Overview

Improving the Quality of Quantitative Real-Time Polymerase Chain Reaction Laboratory Reporting in Chronic Myeloid Leukemia

Carlos E. Vigil, MD,1 Elizabeth A. Griffiths, MD,1 Eunice S. Wang, MD,1 Meir Wetzler, MD, FACP1*

ABSTRACT

Molecular pathologists play a central role in monitoring disease response in patients with chronic myeloid leukemia (CML). Imatinib, nilotinib, and dasatinib regimens have been shown to be highly effective therapies in the management of this disease. Optimal benefit from these agents depends on regular molecular monitoring of disease burden, achieved through measurements of transcript levels of the BCR-ABL chimeric gene. Major molecular response is a key milestone that correlates with improved long-term outcomes and has recently been shown to predict increased survival. Because of the clinical importance of accurate BCR-ABL monitoring, laboratory reports of BCR-ABL levels must be accurate, relevant, and readily understood by physicians. We discuss herein the key areas for optimizing BCR-ABL reports and emerging technologies for standardization and automation of these processes.

Keywords: Chronic myeloid leukemia, tyrosine kinase inhibitors, BCR-ABL transcripts, molecular monitoring, molecular response, International Scale

Tyrosine kinase inhibitors (TKIs) play a central role in the treatment of CML. Currently, 3 TKIs—imatinib, nilotinib, and dasatinib—are approved by the United States Food and Drug Administration for the treatment of CML. The goal of TKI therapy is to prolong survival by preventing transformation of the disease from the chronic phase to an advanced stage known as the blast phase, at which point median survival is 6 months.1 CML treatment guidelines issued by the National Comprehensive Cancer Network (NCCN) and the European LeukemiaNet (ELN) represent an authoritative view on effective treatment and monitoring2,3 and emphasize that favorable outcomes rely on close monitoring of treatment response, with adjustments in therapy when patients’ recovery levels do not meet treatment goals.

The categories of treatment response, in order of increasing sensitivity, are hematologic, cytogenetic, and molecular (Figure 2).2,4 The improved efficacy of TKIs relative to previous standards of care has necessitated the use of increasingly sensitive laboratory monitoring techniques; hence, molecular response has been the focus of much clinical research. Molecular response involves measurement of the transcript levels of BCR-ABL using quantitative real-time polymerase chain reaction (qRT-PCR). Quantitative assessment of BCR-ABL transcript levels by qRT-PCR has been validated as a surrogate marker of response to treatment5 and has become a critical tool in the evaluation of responses to TKI therapy.

The molecular basis of chronic myeloid leukemia (CML) is well understood. Central to CML pathology is the BCR-ABL chimeric gene, formed by a reciprocal translocation that brings the breakpoint cluster gene (BCR; OMIM accession number 151410) of chromosome 22 adjacent to the c-abl oncogene 1 (ABL1; OMIM accession number 189980) on chromosome 9, producing the Philadelphia chromosome (Ph) (Figure 1). The resulting BCR-ABL gene encodes a constitutively active tyrosine kinase, which is the fundamental cause of CML.1

The categories of treatment response, in order of increasing sensitivity, are hematologic, cytogenetic, and molecular (Figure 2).2,4 The improved efficacy of TKIs relative to previous standards of care has necessitated the use of increasingly sensitive laboratory monitoring techniques; hence, molecular response has been the focus of much clinical research. Molecular response involves measurement of the transcript levels of BCR-ABL using quantitative real-time polymerase chain reaction (qRT-PCR). Quantitative assessment of BCR-ABL transcript levels by qRT-PCR has been validated as a surrogate marker of response to treatment5 and has become a critical tool in the evaluation of responses to TKI therapy.

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Abbreviations

CML, chronic myeloid leukemia; ABL1, c-abl oncogene 1; BCR, breakpoint cluster gene; Ph, Philadelphia chromosome; NCCN, National Comprehensive Cancer Network; ELN, European LeukemiaNet; qRT-PCR, quantitative real-time polymerase chain reaction; MMR, major molecular response; IRIS, International Randomized Study of Interferon and STI571; CMR, complete molecular response; EAC, Europe Against Cancer; ABL, Abelson; B2M, beta-2-microglobulin; GUSB, beta-glucuronidase; IS, International Scale; CAP, College of American Pathologists; TKI, tyrosine kinase inhibitor; LOD, limit of detection; NCN, normalized copy number

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Importance of Assessing Molecular Response

The key response milestone, major molecular response (MMR), was defined in the pivotal International Randomized Study of Interferon and STI571 (IRIS) of imatinib versus interferon plus cytarabine as a 3-log or greater reduction in BCR-ABL transcripts from a standardized baseline.6,7 Data continue to be reported supporting the clinical importance and defining the prognostic value of achieving MMR. In the IRIS trial, patients who achieved MMR by 18 months and who did not progress to advanced disease during the subsequent 7 years demonstrated a 7-year event-free survival of 95%.8 In the German CML Study IV, patients who had achieved MMR by 12 months had significantly prolonged progression-free survival and improved overall survival at 3 years.9 Further, in the recently reported frontline studies of nilotinib and dasatinib, no patient who had achieved MMR displayed progression to advanced disease.10,11

Complete molecular response (CMR) is used to describe a response with BCR-ABL transcripts below the level of detection,4 currently a 4.5- to 5-log reduction. The prognostic implications of CMR have not yet been established, to our knowledge; achievement of CMR has been reported only recently12-14 as a clinical trial end point in studies of dasatinib and nilotinib in the frontline setting. Ongoing clinical trial data suggest that CMR may be of considerable value in identifying patients who may be candidates for discontinuation of TKI therapy.15

Serial BCR-ABL monitoring provides a measure of the response to treatment, enabling rapid identification of patients with suboptimal response, loss of response, treatment failure (eg, due to primary or acquired resistance to current therapy), or lack of adherence to treatment and is used to guide clinical decisions for patient management.16-18 The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines for Patients) and ELN recommendations encourage frequent monitoring of BCR-ABL transcript levels using qRT-PCR—that is, every 3 months until patients achieve complete cytogenetic response (NCCN Guidelines) or MMR (ELN recommendations).2,3 Subsequent molecular monitoring is recommended every 3 or 6 months.
Molecular pathologists play a key role in advancing the management of CML. Appropriate medical care for patients with CML can be compromised if molecular monitoring is inaccurate. If this test incorrectly indicates a stable or deepening molecular response while, in fact, the transcript level is rising due to the emergence of resistance or lack of adherence, treatment failures may be missed. Conversely, the inaccurate identification of a rising BCR-ABL transcript level may result in unnecessary use of health care resources, including physician time to evaluate adherence, additional diagnostic procedures such as bone marrow biopsy, and costly evaluations, including cytogenetic and mutation testing. Also, such errors could result in unnecessary alterations to effective therapy.

**Status of Molecular Monitoring**

The frequency of molecular monitoring underscores the importance of accurate assays and clear laboratory reports. To be practical and to provide useful data, these reports must provide health care professionals with routine access to reliable qRT-PCR assays for serial measurements of BCR-ABL transcripts.

Two groups have noted the need for well-vetted housekeeping genes to use as internal controls for qRT-PCR. The Europe Against Cancer (EAC) program evaluated 14 genes and selected 3 of them—Abelson (ABL), beta-2-microglobulin (B2M), and beta-glucuronidase (GUSB; OMIM accession number 611499)—as being most suitable. Wang and colleagues examined 9 genes, selecting GUSB as the best internal control, used with the primer/probe set from Life Technologies Corporation (Carlsbad, CA). In contrast to the EAC group, Wang et al do not recommend ABL; they attribute differences in findings to the use of different primer sets in the 2 studies.

There is a clear need for standardization of BCR-ABL testing because variations exist in the methods and materials used by different laboratories. Such variability may potentially be introduced at every stage of the qRT-PCR assay, from sample preparation and transportation through data analysis and report generation. These factors may make it difficult to compare results between laboratories and even within the same laboratory, which impacts the validity of serial testing. Standardization of molecular monitoring is of great importance. Determination of MMR is dependent on the International Scale (IS) and, notably, the IS facilitates the “portability” of a patient’s molecular response data, allowing comparison of BCR-ABL transcript levels between diagnostic laboratories (eg, if a patient has to relocate and transfers to a new physician).

**Reporting Results from Molecular Monitoring**

Differences in reporting results can limit the ability to compare results between laboratories, as well as between clinical studies and the results obtained in clinical practice. BCR-ABL transcript levels are reported in several different ways (Table 1). The development of the IS for reporting BCR-ABL transcript levels enables all laboratories to report on a single scale. This scale is “anchored” to 2 predefined values: 100% on the IS corresponds to the baseline BCR-ABL level established in the IRIS trial, and a 3-log reduction is defined as 0.10% of the standardized baseline (ie, MMR). To convert locally determined BCR-ABL levels to the IS, a conversion factor specific to each local laboratory may be calculated using a set of certified samples after having verified BCR-ABL levels; these standard samples could potentially be plasmids, cell extracts, or lyophilized cells. Finally, the IS value is determined by BCR-ABL (local value × conversion factor = BCR-ABL (IS)). Ideally, internationally certified, commercially produced reference standards would be used as controls; this may, at least partially, replace the need for the method of aligning data mentioned earlier herein. The first such standards have been made available as a World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL by qRT-PCR. The standards are freeze-dried and consist of a BCR-ABL-positive human erythroleukemia cell line (K562 cells) diluted in a human, BCR-ABL-negative leukemia cell line (HL60). The production of the first reference standard and its eventual increased availability offer hope of increased standardization between laboratories.

Although there are benefits to the harmonization of methodologies, the use of the IS for reporting BCR-ABL transcript levels, as well as the use of standard reference materials, cannot address all potentially important sources of error in laboratory-developed tests. Use of standard materials and an IS conversion factor should help, but cannot ensure, quality control. The efficacy of the IS is fundamentally limited by within-laboratory reproducibility. Also, the conversion factor for each
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laboratory cannot be considered a fixed value. Evidence suggests that it can be unstable, indicating the need for more frequent validation.27 One approach to address these limitations is the development of instrumentation that provides fully automated nucleic acid purification, nested qRT-PCR, and data analysis. This approach reduces intra- and interlaboratory variability and offers the potential for convenient cross-laboratory standardization.28-30

Molecular Monitoring Report

The interface between the laboratory and the treating physician is the molecular pathology report. This report should have a clear layout, sufficient detail about the assay and its limitations, and technical and clinical conclusions.31 Standardization of reports is recommended to ensure that all necessary information is presented.31 This practice, together with the use of the IS, makes it far easier for the physician to compare reports from different laboratories. Moreover, a lack of standardization in a laboratory makes it more difficult to assess whether that laboratory is operating at an optimal quality assurance and quality control level compared with other laboratories.

Guidelines on what constitutes an optimal molecular pathology report have been published by the College of American Pathologists (CAP).31,32 The molecular pathologist provides the interpretation of the test results. This interpretation includes an analytical interpretation, in which the quality or quantity of the sample is assessed and a result is obtained, and a clinical interpretation, whereby the result is interpreted to reach a conclusion about its importance for the patient and his or her treatment. It should not be assumed that health care

<table>
<thead>
<tr>
<th>Value Reported</th>
<th>Method for Determination of Value</th>
<th>How Expressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL copy number</td>
<td>Absolute copy number</td>
<td>Absolute copy number</td>
</tr>
<tr>
<td>BCR-ABL ratio</td>
<td>Ratio of BCR-ABL to a control gene</td>
<td>Percentage or fraction</td>
</tr>
<tr>
<td>Relative to single diagnostic sample</td>
<td>Highest BCR-ABL transcript level from a patient’s single diagnostic sample converted to log_{10} scale and used as the baseline</td>
<td>Log reduction (ie, patient’s current BCR-ABL transcript levels converted to log_{10} scale) from specified baseline</td>
</tr>
<tr>
<td>Relative to laboratory median of diagnostic samples</td>
<td>Median BCR-ABL transcript levels derived from RNA, cDNA, plasmid DNA, or cell line samples converted to log_{10} scale and used as baseline</td>
<td>Log reduction (ie, patient’s current BCR-ABL transcript levels converted to log_{10} scale) from specified baseline</td>
</tr>
<tr>
<td>Relative to lab mean of diagnostic samples</td>
<td>Mean BCR-ABL transcript levels derived from RNA, cDNA, plasmid DNA, or cell line samples converted to log_{10} scale and used as baseline</td>
<td>Log-reduction (ie, patient’s current BCR-ABL transcript levels converted to log_{10} scale) from specified baseline</td>
</tr>
<tr>
<td>Relative to previous patient sample</td>
<td>BCR-ABL transcript levels from patient’s last test result or baseline test result converted to log_{10} scale and used as the baseline</td>
<td>Log-reduction (ie, patient’s current BCR-ABL transcript levels converted to log_{10} scale) from specified baseline</td>
</tr>
<tr>
<td>Relative to diluted and undiluted K562 cells</td>
<td>BCR-ABL transcript levels derived from standard curve based on serial samples of diluted and undiluted K562 cells converted to log_{10} scale and used as the baseline</td>
<td>Log-reduction (ie, patient’s current BCR-ABL transcript levels converted to log_{10} scale) from specified baseline Percentage, per IS</td>
</tr>
<tr>
<td>Per IS</td>
<td>Anchored to a lab-specific pretreatment standardized value baseline BCR-ABL ratio (100% IS) and a 3-log reduction from the same (0.1% IS); mathematical conversion to IS by multiplication of the BCR-ABL/control ratio by a laboratory-specific conversion factor</td>
<td></td>
</tr>
</tbody>
</table>

cDNA, complementary DNA; IS, International Scale.

*OMIM accession number for BCR-ABL: 608232.
Molecular Monitoring: Present and Future

Currently, most molecular pathology diagnostics are carried out using assays tailored to a particular laboratory (known as laboratory-developed tests). Despite the commercial availability of reagents and kits to facilitate the process (Table 2), variation and error may still occur. Hence, the unmet need for an accurate, automated, standardized, and reproducible method to perform BCR-ABL assays has led to the development of the GeneXpert System (Cepheid, Sunnyvale, CA). This instrument automates all steps in the process of measuring BCR-ABL transcript levels directly from a patient sample, producing a result that is converted to the IS and is complete in approximately 2 hours. However, there are several potential limitations of the automated Cepheid system, such as its inflexibility in choice of the housekeeping gene, cost, and low throughput. Also, this instrumentation does not support the use of other laboratory-developed tests. As use of molecular methods becomes a more standard part of clinical practice, development of rapid, accurate, and reproducible assays for molecular markers will continue to be refined.

Further, as technology evolves, the ability to detect BCR-ABL transcripts below the current limit of quantification improves. More sensitive methodologies are in development, such as digital PCR; in the future, these methodologies may provide a definition of CMR that more closely reflects complete eradication of the CML clone. Therefore, with the potential for discontinuation of treatment on the horizon, the identification of patients who exhibit CMR becomes increasingly important. With continuing clinical trials and drug development for the treatment of CML, as well as the ability to detect smaller quantities of BCR-ABL transcripts in the molecular laboratory, a cure may be possible for patients with CML.

Conclusions

Molecular monitoring of BCR-ABL transcript levels through qRT-PCR is central to modern monitoring of patients with CML, providing important clinical information for monitoring patient response to therapy with TKIs and for determining whether a change in treatment is warranted. Conventional methods of qRT-PCR are complex to perform, involve multiple steps, and thus are subject to significant error.
Figure 3

Excerpts from diagnostic laboratory molecular analysis reports. A, Test results from laboratory 1 include test specifications; patient, physician, and test information; interpretation; and cumulative results, represented in graph and table formats. Reproduced with permission from MolecularMD, Portland, OR.
Overview

Figure 3
Excerpts from diagnostic laboratory molecular analysis reports. A, Test results from laboratory 1 include test specifications; patient, physician, and test information; interpretation; and cumulative results, represented in graph and table formats. Reproduced with permission from MolecularMD, Portland, OR.

**Table 1**

<table>
<thead>
<tr>
<th>Specimen #</th>
<th>Collection Date</th>
<th>MMR</th>
<th>BCR-ABL/ABL % fold increase from lowest</th>
<th>Fold Change from last analysis</th>
<th>Fold Change from lowest level</th>
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<tbody>
<tr>
<td>MMD2010-631822</td>
<td>15-Jan-2009</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>25.81</td>
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<tr>
<td>MMD2010-631823</td>
<td>15-Apr-2009</td>
<td>No</td>
<td>No</td>
<td>-2.51</td>
<td>10.20</td>
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<td>MMD2010-631824</td>
<td>15-Jul-2009</td>
<td>Yes</td>
<td>No</td>
<td>-2.89</td>
<td>3.529</td>
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<tr>
<td>MMD2010-631825</td>
<td>15-Oct-2009</td>
<td>Yes</td>
<td>No</td>
<td>-3.52</td>
<td>0</td>
</tr>
</tbody>
</table>

**Specimen # | Collection Date | %BCR-ABL/ABL (IS) | %BCR-ABL/ABL (raw) | ABL Copies | BCR-ABL Copies |
<table>
<thead>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MMD2010-631822</td>
<td>15-Jan-2009</td>
<td>0.716%</td>
<td>0.884%</td>
<td>28975</td>
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<tr>
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<td>15-Apr-2009</td>
<td>0.285%</td>
<td>0.352%</td>
<td>34098</td>
<td>120</td>
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<tr>
<td>MMD2010-631824</td>
<td>15-Jul-2009</td>
<td>0.099%</td>
<td>0.122%</td>
<td>45987</td>
<td>56</td>
</tr>
<tr>
<td>MMD2010-631825</td>
<td>15-Oct-2009</td>
<td>0.028%</td>
<td>0.034%</td>
<td>34785</td>
<td>12</td>
</tr>
</tbody>
</table>

**TABLE KEY**

Specimen Number = Accession number for specimen set
Collection Date = Specimen collection date
MMR Status = report describing the maintenance or loss (confirmed or unconfirmed) of MMR
BCR-ABL (IS) International Scale = BCR-ABL/ABL value multiplied by the correction factor (0.81)
BCR-ABL/ABL (raw) = BCR-ABL Copies divided by ABL copies
ABL = ABL Copies
BCR-ABL = BCR-ABL Copies

**TEST DESCRIPTION(S):**

**BCR-ABL QRT-PCR test:**

The BCR-ABL QRT-PCR test quantitatively measures the RNA blood level of BCR-ABL, a marker for the presence and amount of transcriptionally active Philadelphia chromosome positive leukemia cells. For this test total RNA from whole leukocytes is reverse transcribed with random primers and the cDNA product is quantitated by fluorescent real-time QRT-PCR. The BCR-ABL QRT-PCR is a multiplex reaction containing primers from bcr exons 52 & b3 and abl exon 2, such that the major (p210) translocation breakpoint is detected. ABL cDNA copy numbers are used to control for the quality and quantity of sample RNA. Therefore, the result of this test is expressed in % as a fraction of BCR-ABL expression to that of the control gene ABL (% BCR-ABL/ABL).

*Major Molecular Response (MMR) value at MolecularMD is designated 0.12%, which is equivalent to a 3-log reduction from a standardized baseline value from the International Randomized Inferon versus STI571 (IRIS) study or 0.1% per International Scale (IS). A correction factor of 0.81 is applied to all MolecularMD BCR-ABL/ABL ratios to obtain the International Scale value and MMR is reported when a patient shows a BCR-ABL/ABL% less than or equal to 0.1% IS. This conversion factor is validated for BCR-ABL/ABL ratios of 10% and below.

**Electronically Signed By:**

Test PQTester3
Pathologist
(Case reviewed 01/27/2010)

This test was developed and its performance characteristics determined by this laboratory. It has not been cleared or approved by the FDA. This test is used for clinical purposes. It should not be regarded as investigational or for research. The laboratory is regulated under CLIA of 1988. *False positive or negative results may occur for reasons that include genetic variants, blood transfusions, or somatic heterogeneity of the tissue sample. 1341 SW Custer Drive, Portland, OR 97219 T: 503-459-4974 F: 503-459-4976 www.molecularmd.com
### Figure 3 (cont)

**B.** Test results from laboratory 2 include data from the assessment of BCR-ABL (OMIM accession number 608232) rearrangement and interpretation, as well as a narrative explaining the assay, test sensitivity, and calculations. Reproduced with permission from Quest Diagnostics, Madison, NJ.

<table>
<thead>
<tr>
<th>BCR/ABL1 GENE REARRANGEMENT, QUANTITATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLINICAL INFORMATION</strong></td>
</tr>
<tr>
<td>SPECIMEN SUBMITTED: Bone Marrow</td>
</tr>
<tr>
<td>PREVIOUS CASE:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MUTATION</strong></td>
</tr>
<tr>
<td>BCR-ABL t(9;22) Ratio</td>
</tr>
<tr>
<td>BCR-ABL/ABL Log Reduction</td>
</tr>
</tbody>
</table>

**INTERPRETATION:** The real-time assay shows no evidence of expression of the BCR-ABL fusion transcript, which usually results from t(9;22)(q34;q11) translocation. Negative result in patients being treated for CML represents at least 5 log reduction.

**CLINICAL UTILITY:**
This test assays for the BCR-ABL fusion transcript which usually results from t(9;22)(q34;q11) translocation and is seen in essentially all cases of chronic myelogenous leukemia (CML) and a subset of lymphoblastic leukemias. In our laboratory, the median value for the BCR-ABL/ABL ratio in previously untreated CML patients is 4.13±5 in peripheral blood samples (N=120) and 5.09 in bone marrow samples (N=100). Based on data from the IRIS study, 3-log or higher reduction in BCR-ABL transcript levels by 12 months of therapy is termed major molecular response and is associated with a very low risk of long-term progression in patients receiving continuous imatinib therapy (Blood, 2010:116:3758). Undetectable BCR-ABL transcript is typically the therapeutic goal in t(9;22)-bearing lymphoblastic leukemias.

The log reduction for blood samples is calculated as:

\[
\text{LOG10}(4.13)-\text{LOG10}(\text{BCR-ABL ABL ratio}) \text{ Unit/Log reduct.}
\]

The log reduction for bone marrow samples is calculated as:

\[
\text{LOG10}(5.09)-\text{LOG10}(\text{BCR-ABL ABL ratio}) \text{ Unit/Log reduct.}
\]

**TEST SENSITIVITIES:**
The analytical sensitivity of this test is approximately 1 tumor cell in 100,000 normal cells or 5-log reduction from untreated CML baseline levels. However the level of BCR-ABL transcripts in t(9;22)-bearing tumors may vary widely resulting in a variable assay sensitivity for any given patient.

**METHODOLOGY:**
Extracted RNA is subjected to quantitative reverse transcription real-time PCR to simultaneously measure the quantity of the primary BCR-ABL fusion transcripts b2a2, b3a2 and b1a2. An additional PCR amplification for the ABL gene is performed as a control for sample RNA quality and as a reference for relative quantification. The results are reported as a ratio between the quantity of the BCR-ABL fusion transcript compared to the internal control ABL. This test was developed and its performance characteristics have been determined by Performance characteristics refer to the analytical performance of the test.

**REFERENCES:**
- Hugop M. Karranjan, Moshe Talpaz, Jorge Cortes, etc. Quantitative Polymerase Chain Reaction Monitoring of BCR-ABL during Therapy with Imatinib Mesylate (STI571; Gleevec) in Chronic-Phase Chronic Myelogenous Leukemia. Clinical Cancer Research, 2003; 9: 160-166.
Measurement accuracy and interlaboratory comparisons can be improved through the adoption of harmonized international guidelines and the IS. Also, there is mounting evidence that achievement of CMR may allow some patients to discontinue TKI therapy. The improvements in treatment efficacy and in molecular monitoring are exciting in that curative intent may be possible with TKI therapy but challenging because the onus is on pathologists to ensure accurate reports of molecular response based on validated, standardized methodology. These reports will directly influence clinical decision-making for patient care and will signal whether TKI therapy needs to be adjusted or potentially discontinued. **LM**

**Acknowledgments**
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Financial support for medical editorial assistance was provided by Novartis AG, Basel, Switzerland.

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**References**


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**Table 2. Selected List of Commercially Available RT-PCR Kits and Reagents for Processing Samples to Assay BCR-ABL Levels**

<table>
<thead>
<tr>
<th>Manufacturer and Location</th>
<th>Product Name</th>
<th>Selected Product Claims/Components</th>
<th>Principal Reagents/Components</th>
</tr>
</thead>
</table>
| Ipsogen SA, Marseilles, France | BCR-ABL Mtcr IS-MMR Kit | • qRT-PCR to detect and quantify specific BCR-ABL fusion gene transcripts relative to ABL control gene expression in sample RNA  
• BCR-ABL IS: NCN results converted to the IS  
• Good sensitivity |
| MolecularMD, Portland, OR | One-Step qRT-PCR BCR-ABL Kit | • One-step protocol: reverse transcription and quantitative PCR reactions are performed in the same well, saving time and money  
• Exceptional sensitivity down to 3 copies of BCR-ABL  
• Integrated conversion factor, enabling results on the IS |
| Life Technologies Corporation, Carlsbad, CA | Asuagen BCR/ABL1 Quant Test | • An LOD with >50% positivity was obtained at a 0.001% ratio  
• Sensitive: precise quantification at low BCR/ABL1:ABL1 ratios aids in measuring MMR, minimal residual disease, and estimating risk of relapse  
• IS harmonization: has performance characteristics required for reporting quantitative BCR/ABL1 results on the IS |
| Cepheid, Sunnyvale, CA | GeneXpert System BCR/ABL Assay (for research use only) | • Fully automated RT-PCR system that combines integrated sample preparation with amplification and detection  
• BCR-ABL assay: closed system, nested RT-PCR assay reporting in approximately 2 hours  
• Microfluidic single-use cartridge includes processing chamber with reagents, filters, and capture technologies necessary to extract, purify, amplify, and detect target nucleic acids |

**Abbreviations:** ABL1, c-abl oncogene 1; ARQ, Armored RNA Quant; BCR, breakpoint cluster gene; IS, International Scale; LOD, limit of detection; MMR, major molecular response; NCN, normalized copy number; PCR, polymerase chain reaction; RT-PCR, real-time PCR; qRT-PCR, quantitative RT-PCR.

*OMIM accession number for BCR-ABL: 608232.*


