Nilotinib

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Perspective

One recurrent feature of drug development for hematologic malignancies is that once we have a therapy that works, soon even better agents come along. Examples include hairy cell leukemia, where IFN-α was followed by pentostatin and then 2-chlorodeoxyadenosine or acute promyelocytic leukemia, where all-*trans* retinoic acid has been joined by arsenic trioxide as a second extremely active agent. In chronic myeloid leukemia (CML), the major breakthrough has occurred with the introduction of imatinib (Gleevec), a small-molecule inhibitor of BCR-ABL, the constitutively active tyrosine kinase that causes the disease. Imatinib has completely changed our therapeutic approach to CML. Now, 6 years after its first approval, the therapeutic space is being populated by second-line BCR-ABL inhibitors with the potential to make even better what is already very good. The newest of these agents is nilotinib (Tasigna; ref. 1).

Current Landscape of CML Therapy

A recent update of a trial in CML patients with newly diagnosed chronic-phase patients (IRIS study) showed projected 6-year overall and event-free survival rates of 88% and 83%, respectively (2). Importantly, the annual rate of progression to accelerated phase or blast crisis, after reaching a peak in the second year, has been declining in every subsequent year and there was no progression to advanced disease in year 6 (2). However, despite these excellent results, we are not yet "done" with CML. Some 10% to 15% of patients with chronic-phase CML fail imatinib because of primary or secondary resistance, and in patients with accelerated phase or blast crisis, resistance is the rule rather than the exception (3, 4). Studies into the mechanism of resistance showed that reactivation of BCR-ABL signaling is common in patients who relapse after an initial response, most commonly as a result of mutations in the kinase domain of BCR-ABL that impair drug binding (5). Thus, the pathogenetic principle that initiated the disease regains activity at the time of relapse, implying that BCR-ABL remains the optimal therapeutic target. Paradoxically, the proportion of kinase domain mutant alleles is not always 100% or close. The reason for this is unknown. Hypothetical explanations include the coexistence of various resistance mechanisms; only some of which involve kinase domain mutations. Another intriguing possibility is that kinase domain mutant clones may produce growth factors that permit the survival of bystander cells expressing unmutated BCR-ABL. This has been shown in an *in vitro* model but not yet in patients (6). It should be noted that by far not all patients with resistance have kinase domain mutations, suggesting that other mechanisms must contribute. Despite extensive research, no common theme has emerged, and "kinase domain mutation–negative" resistance may be multicausal. Besides resistance, intolerance to imatinib can pose a clinical challenge. Some patients must stop the drug altogether because of severe adverse events, whereas others experience diminished quality of life due to minor but multiple side effects. This is not trivial because in the perception of many patients CML has metamorphosed from a deadly condition worth accepting the risks of an allogeneic transplant to a chronic ailment, where quality of life issues assume much greater importance. Moreover, current experience indicates that discontinuation of the drug is not advisable: low-level residual disease remains detectable by reverse transcription-PCR in most patients, including those with a complete cytogenetic response (7). Even in reverse transcription-PCR–negative patients, recurrence of active disease is the rule if imatinib is stopped, indicating that the drug fails to eradicate leukemia stem cells (8). This implies that therapy must continue lifelong in spite of side effects and strained health care budgets. For all these reasons, the battle is not yet over, and second-line ABL kinase inhibitors are coming along to address these issues.

Imatinib's Strong Little Brother

Various approaches have been taken by different drug makers to improve on imatinib. Dasatinib (Sprycel), the first of the second-line ABL inhibitors approved by the Food and Drug Administration, is a multikinase inhibitor that targets SRC and many other kinases besides BCR-ABL (9). Compared with imatinib, dasatinib was developed from a completely different chemical scaffold. In contrast, nilotinib (Tasigna) is the result of rational modifications to the imatinib molecule (10). Crystal structure analysis had revealed that much of the binding energy of imatinib was consumed by two energetically unfavorable hydrogen bonds between the *N*-methylpiperazine moiety and Ile^{360} and His^{361} of ABL (Fig. 1). Replacement of the piperazine ring with a trifluorinated imidazole eliminated these two hydrogen bonds. Furthermore, the imidazole ring fits well into a hydrophobic pocket of the kinase. The result is a better topological fit and a molecule that inhibits ABL with 30-fold increased potency compared with the parental compound (11).

Preclinical Profiling

Nilotinib inhibits ABL, KIT, and platelet-derived growth factor receptor with an IC_{50} of 25, 158, and 53 nmol/L,

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Received 1/10/08; revised 3/25/08; accepted 4/1/08.
Grant support: National Heart, Lung, and Blood Institute grant HL082978-01 and The Leukemia and Lymphoma Society.
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doi:10.1158/1078-0432.CCR-07-5015

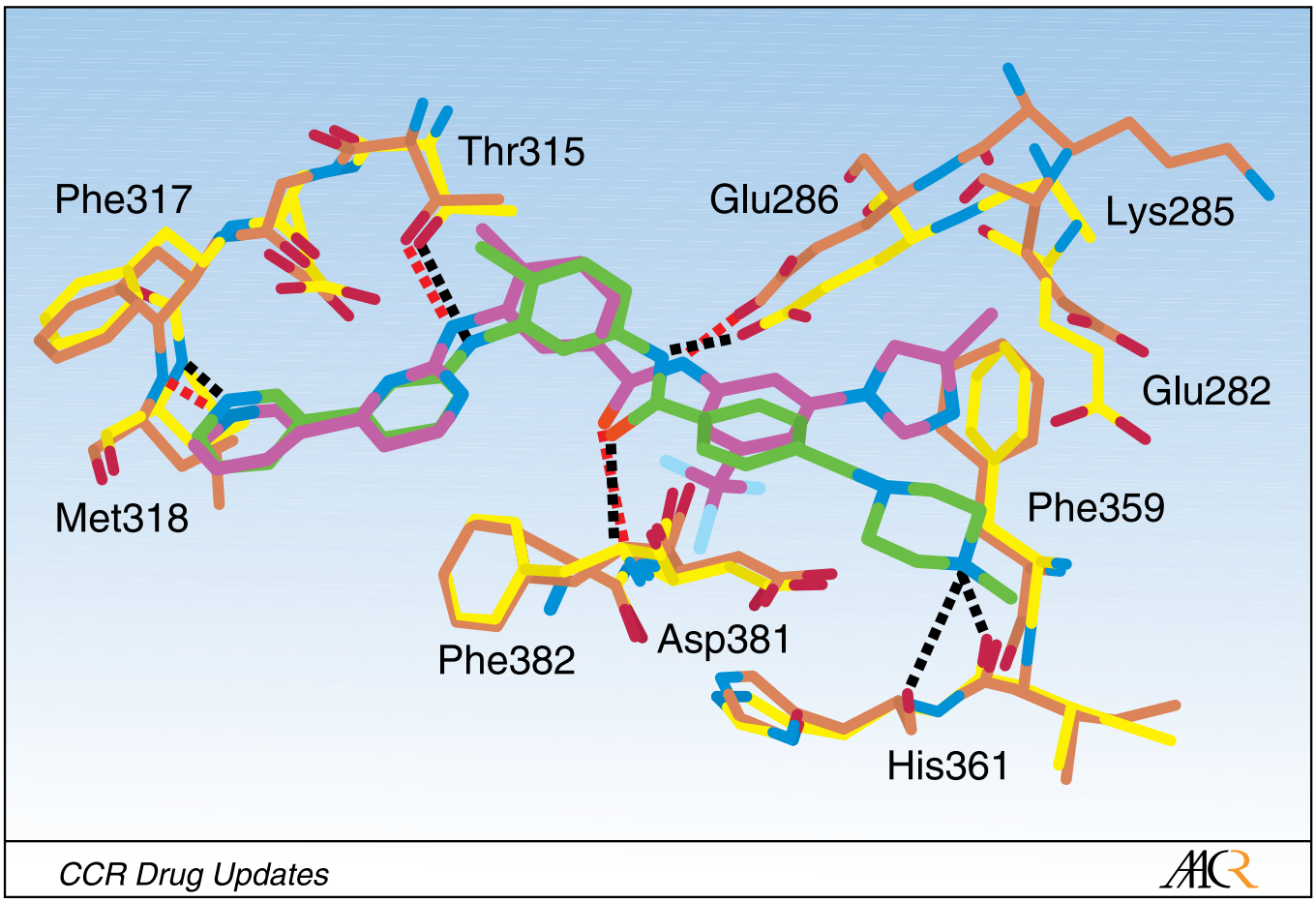

Fig. 1. Superposition of nilotinib (*magenta*) bound to ABL^{M351T} (*orange*), and imatinib (*green*) bound to ABL (*yellow*). Hydrogen bonds within the nilotinib-ABL^{M351T} complex are depicted as dashed red lines, whereas those in the imatinib complex are shown in black. Note that the hydrogen bonds between the piperazine ring of imatinib and Ile^{360} and His^{361} are absent from the nilotinib-ABL complex. The methyl-imidazole group of nilotinib packs in a hydrophobic pocket. Adapted with permission from Weisberg et al. (10).

respectively. In comparison, the respective values for imatinib are 658, 58, and 39 nmol/L. Thus, not only is nilotinib much more potent than imatinib, its activity profile against the main target kinases is also reversed, favoring the primary therapeutic target ABL over KIT and platelet-derived growth factor receptor (10). Because fluid retention, a common side effect of imatinib, is thought to be due to platelet-derived growth factor receptor inhibition, this could be clinically advantageous (12). In cell proliferation assays, nilotinib showed activity against most BCR-ABL mutants associated with imatinib resistance, with the exception of the T315I "gatekeeper" mutant. However, significant differences in activity were apparent also in mutants other than T315I. Notably, the relative activity of nilotinib against the various mutants is similar to imatinib. With mean plasma trough concentrations of 1.7 μmol/L at standard dose of nilotinib (400 mg twice daily) and the increased potency of the drug, many of the mutants come within the reach of clinically achievable drug concentrations, albeit by a variable margin (1). For example, in cell proliferation assays, the IC_{50} for the ATP-binding loop mutants Y253F and E255V are 450 and 430 nmol/L, respectively, compared with 48 and 70 nmol/L for the adjacent G250E and Q252H mutants. Like imatinib, nilotinib binds an inactive conformation of ABL, suggesting that although the binding modes of imatinib and nilotinib are not fundamentally different, the latter "overpowers" most of the BCR-ABL mutants through its greater potency. This is in contrast to dasatinib, which may be able to bind multiple confirmations of the kinase, including the active conformation, and is ~300-fold more potent than imatinib (13). Given that kinases adopt similar conformation in their active state, but rather unique conformations when inactive (14), the different binding modes translate into greater specificity (nilotinib) or greater potency (dasatinib). Thus, the two agents represent divergent approaches to ABL inhibitor development in the post-imatinib era.

Clinical Activity

In a phase I study of nilotinib in CML patients with resistance to imatinib, the rates of complete hematologic response were 92% for chronic phase, 46% for accelerated phase, and 6% for blast crisis (1). Additional advanced-phase patients cleared increased blast counts from blood and marrow but failed to normalize their peripheral blood counts. Complete cytogenetic responses were seen in 35% of chronic phase, 14% of accelerated phase, and 6% of blast crisis patients. Additional

Table 1. Efficacy of nilotinib according to disease phase

	Chronic phase (*n* = 321)	Accelerated phase (*n* = 136)	Myeloid blast crisis (*n* = 105)	Lymphoid blast crisis (*n* = 31)
Imatinib resistant (%)	71	80	NR	NR
Complete hematologic response (%)	77*	26	11	13
Major cytogenetic response (%)	57	31	38	48
Intolerant patients (%)	63	31	NR	NR
Resistant patients (%)	55	29	NR	NR
Complete cytogenetic response (%)	41	19	29	32
Overall survival (%)	91 (18 mo)	81 (12 mo)	NR	NR
Progression-free survival (%)	64 (18 mo)	57 (12 mo)	NR	NR

Abbreviation: NR, not reported.
*Two hundred and six patients were evaluable for complete hematologic response. The figures are based on the update of the phase II studies presented at the 2007 Annual Meeting of the American Society of Hematology (16–18).

13% of patients with accelerated phase and 12% with blast crisis had partial cytogenetic responses (1-35% Philadelphia chromosome–positive metaphases). Responses were seen across all BCR-ABL genotypes, the exception being patients with the T315I mutation, none of whom responded (1). Based on the promising phase I results, larger phase II trials were initiated in patients with chronic phase, accelerated phase, and blast crisis. Unlike in the phase I trial, patients with imatinib intolerance were also included, but stringent criteria were applied for definition of intolerance to avoid selection of a good prognosis cohort (15). The results of the phase II studies confirmed the promising activity seen in the phase I trial and led to the approval of nilotinib for the treatment of imatinib-resistant or imatinib-intolerant patients in chronic- or accelerated-phase CML. The majority of the responses were durable, with superior results in chronic-phase compared with accelerated-phase patients. A recent update of the results is presented in Table 1 (16–18). Although the phase 1 study showed some activity in blast crisis, most responses were short lived and 90% of patients had discontinued therapy at last follow-up, the majority because of disease progression (18). For reasons not yet clear, the response rates in the phase II blast crisis trial are more encouraging, although the results of this study have been presented only in abstract form.

Toxicity

As with imatinib and dasatinib, side effects naturally segregate into two categories: hematologic toxicity and nonhematologic toxicity. Nilotinib-induced myelosuppression is significant but thought to indicate effective therapy in patients with limited normal bone marrow reserve rather than a side effect sensu stricto. Unsurprisingly, grade 3/4 neutropenia and thrombocytopenia were more frequent in accelerated phase and blast crisis than in chronic phase. The most common nonhematologic toxicities were rash, pruritus, headache, nausea, and fatigue (Table 2). Although quite frequent, these side effects were rarely severe. The ''typical'' laboratory abnormalities of nilotinib were elevated levels of lipase, hyperglycemia, hypophosphatemia, and hyperbilirubinemia. Interestingly, hyperbilirubinemia was shown to be associated with certain polymorphisms of the UDP-glucuronyltransferase (19). The etiology of the elevated lipase levels is unknown, but

Table 2. Adverse events in nilotinib patients at 400 mg twice daily

	Grade 1 or 2 (%)	Grade 3 or 4 (%)
Nonhematologic events		
Rash, all types (%)	22	0
Pruritus	6	3
Dry skin	6	0
Constipation	0	0
Nausea, vomiting, or both	13	0
Increase in both total and conjugated bilirubin levels	6	3
Fatigue	16	0
Increase in unconjugated bilirubin level	6	3
Alopecia	0	0
Increase in lipase level	0	9
Increase in level of ALT, AST, or both	3	3
Hematologic events		
Thrombocytopenia	3	25
Neutropenia	0	9
Anemia	0	6

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

in most cases, they were not associated with clinical or radiological evidence of acute pancreatitis. Of some concern has been that nilotinib prolongs cardiac ventricular repolarization, with a dose-dependent prolongation of the QT interval as assessed by surface EKG. In the registration trials, as well as the expanded access program, there was a 0.6% rate of sudden death and a causal relationship between these events and the effect of nilotinib on repolarization cannot be excluded. Therefore, correction of serum potassium and magnesium levels before therapy as well as periodic monitoring of serum electrolyte levels and EKG are mandatory. Overall, the toxicity profile of nilotinib is benign and, apart from myelosuppression, rather different from imatinib. Given the similarities of the structures, this is remarkable and may partially reflect the fact that nilotinib has reduced platelet-derived growth factor receptor inhibitory activity compared with imatinib. An analysis of patients who entered the trials because of imatinib intolerance revealed that recurrence of the imatinib intolerance-defining side effect was very rare. Given this lack of cross-intolerance, nilotinib is a good option for patients who are unable to tolerate imatinib.

Pharmacokinetics

During the dose-escalation portion of the phase I trial, it was noted that plasma concentrations peaked at 600 mg, with no significant increase at higher doses. This suggested saturation of a carrier and prompted institution of a twice daily dosing regimen. Indeed, a dose of 400 mg twice daily resulted in higher plasma levels compared with 800 mg taken as a single dose. At the standard dose of 400 mg twice daily, mean plasma trough levels were 1.7 μmol/L. The apparent plasma half-life of nilotinib is 17 h and some 98% of drug is protein bound. It is not yet clear which intestinal drug transporter is responsible for nilotinib uptake, but interaction studies showed that absorption is increased by food, particularly fatty meals. Given the dose-dependent ability of nilotinib to impair ventricular repolarization, this was the reason to mandate that the drug be taken on an empty stomach to avoid high plasma peak levels. Nilotinib is a substrate for CYP3A4; therefore, caution is warranted when inhibitors of this detoxifying enzyme are used concomitantly with the drug. Conversely, it may be necessary to increase the dose in patients who receive strong inducers of CYP3A4 to achieve sufficient plasma levels.

Which Drug to Choose?

The availability of two second-line agents for patients with imatinib failure raises the question which agent to use in a given patient. In the absence of a prospective study comparing nilotinib and dasatinib, it is impossible to answer this question in a scientifically satisfactory manner. Unfortunately, such a study may be far away, leaving it to the physician to make the call on a semirational basis. One potentially useful guide in terms of efficacy is the type of BCR-ABL mutation that is present in a given patient who fails imatinib. For example, although nilotinib is active *in vitro* against the Y253F and E255V mutants, the IC_{90} concentrations for these mutants are 1,024 and 2,000 nmol/L, which is high considering a mean plasma trough concentration of 1.7 μmol/L with 400 mg twice daily dosing. Consistent with the *in vitro* predictions, although responses were observed in patients with Y253F and E255V, they tended to be less profound and less durable compared with patients with other mutations (20). This suggests that nilotinib may not the best choice for patients with these mutation types. On the other hand, the F317L mutation is relatively resistant to dasatinib *in vitro* and has also been seen in patients who relapsed on dasatinib, implying that nilotinib may be the preferred second-line inhibitor in patients with F317L (11, 21). The correlation between BCR-ABL genotype and response is an impressive validation of the ability of *in vitro* assays to predict *in vivo* responses to tyrosine kinase–targeted therapy (22). In this scenario, the T315I mutant represents the extreme of a spectrum, but relative insensitivity apparently also matters when it comes to the depth and durability of responses. Clearly, because many patients with imatinib resistance, particularly in chronic phase, present without kinase domain mutations, in many cases mutation analysis will not aid in deciding between different inhibitors. One clear-cut scenario is imatinib-resistant blast crisis or Philadelphia chromosome–positive acute lymphoblastic leukemia, where only dasatinib is currently approved. Whether the manufacturer of nilotinib will eventually seek regulatory approval for these indications remains to be seen. An equally important consideration is side effects. Nilotinib has a lower incidence of grade 3/4 myelosuppression and rarely causes fluid retention, which could be an advantage in patients with comorbidities. Evidently, all these speculations must be put to the test of prospective clinical trials. It is likely that the availability of second-line ABL inhibitors with different activity spectra will lead to a new level of molecular sophistication for targeted CML therapy, an important step on the path to individualized cancer therapy.

Prospects for the Future

Will nilotinib or dasatinib eventually become first-line therapy for CML? The stakes are high, given the excellent results with imatinib and the reassuring safety record of this agent. How could a second-line agent make a difference in first-line therapy? Showing an improvement in event-free or overall survival will require a large study and long follow-up. Molecular end points, such as the achievement of a major molecular response, although exciting to the clinician, may not mean much to the patient as long as therapy has to continue anyway. Thus, the real test will be whether nilotinib or any other BCR-ABL–targeted agent will be capable of eradicating the CML clone so that therapy can be stopped. This will depend on whether CML stem cells require BCR-ABL activity for their survival. If they do not, then the question which ABL inhibitor is capable of exterminating residual leukemia cells would be made mute by the biology of the CML stem cells. As of now, we do not know for sure, and regardless of all speculations, the approval of nilotinib is another piece of good news for CML patients.

Disclosure of Potential Conflicts of Interest

M. Deininger is a consultant for Novartis, Bristol-Myers Squibb, and Pharmion. The Deininger lab has received support from Calistoga, Structural Genomix, Targegen, and Cytopia.

References

1. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med 2006;354:2542–51.
2. Hochhaus A, Druker BJ, Larson R, O'Brien SG, Gathmann I, Guilhot F. IRIS 6-year follow-up: sustained survival and declining annual rate of transformation in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. Am Soc Hematol Annu Meet Abstr 2007;110:15.
3. Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 2002;99:1928–37.
4. Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 2002;99:3530–9.
5. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876–80.
6. Liu J, Joha S, Idziorek T, et al. BCR-ABL mutants spread resistance to non-mutated cells through a paracrine mechanism. Leukemia 2008;22:791–9.
7. Lange T, Niederwieser DW, Deininger MW. Residual disease in chronic myeloid leukemia after induction of molecular remission. N Engl J Med 2003;349:1483–4.
8. Deininger MW. Management of early stage disease. Hematology (Am Soc Hematol Educ Program) 2005;:174–82.
9. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 2004;305:399–401.
10. Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant BCR-ABL. Cancer Cell 2005;7:129–41.
11. O'Hare T, Walters DK, Stoffregen EP, et al. *In vitro* activity of BCR-ABL inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res 2005;65:4500–5.
12. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 2005;105:2640–53.
13. Tokarski JS, Newitt JA, Chang CY, et al. The structure of dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. Cancer Res 2006;66:5790–7.
14. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. Science 2000;289:1938–42.
15. Kantarjian HM, Giles F, Gattermann N, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. Blood 2007;110:3540–6.
16. le Coutre P, Giles FJ, Apperley J, et al. Nilotinib is safe and effective in accelerated phase chronic myelogenous leukemia (CML-AP) patients with imatinib resistance or intolerance. Am Soc Hematol Annu Meet Abstr 2007;110:471.
17. Kantarjian HM, Hochhaus A, Cortes J, et al. Nilotinib is highly active and safe in chronic phase chronic myelogenous leukemia (CML-CP) patients with imatinib-resistance or intolerance. Am Soc Hematol Annu Meet Abstr 2007;110:735.
18. Giles FJ, Larson RA, Kantarjian HM, et al. Nilotinib in patients (pts) with Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia in blast crisis (CML-BC) who are resistant or intolerant to imatinib. Am Soc Hematol Annu Meet Abstr 2007;110:1025.
19. Singer JB, Shou Y, Giles F, et al. UGT1A1 promoter polymorphism increases risk of nilotinib-induced hyperbilirubinemia. Leukemia 2007;21:2311–5.
20. Hughes T, Saglio G, Martinelli G, et al. Responses and disease progression in CML-CP patients treated with nilotinib after imatinib failure appear to be affected by the BCR-ABL mutation status and types. Am Soc Hematol Annu Meet Abstr 2007;110:320.
21. Soverini S, Colarossi S, Gnani A, et al. Resistance to dasatinib in Philadelphia-positive leukemia patients and the presence or the selection of mutations at residues 315 and 317 in the BCR-ABL kinase domain. Haematologica 2007;92:401–4.
22. Bradeen HA, Eide CA, O'Hare T, et al. Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an *N*-ethyl-*N*-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. Blood 2006;108:2332–8.

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Clin Cancer Res 2008;14:4027-4031.