Monitoring Chronic Myelogenous Leukemia in the Age of Tyrosine Kinase Inhibitors

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Chronic myeloid leukemia, bcr-abl, treatment monitoring, molecular monitoring, tyrosine kinase, imatinib mesylate

Abstract
Tyrosine kinase inhibitors (TKIs) are now standard up-front therapy for chronic myeloid leukemia (CML). Patients with newly diagnosed chronic-phase CML treated with the TKI imatinib mesylate typically experience a complete cytogenetic remission. Outcomes for patients with advanced-phase disease are distinctly worse. Unfortunately, a small proportion of chronic-phase patients experience relapse during this therapy, and most with advanced-phase disease develop resistance to imatinib mesylate after months of therapy. Hematopoietic cell transplantation remains the only curative approach for CML and can salvage patients with advanced-phase disease. Therefore, physicians must carefully monitor patients with chronic-phase CML treated with TKIs so that they can undergo hematopoietic cell transplant (or treatment with another TKI or experimental therapy) before frank progression occurs. Fortunately, monitoring CML using cytogenetic and molecular methods (i.e., quantitative polymerase chain reaction) effectively defines end points that correlate highly with outcome. [JNCCN 2007;5:497–504]

Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease characterized by the Philadelphia chromosome (Ph), a reciprocal translocation between chromosomes 9 and 22 (Figure 1). CML usually presents in chronic phase, a relatively benign proliferation of (primarily) the myeloid lineage. Without therapy, chronic-phase disease eventually progresses to the aggressive accelerated phase and blast crisis, which are generally fatal secondary to bleeding or infectious complications. The only currently known curative treatment for CML is hematopoietic cell transplant. However, the recent introduction of tyrosine kinase inhibitor (TKI) therapy has dramatically changed the treatment strategy for CML.

CML monitoring is particularly important because several therapeutic approaches are now available. Patient outcomes for all therapies are best in chronic-phase and poorer in advanced-phase disease. Thus, transplantation is associated with long-term survival greater than 75% for patients with chronic-phase disease, but only 40% and 20% for those with accelerated- and blast-phase disease, respectively. Introduction of TKIs has revolutionized the care of CML patients, especially those in chronic phase. Patients with newly diagnosed chronic-phase CML treated with imatinib mesylate (IM) have complete cytogenetic response (CCR) rates greater than 80% and 5-year overall survival rates greater than 90%.[1–4] Relapse in chronic-phase CML, either with a return of chronic-phase CML or progression to more advanced disease, occurs at an estimated annual rate of treatment failure of 3.3% in year 1, 7.5% in year 2, 4.8% in year 3, 1.5% in year 4, and 0.9% in year 5 for patients enrolled in the International Randomized Study of Interferon vs. STI571 (IRIS) trial.[1–4] Unfortunately, responses in accelerated- and blast-phase disease are much poorer, with only approximately 40% and 7% of patients experiencing a CCR, respectively, in phase II clinical trials.[1–7]

The fact that most patients in chronic phase experience an excellent response to IM, whereas some progress underscores the need to identify patients who will ultimately relapse. Early identification of relapse or progression allows for curative or alternative therapy through transplantation or an investigational trial. The quantitative polymerase chain reaction (QPCR) detection of bcr-abl has been shown to be an excellent method for monitoring CML patients.
The Molecular Genetics of CML

The reciprocal translocation of chromosomes 9 and 22 joins the 5' exons of the \textit{bcr} gene from chromosome 22 with the 3' tyrosine kinase exons of \textit{abl} from chromosome 9. This unique fusion gene codes for the chimeric BCR-ABL fusion protein, which is integral to the biology of CML (Figure 2). The exact genetic breakpoint and fusion of BCR and ABL varies. In CML, the breakpoint and fusion of BCR and ABL codes for a 210-kd cytoplasmic fusion protein (p210).

\textbf{Figure 1} Cytogenetic demonstration of the Philadelphia chromosome (Ph). Standard metaphase cytogenetics detect the t(9;22) Ph in more than 95% of chronic myelogenous leukemia (CML) cases; the remaining cases of "cryptic" Ph + CML apparently have translocations too small for detection with cytogenetics, but these cases are positive according to reverse transcription polymerase chain reaction analysis. The reciprocal translocation results in a larger than normal chromosome 9 and a shortened chromosome 22. The arrow indicates the shortened chromosome 22 that is the Ph. (Figure courtesy of Dr. Eileen Bryant, Fred Hutchinson Cancer Research Center, Seattle, Washington)

\textbf{Figure 2} Chromosomal and gene cartoon of the BCR-ABL translocation. The left panel shows the gross structure of the chromosomal reciprocal translocation of chromosomes 9 and 22. The shortened chromosome 22 places the 5' regulatory domains of the BCR gene from chromosome 22 in juxtaposition with the 3' ABL tyrosine kinase domains from the translocated chromosome 9. ABL breakpoints occur anywhere over a 300-kb area upstream of exon Ib, downstream of exon Ia, or most frequently between the 2 (right panel). BCR breakpoints occur in 1 of 3 areas (2 are shown). In chronic myelogenous leukemia (CML), only the p210 is found. Both the p185 and p210 are found in acute lymphoblastic leukemia (ALL). The p210 is generated by alternative splicing between exons 12–16 (p210), resulting in the b2a2 or b3a2 fusion transcripts. (Data from Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood 2000;96:3343–3356.)
Tyrosine Kinase Inhibitor Therapy for CML

In Ph+ acute lymphoblastic leukemia, both the p210 BCR-ABL and a smaller p190 protein can occur. Normal ABL is a nonreceptor tyrosine kinase that shuttles between the nucleus and cytoplasm and appears to regulate apoptosis. The chimeric BCR-ABL protein found in CML is located primarily in the cytoplasm and affects adhesion, proliferation, and apoptosis inhibition. Because BCR-ABL is a unique marker and key regulator of the phenotype of CML disease, it is the obvious target for diagnostic and treatment strategies.

Methods of Monitoring in CML

At diagnosis, a patient may have as many as $10^{12}$ circulating leukemia cells. Therapy decreases the disease burden, and different techniques must be used to monitor disease as the numbers of leukemia cells become progressively lower (Figure 3). The earliest and easiest monitoring checkpoint is a hematologic remission (Table 1), simply defined by the normalization of peripheral white blood cell counts. Most patients with chronic-phase CML experience a hematologic remission within 1 to 2 months after starting IM therapy. The next important milestone relies on cytogenetic response as defined by bone marrow metaphase chromosome analysis. Because of the limited sensitivity of cytogenetics, at least 20 metaphases must be analyzed for proper estimation of disease burden. For chronic-phase disease, the first bone marrow examination after initiation of therapy (all patients should undergo cytogenetic examination before therapy is initiated to accurately document disease stage) should occur at the 6-month mark, with a follow-up at 12 months. This time point is excellent for judging the outcome of therapy and determining prognosis (Table 2). In the IRIS trial of patients with newly diagnosed chronic-phase CML, 86% experienced a CCR after 12 months of therapy. These patients had an excellent prognosis, with 93% of patients experiencing CCR remaining free of disease progression.

More sensitive techniques to monitor disease must be used when a patient experiences a CCR. Fluorescence in situ hybridization (FISH) is a more sensitive measure of the Ph. FISH has 2 inherent advantages over conventional cytogenetics: 1) it does not require dividing cells and therefore more cells can be screened quickly, and 2) it can be performed on peripheral blood. A disadvantage of FISH compared with cytogenetics is that it probes for only the BCR-ABL fusion gene, and therefore other chromosomal changes suggesting advanced-phase disease remain undetected. Its sensitivity generally ranges from approximately 0.5% to 5%, depending on the specific probe used and the laboratory performing the testing. Although FISH has some practical advantages, its level of sensitivity is inadequate for the molecular monitoring strategies that have become important in CML treatment.

Nucleic acid amplification using polymerase chain reaction (PCR) is the most sensitive technique to evaluate minimal residual disease. QPCR assays (disease present or absent) can detect one CML cell in a background of a million normal cells. Real-time quantitative methods of bcr-abl messenger RNA (mRNA) detection are generally approximately one half to one order of magnitude less sensitive than qualitative PCR. mRNA from control genes such as bcr, beta-2 microglobulin, or abl is amplified to ensure the RNA integrity of each patient sample. The details of the QPCR bcr-abl assay and the operating characteristics necessary for a reproducible and robust assay have recently been promulgated in a “white paper” by a panel.
Two subsequent studies post-transplantation in patients who, through cytogenetic examination, seem to be experiencing remission, reliably predicts subsequent relapse, and the assay has been integrated into protocols designed to treat molecular relapse. The PCR assay has now also become a cornerstone of CML monitoring in the TKI era.

**Measuring Response and Resistance in CML Patients Undergoing TKI Therapy**

The landmark IRIS trial showed the first and most convincing evidence of the ability of molecular monitoring to evaluate response to TKI therapy. At the 12-month mark, approximately 70% of patients treated with IM experienced a CCR, compared with only 7% in the interferon (IFN) arm. A separate study examined *bcr-abl* mRNA levels in patients who experienced a CCR, showing 3 important findings. First, patients treated with IM who experienced CCR had significantly greater *bcr-abl* mRNA reductions than similar patients on the IFN/Ara-C arm. A 3-log reduction in *bcr-abl* mRNA expression from baseline (which thereafter was defined as a major molecular response) was obtained in 39% of those treated with IM, compared with only 2% of those treated with IFN/Ara-C.

Secondly, the depth of molecular response at 12 months for the patients treated with IM was associated with progression-free survival. Patients experiencing no CCR had a risk for progression (loss of response and a return to chronic phase, or progression to accelerated-phase disease or blast-crisis) of approximately 25%, and their progression-free survival was 72% at a median of 54 months of follow-up (Figure 4). Patients who experienced a CCR and a less than 3-log reduction in *bcr-abl* at 12 months had a progression-free survival of 89%. Patients who experienced a 3-log or greater reduction in *bcr-abl* by 12 months had a progression-free survival of 97%. Two subsequent studies confirmed the results of the IRIS trial, showing that patients who experienced greater than a 3-log reduction in *bcr-abl* had a very low probability for progression.

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### Table 1 Response Criteria for Monitoring Tyrosine Kinase Inhibitor Therapy

<table>
<thead>
<tr>
<th>Response Assays and Goals</th>
<th>Response Criteria</th>
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<tbody>
<tr>
<td>Complete hematologic response</td>
<td>Complete: Leukocytes &lt; 10 x 10⁹/L, platelets &lt; 450 x 10⁹/L, no immature cells or blasts</td>
</tr>
<tr>
<td>Cytogenetic response</td>
<td>Complete: No Ph+ metaphases</td>
</tr>
<tr>
<td>CCR within 12 months is a predictor of outcome</td>
<td>Partial: 1%–34% Ph+ metaphases</td>
</tr>
<tr>
<td>Minor: 35%–90% Ph+ metaphases</td>
<td></td>
</tr>
<tr>
<td>Molecular responses (RT-PCR)</td>
<td>Major (MMR): &gt; 3-log reduction in <em>bcr-abl</em> ratio</td>
</tr>
<tr>
<td>Evaluated by the <em>bcr-abl</em> control gene ratio</td>
<td>Complete (CMR): poorly defined and not recommended (see text)</td>
</tr>
</tbody>
</table>

Abbreviations: CCR, complete cytogenic response; CMR, complete molecular response; MMR, major molecular response; Ph, Philadelphia; RT-PCR, reverse transcription-polymerase chain reaction.

### Table 2 Recommended Monitoring in Chronic Myelogenous Leukemia on Tyrosine Kinase Inhibitor Therapy

<table>
<thead>
<tr>
<th>Test</th>
<th>Time</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
<td>Diagnosis</td>
<td>Establish disease stage</td>
</tr>
<tr>
<td>Every 6 mo</td>
<td>Assess IM response until CCR</td>
<td></td>
</tr>
<tr>
<td>After CCR</td>
<td>Every 12 mo to look for newly acquired cytogenetic changes anytime <em>bcr-abl</em> increases</td>
<td></td>
</tr>
<tr>
<td>PCR for <em>bcr-abl</em></td>
<td>Diagnosis</td>
<td>Establish breakpoint and <em>bcr-abl</em> level</td>
</tr>
<tr>
<td>After CCR</td>
<td>Monitor for MMR (&gt; 3-log decrease) or increase in <em>bcr-abl</em></td>
<td></td>
</tr>
<tr>
<td>ABL TKD point mutation analysis</td>
<td>Diagnosis</td>
<td>Advanced phase disease</td>
</tr>
<tr>
<td>Anytime</td>
<td>If no/poor response, relapse, or increasing <em>bcr-abl</em></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CCR, complete cytogenic response; IM, imatinib mesylate; MMR, major molecular response; PCR, polymerase chain reaction; TKD, tyrosine kinase domain.
A third interesting result of the IRIS study was that undetectable bcr-abl (rigorously defined as quantitatively and qualitatively undetectable bcr-abl, with confirmation by an outside, second laboratory) was relatively rare, occurring in less than 5% of cases. This finding contrasts with PCR data after allogeneic transplantation, which show that undetectable bcr-abl at 12 months post-transplantation is approximately 75%. 24 This last observation suggests the presence of a small, but real, reservoir of CML cells that may have the potential for disease relapse.

The rate of bcr-abl decline in the initial 3 months of IM therapy also seems to strongly predict subsequent response. 19,21–23 For example, one study showed that only 80% of patients with less than a 1-log response of bcr-abl at 3 months of IM treatment experienced a CCR, compared with 100% in patients with a greater reduction of bcr-abl. 19 Thus, early molecular monitoring after initiating IM therapy may identify patients most likely to experience a poor response to IM therapy.

**Resistance and ABL Tyrosine Kinase Domain Mutations**

Responses to IM are the rule in chronic-phase disease, but responses are relatively short-lived in advanced-phase (accelerated and blast-phase) disease. In chronic-phase disease, approximately 10% of patients treated with the standard 400-mg dose will not experience an adequate cytogenetic response and are classified as primary resistance cases. The mechanism of primary resistance is poorly understood. Relapses that occur after an initial response to IM are known as secondary or acquired resistance and are often caused by the acquisition of point mutations in the ABL tyrosine kinase domain (TKD). These mutations alter the binding kinetics of IM to the ATP-binding pocket of the ABL TKD, thereby reducing BCR-ABL inhibition. 24 TKD point mutations are a function of disease phase. Patients undergoing first-line treatment for chronic-phase disease have the lowest rate of subsequent mutation development, followed by those in late chronic phase (diagnosed > 1 year before starting IM), accelerated phase, and blast crisis. The GIMEMA Working Party on CML examined 256 patients treated with IM, finding ABL TKD point mutations in 26% of those with chronic-phase disease (only 4% in a subset of patients with early chronic-phase disease), 44% with accelerated-phase disease, 73% with myeloid blast crisis CML, and 81% with lymphoid blast crisis CML. 25,26 Point mutations clustered primarily at only 7 amino acid residues and accounted for 85% of the total point mutations detected. 21 Data suggest that not all mutations behave equally. Several of the most common ABL TKD mutations, particularly those in the ATP phosphate-binding loop of the ABL TKD (P-loop) and the T315I mutation, are associated with poor prognosis with progression to advanced disease. 26–28 Although the new TKIs (such as dasatinib and nilotinib) are effective in treating many of these TKD mutations, some, particularly the T315I mutation, are not sensitive to any available agents. 29,30 Recently, MK-0457 has been reported to have efficacy in 3 patients with Ph+ ALL or CML. 31 Patients who develop these mutations should be referred for transplantation or clinical trials of novel investigational therapies.

ABL TKD mutations can be detected with several techniques. 24,27,32,33 The most common is direct nucleotide sequencing, in which the bcr-abl transcript is amplified and the product sequenced to detect the point mutation. Although widely available, this technique is relatively insensitive and can detect mutations at only a frequency of 20% to 25% in a background of wild-type bcr-abl. 31 Direct sequencing has been used to monitor ABL TKD mutations in most...
TKI trials. Denaturing high-performance liquid chromatography is a more sensitive technique that can detect mutations at a frequency of 5% to 15% but it is technically more demanding and not widely available. The limited sensitivity of these assays makes identifying point mutations early in the course of therapy difficult, and therefore most ABL TKD point mutations are detected at relapse. However, serial PCR testing may reveal patients at high risk for having point mutations. Branford et al. found that a greater than twofold rise in bcr-abl mRNA was associated with the emergence of ABL TKD mutations. In 214 patients, 61% of patients with a greater than twofold increase in bcr-abl had detectable mutations compared with less than 1% of patients with stable or decreasing bcr-abl. This reinforces the need for an aggressive monitoring strategy for patients undergoing TKI therapy.

Secondary, Non-Ph+ Clonal Rearrangements in Patients Experiencing CCR
Secondary clonal abnormalities develop in approximately 5% of CCR patients. The most common secondary chromosomal event is trisomy 8, but cytogenetic lesions associated with myelodysplasia (–5 and –7 deletions) also have been described, although progression to myelodysplastics syndrome in these cases is unusual. The long-term consequences of these secondary clonal abnormalities remain unknown, but are another reason periodic bone marrow examination for cytogenetics is indicated.

Testing Recommendations for CML Patients Undergoing TKI Therapy
Guidelines for monitoring patients have been established (Tables 1 and 2), and seem reasonable based on the published literature.
Bone marrow aspirations for pathology and cytogenetics are recommended at 6 and 12 months after the initiation of TKI therapy. A cytogenetic evaluation is required at 6-month intervals until CCR. For example, if CCR has not been achieved by 12 months, another examination at 18 months is necessary. In general, a treatment change should be considered for a suboptimal response and is proposed for patients not experiencing any cytogenetic response by 6 months, those who have not achieved less than 35% Ph cells on routine metaphase cytogenetics by 12 months, and those who have not experienced CCR by 18 months of therapy.

Peripheral blood PCR testing for bcr-abl is recommended every 3 months. Although testing can begin at diagnosis, waiting until the patient experiences a CCR is acceptable. A rising bcr-abl in serial assays prompts a bone marrow evaluation for cytogenetics and testing for an ABL TKD point mutation. In addition, a rising bcr-abl level may support clinical decisions, such as increasing the IM dose, changing to another TKI, or considering allogeneic transplantation.

Ongoing Issues in bcr-abl Molecular Testing
Several issues have limited the use of bcr-abl testing in the United States; it is more routinely used in Europe. A major obstacle is reporting the result. Different laboratories use different control genes, and therefore the ratio of bcr-abl/abl in one laboratory, for example, is not easily compared with that found in another laboratory using bcr-abl/glyceraldehyde-3-phosphate dehydrogenase. In anticipation of this problem, the IRIS trial reported bcr-abl values as a log reduction below a standardized pretreatment baseline. This concept allows any laboratory to use their own preferred control gene, as long as they could report values from a reference baseline in their own laboratory. A misunderstood feature of the IRIS molecular study is that the analysis of log reduction was not performed using each patient’s baseline bcr-abl value; rather, each laboratory established its own baseline using a reference set of 30 diagnostic samples. Unfortunately, most reference laboratories do not report their baseline value for diagnostic samples, and therefore translating a bcr-abl/control gene value to a log reduction scale is impossible. The recent expert consensus on bcr-abl advocates the use of an International Scale based on the log reduction principal.

A second difficulty is that many studies and laboratories use the poorly defined and misleading terms complete molecular remission or PCR-negative. This terminology has 2 problems: 1) a negative bcr-abl assay may simply result from a bad assay with poor sensitivity, and 2) rates of negativity (or complete molecular responses) are often compared with the rates encountered...
in the IRIS trial, but use different assays and less rigorous approaches to define negativity. A bcr-abl-negative assay in the IRIS trial was defined as negative quantitatively and qualitatively (the latter even more sensitive), which was then confirmed as negative at a second reference laboratory. Thus, reports that base bcr-abl negativity on a single quantitative assay cannot properly be compared with the results of the IRIS trial.

Conclusions
Several potentially curative therapies now exist for CML, which is the model disease for integrating high-tech molecular monitoring methods with novel targeted therapies. Standardization of bcr-abl testing is underway that will allow clinicians to carefully monitor disease and help guide decisions about timing of alternative therapies, if necessary. The success of this molecular approach is currently being adapted to the treatment of other hematologic malignancies and solid tumors.

References


