Major Molecular Response in CML Patients Treated with Tyrosine Kinase Inhibitors: The Paradigm for Monitoring Targeted Cancer Therapy

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Chronic myeloid leukemia (CML) is characterized by a reciprocal translocation event [t(9;22)(q34;q11.2)] that generates a shortened chromosome 22 known as the Philadelphia (Ph) chromosome [1]. This pathognomonic chromosomal translocation can be identified by conventional cytogenetics—the historic gold standard for diagnosing and monitoring the treatment of CML. The Ph translocation results in the constitutive overexpression of a chimeric BCR-ABL fusion protein that directly promotes leukemogenesis via its dysregulated tyrosine kinase activity. Targeted therapy with tyrosine kinase inhibitors (TKIs), specifically imatinib, has become the recommended first-line treatment for patients with newly diagnosed CML [2–4]. Most patients with CML treated with TKIs achieve a complete cytogenetic response (CCyR), that is, the absence of detectable Ph chromosomes [5, 6]. Even after this threshold is achieved, the disease burden continues to progressively decrease with continued treatment [7, 8]. Therefore, more sensitive polymerase chain reaction (PCR)–based molecular methods are required to detect and quantify levels of

minimal residual disease, especially at long time points after TKI initiation. Low levels of minimal residual disease, as measured by real-time quantitative PCR (RQ-PCR) of BCR-ABL RNA, have been shown to be an excellent surrogate marker for long-term prognosis [5, 9–12], and BCR-ABL RQ-PCR represents the scientific paradigm for successful molecular diagnostic monitoring of targeted cancer therapy.

RQ-PCR–based molecular monitoring of BCR-ABL transcripts is the most sensitive tool for assessing disease burden in patients with CML. Molecular monitoring entails regular blood (or bone marrow) RQ-PCR assays during the entire course of TKI therapy, and, if transcript levels are rising, simultaneous mutation testing to assess possible drug resistance caused by *BCR-ABL* kinase domain mutations [4, 13–15]. With RQ-PCR, BCR-ABL transcripts are quantified relative to a non-leukemia-associated reference gene to control for sample-specific variations in RNA quantity and quality (often ABL, BCR, G6PDH, or GUSB) [15, 16]. When performed in a high-quality laboratory, RQ-PCR is

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approximately three logs more sensitive than cytogenetic techniques [8], and it is typically able to detect one leukemia cell in a background of approximately 100,000 normal cells. Molecular testing of peripheral blood samples is more convenient than bone marrow sampling, which is usually required for cytogenetic monitoring, and molecular testing correlates well with clinical response [15]. As a result, frequent blood-based molecular testing may reduce the need for invasive bone marrow testing. The European Leukemia-Net and the National Comprehensive Cancer Network recommend RQ-PCR-based molecular monitoring every 3 months until a deep response (i.e., major molecular response [MMR] or CCyR) occurs and every 3–6 months thereafter [2, 4].

Typically in patients with CML, molecular responses progressively increase throughout the duration of imatinib therapy (Fig. 1) [7, 8]. An MMR is defined as a BCR-ABL RNA level $\leq 0.1\%$ on the International Scale—a consensus standardized measurement scale intended to allow direct comparison of BCR-ABL RNA levels in any laboratory adopting its use. The International Scale was specifically designed so that, by definition, 100% is the median pretreatment baseline level of BCR-ABL RNA in early chronic-phase CML (as determined in the pivotal International Randomized Study of Interferon versus STI571 (IRIS) imatinib trial) [15–19], and a 1,000-fold (three-log) reduction from baseline is defined as 0.1% (MMR).

The achievement of an MMR (at 12 or 18 months post-imatinib initiation, or at any time after CCyR) predicts superior long-term clinical outcomes, namely, progression-free survival (PFS) and event-free survival (EFS) (Fig. 2) [5, 9–11]. The achievement of an MMR, reached by approximately 50% of imatinib-treated patients within 12 months, is also associated with a longer duration of CCyR [10, 12]. Because of its proven value as a good prognostic indicator, the achievement of an MMR has become both a consensus goal of CML therapy [2–4] and a proven surrogate response endpoint in CML clinical trials [20]. Conversely, the failure to achieve an MMR after 12 months of imatinib therapy is considered a “warning sign” that these CML patients may require more frequent monitoring [2, 3]. Similarly, the failure to achieve an MMR after 18 months of imatinib therapy has become a consensus criterion for defining a “suboptimal” response and for the consideration of a possible change in therapy [2, 3].

Beyond imatinib, molecular response is also correlated with long-term outcomes in patients treated with second-generation TKIs. For example, a post hoc analysis of phase II and phase III trials showed that attainment of an MMR is a significant, good prognostic indicator for patients treated with the second-generation TKI dasatinib after imatinib

Figure 1. Disease burden is reduced over time with imatinib therapy [8].

Abbreviations: FISH, fluorescence in situ hybridization; RQ-PCR, real-time quantitative polymerase chain reaction.

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Figure 2. Cumulative rate of progression-free survival for patients with a complete cytogenetic response (CCyR) who achieved a major molecular response (MMR) (≥ 3 -log decrease in BCR-ABL RNA from baseline; $n = 42$) compared with those who did not achieve an MMR (< 3 -log decrease in BCR-ABL; $n = 43$) [10].

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failure [21]. The degree of early molecular response at 3 months has also been shown to predict subsequent major cytogenetic response, MMR, and EFS at 24 months in patients treated with nilotinib after imatinib failure [22].

With long-term imatinib therapy, minimal residual disease levels often continue to decline, even after an MMR is achieved, such that some patients eventually achieve a complete molecular response (CMR), defined as “undetectable” BCR-ABL by RQ-PCR [7, 11]. In fact, attainment of

Figure 3. Frequency of *BCR-ABL* kinase domain mutations among patients with chronic myeloid leukemia treated with a tyrosine kinase inhibitor at the Oregon Health & Science University in Portland, Oregon. Of the 355 patients who were tested by DNA sequencing, 153 harbored at least one detectable mutation.

an MMR—a proven response threshold associated with a PFS advantage—also predicts a subsequent CMR [7]. Furthermore, patients who achieve a CMR have a longer PFS interval than patients who achieve an MMR but not a CMR [11], and relapse is rare once a CMR is achieved [7, 11]. The achievement of a CMR, therefore, defines an excellent prognosis and may be an optimal therapeutic goal. However, because PCR negativity is poorly standardized among laboratories and varies depending on the sensitivity of PCR assays, the definition of CMR continues to evolve, and confirming a consistent prognostic value for CMR has been problematic.

A loss of molecular response, as measured by rising *BCR-ABL* RNA levels, can also be a sensitive laboratory method for prognosticating early treatment failure. For ex-

ample, a rise in *BCR-ABL* RNA levels in patients with a CCyR conveys a significantly greater risk of subsequent disease progression [11], although most such patients remain in stable CCyR [11, 23]. Even if an MMR is achieved but is then lost (as evidenced by rising *BCR-ABL* RNA levels), the risk of subsequent disease progression also significantly increases, although again, most such patients do not progress [11]. Consensus recommendations for imatinib-treated patients with rising *BCR-ABL* RNA levels therefore suggest more frequent laboratory monitoring and/or the consideration of a change in therapy [2–4]. Furthermore, once rising *BCR-ABL* RNA levels are documented, screening for *ABL* kinase domain mutations (which cause drug resistance) may also be considered [4]. Because the spectrum of acquired mutations is diverse (Fig. 3), and the

Table 1. Sensitivities, specificities, negative predictive values, and odds ratios for predicting a concomitant kinase domain mutation at various threshold increases in the level of BCR-ABL RNA [14]

RQ-PCR increase (fold change)	Sensitivity (95% CI) (%)	Specificity (%)	Negative predictive value (95% CI) (%)	Odds ratio (95% CI)	p-value
2.0	77 (62–88)	44	88 (80–94)	2.6 (1.2–5.4)	.01
2.5	77 (62–88)	46	89 (81–94)	2.8 (1.4–5.9)	.005
2.6	77 (62–88)	47	89 (81–94)	2.9 (1.4–6.0)	.005
2.6 (include subsequent sample) ^a	94 (82–99)	47	97 (91–99)	13 (3.9–43)	<.0001
3.0	74 (60–86)	48	88 (80–94)	2.7 (1.3–5.5)	.008
3.5	64 (49–77)	54	86 (78–91)	2.1 (1.1–4.1)	.03
4.0	60 (44–74)	60	85 (78–91)	2.2 (1.1–4.2)	.02
4.5	55 (40–70)	64	85 (78–90)	2.2 (1.2–4.2)	.02
5.0	47 (32–62)	68	84 (77–89)	1.9 (1.0–3.6)	.06
10	26 (14–40)	83	82 (75–87)	1.7 (0.8–3.7)	.2

^aExcept for this row, the table includes only those patients with successful RQ-PCR and sequencing performed on the same sample. If samples after the initial genotype determination are included, at the optimal 2.6-fold cutoff, the sensitivity, negative predictive value, and odds ratio each increase.

Abbreviations: CI, confidence interval; RQ-PCR, real-time quantitative polymerase chain reaction.

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functional drug resistance consequences of each mutation are unique, most laboratories use an unbiased direct DNA sequencing approach to screen for the presence of kinase domain mutations [24].

In vitro half-maximal inhibitory concentration (IC_{50}) drug resistance data alone are not sufficient to determine the clinical consequences of particular *BCR-ABL* mutations on patient responses to second-generation TKIs. The T315I mutation confers cross-resistance to imatinib, nilotinib, and dasatinib in vitro and in vivo [25–27], and patients with this mutation should be considered for a clinical trial or for stem cell transplant. Clinical data with other mutations are limited. In the phase II registration trial for nilotinib in imatinib-resistant CML patients, those with E255K/V-, Y253H-, or F359C/V-mutated *BCR-ABL* demonstrated lower and less durable responses and a shorter time to disease progression than patients with other more sensitive mutations ($IC_{50} \leq 150$ nm) or without mutations [26]. Patients with all other *BCR-ABL* mutations (including those with unknown in vitro sensitivity to nilotinib) had outcomes comparable with those of patients with no mutations [26]. Similarly, with dasatinib treatment of imatinib-resistant CML patients, those with Q252H, V299L, or F317L mutations had lower response rates than patients harboring mutations more sensitive to dasatinib ($IC_{50} \leq 3$ nm) and those with no mutations [27]. Thus, for patients with one of these few specific mutations, the choice of second-line therapy is straightforward. For the majority of patients, other factors,

such as patient history and concomitant conditions, must be considered when choosing between nilotinib and dasatinib after imatinib failure.

Currently, the quantitative definition of a significant “rise” in BCR-ABL RNA (to warrant mutation analysis) is controversial and varies among laboratories. To directly address this issue, a 2.6-fold increase in transcript levels was recently shown to be the optimal cutoff for detecting concomitant *BCR-ABL* kinase domain mutations (Table 1) [14]. In that study, increases in BCR-ABL RNA levels of greater than (but not less than) five-fold had poor diagnostic sensitivity because of the many false-negative cases in patients with transcript rises below five-fold who also had mutations. Therefore, the 5- to 10-fold transcript level “rise” definitions provisionally proposed by consensus groups [4, 15] may be too stringent. Although the detection of a kinase domain mutation (compared with a wild-type genotype) has been shown to convey a significantly shorter PFS time in imatinib-treated patients (Fig. 4) [14, 28], the early detection of mutations or loss of response by more stringent molecular monitoring has never been shown to reverse this trend. In fact, given the effectiveness of second-line therapies such as nilotinib and dasatinib, a less stringent approach to mutation monitoring may be sufficient in most patients.

In summary, RQ-PCR–based testing of BCR-ABL transcript levels is the most sensitive method both for assessing minimal residual disease in patients with CML and

Figure 4. Progression-free survival among patients ($n = 41$) treated with imatinib according to the presence or absence of a kinase domain mutation [14].

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for predicting future outcomes. RQ-PCR testing defines “molecular response” and can be done with a simple blood draw, making it safer and more convenient than bone marrow testing. The depth of a patient’s molecular response predicts their long-term outcome and the durability of their CCyR. Once an MMR is achieved, relapse is uncommon and is rarer still in patients who achieve a CMR. Consensus recommendations support an MMR as an important surrogate endpoint for patients with CML being treated with TKIs. These guidelines define failure to achieve MMR by

12 months of imatinib therapy as a “warning sign” for patients who may require more frequent monitoring, and failure to achieve an MMR by 18 months of therapy as a “suboptimal” response for which alternative therapies may be considered. Just as molecularly targeted therapy for CML has become the paradigm for future “personalized” cancer treatments, the regular laboratory monitoring of BCR-ABL RNA with RQ-PCR will likely become the model for successful molecular diagnostic monitoring of cancer patients to both assess and prognosticate the efficacy of these targeted treatments.

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