Molecular monitoring and minimal residual disease in the management of chronic myelogenous leukemia

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The introduction of BCR-ABL1 tyrosine kinase inhibitors (TKIs) in 2001 for treatment of chronic myelogenous leukemia (CML) marked a paradigm shift in management of the disease. With that advance, CML has been largely managed as a chronic condition, with daily medication and frequent monitoring. Optimizing monitoring methods and identifying factors associated with response and long-term outcomes has thus been a major clinical research focus. Given the improved understanding of surveillance techniques in CML and the advent of several recently approved second- and third-generation TKIs, there have been recent updates to clinical practice guidelines. The dramatic change in survival for patients with CML treated with TKIs compared with previous therapies and the subsequent incremental therapeutic improvements have led to uniquely well-supported approaches and surveillance of patients on TKI therapy. Measurement of RNA for *BCR-ABL1* via quantitative PCR (qPCR) is the cornerstone of disease management. Efforts to maximize utility and scheduling of molecular monitoring and the care plans based on results of that monitoring are at the heart of current investigations. Study designs of major clinical studies in CML will incorporate new goals of therapy and molecular monitoring methods.

Treatment of chronic myelogenous leukemia (CML) with BCR-ABL1 tyrosine kinase inhibitors (TKIs) has profoundly altered the natural history of this disease. CML was the first human tumor found to be driven by a necessary and dominant cytogenetic aberration.[1,2] The selective destruction of BCR-ABL1 tyrosine kinase harbors the rationale both for the favorable safety profile and the striking potency of these TKIs. These drugs reduce disease burden – often to undetectable levels – protect against progression of CML to advanced stages of disease and are generally more tolerable compared with previous standard therapy. Most striking is that disease-free survival has improved notably and has led to the treatment of CML as a chronic condition. In turn, that has led to an intensified focus on both the methods by which minimal residual disease (MRD) is monitored over time and how the kinetics of MRD can help predict long-term survival benefit. This review discusses the relevance of recent clinical research on MRD in CML to practice in the community and how these discoveries have informed changes in recent clinical practice guidelines.

Prognostic significance of early molecular response to TKI therapy

Early in the imatinib era, it became clear that achieving deep response within the first 12-18 months of treatment correlated significantly with improved outcomes.[3-16] At the time of diagnosis, the burden of disease is large and response to therapy can be measured by morphologic regression. With effective therapy, however, shrinking disease burden needs to be measured by increasingly sensitive methods. This has been described elsewhere in the literature and is outlined in Figure 1 (p. 174).

Deep molecular responses are tightly correlated with survival. Patients in the International Randomized Study of Interferon and STI571 (IRIS) who achieved major molecular response (MMR; 0.1% *BCR-ABL1* level on the international scale [IS]) at 12 or 18 months had significantly higher rates of event-free survival (EFS) and transformation-free survival at 7 years than did patients who did not achieve such responses.[8] Further landmark analyses evaluating the prognostic value of achieving molecular response at early time points after initiation of TKI therapy found that the achievement of *BCR-ABL1* ≤ 10%

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TABLE Summary of landmark analyses of molecular response at 3 mo of TKI therapy

TKI	Study	Outcome measure	Stratification by BCR-ABL1 (IS), % at 3 mo (no. of patients)		*P* value
Imatinib	IRIS[4,6]		< 1 (12)	≥ 1 (23)	
		MMR by 24 mo	100	54.2	< .001
			≤ 10 (131)	> 10 (43)	
		8-year OS	93	81	.011
	Royal Liverpool University Hospital[74]		< 10 (23)	> 10 (24)	
		MCyR at 6 mo	95.7	4.2	NR
	Hammersmith[12]		≤ 9.84 (211)	> 9.84 (68)	
		OS rate at 8 y	93.3	56.9	< .001
			≤ 9.84 (211)	> 9.84 (66)	
		EFS rate at 8 y	65.1	6.9	< .001
			≤ 9.54 (208)	> 9.54 (71)	
		PFS rate at 8 y	92.8	57.0	< .001
			≤ 8.58 (169)	> 8.58 (79)	
		CCyR rate at 8 y	99.4	21.7	< .001
			≤ 2.81 (141)	> 2.81 (137)	
		MMR rate at 8 y	82.5	21.1	< .001
			≤ 0.61 (57)	> 0.61 (222)	
		CMR[a] rate at 8 y	84.7	1.5	< .001
	German CML Study IV[b] [69]		≤ 10 (501)	> 10 (191)	
		5-year OS	95.2	87.0	< .001
	ENESTnd[c] [18]		≤ 10 (176)	> 10 (88)	
		MR4.5 by 4 y	34	5	< .0001
		PFS at 4 y	98	83	< .0001
		OS at 4 y	99	84	< .0001
	DASISION[c19]		≤ 10 (154)	> 10 (85)	
		AP/BC within 3 y	2.6	12.9	NR
		3-year PFS	95.9	75.3	< .0001
		3-year OS	96.0	88.0	.0036
	BELA[75]		≤ 10 (146)	> 10 (77)	
		MMR by 24 mo	69	17	< .001
		CCyR by 24 mo	95	65	< .001
		EFS at 24 mo	92	85	NS
		OS at 24 mo	99	95	NS
Nilotinib	ENESTnd[d] [18]		≤ 10 (233)	> 10 (24)	
		MR4.5 by 4 y	47	4	< .0001
		PFS at 4 y	95	83	.0061
		OS at 4 y	97	87	.0116
	GIMEMA[76]		≤ 1 (173)	> 1 (23)	
		MMR at 12 mo	79	35	< .001
		FFS at 3 y	92	74	.002
		PFS at 3 y	95	83	.009
		OS at 3 y	96	86	.059

Continued on next page

TABLE *continued*

TKI	Study	Outcome measure	Stratification by BCR-ABL1 (IS), % at 3 mo (no. of patients)			*P* value
Dasatinib	DASISION[19]		≤ 10 (198)		> 10 (37)	
		AP/BC within 3 y	3.0		13.5	NR
		3-year PFS	93.1		68.2	.0003
		3-year OS	95.9		85.9	.0348
	Hammersmith[11]		≤ 10 (117)		> 10 (11)	
		CCyR by 2 y	91.4		58.8	< .001
		MMR by 2 y	79.8		14.3	< .001
		MR4.5 by 2 y	45.7		0.0	< .001
Bosutinib	BELA[75]		≤ 10 (179)		> 10 (29)	
		MMR by 24 mo	74		21	< .001
		CCyR by 24 mo	96		48	< .001
		EFS at 24 mo	93		83	.004
		OS at 24 mo	99		88	.004
Multiple TKIs[e]	MDACC[77]		≤ 1 (300)	> 1-10 (66)	> 10 (13)	
		3-year EFS	96	98	61	< .001
		3-year TFS	99	98	100	NS

AP, accelerated phase; BC, blast crisis; CCyR, complete cytogenetic response; CML, chronic myelogenous leukemia; CMR, complete molecular response; EFS, event-free survival; FFS, failure-free survival; MR4.5, deep molecular remission denoted by 4.5 log reduction of *BCR/ABL* on the International Standardized Ratio; MMR, major molecular response; NR, not reported; NS, not significant; OS, overall survival; PFS, progression-free survival; TFS, transformation-free survival; TKI, tyrosine kinase inhibitor

[a]CMR defined as 2 consecutive samples with no detectable *BCR-ABL1* transcripts and > 40,000 *ABL1* control transcripts. [b]Includes patients treated with imatinib alone (400 mg QD or 400 mg twice daily) and imatinib plus interferon-α. [c]Data for the imatinib arm of the study. [d]Data for the nilotinib 300 mg twice daily arm of the ENESTnd study. [e]Includes patients treated with imatinib (400 mg once daily or 400 mg twice daily), nilotinib, or dasatinib.

(IS) at 3 months correlated significantly with improved long-term outcome [6,7,11,12,16,17] (Table).

The first of these landmark analyses to demonstrate an overall survival (OS) advantage of achieving *BCR-ABL1* ≤ 10% (IS) at 3 months was a retrospective analysis of 282 consecutive patients with newly diagnosed CML in chronic phase (CML-CP) treated with first-line imatinib.[12] That analysis found that for each landmark (3, 6, and 12 months) and each outcome measure (8-year OS, progression-free survival [PFS], EFS, complete cytogenetic response [CCyR], MMR, and complete molecular response [CMR; undetectable *BCR-ABL1* (IS) with ≥ 40,000 copies of *ABL1* control]), a threshold molecular response could distinguish high-risk patients from low-risk patients with maximal sensitivity and specificity. Multivariate Cox regression analysis showed that the 3-month *BCR-ABL1* transcript level (≤ 9.84% vs > 9.84%) was the only independent predictor of 8-year OS, PFS, EFS, and current CCyR survival (c-CCyRS; the probability of being alive and in CCyR at a given time point). For patients with a 3-month *BCR-ABL1* transcript level ≤ 9.84%, predicted 8-year OS was 93%, compared with 57% in patients with a transcript level ≥ 9.84% *(P* < .001). Notably, although patients in this study who developed resistance or intolerance to first-line imatinib (n = 118) during the follow-up period were given second-line treatment (dasatinib, n = 72; nilotinib, n = 37; allogeneic stem cell transplant [SCT], n = 9), the 8-year c-CCyRS remained significantly lower for high-risk patients than for low-risk patients, indicating that the 3-month *BCR-ABL1* measurement retained its prognostic value in this cohort regardless of second-line therapy or subsequent interventions. On the basis of this finding, a single assessment of *BCR-ABL1* transcript level at 3 months was sufficient to predict the clinical outcome of these patients, regardless of second-line or subsequent therapies, or of Hansford or Sokal risk.[12]

As shown in the Table, other landmark analyses that compare future outcomes, including cytogenetic and molecular response outcomes and survival outcomes of patients who do and do not achieve specific molecular responses at 3 months of first-line TKI therapy, consistently demonstrate the prognostic significance of this early molecular response milestone, regardless of the TKI received. Although outcomes are significantly better for patients achieving early response on any first-line TKI, it has been suggested that

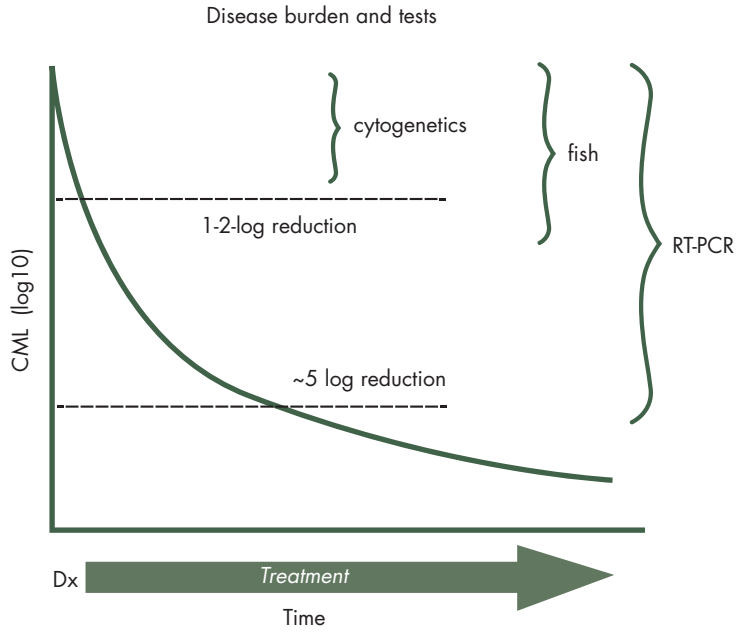

FIGURE Sensitivities of test used in chronic myelogenous leukemia to monitor disease burden and treatment response. (Originally published in Radich JP. How I monitor residual disease in chronic myeloid leukemia. Blood. 2009;114:3376-3381. Reprinted with permission. ©American Society of Hematology.)

treatment with second-generation TKIs may lead to superior outcomes compared with treatment with imatinib. Rates of *BCR-ABL1* (IS) ≤ 10% at 3 months for patients on nilotinib compared with imatinib in the Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients (ENESTnd) study were 91% and 67%, respectively,[18] and rates of *BCR-ABL1* (IS) ≤ 10% at 3 months for patients on dasatinib compared with imatinib in the Dasatinib versus Imatinib in Newly Diagnosed Chronic Phase CML (DASISION) study were 84% and 64%, respectively.[19] The follow-up of the ENESTnd and DASISION studies continues; it remains to be determined whether these improved response rates lead to improved outcomes. In these analyses, patients in the ENESTnd and DASISION studies who achieved ≤ 10% *BCR-ABL1* (IS), regardless of TKI used, have superior survival rates compared with those who did not reach this landmark (Table).

These findings imply that *BCR-ABL1* (IS) ≤ 10% versus >10% at 3 months marks a critical threshold that distinguishes patients with low versus high probability of poor prognosis, respectively. On the basis of these landmark analyses, new guidelines explicitly acknowledge the prognostic significance of achieving rapid molecular response to TKI therapy by revising the expected treatment response at 3 months to *BCR-ABL1* (IS) ≤ 10% (or partial cytogenetic response [PCyR]).[20]

Implications for the future of CML disease management and clinical study design

Management of patients who do not achieve BCR-ABL1 (IS) ≤ 10% at 3 months

For patients without adequate early response, heightened vigilance should be the approach, including closer evaluation of potential patient-related issues such as treatment adherence, and treatment-related issues such as drug interactions or adverse events. In absence of a clinical trial option, switching to another TKI and evaluating for SCT may be warranted.

At present, there are limited clinical data to support any particular approach as the best evidence-based management of patients who do not achieve *BCR-ABL1* (IS) ≤ 10% at 3 months. Although the findings of Marin and colleagues and others already described here have shown that second-line therapy did not significantly improve the prognosis of patients in the study who had *BCR-ABL1* (IS) > 10% at 3 months, it is worth noting that patients in the study were given second-line therapy when they developed resistance or intolerance to first-line imatinib, not at the 3-month assessment. Thus, the potential effect on survival of an "early switch" to second-line therapy in patients with *BCR-ABL1* (IS) > 10% at 3 months is unknown, because there are currently no published studies that prospectively evaluate the effect of switching to second-line therapy in patients who do not achieve *BCR-ABL1* ≤ 10% at 3 months with first-line TKI treatment. Some clinical evidence suggests that an earlier switch to second-line treatment might yield improved response compared with a later switch.[21,22] In the Factors Impacting On Response to Dasatinib in Europe (FORTE) observational study, which assessed the relationship between time elapsed from first detection of imatinib resistance or intolerance to initiation of second-line dasatinib and best response to dasatinib over a 12-month observation period,[23] shorter time to initiation of second-line dasatinib was significantly correlated with improved response in patients who were intolerant to imatinib ($P < .0001$); however, a significant correlation between earlier switch and improved outcomes was not seen in other studies.[24,25] Further investigations of the potential advantages of early switch to second-line TKI therapy are underway.

Clinical studies with long follow-up have established that second-line nilotinib and dasatinib can elicit high rates of cytogenetic and molecular response, and improve PFS and OS in patients who develop resistance or intolerance to first-line imatinib.[21,26-39] Although there is less experience with bosutinib and ponatinib used in the second- or third-line

setting, these TKIs have shown impressive activity in patients for whom imatinib, dasatinib, and nilotinib failed.[28,40,41]

As observed in the first-line setting, the 3-month response milestone also carries prognostic significance in the second-line setting. In a study of 155 patients who were treated with nilotinib or dasatinib after imatinib failure, *BCR-ABL1* level at 3 months after the start of second-line TKI was found to significantly correlate with probabilities of achieving MMR ($P < .0001$) and major cytogenetic response ($P < .0001$) at 24 months.[42] A more recent study yielded corroborative results: Patients with *BCR-ABL1* < 10% at 3 months after starting second-line TKI therapy had significantly better PFS and OS at 3 years than patients with ≥ 10% at 3 months.[43] At present, guidance on criteria for establishing optimal response to second-line TKI therapy is lacking. With longer follow-up of second-line dasatinib and nilotinib, however, this information should become available.[30,38]

Use of the IS in molecular monitoring of BCR-ABL1 levels

The IS was established in the phase 3 IRIS study as a means to facilitate direct comparison of quantitative polymerase chain reaction (qPCR) results across the 3 laboratories that were supporting the study.[44] Investigators at each of the laboratories used a common set of 30 patient samples to calculate the median value of the *BCR-ABL1*: control ratio, which was designated as 100% (IS) and used as the standardized baseline at each laboratory. Using the IS-standardized baseline, the IRIS investigators established a second "anchor" on the IS, MMR, which was defined as ≥ 3-log reduction in the *BCR-ABL1*: control ratio, or 0.1% (IS). Because the IS is independent of an individual patient's baseline *BCR-ABL1*: control value, reductions in transcript levels reported on the IS represent absolute – not relative – values. Publication of IRIS led to demand for molecular testing, but th edevelopment of IS qPCR lagged. Ultimately, the complexity of changing guidelines and local laboratory reporting created inefficiencies in practice and underutilization of proper surveillance during therapy.[45,46]

Over the past decade, great efforts have been made to encourage broader, universal use of the IS,[17,47-49] including the development and validation of IS-standardized reference materials by the World Health Organization that laboratories can use to align their qPCR results to the IS.[49,50] Although there have been improvements in its use, some commercial laboratories in the Unite States do not report *BCR-ABL1* test results on the IS. Nevertheless, adoption of the IS is increasing in the US and Europe.[51] In the absence of established access to IS testing, "homegrown" qPCR assays continue to be used. Because homegrown qPCR assays potentially use laboratory-specific parameters – such as baseline standards, control genes, and test reporting standards – the results from such assays may be difficult to interpret in the context of an individual patient from one time point to another or a cohort of patients in a single practice, or to compare an individual patient's molecular testing results with those reported in clinical studies. In recognition of the potential hurdles of using homegrown, non–IS-standardized qPCR assays, classic metaphase spread from bone marrow should be used in absence of IS-standardized molecular testing to assess treatment response.[52,53]

Use of qPCR assays with adequate level of sensitivity to define complete molecular response

The term "complete molecular response" has come to imply ≥ 4.5-log reduction from baseline in *BCR-ABL1* transcript levels (IS). Often, however, CMR is used to indicate undetectable *BCR-ABL1* transcript levels. This definition is imprecise and untenable, particularly if results are not reported on the IS and are subject to limits of detection of individual qPCR assays. The limitations inherent in the term CMR underscore the importance of establishing universal standardization in test reporting (ie, the IS) and minimum requirements for qPCR assay sensitivity when defining undetectable *BCR-ABL1* transcript levels. This is consistent with a recent shift in the manner in which molecular response is reported, to include the limit of detection of the assay. By this convention, $MR^{4.5}$ would indicate a molecular response of ≥ 4.5-log reduction from baseline in *BCR-ABL1* level, and $CMR^{4.5}$ would indicate undetectable *BCR-ABL1* transcripts using an assay capable of detecting MR.[4.5] When *BCR-ABL1* transcripts are undetectable, the control-gene copy number should be reported as verification that the sample is not degraded and the assay is not technically flawed.[54]

As newer treatment approaches and more powerful assays are developed, MRD will be reported in diminishingly lower levels and the term CMR will become anachronistic. Current clinical trials are testing digital detection (DiD) PCR assays[55] in which qPCR is performed in extremely small volumes on a nanofluidic chip using replicate terminal dilutions of a sample.[56,57] DiD PCR further improves the sensitivity of currently available molecular testing such that it can detect 1 rogue copy of *BCR-ABL1* among 500,000 control *ABL1* copies. An assay sensitivity of ≥ 4.5 log below baseline (IS) is commonly used when determining CMR and it has been adapted in the National Comprehensive Cancer Network guidelines. This specification is an acknowledgement of the advances in qPCR technology that have allowed ever more sensitive detection of *BCR-ABL1* transcripts and, more importantly, the remarkable effectiveness with which modern CML therapy suppresses *BCR-ABL1* expression.

Potential for treatment-free remission

The movement toward standardization of molecular monitoring and specification of the level of molecular response

supports the accurate identification of patients with deep molecular response to first-line TKI therapy, including those with undetectable *BCR-ABL1* transcripts, who may be eligible for enrollment in clinical studies of treatment-free remission. The probability of molecular relapse after stopping TKI therapy is lower in patients who achieve and maintain at least $MR^{4.5}$ for ≥ 2 years before stopping therapy.[58-61] Based on the STop IMatinib (STIM) study, it is estimated that only a minority of patients treated with imatinib would meet the study inclusion criteria.[59] The ENESTnd study was the first phase 3 randomized study in CML to have molecular response as its primary endpoint.[62] By 3 years of follow-up in that study, 32% of patients who received nilotinib 300 mg twice daily, 28% of patients receiving nilotinib 400 mg twice daily, and 15% of patients receiving imatinib 400 mg daily had achieved *BCR-ABL1* ≤ 0.0032% (ie, $MR^{4.5}$).[63] The phase 3 randomized DASISION study revealed similar results in preliminary reports of 3-year data.[64] If molecular response with nilotinib and dasatinib can be sustained, a considerably higher proportion of patients may be eligible for entry into studies of safe TKI cessation.

Delay of disease progression with TKI therapy

Advanced CML, including accelerated phase (AP) and blast crisis (BC), is notoriously difficult to treat. Standard TKI therapies for CML-CP are considerably less effective in advanced disease.[65] The difficulty in treating advanced CML is underscored by the low survival rate in patients with advanced disease, even in the current era of TKI therapy; median survival of patients with CML-BC has improved from 4-6 months before 2001 to 7-9 months since 2001.[65,66] Thus, a primary goal of treating CML is to prevent progression to CML-AP/BC in patients newly diagnosed with CML-CP.[65,67,68]

Landmark analyses of imatinib treatment have shown a significant correlation between achievement of early response to imatinib and reduced likelihood of an event, such as loss of response, progression to CML-AP/BC, or death.[3,5,8,9,12,13,15,69-71] Although preliminary, landmark analyses of nilotinib[72] and dasatinib[73] also show a significant correlation between achievement of early molecular response and survival without disease progression to CML-AP/BC.

Summary and conclusions

Research validating the prognostic value of early molecular response has been remarkably consistent. Achieving *BCR-ABL1* ≤ 10% (IS) at 3 months after the start of either first- or second-line TKI therapy predicts significantly better long-term response and survival outcomes compared with *BCR-ABL1* > 10% (IS) at 3 months. What is not as well established is the optimal course of action in patients who do not meet treatment response goals at 3 months. Although long-term follow-up of clinical studies has confirmed the efficacy and safety of second-line TKI therapy after imatinib failure, questions remain whether switching to second-line TKI therapy earlier (eg, switching at 3 months) yields better outcomes than switching later. In the German CML Study IV, patients who achieved *BCR-ABL1* ≤ 10% (IS) at 6 months had 5-year OS, similar to those who achieved this level of response at 3 months.[70] In the ENESTnd study, by contrast, half of the patients with *BCR-ABL1* > 10% (IS) at 3 months who progressed on study did so between 3 and 6 months.[18]

So what is a clinician to do when patients do not reach the 3-month treatment response goal? As mentioned earlier, heightened vigilance is crucial in these cases. Molecular response data should be considered as one factor among many, including patient history, prior treatment response, comorbidities, and drug efficacy and safety profiles, before making decisions about changes in therapy.

The priorities of recent clinical research suggest the direction in which the management of CML is going. In the future, we can expect IS-standardized qPCR assays with ≥ 4.5-log sensitivity to be used routinely for detecting MRD and monitoring molecular response to TKI therapy; achievement of undetectable *BCR-ABL1* levels (as measured by very sensitive assays) as a goal of therapy; continued exploration of treatment-free remission strategies for patients achieving sustained deep molecular responses on TKI therapy; and molecular response endpoints with more stringent response criteria to be used in clinical studies in CML.

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