation for testing for t(9;22) (q34.1;q11.2) and BCR-ABL1; Recommendation for testing for KMT2A (MLL) translocations may be performed.

European Society for Medical Oncology (ESMO) (Hochhaus et al., 2017; Hoelzer et al., 2016)

Chronic Myeloid Leukemia (CML)

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

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

Test	Baseline (diagnostic work-up)	To assess the response	To monitor the response and the treatment
Blood counts and differential	Yes	Every 15 days until a CHR [complete hematological response] without significant cytopaenias has been achieved	Every 3 months
BM [bone marrow], cytology	Yes	No	No
BM, karyotype	Yes	At 3 and 6 months	Then every 6 months until CCyR [complete

			cytogenetic response] has been achieved
Blood, iFISH	No	No	Only if cytogenetics of BM metaphases cannot be analyzed or is normal and molecular response cannot be assessed
Blood, RT-PCR (qualitative)	Yes	No	No
Blood, qRT-PCR (quantitative, BCR–ABL %)	No	Every 3 months	Every 4–6 weeks in first year after treatment discontinuation
Mutational Analysis	Only in AP [accelerated phase] or BP [blast phase]	No	Only in the case of failure

The ESMO has also stated that a "diagnosis must be confirmed by cytogenetics showing t(9;22)(q34;q11), and by multiplex RT-PCR showing BCR–ABL1 transcripts" (Hochhaus et al., 2017). Other warning signs include "Major route cytogenetic aberrations (+8, iso(17q), +19, +22q-), chromosome 3 aberrations and BM fibrosis at diagnosis signs"; further, a quantification of BCR-ABL mRNA is required every 3 months (Hochhaus et al., 2017). Finally, the ESMO acknowledges that mutation analysis is "due" in case of failure of first-line therapy or if BCR-ABL transcript levels increase. However, ESMO recommends against baseline mutational analysis in patients with newly diagnosed CML-CP (Hochhaus et al., 2017).

Acute Lymphoblastic Leukemia (ALL)

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

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

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

VI. State and Federal Regulations, as applicable

A. Food and Drug Administration (FDA)

A search of the FDA database on 05/05/2021 using the term "*BCR-ABL*" yielded 3 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as

high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

On September 27, 2019 the FDA approved Cepheid's Xpert BCR-ABL Ultra, GeneXpert Dx System which is classified as a BCR-ABL1 monitoring test. The FDA states that this type of test is "a quantitative in vitro diagnostic device used to monitor the BCR/ABL1 to ABL1 ratio by reverse-transcriptase quantitative polymerase chain reaction (RQ-PCR) on whole blood or bone marrow of diagnosed Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML) patients expressing BCR-ABL1 fusion transcripts such as e13a2 and/or e14a2. It is intended for use during monitoring of treatment response by reporting results on the international scale (%IS) and as log molecular reduction (MR) value (FDA, 2019a)."

On July 22, 2016 the FDA approved the QuantideX qPCR BCR-ABL IS Kit as an *in vitro* nucleic acid amplification test for the quantitation of BCR-ABL1 and ABL1 transcripts in total RNA from whole blood of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2 as a class II device with special controls (FDA, 2016). Then, on February 13, 2019, the FDA approved the updated QXDx BCR-ABL %IS Kit from Biorad for use on the QXdx AutoDG ddPCR System to test RNA transcripts from whole blood (FDA, 2019b).

On December 22, 2017 the FDA approved the MolecularMD MRDx BCR-ABL Test as an in vitro diagnostic test for the quantitative detection of BCR-ABL1 transcripts (e13a2/b2a2 and/or e14a2/b3a2) and the ABL1 endogenous control mRNA in peripheral blood specimens from patients previously diagnosed with t(9:22) positive chronic myeloid leukemia (CML) as substantially equivalent (FDA, 2017).

B. Centers for Medicare & Medicaid Services (CMS)

L36044 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease - https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?lcdid=36044&ver=53&bc=0

VII. L38047 MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies - https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?lcdid=38047&ver=9&bc=0Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81170	ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase) (eg, acquired imatinib tyrosine kinase inhibitor resistance), gene analysis, variants in the kinase domain
81206	BCR/ABL1 (t(9:22)) (eg, chronic myelogenous leukemia) translocation analysis; major breakpoint, qualitative or quantitative
81207	Minor breakpoint, qualitative or quantitative
81208	Other breakpoint, qualitative or quantitative

Code Number	Code Description
81401	Molecular pathology procedure, Level 2 (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat) –GENES: ***ABL*** (c-abl oncogene 1, receptor tyrosine kinase) (e.g., acquired imatinib resistance), T315I variant
0016U	Oncology (hematolymphoid neoplasia), RNA, BCR/ABL1 major and minor breakpoint fusion transcripts, quantitative PCR amplification, blood or bone marrow, report of fusion not detected or detected with quantitation **Proprietary test:** QuantideX® qPCR BCR-ABL Test **Lab/Manufacturer:** University of Iowa, Department of Pathology, Asuragen
0040U	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis, major breakpoint, quantitative **Proprietary test:** MRDx® BCR-ABL Test **Lab/Manufacturer:** MolecularMD

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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