Nilotinib: a novel encouraging therapeutic option for chronic myeloid leukemia patients with imatinib resistance or intolerance

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Abstract: Although high rates of complete hematologic and cytogenetic remission have been observed in patients with chronic phase chronic myeloid leukemia (CML) treated with imatinib, a short duration of response with eventual emergence of imatinib resistance has also been reported in a subset of CML patients. The most frequent clinically relevant mechanisms that change imatinib sensitivity in BCR-ABL-transformed cells are mutations within the Abl kinase domain, affecting several of its properties. Crystal structure analysis of the Abl-imatinib complex has proven helpful in identifying potential critical residues that hinder interactions of imatinib with mutated Abl. This has led to the development of a second generation of targeted therapies such as nilotinib and dasatinib, already in phase II clinical trials or SKI-606 and MK-0457 in phase I trials. In this review, we discuss the activity of nilotinib, developed by Novartis using a rational drug design strategy in which imatinib served as the lead compound. Preliminary studies demonstrated that nilotinib has more efficacy than imatinib in inhibiting proliferation of BCR-ABL-dependent cells, a relatively safety profile and clinical efficacy in all phases of CML.

Keywords: Chronic myeloid leukemia, imatinib resistance, nilotinib

Introduction

In the last 5 years many clinical studies have demonstrated that imatinib, the first tyrosine kinase inhibitor introduced in the treatment of chronic myeloid leukemia (CML) (Olsson-Stromberg et al) has high level of activity, low toxicity, and ongoing durability (Druker et al 2001; O'Brien and Deininger 2003; Baccarani et al 2004; Kantarjian et al 2004). Its efficacy is mainly observed in newly diagnosed patients with chronic phase CML, where complete hematological and cytogenetic responses where achieved in about 98% and in more than 80% of cases, respectively (Hughes et al 2003; Muller et al 2003; Kantarjian et al 2006c). The findings of the 5-year update of the International Randomised Study of Interferon versus STI571 (IRIS) comparing the efficacy of imatinib 400 mg/day with interferon-alpha (IFNα) + ara-c in newly diagnosed patients with chronic phase CML showed an estimated rate of complete cytogenetic response (CCR) of 87% and an overall survival rate of 89% at 60 months (Druker et al 2006). With similar follow-up for patients initially studied in "late chronic phase" disease with intolerance or failure during prior IFN, CCR was achieved in 53%, reflecting the remarkable ability of imatinib to salvage such patients (Iacobucci et al 2006). Although the rates of progression for patients with chronic phase CML treated with imatinib remain low, a subset of patients develops resistance to this drug. The most common mechanism of resistance is the expansion of a clone of leukemic cells with a mutation in the kinase domain of Bcr-Abl (Branford et al 2002; Shah 2005; Soverini et al 2005; Branford and Hughes 2006). This mutant clone is partially or

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totally imatinib resistant because of the reduced capacity to imatinib to bind to the mutant Bcr-Abl protein. Therapeutic options in cases of imatinib resistance include increasing the dose of imatinib, switching to a second generation Abl kinase inhibitor and proceeding to an allogenic stem cell transplant. In this review, we focus on the characterization of nilotinib, a novel, oral tyrosine kinase inhibitor developed from its parent compound, imatinib, and we discuss its current status in clinical trials.

Mechanisms of imatinib resistance

Depending on the time of onset, two categories of resistance can be distinguished: if there is no response after initial treatment, resistance is described as *primary* or intrinsic, in contrast, *secondary* or extrinsic resistance refers to loss of a previously established response (Hochhaus 2003). The rate of all progression events, including hematologic and cytogenetic relapse within chronic phase and transformation to advanced phase is 18% after a median of 5 years (Mauro 2006). Although the molecular mechanisms responsible for the rare cases of primary resistance remain poor, the mechanisms of secondary resistance are largely understood. In the majority of cases, resistance is caused by reactivation of BCR-ABL tyrosine kinase activity due to the emergence of specific point mutations within several critical regions of the Abl kinase domain (Hochhaus et al 2002; Shah et al 2002; Branford et al 2003; Soverini et al 2006). Such mutations impair imatinib binding either by affecting critical contact residues or by inducing a BCR-ABL conformation to which imatinib is unable to bind. More than 40 different point mutations encoding for distinct single amino acid substitutions in the Bcr-Abl kinase domain have been identified in relapsed CML patients. Different mutants seem to have different degrees of resistance to imatinib: in vitro data indicate that while some mutations might be overcome by dose escalation (O'Hare et al 2006), others confer a highly resistant phenotype, thereby suggesting withdrawal of imatinib in favor of alternative therapeutic strategies. Indeed, since resistance often coincides with reactivation of the kinase activity within the leukemic clone, either Bcr-Abl itself or Bcr-Abl-triggered downstream signaling pathways remain good targets for molecular therapy.

Mechanisms of imatinib resistance that do not involve ABL mutations but are clinically relevant include amplification of the BCR-ABL fusion gene, transcriptional overexpression of Bcr-Abl, increased multi-drug resistance (MDR) activity (Marull and Rochat 2006; White et al 2006), cytogenetic progression, or possible the involvement of other kinases including members of the Src family (Cowan-Jacob et al 2004; Krause and Van Etten 2005). In patients who achieve a deep reduction in leukemic-cell burden, BCR-ABL transcripts rarely become undetectable and the disease recurs in most of these patients if imatinib is discontinued. This persistence of a molecularly detectable leukemic population is due to CML "stem cell resistance", based in the ability of CML progenitors to exchange between a cycling and resting or "quiescent" state, the latter associated with minimal or no BCR-ABL expression and resulting lack of effect of Abl kinase inhibitors (Goldman and Gordon 2006).

Looking behind imatinib: nilotinib, a novel inhibitor of BCR-ABL

The emergence of imatinib resistance has stimulated the development of new kinase inhibitors that are able to overcome or prevent the development of mechanisms of failure and ultimately eliminate all evidence of disease. Two of these inhibitors are in phase II trials: dasatinib (formerly BMS-354825, Spricel®) (Luo et al 2006; Talpaz et al 2006; Cortes et al 2007; Hochhaus et al 2007; Quintas-Cardama et al 2007) and nilotinib (formerly AMN-107, Tasigna®). Other inhibitors (SKI-606, VX-680) (Golas et al 2005; Coluccia et al 2006; Giles et al 2007;) are currently in phase I trials.

Nilotinib was developed by researchers at Novartis Pharmaceuticals using a rational drug design strategy based on the replacement of the metylpiperazinyl group of imatinib and optimization of drug-like properties (Figure 1).

Like imatinib, nilotinib does not inhibit Src kinase and binds only to the inactive conformation of Bcr-Abl, with P-loop folding over the ATP-binding site, and the activation-loop blocking the substrate binding site, to disrupt the ATP-phosphate-binding site and inhibit the catalytic activity of the enzyme (Weisberg et al 2006). Nilotinib makes 4 hydrogen-bond interactions with the Abl kinase domain, involving the pyridyl-N and the backbone-NH of Met318, the aniline-NH and the side chain hydroxyl of Thr315, the amido-NH and side chain carboxylate of Glu286, as well as the amido-C═O of Asp381 and a fluorine atom in the trifluoromethyl group of nilotinib (Weisberg et al 2006). This close interaction made changes in the core of imatinib prohibitive. The improvement in binding affinity for Bcr-Abl maintains the ability to inhibit also Kit and PDGFR, but with less affinity of imatinib (Bcr-Abl > PDGFR > Kit).

Preclinical studies

Nilotinib is approximately 30-fold more sensitive than imatinib in the killing of BCR-ABL-dependent cells derived

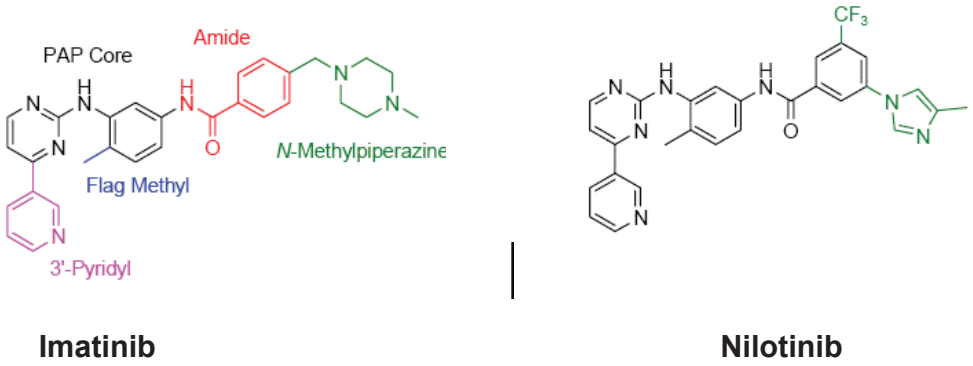

Figure 1 Modular structure of imatinib and nilotinib.

from patients with CML (K562 and Ku-812F cells) and cell lines (32D and baF3), and it is active against 32/33 imatinib-resistant cell lines with BCR-ABL mutations. The sensitivity of Bcr-Abl mutants to AMN107 can be summarized into 4 categories: high ($IC_{50} \leq 70$ nmol/L: M244V, G250E, Q252H, F3llL, F317L, M351T, V379I, L387M, H396P, H396R), medium ($IC_{50} \leq 200$ nmol/L: Y253F, E255K, F359V), low ($IC_{50} \leq 450$ nmol/L: Y253H, E255V), and insensitive ($IC_{50} > 2$ µmol/L: T315I) (Table 1) (O'Hare et al 2005). Nilotinib does not inhibit T315I (also resistant to imatinib and dasatinib), due to the loss of the hydroxyl side chain and the introduction of the methyl group of the isoleucine in the "gatekeeper" region of the ATP binding site, which inhibits binding (Pricl et al 2005). On the other hand, M351I is more sensitive to nilotinib than would be predicted. This residue makes close contact with imatinib, but not with nilotinib, due to the differences in their chemical structure. Therefore, M351I affects the binding of imatinib, but has little effect on the binding of nilotinib (O'Hare et al 2005) (Table 1). In vivo studies demonstrated that nilotinib increased survival in mouse models with native Bcr-Abl and imatinib-resistant Bcr-Abl (Golemovic et al 2005). Nilotinib is also approximately 30–40 times more potent than imatinib in inhibiting the proliferation of p190 Bcr-Abl – expressing Ph-positive ALL cell lines and it is also consistently (more than 20 times) more potent than imatinib in inhibiting phosphorylation of p190 Bcr-Abl kinase in these cell lines (Verstovsek et al 2005).

Phase I study

In a dose-escalating phase I study (Kantarjian et al 2006a), nilotinib was administered orally to 119 patients with imatinib-resistant CML or acute lymphoblastic leukemia (ALL) according to the following dosing schedule: 50 mg (7 patients), 100 mg (7), 200 mg (10), 400 mg (10), 600 mg (6), 800 mg (19), and 1200 mg (10) or to receive 400 mg twice daily (32) or 600 mg twice daily (18). Plasma level saturation was observed at more than 400mg/day. Among 119 patients, 17 were in chronic phase disease, 56 in accelerated phase (10 with clonal evolution only), 24 in myeloid blast phase, 9 in lymphoid blast phase, and 13 had Ph-positive ALL. Significant clinical activity was identified in all CML phases. Overall, of 33 patients with the blastic phase, 13 had a hematologic response to nilotinib (39%) and 9 patients (27%) had a cytogenetic response, 6 of whom had a major cytogenetic response (Ph-positive cells in metaphase, ≤35%). Of 46 patients with accelerated-phase CML (excluding those with clonal evolution only), 33 had a hematologic response and 22 had a cytogenetic response; 9 of these responses were major. Among the 10 patients who had clonal evolution as the only feature of the accelerated phase of CML, 5 had active disease and 5 were in complete hematologic remission. All 5 patients with clonal evolution and hematologic disease had a complete hematologic response; 6 of 10 had a major cytogenetic response. Among 17 patients with the chronic phase of disease, 11 of 12 patients with active disease have had a complete hematologic remission. There were cytogenetic responses in 9 of 17 patients who could be evaluated, including 6 responses that were complete. One of 10 patients with Ph-positive ALL (hematologic relapse) had a partial hematologic response, and 1 of 3 patients with Ph-positive ALL and persistent molecular signs of ALL had a complete molecular remission (Table 2). Response rates to nilotinib were similar in patients with and those without BCR-ABL mutations. Two patients with a mutation of T315I did not have a response to nilotinib, as predicted from preclinical studies.

The treatment was well tolerated. Most frequent grade 3–4 adverse events were hematologic, with thrombocytopenia and neutropenia seen in 20% and 12%, respectively.

Table 1 Comparison between imatinib, nilotinib IC_{50} values (nmol/L) for cellular proliferation and cellular Bcr-Abl tyrosine phosphorylation assay

	Ba/F3 cellular assays			
	Imatinib		Nilotinib	
	Celular proliferation IC_{50} (nmol/L)	Bcr-Abl tyrosine phosphorylation IC_{50} (nmol/L)	Celular proliferation IC_{50} (nmol/L)	Bcr-Abl tyrosine phosphorylation IC_{50} (nmol/L)
WT Bcr-Abl	260	280	13	10
M244V	2,000	500	38	8
G250E	1,350	1,000	48	7
Q252H	1,325	1,500	70	15
F311L	480	600	23	44
F317L	1,050	400	50	47
M351T	880	500	15	8
V397I	1,630	800	51	15
L387M	1,000	2,700	49	33
H396P	850	2,700	41	70
H396R	1,750	1,000	41	22
Y253F	3,475	4,200	125	55
E255K	5,200	5,000	200	70
F359V	1,825	3,100	175	43
Y253H	>6,400	>5,000	450	155
E255V	>6,400	>5,000	430	250
T315I	>6,400	>5,000	>2,000	>5,000

Other adverse events were less frequent and were primarily grade 1–2. Neutropenia and indirect self-limited reversible hyperbilirubinemia were observed mostly at the dose of 600 mg twice daily. A nilotinib dose of 400 mg twice daily was selected for the phase II studies based on efficacy and long-term side effects (Jabbour et al 2006). Nilotinib prolongs the QTcF interval in some patients. One unexplained sudden death was reported beyond the follow-up time analysis (Kantarjian et al 2006a). This finding indicates the need for careful monitoring for cardiac events and arrhythmias in all patients who are receiving nilotinib and a strict avoidance of medications that may prolong the QTcF interval.

Phase II studies

The phase II study was designated to evaluate the safety and efficacy, as defined by hematologic (normalization of white blood cell counts) and cytogenetic (reduction or elimination of the Ph+ chromosome) response rates of nilotinib administered to imatinib-resistant or intolerant patients with Ph+ CML in chronic phase, accelerated phase, blastic phase and Ph-positive ALL. The 316 chronic-phase patients in the phase II study were heavily pre-treated for Ph+ CML, with a significant majority (72%) having received at least 600 mg of imatinib as well as having been treated earlier with interferon (65%) and hydroxyurea (83%).

Among 279 assessable patients (ie, those patients with at least six months of follow up) with chronic-phase disease, major cytogenetic response was observed in 145 (52%) of which 96 (34%) were complete. Complete hematologic response was reported in 137 (74%) of 185 assessable patients. In patients with at least 10 months follow-up, the median time to cytogenetic response was 2.8 months (range 1–11), and the median time to complete hematologic response was 1.0 (range 1–8) months (Giles et al 2006; le Coutre et al 2006).

Among 64 patients with accelerated-phase disease, major cytogenetic response was observed after at least 8 months follow-up in 23 patients (36%), of which 14 (22%) were complete. Confirmed hematologic response occurred in 38 (59%), of which 15 (23%) were complete. The median time to cytogenetic response was 2.0 months (range 1–8), and the median time to complete hematologic response was 1.0 (range 1–3) months (Kantarjian et al 2006b).

Preliminary data have also been presented for 96 blast phase and 34 Ph-positive ALL patients. Complete hematological response was reported in 12 (13%) blast phase patients, marrow response in 6 (6%), return to chronic phase and stable disease in 17 (18%) patient each. Complete responses were reported in 2 (6%) Ph+ ALL patients (1 relapsed/refractory and 1 with minimal residual disease) (Table 3) (Ottmann et al 2006).

Table 2 Results from a phase I study: response to nilotinib in patients with imatinib resistance

Disease phase	Patients	Hematological response		Cytogenetic response	
	(n)	Total (%)	Complete (%)	Total (%)	Major (%)
Chronic phase CML	17	92	92	53	35
Accelerated phase CML					
Hematological disease	46	72	46	48	20
Clonal evolution only	10	100	100	90	60
Myeloid blastic phase CML	24	42	8	29	21
Lymphoid blastic phase CML	9	33	0	22	11

Abbreviations: CML, chronic myeloid leukemia.

The phase II study showed an acceptable tolerability profile with a low incidence of events related to fluid retention such as edema, a side effect common with other tyrosine kinase inhibitors. The most frequent Grade 3 or 4 adverse events were primarily hematological in nature and include neutropenia and thrombocytepenia. Elevations were seen in bilirubin, liver function tests, lipase enzymes, and blood sugar, which were mostly transient and resolved over time. These cases were easily managed and rarely led to discontinuation. Pancreatitis was reported in less than 1% of cases. The study also showed virtually no non-hematologic cross-intolerance between imatinib and nilotinib (cross-intolerance occurs when patients cannot tolerate two different drugs because of the same side effects). Causes of non-hematologic intolerance to imatinib, which occurred in 95 patients, included Grade 3 or 4 rash/skin toxicity, fluid retention, gastrointestinal intolerance, liver toxicity, and myalgia/arthralgia. When treated with nilotinib, none of these patients experienced severe rash/skin toxicity, fluid retention or myalgia/arthralgia. One patient each experienced severe gastrointestinal intolerance and liver toxicity. Further follow-up of the phase II studies is ongoing.

Nilotinib as first-line therapy

Based on Phase II experience, a study of nilotinib as first line therapy for patients with CML in early chronic phase has been started with the objective to improve the overall molecular response and to achieve responses earlier since early achievement of response has been correlated with improved long-term outcome. The preliminary data in 14 patients treated suggest that indeed the higher potency is translating into better responses, with 100% of patients in complete hematologic response after 3 months of therapy, and 100% of patients in complete cytogenetic response within 6 months. A major molecular response was achieved in 54% by 6 months. These responses appeared to be more rapid than observed with imatinib 800 mg daily (Jabbour et al 2006).

In addition, considering the efficacy of nilotinib after failure to imatinib, there is interest in exploring earlier intervention in patients who have not met the criteria for failure but have a suboptimal response as defined by the recommendations of a panel of CML experts (Baccarani et al 2006).

Future strategies

There is an urgent need for the development of compounds active against the T315I-Bcr-Abl mutant because it might drive the majority of cases who acquire resistance to novel second-generation inhibitors. The T315I is responsible for approximately 15% of the cases of relapse in CML and Ph+ ALL patients on imatinib therapy. Structural analyses indicate that the substitution of threonine with isoleucine

Table 3 Results from a phase II study: response to nilotinib in patients with imatinib failure

Disease phase	Patients	Hematological response	Cytogenetic response
	(n)	Complete (%)	Major (%)
Chronic phase CML	279	74	52
Accelerated phase CML	64	23	36
Blast phase CML	96	13	NA
Ph-positive ALL	34	6	NA

Abbreviations: ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukemia; NA, not available.

at residue 315 eliminates a crucial hydrogen-bonding interaction and introduces a steric clash which abrogates binding and effective inhibition of Bcr-Abl by imatinib as well as by several novel inhibitors (Pricl et al 2005). A different approach to tyrosine kinase inhibition is to design inhibitors binding regions of Bcr-Abl other than the ATP binding pocket. This is the case of ON012380 (Gumireddy et al 2005), which binds to the substrate binding site. ON01910 is a similar agent that also inhibits Plk1, a kinase that plays a critical role in cell cycle progression. Adaphostin is a non-ATP competitive TKI that inhibits Bcr-Abl and induces apoptosis in both imatinib-sensitive and imatinib-resistant cell lines and acts synergistically with imatinib. Adaphostin also increases levels of intracellular reactive oxygen species and can stimulate apoptosis in Ph-negative malignant cells (Mow et al 2002).

An intriguing alternative is to explore the possibility of whether molecules that have been developed as inhibitors for other protein kinases and are already undergoing clinical trials might inhibit the T315I-Bcr-Abl mutant. MK-0457 (VX-680) is a potent multikinase inhibitor with activity against aurora kinases (Carter et al 2005; Shah 2006). The biochemical structure of MK-0457 makes it able to bind and inhibit Bcr-Abl kinase despite the presence of T315I mutation that prevents the inhibitory activity of all other kinase inhibitors tested in the clinic including imatinib, dasatinib and nilotinib. A phase I study of MK-0457 is being conducted and the preliminary analysis has shown responses in some of the patients with CML who had failed imatinib and had the T315I mutation, as well as patients with myeloproliferative disorders with the JAK-2 mutation. Phase II studies of this agent, with particular attention to these patient populations, are starting (Giles et al 2007).

Conclusion

Since Bcr-Abl signaling remains central to CML pathogenesis, the selective inhibition of wild type and imatinib resistant Bcr-Abl signaling is still the therapeutic strategy of choice to overcome imatinib resistance. This approach led to the development of novel tyrosine kinase inhibitors more potent than imatinib, such as dasatinib and nilotinib. Preclinical and early-phase clinical findings indicate that nilotinib, a "cousin" of imatinib, may be useful in the treatment of imatinib-refractory CML, due to its strong binding affinity to Abl, its activity against imatinib-resistant BCR-ABL mutants and its efficacy and tolerability in clinical studies. However, the durability of responses remains undefined. Furthermore, nilotinib (like imatinib and dasatinib) do not overcome the T315I-Bcr-Abl mutation, which may drive the majority of acquired resistance cases to these compounds. Strategies to override resistance mediated by the T315I mutation represent the next major goal in the targeted treatment of CML. Compounds such as VX-680, an aurora kinase inhibitor, capable of binding to and inhibiting kinase activity of the T315I-Bcr-Abl mutation, are very encouraging.

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References

Baccarani M, Martinelli G, Rosti G, et al. 2004. Imatinib and pegylated human recombinant interferon-alpha2b in early chronic-phase chronic myeloid leukemia. *Blood*, 104:4245–51.

Baccarani M, Saglio G, Goldman J, et al. 2006. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*, 108:1809–20.

Branford S, Hughes T. 2006. Detection of BCR-ABL mutations and resistance to imatinib mesylate. *Methods Mol Med*, 125:93–106.

Branford S, Rudzki Z, Walsh S, et al. 2002. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood*, 99:3472–5.

Branford S, Rudzki Z, Walsh, et al. 2003. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood*, 102:276–83.

Carter TA, Wodicka LM, Shah, et al. 2005. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci USA*, 102:11011–16.

Coluccia AM, Benati D, Dekhil, et al. 2006. SKI-606 decreases growth and motility of colorectal cancer cells by preventing pp60(c-Src)-dependent tyrosine phosphorylation of beta-catenin and its nuclear signaling. *Cancer Res*, 66:2279–86.

Cortes J, Rousselot P, Kim DW, et al. 2007. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. *Blood*, 109:3207–13.

Cowan-Jacob SW, Guez V, Fendrich G, et al. 2004. Imatinib (STI571) resistance in chronic myelogenous leukemia: molecular basis of the underlying mechanisms and potential strategies for treatment. *Mini Rev Med Chem*, 4:285–99.

Druker BJ, Guilhot F, O'Brien SG, et al. 2006. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*, 355:2408–17.

Druker BJ, Talpaz M, Resta DJ, et al. 2001. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*, 344:1031–7.

Giles F, le Coutre P, Bhalla B, et al. 2006. A phase II study of Nilotinib, a novel tyrosine kinase inhibitor administered to patients with imatinib resistant or intolerant chronic myelogenous leukemia (CML) in chronic phase (CP), accelerated phase (AP) or blast crisis (BC) who have also failed dasatinib therapy. *Blood (ASH Annual Meeting Abstracts)*, 108:2170.

Giles FJ, Cortes J, Jones D, et al. 2007. MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. *Blood*, 109:500–2.

Golas JM, Lucas J, Etienne C, et al. 2005. SKI-606, a Src/Abl inhibitor with in vivo activity in colon tumor xenograft models. *Cancer Res*, 65:5358–64.

Goldman J, Gordon M. 2006. Why do chronic myelogenous leukemia stem cells survive allogeneic stem cell transplantation or imatinib: does it really matter? *Leuk Lymphoma*, 47:1–7.

Golemovic M, Verstovsek S, Giles F, et al. 2005. AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, has in vitro activity against imatinib-resistant chronic myeloid leukemia. *Clin Cancer Res*, 11:4941–7.

Gumireddy K, Baker SJ, Cosenza SC, et al. 2005. A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. *Proc Natl Acad Sci USA*, 102:1992–7.

Hochhaus A. 2003. Cytogenetic and molecular mechanisms of resistance to imatinib. *Semin Hematol*, 40:69–79.

Hochhaus A, Kantarjian HM, Baccarani M, et al. 2007. Dasatinib induces notable hematologic and cytogenetic responses in chronic phase chronic myeloid leukemia after failure of imatinib therapy. *Blood*, 109:2303–9.

Hochhaus A, Kreil S, Corbin AS, et al. 2002. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia*, 16:2190–6.

Hughes TP, Kaeda J, Branford S, et al. 2003. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med*, 349:1423–32.

Iacobucci I, Rosti G, Amabile M, et al. 2006. Comparison between patients with Philadelphia-positive chronic phase chronic myeloid leukemia who obtained a complete cytogenetic response within 1 year of imatinib therapy and those who achieved such a response after 12 months of treatment. *J Clin Oncol*, 24:454–9.

Jabbour E, Cortes J, Giles F, et al. 2006. Preliminary activity of nilotinib (AMN107), a novel selective potent oral Bcr-Abl tyrosine kinase inhibitor, in newly diagnosed Philadelphia chromosome (Ph)-positive chronic phase chronic myelogenous leukemia (CML-CP). *Blood (ASH Annual Meeting Abstracts)*, 108:2172.

Kantarjian H, Giles F, Wunderle L, et al. 2006a. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N Engl J Med*, 354:2542–51.

Kantarjian HM, Cortes JE, O'Brien S, et al. 2004. Long-term survival benefit and improved complete cytogenetic and molecular response rates with imatinib mesylate in Philadelphia chromosome-positive chronic-phase chronic myeloid leukemia after failure of interferon-alpha. *Blood*, 104:1979–88.

Kantarjian HM, Gattermann N, Hochhaus A, et al. 2006b. A phase ii study of nilotinib a novel tyrosine kinase inhibitor administered to imatinib-resistant or intolerant patients with chronic myelogenous leukemia (CML) in accelerated phase (AP). *Blood (ASH Annual Meeting Abstracts)*, 108:2169.

Kantarjian HM, Talpaz M, O'Brien S, et al. 2006c. Survival benefit with imatinib mesylate versus interferon-alpha-based regimens in newly diagnosed chronic-phase chronic myelogenous leukemia. *Blood*, 108:1835–40.

Krause DS, Van Etten RA. 2005. Tyrosine kinases as targets for cancer therapy. *N Engl J Med*, 353:172–87.

le Coutre P, Bhalla K, Giles F, et al. 2006. A phase ii study of nilotinib, a novel tyrosine kinase inhibitor administered to imatinib-resistant and -intolerant patients with chronic myelogenous leukemia (CML) in chronic phase (CP). *Blood (ASH Annual Meeting Abstracts)*, 108:165.

Luo FR, Yang Z, Camuso A, et al. 2006. Dasatinib (BMS-354825) pharmacokinetics and pharmacodynamic biomarkers in animal models predict optimal clinical exposure. *Clin Cancer Res*, 12:7180–6.

Marull M, Rochat B. 2006. Fragmentation study of imatinib and characterization of new imatinib metabolites by liquid chromatography-triple-quadrupole and linear ion trap mass spectrometers. *J Mass Spectrom*, 41:390–404.

Mauro MJ. 2006. Defining and managing imatinib resistance. *Hematology (Am Soc Hematol Educ Program)*, 219–25.

Mow BM, Chandra J, Svingen PA, et al. 2002. Effects of the Bcr/abl kinase inhibitors STI571 and adaphostin (NSC 680410) on chronic myelogenous leukemia cells in vitro. *Blood*, 99:664–71.

Muller MC, Gattermann N, Lahaye T, et al. 2003. Dynamics of BCR-ABL mRNA expression in first-line therapy of chronic myelogenous leukemia patients with imatinib or interferon alpha/ara-C. *Leukemia*, 17:2392–400.

O'Brien SG, Deininger MW. 2003. Imatinib in patients with newly diagnosed chronic-phase chronic myeloid leukemia. *Semin Hematol*, 40:26–30.

O'Hare T, Corbin AS, Druker BJ. 2006. Targeted CML therapy: controlling drug resistance, seeking cure. *Curr Opin Genet Dev*, 16:92–9.

O'Hare T, Walters DK, Stoffregen EP, et al. 2005. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res*, 65:4500–5.

Olsson-Stromberg U, Hoglund M, Bjorkholm M, et al. 2006. Successful mobilization of Ph-negative blood stem cells with intensive chemotherapy + G-CSF in patients with chronic myelogenous leukemia in first chronic phase. *Leuk Lymphoma*, 47:1768–73.

Ottmann O, Kantarjian H, Larson R, et al. 2006. A phase ii study of nilotinib, a novel tyrosine kinase inhibitor administered to imatinib resistant or intolerant patients with chronic myelogenous leukemia (CML) in blast crisis (BC) or relapsed/refractory Ph+ acute lymphoblastic leukemia (ALL). *Blood (ASH Annual Meeting Abstracts)*, 108:1862.

Pricl S, Fermeglia M, Ferrone, et al. 2005. T315I-mutated Bcr-Abl in chronic myeloid leukemia and imatinib: insights from a computational study. *Mol Cancer Ther*, 4:1167–74.

Quintas-Cardama A, Kantarjian H, et al. 2007. Dasatinib (BMS-354825) is active in Philadelphia chromosome-positive chronic myelogenous leukemia after imatinib and nilotinib (AMN107) therapy failure. *Blood*, 109:497–9.

Shah NP. 2005. Loss of response to imatinib: mechanisms and management. *Hematology (Am Soc Hematol Educ Program)*, 183–7.

Shah NP. 2006. Improving upon the promise of targeted therapy of human malignancy: chronic myeloid leukemia as a paradigm. *Cancer Chemother Pharmacol*, 58(Suppl 7):49–53.

Shah NP, Nicoll JM, Nagar B, et al. 2002. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell*, 2:117–25.

Soverini S, Colarossi S, Gnani, et al. 2006. Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. *Clin Cancer Res*, 12:7374–9.

Soverini S, Martinelli G, Rosti, et al. 2005. ABL mutations in late chronic phase chronic myeloid leukemia patients with up-front cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. *J Clin Oncol*, 23:4100–9.

Talpaz M, Shah NP, Kantarjian H, et al. 2006. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N Engl J Med*, 354:2531–41.

Verstovsek S, Golemovic M, Kantarjian H, et al. 2005. AMN107, a novel aminopyrimidine inhibitor of p190 Bcr-Abl activation and of in vitro proliferation of Philadelphia-positive acute lymphoblastic leukemia cells. *Cancer*, 104:1230–6.

Weisberg E, Manley P, Mestan J, et al. 2006. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer*, 94:1765–9.

White DL, Saunders VA, Dang P, et al. 2006. OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. *Blood*, 108:697–704.