

Imatinib PCR testing for CML

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EUTOS for CML



European Treatment and Outcome Study

Dramatic improvements achieved with the targeted therapy imatinib (formerly STI571) for treatment of Philadelphia chromosome-positive chronic myeloid leukemia (Ph+ CML) have spearheaded a revolution in cancer therapy. More than 8 years of experience with imatinib in clinical trials and 5 years in the post-approval setting have established that imatinib is easy to administer and is safe and tolerable. Imatinib has not only produced unprecedented response rates in Ph+ CML that are durable for years, but has also raised response assessment to the molecular standard. This backgrounder provides information for understanding the role of molecular response assessment for optimizing therapy with imatinib.

More than 95% of patients with CML exhibit the Ph chromosome, which is generated by a balanced reciprocal translocation between the long arms of chromosomes 9 and 22, namely the t(9;22)(q34;q11).¹ This aberrant chromosome encodes a fusion gene, *BCR-ABL*. Depending on the position of the *BCR* breakpoint, various *BCR-ABL* fusion gene transcripts are translated into functional BCR-ABL proteins (p190, p210, and p230). Most common of these are the 210-kDa proteins, usually referred to as p210 BCR-ABL. Constitutive activity of the ABL tyrosine kinase portion of the p210 BCR-ABL fusion proteins has been shown to be critical in the pathogenesis of Ph+ CML.¹

IMATINIB

BCR-ABL served as a rational target for the development of imatinib, a 2-phenylaminopyrimidine derivative that selectively inhibits BCR-ABL kinase activity.^{2,3} Based on safety, tolerability, and efficacy results from phase 2 and 3 trials, imatinib has become the standard of therapy for all phases of Ph+ CML.⁴⁻⁸

RESPONSE ASSESSMENT IN CML

Response to Ph+ CML therapy is monitored by 3 different methods with increasing sensitivity for detection of residual disease. **(Figure 1)** Hematological response is assessed by peripheral blood cell counts and by spleen size; cytogenetic response is assessed by determining the percentage of the Ph-chromosome positive metaphases in the bone marrow karyotype, and molecular response is assessed by measuring levels of *BCR-ABL* gene transcripts.

Figure 1. Monitoring Methods in Ph+ CML⁹

Haematological Response (HR)		Cytogenetic Response (CyR)		Molecular Response (MR) <small>[BCR-ABL to control gene ratio according to International Scale (IS)]³⁵</small>	
Complete (CHR)	<ul style="list-style-type: none"> • Platelets: <450 x 10⁹/L • WBCC: <10 x 10⁹/L • Differential without immature granulocytes and <5% basophils • Nonpalpable spleen 	Complete (CCyR)	Ph+ 0%	Complete	Transcripts nonquantifiable and nondetectable
		Partial (PCyR)	Ph+ 1%-35%	Major (MMR)	≤0.1%*
		Minor	Ph+ 36%-65%		
		Minimal	Ph+ 66%-95%		
		None	Ph+ >95%		
Major = partial + complete					

WBCC, white blood cell count.

*Standardized baseline represents 100% on IS; 0.1% ≅ 3-log reduction from standard baseline.

Table 1. Recommendations for Monitoring Response⁹

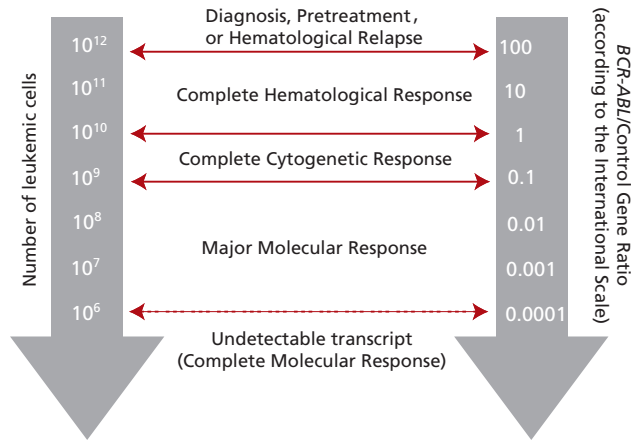
	Hematological Response	Cytogenetic Response	Molecular Response <small>(BCR-ABL to control gene ratio according to the International Scale)³⁵</small>
Frequency	<ul style="list-style-type: none"> • Every 2 weeks until a complete response has been achieved and confirmed • Every 3 months unless otherwise 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
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

FISH, fluorescence in situ hybridization.

RQ-PCR, real-time quantitative polymerase chain reaction.

Quantitative detection of *BCR-ABL* transcripts was made routine by the introduction of ‘real-time’ quantitative polymerase chain reaction methodology (RQ-PCR).¹⁰⁻¹³ *BCR-ABL* transcript levels measured in both bone marrow and peripheral blood samples from Ph+ CML patients have been shown to correlate with the number of the residual leukemic cells in the sample as established by cytogenetic analysis. **(Figure 2)** RQ-PCR can also be very sensitive as it can detect a single *BCR-ABL*-positive cell among 10^5 - 10^6 normal cells, although the most commonly reached sensitivity is in the range of 10^4 - 10^5 .

Figure 2. The *BCR-ABL* Transcript Percent Parallels the Number of Leukemic Cells⁹



MOLECULAR RESPONSE

The value of molecular response assessment in evaluating therapeutic efficacy for Ph+ CML therapy was demonstrated in the phase 3 International Randomized Study of Interferon and STI571 (IRIS) trial.⁸ This prospective, multicenter, open label, randomized study enrolled 1106 patients with newly diagnosed chronic-phase (CP) Ph+ CML. Patients were randomized (553 in each arm) to receive either imatinib at 400 mg orally once daily or gradually escalating doses of interferon alfa (target dose, 5 million U per square meter of body surface area per day) plus subcutaneous low-dose cytarabine at a dose of 20 mg per square meter per day (maximal daily dose, 40 mg) for 10 days every month.

Patients in the IRIS trial treated first-line with imatinib achieved significantly higher overall rates of complete hematological and cytogenetic responses compared with the combination therapy.⁸ The degree of imatinib efficacy was further explored by measuring *BCR-ABL* transcripts to assess the residual disease burden in patients who achieved a complete cytogenetic remission.¹⁴

Molecular responses to imatinib in the IRIS trial were evaluated using RQ-PCR methodology.¹⁴ *BCR-ABL* transcript levels were expressed as a percentage ratio of *BCR-ABL* to *BCR* transcripts. *BCR* served as a control to compensate for variations in the quality of the RNA and for differences in the efficiency of the reverse transcription reaction. To standardize the results generated in the three testing laboratories, a pretreatment baseline value was established. *BCR-ABL* transcripts were measured in blood samples from 30 patients with newly diagnosed Ph+ CML-CP prior to treatment in each of the testing laboratories. The median *BCR-ABL/BCR* percent value in each laboratory was designated as the standard baseline for that laboratory. A reduction in *BCR-ABL* transcript levels of ≥ 3 log (1000-fold reduction) from the laboratory-specific pretreatment standard baseline was defined as a major molecular response (MMR). Thus molecular responses were reductions from an absolute baseline (common to all) rather than a relative baseline (individualized). This ensures that patients with the same level of response have the same degree of residual disease. Moreover, under- or overestimation of the extent of response due to individual variations in pretreatment disease levels is avoided by using a common standard baseline.

The IRIS study avoided the term complete molecular response (CMR) but rather determined the number of patients with undetectable *BCR-ABL* with a sensitivity of 4.5 log below the standardized baseline.¹⁴ Recognizing that this level of response reflects the limits of current detection methods, undetectable *BCR-ABL* should not be equated with eradication of *BCR-ABL* expression or cure. Cases in which patients with undetectable *BCR-ABL* discontinued imatinib therapy and then subsequently relapsed reinforce that the lack of detection of *BCR-ABL* transcripts is neither a cure nor a reason to discontinue imatinib therapy.¹⁵⁻¹⁷

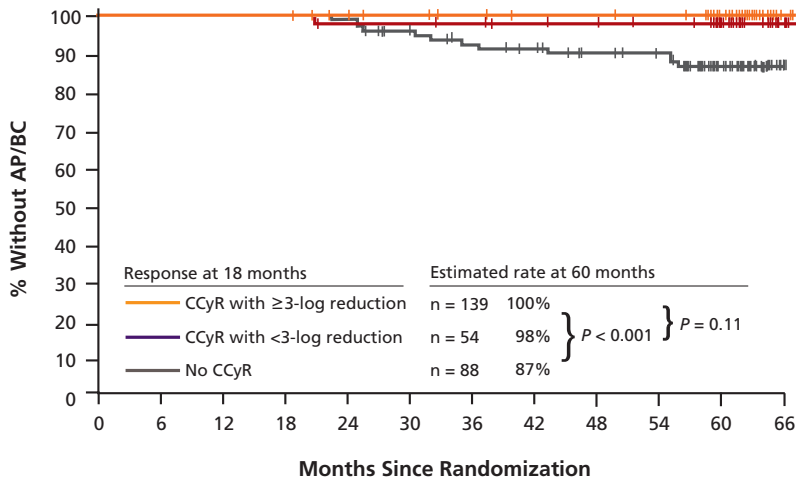
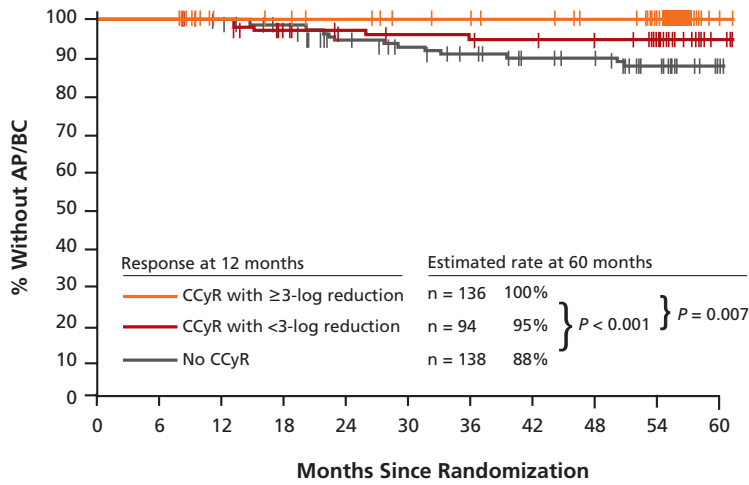
Molecular Response Correlates With Cytogenetic Response

In the IRIS trial, patient blood samples were monitored for *BCR-ABL* transcript levels within 2 weeks after achieving complete cytogenetic response (CCyR) and then every 3 months.¹⁴ Patients treated first-line with imatinib had significantly higher rates of both cytogenetic and molecular responses compared with patients treated with combination therapy, suggesting that cytogenetic responses correlate with molecular responses. There is a strong association between an approximately 2-log reduction in *BCR-ABL* transcript levels from the standardized baseline and the achievement of a CCyR.^{18, 19}

Molecular Response Predicts Progression-Free Survival

Cytogenetic responses are an established prognostic indicator of progression-free survival in CML.^{20, 21} Furthermore, long-term analysis of IRIS data also supports for the first time the prognostic significance of molecular responses in Ph+ CML therapy.²² Overall, patients with CCyR and MMR at 12 months on first-line imatinib therapy had the lowest rate of progression (zero) to accelerated phase (AP) or blast crisis (BC) at 60 months compared with patients with CCyR but without MMR. **(Figure 3A)** It is noteworthy, however, that at 18 months patients with CCyR have more stable responses and a significantly higher rate of freedom from progression to AP/BC (98%-100%) with respect to patients without CCyR (87%), independent of the degree of molecular remission. This implies that evaluation of MMR at 12 months of therapy is indeed of high prognostic significance and, on the other hand, that 18 months may be too early to be concerned about lack of MMR during imatinib therapy. **(Figure 3B)**

Figure 3A & 3B. Survival Without Progression to AP/BC at 60 Months According to Molecular Response at 12 and 18 Months of Imatinib Therapy²³



CONSENSUS RECOMMENDATIONS FOR RESPONSE ASSESSMENT IN Ph+ CML

Evidence obtained in clinical trials and post-approval setting with imatinib has prompted experts to formulate consensus recommendations for response assessment and treatment of patients with Ph+ CML.^{9, 24-26} The European LeukemiaNet recommendations propose a schedule of response expectations at various time points of imatinib therapy. **(Figure 4)**

Figure 4. Goals of Therapy: Criteria for Satisfactory Response to Imatinib Treatment

	3 months	6 months	12 months	18 months	At any time
Treatment Failure	No HR	<CHR No CyR	<PCyR	<CCyR	<ul style="list-style-type: none"> • Loss of CHR[*] • Loss of CCyR[†] • Mutation with a high level of insensitivity to IM[‡]
Suboptimal Response	<CHR	<PCyR	<CCyR	<MMR	<ul style="list-style-type: none"> • ACA in Ph+ cells[§] • Loss of MMR[§] • Mutation with a low level of insensitivity to IM[‡]
<hr/>					
Warnings	At diagnosis		12 months	At any time	
	<ul style="list-style-type: none"> • High risk • Del 9q+ • ACA in Ph+ cells 		<MMR	<ul style="list-style-type: none"> • Any rise in transcript level • Other chromosomal abnormalities in Ph- cells 	

ACA, additional chromosome abnormalities; CCyR, complete cytogenetic response; CyR, cytogenetic response; CHR, complete hematological response; HR, hematological response; MMR, major molecular response; PCyR, partial cytogenetic response.

*To be confirmed on 2 occasions, unless associated with progression to AP/BC.

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

‡Mutations need to be interpreted within clinical context.

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

MOLECULAR MONITORING

Molecular monitoring can assist in identification of imatinib refractory patients as well as predict relapse. Refractoriness to imatinib can be considered infrequent in patients with newly diagnosed Ph+ CML-CP, and the risk for progression decreases with time on therapy.²² **(Table 2)** The annual progression rate, including all events, of patients treated first-line with imatinib in the IRIS trial became <1% during the 5th year of therapy. However, an increasing proportion of patients in advanced Ph+ CML disease phases are either initially refractory to imatinib treatment or lose sensitivity over time and experience relapse.²⁷

Table 2. Annual Event Rates on First-line Imatinib

Year	All Events*	AP/BC
1st	3.3%	1.5%
2nd	7.5%	2.8%
3rd	4.8%	1.6%
4th	1.5%	0.9%
5th	0.9%	0.6%

*All deaths or loss of response including progression to AP/BC

Molecular Analysis at Diagnosis

Assessment of *BCR-ABL* transcript by RQ-PCR at diagnosis is necessary to confirm disease and establish the type of *BCR-ABL* fusion present. This is most frequently e13a2 or e14a2 (corresponding to P210), but occasionally e1a2, (corresponding to P190) e19a2 (corresponding to P230) or a range of other rare variants.²⁸ Fluorescence in situ hybridization (FISH) can be used to detect the presence of gene deletions on the 9q chromosome, but there is little or no evidence that these are associated with an adverse prognosis in imatinib-treated patients.

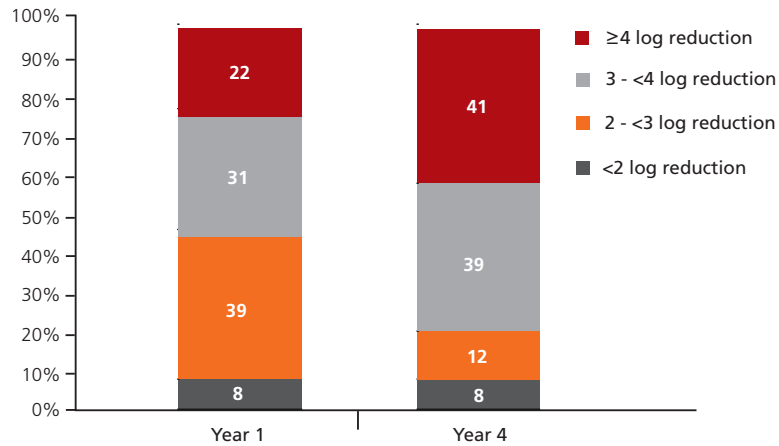
Molecular Monitoring During Therapy

European LeukemiaNet experts recommend continued molecular monitoring during imatinib therapy. Patients with Ph+ CML should have molecular response assessment performed at regular 3-month intervals.^{9, 26} A steady decline in *BCR-ABL* transcripts indicates an ideal response to therapy.

The IRIS trial demonstrated that molecular responses continue to improve with time on imatinib therapy. **(Figure 5)** A substantial portion of patients without CCyR or MMR at 12 or 18 months eventually achieved these levels of response during continued therapy.²⁹ In a recent study, a number of patients without CCyR with imatinib at 1 year could reach the same levels of molecular response as those patients with CCyR at 12 months after 3 or 4 years.³⁰ Together these results indicate that the time at which patients may achieve molecular responses varies and some patients require a year

or more to achieve MMR. Patients who achieve CCyR and MMR have the best prognosis compared with patients who achieve only CCyR without MMR or no CCyR.²² A recent study suggests, however, that MMR achieved any time within the 15 month observation time after CCyR was obtained, is a prognostic marker for progression-free survival. Until unequivocal evidence confirms that the rate of decline in *BCR-ABL* transcripts has prognostic significance for survival, a steady decline in *BCR-ABL* transcripts is the current criterion for molecular response during imatinib in Ph+ CML therapy.

Figure 5. Degree of *BCR-ABL* Log Reduction in 124 Patients With CCyR at 1 and 4 Years (in Percent)³¹



Molecular Monitoring Predicts Relapse

A rise in *BCR-ABL* transcript levels detected during imatinib therapy for Ph+ CML is a warning sign and should trigger a subsequent, more stringent, RQ-PCR analysis.⁹ A single ≥ 2 -fold rise in *BCR-ABL* transcript levels using an RQ-PCR method that can reliably detect a 2-fold change in consecutive analyses has been shown to be strongly associated with the detection of imatinib-resistant *BCR-ABL* mutations.³² However, other studies found that only confirmation of such a rise with a repeat test accurately predicts imatinib resistance due to mutations.³³

This may reflect the variability in RQ-PCR assay measurement reliability. A 5-fold to 10-fold (0.5 or 1 log) increase in *BCR-ABL* transcript levels is currently proposed as the threshold for molecular relapse or resistance.²⁵ This avoids the potential for raising concern over potentially clinically irrelevant fluctuations in *BCR-ABL* transcript levels. Such fluctuations, however, indicate that the measurement reliability of the RQ-PCR assay should be optimized. A rise in *BCR-ABL* transcript levels can be used to trigger analysis for imatinib-resistant *BCR-ABL* mutations. At present, direct gene sequencing for mutation detection is a commonly used approach. Finally, it must be considered that a meaningful rise in *BCR-ABL* transcripts could also be due to inappropriate imatinib dosing or lack of patient adherence with therapy.

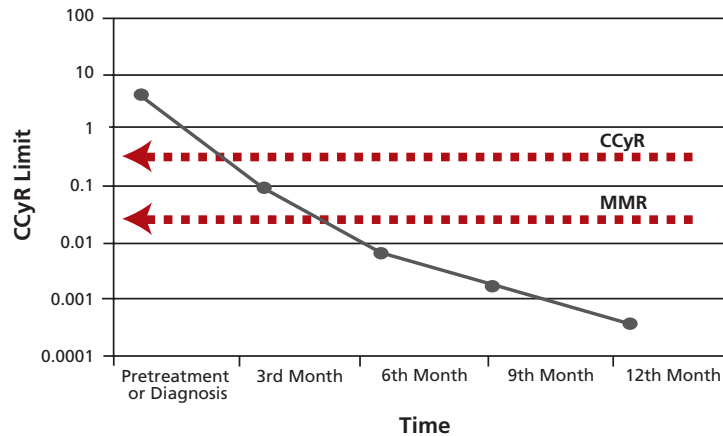
Molecular Monitoring Methodology

The Ph+ CML community has recognized a need to harmonize methodology for detecting and reporting *BCR-ABL* transcript levels. To that end, a consensus meeting of leading investigators in Ph+ CML was held in October 2005 at The National Institutes of Health (NIH) to harmonize RQ-PCR methodology worldwide.^{34, 35} The discussion included key suggestions for stabilizing RNA samples, selection of a suitable control gene, as well as optimizing the methodology.

Another important consideration discussed at the NIH meeting was the need for a *BCR-ABL* standard for quantitation. *BCR-ABL* transcript levels in the IRIS trial were plotted with respect to a standard baseline derived from the median value obtained from samples from 30 patients with newly diagnosed Ph+ CML-CP.¹⁴ Unfortunately, the patient samples used in the IRIS trial to establish the standard baseline are no longer available. The advent of commercially prepared *BCR-ABL* standards is likely to address this problem.

Recommendations for uniform expression of molecular response data were also formulated at this meeting.³⁵ An International Scale for expressing molecular response in Ph+ CML that is directly traceable to the IRIS scale, was proposed.^{34, 35} To harmonize the scale across laboratories worldwide, *BCR-ABL*/control gene transcript ratios obtained in separate laboratories and known to correspond to a 3-log reduction in *BCR-ABL* transcripts from the IRIS standard baseline will each be converted to 0.10% by arithmetic conversion factors. The International Scale will therefore be anchored to a critical value with known prognostic significance.³⁶ **(Figure 6)** Uniform expression of molecular monitoring data is anticipated to facilitate the understanding and use of this information for clinicians and scientists worldwide.

Figure 6. Typical Molecular Response Profile on the International Scale of a Patient With Ph+ CML on Imatinib Therapy³⁷



SUMMARY

Molecular monitoring is clearly an important, and indisputably the most sensitive, tool for evaluating responses as well as for predicting progression-free survival or relapse during imatinib therapy for Ph+ CML. Although peripheral blood sampling is relatively easy to perform, obtaining reliable molecular monitoring data can be a challenge for clinicians not associated with institutions routinely performing these assays. Lack of access to or unreliable molecular response data can compromise therapy for patients with Ph+ CML on imatinib. Reliable and timely molecular monitoring during therapy has the potential to guide clinical decision making toward optimizing imatinib for Ph+ CML.

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CLEAR ADHESIVE CD POCKET

