The importance of hematologic, cytogenetic, and molecular testing and mutational analysis in chronic myeloid leukemia

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The introduction of *BCR-ABL1* tyrosine kinase inhibitors (TKIs) for treatment of chronic myeloid leukemia (CML) has made it possible for this cancer to be controlled in many patients for long periods with chronic medication and regular monitoring of disease status. Hematologic and cytogenetic testing, molecular monitoring, and *BCR-ABL1* mutational analysis have become integral to the routine management of CML. The information that each type of test provides is essential to confirm a diagnosis, determine the disease stage, assess response to treatment, and monitor for signals of disease progression – all of which can be used to identify patients who might require further evaluation, closer follow-up, and additional intervention, and to guide clinical decisions. This review describes how each type of test is performed, the information it provides, and the clinical importance of such information. It also uses actual patient case studies to illustrate important points. The goal of this review is to provide health care providers, particularly nursing professionals, with a clear understanding of the method and purpose of each type of test required in the management of patients with CML.

Chronic myeloid leukemia (CML) is a hematologic cancer marked by the overgrowth of myeloid cells in the bone marrow and the accumulation of myeloid cells in peripheral blood. Almost all patients with CML have a chromosomal abnormality, the Philadelphia (Ph) chromosome,[1] which is the hallmark of this cancer. Formation of the Ph chromosome through a translocation between chromosomes 9 and 22 generates an aberrant fusion gene, *BCR-ABL1*, that encodes a constitutively active tyrosine kinase that has been shown to be the pathologic cause of CML.[2]

CML in chronic phase (CP) is highly treatable. In most patients who have been newly diagnosed with CML-CP, disease control can be maintained for years under long-term, continuous treatment with BCR-ABL1 tyrosine kinase inhibitors (TKIs). Five TKIs are currently approved for treatment of CML: imatinib, nilotinib, and dasatinib are approved for first- and second-line use, and bosutinib and ponatinib are approved for second- or third-line use. In addition to these TKIs, a protein synthesis inhibitor, omacetaxine mepesuccinate, derived from the extract of the yew tree, is also approved for third-line treatment of CML after treatment with at least two TKIs. Imatinib (originally known as STI571) was the first TKI approved for the treatment of CML. Eight years of follow-up of the phase 3 International Randomized Study of Interferon vs STI571 (IRIS) in the treatment of patients with newly diagnosed CML-CP have documented annual rates of disease progression with imatinib ranging from 0% to 2.8%.[3-5] In head-to-head comparative studies with imatinib, nilotinib and dasatinib are associated with even lower annual rates of progression. Four years of follow-up of the Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients (ENESTnd) study found that treatment with nilotinib results in significantly lower annual rates of disease progression each year compared with imatinib.[6-9] Three years of follow-up of the Dasatinib versus Imatinib Study in Treatment-Naive CML Patients (DASISION) found that treatment with dasatinib also resulted in lower annual rates of disease progression compared with imatinib.[10-12]

Both bosutinib and ponatinib were approved for second- or third-line treatment of CML on the basis of findings of phase 2 studies. The bosutinib phase 2 study showed rates of 2-year progression-free survival (PFS) of 79% in patients with CML-CP resis-

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tant to or intolerant of prior imatinib[13] and 73% in patients with advanced CML who failed treatment with ≥ 2 TKIs.[14] Bosutinib has also been evaluated against imatinib as an initial therapy in patients with CML-CP.[15] In the first-line setting, although bosutinib was associated with a lower rate of disease progression, it did not significantly improve the rate of complete cytogenetic response (CCyR) compared with imatinib.

In the phase 2 PACE (Ponatinib Ph+ acute lymphoblastic leukemia [ALL] and CML Evaluation) study, cytogenetic and molecular responses were observed in patients with CML-CP or advanced CML who were resistant to or intolerant of ≥ 1 TKI. This included patients harboring the T315I *BCR-ABL1* mutation, which is resistant to other TKIs approved for CML.[16] A clinical study of first-line ponatinib versus imatinib is currently underway. No results are available yet.

Essential components of the management of patients with CML that is controlled with TKI therapy are the routine monitoring of treatment response and the assessment of minimal residual disease. Performing the recommended tests at regular intervals provides information that can help guide treatment decisions, predict prognosis, and herald the occurrence of disease relapse before the appearance of clinical symptoms. Despite the importance and necessity of routine testing in CML, surveys show that many health care providers do not perform such testing at the recommended frequencies.[17-20]

In this review, I describe the types of testing that are performed in patients with CML, outline recommendations for the frequency of testing, and discuss the clinical data that support the importance of each test in optimizing clinical outcomes for patients with CML.

Common tests performed in the management of CML

The National Comprehensive Cancer Network (NCCN) provides clinical practice guidelines that include recommendations for health care providers on the timing and frequency of the tests used in the management of CML.[21] These recommendations are summarized in Table 1. The criteria that define levels of response as assessed by various types of testing are provided in Table 2 (p. 182).

Hematologic testing

Hematologic testing, which includes a complete blood cell count with white blood cell (WBC) differential, is done routinely as part of the patient work-up at the time of diagnosis. Hematologic test results are used to determine the stage of disease that patients are in, either early (CP) or advanced (accelerated phase [AP] or blast phase [BP]). Several sets of criteria that define CML-AP and CML-BP disease are commonly used (eg, those developed by MD Anderson Cancer Center, the World Health Organization, and the International Bone Marrow Transplant Registry). Although these sets of criteria include distinct clinical and pathologic factors, they all rely at least in part on cell counts (WBC differential and platelets) to determine disease stage.[21]

In the past, hematologic testing was used as a measure of early response to TKI treatment and the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) previously included achievement of complete hematologic response (CHR) as the expected level of response after 3 months of TKI therapy. However, clinical experience has now demonstrated that nearly all patients on first-line TKI therapy achieve CHR by 3 months. Therefore, this criterion was not stringent enough to distinguish high-risk patients – who might benefit from further evaluation, closer follow-up, or a change in treatment plan – from low-risk patients, who do not need additional intervention. In recent NCCN Guidelines, the NCCN revised the 3-month response milestone to the achievement of deeper levels of response (ie, cytogenetic or molecular response).[21]

Hematologic testing is also performed routinely during TKI therapy as a means to monitor loss of CHR and the emergence of myelosuppression. Because loss of hematologic response may be a sign of TKI resistance and/or disease relapse or progression, patients who lose CHR require further evaluation, including assessment of treatment adherence and drug interactions and *BCR-ABL1* mutational analysis (see p. 6-7). A change in treatment plan should be considered for these patients, with the goal of recapturing previously achieved levels of response. Myelosuppression is a frequent and predictable side effect of TKI therapy. Patients who develop severe myelosuppression during TKI therapy may require dose adjustment, brief interruption of treatment or addition of growth factors to TKI therapy.[21]

Cytogenetic testing

Cytogenetic testing usually involves either karyotyping or fluorescence in situ hybridization (FISH). Of the 2 techniques, cytogenetic testing by karyotyping is more commonly used in routine monitoring in CML. Karyotyping allows the visualization of the full complement of chromosomes at once and is suitable for detecting gross chromosomal abnormalities. Karyotyping is typically performed using cells from a bone marrow sample that are induced to undergo mitosis. Cells are arrested in metaphase and burst open and nuclei are fixed. Stains are added to reveal structural features of chromosomes (Figure 1, p.182).[22] At least 20 cells must be analyzed to be considered an adequate sample.[23] Karyotyping has the resolution to detect such chromosomal abnormalities as translocations, aneuploidy, deletions, and insertions. In fact, karyotyping was the technique used to determine that the Ph chromosome

TABLE 1 Recommended timing and frequency of hematologic, cytogenetic, and molecular testing, and mutational analysis[a]

Testing	Recommended timing and frequency of testing
Hematologic	At diagnosis, to obtain a complete blood count with differential and platelets
Cytogenetic	At diagnosis, for karyotypic analysis to confirm the diagnosis[b] and for morphological review to establish the disease phase During TKI therapy ■ At 3 mo, if qRT-PCR (IS) is not available ■ At 12 mo, if neither CCyR nor MMR is achieved ■ At 18 mo, if MMR is not achieved and CCyR was not achieved at 12 mo ■ At any time, if ≥ 1-log increase in *BCR-ABL1* level without MMR
qRT-PCR (IS)	At diagnosis to establish a baseline *BCR-ABL1* level During TKI therapy ■ Every 3 mo, if patient is responding to treatment ■ After achievement of CCyR, every 3 mo for 3 y; every 3-6 mo thereafter ■ At any time, if ≥ 1-log increase in *BCR-ABL1* level with MMR, repeat testing in 1-3 mo
BCR-ABL1 kinase domain mutation analysis	During TKI therapy ■ If there is failure to achieve *BCR-ABL1* (IS) ≤ 10% or PCyR at 3 mo, or CCyR at 12 and 18 mo ■ If there is loss of response, namely hematologic or cytogenetic relapse, or ≥ 1-log increase in *BCR-ABL1* level and loss of MMR ■ At any time, if there is disease progression to CML-AP or CML-BP

AP, accelerated phase; BP, blast phase; CCyR, complete cytogenetic response; FISH, fluorescence in situ hybridization; IS, international scale; MMR, major molecular response; PCyR, partial cytogenetic response; qRT-PCR (IS), quantitative reverse transcription polymerase chain reaction assay per the international scale; TKI, tyrosine kinase inhibitor

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was a result of a reciprocal translocation between chromosomes 9 and 22.[2]

Karyotyping is recommended at the time of initial patient work-up as a means to confirm a diagnosis of CML. The importance of this testing to proper diagnosis is illustrated by the following actual patient case of a 72-year-old woman who initially came under our care showing a lack of response to treatment for acute myeloid leukemia (AML). Hematologic testing revealed > 30% blasts on peripheral blood counts, a result that was subsequently confirmed by a bone marrow sample. A thorough review of her medical records of the previous 2 years raised the possibility that the patient could have CML-BP, not AML. A karyotype was done, the presence of the Ph chromosome was confirmed, and a diagnosis of CML-BP was made. The patient was then immediately started on TKI therapy for CML-BP. She responded well and has remained in remission for more than 14 months. In this case, the karyotype was critical to making an accurate diagnosis, which explained the lack of response to AML therapy and allowed more effective treatment to be given.

During TKI therapy, the percentage of Ph-positive cells, as determined by karyotyping, is a measure of response to treatment (Table 2). The detection of additional cytogenetic aberrations (ACAs), either at diagnosis or during treatment, is considered a high-risk warning sign that predicts poorer prognosis or disease progression (ie, clonal evolution). Studies have shown that rates of 5-year overall survival (OS) and PFS are lower for patients who show ACAs at diagnosis.[24,25] In addition, the detection of clonal evolution is a correlate of disease progression, as suggested by the greater prevalence of ACAs in patients with CML-BP compared with those with CML-CP/AP.[26] Because clonal evolution results in the nonrandom accumulation of chromosomal abnormalities, it is viewed as an early step in the process of disease progression.[27]

Patients who have ACAs should be considered for further evaluation and closer follow-up. We have in our care a 68-year-old woman diagnosed with both CML and low-grade myelodysplastic syndrome (MDS). At 6 months after her initial diagnosis, a bone marrow cytogenetic analysis was performed and trisomy 8 with no additional cytogenic aberrations was detected. TKI therapy was initiated for treatment of the CML and she achieved undetectable levels of residual disease (as measured by molecular testing [see p. 183]) within 8 months of starting TKI therapy for CML. Although the patient has maintained her response on dasatinib for over 4 years, we continue to monitor her closely because the presence of MDS, a second malignancy, requires weekly treatment of erythropoietin injection and

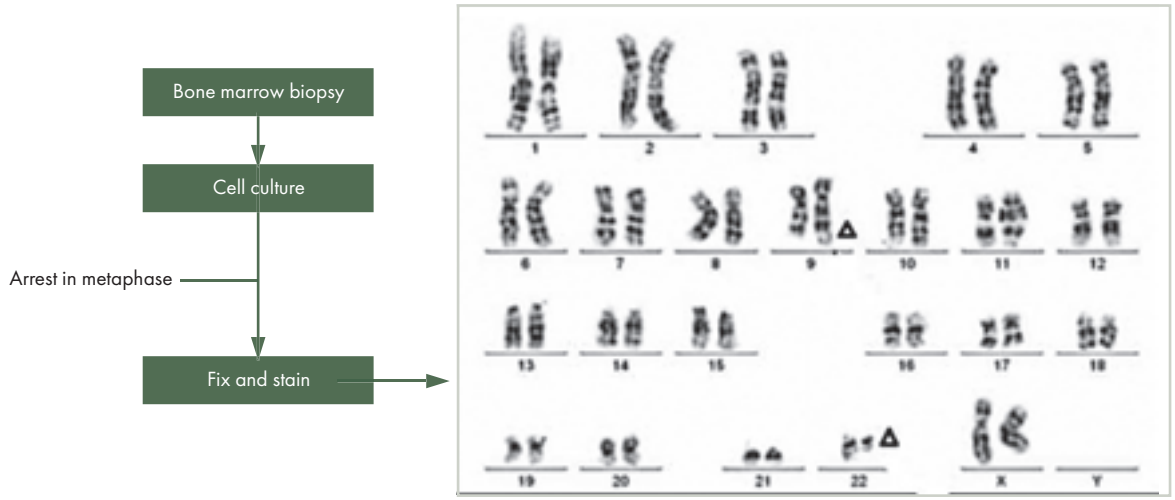

FIGURE 1 Conventional bone marrow cytogenetics – karyotyping. The karyotype of a typical patient with chronic myeloid leukemia shows the translocation (black and white arrowheads) between chromosomes 9 and 22. The altered chromosome 22q- is also known as the Philadelphia (Ph) chromosome, the hallmark of CML.[22] Image source: Pieńkowska-Grela B, Rygier J, Woroniecka R, et al. Karyotype changes during long-term targeted therapy of chronic myeloid leukemia with imatinib. Leuk Lymphoma. 2009;50:952-965. Reproduced with permission.

because trisomy 8 is a very common cytogenetic abnormality arising in patients with clonal evolution,[27] which may predispose them to disease progression.

FISH is a technique that allows the visualization of specific DNA sequences on chromosomes. FISH can be performed on cells from bone marrow or a peripheral blood sample. Fluorescent probes that are designed to bind to specific DNA sequences are hybridized to DNA on a slide and a specialized fluorescence microscope is used to detect the position of the fluorescent probes bound to their target DNA sequences[28] (Figure 2). At least 100 cells are usually analyzed with this technique. Because FISH uses probes that recognize specific, often unique DNA sequences, it is unsuitable for visualizing or detecting gross chromosomal abnormalities or surveying overall chromosomal structure. As such, FISH is used in limited circumstances in patients

TABLE 2 Criteria for hematologic, cytogenetic, and molecular response[a,b]

Testing	Level of response	Response criteria
Hematologic	Complete (CHR)	■ Complete normalization of peripheral blood counts ■ Leukocyte count < 10 × 10^9/L ■ Platelet count < 450 × 10^9/L ■ No immature cells (myelocytes, promyelocytes, or blasts) in peripheral blood ■ Disappearance of palpable splenomegaly ■ No signs and symptoms of disease
Cytogenetic	Complete (CCyR) Partial (PCyR) Major (MCyR) Minor (mCyR)	0% Ph+ metaphases 1%-35% Ph+ metaphases 0%-35% Ph+ metaphases (CCyR + PCyR) > 35% Ph+ metaphases
Molecular	Complete (CMR) Major (MMR)	No detectable *BCR-ABL1* transcripts by qRT-PCR (IS) using an assay with ≥4.5-log sensitivity below the standardized baseline ≥ 3-log reduction in *BCR-ABL1* level by qRT-PCR (IS)

Ph+, Philadelphia chromosome–positive; qRT-PCR (IS), quantitative reverse transcription polymerase chain reaction assay per the international scale

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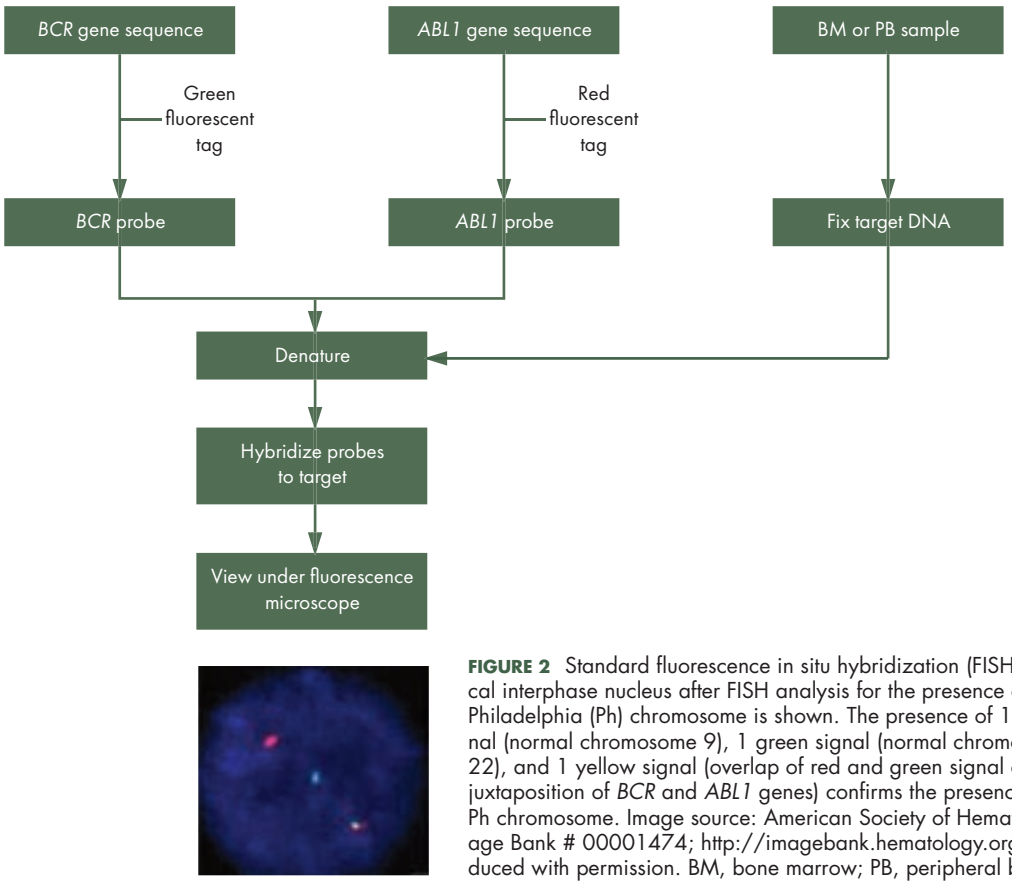

FIGURE 2 Standard fluorescence in situ hybridization (FISH). A typical interphase nucleus after FISH analysis for the presence of the Philadelphia (Ph) chromosome is shown. The presence of 1 red signal (normal chromosome 9), 1 green signal (normal chromosome 22), and 1 yellow signal (overlap of red and green signal due to juxtaposition of *BCR* and *ABL1* genes) confirms the presence of the Ph chromosome. Image source: American Society of Hematology Image Bank # 00001474; http://imagebank.hematology.org/. Reproduced with permission. BM, bone marrow; PB, peripheral blood

with CML. At diagnosis, FISH can be performed with dual color probes to detect *BCR* and *ABL1*, if bone marrow cytogenetic testing is not available to confirm diagnosis.[21] Furthermore, FISH has not been adequately studied as a method to monitor response to treatment, so regular FISH testing during treatment is not recommended.[21]

Molecular testing

Molecular testing is now most commonly done by quantitative reverse transcription polymerase chain reaction (qRT-PCR; Figure 3, p. 185). qRT-PCR uses RNA extracted from either a peripheral blood or a bone marrow sample. The use of peripheral blood rather than bone marrow for cytogenetic testing is preferred because of the less invasive nature of blood sampling. In this type of molecular testing, RNA is converted to DNA by reverse transcription and DNA is amplified by PCR.[29] The qRT-PCR technique is both specific and sensitive, because amplification of the target sequence only occurs when the PCR primers and the qRT-PCR fluorescent probe bind to the correct sites. Typical assays based on this technique can detect a single CML cell among 10^5-10^6 normal cells.[30] Therefore, qRT-PCR is the most suitable method for regular monitoring of response to TKI therapy.

Current NCCN Guidelines specify the use of qRT-PCR assays aligned with the international scale (IS).[21] Because the IS defines a standardized baseline that is not dependent on the baseline *BCR-ABL1* levels of a specific patient,[31] qRT-PCR test results reported on the IS can be directly compared across laboratories that use assays standardized to the IS and results can be interpreted more accurately, consistently, and reproducibly. If qRT-PCR (IS) is not available, the NCCN Guidelines recommend the use of bone marrow cytogenetic testing with karyotyping[21] as the means to monitor TKI treatment response. Use of non–IS-standardized qRT-PCR is not recommended. Laboratories that do not report qRT-PCR results on the IS can convert to the IS through a validation process that uses a reference set of samples for calibration of test results to 2 "anchor" points on the IS, baseline (100% IS) and major molecular response (MMR, 0.1% IS).[32]

Molecular monitoring during TKI therapy is recommended once every 3 months. Maintaining regular molecular monitoring frequency is important for several reasons. First, achievement of early molecular response to TKI therapy predicts favorable long-term outcomes, including improved rates of future treatment response and rates of OS, PFS, and disease transformation to CML-AP/BP.[33-40] Current NCCN

TABLE 3 Satisfactory and unsatisfactory response to TKI therapy by time on TKI therapy[a]

	Response	
Time on TKI therapy, mo	**Satisfactory[b]**	**Unsatisfactory[c]**
3	*BCR-ABL1* ≤ 10% (IS), or PCyR	*BCR-ABL1* >10% (IS), or < PCyR
12	CCyR	< CCyR
18	CCyR	< CCyR

CCyR, complete cytogenetic response; IS, international scale; PCyR, partial cytogenetic response; TKI, tyrosine kinase inhibitor

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Guidelines consider *BCR-ABL1* ≤ 10% (IS) to be the desired level of response at 3 months[21] (Table 3). A change in treatment plan may be considered for patients who do not achieve desired levels of response. In this way, the results of molecular monitoring at early time points can provide prognostic information that health care providers can apply to their clinical decisions about a patient.

Second, rises in *BCR-ABL1* level may occur as a result of reduced treatment adherence.[41] Patients who have achieved MMR and demonstrate a 1-log increase in *BCR-ABL1* level should have repeat testing done in 1-3 months.[21] If the *BCR-ABL1* level continues to rise, further evaluation is warranted. Patients also should be queried about their adherence to treatment. In my experience, younger patients exhibit overall better adherence to TKI therapy than do middle-aged patients, particularly younger patients who are still under parental care and thus have a strong support system. Other researchers have also identified a significant correlation between a greater level of social support and better treatment adherence, although this was not dependent on patient age.[42] Based on my clinical observations, adherence is more of a problem in middle-aged patients, who may be juggling many responsibilities (eg, work, children, aging parents) in addition to their own care. Their divided attention may contribute to forgetfulness or to the sense that other areas of their lives take priority over self-care.

Consider the case of a 35-year-old family man diagnosed with CML and treated at our practice. He began first-line TKI therapy and achieved CHR after 2 months. Unbeknown to us, the patient started skipping doses because he no longer felt sick. After 6 months, he showed signs of a response plateau, but his *BCR-ABL1* transcript levels continued to decline, although at a slower pace. At 1 year, the patient had not achieved MMR. Subsequent cytogenetic testing (karyotyping) showed 90% Ph-positive cells, indicating failure of first-line therapy. In light of these findings, the patient admitted to skipping doses, although cytogenetic testing in this case had already confirmed our suspicion that the patient had not been adherent to TKI therapy. We worked with the patient to address issues in his personal life that interfered with his taking daily medication and we switched him to second-line TKI treatment. Following this change in treatment, we monitored his response to treatment more closely, with qRT-PCR testing every month for the first 6 months, then every 3 months thereafter. At each clinic visit, we make it a point to ask if he has been taking his daily medication. Now, 6 months after starting second-line therapy, the patient is doing well.

Rises in *BCR-ABL1* level also may result from the development of resistance, marking an early sign of disease relapse or disease progression.[43,44] Disease progression may occur for a time before patients experience outward symptoms. In such instances, regular molecular monitoring can be one of the earliest ways to detect worsening disease severity.

Regular molecular monitoring is an essential component of care, not only during periods of active treatment but also when TKI therapy is discontinued for medical reasons. It should be emphasized that discontinuation of TKI therapy is not routine practice and should not be undertaken at this time outside the context of a clinical study or the most dire circumstances. We had no choice but to prematurely discontinue treatment when a 34-year-old woman in our practice became pregnant while on TKI therapy. The patient had been on TKI treatment for 15 months and had achieved CCyR when she intentionally became pregnant. When her pregnancy was brought to our attention, TKI therapy was immediately discontinued and she was placed on interferon alfa (IFN-α) for the duration of her pregnancy. Monthly qRT-PCR (IS) testing was performed to monitor her *BCR-ABL1* level. The patient showed minimal response to IFN-α therapy and her *BCR-ABL1* level began to rise in the 24th week of pregnancy. Over the next several weeks, we adjusted the IFN-α dose and continued monitoring *BCR-ABL1* levels for response. The patient gave birth to a healthy baby girl and, soon after, was restarted on TKI treatment. Three months after restarting TKI therapy, testing indicated that our patient had achieved a 3-log reduction in her *BCR-ABL1* levels, 0.1% as determined by qRT-PCR (IS). We plan to follow

up at 1 year with bone marrow cytogenetics to determine whether she has achieved CCyR once again.

It is important to note that pregnancy during TKI therapy for CML is not recommended because of the potential harm that the medication may pose to the developing fetus and because of the unknown possible effects of in utero exposure to TKI therapy on infant/child development. The TKIs are considered pregnancy category D drugs.[16,45,48] A global imatinib and nilotinib pregnancy exposure registry was opened in 2011 to assess effects of TKI discontinuation on maternal health and TKI exposure on fetal and infant development (NCT01289054), and an observational study of conception and pregnancy in patients with CML on TKI therapy is currently open for enrollment (NCT01752062).

The case described above highlights the struggles that younger patients face in grappling with the effects of their disease on their fertility, sexuality, and family planning. These are important issues to patients (as they are to the general population), and health care providers might find that their patients seek information and guidance on how to handle issues arising when the disease intersects with personal hopes and dreams. When this happens, health care providers might find that a straightforward approach to open discussion of these topics yields the best results.

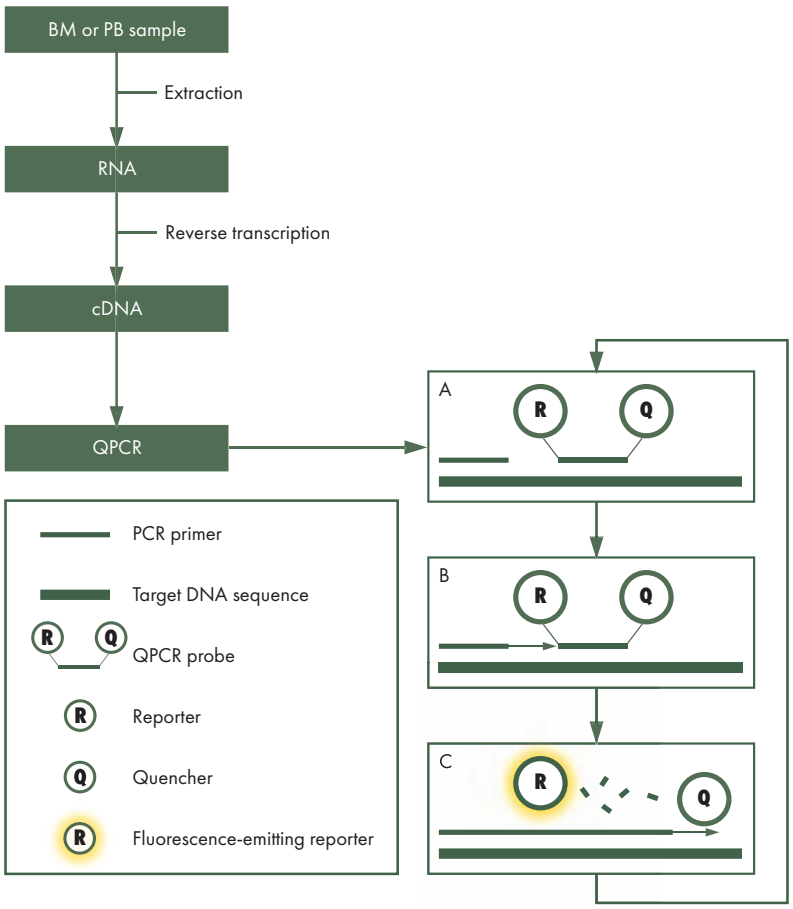

FIGURE 3 Quantitative reverse transcription polymerase chain reaction (qRT-PCR). The typical qRT-PCR technique used to measure *BCR-ABL1* transcript level is shown. A, PCR primers and the qRT-PCR probe anneal to target DNA sequence. No fluorescence is emitted when the quencher (Q) is proximal to the reporter (R). B, DNA polymerase extends the sequence from the primer toward the probe. C, The exonuclease function of DNA polymerase cleaves the probe and releases the reporter from its association with the quencher, and a fluorescent signal is emitted. Image courtesy of A. Lau. BM, bone marrow; PB, peripheral blood

Mutational analysis

Mutational analysis refers to testing that detects the presence of genetic mutations in the *BCR-ABL1* gene. Various techniques are used to detect genetic mutations, and they are generally based on DNA sequencing,[49] denaturing high-performance liquid chromatography,[50,52] or PCR.[53,54]

The development of *BCR-ABL1* kinase domain mutations may confer resistance to treatment with 1 or more TKIs. The 5 approved TKIs differ in the spectrum of *BCR-ABL1* mutants to which they bind.[55,57] In addition to the TKI safety profile, this information may help to inform TKI treatment choice,[21] depending on what specific *BCR-ABL1* mutation or mutations are detected. For example, ponatinib is the only approved TKI that binds to the T315I *BCR-ABL1* mutant protein.[55] It should be noted that factors other than in vitro binding affinity can affect TKI activity in patients.[58,60]

Summary and conclusions

TKI therapy for CML has changed the way patients with this cancer are managed. The longer life expectancies of patients diagnosed with CML in the current era of TKI therapy have allowed this once rapidly fatal cancer to be controlled as a chronic condition with continuous, long-term medication. An essential component of CML management therefore involves regular assessments of treatment response and minimal residual disease to monitor the stability of disease control over long periods of active treatment.

Hematologic and cytogenetic testing, molecular monitoring, and mutational analysis are integral assessment tools in the management of CML. Each type of testing serves an important purpose in assessing and monitoring disease status. Hematologic testing is the most suitable method to assess the presence of myelosuppression and loss of response. Cytogenetic testing is the most suitable method to determine clonal evolution and possible disease progression. qRT-PCR is the most expedient method available to determine treatment response and track minimal residual disease. qRT-PCR testing may also reveal signs of reduced treatment adherence or disease progression. *BCR-ABL1* kinase domain mutational analysis may offer insight into mechanisms of TKI resistance and inform subsequent treatment selection. What all of these tests have in common is that their results can identify patients in need of further evaluation or closer follow-up. Early identification of patients who have not reached treatment milestones or have lost a response allows early intervention, which may reduce the likelihood of disease progression.

In conclusion, each type of test used in the evaluation of patients with CML serves an important function, and optimal management of patients with CML depends in large part on the appropriate implementation of these tests at the recommended intervals. Furthermore, with a clear understanding of the method and purpose of these common tests, nursing professionals can educate their patients and manage their patients' expectations about what the management of CML as a chronic disease will entail. Getting patients "on board" with the CML management plan from the very start of care could go a long way toward instilling in patients good habits of regular testing and clinic visits, and adhering to treatment – habits that bode well for achieving good patient outcomes.

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References

1. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 1960;25:85-109.
2. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243:290-293.
3. Deininger M, O'Brien SG, Guilhot F, et al. International randomized study of interferon versus STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. Blood (ASH Annual Meeting Abstracts). 2009;114:1126.
4. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355:2408-2417.
5. Hochhaus A, O'Brien SG, Guilhot F, et al. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia. 2009;23:1054-1061.
6. Kantarjian HM, Hochhaus A, Saglio G, et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. Lancet Oncol. 2011;12:841-851.
7. Larson RA, Hochhaus A, Hughes TP, et al. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENESTnd 3-year follow-up. Leukemia. 2012;26:2197-2203.
8. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362:2251-2259.
9. Kantarjian HM, Kim D-W, Issaragrisil S, et al. Enestnd 4-year (y) update: continued superiority of nilotinib vs imatinib in patients (pts) with newly diagnosed Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia in chronic phase (CML-CP). Blood (ASH Annual Meeting Abstracts). 2012;120:1676.
10. Hochhaus A, Shah NP, Cortes JE, et al. Dasatinib versus imatinib (IM) in newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP): DASISION 3-year follow-up. J Clin Oncol. 2012;30:6504.
11. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362:2260-2270.
12. Kantarjian HM, Shah NP, Cortes JE, et al. Dasatinib or imatinib in newly diagnosed chronic phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). Blood. 2012;119:1123-1129.
13. Cortes JE, Kantarjian HM, Brummendorf TH, et al. Safety and efficacy of bosutinib (SKI-606) in chronic phase Philadelphia chromosome-positive chronic myeloid leukemia patients with resistance or intolerance to imatinib. Blood. 2011;118:4567-4576.
14. Khoury HJ, Cortes JE, Kantarjian HM, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. Blood. 2012;119:3403-3412.
15. Cortes JE, Kim DW, Kantarjian HM, et al. Bosutinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: results from the BELA trial. J Clin Oncol. 2012;30:3486-3492.
16. Iclusig (ponatinib), prescribing information. Cambridge, MA: ARIAD Pharmaceuticals Inc; 2012.
17. Kantarjian HM, Cortes J, Guilhot F, Hochhaus A, Baccarani M, Lokey L. Diagnosis and management of chronic myeloid leukemia: a survey of American and European practice patterns. Cancer. 2007;109:1365-1375.
18. Kantarjian HM, Larson RA, Cortes JE, Deering KL, Mauro MJ. Current practices in the management of chronic myeloid leukemia. Clin Lymphoma Myeloma Leuk. 2013;13:48-54.
19. Fogarty M. Letter from the Editor: How cancer treatment benefits from the industry's input. Oncology Times. 2008;5:3.
20. Chen L, Guerin A, Xie J, et al. Monitoring and switching patterns of patients with chronic myeloid leukemia treated with imatinib in community settings: a chart review analysis. Curr Med Res Opin. 2012;28:1831-1839.
21. NCCN. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Chronic Myelogenous Leukemia V.3.2014. National Comprehensive Cancer Network Inc, 2014. All rights reserved. www.nccn.org. Accessed April 14, 2014.
22. Pienkowska-Grela B, Rygier J, Woroniecka R, et al. Karyotype changes during long-term targeted therapy of chronic myeloid leukemia with imatinib. Leuk Lymphoma. 2009;50:952-965.
23. O'Connor C. Karyotyping for chromosomal abnormalities. Nature Education. 2008;1:1.
24. Fabarius A, Leitner A, Hochhaus A, et al. Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML Study IV. Blood. 2011;118:6760-6768.
25. Verma D, Kantarjian H, Shan J, et al. Survival outcomes for clonal evolution in chronic myeloid leukemia patients on second generation

tyrosine kinase inhibitor therapy. Cancer. 2010;116:2673-2681.
26. Lahaye T, Riehm B, Berger U, et al. Response and resistance in 300 patients with BCR-ABL-positive leukemias treated with imatinib in a single center: a 4.5-year follow-up. Cancer. 2005;103:1659-1669.
27. Johansson B, Fioretos T, Mitelman F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol. 2002;107:76-94.
28. O'Connor C. Fluorescence in situ hybridization (FISH). Nature Education. 2008;1:1.
29. Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. Genome Res. 1996;6:995-1001.
30. Radich JP. How I monitor residual disease in chronic myeloid leukemia. Blood. 2009;114:3376-3381.
31. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. New Engl J Med. 2003;349:1423-1432.
32. Branford S, Fletcher L, Cross NC, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood. 2008;112:3330-3338.
33. Branford S, Rudzki Z, Harper A, et al. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia. 2003;17:2401-2409.
34. Hehlmann R, Hanfstein B, Müller MC, et al. The prognostic significance of early molecular and cytogenetic response for long-term progression-free and overall survival in imatinib-treated chronic myeloid leukemia (CML). J Clin Oncol. 2012;30:6510.
35. Hochhaus A, Boqué C, Bradley Garelik M, Manos G, Steegmann JL. Molecular response kinetics and BCR-ABL reductions in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) receiving dasatinib vs imatinib: DASISION 3-year follow-up [Congress of the European Hematology Association abstract 0192]. https://www.eventure-online.com/eventure/publicAbstractView.do?id=193373&congressId=5650. Posted April 23, 2013. Accessed April 14, 2014.
36. Hochhaus A, Guilhot F, Kathrin Haifa AA, et al. Early BCR-ABL transcript levels predict future molecular response and long-term outcomes in newly diagnosed patients with chronic myeloid leukemia in chronic phase: analysis of ENESTnd 3-year data [Congress of the European Hematology Association abstract 0584]. https://www.eventure-online.com/eventure/publicAbstractView.do?id=191939&congressId=5650. Posted April 23, 2013. Accessed April 14, 2014.
37. Hochhaus A, Saglio G, Chuah C, et al. Dasatinib and imatinib-induced reductions in BCR-ABL transcript levels below 10% at 3 months are associated with improved responses in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP): analysis of molecular response kinetics in the DASISION Trial [ASH abstract 2767]. Blood. 2011;118:2767.
38. Marin D, Hedgley C, Clark RE, et al. Predictive value of early molecular response in patients with chronic myeloid leukemia treated with first line dasatinib. Blood. 2012;120:291-294.
39. Marin D, Ibrahim AR, Lucas C, et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012;30:232-238.
40. Quintas-Cardama A, Kantarjian H, Jones D, et al. Delayed achievement of cytogenetic and molecular response is associated with increased risk of progression among patients with chronic myeloid leukemia in early chronic phase receiving high-dose or standard-dose imatinib therapy. Blood. 2009;113:6315-6321.
41. Branford S, Yeung DT, Prime JA, et al. BCR-ABL1 doubling times more reliably assess the dynamics of CML relapse compared with the BCR-ABL1 fold rise: implications for monitoring and management. Blood. 2012;119:4264-4271.
42. Efficace F, Baccarani M, Rosti G, et al. Investigating factors associated with adherence behaviour in patients with chronic myeloid leukemia: an observational patient-centered outcome study. Br J Cancer. 2012;107:904-909.
43. Kantarjian HM, Shan J, Jones D, et al. Significance of increasing levels of minimal residual disease in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in complete cytogenetic response. J Clin Oncol. 2009;27:3659-3663.
44. Press RD, Galderisi C, Yang R, et al. A half-log increase in BCR-ABL RNA predicts a higher risk of relapse in patients with chronic myeloid leukemia with an imatinib-induced complete cytogenetic response. Clin Cancer Res. 2007;13:6136-6143.
45. Bosulif (bosutinib), prescribing information. New York, NY: Pfizer Labs; 2012.
46. Gleevec (imatinib mesylate), prescribing information (US). East Hanover, NJ: Novartis Pharmaceuticals Corp; 2012.
47. Sprycel (dasatinib), prescribing information (US). Princeton, NJ: Bristol-Myers Squibb Co; 2013.
48. Tasigna (nilotinib), prescribing information (US). East Hanover, NJ: Novartis Pharmaceuticals Corp; 2012.
49. Hochhaus A, Kreil S, Corbin AS, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia. 2002;16:2190-2196.
50. Deininger MW, McGreevey L, Willis S, Bainbridge TM, Druker BJ, Heinrich MC. Detection of ABL kinase domain mutations with denaturing high-performance liquid chromatography. Leukemia. 2004;18:864-871.
51. Irving JA, O'Brien S, Lennard AL, Minto L, Lin F, Hall AG. Use of denaturing HPLC for detection of mutations in the BCR-ABL kinase domain in patients resistant to Imatinib. Clin Chem. 2004;50:1233-1237.
52. Soverini S, Martinelli G, Amabile M, et al. Denaturing-HPLC-based assay for detection of ABL mutations in chronic myeloid leukemia patients resistant to Imatinib. Clin Chem. 2004;50:1205-1213.
53. Gruber FX, Lamark T, Anonli A, et al. Selecting and deselecting imatinib-resistant clones: observations made by longitudinal, quantitative monitoring of mutated BCR-ABL. Leukemia. 2005;19:2159-2165.
54. Pelz-Ackermann O, Cross M, Pfeifer H, et al. Highly sensitive and quantitative detection of BCR-ABL kinase domain mutations by ligation PCR. Leukemia. 2008;22:2288-2291.
55. O'Hare T, Shakespeare WC, Zhu X, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell. 2009;16:401-412.
56. Redaelli S, Piazza R, Rostagno R, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. J Clin Oncol. 2009;27:469-471.
57. Soverini S, Hochhaus A, Nicolini FE, et al. Bcr-Abl kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118:1208-1215.
58. Laneuville P, Dilea C, Yin OQ, Woodman RC, Mestan J, Manley PW. Comparative In vitro cellular data alone are insufficient to predict clinical responses and guide the choice of BCR-ABL inhibitor for treating imatinib-resistant chronic myeloid leukemia. J Clin Oncol. 2010;28:e169-171; author reply e172.
59. Lange T, Ernst T, Gruber F, et al. The quantitative level of T315I mutated BCR-ABL predicts for major molecular response to second line nilotinib or dasatinib treatment in patients with chronic myeloid leukemia. Haematologica. 2013;98:714-717.
60. Soverini S, Rosti G, Iacobucci I, Baccarani M, Martinelli G. Choosing the best second-line tyrosine kinase inhibitor in imatinib-resistant chronic myeloid leukemia patients harboring Bcr-Abl kinase domain mutations: how reliable is the IC_{50}? Oncologist. 2011;16:868-876.