Template for Reporting Results of Monitoring Tests for Patients With Chronic Myelogenous Leukemia (*BCR-ABL1+*)

This biomarker template is NOT required for accreditation purposes

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With guidance from the CAP Cancer Biomarker Reporting Committee

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Summary of Changes

Added note:

All cytogenetic variations should be reported using the International System for Human Cytogenetic Nomenclature (ISCN) and gene sequence variations should be reported following the recommendations of the Human Genome Variation Society (http://varnomen.hgvs.org/; accessed May 9, 2017).

CML Biomarker Reporting Template

Template posting date: June 2017

Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team. **This template is not required for accreditation purposes.**

CHRONIC MYELOGENOUS LEUKEMIA, (*BCR-ABL1+*) MONITORING

Report a single response to each data element unless otherwise indicated.

+ SPECIMEN TYPE
+ ___ Peripheral blood
+ ___ Bone marrow
+ ___ Other (specify): _____

+ RESULTS
Note: If a marker is tested by more than one method (eg, polymerase chain reaction and immunohistochemistry), please document the additional result(s) and method(s) in the Comments section of the report.

+ Cytogenetic Testing Results (karyotype) (select all that apply)
+ ___ No abnormalities detected
+ ___ t(9;22)(q34;q11.2); *BCR-ABL1* (Philadelphia chromosome [Ph]) detected
    + Total number of metaphases examined: _____
    + Number of Ph+ metaphases: _____
+ ___ Other abnormalities detected (specify): _____

+ *BCR-ABL1* Testing by Fluorescence In Situ Hybridization (FISH) (select all that apply)
+ ___ No *BCR-ABL1* fusions detected
+ ___ *BCR-ABL1* fusions detected
    + Total number of cells examined: _____
    + Number of *BCR-ABL1* positive cells: _____
+ ___ *BCR-ABL1* amplification (duplication) detected (copies/cell): _____

+ Quantitative *BCR-ABL1* Transcript Reverse Transcription Polymerase Chain Reaction (RT-PCR) Testing (select all that apply)
+ ___ No *BCR-ABL1* transcripts detected
+ ___ *BCR-ABL1*, p210 type (e13/14a2) transcripts detected
    + Normalized copy number (e13/14a2 transcripts/reference gene): _____
    + Percent *BCR-ABL1* (International Scale [IS]): _____%
+ ___ *BCR-ABL1*, p190 type (e1a2) transcripts detected
    + Normalized copy number (e1a2 transcripts/reference gene): _____

+ ___ Other *BCR-ABL1* transcripts detected (ie. e19a2; p230 type)
    (specify): _____
    + Normalized copy number (other *BCR-ABL1* transcripts/reference gene): _____

+ *BCR-ABL1* Mutation Analysis
+ ___ No mutation detected

+ Data elements preceded by this symbol are not required for accreditation purposes.

+ ___ Mutation(s) detected
  + ___ p.T315I mutation
  + ___ Other (specify): _____
  + Significance of mutation:
      + ___ Reported to confer resistance to tyrosine kinase inhibitors (TKIs)
      + ___ Unknown resistance profile
      + ___ Normal sequence variant and/or not associated with resistance

+ Comparison to Prior Studies
+ Date of most recent cytogenetic study: _____
+ Most recent cytogenetic results (select all that apply):
  + ___ No abnormalities detected
  + ___ t(9;22)(q34;q11.2); *BCR-ABL1* (Philadelphia chromosome [Ph]) detected
      + Total Number of metaphases examined: _____
      + Number of Ph+ metaphases: _____
  + ___ Other abnormalities detected (specify): _____

+ Date of most recent FISH study: _____
+ Most recent FISH results (select all that apply):
  + ___ No *BCR-ABL1* fusions detected
  + ___ *BCR-ABL1* fusions detected
      + Total number of cells examined: _____
      + Number of *BCR-ABL1* positive cells: _____
  + ___ *BCR-ABL1* amplification (duplication) detected (copies/cell): _____

+ Date of most recent *BCR-ABL1* quantitative RT-PCR study: _____
+ Most recent *BCR-ABL1* quantitative RT-PCR results (select all that apply):
  + ___ No *BCR-ABL1* transcripts detected
  + ___ *BCR-ABL1* (e13/14a2; p210 type) transcripts detected
      + e13/14a2 normalized copy number: _____
      + Percent *BCR-ABL1* (International Scale [IS]): _____%
  + ___ *BCR-ABL1* (e1a2; p190 type) transcripts detected
      + e1a2 normalized copy number: _____
  + ___ Other *BCR-ABL1* transcripts detected (specify type): _____
      + Normalized copy number: _____

+ METHODS

+ Quantitative *BCR-ABL1* Transcript RT-PCR Testing
+ *BCR-ABL1* RT-PCR assay sensitivity: _____
+ Fusion transcripts covered
  + ___ e13/14a2 (p210)
  + ___ e1a2 (p190)
  + ___ Other (specify): _____

+ *BCR-ABL1* Mutation Analysis
+ *BCR-ABL1* mutation analysis assay sensitivity: _____
+ *BCR-ABL1* mutation analysis assay coverage
  +*ABL1* codons _____ through _____ or list: _____
+ *BCR-ABL1* mutation analysis method:
  + ___ Sanger sequencing
  + ___ Pyrosequencing
  + ___ Allele specific-PCR
  + ___ DHPLC
  + ___ Next-generation (massively parallel) sequencing
  + ___ Other (specify): _____

+ *BCR-ABL1* reference sequence accession number: _____

+ COMMENT(S)

_____

_____

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed February 10, 2015).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed February 10, 2015).

All cytogenetic variations should be reported using the International System for Human Cytogenetic Nomenclature (ISCN) and gene sequence variations should be reported following the recommendations of the Human Genome Variation Society (http://varnomen.hgvs.org/; accessed May 9, 2017).

Explanatory Notes

Chronic myelogenous leukemia (CML) is characterized by the presence of an abnormal clonal myeloid population harboring t(9;22)(q34;q11.2) (known as the Philadelphia chromosome [Ph]) resulting in the presence of *BCR-ABL1* mRNA transcripts and an abnormal fusion protein with constitutive ABL1 tyrosine kinase activity. Detection and monitoring of t(9;22)(q34;q11.2) or *BCR-ABL1* fusion transcripts by a variety of laboratory methods, including classical cytogenetic karyotyping, fluorescence in situ hybridization (FISH), and quantitative reverse transcription polymerase chain reaction (qRT-PCR) provides an effective way to assess the response to tyrosine kinase inhibitor (TKI) therapy. Furthermore, these techniques provide a mechanism for the early detection of emerging TKI resistance and for identifying newly acquired genetic abnormalities that may be associated with transformation to a more aggressive phase of disease or with resistance to particular TKIs. Clear, concise, and accurate reporting of results is extremely important for effective clinical management. The National Comprehensive Cancer Network (NCCN) publishes extensive clinical guidelines for appropriate laboratory monitoring of CML patients to ensure accurate characterization of the hematologic, cytogenetic and molecular response to therapy.[1] The NCCN guidelines continue to evolve and should be consulted for the most up-to-date recommendations.

Cytogenetic analysis is typically performed at diagnosis and at certain intervals during treatment with TKIs, particularly if there is evidence of a suboptimal therapeutic response or evidence of emerging resistance. Although karyotyping is the least sensitive method for detecting t(9;22)(q34;q11.2), it is essential for establishing the depth of the cytogenetic response to therapy and for assessing whether or not important therapeutic milestones have been met. Cytogenetic analysis is also critical for the detection of additional abnormalities that are commonly present at disease progression such as trisomy 8 (+8) or isochromosome 17q [i(17q)], among others. Reporting of the cytogenetic results should include both the total number of metaphases examined and the number of Ph+ metaphases as well as any additional abnormalities that are identified. FISH for *BCR-ABL1* fusions is often used as an adjunct to karyotyping due to the increased sensitivity of the technique. It may also allow for the detection of rare cryptic translocations that are otherwise undetectable by karyotyping. It is important to report FISH results with the total number of cells analyzed along with the number of *BCR-ABL1*-positive cells. FISH is also important for detecting genomic duplication or amplification of the *BCR-ABL1* locus, which may contribute to TKI resistance in a subset of CML patients.

BCR-ABL1 qRT-PCR testing is the most sensitive method for the detection and monitoring of the abnormal fusion transcripts and may be performed on peripheral blood or bone marrow samples. Unless otherwise clinically indicated, it is not necessary to obtain bone marrow specifically for molecular testing. In an effort to promote the standardization of qRT-PCR reporting and the interlaboratory comparison of test results, a standardized reporting scale, known as the International Scale (IS) was introduced and has been widely adopted by laboratories worldwide.[2] Serial testing of patients by qRT-PCR during TKI therapy allows for the accurate assessment of important molecular treatment milestones. Importantly, both the depth and the kinetics of the response are critical for the evaluation of therapeutic efficacy and for the assignment of overall prognosis.[3] A major molecular response (MMR) is defined as *BCR-ABL1* qRT-PCR values ≤0.1% IS, a 3-log reduction from the standardized baseline. A complete molecular response (CMR) is defined as undetectable *BCR-ABL1* levels using a test with 4.5-log sensitivity. The definition of CMR highlights the importance of test performance characteristics such as sensitivity. In order to evaluate the response kinetics it is necessary to place current results in the appropriate clinical context using the clinical history and the results of prior testing. For simplicity, this reporting template includes space for only a single prior test result, but this issue may be revisited in future template updates.

CML patients undergoing treatment with TKIs may manifest signs of therapeutic resistance in a variety of ways, including progression to accelerated or blast phase, failure to achieve timely cytogenetic or molecular milestones, or with signs of the loss of a previously achieved response. A subset of patients may acquire resistance to TKI therapy due to substitution mutations in the translocated *ABL1* kinase domain. Specific mutations may impart resistance to certain, but not other, kinase inhibitors. Because the choice of subsequent TKI therapy depends on the identity of the mutation detected, it is important to report this information clearly in terms of the amino acid change (ie, p. F359V). In most current studies, the most commonly detected mutation in resistant CML patients is p.T315I,[4] an abnormality which promotes resistance to all but one of the currently approved TKIs (as of January 2014). A number of germline polymorphisms also occur in the *ABL1* kinase domain and should not be confused

with true resistance mutations.[5] Insertion and deletion type mutations (in/dels) occur as well but have uncertain clinical significance.[6] Rare mutations are identified in signaling domains in the translocated *ABL1* sequence called the Src homology-2 (SH2) and Src homology-3 (SH3) domains. Most current *BCR-ABL1* mutation tests are focused on the kinase domain and do not provide information on potential SH2/SH3 mutations, but certain rare mutations in these domains have also been reported to confer resistance to TKI therapy.[7]

References

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology – Chronic Myelogenous Leukemia. Version 2.2014. www.nccn.org. Accessed January 7, 2014.
2. Muller MC, Cross NC, Erben P, et al. Harmonization of molecular monitoring of CML therapy in Europe. *Leukemia*. 2009;23:1957-1963.
3. Marin D, Hedgley C, Clark RE, et al. Predictive value of early molecular response in patients with chronic myeloid leukemia treated with first-line dasatinib. *Blood*. 2012;120:291-294.
4. Branford S, Melo JV, Hughes TP. Selecting optimal second-line tyrosine kinase inhibitor therapy for chronic myeloid leukemia patients: does the BCR-ABL mutation status really matter? *Blood*. 2009;114:5426-5435.
5. Ernst T, Hoffman J, Erben P, et al. ABL single nucleotide polymorphisms may masquerade as BCR-ABL mutations associated with resistance to tyrosine kinase inhibitors in patients with chronic myeloid leukemia. *Haematologica*. 2008;93:1389-1393.
6. O'Hare T, Zabriskie MS, Eide CA, et al. The BCR-ABL35ins insertion/truncation mutant is kinase-inactive and does not contribute to tyrosine kinase inhibitor resistance in chronic myeloid leukemia. *Blood*. 2011;118:5250-5254.
7. Sherbenou DW, Hantschel O, Kaupe I, et al. BCR-ABL SH3-SH2 domain mutations in chronic myeloid leukemia patients on imatinib. *Blood*. 2010;116:3278-3285.