Impact of Baseline *BCR-ABL* Mutations on Response to Nilotinib in Patients With Chronic Myeloid Leukemia in Chronic Phase

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ABSTRACT

Purpose

Nilotinib is a second-generation tyrosine kinase inhibitor indicated for the treatment of patients with chronic myeloid leukemia (CML) in chronic phase (CP; CML-CP) and accelerated phase (AP; CML-AP) who are resistant to or intolerant of prior imatinib therapy. In this subanalysis of a phase II study of nilotinib in patients with imatinib-resistant or imatinib-intolerant CML-CP, the occurrence and impact of baseline and newly detectable *BCR-ABL* mutations were assessed.

Patients and Methods

Baseline mutation data were assessed in 281 (88%) of 321 patients with CML-CP in the phase II nilotinib registration trial.

Results

Among imatinib-resistant patients, the frequency of mutations at baseline was 55%. After 12 months of therapy, major cytogenetic response (MCyR) was achieved in 60%, complete cytogenetic response (CCyR) in 40%, and major molecular response (MMR) in 29% of patients without baseline mutations versus 49% ($P = .145$), 32% ($P = .285$), and 22% ($P = .366$), respectively, of patients with mutations. Responses in patients who harbored mutations with high in vitro sensitivity to nilotinib (50% inhibitory concentration [IC_{50}] ≤ 150 nM) or mutations with unknown nilotinib sensitivity were equivalent to those responses for patients without mutations (not significant). Patients with mutations that were less sensitive to nilotinib in vitro ($IC_{50} > 150$ nM; Y253H, E255V/K, F359V/C) had less favorable responses, as 13%, 43%, and 9% of patients with each of these mutations, respectively, achieved MCyR; none achieved CCyR.

Conclusion

For most patients with imatinib resistance and with mutations, nilotinib offers a substantial probability of response. However, mutational status at baseline may influence response. Less sensitive mutations that occurred at three residues defined in this study, as well as the T315I mutation, may be associated with less favorable responses to nilotinib.

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INTRODUCTION

Imatinib (Glivec®, Gleevec®; Novartis Pharmaceuticals, East Hanover, NJ), a *BCR-ABL* kinase inhibitor, has revolutionized the treatment of chronic myeloid leukemia (CML) and is the current standard of care in the treatment of patients with newly diagnosed CML. Although the introduction of imatinib for the treatment of Philadelphia-positive (Ph+) CML has been an unprecedented advance in the field of oncology, the 5-year follow-up data from the International Randomized Study of Interferon and STI571 (IRIS) trial show that approximately 15% to 17% of patients become resistant to imatinib

at some point during the course of treatment.¹ Imatinib resistance rates are higher in more advanced phases of CML than in the chronic phase (CP; CML-CP).^{2,3} Although the mechanism of clinical resistance to imatinib in CML varies widely, the most common cause is *BCR-ABL* kinase domain point mutations. The binding of imatinib to these *BCR-ABL* mutants often is impaired, which leads to inadequate response or loss of response.

To date, nearly 100 mutations have been discovered within the *BCR-ABL* gene that were detected at the time of disease progression or resistance to imatinib.⁴⁻⁸ Although clinical resistance to imatinib can be attributed in most instances to the presence of

mutations (ie, continued proliferation by the mutant clone to become the dominant clone among the Ph⁺ cells), the detection of mutations using high-sensitivity methods alone does not necessarily predict future resistance to imatinib.^{9,10}

Nilotinib (Tasigna[®]; Novartis Pharmaceuticals) is a second-generation tyrosine kinase inhibitor (TKI) designed with enhanced selectivity and potency for *BCR-ABL* compared with imatinib. In vitro studies demonstrate that nilotinib is 20- to 50-fold more potent than imatinib.^{11,12} Nilotinib exhibits in vitro inhibitory activity against the majority of mutant *BCR-ABL* kinases that may be present after imatinib resistance, with the exception of the T315I mutation.¹¹⁻¹³ The approval of nilotinib in imatinib-resistant and imatinib-intolerant patients with CML-CP and CML in accelerated phase (AP; CML-AP) was based on the results of a pivotal phase II registration trial.¹⁴⁻¹⁶ In this trial, 280 patients with CML-CP who were resistant to or intolerant of imatinib were treated with nilotinib 400 mg twice daily. After 6 months of therapy, 48% of patients achieved a major cytogenetic response (MCyR), 66% of which were complete cytogenetic response (CCyRs).¹⁴ The estimated overall 1-year survival rate for these patients was 95%.¹⁴

Although preclinical data demonstrates that nilotinib can overcome imatinib resistance, the impact of *BCR-ABL* kinase domain mutations (occurring before and during nilotinib therapy) on the clinical efficacy of nilotinib has not been assessed. Indeed, across several studies, the rates of patients harboring *BCR-ABL* mutations detected after imatinib failure have varied from 19% to 90%, depending on the methodology applied.^{3,6,8,17-22}

The objectives of this analysis were to investigate the prevalence, pattern, and evolution of *BCR-ABL* kinase domain mutations in peripheral-blood samples before and during nilotinib therapy in patients with CML-CP who were resistant to prior imatinib therapy. Patients with T315I mutations at baseline (five [3%] of 192) were excluded from clinical response analyses. The impact of baseline *BCR-ABL* mutations on clinical efficacy was also determined by examining multiple clinical end points, including complete hematologic response (CHR), MCyR, CCyR, major molecular response (MMR), and progression. During nilotinib treatment, the occurrence of newly detectable *BCR-ABL* mutations was assessed, and the impact of mutations on treatment outcome was determined after 12 months of nilotinib therapy.

An initial analysis of nilotinib in patients with *BCR-ABL* mutations was published by Kantarjian et al¹⁴ and showed the previous rates of hematologic and cytogenetic response to nilotinib by specific mutation. This analysis complements the initial publication by providing longer follow-up, nearly 100 more patients with mutation data available (183 v 281 patients), molecular response data, data on newly detectable mutations, and data on mutations associated with progression.

PATIENTS AND METHODS

Patients

Peripheral-blood samples were collected from patients aged ≥ 18 years with imatinib-resistant or imatinib-intolerant, Ph⁺ CML-CP who were enrolled on the open-label, phase II registration trial.¹⁴ Adult patients with imatinib-resistant or imatinib-intolerant Ph⁺ CML-CP; adequate performance status (WHO performance score ≤ 2); normal hepatic, renal, and cardiac functions; and no known T315I mutation before the start of study were

eligible. Despite exclusion of patients with previously detected T315I mutations, five patients did exhibit the T315I mutation at baseline and continued on study. Nilotinib 400 mg was administered orally twice daily (ie, 800 mg/d). Dose escalation to 600 mg twice daily was allowed if a lack or loss of response was observed.

This study was conducted in accordance with the Declaration of Helsinki. The protocol was reviewed and approved by ethics committees or institutional review boards of all participating centers, and all patients gave written informed consent according to the individual institutional guidelines.

Assessment of *BCR-ABL* Mutation Status

Peripheral-blood samples were collected from the patients at baseline and once every 3 months after nilotinib therapy. RNA was extracted from total blood leukocytes and was reverse transcribed. cDNA was used as a template to amplify the *BCR-ABL* kinase domain (amino acids 230 to 490) by nested polymerase chain reaction (PCR) using primers located in the *BCR* and *ABL* regions of the *BCR-ABL* gene. Mutations within this region were identified by direct sequencing, which allows for the reliable detection of mutant alleles when they represent at least 20% of the total leukemic clone. Patients with baseline *BCR-ABL* mutations were observed every 3 months during nilotinib therapy. Patients without baseline mutations were analyzed for mutations when a significant increase in *BCR-ABL* transcript levels was observed, as defined by prior published literature.^{23,24} *BCR-ABL* mutations were classified according to their sensitivity to nilotinib after transfection of the mutants into Ba/F3 cells; cell viability was assessed by utilizing an in vitro cell proliferation assay, as published previously by Weisberg et al.¹² Although blood samples were collected at baseline for mutational analysis, no mutational screening was performed before enrollment. Mutational analysis also was performed on all patients who discontinued study and at the time of progression. Mutational analysis was performed by six regional laboratories: Hanson Institute, Adelaide, Australia; University of Turin, Torino, Italy; Institute of Hematology and Medical Oncology, Bologna, Italy; The Catholic University of Korea, Seoul, Korea; Universitätsmedizin Mannheim, University of Heidelberg, Germany; and Fred Hutchinson Cancer Research Center, Seattle, WA.

RESULTS

Frequency of Baseline Mutations in Imatinib-Resistant and Imatinib-Intolerant Patients

Baseline mutation data were available for 281 (88%) of 321 patients with CML-CP who were enrolled on and evaluated for efficacy in the phase II nilotinib registration study. Of the 281 patients, 192 (68%) were imatinib resistant, and 89 (32%) were imatinib intolerant (Table 1). One hundred fourteen (41%) of 281 patients had detectable

Table 1. Incidence of Baseline *BCR-ABL* Mutations

Patient Group	Patients Overall		Patients With Any Mutation	
	No.	%	No.	%
All	281		114	41
Imatinib intolerant	89	32	9	10
Imatinib resistant	192	68	105	55
Primary resistance*	46	24	21	46
Secondary resistance†	146	76	84	58

*Primary imatinib resistance was defined as no complete hematologic response at or after 3 months of therapy; as no minimal cytogenetic response at or after 6 months; or as no major cytogenetic response at or after 12 months.

†Secondary imatinib resistance was defined as loss of complete hematologic response, loss of minimal cytogenetic response, loss of major cytogenetic response, or development of clonal evolution.

Fig 1. Baseline mutations in patients with imatinib-resistant chronic myeloid leukemia in chronic phase (n = 192). *Mutations without 50% inhibitory concentration (IC₅₀) data available.

BCR-ABL kinase domain mutations. The frequency of *BCR-ABL* mutations at baseline was considerably higher in imatinib-resistant patients (105 [55%] of 192) than that observed in imatinib-intolerant patients (nine [10%] of 89). The majority of the imatinib-resistant patients had secondary resistance to imatinib (76%; Table 1). The proportion of patients with baseline mutations was comparable among patients with primary (46%) versus secondary (58%) imatinib resistance.

Thirty-five different baseline mutations affecting 27 amino acid residues within the *BCR-ABL* kinase domain were identified among the imatinib-resistant patients in this analysis. These mutations are grouped according to their in vitro sensitivity to nilotinib (Fig 1).¹² Of the 192 imatinib-resistant patients, 45% had no *BCR-ABL* mutations at baseline. Twenty-three percent of imatinib-resistant patients had mutations that were sensitive to nilotinib in the in vitro proliferation assay (50% inhibitory concentration [IC₅₀] ≤ 150 nM). These 12 mutations (M244V, L248V, G250E, Q252H, E275K, D276G, F317L, M351T, E355A/G, L387F, F486S) spread across the entire *BCR-ABL* kinase domain, including P-loop, A-loop, and other regions. An ad-

ditional 14% of imatinib-resistant patients had mutations that were less sensitive to nilotinib (IC₅₀ > 150 nM). These less sensitive mutations affect three amino acid residues (Y253H, E255K/V, and F359C/V). Another 15% of the imatinib-resistant patients had mutations with unknown in vitro sensitivity to nilotinib. Three percent of imatinib-resistant patients had the T315I mutation, which had been resistant to nilotinib in the in vitro assay, with an IC₅₀ value of greater than 10,000 nM.²⁰ The T315I mutation was identified less frequently in this study than in previous reports of imatinib-resistant patients, because patients with known T315I were not eligible for enrollment in this study.

Responses to Nilotinib Stratified by Baseline *BCR-ABL* Mutation Status

The overall response to nilotinib was assessed after 12 months of therapy. The response rates to nilotinib in patients with and without baseline mutations are listed in Table 2 and were determined by examining the following clinical end points: CHR, MCyR, CCyR, and MMR. CHR was achieved in 35 (80%) of 44 patients, MCyR in 52

Table 2. Best Responses Within 12 Months of Therapy by Baseline *BCR-ABL* Mutation Status

Mutation*	In Vitro IC ₅₀ to Nilotinib (nM)	Response											
		CHR			MCyR			CCyR			MMR		
		No.	Total No.	%	No.	Total No.	%	No.	Total No.	%	No.	Total No.	%
None		35†	44	80	52‡	87	60	35§	87	40	22	76	29
Any mutation		57	80	71	49	100	49	32	100	32	19	87	22
IC ₅₀ ≤ 150 nM		31	37	84	26	45	58	18	45	40	12	41	29
IC ₅₀ > 150 nM													
Y253H	700	0	6	0	1	8	13	0	8	0	0	7	0
E255K/E255V	548/791	5	7	71	3	7	43	0	7	0	1	7	14
F359C/F359V	258/161	3	10	30	1	11	9	0	11	0	0	10	0
Other¶		18	20	90	18	29	62	14	29	48	6	22	27

Abbreviations: IC₅₀, 50% inhibitory concentration; CHR, complete hematologic response; MCyR, major cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response.

*Patients with T315I mutation (3% of total population) were excluded; patients with multiple mutations were grouped in the known and higher IC₅₀ group.

†Comparison of CHR in patients with or without mutations ($P = .393$).

‡Comparison of MCyR in patients with or without mutations ($P = .145$).

§Comparison of CCyR in patients with or without mutations ($P = .285$).

||Comparison of MMR in patients with or without mutations ($P = .366$).

¶Mutations without in vitro IC₅₀ data.

(60%) of 87 patients, CCyR in 35 (40%) of 87 patients, and MMR in 22 (29%) of 76 patients without baseline mutations; in comparison, CHR was achieved in 57 (71%) of 80 patients, MCyR in 49 (49%) of 100 patients, CCyR in 32 (32%) of 100 patients, and MMR in 19 (22%) of 87 patients with non-T315I mutations. Although response rates were slightly lower in patients with baseline mutations than in those without mutations, the clinical efficacy of nilotinib was demonstrated in most imatinib-resistant patients with mutations, with the exception of patients who harbored Y253H, E255K/V, and F359C/V (as well as T315I) baseline *BCR-ABL* mutations.

Among patients with mutations, those who harbored mutations with high (ie, $IC_{50} \leq 150$ nM) or unknown in vitro sensitivity to nilotinib achieved similar rates of responses compared with those patients without baseline mutations (Table 2). The rates of CHR, MCyR, CCyR, and MMR achieved were 84% (31 of 37 patients), 58% (26 of 45 patients), 40% (18 of 45 patients), and 29% (12 of 41 patients) in patients with mutations sensitive to nilotinib in vitro (ie, $IC_{50} \leq 150$ nM); these rates were 90% (18 of 20 patients), 62% (18 of 29 patients), 48% (14 of 29 patients), and 27% (six of 22 patients), respectively, in patients who harbored mutations with unknown in vitro sensitivity to nilotinib.

However, response rates in patients with mutations less sensitive to nilotinib in vitro (ie, $IC_{50} > 150$ nM) were less favorable. Of 26 patients with either the Y253H, E255K/V, or F359C/V mutations at baseline, only eight (35%) of 23 patients without CHR at baseline achieved CHR, and five patients (19%) achieved MCyR during 12 months of nilotinib therapy. None of these 26 patients achieved CCyR (Table 2).

Dose escalation to nilotinib 600 mg twice daily was allowed in the absence of safety concerns. Fourteen of 26 patients with mutations less sensitive to nilotinib (ie, Y253H, E255K/V, and F359C/V) received dose escalation, but only one patient (with E255K and E255V mutations) responded to dose escalation and achieved CHR and MCyR. Thus, dose escalation of nilotinib did not provide improved responses in patients with less sensitive mutations in this study.

Progression During Nilotinib Therapy Stratified by Baseline *BCR-ABL* Mutation Status

Among patients with baseline mutation data available, the median durations of follow-up were 16.1 months (range, 0.03 to 26.8

months) for all patients ($N = 281$) and 15.5 months (range, 0.1 to 26.4 months) for imatinib-resistant patients ($n = 192$). Progression, defined as loss of CHR, loss of MCyR, progression to AP/blast crisis (BC), or death as a result of any cause, was assessed during the study. The rate of progression events, and progression specifically to AP/BC, stratified by baseline mutation status are listed in Table 3. A higher proportion of patients with baseline mutations had disease progression compared with patients without mutations at baseline (46 [46%] of 100 patients v 23 [26%] of 87 patients). Rates of progression also varied with mutation types. Although only 16 (36%) of 45 patients who harbored mutations with in vitro sensitivity to nilotinib (ie, $IC_{50} \leq 150$ nM) progressed, 18 (69%) of 26 patients with mutations less sensitive to nilotinib in vitro (ie, $IC_{50} > 150$ nM) progressed during the course of nilotinib treatment. The baseline mutations most frequently associated with progression during nilotinib therapy were the E255K/V mutations (six [86%] of seven patients) and F359C/V (nine [92%] of 11 patients), of which both had in vitro IC_{50} greater than 150 nM (Table 3). Three (38%) of eight patients with the Y253H mutation progressed on nilotinib therapy. Progression to AP/BC only occurred in eight (4%) of 192 imatinib-resistant patients on nilotinib therapy. Although the low rate of progression to AP/BC is promising, it is difficult to assess the impact of the mutational status given the small number of patients who progressed.

Mutational Status During Nilotinib Therapy and at Time of Progression

The occurrence of newly detectable *BCR-ABL* mutations during nilotinib therapy was assessed. Fifty-three (19%) of all 281 patients included in this analysis had new mutations detected during nilotinib therapy that were either different from, or in addition to, existing baseline mutations. Imatinib-resistant patients had a considerably higher frequency of newly detectable mutations than imatinib-intolerant patients (47 (24%) of 192 patients v six (7%) of 89 patients). Moreover, newly detectable mutations were found in 34 (30%) of all 114 patients with baseline mutations compared with 19 (11%) of all 167 patients without baseline mutations (Table 4). The newly detectable mutations identified in all imatinib-resistant patients ($n = 192$) include E255K/V in 13 (7%), T315I in 12 (6%), F359C/V in seven (4%), G250E in seven (4%), and Y253 H in six (3%) patients.

Table 3. Progression in Imatinib-Resistant Patients With and Without Baseline Mutations

Status*	In Vitro IC_{50} to Nilotinib (nM)	No. of Patients	Progressed		Progressed to AP/BC	
			No.	%	No.	%
No mutation		87	23	26	4	5
Any mutation		100	46	46	4	4
$IC_{50} \leq 150$ nM		45	16	36	1	2
$IC_{50} > 150$ nM						
Y253H	700	8	3	38	1	14
E255K/E255V	548/791	7	6	86	0	0
F359C/F359V	258/161	11	9	92	1	9
Other†	NA	29	12	41	1	3

Abbreviations: IC_{50} , 50% inhibitory concentration; AP, accelerated phase; BC, blast crisis; NA, not applicable.

*Patients with T315I mutation (3% of total population) were excluded; patients with multiple mutations were grouped in the known and higher IC_{50} group.

†Mutations without in vitro IC_{50} data.

Table 4. Newly Detectable Mutations During Therapy

Status	All Patients			Imatinib Resistant			Imatinib Intolerant		
	No.	Total No.	%	No.	Total No.	%	No.	Total No.	%
Newly detectable mutation	53	281	19	47	192	24	6	89	7
With baseline mutation	34	114	30	33	105	31	1	9	11
Without baseline mutation	19	167	11	14	87	16	5	80	6

Of the 64 patients who experienced progression, 25 patients (39%) had newly detectable mutations at the time of progression (Table 5). Of the remaining patients, 61% had no newly detectable mutations on progression; 19 (30%) of 64 were bearing no mutations, and 20 (31%) of 64 were bearing the same mutation as at baseline. Moreover, a majority of the patients (14 [74%] of 19) who progressed without a detectable mutation did not have a mutation at baseline. In addition, 20 (80%) of the 25 patients who progressed with newly detectable mutations had baseline mutations before therapy. Individual mutations present at the time of progression are listed in Table 6. The most common mutations associated with progression were the E255K/V, F359C/V, Y253H, and T315I mutations.

The efficacy analyses described in this report excluded patients with the highly resistant T315I *BCR-ABL* mutation. There were five imatinib-resistant patients and one imatinib-intolerant patient with the T315I mutation at baseline. Two of these patients showed responses during nilotinib therapy, but none exhibited sustained responses. There were also 12 patients who exhibited multiple baseline mutations before the initiation of nilotinib therapy. Two patients had three mutations. Four of the 12 patients exhibited the T315I mutation. Of these 12 patients, five (46%) of 11 patients without CHR at baseline achieved CHR, four (33%) achieved MCyR, and two (17%) achieved CCyR.

DISCUSSION

In this analysis, significant responses were achieved in patients without baseline mutations and in those who harbored mutations with high in vitro sensitivity to nilotinib. Patients with mutations of unknown in vitro sensitivity to nilotinib exhibited response rates similar to patients with no mutations. However, in this study, we have identified mutations at three non-T315I residues (Y253H, E255K/V, and F359C/V) that were less sensitive to nilotinib in vitro and that were associated with lower response rates and a higher risk of progression in vivo. These less sensitive mutations (including T315I) were detected in 17%

of the imatinib-resistant patients at baseline and might be slightly higher in clinical practice, because patients with the T315I mutation were excluded from study entry. Thus, the presence of these specific mutations may impact the response to nilotinib. Moreover, patients with mutations at baseline were more prone to developing new detectable mutant clones and to experiencing disease progression on nilotinib.

Of the mutations with less favorable responses to nilotinib, Y253H and E255K/V are P-loop mutations, but F359 is located outside the P-loop region. In contrast, other P-loop mutations, such as L248V, G250E, and Q252H, demonstrated in vitro sensitivity to nilotinib (ie, $IC_{50} \leq 150$ nM). Moreover, patients with G250E (in vitro $IC_{50} = 120$ nM) achieved MCyR of 60% (three of five patients), CCyR of 60% (three of five patients), and MMR of 40% (two of five patients). Thus, the P-loop-versus-non-P-loop categorization that has been used commonly in the field of CML might be an oversimplification, and less relevant, when assessing the clinical efficacy of nilotinib.

In this analysis, we sought to establish a relationship between in vitro sensitivity of different *BCR-ABL* mutations and clinical efficacy to nilotinib. Because of the small sample size for individual mutant types, we grouped different mutations into the highly sensitive (ie, $IC_{50} \leq 150$ nM) and less sensitive (ie, $IC_{50} > 150$ nM) nilotinib groups, and we observed differences in responses rate and progression between the two groups. The IC_{50} of 150 nM was chosen through careful examination of clinical efficacy data to identify the best division between the two groups. However, within each group, we did not find that the responses were always inversely correlated to the in vitro IC_{50} value in a linear fashion. For example, although the F359C/V

Table 5. Mutation Status at Time of Progression

Status	Patients (n = 64)		Baseline Mutation	
	No.	%	Yes	No
No mutation	19	30	5	14
Same baseline mutation	20	31	20	0
Newly detectable mutation	25	39	20	5

NOTE. Data reflect patients who progressed and who had mutational data available at the time of progression.

Table 6. Specific Mutations Present at Time of Progression

Mutation	Mutation Present at Baseline	Mutation Newly Detected During Therapy	Patients (N = 64)	
			No.	%
E255K/E255V	6/0	4/3	13	20
T315I	1	7	8	13
F359C/F359I/F359V	1/2/4	1/0/1	9	14
Y253F/Y253H	1/2	1/2	6	9
G250E	1	2	3	5
E459G/E459K	—/—	1/2	3	5
M351T	1	1	2	3
H396R	1	1	2	3
M244V	1	—	1	2
E275K	1	—	1	2
S438C	1	—	1	2

NOTE. Some mutations that were not associated with progression did emerge on nilotinib therapy. Data reflect patients who progressed and who had mutational data available at the time of progression.

mutations had lower in vitro IC₅₀ values compared with the E255K/V mutations, they showed lower CHR and MCyR rate and equally high rate of progression. As noted above, many of these seemingly imperfect correlations might be caused by the small sample size of individual mutant types.

Interestingly, 61% of patients who progressed on nilotinib had no newly detectable mutation at progression, which suggests the involvement of alternative mechanisms of resistance in these patients. Because mutations were examined only between residues 230 to 490 of the BCR-ABL gene, the presence of other mutations, including insertions or deletions, cannot be ruled out and could be one possible explanation for these results. Other potential explanations for nilotinib resistance that have recently been described in vitro and that could be a factor in vivo include overexpression of BCR-ABL, P-glycoprotein, Lyn kinase, and Src kinase.²⁵

It is clear that the integration of mutational analysis into the CML treatment algorithm has already become a reality. The results presented herein support the concept of mutational analysis as an important component of the clinical decision-making process and provide additional guidance to clinicians on how best to integrate mutational analysis successfully. These data suggest that nilotinib is effective in patients with most mutations, but that it may not be the best treatment option for patients with the E255K/V, F359C/V, Y253H, or T315I mutations. Although the clinical data are compelling, it is important to note that mutational analysis should not be used in isolation but should be considered in the wider clinical context for each individual patient.

Clear recommendations for second-line therapy on the basis of the mutation findings at baseline are possible currently only in patients in whom resistant mutations occur. We still lack the necessary information to make evidence-based recommendations in patients who have rare mutations or multiple mutations. As has been reported with dasatinib, there are a limited number of mutations that appear resistant to initial second-generation TKI therapy.²⁶⁻²⁸

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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