Initial Molecular Response at 3 Months May Predict Both Response and Event-Free Survival at 24 Months in Imatinib-Resistant or -Intolerant Patients With Philadelphia Chromosome–Positive Chronic Myeloid Leukemia in Chronic Phase Treated With Nilotinib

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ABSTRACT

Purpose

The association between initial molecular response and longer-term outcomes with nilotinib was examined.

Patients and Methods

Patients with imatinib-resistant or -intolerant chronic myeloid leukemia in chronic phase from the phase II nilotinib registration study with available postbaseline *BCR-ABL1* transcript assessments were included (N = 237).

Results

BCR-ABL1 transcript levels (International Scale [IS]) at 3 months correlated with complete cytogenetic response (CCyR) by 24 months. Patients with *BCR-ABL1* (IS) of $> 1\%$ to $\leq 10\%$ at 3 months with nilotinib had higher cumulative incidence of CCyR by 24 months than patients with *BCR-ABL1* (IS) of $> 10\%$ (53% v 16%). *BCR-ABL1* (IS) at 3 months predicted major molecular response (MMR) by 24 months. Cumulative incidence of MMR by 24 months for patients with *BCR-ABL1* (IS) of $> 0.1\%$ to $\leq 1\%$, $> 1\%$ to $\leq 10\%$, and $> 10\%$ was 65%, 27%, and 9%, respectively. These differences were observed for patients with or without baseline *BCR-ABL1* mutations and for those with imatinib resistance or intolerance. Estimated event-free survival (EFS) rates at 24 months decreased with higher transcript levels at 3 months; patients with *BCR-ABL1* (IS) of $\leq 1\%$ had an estimated 24-month EFS rate of 82%, compared with 70% for patients with *BCR-ABL1* (IS) of $> 1\%$ to $\leq 10\%$ and 48% for patients with *BCR-ABL1* (IS) of $> 10\%$.

Conclusion

Patients with *BCR-ABL1* (IS) of $> 10\%$ at 3 months had a lower cumulative incidence of CCyR and MMR and lower rates of EFS versus patients with *BCR-ABL1* (IS) of $\leq 10\%$. Prospective studies may determine whether close monitoring or alternative therapies are warranted for patients with minimal initial molecular response.

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INTRODUCTION

Resistance to the *BCR-ABL1* tyrosine kinase inhibitor imatinib can develop through several mechanisms, but the most frequently identified acquired resistance results from point mutations in the *BCR-ABL1* kinase domain. Nilotinib (Tasigna; Novartis, Basel, Switzerland) is an inhibitor of *BCR-ABL1* kinase that was rationally designed to have greater specificity and potency than imatinib.¹⁻³ Nilotinib is 30-fold more potent at

inhibiting *BCR-ABL1* in vitro than imatinib and is a highly selective *BCR-ABL1* kinase inhibitor.^{1,2} Results from a single-arm, open-label phase II registration study demonstrated the efficacy and safety of nilotinib in patients with imatinib-resistant or -intolerant Philadelphia chromosome–positive chronic myeloid leukemia in chronic phase (Ph+ CML-CP)^{4,5} or Ph+ CML in accelerated phase (AP).⁶ Nilotinib was recently approved in more than 40 countries, including the United States, the European Union, Switzerland, and Japan, for treatment

Fig 1. CONSORT diagram of patients included in the analysis. CCyR, complete cytogenetic response; EFS, event-free survival; MCyR, major cytogenetic response; MMR, major molecular response; PCR, polymerase chain reaction.

of patients with newly diagnosed Ph+ CML-CP based on results from the phase III randomized ENESTnd (Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients) trial, which showed the good tolerability and superior efficacy of nilotinib over imatinib.⁷

While undergoing treatment, patients with Ph+ CML-CP must be continually monitored to assess possible disease progression. Molecular monitoring provides important prognostic information. Achievement of a major molecular response (MMR; $\leq 0.1\%$ according to the International Scale [IS]) within 18 months of imatinib treatment has been associated with delayed progression to AP/blast crisis (BC)^{8–10} and is considered an optimal response.¹¹

The 3-month *BCR-ABL1* level has been shown to be predictive of MMR in imatinib-treated patients, and most newly diagnosed patients who achieve *BCR-ABL1* (IS) of $\leq 1.0\%$ with imatinib subsequently achieve MMR.¹² The value of molecular monitoring in the setting of nilotinib therapy after imatinib failure has not been defined. We conducted a post hoc analysis of the nilotinib phase II registration trial to explore the association between initial molecular response to nilotinib after imatinib failure and achievement of complete cytogenetic response (CCyR), MMR, and event-free survival (EFS) during therapy.

PATIENTS AND METHODS

Patient Population

This post hoc analysis included adults (age ≥ 18 years) with imatinib-resistant or -intolerant Ph+ CML-CP enrolled onto a phase II open-label, single-treatment arm study with nilotinib who had any available postbaseline *BCR-ABL1* transcript assessment, measured by real-time quantitative polymerase chain reaction (PCR), after initiation of nilotinib therapy (N = 294). Fifty-seven of the 294 patients enrolled had a missing PCR assessment at month 3 (no PCR assessment between study days 56 and 112) and were excluded from all landmark analyses performed on *BCR-ABL1* transcript

levels at month 3. Among these patients, 19 discontinued treatment before the 3-month PCR assessment (14 because of adverse events; three, disease progression; one, protocol violation; and one, consent withdrawal), seven had a PCR assessment before month 3 but no assessment afterward, and the remaining 31 did not have the 3-month PCR assessment but had assessments after 3 months. Hence, the patient population for the 3-month PCR landmark analyses consisted of 237 patients (Fig 1). Patient eligibility and trial design were previously described.^{4,5} This trial was registered at <http://www.clinicaltrials.gov> as NCT00471497.

The current landmark analyses of response based on *BCR-ABL1* transcript levels at month 3 also excluded patients who had already attained that response (Fig 1). Analysis of major CyR (MCyR) excluded 106 patients (45%; 106 of 237) who attained MCyR at month 3, analysis of CCyR excluded 77 patients (32%; 77 of 237) who attained CCyR at month 3 or had *BCR-ABL1* (IS) of $\leq 1\%$ at month 3, and analysis of MMR excluded 28 patients (12%; 28 of 237) who achieved MMR at month 3. A *BCR-ABL1* value of $\leq 1\%$ correlates closely with the achievement of CCyR and was considered an appropriate surrogate in patients with missing cytogenetic data at 3 months.^{13,14} Additionally, analysis of EFS excluded 22 patients (9%; 22 of 237) with an event within the first 3 months.

Study Design

The overall design of the nilotinib registration study was described previously.^{4,5} After our exclusion criteria were applied, patients were grouped according to their level of *BCR-ABL1* transcripts. *BCR-ABL1* transcript levels at 3 months were used to perform landmark analyses to assess the association between initial molecular response and achievement of MCyR, CCyR, and MMR by 24 months as well as EFS. The categories for *BCR-ABL1* transcript levels used for landmark analysis were: one, $> 0.1\%$ to $\leq 1\%$; two, $> 1\%$ to $\leq 10\%$; and three, $> 10\%$. These categories are consistent with previous landmark analyses and were chosen because a *BCR-ABL1* level of $\geq 10\%$ at 3 months has been shown to be associated with poorer outcomes with imatinib.^{15,16} A *BCR-ABL1* level of 1% is considered equivalent to CCyR,^{13,14} and the definition of MMR is *BCR-ABL1* of $\leq 0.1\%$ according to the IS.^{17–19} Thus, CCyR by 24 months was evaluated only in categories two and three because patients with *BCR-ABL1* of $\leq 1\%$ had already achieved CCyR.

Events were defined as loss of complete hematologic response or MCyR, progression to AP/BC, discontinuation because of progression to AP/BC, and death resulting from any cause during nilotinib therapy. *BCR-ABL1* transcript

levels at 1 month were also used to perform a landmark analysis to assess the association between initial molecular response and achievement of MCyR by 12 months. The 1-month landmark was chosen for this analysis because a large proportion of patients had achieved MCyR at month 3 of therapy and were excluded from the 3-month landmark analysis. Thirteen of 250 patients without MMR at month 1 achieved MCyR at month 1 and were excluded from the 1-month landmark analyses. In addition, patient response was stratified by *BCR-ABL1* mutation status at baseline and by response to imatinib (resistant v intolerant).

Assessment of *BCR-ABL1* Mutation Status

Peripheral blood samples were collected from patients at baseline before the start of treatment; at months 1, 2, and 3; and then every 3 months after nilotinib therapy was initiated. Patients with a baseline mutation were assessed every 3 months. For patients who did not have a baseline mutation, mutation assays were performed only when there was a significant ($>$ two-fold to five-fold) rise in *BCR-ABL1* transcript levels, based on the variability of the assay in each of the participating laboratories. RNA was extracted from total blood leukocytes and reverse transcribed. The *BCR-ABL1* kinase domain (amino acids 230 to 490) was amplified by nested PCR using primers located in the *BCR* and *ABL* regions of the *BCR-ABL1* gene. Mutations within this region were identified by direct sequencing, which allows for the reliable detection of mutant clones when they represent at least 20% of the total leukemic clone.²⁰

Statistical Analysis

BCR-ABL1 transcript levels at 3 months were used to perform landmark analyses to assess the association between initial molecular response and cumulative incidence of MCyR, CCyR, and MMR by 24 months, as well as the association between initial molecular response and EFS. The cumulative incidence of response was displayed by an increasing step function. This curve increased each time a new responder was observed up to the best observed response rate by a certain time point. Event-free survival was estimated based on Kaplan-Meier analysis. For patients not experiencing an event, EFS was censored at the data analysis cutoff date for patients still receiving treatment or at the treatment discontinuation date.

The Cox proportional hazards model was used to provide the hazard ratio (HR) for the likelihood of response between groups of patients with different *BCR-ABL1* transcript levels at 3 months, and the log-rank test was used for statistical comparisons. A test of the proportionality assumption was used by fitting a Cox model with *BCR-ABL1* group indicator, and an artificial time-dependent covariate was created from the *BCR-ABL1* indicator $\times$ time (t). Thus, the Wald test of H_0 : no effect on the time-dependent covariate was used to test the proportional hazards assumption. The log of negative log of estimated survivor function versus log of time was also used as a graphical method for checking the proportionality assumption. A similar approach was used in the 1-month landmark analysis.

RESULTS

Overall Efficacy

Patients were recruited to the study between April 21, 2005, and April 26, 2006. Data cutoff for this analysis was April 20, 2008. The overall population of the registration study had a median follow-up of 27.3 months (range, 0.7 to 36.0 months) at data cutoff. Nilotinib remained an effective and well-tolerated therapy for patients with imatinib-resistant or -intolerant Ph+ CML-CP at 24 months, with MCyR and CCyR rates of 59% and 44%, respectively; the 24-month Kaplan-Meier estimate of overall survival (OS) was 87%.⁴ Recently presented results demonstrated that the estimated OS rate was 78% at 48 months.²¹

Correlation Between *BCR-ABL1* Transcript Levels and CyR

BCR-ABL1 levels at baseline and 3 months are shown in Appendix Table A1 (online only). There was a significant correlation

between *BCR-ABL1* transcript levels at 3 months and achievement of CCyR (Fig 2A). Patients with *BCR-ABL1* (IS) of $> 1\%$ to $\leq 10\%$ at 3 months had a higher cumulative incidence rate of CCyR by 24 months than patients with *BCR-ABL1* (IS) of $> 10\%$ (Table 1; $P < .001$). On the basis of an analysis of time to CCyR, the likelihood of achieving CCyR at any time after 3 months was about 5 $\times$ higher for patients with *BCR-ABL1* (IS) of $> 1\%$ to $\leq 10\%$ at 3 months than for patients with *BCR-ABL1* (IS) of $> 10\%$ (HR, 4.9; 95% CI, 2.7 to 9.0; log-rank $P < .001$). Differences in the likelihood of achieving CCyR according to *BCR-ABL1* levels at 3 months

Fig 2. Cumulative incidence of complete cytogenetic response (CCyR) by *BCR-ABL1* ratio at 3 months in (A) all patients, (B) imatinib-resistant patients, and (C) patients with a baseline mutation.

Table 1. Cumulative Incidence of CCyR and MMR by 24 Months Based on Molecular Response at 3 Months

Response	No. of Patients	BCR-ABL1 at 3 Months (%)*			HR†	95% CI	P‡
		> 0.1% to ≤ 1%	> 1% to ≤ 10%	> 10%			
Patients achieving CCyR by 24 months§							
All	160		53	16			
> 10% v > 1% to ≤ 10%					4.9	2.7 to 9.0	< .001
With baseline mutation	72		53	11			
> 10% v > 1% to ≤ 10%					8.2	2.9 to 23	< .001
Without baseline mutation	75		50	19			
> 10% v > 1% to ≤ 10%					3.5	1.5 to 8.1	.002
Resistant	122		53	17			
> 10% v > 1% to ≤ 10%					4.7	2.4 to 9.3	< .001
Intolerant	38		50	11			
> 10% v > 1% to ≤ 10%					6.1	1.5 to 26	.006
Patients achieving MMR by 24 months							
All	209	65	27	9			
> 1% to ≤ 10% v > 0.1% to ≤ 1%					2.9	1.5 to 5.6	.001
> 10% v > 1% to ≤ 10%					3.8	1.8 to 8.2	< .001
With baseline mutations	83	86	32	9			
> 1% to ≤ 10% v > 0.1% to ≤ 1%					3.8	1.2 to 12.1	.018
> 10% v > 1% to ≤ 10%					4.5	1.4 to 14.8	.007
Without baseline mutations	108	67	24	10			
> 1% to ≤ 10% v > 0.1% to ≤ 1%					3.5	1.5 to 8.5	.003
> 10% v > 1% to ≤ 10%					2.9	1.0 to 8.5	.036
Resistant	150	63	28	9			
> 1% to ≤ 10% v > 0.1% to ≤ 1%					2.9	1.2 to 7.0	.011
> 10% v > 1% to ≤ 10%					3.8	1.5 to 9.5	.002
Intolerant	59	67	27	10			
> 1% to ≤ 10% v > 0.1% to ≤ 1%					2.7	0.9 to 8.0	.064
> 10% v > 1% to ≤ 10%					3.7	0.9 to 15.6	.053

Abbreviations: CCyR, complete cytogenetic response; HR, hazard ratio; IS, International Scale; MMR, major molecular response.

*According to IS.

†Cox proportional hazard model.

‡Log-rank test.

§Excludes 32% of patients (77 of 237) who achieved CCyR at 3 months or had *BCR-ABL1* (IS) of ≤ 1% at 3 months.

||Excludes 12% of patients (28 of 237) who achieved MMR at 3 months.

remained statistically significant when patients were stratified by previous imatinib treatment outcome (ie, resistance or intolerance; Table 1; Fig 2B) or presence of baseline mutation (ie, present or absent; Table 1; Fig 2C).

Similarly, there was a significant correlation between *BCR-ABL1* transcript levels at 3 months and achievement of MCyR. However, a large proportion of patients (45%; 106 of 237) had achieved MCyR at month 3 and were excluded from this analysis. As a result, landmark analysis of MCyR was performed based on *BCR-ABL1* transcript levels at month 1 of nilotinib therapy. Patients with *BCR-ABL1* (IS) of ≤ 10% had a higher cumulative incidence rate of MCyR by 12 months compared with patients with *BCR-ABL1* (IS) of > 10% (78% v 53%; Fig 3). On the basis of an analysis of time to MCyR, the likelihood of achieving MCyR at any time after 1 month was 2.4× higher for patients with *BCR-ABL1* (IS) of ≤ 10% at 1 month than patients with *BCR-ABL1* (IS) of > 10% (HR, 2.4; 95% CI, 1.6 to 3.6; log-rank $P < .001$).

Correlation Between 3-Month *BCR-ABL1* Transcript Levels and MMR

BCR-ABL1 transcript levels at 3 months were highly predictive of MMR by 24 months (Fig 4A; Table 1). On the basis of an analysis of time to MMR, the likelihood of achieving MMR at any time after 3 months was about 4× higher for patients with *BCR-ABL1* (IS) of > 0.1% to ≤ 1% at 3 months than patients with *BCR-ABL1* (IS) of

> 1% to ≤ 10% (HR, 3.8; log-rank $P < .001$) and approximately 3× higher for patients with *BCR-ABL1* (IS) of > 1% to ≤ 10% at 3 months than patients with *BCR-ABL1* (IS) of > 10% (HR, 2.9; log-rank $P = .001$). Differences in the likelihood of achieving MMR according to *BCR-ABL1* levels at 3 months remained statistically significant when patients were stratified by resistance to previous imatinib treatment (Table 1; Fig 4B) or baseline mutation (ie, present or absent; Table 1; Fig 4C).

Correlation Between 3-Month *BCR-ABL1* Transcript Levels and EFS Rates

The probability of EFS was also correlated with *BCR-ABL1* transcript levels at 3 months. Estimated EFS rates at 24 months decreased with higher transcript levels at 3 months (Table 2). A statistically significant difference between *BCR-ABL1* transcript levels and the estimated probability of EFS was observed for four subsets: one, patients with *BCR-ABL1* (IS) of > 1% to ≤ 10% versus *BCR-ABL1* (IS) of > 10% (log-rank $P = .030$); two, patients with a baseline mutation with *BCR-ABL1* (IS) of ≤ 1% versus *BCR-ABL1* (IS) of > 1% to ≤ 10% (log-rank $P = .011$); three, patients without a baseline mutation with *BCR-ABL1* (IS) of > 1% to ≤ 10% versus *BCR-ABL1* (IS) of > 10% (log-rank $P = .052$; borderline significance); and four, patients with imatinib intolerance with *BCR-ABL1* (IS) of > 1% to ≤ 10% versus *BCR-ABL1* (IS) of > 10% (log-rank $P = .015$).

Fig 3. Cumulative incidence of major cytogenetic response (MCyR) by *BCR-ABL1* ratio at 1 month (all patients).

DISCUSSION

In imatinib-resistant and -intolerant patients with CML-CP treated with nilotinib, *BCR-ABL1* transcript levels at 3 months correlated significantly with CCyR and MMR by 24 months, as well as with EFS. Generally, patients with *BCR-ABL1* transcript levels $< 10\%$ at 3 months achieved higher rates of MMR, CCyR, and MCyR by 24 months. The poorer responses for patients with *BCR-ABL1* (IS) of $> 10\%$ was independent of *BCR-ABL1* mutation status and of whether patients commenced nilotinib because of imatinib intolerance or resistance. This suggests that initial molecular response is a compelling predictor of later response. Furthermore, with an EFS rate of approximately 50% at 24 months, *BCR-ABL1* (IS) of $> 10\%$ at 3 months may be an indicator for consideration of a search for an appropriate donor for allogeneic stem-cell transplantation.

Because a considerable number of the best-responding patients (ie, those who achieved responses within the first 3 months of nilotinib therapy) were excluded from these landmark analyses, the effect of early molecular responses on longer-term outcomes, especially MCyR, may be underestimated at the 3-month landmark. To address this, we performed a landmark analysis according to *BCR-ABL1* transcript levels at 1 month. The patients who failed to achieve a *BCR-ABL1* value of at least 10% by 1 month had a significantly lower probability of achieving MCyR by 12 months. This is an important finding because it was demonstrated in an analysis of 113 patients receiving nilotinib (n = 43) or dasatinib (n = 70) after imatinib failure that patients achieving MCyR by 12 months had a significant survival advantage.²²

The finding that early reduction of *BCR-ABL1* to $< 1\%$ correlated with early achievement of MMR during nilotinib therapy is consistent with previous reports examining the prognostic significance of early molecular response to imatinib therapy. Early molecular response to imatinib therapy is strongly associated with a higher probability of achieving MMR.²³ Furthermore, the degree of molecular response at early time points was associated with a high MMR rate and low rate of disease-related events. In a study conducted at the MD Anderson Cancer Center, for example, patients who achieved a *BCR-ABL1* ratio of $\leq 1\%$ by 3 months had higher MMR rates and lower event rates than those who did not achieve this milestone.²⁴ Other analyses of large randomized trials

Fig 4. Cumulative incidence of major molecular response (MMR) by *BCR-ABL1* ratio at 3 months in (A) all patients, (B) imatinib-resistant patients, and (C) patients with a baseline mutation.

have confirmed the relationship between the degree of molecular response at early time points and progression to both AP/BC and EFS.^{13,25} Importantly, early achievement of MMR with imatinib therapy was associated with more durable responses, which in turn were associated with prolonged progression-free survival.²⁶⁻³⁰

In this post hoc analysis of the nilotinib registration study, we demonstrated that patients who achieve early molecular responses during nilotinib therapy may have an increased probability of improved long-term outcomes. The benefit of early molecular response has also been observed in results from the phase III ENESTnd trial. In this study, nilotinib therapy provided earlier and deeper molecular responses than did imatinib treatment, resulting in lower rates of

Table 2. EFS at 24 Months Based on Molecular Response at 3 Months

Estimated EFS Rate for Patients at 24 Months*	No. of Patients	BCR-ABL1 at 3 Months (%)†			P‡
		≤ 1%	> 1% to ≤ 10%	> 10%	
All	215	82	70	48	
≤ 1% v > 1% to ≤ 10%					.075
> 1% to ≤ 10% v > 10%					.030
With baseline mutations	74	100	54	36	
≤ 1% v > 1% to ≤ 10%					.011
> 1% to ≤ 10% v > 10%					.58
Without baseline mutations	123	81	82	59	
≤ 1% v > 1% to ≤ 10%					.91
> 1% to ≤ 10% v > 10%					.052
Resistant	152	75	64	47	
≤ 1% v > 1% to ≤ 10%					.14
> 1% to ≤ 10% v > 10%					.19
Intolerant	63	89	91	54	
≤ 1% v > 1% to ≤ 10%					.71
> 1% to ≤ 10% v > 10%					.015

Abbreviations: EFS, event-free survival; IS, International Scale.

*Excludes 9% (22 of 237) of patients who had an event within the first 3 months.

†According to IS.

‡Log-rank test.

progression to AP/BC by 12 months. Longer follow-up at 24³¹ and 36³² months confirmed the benefits of early molecular response, including a reduced incidence of mutations. The study is ongoing.

It should be noted that some baseline factors may influence the *BCR-ABL1* value at 3 months. For example, patients with mutations less sensitive to nilotinib (T315I, Y253H, E255V/K, and F359V/C) at baseline have lower response rates and higher progression rates compared with patients without a baseline mutation or patients with other baseline mutations, and clinicians are advised against selecting nilotinib therapy for these patients.³³ In addition, patients who entered the nilotinib registration study because of imatinib intolerance generally had better outcomes than those who entered because of imatinib resistance.^{4,5} It was for these reasons that we chose to independently analyze the effect of *BCR-ABL1* (IS) levels at 3 months on longer-term outcomes in four patient subgroups (with baseline mutation, without baseline mutation, resistant, and intolerant). In all of these groups, our study demonstrated that *BCR-ABL1* values of > 10% (IS) at 3 months identified those with subsequent poor response.

Ultimately, the exact clinical importance of these findings in patients with imatinib resistance and intolerance needs confirmation. This study did not evaluate the impact of changing therapy at 3 months, and this question can only be properly addressed in a prospective clinical trial. Longer follow-up is required to assess the association between 3-month *BCR-ABL1* transcript levels and outcomes such as progression-free survival and OS. Nevertheless, this study indicates that molecular analysis is an important assessment for patients treated with kinase inhibitor therapy. Molecular analysis provides significant prognostic information when measured within the first few months of initiation of nilotinib therapy after imatinib failure, which is independent of *BCR-ABL1* mutation status at commencement of nilotinib. Patients who do not have a reduction of *BCR-ABL1* (IS) to ≤ 10% at 3 months during nilotinib therapy, after imatinib failure, may benefit from close

monitoring or alternative therapy, which should be prospectively investigated.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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