Molecular Monitoring of Response In Patients With Chronic Myeloid Leukemia

Although this practice can optimize long-term patient outcomes, it is not routine in clinical practice

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Abstract

Purpose: To discuss the importance of regular, consistent use of molecular testing to monitor treatment response and minimal residual disease in patients with chronic myeloid leukemia (CML), as recommended in established practice guidelines.

Design: This review outlines the efficacy of BCR-ABL1 tyrosine kinase inhibitors (TKIs) in eliciting significant treatment responses in patients with CML; describes the positive effect of achieving molecular responses on long-term outcomes; discusses the importance of regular, consistent molecular monitoring in CML; and highlights issues critical to the implementation of molecular monitoring in routine practice.

Methods: Published literature pertaining to molecular monitoring of the response to BCR-ABL1 TKI therapy for CML was searched and reviewed.

Results: BCR-ABL1 TKI therapy for CML can reduce the disease burden to a level detectable only by molecular methods. Although practice guidelines recognize the importance of molecular monitoring of disease as a means to optimize long-term patient outcome, standardization of methods and appropriate use of molecular monitoring in routine clinical practice are not ideal.

Conclusions: Without accurate, reproducible methods to measure treatment response, the clinical course of disease cannot be adequately monitored during treatment, leaving open the potential to miss patients who might benefit from a change in their treatment plan.

Introduction

The last decade has witnessed a paradigm shift in the treatment of patients with chronic myeloid leukemia (CML). The approval of imatinib, a BCR-ABL1 tyrosine kinase inhibitor (TKI), for CML marks one of the earliest successes of rational drug design based on knowledge of a specific underlying molecular defect. Imatinib — and the newer BCR-ABL1 TKIs nilotinib, dasatinib, bosutinib, and ponatinib — reduce the disease burden in CML so effectively that highly sensitive techniques are needed to adequately monitor treatment responses and minimal residual disease. Evidence is mounting that the achievement of a reduced disease burden early in treatment with TKIs predicts a favorable long-term outcome, and that patients who show suboptimal responses to TKI therapy may benefit from treatment modifications. Therefore, the consistent use of accurate and reproducible techniques to monitor treatment response becomes a critical component of care in the management of patients with CML. Efforts are now focused on the standardization of monitoring techniques and methods, as standardization is expected to affect both the use of health care resources and long-term patient outcome.

This review discusses the rationale behind the current need for standardized methods to measure accurately and reproducibly the level of response to TKI treatment, and addresses the

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>alloHSCT</td>
<td>Allogeneic hematopoietic stem cell transplant</td>
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<tr>
<td>AP</td>
<td>Accelerated phase</td>
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<tr>
<td>BC</td>
<td>Blast crisis</td>
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<tr>
<td>CoR</td>
<td>Complete cytogenetic response</td>
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<tr>
<td>CML</td>
<td>Chronic myeloid leukemia</td>
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<tr>
<td>CMR</td>
<td>Complete molecular response</td>
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<tr>
<td>CP</td>
<td>Chronic phase</td>
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<tr>
<td>ELN</td>
<td>European LeukemiaNet</td>
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<tr>
<td>IFN-α</td>
<td>Interferon-α</td>
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<tr>
<td>MGyR</td>
<td>Major cytogenetic response</td>
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<tr>
<td>MMR</td>
<td>Major molecular response</td>
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<tr>
<td>NCCN</td>
<td>National Comprehensive Cancer Network</td>
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<tr>
<td>OS</td>
<td>Overall survival</td>
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<tr>
<td>Ph+</td>
<td>Philadelphia chromosome-positive</td>
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<tr>
<td>qRT-PCR</td>
<td>Quantitative reverse-transcription polymerase chain reaction</td>
</tr>
<tr>
<td>TKI</td>
<td>Tyrosine kinase inhibitor</td>
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implications that the use of substandard monitoring methods may have for health care resource utilization and patient outcome.

**CML disease background and current standard of care**

CML is a myeloproliferative disorder characterized by the unregulated growth of myeloid cells in the bone marrow and their accumulation in the blood. CML is caused by the constitutive expression of an aberrant fusion gene, **BCR-ABL** (also referred to as **BCR-ABL1**), that is formed through a reciprocal translocation between chromosomes 9 and 22 (the Philadelphia chromosome [Ph]) (Bernards 1987; Nowell 1960). It has been estimated that 5,920 new cases of CML will be diagnosed in 2013 and that 610 people will die from the disease (American Cancer Society 2013). Without treatment, CML typically progresses from an initial chronic phase (CP) to the advanced accelerated phase (AP) within 4 to 6 years, and then to a final, fatal phase — blast crisis (BC) — within 1 year (Kalidas 2001).

In CML, treatment response is measured on several levels: hematologic response, cytogenetic response, and molecular response (Table 1) (National Comprehensive Cancer Network 2012). In terms of detecting minimal residual disease in CML, monitoring molecular responses by measuring **BRC-ABL1** expression offers the highest degree of sensitivity compared with monitoring hematologic or cytogenetic responses (Radich 2009). Current standard first-line treatment for CML includes TKI therapy with imatinib, nilotinib, or dasatinib (National Comprehensive Cancer Network 2012), all of which are effective in eliciting molecular responses through the inhibition of **BRC-ABL1** expression.

Imatinib was the first TKI to be approved for Ph-positive (Ph+) CML, based on the findings of the phase 3 International Randomized Study of Interferon and STI571 (IRIS), in which imatinib significantly improved the rates of major (MCyR) and complete (CCyR) cytogenetic responses, and the rate of freedom from progression to AP or BC compared with interferon-α (IFN-α) plus cytarabine (O’Brien 2003). Continued follow-up of the IRIS study confirmed the efficacy and safety of imatinib over 8 years of treatment (Deininger 2009; Druker 2006; Hochhaus 2009). Four newer TKIs more potent than imatinib have been approved: nilotinib and dasatinib for first-line and second-line treatment of Ph+ CML (Sprycel 2011; Tasiguna 2012), and bosutinib and ponatinib for second- and third-line treatment (Bosulif 2012; Iclusig 2012). Two- and 3-year follow-up of the phase 3 Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients (ENESTnd) study confirmed initial findings that nilotinib 300 mg twice daily (BID) was significantly more effective than imatinib in improving the rates of CCyR (87% vs. 77%, respectively; \( P = .018 \)) (Kantarjian 2011; Saglio 2010), major molecular response (MMR; 85% vs. 64%; \( P < .0001 \)), and complete molecular response (CMR; reported as MR4.5; 32% vs. 15%; \( P < .0001 \)) at 3 years (Larson 2012), and in reducing the number of progressions to AP or BC (2 vs. 17; \( P = .0003 \)) by 3 years (Larson 2012). A 4-year follow-up report on the ENESTnd trial further supported the efficacy of first-line nilotinib (Kantarjian 2012). Two-year follow-up of the phase 3 Dasatinib vs. Imatinib Study in Treatment-Naive CML Patients (DASISION) also showed that dasatinib significantly improved the rates of CCyR (86% vs. 82%; \( P = .0002 \)), MMR (64% vs. 46%, \( P < .0001 \)), and CMR (17% vs. 8%; \( P = .002 \)), and resulted in a lower rate of transformation to AP or BC (2.3% vs. 5%), compared with imatinib (Kantarjian 2010; Kantarjian 2012). Three-year follow-up data from the DASISION trial showed durable responses with dasatinib over time (Hochhaus 2012).

The advent of BCR-ABL1 TKI therapy represents a major advance in the treatment of CML. That TKI therapy can significantly reduce the frequency of progression of CML to advanced stages is important because the median overall survival (OS) of patients who progress to CML-AP/BC while being treated with imatinib or nilotinib is short (10.5 months) (Larson 2012). CML-AP/BC is considerably more difficult to treat than is CML-CP, and few effective therapeutic options are available (National Comprehensive Cancer Network 2012). Although allogeneic hematopoietic stem cell transplant (alloHSCT) — considered a potentially curative treatment for patients with CML — is standard treatment for patients with advanced disease, those with CML-AP/BC fare much worse following alloHSCT than do patients with CML-CP; 3-year OS rates after alloHSCT were 91% and 59% for patients with CML-CP and CML-AP/BC, respectively (Saussele 2010). The ability of TKIs to elicit response rates that are markedly higher than previously reported rates has driven the use of more rigorous clinical endpoints and has made it necessary to modernize the methods needed to measure treatment response. The end result has been updates to clinical practice guidelines that include expectations for milestone responses and that reflect the use of current technologies.

**Guidelines for molecular monitoring of treatment response**

Because TKI therapy for CML has the potential to reduce the disease burden to below the threshold of
detection of hematologic and cytogenetic testing, molecular monitoring using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) is the method best suited to discriminate among degrees of response. Guidelines issued by the National Comprehensive Cancer Network (NCCN) (National Comprehensive Cancer Network 2012) and by the European LeukemiaNet (ELN) (Baccarani 2009) recommend molecular monitoring of BRC-ABL1 expression using qRT-PCR to monitor treatment response. Notably, both

<table>
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<tr>
<th>Type of testing</th>
<th>Technique</th>
<th>Response</th>
<th>NCCN response criteria</th>
<th>ELN response criteria</th>
</tr>
</thead>
</table>
| **Hematologic** | Blood cell count using blood sample | Complete hematologic response | • Complete normalization of peripheral blood counts  
• Leukocyte count <10 × 10⁹/L  
• No immature cells, such as myelocytes, promyelocytes, or blasts in peripheral blood  
• Platelet count <450 × 10⁹/L  
• No sign or symptoms of disease with disappearance of palpable splenomegaly | • WBC count <10 × 10⁹/L  
• Basophils <5%  
• No myelocytes, promyelocytes, or myeloblasts in the differential  
• Platelet count <450 × 10⁹/L  
• Spleen nonpalpable |
| **Cytogenetic** | Karyotyping or FISH analysis using bone marrow aspirate | Complete cytogenetic response  
Partial cytogenetic response  
Minor cytogenetic response  
Minimal cytogenetic response  
No cytogenetic response | • No Ph+ metaphases  
• 1%–35% Ph+ metaphases  
• >35% Ph+ metaphases  
• Undetectable BCR-ABL1 mRNAs by qRT-PCR using an assay with a sensitivity of ≥4.5 logs below the standardized baseline  
• ≥3-log reduction (IS) of BCR-ABL1 mRNA  
• Ratio of BCR-ABL1 to ABL1 (or other housekeeping genes) ≤0.1% (IS) |  |
| **Molecular** | qRT-PCR using blood sample or bone marrow aspirate | Complete molecular response  
Major molecular response | • No detectable BCR-ABL1 mRNA by qRT-PCR (IS) using an assay with a sensitivity of ≥4.5 logs below the standardized baseline |  |

ELN, European LeukemiaNet; FISH, fluorescence in situ hybridization; IS, International Scale; NCCN, National Comprehensive Cancer Network; Ph+, Philadelphia chromosome-positive; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; WBC, white blood cell.


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sets of guidelines recommend reporting levels of BRC-ABL1 expression on the International Scale (IS), which uses a standardized baseline that was meant to be applicable across laboratories. Both the NCCN and ELN guidelines also establish response milestones — responses expected within specified timeframes — for first-line (Baccarani 2009; National Comprehensive Cancer Network 2012) and second-line (Baccarani 2009) TKI treatment (Tables 2 and 3). The two sets of guidelines have considerable overlap as well as differences with respect to treatment-response milestones. Nevertheless, it is important that the ELN guidelines be considered in the management of patients with CML in the United States because the ELN response milestones form the foundation for the response criteria and clinical endpoints used in modern clinical studies of CML (Guilhot 2012).

**Achievement of molecular response and improved long-term outcomes**

Evidence supports the concept that the achievement of an optimal response — that is, a level of response that meets or exceeds response milestones defined by the ELN or NCCN guidelines — within the first 18 months of treatment with imatinib significantly predicts favorable long-term outcomes. Applying ELN-defined criteria for treatment response, one study found that patients achieving optimal treatment responses at 6, 12, and 18 months had significantly higher rates of CCyR and MMR, and lower rates of disease transformation to AP or BC, after 24 and 48 months of treatment, compared with patients who failed treatment (Alvarado 2009). Another study found that treatment failure (as defined by ELN criteria) at 3, 6, 12, or 18 months correlated significantly with a lower 5-year probability of CCyR, OS, and progression-free survival (PFS) (Marin 2008).

Studies that designate a specific level of response — not necessarily tied to ELN or NCCN criteria — as a milestone have also found significant correlations between the achievement of a treatment response and long-term outcome. One such study found that CCyR at 3, 6, or 12 months correlated with improved 3-year event-free survival (EFS) and OS (Jabbour 2011). An analysis of the IRIS study showed that achievement of MMR by 12 and 18 months of imatinib therapy correlated significantly with a higher rate of EFS at 7 years (Hughes 2010). With respect to PFS, however, achievement of MMR at 12 and 18 months in patients with CCyR did not significantly improve the rate of 5-year PFS, compared with patients who had achieved only CCyR (Druker 2006). An analysis of the German CML IV study showed a significant correlation between MMR

<table>
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<tr>
<th>Recommendation</th>
<th>Favorable response</th>
<th>Time on therapy</th>
<th>Unfavorable response</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continue same dose</td>
<td>BCR-ABL1(IS) ≤10%</td>
<td>3 months (IM)</td>
<td>BCR-ABL1(IS) &gt;10%</td>
<td>• Evaluate for adherence and drug interactions</td>
</tr>
<tr>
<td></td>
<td>BCR-ABL1(IS) ≤10%</td>
<td>3 months (NIL or DAS)</td>
<td>≤PCyR or cytogenetic relapse</td>
<td>• Conduct mutational analysis</td>
</tr>
<tr>
<td></td>
<td>CCyR</td>
<td>12 months (IM)</td>
<td>≤mCyR or cytogenetic relapse</td>
<td>• For patients with PCyR at 12 months, increase imatinib dose up to 800 mg, as tolerated, if patient is not a candidate for other TKIs</td>
</tr>
<tr>
<td></td>
<td>≥PCyR</td>
<td>12 months (NIL or DAS)</td>
<td>≤mCyR or cytogenetic relapse</td>
<td>• Switch to another TKI</td>
</tr>
<tr>
<td></td>
<td>CCyR</td>
<td>18 months (IM)</td>
<td>≤PCyR or cytogenetic relapse</td>
<td>• Evaluate for allogeneic HSCT</td>
</tr>
<tr>
<td></td>
<td>CCyR</td>
<td>18 months (NIL or DAS)</td>
<td>≤PCyR or cytogenetic relapse</td>
<td>• Consider enrollment in clinical trials</td>
</tr>
</tbody>
</table>

*Please refer to NCCN Guidelines for more detailed description of treatment recommendations.

CCyR, complete cytogenetic response; CHR, complete hematologic response; DAS, dasatinib; HSCT, hematopoietic stem cell transplant; IM, imatinib; IS, International Scale; mCyR, minor cytogenetic response; NIL, nilotinib; PCyR, partial cytogenetic response

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at 12 months and improved PFS and OS at 3 years (Hehlmann 2012). Another study showed that achievement of CCyR at any time correlated significantly with an improved rate of 5-year EFS, and that achievement of both CCyR and MMR at 18 and 24 months correlated significantly with higher rates of 5-year and 7-year EFS (Furukawa 2011).

Several studies have found that achievement of specific BRC-ABL1 levels, generally ≤10% (IS), after 3 months of imatinib therapy was a highly significant predictor of long-term outcomes, such as the 12-month probability of MMR and CMR (Nagvi 2011), the 12-month probability of CCyR, the 24-month probability of MMR and PFS, and the progression to AP or BC at any time (Hochhaus 2011); 5-year OS and PFS (Hanfstein 2012); 8-year OS, PFS, and EFS; and the probability of CCyR, MMR, and

<table>
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<tr>
<th>TABLE 3 European LeukemiaNet (ELN) definitions of response milestones for treatment with first-line imatinib (white rows) and provisional definitions of response with second-line nilotinib or dasatinib (gray rows) (Baccarani 2009)</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>Imatinib</td>
</tr>
<tr>
<td>Nilotinib or dasatinib</td>
</tr>
<tr>
<td><strong>3 months</strong></td>
</tr>
<tr>
<td>Imatinib</td>
</tr>
<tr>
<td>Nilotinib or dasatinib</td>
</tr>
<tr>
<td><strong>6 months</strong></td>
</tr>
<tr>
<td>Imatinib</td>
</tr>
<tr>
<td>Nilotinib or dasatinib</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
</tr>
<tr>
<td>Imatinib</td>
</tr>
<tr>
<td>Nilotinib or dasatinib</td>
</tr>
<tr>
<td><strong>Any time</strong></td>
</tr>
<tr>
<td>Imatinib</td>
</tr>
<tr>
<td>Nilotinib or dasatinib</td>
</tr>
</tbody>
</table>

CCyR, complete cytogenetic response; CHR, complete hematologic response; CML-CP, chronic myeloid leukemia in chronic phase; minCyR, minimal cytogenetic response; mCyR, minor cytogenetic response; PCyR, partial cytogenetic response; MMR, major molecular response; Ph+, Philadelphia chromosome–positive; Ph–, Philadelphia chromosome–negative, TKI, tyrosine kinase inhibitor

CMR (Marin 2012). Based on the strength of these clinical data, the NCCN recently updated the criteria for the 3-month treatment response milestone to include a BRC-ABL1 level of ≤10% (IS) (Table 3).

At present, only preliminary data are available regarding early responses to the newer TKIs, but these data indicate a significant correlation between a favorable response to nilotinib, dasatinib, and bosutinib at 3 months and improved long-term outcomes (Brümmendorf 2012; Hochhaus 2012; Hochhaus 2011; Jabbour 2011; Marin 2012; Nicolinii 2011).

Overall, these clinical findings highlight the importance of accurate molecular monitoring as a means of measuring treatment responses. The NCCN and ELN guidelines provide management recommendations for patients who fail to meet specific treatment-response milestones, including the evaluation of patients for adherence or intolerability, BRC-ABL1 mutational analysis, dose escalation, or a change in drug therapy (Table 2) (Baccarani 2009; National Comprehensive Cancer Network 2012). Because the guidelines recommend treatment modifications are based on the patient’s response to treatment, consistent implementation of accurate and reproducible response measurements is crucial and must be an integral part of patient management. Despite the importance of molecular monitoring to assess treatment response, nearly half of clinicians surveyed do not follow practice guidelines in this respect (Chen 2012; Fogarty 2008; Quintas-Cardama 2011), indicating an important gap in their knowledge of CML that may have negative implications for patient outcome.

**Important issues to consider in the molecular assessment of treatment response**

**Frequency of monitoring and implications of increases in BCR-ABL1 levels**

Both the NCCN and the ELN guidelines recommend regular molecular monitoring of BRC-ABL1 transcript levels by qRT-PCR during TKI treatment (Tables 4 and 5). The NCCN recommends testing at baseline, every 3 months for 3 years, and every 3 to 6 months thereafter if CCyR has been achieved and maintained (National Comprehensive Cancer Network 2012). Similarly, the ELN recommends testing every 3 months until MMR is achieved and maintained, and at least every 6 months thereafter (Baccarani 2009). This testing frequency allows clinicians to monitor 1) the achievement and maintenance of optimal treatment response, and 2) increases in BRC-ABL1 levels (a sign of potential relapse or emerging resistance). Early detection of events, such as a loss of response or possible signs of disease progression, may signal to the clinician that patients are having problems with their medication and allow the clinician to intervene in a timely manner to minimize the potential impact that these issues may have on long-term outcomes.

There is evidence that the loss of response during treatment, as determined by an increase in the BRC-ABL1 level, precedes and predicts the emergence of clinical symptoms of disease relapse. In one study, a third of patients with CCyR and MMR who subsequently lost MMR (i.e., they had an increase in BRC-ABL1 levels) experienced disease relapse a median of 14 months later (Press 2007). In another study, 10% of patients with stable CCyR who had lost MMR and/or had a >1-log increase in BRC-ABL1 level subsequently experienced disease progression (Kantarjian 2009).

These findings underscore the importance of regular molecular monitoring of BRC-ABL1 levels, as outlined in practice guidelines, as a means to identify patients at risk of disease progression, who may benefit most from closer follow-up and a reassessment of their treatment strategy.

The loss of a treatment response or failure to achieve a response may indicate that patients are having difficulty with medication adherence. Studies have found that patients on long-term imatinib therapy with lower adherence rates had a higher risk of losing CCyR (Ibrahim 2011) and a lower probability of achieving MMR and CMR (Marin 2010) compared with patients with better adherence. In addition, lower rates of adherence to TKI therapy for CML have been found to correlate with higher health care and medical costs, excluding the cost of the drug (Darkow 2007), and higher health care utilization, including inpatient hospitalizations and emergency room visits (Wu 2010). According to one study, a major reason for nonadherence in patients with CML receiving imatinib therapy is the presence of side effects (Eliasson 2011). Therefore, detection of a lost response may help clinicians to identify tolerability and/or adherence issues that may require additional patient support, such as education or ancillary medications.

In my clinical experience, poor adherence to TKI therapy can be a manifestation of broader psychosocial issues. In an outcomes study currently ongoing in our practice, we have found that patients with insufficient or no health insurance often face an array of psychosocial challenges, such as financial troubles and transportation issues, that can negatively affect clinical outcomes. These patients often miss clinic appointments, make unauthorized reductions in the medication dose, or discontinue treatment without the
clinician’s consent or knowledge. Such “intentional nonadherence” — defined as a patient’s decision to alter or discontinue treatment (Eliasson 2011) — is not uncommon among patients with CML. More important, patients without adequate health insurance have worse cancer survival rates than patients with adequate coverage (American Cancer Society 2008). It is important for payers to understand that regular monitoring of BRC-ABL1 levels tracks TKI treatment response and provides opportunities for clinicians to connect regularly with patients to assess any problems — such as unsatisfactory treatment adherence or the inability to cover medication copayments — that might be improved by educational reinforcement or by enrollment in a drug-company-sponsored patient assistance program.

An increase in the BRC-ABL1 level detected on molecular monitoring may also signal the emergence of new BRC-ABL1 mutations that can confer resistance to TKI therapy. It should be noted, however, that BRC-ABL1 mutations may account for about half the cases of acquired resistance (as few as 19% and as many as 91%), depending on the detection method, patient population, and stage of disease (Branford 2003; Gorre 2001; Hochhaus 2002; Shah 2002). Other BRC-ABL1–dependent (e.g., BRC-ABL1 gene amplification) and BRC-ABL1–independent (e.g., clonal evolution, drug efflux) mechanisms may also confer TKI resistance. Both the NCCN and the ELN recognize the link between BRC-ABL1 mutations and TKI resistance, and their guidelines recommend performing mutational analysis in cases of inadequate response, loss of response (including loss of MMR with ≥1-log increase in BRC-ABL1 level), or disease progression to AP or BC (National Comprehensive Cancer Network 2012), or in cases of suboptimal response or treatment failure (Baccarani 2009).

The detection of a new mutation warrants a reassessment of the treatment strategy (Soverini 2011). The choice of subsequent therapy may be influenced in part by the specific mutation detected (Table 6) because the most commonly detected mutations vary in their in vitro sensitivities to nilotinib and dasatinib (Soverini 2011). Clinicians should keep in mind, however, that treatment selection should also take into account clinical and pathological factors of

<table>
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<th>Disease / treatment course</th>
<th>Disease monitoring</th>
<th>Frequency of monitoring</th>
</tr>
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<tbody>
<tr>
<td>At diagnosis</td>
<td>• Bone marrow cytogenetics</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>• Peripheral blood FISH (if bone marrow collection is not feasible)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>• BCR-ABL1 transcript levels by qRT-PCR</td>
<td>—</td>
</tr>
<tr>
<td>After treatment initiation</td>
<td>• Bone marrow cytogenetics</td>
<td>• At 3 months, if qRT-PCR (IS) is not available to assess response to TKI therapy</td>
</tr>
<tr>
<td></td>
<td>• BCR-ABL1 transcript levels by qRT-PCR</td>
<td>• At 12 months, if neither CCyR nor MMR is achieved</td>
</tr>
<tr>
<td></td>
<td>• BCR-ABL1 transcript levels by qRT-PCR</td>
<td>• At 18 months, if not in MMR and no CCyR at 12 months</td>
</tr>
<tr>
<td>After achievement of CCyR</td>
<td>• Bone marrow cytogenetics</td>
<td>• No recommendation</td>
</tr>
<tr>
<td></td>
<td>• BCR-ABL1 transcript levels by qRT-PCR</td>
<td>• Every 3 months for 3 years</td>
</tr>
<tr>
<td></td>
<td>• BCR-ABL1 transcript levels by qRT-PCR</td>
<td>• Every 3 to 6 months thereafter</td>
</tr>
<tr>
<td>Increase (≥1 log) in BCR-ABL1 transcript levelsb</td>
<td>• Bone marrow cytogenetics</td>
<td>• If no MMR</td>
</tr>
<tr>
<td></td>
<td>• BCR-ABL1 transcript levels by qRT-PCR</td>
<td>• If MMR, repeat in 1-3 months</td>
</tr>
</tbody>
</table>

*Please refer to NCCN Guidelines for more detailed description of disease monitoring recommendations.

†Adherence evaluation and mutational analysis are also recommended.

CCyR, complete cytogenetic response; FISH, fluorescence in situ hybridization; MMR, major molecular response; qRT-PCR, quantitative reverse-transcription polymerase chain reaction

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the patient and disease (Soverini 2011). Mutational analyses conducted for the ENESTnd and DASISION studies found that treatment with nilotinib and dasatinib, respectively, did not increase the frequency of new mutations in \textit{BRC-ABL1} compared with imatinib therapy (Kantarjian 2011; Kantarjian 2012).

\textbf{Choice of control genes}

The use of control genes in qRT-PCR testing is essential for the generation of reliable and reproducible results, and compensates for factors that can cause sample variation, such as the amount of total sample RNA, sample degradation, and the efficiency of the reverse transcription reaction (Hughes 2006). The three most studied control genes suitable for \textit{BRC-ABL1} qRT-PCR testing (Hughes 2006) are \textit{BCR}, which was used in the IRIS study (Hughes 2003), and \textit{ABL1} and the β-glucuronidase gene (\textit{GUSB}), which were used by a consortium of labs in Europe (Gabert 2003). These control genes are suitable for \textit{BRC-ABL1} testing because their expression levels are broadly similar to that of \textit{BRC-ABL1} at diagnosis of CML; their transcript stability is similar to that of \textit{BRC-ABL1}; and primers for these control genes do not inadvertently amplify other sequences in genomic DNA (Hughes 2006). The use of other genes as controls is problematic because they may not satisfy one or more of the above criteria, thus rendering the qRT-PCR results obtained with these genes potentially unreliable and unsuitable for comparison with other data.

\textit{The International Scale (IS)}

The IS was established in the IRIS study as a means to standardize the results of \textit{BRC-ABL1} qRT-PCR testing and to make data obtained in multiple laboratories suitable for comparison (Hughes 2006; Hughes 2003). In addition, because the IS is anchored to two defined values — the standardized baseline, representing 100%, and MMR, representing 0.1% (i.e., a 3-log reduction from the standardized baseline) — qRT-PCR results reported on the IS are absolute values and therefore do not depend on an individual patient’s baseline \textit{BRC-ABL1} level (Hughes 2006).

A reference laboratory that uses the IS has determined a laboratory-specific conversion factor based on the \textit{BRC-ABL1} control gene ratio used in that laboratory that is equivalent to the MMR established in the IRIS study, which allows the laboratory to convert its \textit{BRC-ABL1} results to the IS (Hughes 2006). In practical terms, working with reference laboratories that use the IS can simplify certain aspects of patient management, such as allowing clinicians to use a common set of clinical decision

\begin{table}[h]
\begin{tabular}{|l|l|l|}
\hline
Type of monitoring & Disease / treatment course & Frequency of monitoring \\
\hline
Hematologic & At diagnosis & — \\
 & During treatment & • Every 15 days until CHR is achieved and confirmed  \\
 & & • At least every 3 months thereafter, or as required \\
\hline
Cytogenetic & At diagnosis & — \\
 & During treatment & • At 3 and 6 months  \\
 & & • Every 6 months until CCyR is achieved and confirmed  \\
 & & • Every 12 months, if regular molecular monitoring cannot be assured  \\
 & & • Always for occurrences of treatment failure and unexplained anemia, leukopenia, or thrombocytopenia \\
\hline
Molecular by qRT-PCR & During treatment & • Every 3 months until MMR is achieved and confirmed  \\
 & & • At least every 6 months thereafter \\
\hline
Mutational analysis (molecular) & During treatment & • For occurrences of suboptimal response or failure  \\
 & & • Always before switching to other TKIs or other therapies \\
\hline
\end{tabular}
\end{table}

\textit{a}Cytogenetics should be performed by chromosome banding analysis of marrow-cell metaphases until CCyR has been achieved and confirmed. Interphase fluorescent \textit{in situ} hybridization cannot be used to assess a less-than-complete response, but it can substitute for chromosome banding analysis to monitor the completeness of a CCyR, provided that \textit{BCR-ABL1} extrasignal, dual color, dual fusion, or \textit{in situ} hybridization probes are used and that at least 200 nuclei are scored.

\textit{CCyR}, complete cytogenetic response; \textit{CHR}, complete hematologic response; \textit{MMR}, major molecular response; \textit{qRT-PCR}, quantitative reverse-transcription polymerase chain reaction; \textit{TKI}, tyrosine kinase inhibitor

values (e.g., criteria for optimal and suboptimal treatment response) and allowing more seamless follow-up of patients who transfer from one clinic to another (Branford 2008).

**Use of a single reference laboratory**

The use of a single reference laboratory, particularly one that uses the IS, can further simplify the process of molecular monitoring. The likelihood of interlaboratory differences is eliminated (Hughes 2006), and intralaboratory variations are reduced, since variables such as the choice of the control gene, sample handling, the qRT-PCR technique, and the reporting of results should be consistent. In practical terms, working with a single reference laboratory could yield potential time and cost efficiencies. It is often the case, however, that the choice of a laboratory may vary as insurance contracts are changed, which can make it difficult to work with only one reference laboratory (Radich 2009).

**Automation of qRT-PCR testing**

Interlaboratory variability can include differences in the choice of the control gene, sample handling, and the qRT-PCR technique. Although not yet available for routine clinical use, automated testing of BRC-ABL1 transcript levels can potentially increase access to reproducible BRC-ABL1 monitoring by qRT-PCR and may improve the accuracy and efficiency of monitoring. In a cost-feasibility study, the use of an automated system that executes RNA extraction and qRT-PCR was found to yield highly reproducible results and to be a cost-effective option for laboratories that perform fewer than 300 BRC-ABL1 tests annually (Cayuela 2011). In a separate analysis, a comparative cost model of automated versus laboratory-developed testing to monitor BRC-ABL1 levels showed a 3.2% improvement in overall accuracy with automated testing over 5 years. In addition, automated testing provided a 19% cost reduction for every 100 patients monitored per laboratory according to NCCN guidelines (Ratcliffe 2011). Although self-contained automated assays are under review by the U.S. Food and Drug Administration for marketing approval, they are not currently approved (Giles 2011) and therefore are not widely available.

### Remaining questions about molecular monitoring

**Definition of CMR**

CMR is variously defined in practice guidelines as “no detectable BRC-ABL1 mRNA by qRT-PCR (IS) using an assay with a sensitivity of ≥4.5 logs below the standardized baseline” (National Comprehensive Cancer Network 2012) or as “undetectable BRC-ABL1 mRNA transcripts by real-time quantitative and/or nested PCR in two consecutive blood samples of adequate quality (sensitivity >10⁴)” (Baccarani 2009). In the European Treatment Outcome Study (EUTOS), the extent of a molecular response was defined either by a threshold level of detection (e.g., MR4.0 = detectable disease ≤0.01% BRC-ABL1) or as a function of the control gene’s transcript level (e.g., MR4.0 = undetectable disease in cDNA with ≥10,000 ABL1 transcripts) (Cross 2012). Because the definition of CMR is variable and depends on the assay’s level of sensitivity and on the reliability of the results, establishing a universally accepted definition of CMR remains an important goal of standardization.

The ability to accurately determine and to monitor stable CMR has implications for potential treatment dis-

---

**TABLE 6**

Summary of treatment options based on BRC-ABL1 kinase domain mutation status (National Comprehensive Cancer Network 2012; Soverini 2011)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Treatment recommendation(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T315I</td>
<td>Hematopoietic stem cell transplant or clinical trial or omacetaxine</td>
</tr>
<tr>
<td>V299L</td>
<td>Consider nilotinib or omacetaxine rather than dasatinib or bosutinib</td>
</tr>
<tr>
<td>T315A</td>
<td>Consider nilotinib, imatinib, b bosutinib, or omacetaxine rather than dasatinib</td>
</tr>
<tr>
<td>F317L/V/I/C</td>
<td>Consider nilotinib, bosutinib, or omacetaxine rather than dasatinib</td>
</tr>
<tr>
<td>Y253H, E255K/V, and F359V/C/I</td>
<td>Consider dasatinib, bosutinib, or omacetaxine rather than nilotinib</td>
</tr>
<tr>
<td>Any other mutation</td>
<td>Consider high-dose imatinib, c or dasatinib, nilotinib, bosutinib, or omacetaxine</td>
</tr>
</tbody>
</table>

*Ponatinib was approved in December 2012 for the treatment of adult patients with chronic-phase, accelerated-phase, or blast-phase chronic myeloid leukemia that is resistant to or intolerant of prior tyrosine kinase inhibitor therapy, after the current version of the NCCN Guidelines (v3.2013) was published. Inclusion of ponatinib in the NCCN Guidelines is expected in a future version.

bIf mutation is detected following treatment with dasatinib.

cInsufficient data are available on dose escalation to indicate whether mutations with lower IC₅₀ values are sensitive to high-dose imatinib.

continuation in patients with CML. Numerous case reports (Ali 2005; Breccia 2006; Cortes 2004; Guastafierro 2009; Mauro 2004; Merante 2005; Okabe 2007; Verma 2008) and clinical studies (Goh 2011; Goh 2009; Mahon 2010; Mahon 2011; Matsuki 2011; Rousselot 2007; Takahashi 2012; Yhim 2012) describe patients who have discontinued imatinib therapy after achieving and maintaining stable CMR before discontinuation. After discontinuing imatinib, about half of the patients in clinical studies who discontinued—as as few as 13% (Goh 2009) and as many as 90% (Goh 2011) — were found to have remained in remission for extended periods (Goh 2011; Goh 2009; Mahon 2010; Mahon 2011; Matsuki 2011; Rousselot 2007; Takahashi 2012; Yhim 2012). Clinical studies of dasatinib and nilotinib discontinuation are also underway (Rea 2011).

The factors associated with successful imatinib discontinuation have not been definitively identified, but factors such as a shorter period to BRC-ABL1 negativity (Rousselot 2007), male gender (Mahon 2010), a low Sokal risk score (Mahon 2010), longer duration of imatinib therapy (Mahon 2010; Takahashi 2012), prior IFN-α therapy (Takahashi 2012), greater imatinib dose intensity (Takahashi 2012), and longer duration of CMR before discontinuation (Takahashi 2012) may be important. At this time, treatment discontinuation for CML patients remains an important issue worthy of continued investigation and should not be attempted outside of a clinical study.

The prospect of treatment discontinuation, whether temporary or permanent, could have important implications for patients (Mattison 2009; Smith 2011), clinicians, and payers. For patients, the possibility of treatment discontinuation could bring relief to those dealing with persistent side effects (Cortes 2004; Ghanima 2004); allow patients to start families (Ali 2005; Cortes 2004; Kobayashi 2009), cope with a concomitant illness (Breccia 2006), or be free from continuous medication. It is likely, however, that patients who are able to discontinue treatment may need more frequent disease monitoring and follow-up.

For clinicians, treatment discontinuation may affect the frequency of disease monitoring and patient follow-up.

For payers, treatment discontinuation could affect both medical costs (e.g., drug costs) and health care costs (e.g., potentially necessitating a different schedule for follow-up clinic visits and molecular monitoring, as well as future management and treatment of patients who relapse). At present, however, no published studies have described health care utilization in patients who have discontinued TKI treatment, although this type of study would be valuable.

**Use and understanding of molecular monitoring in routine clinical practice**

Currently, many health care providers do not appear to appreciate fully the need for regular, consistent monitoring of BRC-ABL1 levels (Kantarjian 2007; Radich 2009). They do not understand why monitoring is needed after CCyR has been achieved (Radich 2009); how often molecular monitoring should be performed (Kantarjian 2007; Radich 2009); or how to interpret and act on test results that show rising BRC-ABL1 levels (Radich 2009). It should be impressed upon treating clinicians that molecular monitoring of BRC-ABL1 can be used to rapidly assess treatment response, to predict outcomes, and to plan for contingencies, particularly when the test results suggest that therapeutic modifications may be expected. As I have witnessed in clinical practice, being proactive rather than reactive in the management of patients can be life-saving. The sooner potential problems with treatment and the patient’s response are identified, the greater the opportunity clinicians have to intervene before disease progression.

**Conclusion**

The advent of BCR-ABL1 TKIs for CML has made possible the achievement of deep molecular responses in a high proportion of patients. Because evidence shows that CML patients who remain in remission have a greater likelihood of favorable long-term outcomes, practice guidelines recommend regular and consistent molecular monitoring of BRC-ABL1 transcript levels as a method to regularly assess the patient’s response to treatment and disease progression. Clinicians’ adherence to guidelines ensures the early detection of any changes in response or disease progression, which allows reassessment of and modifications to treatment plans to meet the patient’s changing disease status.

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