Chronic Myeloid Leukemia

Session Chair: Armand Keating, MD

Speakers: Timothy Hughes, MD, MBBS; Michael J. Mauro, MD; and Jane F. Apperley, MBChB

ABL Kinase Inhibitor Therapy for CML: Baseline Assessments and Response Monitoring

Timothy Hughes

For chronic phase chronic myeloid leukemia (CML) patients treated with imatinib, the essential pre-therapy assessments include bone marrow morphology and cytogenetics as well as a baseline real-time quantitative PCR (RQ-PCR) for BCR-ABL. Baseline assessments of clinical relevance include Sokal and Hasford prognostic scores. Several other baseline assays are likely to be predictive of response, including IC50imatinib, organic cation transporter-1 (OCT-1) mRNA level, and gene expression profiles, but further confirmation is required. RQ-PCR assays of blood at least every 3 months once patients have commenced imatinib is recommended. This will facilitate early identification of suboptimal responders who may benefit from higher doses of imatinib or alternative therapy, and identify at an early stage patients with acquired resistance. Management of the latter group can be further guided by the findings from cytogenetics and BCR-ABL kinase domain mutation screening. Bone marrow cytogenetics is indicated at least every 6 months until the patient achieves major molecular response. RQ-PCR is only clinically useful if it is conducted under a rigorous quality control regimen so that fluctuations in the BCR-ABL level can be confidently attributed to a biological cause rather than assay variation. To further improve the clinical value of RQ-PCR monitoring, expression of results on an international scale is needed. This will facilitate a more uniform and rational approach to management of suboptimal response and loss of response.

Most chronic phase chronic myeloid leukemia (CML) patients who receive imatinib as first-line therapy will achieve good cytogenetic and molecular responses.1,2 In the IRIS trial complete cytogenetic remission (CCR) was observed in 69% and major molecular response (MMR) in 40% of patients by 12 months.2 MMR is defined as the achievement of ≥3 log reduction in BCR-ABL from the standardized baseline, which was established by calculating the median BCR-ABL level of 30 pre-therapy samples. The achievement of MMR at 12 months is associated with 100% probability of transformation-free survival at 60 months.3 However, long-term molecular studies suggest that around 25-30% of patients will not achieve MMR even after 60 months of therapy.4 Furthermore, 10-15% of CML patients treated with imatinib as first-line therapy will have disease progression. Major causes of imatinib resistance include the emergence of leukemic clones with mutations in the kinase domain of BCR-ABL and clonal evolution.5,6 Several pretherapy and early therapy parameters have been identified that are predictive of response to imatinib. If prospectively confirmed these pretherapy markers could be used to identify patients who require higher doses of imatinib up front or a more potent ABL kinase inhibitor. Once imatinib therapy commences, frequent and accurate monitoring may identify poor responders who are unlikely to achieve prolonged progression-free survival (PFS) with ongoing imatinib therapy at the current dose. Some of these patients may respond to higher doses of imatinib,9 while others may require either a second-generation ABL kinase inhibitor or an allogeneic stem cell transplant. Whatever second-line therapy is selected it should be applied before the disease transforms into the accelerated or blast phase. Once the disease has entered advanced phase, long-term responses to second-line therapy are unlikely. On the positive side, the majority of patients receiving first-line imatinib therapy achieve major molecular responses by 18-

Correspondence: Timothy Hughes, MD, MBBS; Institute of Medical Veterinary Science, Frome Road, Adelaide, 5000, Australia; Phone +61 (8) 82223330; Fax +61 (8) 82223139; Email timothy.hughes@imvs.sa.gov.au
24 months, and regular RQ-PCR assays will provide these patients with reassurance that standard-dose imatinib is the appropriate therapy for the long term.

Pretherapy Predictors of Response to Imatinib in de-novo CML

Cytogenetic analysis
Cytogenetic analysis of bone marrow metaphases has been the standard technique for the diagnosis of CML and also identifies any cytogenetic abnormalities, in addition to the Philadelphia chromosome, that are present in the leukemic population. While the appearance of additional chromosomal abnormalities in the Ph-positive clone over time is associated with poorer responses to imatinib and inferior outcome, the significance of additional changes at diagnosis is less clear. Given the more favorable responses observed using imatinib at 600 mg/day in patients with clonal evolution as their only manifestation of accelerated phase, it would be reasonable to start imatinib therapy with 600 or 800 mg/day in these patients. The prognostic significance of deletions in 9q in the imatinib era remains uncertain.

Sokal/Hasford prognostic scores
Both the Sokal and Hasford prognostic scores were developed in the pre-imatinib era. However, they still have some value in predicting response to imatinib. CCR and MMR are more frequently observed in low-risk Sokal patients and the degree of improvement in molecular response over time in patients achieving CCR may also be linked to the Sokal score. High-risk Sokal patients achieving CCR at 12 months have a 90% probability of survival at 54 months compared to 94% and 97% in intermediate- and low-risk Sokal patients (p = 0.054). However, even the most unfavorable prognostic score is not sufficiently adverse to justify a recommendation that a patient go straight to an allograft without a trial of imatinib.

mRNA expression profiles
The potential to identify pathways and genes in primary CML cells that are disrupted in individual CML patients has stimulated many groups to conduct microarray studies using pretherapy CML blood or marrow cells. There is an expectation that these studies will provide a patient-specific profile that will be predictive of response and toxicity, facilitating a personalized approach to therapy rather than a uniform approach for all. At this stage no expression profile has been sufficiently characterized to be incorporated into treatment guidelines. In a recent study Yong et al applied a multivariate Cox regression model to microarray data from CD34+ cells collected at diagnosis. They identified low expression of CD7 combined with high expression of proteinase 3 or elastase in CD34+ cells as strong predictors of long survival. However, these data were derived from patients in the pre-imatinib era and have not yet been tested for predictive value in patients receiving imatinib. Several gene expression studies have been performed in patient cohorts who received imatinib therapy. In these studies the main focus was the prediction of short-term cytogenetic response. Thirty-one genes were identified in patients on the IRIS study that were strongly predictive of the achievement of a major cytogenetic response (MCR); however, the predictive value of these genes has not been tested prospectively. A study looking at expression profiles in white blood cells taken from patients divided according to whether they achieved cytogenetic response to imatinib or not found no consistent pattern predictive of failure to achieve CCR. However, a more recent study from the same group found some significant differences in the expression profile in CD34+ cells according to cytogenetic response. A study of chronic, accelerated and blastic phase patients showed a distinct expression profile involving 3000 genes that were differentially expressed in blast crisis. Larger patient cohorts now need to be tested prospectively to define more precisely whether an expression profile can be identified that is sufficiently adverse to indicate the need for a different initial therapeutic approach in appropriate cases.

Intrinsic sensitivity to ABL kinase inhibitors
The sensitivity of CML cells to Bcr-Abl kinase inhibition by imatinib can be calculated by determining the concentration of imatinib needed in vitro to inhibit Bcr-Abl kinase activity by 50% (IC50imatinib). There is significant interpatient variability in IC50imatinib values in mononuclear cells taken from CML patients prior to therapy. Furthermore, the variability in sensitivity to imatinib is biologically relevant because patients with high intrinsic sensitivity (low IC50imatinib) had superior molecular responses. IC50imatinib does not correlate with Sokal prognostic scores. Combining the two prognostic indicators provides an even stronger predictor of molecular response. Patients who have Sokal scores below the median as well as low IC50imatinib have a 73% probability of achieving MMR by 12 months (Figure 1). Why is there such a range of sensitivity among CML patients at diagnosis? It appears that differences in IC50imatinib are mainly due to differences in the efficiency of intracellular uptake and retention (IUR) of imatinib. This can be measured in vitro by adding radiolabeled 14C imatinib to mononuclear cells from CML patients and measuring the intracellular drug concentration at defined timepoints. Significantly higher intracellular concentrations of imatinib (IUR) are observed in low-IC50 patients. Imatinib uptake into cells is dependent on active influx mediated mainly by the OCT-1 pump. The cause of interpatient differences in imatinib uptake may be a variable function of OCT-1. Interestingly, AMN107 (nilotinib) does not depend on OCT-1 for its uptake into cells. These observations were all made within the context of the Australian TIDEL trial, in which higher doses of imatinib were used in newly diagnosed chronic phase patients and dose
increases were mandated for suboptimal response. The prognostic impact of IC50\textsuperscript{imatinib} and IUR has not yet been tested in the setting of more conventional dosing schedules. The notion that variable cellular uptake of imatinib is a key factor in determining response is further supported by a study looking at the prognostic value of \textit{OCT-1} mRNA levels in CML cells. This study found that patients with high \textit{OCT-1} expression were more likely to achieve good cytogenetic responses.\textsuperscript{22} Another study found that imatinib-induced reductions in total phosphotyrosine levels in CD34\textsuperscript{+} cells, measured by flow cytometry, were predictive of cytogenetic response to imatinib.\textsuperscript{23} The reduction in expression of WT1 in CML cells exposed \textit{in vitro} to imatinib pretherapy was also found to predict cytogenetic response.\textsuperscript{24} Variable expression of WT1 may reflect differences in the intracellular concentration of imatinib. Putting all these observations together, it would appear that a subset of patients have poor Oct-1 function and will have lower intracellular concentrations of imatinib relative to blood levels. This might in some cases be overcome by higher doses of imatinib or with a second-generation Abl kinase inhibitor. If these observations are confirmed, some or all of these assays could be incorporated into the standard baseline assessment to provide a guide to select the appropriate Abl kinase inhibitor and the appropriate dose.

**Baseline level of BCR-ABL mRNA**

There is currently no evidence that the amount of BCR-ABL in the blood at diagnosis has predictive value. When we followed a group of patients with high baseline BCR-ABL levels and a group with low baseline levels we could see no difference in their median BCR-ABL levels by 3 months of imatinib therapy or at later time points. The importance of undertaking a baseline study of BCR-ABL is to confirm the presence of either the B2A2 or B3A2 transcripts (or both). This is necessary to identify rare patients who have an alternative BCR-ABL fusion transcript that cannot be monitored by the standard RQ-PCR assay.

**Sensitive baseline screen for BCR-ABL mutations**

In chronic phase CML patients receiving first-line imatinib therapy, early progression to the acute phase with evidence of an emerging BCR-ABL mutant clone is sometimes observed (1-5%).\textsuperscript{25} It would clearly be desirable to identify these patients pretherapy and enroll them in a trial of a different therapeutic approach. Unfortunately, there is currently no evidence that the early emergence of an aggressive mutant clone can be predicted by high-sensitivity screening for mutations prior to commencing imatinib.\textsuperscript{26} However, the risk factors for emerging mutations in chronic-phase patients have been identified. Duration of disease prior to imatinib therapy, pretherapy peripheral blood blast count and the cytogenetic response at 6 months are all predictive of the risk of developing resistance associated with mutations.\textsuperscript{27} For patients with sudden transformation without evidence of resistant mutations, baseline features have not proved predictive. Indeed, a study of the rare occurrence of sudden blast crisis in chronic-phase patients after achieving CCR found that low-risk features were evident at the time of disease presentation.\textsuperscript{28}
Early Response Indicators

Hematological response
Achievement of early hematological response is a prerequisite to achieving cytogenetic response, but very few patients fail to achieve complete hematological response (CHR) on imatinib. Failure to achieve CHR by 3 months is generally regarded as imatinib failure, indicating the need to consider second-line therapy. A related issue is whether cytopenia is a poor prognostic indicator. Cytopenia has not been associated with poor prognosis in the first-line setting; however, dose reduction or prolonged treatment interruption, which may be required in the cytopenic patient, has been associated with a lower probability of achieving MMR.25

Cytogenetic response
Traditionally, treatment response has been assessed using cytogenetic determination of the number of Ph-positive metaphases in the bone marrow. A partial cytogenetic response represents 1% to 35% Ph-positive metaphases and 0% indicates a CCR. An MCR includes complete and partial responses. In a comparison of cytogenetics and RQ-PCR, 98% of patients who had achieved a 1-2 log reduction were in a MCR and 91% of patients who had achieved 2-3 log reduction were in CCR.29 Therefore, at early time points the molecular response is a good indicator of the level of leukemia reduction and correlates strongly with cytogenetic status. While RQ-PCR can substitute quite well as an early response indicator, only cytogenetic studies can identify the emergence of additional chromosomal abnormalities in the leukemic clone. The progressive accumulation of additional chromosomal abnormalities is an important indicator of disease progression. Thus cytogenetic studies are important as long as the leukemic clone is detectable but of limited value once the patient achieves CCR. Recent analysis of 828 simultaneous RQ-PCR and bone marrow cytogenetic studies from 183 imatinib-treated patients in morphological chronic phase was conducted to assess what additional clinical information was provided by simultaneous cytogenetic analysis. No Ph-positive cells were observed in any of the patients who achieved and maintained a MMR.29 A reasonable monitoring policy would be to conduct cytogenetic analysis of the bone marrow at baseline and at 6 months and then 6 monthly until a patient achieves MMR. Patients who subsequently lose MMR with a significant rise in BCR-ABL level should recommence marrow cytogenetic studies.29

For patients who maintain MMR the only possible value of cytogenetics is the identification of other chromosomal abnormalities (OCA) in the Ph-negative cells. These have been reported in about 5% of patients who achieve CCR on imatinib.30 The most common abnormality is trisomy 8, but deletion of chromosome 7 is also observed. These changes may be transient. In a few cases OCA has been associated with the development of myelodysplasia or acute myeloid leukemia.30 The presence of OCA with no dysplastic features in the blood is probably not an indication for a change in therapy. On this basis routine marrow cytogenetics in patients with normal blood parameters who are maintaining MMR is probably not indicated.

Fluorescent in situ hybridization (FISH)
Some clinicians rely on FISH studies from blood or bone marrow to follow response to imatinib therapy. Co-hybridization of BCR and ABL probes to interphase nuclei (I-FISH) can be used to identify BCR-ABL-positive cells. A false positive rate of 5-10% and its relative insensitivity make it unsuitable for the analysis of minimal residual disease. I-FISH on total blood cells correlates poorly with marrow cytogenetics in imatinib-treated patients, whereas I-FISH of blood neutrophils correlated much better.31 Dual color FISH (D-FISH) provides much lower levels of false positivity (≤ 0.8%), although its sensitivity is still suboptimal. Furthermore, D-FISH is unreliable in patients with 9q deletions. Hypermetaphase FISH provides better sensitivity as well as a low false-positive rate and may be useful for following patients while their leukemia level is still relatively high.31 It is not a substitute for conventional cytogenetics, however, because it will not detect additional cytogenetic abnormalities. FISH may be useful in cases where cytogenetics is not informative. However, once MMR has been established, no FISH method will be sufficiently sensitive to monitor response.

In vivo kinase inhibition
Studies of predictive assays from the TIDEL trial suggest that patients who do not achieve good ABL kinase inhibition in the first 28 days (imatinib dose used was 600 mg/day), as measured by the level of phosphorylated CrkI in blood-derived mononuclear cells, are less likely to achieve MMR by 12 and 24 months.32 Factors found to impact on the level of in-vivo kinase inhibition achieved included actual dose received and the IC50 mutant. These findings need to be prospectively tested in the setting of conventional doses of imatinib before they can be accepted as a valid early warning of suboptimal response.

Molecular response
The amount of BCR-ABL transcript in the peripheral blood, measured as a ratio of BCR-ABL to a control gene, provides an estimate of the number of terminally differentiated cells that are leukemic. Serial studies in which the level of BCR-ABL is tracked in individual patients suggest that it is a good indicator of the total leukemic cell mass. A temporary interruption to imatinib therapy is typically associated with a steady increase in BCR-ABL level.33 Patients who develop mutations in the kinase domain of BCR-ABL almost always have a significant rise in the BCR-ABL level consistent with proliferation of resistant leukemic cells.34 The level of BCR-ABL is also a good predictor of PFS.7 In the IRIS study, serial RQ-PCR assays indicated that the log
reduction in BCR-ABL, measured from the standardized baseline, was a good predictor of subsequent response and risk of progression. The achievement of MMR by 12 months in the IRIS trial was associated with 100% probability of transformation-free survival at 60 months. MMR was achieved by 40% in the IRIS trial by 12 months and by 55% and 75% at 24 and 44 months, respectively. It is not known whether achieving MMR beyond 12 months is equally predictive of a low risk of disease progression. It is not yet known whether achieving MMR at an earlier time-point by using an increased imatinib dose leads to an improved PFS. In both the IRIS trial and the TIDEL trial the molecular response at 3 months proved to be a good predictor of achievement of MMR by 24 months. Another approach to molecular monitoring is to measure the level of BCR-ABL once CCR is achieved, rather than at 3 monthly intervals from the start of therapy. The problem with this approach is that the predictive value of the early molecular response will not be realized. Furthermore, patients who do not achieve CCR by 6 months are the group most likely to develop resistance and are therefore most likely to benefit from close molecular monitoring at these early time points.

Using RQ-PCR as the primary screening strategy
Regular RQ-PCR monitoring with selective bone marrow cytogenetic analysis and mutation screening provides the critical information required for clinical decision-making in CML. A practical approach is to monitor RQ-PCR levels monthly until a 1 log reduction is achieved and then at 3 monthly intervals. This will enable early recognition of imatinib resistance in most cases. If there is a significant rise in BCR-ABL level, the assay should be repeated as soon as possible. The magnitude of increase designated “significant” will depend on the measurement reliability of the assay in the local laboratory. Ideally, these assay-specific variables should be clearly set out in the laboratory report (Tables 1 and 2). In unstable situations, when a higher dose is being tested or the patient has temporarily ceased imatinib, monthly RQ-PCR may be appropriate.

Regular mutation monitoring
Regular mutation screening would identify emerging mutant clones at the earliest time-point. This is appropriate for patients in the advanced phases of CML, when emerging mutations are frequent (Figure 2). However, given the relatively low incidence of mutations in chronic-phase patients receiving imatinib as first-line therapy (5-10% over 2 years), it is probably not cost effective as a general policy. Nevertheless selected patients who have suboptimal cytogenetic and molecular responses and those who have significant increases in the BCR-ABL level should be targeted for mutation studies. The use of more sensitive

Table 1. Example of how serial results of RQ-PCR assays can be presented with local and international scale values shown. Codes can be used to clarify interpretation.

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Source of Material</th>
<th>BCR-ABL Copy Number</th>
<th>BCR Control Gene Copy Number</th>
<th>Ratio (%)</th>
<th>International Scale Conversion (%)</th>
<th>Code</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>02/17/2003</td>
<td>Blood</td>
<td>66,235</td>
<td>80,950</td>
<td>82</td>
<td>105</td>
<td></td>
<td>Pre-imatinib</td>
</tr>
<tr>
<td>05/29/2003</td>
<td>Blood</td>
<td>6,665</td>
<td>173,920</td>
<td>3.8</td>
<td>4.8</td>
<td>A</td>
<td>3 months on imatinib</td>
</tr>
<tr>
<td>08/21/2003</td>
<td>Blood</td>
<td>105</td>
<td>106,915</td>
<td>0.10</td>
<td>0.13</td>
<td>B</td>
<td>6 months on imatinib</td>
</tr>
<tr>
<td>11/13/2003</td>
<td>Blood</td>
<td>95</td>
<td>120,035</td>
<td>0.08</td>
<td>0.10</td>
<td>C</td>
<td>9 months on imatinib</td>
</tr>
<tr>
<td>02/05/2004</td>
<td>Blood</td>
<td>200</td>
<td>90,625</td>
<td>0.22</td>
<td>0.28</td>
<td>D</td>
<td>12 months on imatinib</td>
</tr>
<tr>
<td>05/05/2004</td>
<td>Blood</td>
<td>565</td>
<td>204,000</td>
<td>0.28</td>
<td>0.35</td>
<td>D</td>
<td>15 months on imatinib</td>
</tr>
</tbody>
</table>

Codes:
A: BCR-ABL at a level > 1% on international scale suggests patient has not achieved CCR
B: BCR-ABL at a level < 1% on international scale suggests patient has achieved CCR
C: Major molecular response achieved
D: Significant rise in BCR-ABL level from nadir and mutation analysis is pending

Sensitivity indicator:
BCR level 100,000-400,000 sensitivity at least 0.01% on international scale.
BCR level > 400,000 sensitivity at least 0.003% on international scale.

Measurement reliability:
BCR-ABL values < 0.1% CV = 27.7%
BCR-ABL values 0.11-1% CV = 19.8%
BCR-ABL values > 1% CV = 15.3%
assays for mutation screening is of unproven value at present, mainly because not all mutations identified by sensitive assays (HPLC, ASO-PCR) will lead to resistance over the subsequent 12-24 months. We have previously shown that a significant rise in BCR-ABL level (> 2-fold with the Adelaide assay) is associated with a 61% incidence of mutations by direct sequencing at the time of the significant rise.34 In cases where the > 2-fold rise was still below the level of MMR, the incidence was 40%. Mutation analysis is particularly relevant for those patients with the T315I mutation or P-loop mutations as these have been associated with a poorer prognosis in some, but not all studies.38-41 An explanation for this discrepancy may be that poor prognosis is associated with some but not all of the P-loop mutants. Figure 3 shows our current monitoring policy.

**International Standardization of Molecular Monitoring**

It is highly desirable that an international scale of measurement for BCR-ABL transcript levels is established. This would enable molecular responses to be compared in trials assessing different drug regimens where the RQ-PCR assays have been performed in different laboratories using various techniques. It would also allow the clinician relying on RQ-PCR results from a local laboratory to determine with confidence whether their patient had achieved MMR. A consensus meeting in October 2005 in Bethesda proposed the establishment of an international scale that could be applied at individual centers. It was agreed that the international scale would be anchored to the MMR level, which would be expressed as a value of 0.1%. The process for the local laboratory to convert their in-house results to the international scale involves (1) adoption of

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Source of Material</th>
<th>BCR-ABL Copy Number</th>
<th>BCR Control Gene Copy Number</th>
<th>Ratio (%)</th>
<th>International Scale Conversion (%)</th>
<th>Code</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>02/11/2003</td>
<td>Blood</td>
<td>79,860</td>
<td>50,950</td>
<td>156</td>
<td>195</td>
<td>Pre-imatinib</td>
<td></td>
</tr>
<tr>
<td>05/20/2003</td>
<td>Blood</td>
<td>415</td>
<td>144,760</td>
<td>0.29</td>
<td>0.36</td>
<td>B</td>
<td>3 months on imatinib</td>
</tr>
<tr>
<td>08/11/2003</td>
<td>Blood</td>
<td>115</td>
<td>252,755</td>
<td>0.05</td>
<td>0.06</td>
<td>C</td>
<td>6 months on imatinib</td>
</tr>
<tr>
<td>10/27/2003</td>
<td>Blood</td>
<td>10</td>
<td>215,715</td>
<td>0.006</td>
<td>0.008</td>
<td></td>
<td>9 months on imatinib</td>
</tr>
<tr>
<td>01/19/2004</td>
<td>Blood</td>
<td>20</td>
<td>236,310</td>
<td>0.008</td>
<td>0.010</td>
<td></td>
<td>12 months on imatinib</td>
</tr>
<tr>
<td>04/19/2004</td>
<td>Blood</td>
<td>0</td>
<td>241,555</td>
<td>0.009</td>
<td>0.011</td>
<td></td>
<td>15 months on imatinib</td>
</tr>
<tr>
<td>07/08/2004</td>
<td>Blood</td>
<td>0</td>
<td>330,100</td>
<td>0</td>
<td>0</td>
<td>F</td>
<td>18 months on imatinib</td>
</tr>
<tr>
<td>10/11/2004</td>
<td>Blood</td>
<td>0</td>
<td>320,450</td>
<td>0</td>
<td>0</td>
<td>F</td>
<td>21 months on imatinib</td>
</tr>
<tr>
<td>01/04/05</td>
<td>Blood</td>
<td>15</td>
<td>914,160</td>
<td>0.002</td>
<td>0.003</td>
<td>G</td>
<td>24 months on imatinib</td>
</tr>
<tr>
<td>03/08/05</td>
<td>Blood</td>
<td>0</td>
<td>567,340</td>
<td>0</td>
<td>0</td>
<td></td>
<td>27 months on imatinib</td>
</tr>
</tbody>
</table>

**Codes:**

B: BCR-ABL at a level <1% on international scale suggests patient has achieved CCR
C: Major molecular response achieved
F. BCR-ABL undetectable at a sensitivity of at least 0.01% on international scale, which is equivalent to > 4 logs below the standardized baseline as defined in the IRIS trial
G. BCR-ABL undetectable at a sensitivity of at least 0.003% on international scale, which is equivalent to > 4.5 logs below the standardized baseline as defined in the IRIS trial

**Sensitivity indicator:**

BCR level 100,000-400,000 sensitivity at least 0.01% on international scale.
BCR level > 400,000 sensitivity at least 0.003% on international scale.

**Measurement reliability:**

<table>
<thead>
<tr>
<th>BCR-ABL values</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.1%</td>
<td>27.7%</td>
</tr>
<tr>
<td>0.11-1%</td>
<td>19.8%</td>
</tr>
<tr>
<td>&gt; 1%</td>
<td>15.3%</td>
</tr>
</tbody>
</table>

**International Standardization of Molecular Monitoring**

It is highly desirable that an international scale of measurement for BCR-ABL transcript levels is established. This would enable molecular responses to be compared in trials assessing different drug regimens where the RQ-PCR assays have been performed in different laboratories using various techniques. It would also allow the clinician relying on RQ-PCR results from a local laboratory to determine with confidence whether their patient had achieved MMR. A consensus meeting in October 2005 in Bethesda proposed the establishment of an international scale that could be applied at individual centers. It was agreed that the international scale would be anchored to the MMR level, which would be expressed as a value of 0.1%. The process for the local laboratory to convert their in-house results to the international scale involves (1) adoption of

**Figure 3. Current monitoring policy for chronic-phase chronic myeloid leukemia (CML) patients treated with imatinib in Adelaide. Patients continue 3 monthly molecular monitoring by RQ-PCR beyond 24 months.**
the consensus principles established by the Bethesda group; (2) testing a set of reference standards multiple times to establish a laboratory-specific conversion factor; and (3) multiplying all local BCR-ABL values by the conversion factor to express results on the international scale. To test the accuracy of this approach, a set of clinical samples assayed in the local laboratory would then need to be sent to an international reference laboratory to verify that the conversion factor provided an accurate conversion. Tables 1 and 2 demonstrate conversion of BCR-ABL values to the international scale by using results from 2 patients who were followed by the Adelaide group.

**Conclusion**

The development of predictive assays for molecular response and PFS may enable ABL kinase inhibitor therapy to be specifically tailored for the individual patient rather than the current “one dose for all” practice. Patients assessed as sensitive could receive a standard dose while less sensitive patients receive a higher dose initially or a more potent ABL kinase inhibitor. Patients predicted to be refractory to ABL kinase inhibitors could proceed straight to allogeneic transplant. Harmonization of RQ-PCR assays internationally and the preparation of certified reference materials has been identified as a major priority. Once established, the adoption of RQ-PCR monitoring internationally will facilitate early and rational therapeutic decisions in cases of suboptimal response and acquired resistance.

**References**


newly diagnosed patients with CML is predictive of molecular response, independent of the ability to increase dose at a later point. ASH Annual Meeting Abstracts. 2005;106:164a.


27. Branford S, Rudzki Z, Lynch K, Hughes T. Pre-imatinib factors can be used to define the risk of BCR-ABL mutations for patients with CML in chronic phase and identify a minority who should have regular mutation screening. ASH Annual Meeting Abstracts. 2005;106:1079a.


