How to Monitor Patients with Chronic Myelogenous Leukemia

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CML is associated with the presence of a specific chromosomal translocation (9;22) (q34;q11). This reciprocal translocation between the long arms of chromosomes 9 and 22 results in a shortened chromosome 22, commonly known as the Philadelphia chromosome (Ph). The molecular consequence of this translocation event is the fusion of the c-abl oncogene from chromosome 9 to sequences from chromosome 22, the breakpoint cluster region (bcr), giving rise to a chimeric bcr-abl gene.

This gene encodes a fusion protein, of varying size, depending on the site of the breakpoint in bcr. The two most common fusion proteins produced are termed p185 (185 kd) and p210 (210 kd). The p210 protein is seen in approximately 95% of patients with CML and up to 20% of adult patients with de novo acute lymphocytic leukemia (ALL), whereas the p185 form is seen in approximately 10% of adult patients with ALL and in the majority of pediatric patients with Ph-positive ALL (5% of pediatric ALL cases). These fusion proteins have constitutive tyrosine kinase activity, which is essential for their transforming ability.

Conclusive evidence for their role in leukemogenesis is the demonstration that introduction of bcr-abl into murine hematopoietic stem cells followed by transplantation of these stem cells into syngeneic mice produces a CML-like syndrome. Although bcr-abl is thought to be the initial disease-transforming event in CML, the acquisition of other molecular and cytogenetic abnormalities is likely to be responsible for disease progression.

Treatment

Until recently, standard treatment options for patients in the chronic phase of CML were allogeneic stem cell transplantation, hydroxyurea, busulfan, or interferon–α–based regimens. The understanding that the deregulated tyrosine kinase activity of bcr-abl is the essential transforming event in CML provided the rationale for...
the development of imatinib. Based on the encouraging preliminary results of the International Randomized Study of Interferon vs STI571 (IRIS) study, imatinib has now been licensed by the Food and Drug Administration as first-line treatment for CML. This large randomized study compared imatinib with interferon and ara-C (the previous standard of care) in newly diagnosed patients. For all parameters assessed, including cytogenetic response rates, progression-free survival, and tolerability, imatinib was clearly superior, establishing it as the new nontransplantation therapy of choice.

Goals of Treatment
Regardless of the mode of treatment, the goal should be to reduce the number of \( bcr-abl \)-expressing cells to as low a level as possible. As such, successful therapy should achieve, in consecutive order, a complete hematologic response, a cytogenetic response, and, ultimately, molecular remission, with no remaining evidence of \( bcr-abl \) transcripts. Response definitions for these different categories of response are shown in the NCCN Chronic Myelogenous Leukemia Clinical Practice Guidelines in Oncology (see page 514; guidelines are available online at www.nccn.org). The degree of tumor load reduction is an important prognostic factor and, thus, using appropriate techniques to monitor patients at regular intervals to judge the efficacy of treatment is critical.

Techniques for Monitoring
Techniques generally available for monitoring include standard metaphase cytogenetics, fluorescent in situ hybridization (FISH), and, perhaps to a lesser extent, quantitative real-time polymerase chain reaction (QRT-PCR). Standard metaphase cytogenetics assesses the proliferative fraction of marrow cells. Several disadvantages are associated with this technique. Because analysis requires dividing cells, a bone marrow sample is required. Sometimes adequate numbers of cells fail to grow in culture, precluding full assessment. Marrow culture and metaphase analysis is a laborious and time-consuming process, and this can lead to significant delays in obtaining results. Moreover, a small number of metaphases, usually only 20, are analyzed, limiting the sensitivity of this technique to approximately 5%.

Nevertheless, metaphase cytogenetics remains the gold standard test for monitoring patients. Achievement of a cytogenetic response as assessed by metaphase cytogenetics has long been recognized as a surrogate for survival in interferon-treated patients. Data are now emerging that achievement of a major cytogenetic response may also be associated with a survival advantage in imatinib-treated patients. An advantage of this technique is that it also can detect the presence of additional chromosomal abnormalities in the \( Ph \)-positive cell, which can be a feature of disease progression. More recently, additional abnormalities have been described in \( bcr-abl \) negative cells after treatment with imatinib.

Fluorescence in Situ Hybridization
Interphase fluorescence in situ hybridization (FISH) analyzes cells using 2 sets of fluorescent probes, a 3′ \( abl \) probe and a 5′ \( bcr \) probe, which give red and green fluorescent signals, respectively. The older single fusion technique (S-FISH) gives a signal pattern 2 red and 2 green in normal cells, corresponding to the 2 normal \( abl \) and \( bcr \) genes, respectively. In cells containing \( bcr-abl \), the signal pattern is one red, one green, and one yellow fusion signal, representing the derivative chromosome 22.

Unfortunately, this technique is associated with a significant false-positive rate of 5% to 10%. This arises from the random three-dimensional superimposition of red and green signals in normal cells, mimicking a \( bcr-abl \) fusion. This problem has been addressed by the newer double fusion (D-FISH) probes, which flank the breakpoints of the \( bcr \) and \( abl \) genes. The normal signal pattern with these probes is 2 red and 2 green. The common abnormal pattern for D-FISH is one red, one green, and 2 yellow signals (representing both the derivative 9 and derivative 22). Observation of a single interphase cell with one red, one green, and 2 yellow signals in normal cells is considered to be positive for the \( Ph \) arrangement, with a false-positive rate of 0.2%. Variations in the signal pattern may reflect underlying complex karyotypes, and when a single fusion pattern is seen, as can be the case with variant fusions or chromosome 9 deletions, the false positive rate is 10% to 15%, as previously described.

A major advantage of FISH is that a large number of cells can be rapidly analyzed (usually 100 to 500). In addition, this technique can be performed on peripheral blood and the results correlate well with marrow samples, at least in patients treated with interferon. However, discrepancies between conven-
tional cytogenetics and FISH are occasionally seen. At Oregon Health & Science University, we noted an inconsistent correlation in imatinib-treated patients between FISH and cytogenetics when FISH was greater than 35%. Potential explanations for this discrepancy include: 1) scoring of T-lymphocytes, which are predominantly bcr-abl negative, may underestimate residual disease; 2) metaphase cytogenetics analyzes proliferating cells, and interphase FISH analyzes non-dividing cells; and finally, 3) when cells are placed in metaphase culture, they are washed and may be grown for up to 48 hours in culture medium in the absence of imatinib. This may lead to the outgrowth of a highly proliferative fraction of Ph-positive cells. Other disadvantages of FISH include the inability to detect clonal evolution and, depending on the technique, a false-positive rate of between 0.2% (D-FISH) and 10% (S-FISH). In conclusion, this author’s opinion is that interphase FISH is a useful back-up technique in situations in which obtaining adequate metaphases for analysis is not feasible. However, it does not replace the need for periodic metaphase examination.

Hypermetaphase FISH (HM-FISH) combines a modified preparation of metaphases with FISH. Because a far greater number of metaphases (up to 500) are analyzed, the sensitivity is increased compared with conventional cytogenetics. Because metaphases are directly analyzed, the false-positive rate is also low. However, whether the prognosis of complete responders by HM-FISH is significantly better than that of complete responders by conventional cytogenetics remains to be evaluated in a clinical trial.

This technique is not widely available.

Polymerase Chain Reaction
Reverse transcriptase-polymerase chain reaction (RT-PCR), using primers corresponding to bcr and abl exons, can be used to amplify very low levels of bcr-abl mRNA, allowing detection of one leukemic cell in a background of 10^6 to 10^8 normal cells. As such, this is the most sensitive method for measuring residual disease. The most sensitive qualitative method, “nested” PCR, uses a second set of primers, located within the sequence amplified by the primary set, in a second round of amplification, thus enhancing the sensitivity and specificity of the reaction. This enables detection of up to 1 in 10^6 cells. Although useful diagnostically and in monitoring patients after allogeneic stem cell transplantation, the major limitation of these qualitative methods is that they cannot quantify changes in disease burden over time.

Quantitative real-time PCR (QRT-PCR) allows the rapid quantification of bcr-abl mRNA. Two main technologies are available: the TaqMan system (Applied Biosystems, Foster City, CA) and the LightCycler system (Roche Diagnostics, Basel, Switzerland). As increasing PCR product is formed, a fluorescent signal is emitted that is proportional to the amount of PCR product. In addition to bcr-abl, mRNA from a normal gene, such as abl or G6PD, is amplified as an internal control. Results are usually expressed as a percentage ratio of bcr-abl: internal control (e.g., bcr-abl:abl%). QRT-PCR may not be as sensitive as nested PCR and it is usual to confirm any negative tests with nested PCR.

In addition to helping to predict relapse after allogeneic stem cell transplantation, QRT-PCR is also very effective at predicting durability of complete cytogenetic response in interferon-treated patients. Those with bcr-abl:abl ratios less than 0.045% have been shown to have low risk of cytogenetic relapse. As clinical experience with imatinib grows, QRT-PCR probably will play an increasing role in patient management. Reports that a rapid reduction in bcr-abl transcripts may predict subsequent cytogenetic response already have been published.

In the IRIS study, a correlation was seen between decline in bcr-abl transcript number and progression-free survival (PFS). In patients in whom a complete cytogenetic response was not achieved within 12 months, the 24-month PFS was 90%. Patients in complete cytogenetic response with a bcr-abl log reduction less than 3 showed a PFS of 96%, and those with a greater than 3 log reduction (38% of imatinib-treated patients) had 100% PFS. A major advantage is that quantitative QRT-PCR can be performed on peripheral blood; advocates argue that this test could replace bone marrow examinations in the future. Disadvantages include the current lack of standardization, with considerable interlaboratory variability; the inability to detect clonal evolution; and, perhaps, lack of widespread availability and cost.

How Successful is Imatinib in Achieving Treatment Goals?
Allogeneic stem cell transplantation achieves all of these goals in a majority of patients. Although imatinib has achieved impressive cytogenetic responses,
very few patients have shown molecular negativity. In the IRIS study, major and complete cytogenetic response rates at 18 months with imatinib were 85% and 74%, respectively. In contrast, fewer than 25% of interferon-treated patients showed a major cytogenetic response. However, despite these encouraging response rates, only 3% showed molecular negativity as assessed by QRT-PCR. In contrast, allogeneic stem cell transplantation induces molecular negativity in a majority of patients. Whether molecular remission is necessary for long-term disease control in imatinib-treated patients remains an unanswered question. As noted previously, achievement of a cytogenetic response is an important surrogate for survival in interferon-treated patients, and evidence is growing that this also may be the case with imatinib, with lack of response associated with disease progression.

**Suggested Monitoring**

Repeating a bone marrow aspirate every 6 months after starting imatinib therapy is appropriate. Samples should be sent for routine metaphase cytogenetics with FISH, a useful back-up test where available. In addition to assessment of cytogenetic response (residual Ph positivity), metaphase analysis permits detection of new clonal abnormalities in the CML clone, which may be associated with resistance and disease progression. Marktel et al. recently reported that development of new clonal abnormalities during treatment with imatinib is associated with subsequent disease progression, particularly in patients with recurrent imatinib-induced neutropenia. The authors hypothesize that these patients may have minimal residual Ph-negative stem cells, leading to the selection of a population of “more transformed” Ph-positive cells, which are relatively resistant to the drug. In keeping with this hypothesis, Hochhaus et al. recently reported the presence of clonal evolution in a significant number of patients with established resistance. The authors showed that, in some cases, resistance occurred despite continuous inhibition of bcr-abl by imatinib. Marktel et al. concluded that the early identification of new clonal abnormalities may be an indication to adopt alternative therapeutic approaches.

After a patient shows complete cytogenetic response, monitoring minimal residual status using Q-PCR for bcr-abl is appropriate where possible. Although still considered investigational and lacking standardization, this is a useful test. As noted, a major advantage is that quantitative Q-PCR can be performed on peripheral blood. However, given the increasing reports of clonal abnormalities in Ph-negative cells in patients on imatinib, periodic monitoring of marrow metaphases (perhaps annually) is still warranted.

For treatment with imatinib to be considered successful, a patient should achieve a complete hematologic response within 3 months, major cytogenetic response within 6 months, and complete cytogenetic response within 12 months. Absence of complete hematologic response within 3 months, lack of any cytogenetic response after 6 months, lack of major cytogenetic response after 12 months, lack of complete cytogenetic response after 18 months, loss of complete hematologic response or major cytogenetic response, or a sharp rise in bcr-abl transcripts to a level consistent with cytogeneticrelapse all should be considered an indication for a change of therapy. Options at this stage could include escalation of the dose of imatinib to 800 mg, imatinib in combination with novel agents such as farnesyl transferase inhibitors or arsenic trioxide, or allogeneic stem cell transplantation.

**References**


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