Monitoring the response and course of chronic myeloid leukemia in the modern era of BCR-ABL tyrosine kinase inhibitors: practical advice on the use and interpretation of monitoring methods

Hagop Kantarjian,1 Charles Schiffer,2 Dan Jones,3 and Jorge Cortes1

Departments of 1Leukemia and 2Hematopathology, The University of Texas M. D. Anderson Cancer Center, Houston; and 3Wayne State University School of Medicine, Karmanos Cancer Institute, Division Hematology/Oncology, Detroit, MI

Introduction

The management of hematologic malignancies, including chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), and acute lymphocytic leukemia (ALL), has led the way in establishing the use of regular laboratory testing to assess for residual disease on an ongoing basis. The goals of minimal residual disease (MRD) monitoring for leukemias include (1) demonstration of the effectiveness of initial therapy; (2) monitoring for treatment resistance or relapse; and (3) dissecting the mechanisms of treatment failure to help in selection of alternative therapies. The underlying biology and range of treatment options for each leukemia type influence the methodologies used for MRD monitoring and the timing and duration of such monitoring. For example, flow cytometric monitoring plays a large role in MRD monitoring for ALL and CLL, whereas it is not used currently in CML. Here, we present an overview of the various approaches to monitoring CML, and a practical summary of the current uses of molecular methodologies and their routine applications in clinical practice.

The treatment of patients with Philadelphia chromosome (Ph)−positive CML with imatinib mesylate has resulted in complete cytogenetic response rates of 65% to 85%, major molecular response rates (ie, 3-log reduction in transcript levels from a standardized baseline) of 40% to 70%, and complete molecular response rates (ie, undetectable BCR-ABL transcripts) of 10% to 40%.1−3 Routine cytogenetic analysis is still considered the gold standard for evaluating response in CML.4−7 As most patients achieve complete cytogenetic responses with modern therapy, there is a need to develop more sensitive and accurate monitoring tools to measure residual disease.1−3 It has been assumed that such increased accuracy might help better predict outcome.

Monitoring methods now include fluorescent in situ hybridization (FISH),8−13 and qualitative and quantitative polymerase chain reaction (QPCR).14−25 Molecular studies (ie, PCR) quantify the amount of disease with an increased sensitivity of up to 10−8.26 Better molecular responses may correlate with improved outcome. The rapidity of molecular response may also have additional prognostic significance.25−28 Achievement of a major molecular response after 12 months of imatinib therapy was associated with the best progression-free survival.1,24 Thus, many specialists use today molecular studies in lieu of cytogenetic analysis to monitor response.29,31

Resistance to imatinib and to other BCR-ABL tyrosine kinase inhibitors has been associated with the development of mutations in the BCR-ABL kinase domain in 40% to 50% of instances.

Mutations prevent the activity of imatinib in different ways. Some may prevent the binding of the tyrosine kinase inhibitor to BCR-ABL; others may favor the active conformation of the kinase, a conformation to which imatinib cannot bind.32−34 Different mutations may confer different levels of resistance, from an absolute resistance, to minimal or no resistance, depending on their location and their effect on the kinase. Molecular and mutational analyses have figured prominently in recent scientific studies, but have also been used in clinical practice to monitor the course of CML. It is unclear whether these more sensitive assays are of value in the management of individual patients; misinterpretation of these tests could lead to potentially harmful clinical decisions. Frequently noted examples are (1) referral of patients in complete cytogenetic response for allogeneic stem-cell transplantation because of “increasing” BCR-ABL transcript levels; (2) changing therapy to new tyrosine kinase inhibitors for similar reasons; and (3) declaring resistance to therapy in a patient who had been reported to be molecularly “negative” and who subsequently shows positive transcript levels.

Because of the confusion about the appropriate use and interpretation of these studies, we elected to review the pertinent information in the context of current clinical practice, and to recommend reasonable monitoring steps. This review emphasizes the practical rather than the technical aspects of methods, and should be considered along with recently published recommendations.30,31

First, a brief primer on available tests

There are several tools to identify and quantify the abnormally rearranged gene. Advantages and disadvantages of these monitoring methods are shown in Table 1.

Conventional cytogenetic studies using chromosome Giemsa banding

These are still the gold standard of treatment monitoring. The current definitions of cytogenetic responses were derived from cytogenetic analysis in at least 20 evaluable metaphases. These include the following: complete cytogenetic response—0% Ph-positive metaphases; partial cytogenetic response—1% to 35% Ph-positive metaphases; and minor-minimal (or simply minor)
cytogenetic response—36% to 90% or 95% Ph-positive metaphases. A major cytogenetic response refers to Ph-positive metaphases 0% to 35%.5,6,35 These definitions were established based on the strong association of major and complete cytogenetic response with improved long-term survival with interferon-alpha therapy.5,7 These prognostic associations have also been confirmed with imatinib therapy,13 suggesting that the relationship between response and survival may be independent of the treatment that produced the response.2 Cytogetic studies analyze only cells in metaphase, require a marrow sample, are time consuming, and have a wide confidence interval. In a patient who demonstrates 5 of 20 Ph-positive metaphases (25% Ph-positive = partial cytogenetic response), the 95% confidence interval of such positivity is 9% to 49%. Studies of the association of cytogenetic response with long-term prognosis have so far relied on classic cytogenetic analysis, not on FISH-based results.

**Fluorescent in situ hybridization studies**

Several FISH methodologies have been developed and are reviewed elsewhere.8-14 Traditional FISH uses a 5′ BCR and a 3′ ABL fluorescent probe of different colors and has a false-positive rate of 1% to 10%.9 Newer FISH techniques use 3 or 4 probes, with imatinib therapy,1-3 suggesting that the relationship between response and survival may be independent of the treatment that produced the response.2 Cytogetic studies analyze only cells in metaphase, require a marrow sample, are time consuming, and have a wide confidence interval. In a patient who demonstrates 5 of 20 Ph-positive metaphases (25% Ph-positive = partial cytogenetic response), the 95% confidence interval of such positivity is 9% to 49%. Studies of the association of cytogenetic response with long-term prognosis have so far relied on classic cytogenetic analysis, not on FISH-based results.

While many oncologists use FISH interchangeably with (or in place of) cytogenetic studies, few studies have investigated the association of long-term outcome with cytogenetic response assessed by FISH. However, it is reasonable to expect that responses assessed by FISH may have similar long-term implications.36-40 In fact, FISH is beginning to be incorporated into current studies of tyrosine kinase inhibitors in CML.41 although most current trials exploring the efficacy of the new tyrosine kinase inhibitors and phase 3 trials comparing imatinib to the new tyrosine kinase inhibitors still rely on conventional cytogenetic analysis for cytogenetic response. Whether patients in complete cytogenetic response (ie, 0% Ph-positive by cytogenetic analysis) but FISH positive would have a similar or worse prognosis to FISH-negative patients is unknown. Clinicians should also be cautious in establishing failure to treatment based on low levels of positivity by FISH.

In clinical practice, it may be reasonable to use peripheral blood FISH as an alternative to routine cytogenetics until the FISH levels are less than 5% to 10%. At that point, a cytogenetic analysis should be used to confirm a complete cytogenetic response. Patients can subsequently be monitored with FISH studies, as long as they are reported to be negative or within the range of background positive results reported by the laboratory performing the test. Persistent low levels of FISH positivity require additional testing (discussed in “Molecular studies”).

**Molecular studies, polymerase chain reaction**

In earlier studies of patients after stem-cell transplantation, qualitative reverse-transcriptase polymerase chain reaction (RT-PCR) was used. More recently, quantitative techniques have been developed, now most frequently performed by real-time RT-PCR.14 Results usually report the ratio of BCR-ABL to a reference gene (recommended genes include ABL, BCR, and GUSB).17,29,31 Several technical aspects have not been standardized, including the sample source (blood or marrow), the effects of shipping and storage, the amount of sample required, the techniques for extraction of RNA, and the preferred reference gene. A detailed description of the

### Table 1. Comparison of conventional cytogenetics, FISH, and molecular studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conventional cytogenetics</th>
<th>FISH</th>
<th>QPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, % tumor</td>
<td>5%-10%</td>
<td>1%-10%</td>
<td>0.001-0.01</td>
</tr>
<tr>
<td>Accuracy of measurement</td>
<td>± 15%</td>
<td>± 2%-5%</td>
<td>± 2- to 5-fold</td>
</tr>
<tr>
<td>Metaphases required</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Marrow sample required</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Equivalence of blood and marrow results</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>False negativity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>False positivity</td>
<td>Rare</td>
<td>Yes, at ≤ 10% level</td>
<td>Yes, at ≤ 0.1% level</td>
</tr>
<tr>
<td>Detection of other chromosomal abnormalities</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Detection of deletions of derivative chromosome 9</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Data from Testoni et al.36

### Table 2. Correlation between conventional cytogenetics and FISH studies

<table>
<thead>
<tr>
<th>Cytogenetic response</th>
<th>No.</th>
<th>FISH 0%</th>
<th>FISH 1% to 5%</th>
<th>FISH more than 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete, 20 or more metaphases</td>
<td>217</td>
<td>177 (82)</td>
<td>33 (15)</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Complete, fewer than 20 metaphases</td>
<td>94</td>
<td>67 (71)</td>
<td>20 (21)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Partial</td>
<td>43</td>
<td>0</td>
<td>21 (49)</td>
<td>22 (61)</td>
</tr>
<tr>
<td>Complete + major molecular response</td>
<td>179</td>
<td>164 (92)</td>
<td>12 (7)</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>

Data from Testoni et al.36
techniques is beyond the scope of this review, but has been discussed previously, together with the variability and reproducibility/reliability of such molecular studies.29,30

As more patients achieve complete cytogenetic responses with imatinib and other tyrosine kinase inhibitors, there is interest in obtaining better molecular responses. A major molecular response has been defined as a reduction in transcript levels (BCR-ABL/reference gene) of at least 3-logs compared with a standardized baseline obtained from patients with untreated newly diagnosed CML. The standardized baseline in the IRIS trial defined as the average ratio from 30 patients was 36%; thus, a major molecular response was defined as achieving levels of 0.036% or less. The considerable range in the values among these patients lends some imprecision to the calculation of the log reduction in an individual patient. This standardized baseline value has to be defined by individual laboratories; unfortunately, this has not been done by many commercial laboratories. The baseline value can also vary considerably from laboratory to laboratory and is not always included in the laboratory report.17,24,25 There is currently an effort to establish a harmonization of results from different laboratories using predefined control samples to adjust the results from each laboratory, such that a conversion factor can be established. Then, all laboratories can adjust their values uniformly to define a major molecular response as a value of 0.1% or less on the adjusted scale.30

A complete molecular response is achieved when transcripts are not detectable (better referred to as undetectable transcript levels), in an assay that has at least 4- to 5-log range of detection. Unfortunately, when negative results for BCR-ABL are reported, the sensitivity is not always reported.

Molecular studies have several advantages including (1) good correlation between marrow and blood levels and (2) detection of very low levels of residual disease. Limitations include (1) a substantial incidence of false-negative tests (RNA degradation, low sensitivity of a given assay); (2) a coefficient of variability, which may be up to 0.5 log; and (3) poor reproducibility of results. Wide variations of transcript levels may be due simply to technical reasons rather than real changes in CML burden.

**Mutation analysis**

Analysis of mutations of the BCR-ABL kinase domain use different techniques: (1) direct sequencing of DNA following nested RT-PCR (detect mutations in 10% to 25% of tumor cells);30,42 (2) denaturing-high performance liquid chromatography analysis (DHPLC; sensitivity of 5% to 10%);43 and (3) fluorescent-based allele-specific oligonucleotide PCR (ASO-PCR) assays (detect mutations in 0.1% to 1% of tumor cells).44 Sequencing of DNA clones increases the sensitivity of the assays in some studies. Direct sequencing is the technique most frequently available to clinicians. Mutation studies have been performed in peripheral blood or bone marrow; a correlation between samples has not been reported.

**Second, an assessment of the use of these methodologies in CML monitoring**

**Cytogenetic analysis**

Routine karyotype is the only routinely available technique that assesses all chromosomes. This is important because additional chromosomal abnormalities besides Ph (ie, clonal evolution) are a criterion for accelerated phase. In addition, some patients treated with tyrosine kinase inhibitors develop chromosomal abnormalities in Ph-negative cells. Most abnormalities are transient or clinically innocuous. Rare instances of transition to myelodysplastic syndromes or acute leukemias have been reported. Thus, performing a marrow aspiration with cytogenetic analysis is recommended at the time of diagnosis, to confirm complete cytogenetic response, and every 12 months thereafter. Conventional cytogenetic studies at 3, 6, and 12 months are performed more often in the setting of research programs. In routine practice, the 3- and 6-month evaluations often use FISH studies that give reasonable estimates of cytogenetic response at these time points, without resorting to a painful bone marrow aspiration procedure.

**Fluorescent in situ hybridization studies**

Pretreatment FISH studies provide some potential benefits: (1) In an occasional patient with a morphologic picture of CML but no detectable Ph-positive metaphases by routine cytogenetics, a FISH or a molecular analysis may detect BCR-ABL–mediated disease.45 (2) Peripheral blood FISH can confirm a diagnosis of CML (although avoiding marrow analysis is not recommended, as the percentage of blasts and basophils in the bone marrow is needed to establish the disease staging). Conversely, FISH or PCR studies can exclude the diagnosis of CML in patients with leukocytosis, obviating the need for a marrow biopsy. (3) They allow for the detection of deletions around the breakpoint of the derivative chromosome 9.46,47 These had been associated with worse prognosis in the imatinib era,46,47 but this is now controversial48 and may not be evident with imatinib therapy.49

FISH studies pretreatment are useful to establish a pretreatment baseline to monitor response by FISH.30 FISH studies are a useful alternative to cytogenetic studies during early Ph suppression, and are informative in a state of complete cytogenetic response if the FISH results are negative.

**Molecular studies**

Molecular studies before treatment may identify Ph-negative BCR-ABL–positive CML, and establish a baseline level for subsequent monitoring. Failure to achieve a cytogenetic response confers a poor prognosis. Whether molecular results can define patients in complete cytogenetic response who are destined to relapse is unclear. The results from the IRIS trial, the most widely cited in this regard, showed that patients who achieved a major molecular response at 12 months had a small but statistically significantly better probability of survival free from transformation to accelerated or blastic phases. The 5-year transformation-free survival was 100% for patients who achieved a major molecular response (± 3-log reduction in transcript levels) versus 95% if they had achieved a complete cytogenetic remission but not a major molecular response ($P = .01$).1,24 Although a similar analysis for survival was not reported, it is likely that the difference would be even smaller. Thus, not achieving a major molecular response in a patient in complete cytogenetic remission at 12 months should not trigger a major change of therapy, particularly given the variability of the assays.

Other studies have not shown a significant difference in progression-free survival or in survival in patients achieving a complete cytogenetic response at 12 months by the depth of molecular response (major vs less than major).2 Similar results were obtained using landmark analyses at 6, 18, and 24 months (H.K., unpublished data, October 2007). The proposed definitions of failure to imatinib therapy do not include failure to achieve molecular response.31 Whether any change in treatment strategy...
cytogenetic relapse. Mutations did not correlate with response to imatinib, event-free survival, or other outcomes. Mutations were not detected in any of the 20 patients in chronic phase. Mutations were detected in 10% of patients in accelerated or blast phase, and in 4% of patients with chronic-phase CML who developed resistance to imatinib, 30% to 50% of patients exhibit imatinib-resistant BCR-ABL KD mutations. New, more potent tyrosine inhibitors, some of which also inhibit SRC family of kinases, overcome resistance in most such cases as well as in instances where no mutations are detected.

In the setting of clinical resistance to imatinib, mutational analyses may be helpful in several ways. Identifying T315I mutations, sometimes referred to as a “gatekeeper” mutation, predicts for resistance to the tyrosine kinase inhibitors imatinib, nilotinib, and dasatinib. Such patients may be referred earlier for allogeneic stem cell transplantation (SCT) or offered investigational therapies with drugs that can inhibit T315I-bearing BCR-ABL. Since resistance in chronic phase occurs in 4% of patients, and T315I mutations account for 10% to 20% of the 40% of resistant patients who develop mutations, the development of the T315I mutations is predicted to occur in approximately 0.16% to 0.32% of newly diagnosed patients in chronic phase on therapy (prevalence of CML approximately 70,000 cases in the United States; annual number of cases with T315I mutations approximately 150 to 300).

Mutations of the BCR-ABL KD have been categorized by their location into mutations occurring in the ATP-binding domain (P-loop), the critical sites of direct contact with imatinib and other kinase inhibitors, the activation loop (A-loop), and the catalytic domain. The definition of P-loop includes amino acids 248 to 255, although some have suggested it should be restricted to amino acids 250 to 255. Earlier studies have associated T315I and P-loop mutations with worse outcomes.

A study by Branford et al reported a very poor survival in patients with P-loop mutations. The 1-year survival rate of 13 patients with P-loop mutations was 15% versus 80% with non-P-loop mutations (P = 0.002). This was corroborated by a multicentric study reported by Nicolini et al. An Italian study by Soverini et al analyzed mutational status by D-HPLC and sequencing among patients who received imatinib and had less than a major cytogenetic response (Ph-positive disease > 35%) after 12 months of therapy. Among 40 such patients, 19 (48%) had detectable mutations. The presence of any mutation, or of P-loop mutations, correlated with worse time to progression and with worse survival and progression-free survival rates. Other studies could not confirm these findings. Jabbour et al analyzed 171 patients with CML after imatinib failure: 62 patients (36%) had mutations (P-loop in 24). They found a similar survival in patients with chronic-phase CML and imatinib resistance whether or not they had mutations, and whether the mutation was located in T315I, the P-loop, or other areas. Possible reasons behind the differences in the results were reviewed. Prognosis in CML after imatinib failure may be dependent on several factors including the therapeutic interventions used after imatinib failure. The geographic grouping of mutations is probably not appropriate for purposes of defining outcome and therapy. Different mutations may alter the kinase activity and proliferation potency of BCR-ABL differently, which may be more relevant than the location of the mutation. Thus, mutations are better described in terms of their relative sensitivity to specific therapies such as imatinib, nilotinib, and dasatinib (ie, the IC50 for each drug to inhibit BCR-ABL with the specific mutation in vitro). With further clinical experience with the newer tyrosine kinase inhibitors, mutational studies may allow selection of particular treatments based on relative sensitivities and clinical responses to different inhibitors, although the accuracy of selecting therapy based on the specific mutation has not been tested prospectively (Table 3). Furthermore, whether finding a mutation in patients with rising transcript levels who are still in complete cytogenetic response should prompt a change in therapy remains to be investigated. The risks and benefits of the alternative therapy should also be carefully evaluated.
Finally, a proposal for a practical monitoring approach in CML

When the authors discussed this section, there was only partial consensus on the optimal monitoring approach with some disagreements on the details of the sequence of tests to be used for patients in chronic phase. We all agreed that adequate monitoring is important to optimize outcome of patients with CML. We therefore elected to present several monitoring approaches as proposed and practiced by CML experts.

The “conservative” approach

Some investigators contend that the best current information relating response and prognosis derives from routine cytogenetic studies. Based on this, they suggest that cytogenetic analysis should still be a central element of monitoring. Thus, a conservative approach would be to monitor patients by bone marrow cytogenetic analysis every 6 months until a complete cytogenetic response is achieved, and then every 6 to 12 months.

The “FISHer” approach

As valuable and historically important as they may be, cytogenetic studies usually require a marrow aspiration, which is painful. Some physicians rely only on peripheral blood FISH to follow response to avoid doing marrows. The extreme proponents of this approach may use only FISH as a surrogate for “molecular” responses, either erroneously interpreting the FISH as sensitive enough to evaluate such responses, or not being convinced that molecular responses may add much information in patients in complete cytogenetic remission (ie, those with repeatedly “negative” results) as measured by FISH.

The “molecular enthusiast” approach

Some CML experts have endorsed the molecular analysis as a substitute to cytogenetics for monitoring. In this approach, PCR studies are performed before treatment, then every 3 months in the first year, and every 3 to 6 months subsequently. This approach relies purely on peripheral blood monitoring and aims to achieve and maintain QPCR levels at a major or better molecular response (eg, BCR-ABL/ABL ratio of 0.1% or less). This response would be associated with a very low relapse rate and excellent survival. Cytogenetic responses may not even be reported, instead referring to a 2-log reduction in transcript levels (or BCR-ABL/ABL ratio of 1% in the international scale) as the equivalent of such a response. Disadvantages to this approach include (1) wide variability of the test (up to 0.5-log or 2- to 5-fold range variation) in commercial and even some “reference” laboratories; (2) disregard of any information from marrow procedures (eg, percentage of basophilis and blasts, presence of myelofibrosis, cytogenetic abnormalities in Ph-positive or Ph-negative cells); (3) false-negative results in laboratories using assays of low sensitivity; and (4) unfamiliarity with the possible variability in results that may not reflect a clinically significant change in CML burden. Physicians are seldom provided with the standardized baseline for the laboratory reporting the results, making interpretation difficult. The proposed international standardization is unfortunately not yet universal. Under these circumstances, molecular monitoring may lead to improper therapeutic decisions.

The “I-don’t-care-about-any-such-studies” approach

It is interesting that some oncologists and hematologists do not use any of these studies, particularly in elderly patients (eg, 70 years or older). The proponents of this approach perceive the benefit-cost ratio to be low, since they may not routinely change therapy unless there is clear-cut evidence of hematologic relapse, which can be detected by inexpensive peripheral blood count studies. The authors believe this approach not to be in the patient’s best interest, as it would delay the implementation of beneficial therapeutic interventions until settings where they may be less effective (eg, advanced-stage disease). At a minimum, some assessment of the degree of CML response is mandatory, given the different options available to patients not responding to imatinib.

A “hybrid” approach

This approach mixes the relative strengths of different monitoring procedures and variations of this approach are favored by the authors. The weight given to each method, and some of the details (eg, frequency, methodology) may vary by investigator.

(a) Since a bone marrow analysis before treatment is useful to assess prognostic factors such as the percentage of marrow blasts and basophilis and clonal evolution, a pretreatment cytogenetic analysis is advisable. FISH or molecular studies on the peripheral blood may not be necessary unless the physician wishes to rely on them solely for monitoring. However, they can help establish that the transcripts can indeed be detected in the individual patient by such tests. Subsequently, a marrow analysis and cytogenetic studies are ordered at 6 and 12 months, then once every 1 to 2 years after a patient has achieved a complete cytogenetic response. If, with further research, the chromosomal abnormalities in Ph-negative metaphases prove to be clinically insignificant, then marrow analyses can be performed less frequently (eg, every 2+ years).

(b) FISH studies would be performed every 3 months in the first 12 months until repeated values are negative. FISH studies would be omitted when marrow analyses are performed (cytogenetic studies done instead). After cytogenetic complete response is confirmed, regular monitoring by QPCR should be done.

(c) Patients can be monitored using real-time PCR every 3 months in the first 12 months, then every 6 months once a stable complete cytogenetic response is achieved. As long as BCR-ABL/CONTROL gene ratios are within the range of major molecular response (0.1% or less in the international scale), this indicates a solid response. If there is an increase in the ratio (greater than the coefficient of variability), this should trigger a repeat analysis, more frequent monitoring (eg, every 3 months), and repeating a bone marrow analysis and cytogenetics, particularly if QPCR levels rise to higher than 1%. Variations in BCR-ABL/ABL levels in a patient in complete cytogenetic response would not trigger a major change in therapy (eg, allogeneic stem-cell transplantation). In a patient with repeatedly rising molecular levels (eg, to higher than 0.1% and a 5-fold increase) who remains in complete cytogenetic response, it may be reasonable to increase the dose of imatinib with a switch to a new TK inhibitor should the dose...
increase not be effective, or to offer a clinical trial. It is also important to determine that the patient was compliant with taking his/her imatinib. Some investigators also advocate that a rise of QPCR levels by 2- to 5-fold should trigger a mutational analysis.

So, when do we do the mutational studies?

In our opinion, mutational studies should be performed only with evidence of hematologic or cytogenetic resistance relapse. Whether they should be performed in patients responding to imatinib therapy if PCR transcript levels increase by 2- to 5-fold remains an open question. But if they are performed, the results should be interpreted with caution, particularly as it relates to any proposed change in therapy. Risks and benefits based on available data should be carefully weighed. Mutational studies may also be helpful any time a change of therapy is considered: identification of specific mutations may help in the choice of one treatment approach over another (eg, allogeneic SCT if T315I mutation identified) or of a specific BCR-ABL inhibitor depending on the relative sensitivity of the particular mutation to these inhibitors (Table 3).55-57

Conclusions

The outcome of patients with CML has improved significantly, and the availability of an increasing number of effective treatment options makes adequate monitoring of patients increasingly important. While some research trials are proposing to evaluate the incidences of major or complete molecular response in patients in complete cytogenetic response as the comparative efficacy end point, the prognostic significance of such molecular (major, complete) end points for long-term survival is unknown. Just as the treatment of CML is rapidly changing, concepts related to monitoring, including available techniques, the interpretation of the results, and the best use of this information are constantly and rapidly evolving. Table 4 proposes a reasonable monitoring treatment approach for CML.

Table 4. Proposed approach to monitoring CML

<table>
<thead>
<tr>
<th>1. At diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG: part of marrow evaluation; detects clonal evolution</td>
</tr>
<tr>
<td>FISH: detects Ph-negative BCR-ABL-positive disease; detects deletions of derivative chromosome 9; also if further follow up before CGCR by FISH</td>
</tr>
<tr>
<td>QPCR: only if subsequent monitoring is based solely on QPCR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. On therapy until cytogenetic complete remission (or equivalent disease level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG: every 3-6 months</td>
</tr>
<tr>
<td>FISH: alternative to CG; every 3 months</td>
</tr>
<tr>
<td>QPCR: alternative to CG; every 3 months</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Documentation of cytogenetic complete remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG: FISH alternative to CG; QPCR as baseline for subsequent comparisons</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. Following attainment of cytogenetic complete remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG: every 12-24 mo</td>
</tr>
<tr>
<td>FISH: alternative to CG; every 6 mo</td>
</tr>
<tr>
<td>QPCR: alternative to CG or FISH; every 3-6 mo</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. At time of suspected resistance/relapse (cytogenetic or hematologic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeat CG</td>
</tr>
<tr>
<td>QPCR</td>
</tr>
<tr>
<td>Mutational studies to direct choice of subsequent therapy</td>
</tr>
</tbody>
</table>

CG indicates cytogenetics; and CGCR, complete CG response.

Authorship

Contribution: H.K., C.S., and J.C. designed research, performed research, analyzed data, and wrote the paper; D.J. performed research, analyzed data, and wrote the paper.

Conflict-of-interest disclosure: H.K., C.S., and J.C. received research grants from BMS and Novartis. D.J. declares no competing financial interests.

Correspondence: Hagop Kantarjian, Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Unit 428, Houston, TX 77030; e-mail: hkantarj@mdanderson.org.

References

19. Luthra R, Sanchez-Vega B, Medeiros LJ. Quantitative TaqMan 1 (9:22) (q34;q11) PCR coupled


Monitoring the response and course of chronic myeloid leukemia in the modern era of BCR-ABL tyrosine kinase inhibitors: practical advice on the use and interpretation of monitoring methods

Hagop Kantarjian, Charles Schiffer, Dan Jones and Jorge Cortes