

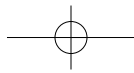
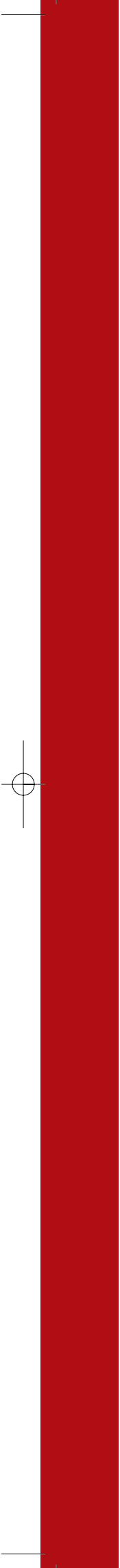
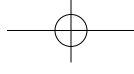
Using Molecular Monitoring to Optimize Therapy for Chronic Myeloid Leukemia

A Case-Based Review

EUTOS for CML



European Treatment and Outcome Study



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Using Molecular Monitoring to Optimize Therapy for Chronic Myeloid Leukemia

A Case-Based Review

Editorial Reviewers

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INTRODUCTION

Background

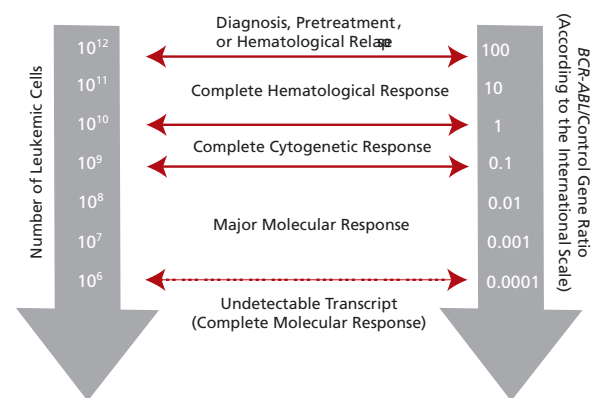
Chronic myeloid leukemia (CML) is characterized in 95% of patients by the presence of the Philadelphia (Ph) chromosome, which is generated by a reciprocal translocation between chromosomes 9 and 22 designated t(9;22)(q34;q11).¹ This translocation creates an aberrant *BCR-ABL* fusion gene on chromosome 22 and leads to expression of a constitutively active BCR-ABL protein tyrosine kinase that is oncogenic for CML.

Recognition that nearly all cases of CML are attributable to the Ph chromosome provided a rational target for a novel therapeutic approach: specific inhibition of BCR-ABL tyrosine kinase activity.² The introduction of the oral tyrosine kinase inhibitor imatinib (STI571) has revolutionized CML treatment. During 8 years of clinical development and more than 5 years of worldwide clinical use, imatinib has been shown to be safe, well tolerated, and more effective than previous CML therapies, generating unprecedented rates of highly durable responses.³⁻⁸ This experience has led to the adoption of imatinib as the standard of care in CML.^{9,10}

Response Assessment and Molecular Response

The success of imatinib therapy has driven the assessment of response to the molecular level. Molecular response assessment is a more sensitive and precise measurement of minimal residual disease based on quantitation of intracellular *BCR-ABL* messenger RNA (mRNA) transcripts using real-time quantitative polymerase chain reaction (RQ-PCR) methodology.¹¹⁻¹³ *BCR-ABL* transcripts present at extremely low levels can be accurately detected and quantitated by RQ-PCR. RQ-PCR is able to detect a single Philadelphia chromosome-positive (Ph+) cell producing *BCR-ABL* in a population of 10^5 - 10^6 normal cells (although sensitivity is more typically in the range of 1 per 10^4 to 10^5 cells) (Figure 1).¹⁰

Figure 1. The *BCR-ABL* Transcript Percentage Parallels the Number of Leukemic Cells



Adapted with permission from Baccarani M et al. Blood. 2006;108:1809-1820.¹⁰

Molecular response assessment was introduced in the International Randomized Interferon Versus STI571 (IRIS) study to further investigate the level of residual disease in patients who had achieved a complete cytogenetic response (CCyR) with imatinib therapy.^{14,15} In this study, a standard baseline level of *BCR-ABL* transcripts was established using the median *BCR-ABL/BCR* ratio in pretreatment samples from 30 patients with newly diagnosed CML in chronic phase (CML-CP). The use of transcript ratios compensates for RNA sample degradation and variation in RNA quantity, as well as variations in PCR efficiency in different laboratories.¹⁴ The 30 patient samples were used in each laboratory participating in the IRIS study to establish a baseline, and molecular responses were expressed as log reductions from this baseline. A new response standard, major molecular response (MMR), was defined as a ≥ 3 -log reduction in the *BCR-ABL/BCR* ratio from the baseline obtained in each laboratory. In this way, a 3-log reduction in transcript levels represents a reduction from a standard baseline, rather than an individual patient baseline, and indicates an absolute, and not a relative, value for residual disease.

There is currently an effort to standardize molecular response assessment methodology and the reporting of results worldwide.¹⁴ An

International Scale (IS) has been proposed that defines a 3-log reduction in the transcript ratio from the IRIS trial baseline as 0.10% and the baseline as 100%. The commercial production of *BCR-ABL* reference standards will assist laboratories worldwide in absolute *BCR-ABL* quantitation.

An additional level of molecular response, complete molecular response (CMR), is defined as achievement of undetectable *BCR-ABL* transcripts. It should be noted that a finding of CMR must be based on RQ-PCR methodology capable of reliably detecting transcripts at the 0.001% (10^{-5}) level.^{10,16} CMR implies an

additional reduction of at least 1.5-2 log in the level of residual disease from MMR (Table 1).¹⁰

Prognostic Value of Molecular Response

Achievement of MMR during imatinib therapy has been correlated with a good outcome in CML.¹⁷ Five-year follow-up data from the IRIS study have shown that 100% of patients who achieved both CCyR and MMR at 12 months remained free from progression to accelerated phase (AP) or blast crisis (BC), compared with 95% of patients who achieved CCyR but not MMR ($P = 0.007$). Patients who achieved neither

Table 1. Response Definitions and Recommendations for Monitoring¹⁰

	Hematological Response (HR)	Cytogenetic Response (CyR)	Molecular Response (MR)*
Response Definitions	Complete (CHR): Platelets <450 x 10 ⁹ /L; WBC count <10 x10 ⁹ /L; differential without immature granulocytes and <5% basophils; nonpalpable spleen	Complete (CCyR): Ph+ 0% Partial (PCyR): Ph+ 1%-35% Minor: Ph+ 36%-65% Minimal: Ph+ 66%-95% None: Ph+ >95% Major: PCyR + CCyR	Complete: Transcripts non-quantifiable and undetectable Major: 0.1%†
Monitoring Frequency	<ul style="list-style-type: none"> ■ Every 2 weeks until a complete response has been achieved and confirmed ■ Every 3 months unless otherwise required 	<ul style="list-style-type: none"> ■ Every 6 months until a complete response has been achieved and confirmed ■ Then every 12 months 	<ul style="list-style-type: none"> ■ Every 3 months
Monitoring Methods	<ul style="list-style-type: none"> ■ Complete blood count (CBC) with differential 	<ul style="list-style-type: none"> ■ Conventional cytogenetic examination ■ FISH (only before treatment) 	<ul style="list-style-type: none"> ■ RQ-PCR

*Ratio of BCR-ABL to control gene transcripts according to International Scale.¹⁴
 †Level of BCR-ABL transcripts according to the proposed International Scale.¹⁴
 FISH, fluorescence in situ hybridization; RQ-PCR, real-time quantitative polymerase chain reaction; WBC, white blood cell.
 Based on recommendations from European LeukemiaNet.¹⁰

CCyR nor MMR at 12 months had a 5-year rate of freedom from progression to AP/BC of 88% ($P < 0.001$ vs CCyR + MMR).¹⁷

In many patients, the level of BCR-ABL transcripts continues to decline with time on imatinib therapy. Among patients who fail to achieve CCyR and/or MMR at 12 months, many finally achieve these levels of response after receiving imatinib for 3-4 years.^{18,19} As the long-term durability of response after imatinib discontinuation remains unclear, it is recommended that patients who maintain CCyR, MMR, or CMR remain on imatinib indefinitely. Continued decline in *BCR-ABL* transcript level should be interpreted as continued response.^{1,9,10}

Conversely, increasing *BCR-ABL* transcript levels have been correlated with imatinib-resistant *BCR-ABL* mutations and relapse.²⁰ However, because of inherent variability in RQ-PCR reliability, confirmation with a second test is more predictive of mutational resistance than a single finding. RQ-PCR detection of an increase in *BCR-ABL*

transcripts should trigger more stringent RQ-PCR evaluation, rather than an immediate change in treatment.²¹ It has been suggested that the threshold for molecular resistance be set at an increase in transcript levels of 0.5-1.0 log, to avoid unnecessary concern about routine fluctuations in RQ-PCR findings.²²

Consensus Guidelines for Assessment and Response

The European LeukemiaNet has developed consensus recommendations that have defined the various levels of hematological, cytogenetic, and molecular response in CML therapy (**Table 1**).¹⁰

In addition, these guidelines include a recommended monitoring schedule for each of the defined responses.^{9,10} Regular monitoring is required to determine whether a patient with CML has achieved a defined level of response at landmark time points, which is associated with optimal outcome, as well as to monitor relapse.

Table 2 summarizes the consensus

Table 2. Criteria for Suboptimal Response and Treatment Failure With Imatinib¹⁰

	Diagnosis	3 Months	6 Months	12 Months	18 Months	At Any Time
Suboptimal Response	—	<CHR	<PCyR	<CCyR	<MMR	<ul style="list-style-type: none"> • ACA in Ph+ cells* • Loss of MMR* • Mutation conferring low level of imatinib resistance†
Treatment Failure	—	No HR	<CHR No CyR	<PCyR	<CCyR	<ul style="list-style-type: none"> • Loss of CHR‡ • Loss of CCyR§ • Mutation conferring high level of imatinib resistance†
Warnings	High risk Del 9+			<MMR		<ul style="list-style-type: none"> • Any rise in transcript level • Other chromosomal abnormalities in Ph-negative cells

*To be confirmed on 2 occasions unless associated with CHR or CCyR loss.

†Mutations should be interpreted within clinical context.

‡To be confirmed on 2 occasions unless associated with progression to AP or BC.

§To be confirmed on 2 occasions unless associated with CHR loss or progression to AP or BC.

ACA, additional chromosomal abnormalities; AP, accelerated phase; BC, blast crisis; CCyR, complete cytogenetic response;

CHR, complete hematologic response; CyR, cytogenetic response; Del 9q+, deletion of the *ABL-BCR* rearrangement on the derivative chromosome 9q+; HR, hematologic response; MMR, major molecular response; PCyR, partial cytogenetic response.

recommendations for levels of response that indicate a suboptimal response or treatment failure. RQ-PCR should be repeated every 3 months to assess continued reduction of *BCR-ABL* transcripts and to detect signs of loss of response.

Cytogenetic analysis is essential during or at diagnosis to assess for additional chromosomal abnormalities associated with more aggressive disease.^{10,22} CCyR is roughly equivalent to a transcript ratio of 1% on the IS.^{10,16} It has been suggested that because bone marrow sampling is uncomfortable and inconvenient, its use for cytogenetic response assessment should at some point be limited to patients who have failed to achieve or have lost MMR.²³

There is evidence that molecular response assessment during therapy can provide critical information that can guide clinical decision making to improve patient outcomes. This monograph provides 4 case histories of patients with CML treated with imatinib that illustrate the value of molecular monitoring during therapy.

Case 1:

Suboptimal Response

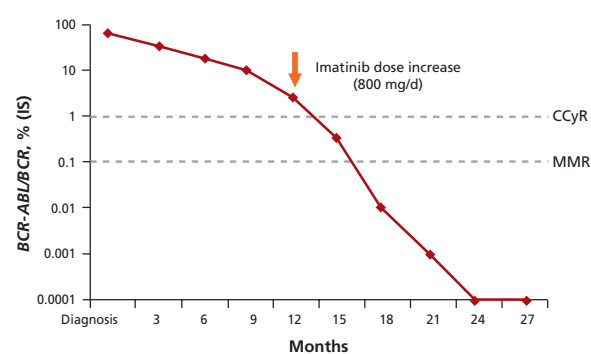
Synopsis

A 42-year-old male was diagnosed with CML-CP in October 2004; both his Sokal risk score²⁴ and his Hasford risk score²⁵ were low at diagnosis. A cytogenetic analysis on bone marrow aspirate revealed the presence of a classic Ph+ chromosome; however, subsequent fluorescence in situ hybridization (FISH) analysis showed that a large deletion of the *ABL-BCR* rearrangement was present on the derivative chromosome 9q+ (del 9q+). On qualitative PCR analysis, an e14a2 (b3a2) *BCR-ABL* transcript was detected.

Although a human leukocyte antigen (HLA)-identical potential sibling donor was identified (the patient's sister), first-line allogeneic hematopoietic stem-cell transplantation (HSCT)

was excluded because the patient's European Cooperative Group for Bone Marrow Transplantation (EBMT) risk score of 3 suggested substantial risk of transplant mortality.^{26,27} The patient was initiated on imatinib 400 mg/day, which was well tolerated. The patient achieved complete hematological response (CHR) at 3 months, minor cytogenetic response at 6 months, and partial cytogenetic response (PCyR) at 12 months. At 12 months, the *BCR-ABL/ABL* transcript ratio by RQ-PCR analysis was 2.3% (IS units) (Figure 2).

Figure 2. Case 1: Change in *BCR-ABL* Transcript Levels With Imatinib



Clinical Discussion

According to European LeukemiaNet recommendations, the patient's response to imatinib was suboptimal after 6 months, based on failure to achieve PCyR (Table 2).¹⁰ The coexistence of a 9q+ deletion was until recently considered a warning of poor prognosis.^{28,29} However, currently there is little or no evidence that 9q+ deletions are associated with an adverse prognosis in imatinib-treated patients.

Given the patient's baseline risk and response, a change in therapy would have been appropriate to consider at 6 months but became compelling with persistent suboptimal response (<CCyR) at 12 months. Three options were available at that time: (1) increase imatinib dose to 800 mg/day; (2) shift the patient to alternative tyrosine kinase inhibitor therapy such as dasatinib or an

investigational protocol using another next-generation tyrosine kinase inhibitor, nilotinib; or (3) initiate allogeneic HSCT using the available sibling donor. After consideration and consultation with the patient, the first option was chosen, primarily because, despite suboptimal response at 6 and 12 months, molecular analysis using RQ-PCR at 3-month intervals showed continuous decline over time in *BCR-ABL* transcript levels (**Figure 2**). These results suggest the possibility of a delayed response to a suboptimal imatinib dose, rather than the presence or emergence of an imatinib-resistant clone. This idea was further reinforced by the absence of *BCR-ABL* mutations and of additional cytogenetic abnormalities in the Ph+ cells.

Follow-up

The imatinib dose was increased to 800 mg/day and the patient achieved CCyR at 15 months (**Figure 2**). At 18 months, molecular analysis documented the achievement of MMR, with a *BCR-ABL* transcript level below the 0.1% threshold. Subsequent to these events, a 5-year follow-up study of patients in the IRIS study showed that once MMR is achieved, there is minimal risk of losing this response and of disease progression.⁴ Moreover, the level of residual disease, measured by *BCR-ABL* transcript levels, continues to decline with continued imatinib treatment in imatinib-responsive patients after achieving MMR.¹⁹ Consistent with these data, *BCR-ABL* transcript levels continued to decline in this patient, who remains on imatinib at 800 mg/day. During the past 6 months, *BCR-ABL* transcripts have reached undetectable levels by both quantitative and qualitative nested PCR analysis.

Molecular monitoring demonstrated continued activity of imatinib in the context of suboptimal cytogenetic response and provided therapeutic guidance not possible with cytogenetic analysis alone

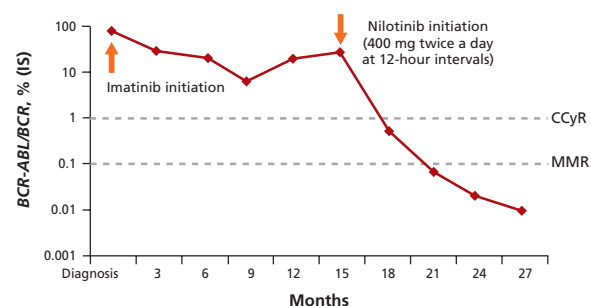
Case 2:

Stalled Response

Synopsis

A 55-year-old female was diagnosed with CML-CP in July 2004, with an intermediate Sokal risk score at diagnosis. A cytogenetic analysis of bone marrow aspirate revealed a classic Philadelphia chromosome in all analyzed metaphases, and qualitative reverse-transcription PCR analysis demonstrated the presence of *BCR-ABL* transcripts with an e13a2 (b2a2) junction. The patient was initiated on imatinib 400 mg/day. She tolerated imatinib well and achieved CHR after only 1 month of therapy. At 6 months, PCyR was achieved and molecular analysis on peripheral blood found that the *BCR-ABL/BCR* transcript ratio was 7% (**Figure 3**). At 9 months, the transcript ratio seemed to plateau at 8% but increased to 19% at 12 months. At the same time, cytogenetic analysis demonstrated persistent PCyR, but the proportion of Ph+ metaphases had risen from 10% to 25%, and trisomy-8 appeared in 80% of the Ph+ cells.

Figure 3. Case 2: Pattern of Change in *BCR-ABL* Transcript Levels



Clinical Discussion

After a rapid and encouraging initial hematological response, the patient's molecular response appeared to be decelerating between months 6 and 9. This observation was confirmed

at 12 months, when analyses showed that both molecular and cytogenetic disease measures had reversed course and begun to increase. Although the patient's response at 12 months continued to meet criteria for suboptimal response rather than treatment failure, the observed increases in Ph+ metaphases as well as percentage of *BCR-ABL* transcripts indicated a decline in therapeutic effect and a strong likelihood of relapse (**Figure 3**). This possibility is further reinforced by the emergence of a new clone characterized by trisomy 8 in addition to the Ph+ metaphases found at diagnosis. Mutation analysis did not identify any new *BCR-ABL* mutations. The imatinib dose was increased to 800 mg/day based on the RQ-PCR and cytogenetic findings. However, a new molecular response analysis 1 month after the dosage increase showed continued increase in *BCR-ABL/BCR* transcript ratio to 25%. Given the absence of *BCR-ABL* mutations, it was recognized that emergence of resistance was likely a consequence of clonal evolution. Clonal evolution is another important cause of imatinib resistance.³⁰ As a result, the patient was enrolled in an experimental protocol for CML patients resistant to or intolerant of imatinib, and treatment with nilotinib 400 mg twice daily at 12-hour intervals was initiated.

Follow-up

The patient tolerated the new treatment regimen extremely well. After 3 months of nilotinib therapy, bone marrow cytogenetic analysis documented the achievement of CCyR and a peripheral blood molecular analysis showed a *BCR-ABL/BCR* transcript ratio of 0.62% (**Figure 3**). After 6 months, the patient achieved MMR, with a transcript ratio of 0.08%. At last follow-up, the patient remained in MMR with molecular assessment every 3 months confirming a continuous trend toward decreasing transcript levels.

Assessment of molecular response provided an early indication of imatinib resistance, and in combination with mutational analysis, helped identify the likely cause of resistance level.

Case 3:

Lost Response

Synopsis

A 17-year-old male was diagnosed with CML-CP in February 2006. At diagnosis, the patient had relatively high Sokal and Hasford risk scores, primarily because of significant spleen enlargement and 3% blasts in peripheral blood. Cytogenetic analysis of bone marrow aspirate revealed classic Ph+ chromosome in 97% of metaphases analyzed, and qualitative PCR analysis demonstrated the presence of *BCR-ABL* transcripts.

An HLA-identical sibling donor (the patient's 20-year-old brother) was available, and the EBMT risk score for HSCT was favorable. Even low-risk HSCT carries a 5-year transplant-related mortality risk of 22%. The patient was initiated on imatinib at 400 mg/day, which later was increased to 600 mg/day.²⁶

The patient initially responded well to imatinib and achieved CCyR at 6 months. Molecular assessment showed that the *BCR-ABL* transcript level was approaching 0.22% (**Figure 4**). At 9 months, the level of transcripts was slightly elevated, although the patient still met criteria for CCyR. Twelve-month RQ-PCR again showed a drop in transcript level to nearly the 0.1% level. At 15 months, a 3-fold increase in transcript levels by RQ-PCR analysis was deemed significant and triggered mutation analysis. Some experts recommend that a 5- to 10-fold rise in *BCR-ABL* transcript levels, observed on at least two consecutive occasions, should be considered significant, depending on the reliability of the

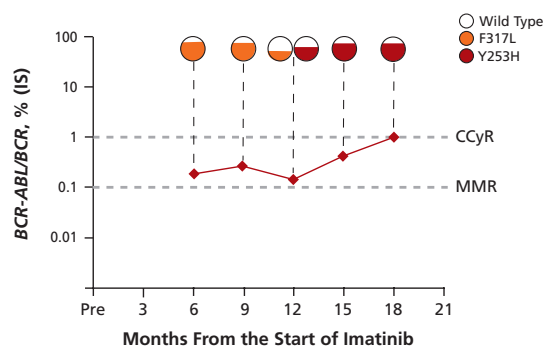
laboratory results.²² At 18 months, a mutation encoding one of the least imatinib-sensitive mutants (Y253H) was identified (**Figure 4**). Examination of earlier samples indicated that at 15 months the Y253H mutant was present as the dominant clone, but at 12 months a mixture of Y253H and F317L mutants was detected. Samples at 6 and 9 months revealed only the F317L mutant.

Clinical Discussion

The patient's initial strong response to imatinib, with achievement of CCyR at 6 months, was compromised by indications that molecular response was leveling off, as *BCR-ABL* transcript levels fluctuated between 6 and 12 months. Mutational analysis is indicated in patients with

signs of declining response to imatinib.^{9,30} Even if analysis fails to identify *BCR-ABL* mutations, the exclusion of mutation as a cause of acquired resistance is frequently useful in charting a change in therapeutic course (e.g. imatinib dose increase or HSCT). CML treatment algorithms suggest 3 responses to acquired imatinib resistance resulting from mutation: (1) increase imatinib to maximum dose; (2) initiate dasatinib therapy; or (3) consider enrollment in a clinical trial for nilotinib.^{9,22} The coexistence of two mutations in this patient, along with the use of 600 mg imatinib during therapy, suggests that an increase in imatinib dose may not be an optimal strategy. The presence of F317L is a concern as it is one of the mutants least responsive to dasatinib. Likewise, the Y253H mutant is one of the least responsive mutants to nilotinib in vitro. In a 17-year-old patient with a sibling donor, the presence of 2 mutants argues in favor of an allograft as a second-line therapy.

Figure 4. Case 3: Pattern of Change in *BCR-ABL* Transcript Levels



Follow-up

After 3 months of dasatinib therapy this patient was referred to HSCT (**Figure 4**).

Molecular monitoring promptly identified possible loss of response to imatinib, as well as the need for mutational analysis

Case 4:

Suboptimal Response and Subsequent BCR-ABL Mutation

Synopsis

A 60-year-old female was diagnosed with CML-CP in July 2003 and was referred to a clinical trial; at diagnosis, both Sokal and Hasford risk scores were low. Cytogenetic analysis of bone marrow aspirate demonstrated the presence of the characteristic Ph+ reciprocal translocation, with no evidence of additional cytogenetic abnormalities. In addition, qualitative PCR analysis demonstrated the presence of *BCR-ABL* transcripts.

The patient was initiated on 400 mg/day imatinib. The patient initially responded well to imatinib, achieving CCyR at 6 months. Continued molecular monitoring at 3-month intervals showed the patient reached MMR after 24 months of therapy, and the level of *BCR-ABL* transcripts continued to decrease over the next 3 months (Figure 5).¹⁴ However, molecular monitoring at 30 and 33 months documented

a 2.1-fold rise in transcript level, and 3 months later, transcript levels had risen above the threshold for MMR (Figure 5).

Although this patient remained in CCyR, mutational analysis was conducted at the same time as molecular monitoring because of the consistent rise in transcript levels. These analyses showed no evidence of mutations until 30 months, when a *BCR-ABL* mutation (E453G) was detected. This mutation was shown to be the predominant *BCR-ABL* species at 33 and 36 months.

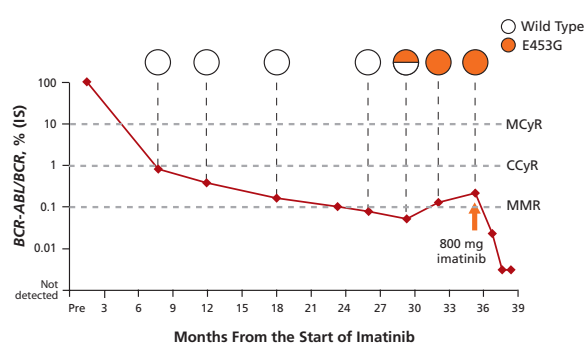
Clinical Discussion

Despite the encouraging initial response to imatinib, documented by the achievement of CCyR at 6 months, there was evidence of inadequate molecular response at 12 months. The failure to achieve MMR by this time is considered a warning in the European LeukemiaNet recommendations, and the persistent lack of MMR at 18 months is considered suboptimal response.¹⁰ This patient did, however, achieve MMR at 24 months.

Given this pattern of response, it might have been reasonable to consider a higher imatinib dose (800 mg/day) at 18 months, because there is some evidence that higher doses may accelerate the achievement of molecular response thresholds.² The pattern of continued decline in *BCR-ABL* transcript levels, however, indicated that the patient was continuing to respond to imatinib and could be expected to achieve additional response milestones, albeit slower than is optimal.^{18,19} Consistent with this view, the patient reached MMR at 24 months, and transcript levels continued to decline at 27 months.

However, the detection of an E453G *BCR-ABL* mutation after 30 months of therapy, followed by the RQ-PCR documentation of an increase in *BCR-ABL* transcript levels at 33 months, heralded a possible loss of response to imatinib at 400 mg/day. This was confirmed by the loss of MMR at the subsequent molecular analysis.

Figure 5. Case 4: Pattern of Change in *BCR-ABL* Transcript Levels and Mutational Analysis



Adapted with permission from Hughes T et al. Blood. 2006;108:28-37.¹⁴

Follow-up

The loss of MMR after this response threshold had been reached called for a reconsideration of clinical strategy. Because some *BCR-ABL* point mutations appear to respond to higher doses of imatinib,³³ the patient was dose-escalated to imatinib at 800 mg/day at 36 months. The patient responded immediately to the higher dose; molecular assessment at 3 months after the dose increase demonstrated a return to MMR.

Molecular response assessment over the following months documented a continuing reduction in *BCR-ABL* transcript levels, as transcripts approached the limit of detection (**Figure 5**).

RQ-PCR analysis can identify patients with suboptimal response and risk for relapse

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