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Development of NS-187, a potent and selective dual Bcr-Abl/Lyn tyrosine kinase inhibitor

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Abstract Imatinib mesylate (Gleevec™) has improved the treatment of Bcr-Abl-positive leukemia. However, resistance is often reported in patients with advanced-stage disease. Chemical modifications of imatinib made with the guidance of molecular modeling have yielded several promising compounds that could override imatinib resistance. Among them, we selected a compound denoted NS-187. The most striking structural characteristic of NS-187 is its trifluoromethyl group at position 3 of the benzamide ring, which strengthens the hydrophobic interactions and fixes the conformation of the compound. NS-187 was 25–55 times more potent than imatinib against wild-type Bcr-Abl in vitro. At physiological concentrations, NS-187 also inhibited the phosphorylation and growth of all Bcr-Abl mutants tested except T315I. In addition to Bcr-Abl, NS-187 also inhibited Lyn, which might be involved in imatinib resistance, without affecting the phosphorylation of Src, Blk, or Yes. This indicates that NS-187 acts as a dual Bcr-Abl/Lyn inhibitor. Our proposed docking models of the NS-187/Abl complex support the notion that NS-187 is more specific for Lyn than for Src. In Balb/c-nu/nu mice, which were injected subcutaneously with Bcr-Abl-positive KU812 cells, NS-187 showed at least tenfold more potency than imatinib. We also tested the ability of NS-187 to suppress tumor growth in another murine tumor model, namely, Balb/c-nu/nu mice intravenously transplanted with BaF3 cells harboring wild-type or several mutations of Bcr-Abl (M244V, G250E, Q252H, Y253F, E255K, T315I, M351T, and H396P). NS-187 prolonged the survival of mice injected with leukemic cells expressing wild-type or all mutated Bcr-Abl except T315I, and its efficacy correlated well with its in vitro effects. NS-187 also inhibited leukemic cells harboring wild-type Bcr-Abl growth in the central nervous system, which sometimes becomes a sanctuary for leukemic cells under imatinib treatment. These results suggest that NS-187 may be a potentially valuable novel agent to combat imatinib-resistant Bcr-Abl-positive leukemia. A phase I study of NS-187 will start in 2006.

Keywords NS-187 · INNO-406 · Imatinib · Chronic myeloid leukemia · Bcr-Abl · Lyn

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Introduction

Chronic myeloid leukemia (CML) is a malignant clonal disorder of hematopoietic stem cells that increases not only myeloid cells, but also erythroid cells and platelets in the peripheral blood and induces marked myeloid hyperplasia in the bone marrow. The diagnosis of CML is usually confirmed by the detection of the Philadelphia (Ph) chromosome, which results from the reciprocal translocation *t*(9;22). The Ph chromosome was the first specific chromosome abnormality to be identified

in cancer and is found in 95% of patients with CML and in a subset of patients with acute lymphoblastic leukemia (ALL). The molecular consequence of the *t*(9;22) translocation is the creation of the fusion protein Bcr-Abl, which is a constitutively active tyrosine kinase that causes leukemias [30]. Transgenic expression of the 190-kd Bcr-Abl protein in mice causes acute leukemia at birth, suggesting that it confers a potent oncogenic signal in hematopoietic cells [15]. Another model of CML involves the retroviral transfer of the *bcr-abl* gene into hematopoietic stem cells of normal mice. Depending on their genetic backgrounds, these animals develop hematologic malignancies that range from ALL to CML [5, 11]. Thus, eradication of leukemic clones harboring *bcr-abl* is essential for CML and Ph^+ALL treatment.

For many years, the principal anticancer agents for CML had been busulfan and hydroxyurea. However, while busulfan and hydroxyurea can reduce the number of leukemic cells, they do not significantly prolong the survival of CML patients. In the 1980s, interferon-α (IFN-α) became the preferred treatment for CML when it was shown that IFN-α could induce hematologic and cytogenetic remission, and that survival was prolonged in comparison with conventional chemotherapy. However, not all patients respond to IFN-α and not all patients have their survival prolonged [18, 28]. Another important option for CML treatment is allogeneic hematopoietic stem cell transplantation (allo-HSCT), which was introduced for the treatment of CML in the late 1970s. Unlike any of the other treatments described above, allo-HSCT is potentially curative. However, the applicability of this method is limited by its high toxicity and by donor limitations [32].

Imatinib mesylate (Gleevec™, Glivec™, formerly STI571, and CGP57148), which was introduced near the beginning of this century, specifically inhibits the autophosphorylation of Abl tyrosine kinase and is not only highly efficacious in treating CML, but also generally produces only mild side effects [25]. As a result, the first-line therapy for CML was dramatically altered within a few years of the introduction of imatinib to the clinic [13], and CML therapy is now described as being in the "*imatinib era*" [10]. However, a small percentage of CML in chronic phase (CML-CP) as well as most advanced-phase patients relapse on imatinib therapy [9]. Bcr-Abl-dependent mechanisms of resistance to imatinib include overexpression of Bcr-Abl and amplification of the *bcr-abl* gene, and, most intriguingly, point mutations within the Abl kinase domain that interfere with imatinib binding [7, 14, 16, 24]. To overcome imatinib resistance, higher doses of imatinib and combination therapy with other agents have been used, with some efficacy. However, these strategies are limited in their application and effectiveness, especially for patients with Abl point mutations [3, 19, 26]. Therefore it is necessary to develop more effective Abl tyrosine kinase inhibitors. Several Src inhibitors from various chemical classes, including PD166326 [36], SKI-606 [12], AP23464 [27], and dasatinib (formerly BMS-354825) [31] have been found to be 100–300 times more effective than imatinib in blocking Bcr-Abl tyrosine kinase autophosphorylation, and these effects extend to point mutants of Bcr-Abl. However, while imatinib binds to only the inactive form of Abl, these Src inhibitors also bind to the active form, which shares considerable conformational similarity with the active forms of diverse tyrosine kinases, including the Src-family proteins [21]. This characteristic of Src inhibitors has some advantage with respect to Lyn kinase, an Src-family protein, because overexpression of Lyn is associated with imatinib resistance [4, 8, 29]. However, the effect of lower specificity against Src-family proteins is not yet fully understood, because these kinases play many important roles in vivo [2, 6, 33, 34]. In addition to these Src inhibitors, AMN107 has been developed as a novel Abl tyrosine kinase inhibitor. The in vitro inhibitory effect of AMN107 is at most 10–30 times more potent than imatinib, but weaker than Src inhibitors [35].

We attempted to develop a novel agent that had a sufficiently higher affinity for Abl than imatinib and AMN107, and was more specific than Src-Abl inhibitors in inhibiting Lyn at clinically relevant concentrations, without affecting the phosphorylation of other Src-family proteins.

Design and synthesis of 3-substituted benzamide derivatives

To develop a novel agent that can override imatinib resistance, we comprehensively investigated the crystal structure of the kinase domain of c-Abl bound with imatinib, which had been reported by Nagar et al. [23]. Imatinib forms six hydrogen bonds with the protein, and the majority of contacts are mediated by van der Waals interactions, which lock the c-Abl kinase into its inactive conformation. We found a hydrophobic pocket formed by amino acid residues Ile-293, Leu-298, Leu-354, and Val-379 around the phenyl ring adjacent to the piperazinylmethyl group of imatinib. We focused on this hydrophobic pocket and introduced various hydrophobic substituents at the phenyl ring of imatinib. We found that 3-halogenated and 3-trifluoromethylated benzamides displayed significantly increased activity compared to unsubstituted imatinib.

The antiproliferative activities of the 3-substituted benzamides synthesized were evaluated in vitro against Bcr-Abl-positive (K562) and -negative (U937) leukemia cells (Table 1). All compounds tested showed more potent antiproliferative activity against K562 cells than did imatinib, whereas they had only weak antiproliferative activity against U937 cells.

Among these compounds, we finally selected 9b, which was later denoted NS-187, as a promising candidate for development, judging from its overall characteristics, including its pharmacokinetics and safety profile as determined in animal studies. The most striking structural characteristic of NS-187 is its trifluoromethyl (CF_3) group at position 3 of the benzamide ring. The presence of the CF_3 group strengthened the hydrophobic interactions of the molecule with the hydrophobic pocket of Abl (Fig. 1). Another possible merit of the CF_3 group is that it may fix the conformation of the drug by hindering its rotation at the 4-position of the benzamide ring (Fig. 2). As a result, a CF_3-bearing molecule may be more potent than more flexible compounds such as imatinib [1].

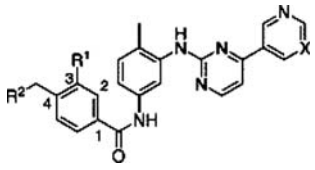
NS-187 blocks wild-type Bcr-Abl signaling

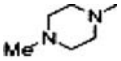
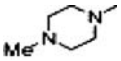
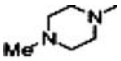
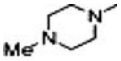
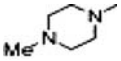
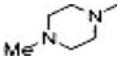
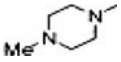
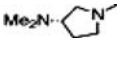
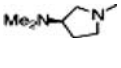
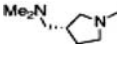
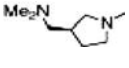
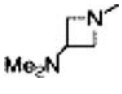
The IC_{50} values of NS-187 against wild-type Bcr-Abl in K562 and 293T cells were 11 and 22 nM, respectively. The corresponding values for imatinib were 280 and 1,200 nM. Thus, NS-187 was 25 and 55 times more potent than imatinib, respectively, in blocking Bcr-Abl autophosphorylation. NS-187 suppressed platelet-derived growth factor receptor (PDGFR) and c-Kit phosphorylation with IC_{50} values similar to those of imatinib. The ranking of IC_{50} with respect to imatinib is PDGFR > c-Kit > Bcr-Abl, while the ranking with respect to NS-187 is Bcr-Abl > PDGFR > c-Kit. Examination of the intracellular phosphorylation status of CrkL and ERK, the downstream mediators of Bcr-Abl, revealed that NS-187 inhibited the phosphorylation of these proteins in K562 cells at much lower concentrations than did imatinib. The inhibition of phosphorylation was also observed in mouse ProB cell line BaF3 expressing wild-type Bcr-Abl cells (BaF3/wt) [20]. These findings indicate that NS-187 is much more potent and specific against Bcr-Abl than imatinib was.

In vitro effects of NS-187 against wild-type Bcr-Abl and mutated Bcr-Abl

More than 30 point mutations within the Abl kinase domain have been reported [17]. NS-187 at physiologi-

Table 1 Activity of 3-substituted benzamide derivatives



Com.	X	R1	R2	IC_{50}(nM)	
				K562	U937
IM	CH	H		182	14,000
5a	CH	F		63	8,000
5b	CH	Cl		10	9,000
5c	CH	Br		7	5,000
5d	CH	I		10	4,000
5e	CH	CF_3		5	5,000
9a	N	CF_3		4	5,000
9b	N	CF_3		11	10,000
9c	N	CF_3		4	9,000
9d	N	CF_3		11	20,000
9e	N	CF_3		9	>100,000
9f	N	CF_3		17	>100,000

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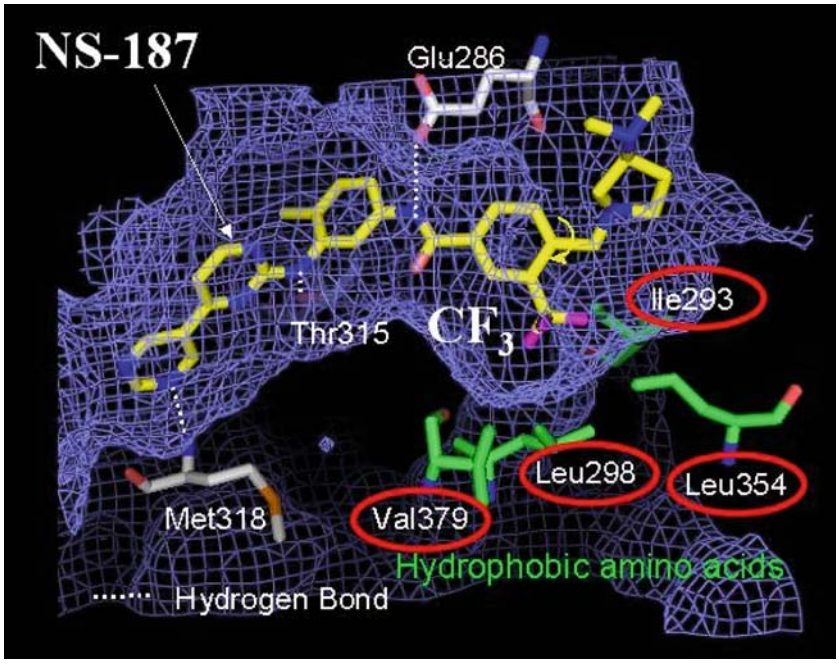

Fig. 1 Docking model of NS-187 with Abl. Hydrophobic amino acids are circled while hydrogen-bonding interactions are indicated by the *dotted lines*. The amino acids in front of NS-187 are not depicted for the sake of clarity

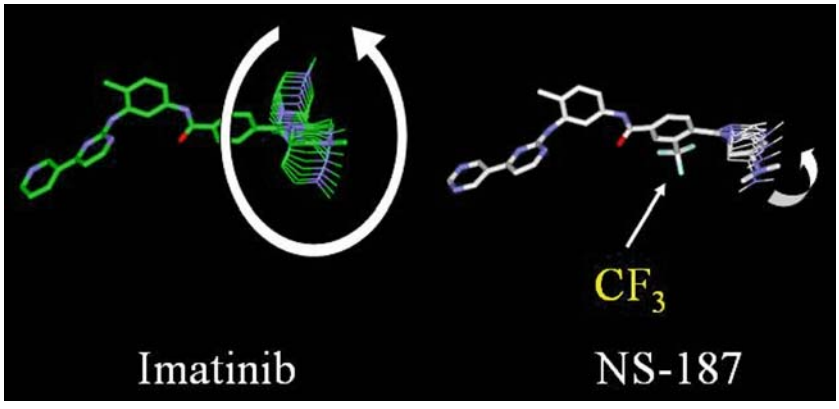

Fig. 2 The rotational barrier around the bond connecting the benzene of imatinib or NS-187 and cyclic amine groups

cally obtainable concentrations inhibited the phosphorylation of Bcr-Abl expressing the M244V, G250E, Q252H, Y253F, E255K, E255V, F317L, M351T, E355G, F359V, H396P, or F486S mutants, but it did not inhibit the phosphorylation of the T315I mutant. For all mutants except T315I, the IC_{50} for imatinib was at least 5 times as high as the corresponding value for NS-187 (Fig. 3) [20].

NS-187 suppressed the growth of the Bcr-Abl-positive cell lines K562, KU812, and BaF3/wt much more potently than did imatinib, but neither drug affected the proliferation of the Bcr-Abl-negative U937 cell line. As to Bcr-Abl point mutants, NS-187 exhibited a concentration-dependent antiproliferative effect against BaF3 cell lines expressing Bcr-Abl/M244V, G250E, Q252H, Y253F, E255K, M351T, or H396P, but had no effect on BaF3/T315I cells within the concentration range tested. Bcr-Abl/wt, Q252H, and M351T were especially sensitive to NS-187. Imatinib, meanwhile, was much less active against all cell lines tested [22, 25]. These data indicate that NS-187 greatly inhibited not only the intracellular phosphorylation of most mutated Bcr-Abl kinases except T315I, but also the proliferation of cells expressing these kinases.

Effects of NS-187 against phosphorylated Abl

Imatinib inhibited the Tyr393-unphosphorylated form of Bcr-Abl with an IC_{50} value of 35 nM, but had little effect on the phosphorylated form. In contrast, NS-187 effectively inhibited the kinase activity of both Tyr393-phosphorylated and Tyr393-unphosphorylated forms of Bcr-Abl with respective IC_{50} values of 72 and 30 nM, suggesting that NS-187 may have a sufficiently high affinity for Bcr-Abl to enable it to bind even to an unfavorable conformation [22].

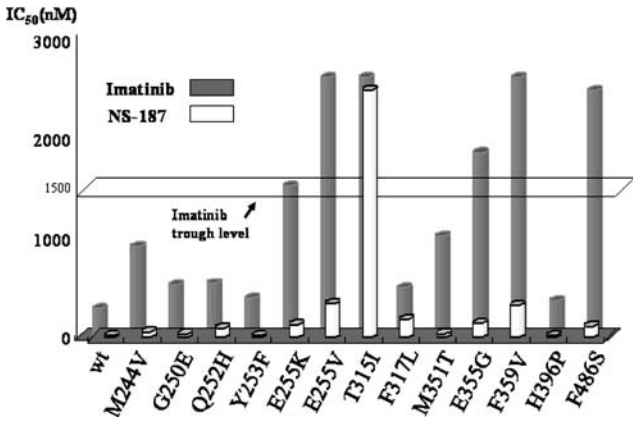

Fig. 3 NS-187 inhibits the tyrosine phosphorylation of 12 of 13 known kinase domain point mutants. IC_{50} values of imatinib (*filled square*) and NS-187 (*open square*) against wild-type or each mutated Bcr-Abl are presented

Inhibitory effects of NS-187 against 79 tyrosine kinases

Seventy-nine tyrosine kinases including the five Src-family proteins Blk, Src, Fyn, Lyn, and Yes were assayed with KinaseProfiler™ (Upstate, Dundee, UK). The effects of NS-187 0.1 μM and imatinib 10 μM were tested, because these concentrations gave equal inhibition of Abl. At a concentration of 0.1 μM, NS-187 inhibited 4 of 79 tyrosine kinases—Abl, Arg, Fyn, and Lyn. Notably, at 0.1 μM, NS-187 did not inhibit PDGFRα, PDGFRβ, Blk, Src, or Yes. In contrast, imatinib 10 μM inhibited nine tyrosine kinases—Abl, Arg, Blk, Flt3, Fyn, Lyn, PDGFRα, PDGFRβ, and p70S6K, indicating that NS-187 inhibited Abl more specifically than did imatinib. The IC_{50} values of NS-187 for Abl, Src, and Lyn were 5.8, 1,700, and 19 nM, respectively, and those of imatinib were 106, >10,000, and 352 nM, respectively [25]. These findings suggest that NS-187 acts as an Abl-Lyn inhibitor, while otherwise remaining highly specific for Abl.

In vivo effects of NS-187

Balb/c-nu/nu mice were injected subcutaneously with KU812 cells on Day 0 and given NS-187 or imatinib orally twice a day from Days 7 to 17. At 20 mg/kg/day, imatinib inhibited tumor growth slightly, while at 200 mg/kg/day, it inhibited tumor growth almost completely. In contrast, at only 0.2 mg/kg/day NS-187 significantly inhibited tumor growth, while at 20 mg/kg/day it completely inhibited tumor growth without any adverse effects. When mice were treated with 0.2 or 20 mg/kg/day of NS-187, the estimated C_{max} was 4 or 400 nM, respectively, suggesting that the in vivo effects of NS-187 were comparable to its in vitro effects. Thus, NS-187 was at least tenfold more potent than imatinib in vivo with complete inhibition of tumor growth as the end-point and at least 100-fold more potent with partial inhibition as the end-point. NS-187 was well tolerated by the mice.

We also tested the ability of NS-187 to suppress tumor growth in another murine tumor model, namely, Balb/c-nu/nu mice intravenously transplanted with BaF3/wild-type cells. The mice were treated orally with NS-187 or imatinib for 11 days starting on Day 1. All seven untreated mice had died by Day 23 due to leukemic cell expansion, while all mice treated with imatinib 400 mg/kg/day had died by Day 25. Thus, imatinib had little effect on tumor growth in this model, whereas NS-187 significantly prolonged the survival of the mice in a dose-dependent manner compared with untreated mice [25].

We next examined the ability of NS-187 to block the in vivo growth of BaF3 cells expressing mutated Bcr-Abl in Balb/c-nu/nu mice. BaF3/M244V, G250E, Q252H, Y253F, E255K, T315I, M351T, or H396P-bearing mice were treated with NS-187 or imatinib. Mice that received BaF3 cells expressing Bcr-Abl/wild-type or any mutant form of Bcr-Abl except Bcr-Abl/T315I showed significant prolongation of survival by NS-187 at a dosage of 200 mg/kg/day without any apparent signs of toxicity. This is consistent with the in vitro results. In contrast, imatinib even at a dosage of 400 mg/kg/day was much less effective. NS-187 resulted in the highest observed *T*/*C* (%) values in BaF3 cells expressing Bcr-Abl/wild-type, Q252H, or M351T, in good agreement with the in vitro data. Moreover, the rank-order of the IC_{50} values for cell growth inhibition was clearly inversely correlated with *T*/*C* (%) in NS-187-treated mice. Thus, the in vitro activity of NS-187 mirrored its efficacy in the in vivo mouse model of leukemia, a result that suggests that NS-187 will be clinically effective [22].

NS-187 against central nervous system leukemia

The penetration of imatinib into the central nervous system (CNS) is poor. Hence the CNS becomes a sanctuary site for leukemic cells in patients who are on prolonged imatinib therapy. P-glycoprotein (P-gp) plays an important role in limiting the distribution of imatinib to the CNS, and it is well-known that imatinib is a substrate of P-gp. In our preliminary pharmacokinetic study, the intracranial concentration of NS-187 was 10% of its serum concentration, suggesting the involve-

ment of P-gp. Even though NS-187 was found to be a substrate for P-gp, it inhibited the proliferation of leukemic cells in the brain, whereas imatinib did not. Moreover, NS-187 significantly prolonged the survival of the mice in a dose-dependent manner in both murine models compared with imatinib, suggesting that NS-187 may prevent or cure CNS leukemia in patients [37].

Conclusions

NS-187 more potently inhibits Abl kinase activity than does imatinib, and its inhibitory activity is less affected by point mutations in the Abl kinase domain. Moreover, NS-187 also inhibits Lyn while otherwise maintaining a high specificity for Bcr-Abl. There are other Bcr-Abl inhibitors being developed for imatinib-resistant CML including dasatinib by Bristol-Myers Squibb and AMN107 by Novartis (East Hanover, NJ, USA), which are currently in Phase II studies. Because there are differences in the profile of kinase inhibition and affinity to Abl among dasatinib, AMN107, and NS-187, we think that patients will require multiple drugs over the course of CML and Ph^+ALL treatment. Due to its significant effects, NS-187 should be effective not only in treating imatinib-resistant CML patients, but also those who have been previously untreated. The ability of NS-187 specifically to target the Bcr-Abl and Lyn kinases may result in an improved side-effect profile compared with agents that target multiple kinases, such as Src inhibitors. A multi-center phase I clinical study of NS-187 (NS-187 has been renamed as INNO-406) will be initiated in the USA in the middle of 2006. The study will include patients with all stages of imatinib-resistant CML. The efficacy and safety of NS-187 for Ph^+ leukemias is expected to be verified by early-phase clinical trials.

References

1. Asaki T, Sugiyama Y, Hamamoto T, Higashioka M, Umehara M, Naito H, Niwa T (2006) Design and synthesis of 3-substituted benzamide derivatives as Bcr-Abl kinase inhibitors. Bioorg Med Chem Lett 16:1421–1425
2. Cary LA, Klinghoffer RA, Sachsenmaier C, Cooper JA (2002) SRC catalytic but not scaffolding function is needed for integrin-regulated tyrosine phosphorylation, cell migration, and cell spreading. Mol Cell Biol 22:2427–2440
3. Cortes J, Giles F, O'Brien S, Thomas D, Garcia-Manero G, Rios MB, Faderl S, Verstovsek S, Ferrajoli A, Freireich EJ, Talpaz M, Kantarjian H (2003) Result of high-dose imatinib mesylate in patients with Philadelphia chromosome-positive chronic myeloid leukemia after failure of interferon-alpha. Blood 102:83–86
4. Dai Y, Rahmani M, Corey SJ, Dent P, Grant S (2004) A Bcr/Abl-independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2. J Biol Chem 279:34227–34229
5. Daley GQ, Van Etten RA, Baltimore D (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247:824–830
6. Davis ME, Cai H, McCann L, Fukai T, Harrison DG (2003) Role of c-Src in regulation of endothelial nitric oxide synthase expression during exercise training. Am J Physiol Heart Circ Physiol 284:1449–1453
7. Deininger M, Buchdunger E, Druker BJ (2005) The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 105:2640–2653
8. Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, Talpaz M (2003) BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. Blood 101:690–698
9. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344:1038–1042
10. Druker BJ (2003) Chronic myeloid leukemia in the imatinib era. Semin Hematol 40(2 Suppl 2):1–3
11. Elefanty AG, Hariharan IK, Cory S (1990) bcr-abl, The hallmark of chronic myeloid leukaemia in man, induces multiple haematopoietic neoplasms in mice. EMBO J 9:1069–1078
12. Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, Frost P, Ye F, Boschelli DH, Boschelli F (2003) SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. Cancer Res 63:375–381
13. Goldman JM, Melo JV (2003) Chronic myeloid leukemia—advances in biology and new approaches to treatment. N Engl J Med 349:1451–1464
14. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293:876–880
15. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J (1990) Acute leukaemia in bcr/abl transgenic mice. Nature 344:251–253
16. Hofmann WK, Jones LC, Lemp NA, de Vos S, Gschaidmeier H, Hoelzer D, Ottmann OG, Koeffler HP (2002) Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. Blood 99:1860–1862
17. Hochhaus A, La Rosee P (2004) Imatinib therapy in chronic myelogenous leukemia: strategies to avoid and overcome resistance. Leukemia 18:1321–1331
18. Kantarjian HM, Smith TL, O'Brien S, Beran M, Pierce S, Talpaz M (1995) Prolonged survival in chronic myelogenous leukemia after cytogenetic response to interferon-α therapy. Ann Intern Med 122:254–261
19. Kantarjian H, Talpaz M, O'Brien S, Garcia-Manero G, Verstovsek S, Giles F, Rios MB, Shan J, Letvak L, Thomas D, Faderl S, Ferrajoli A, Cortes J (2004) High-dose imatinib mesylate therapy in newly diagnosed Philadelphia chromosome-positive chronic phase chronic myeloid leukemia. Blood 103:2873–2878
20. Kimura S, Naito H, Segawa H, Kuroda J, Yuasa T, Sato K, Yokota A, Kamitsuji Y, Kawata E, Ashihara E, Nakaya Y, Naruoka H, Wakayama T, Nasu K, Asaki T, Niwa T,

Hirabayashi K, Maekawa T (2005) NS-187, a potent and selective dual Bcr-Abl/Lyn tyrosine kinase inhibitor, is a novel agent for imatinib-resistant leukemia. Blood 106:3948–3954
21. Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, Castaneda S, Cornelius LA, Das J, Doweyko AM, Fairchild C, Hunt JT, Inigo I, Johnston K, Kamath A, Kan D, Klei H, Marathe P, Pang S, Peterson R, Pitt S, Schieven GL, Schmidt RJ, Tokarski J, Wen ML, Wityak J, Borzilleri RM (2004) Discovery of N-(2-chloro-6-methyl-phenyl)-2- (6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino) thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem 47:6658–6661
22. Naito H, Kimura S, Nakaya Y, Naruoka H, Kimura S, Ito S, Wakayama T, Maekawa T, Hirabayashi K (2006) In vivo inhibitory effect of NS-187, a dual Bcr-Abl/Lyn tyrosine kinase inhibitor, on the proliferation of leukemic cells harbouring Abl kinase domain mutations. Leuk Res 30:1443–1446
23. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, Clarkson B, Kuriyan J (2002) Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). Cancer Res 62:4236–4243
24. Nardi V, Azam M, Daley GQ (2004) Mechanisms and implications of imatinib resistance mutations in BCR-ABL. Curr Opin Hematol 11:35–43
25. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ IRIS Investigators (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348:994–1004
26. O'Brien S, Giles F, Talpaz M, Cortes J, Rios MB, Shan J, Thomas D, Andreeff M, Kornblau S, Faderl S, Garcia-Manero G, White K, Mallard S, Freireich E, Kantarjian HM (2003) Results of triple therapy with interferon-alpha, cytarabine, and homoharringtonine, and the impact of adding imatinib to the treatment sequence in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in early chronic phase. Cancer 98:888–893
27. O'Hare T, Pollock R, Stoffregen EP, Keats JA, Abdullah OM, Moseson EM, Rivera VM, Tang H, Metcalf CA III, Bohacek RS, Wang Y, Sundaramoorthi R, Shakespeare WC, Dalgarno D, Clackson T, Sawyer TK, Deininger MW, Druker BJ (2004) Inhibition of wild-type and mutant Bcr-Abl by AP23464, a potent ATP-based oncogenic protein kinase inhibitor: implications for CML. Blood 104:2532–2539
28. Ohnishi K, Ohno R, Tomonaga M, Kamada N, Onozawa K, Kuramoto A, Dohy H, Mizoguchi H, Miyawaki S, Tsubaki K, Miura Y, Omine M, Kobayashi T, Naoe T, Ohshima T, Hirashima K, Ohtake S, Takahashi I, Morishima Y, Naito K, Asou N, Tanimoto M, Sakuma A, Yamada K, The Kouseisho Leukemia Study Group (1995) A randomized trial comparing interferon-alpha with busulfan for newly diagnosed chronic myelogenous leukemia in chronic phase. Blood 86:906–916
29. Ptasznik A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM (2004) Short interfering RNA (siRNA) targeting the lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL1(+) leukemia cells. Nat Med 10:1187–1189
30. Sawyers CL (1999) Chronic myeloid leukemia. N Engl J Med 340:1330–1340
31. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL (2004) Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 305:399–401
32. Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C, Goldman JM, Guilhot F, Kantarjian HM, Lichtin AE, Talpaz M, Tura S (1999) An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. Blood 94:1517–1536
33. Tanaka S, Amling M, Neff L, Peyman A, Uhlmann E, Levy JB, Baron R (1996) c-Cbl is downstream of c-Src in a signalling pathway necessary for bone resorption. Nature 383:528–531
34. Touyz RM, Wu XH, He G, Park JB, Chen X, Vacher J, Rajapurohitam V, Schiffrin EL (2001) Role of c-Src in the regulation of vascular contraction and Ca^{2+} signaling by angiotensin II in human vascular smooth muscle cells. J Hypertens 19:441–449
35. Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A, Huntly B, Fabbro D, Fendrich G, Hall-Meyers E, Kung AL, Mestan J, Daley GQ, Callahan L, Catley L, Cavazza C, Azam M, Neuberg D, Wright RD, Gilliland DG, Griffin JD (2005) Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell 7:129–141
36. Wisniewski D, Lambek CL, Liu C, Strife A, Veach DR, Nagar B, Young MA, Schindler T, Bornmann WG, Bertino JR, Kuriyan J, Clarkson B (2002) Characterization of potent inhibitors of the Bcr-Abl and the c-kit receptor tyrosine kinases. Cancer Res 62:4244–4255
37. Yokota A, Kimura S, Oyama T, Ashihara E, Naito H, Urasaki Y, Ueda T, Terui Y, Hatake K, Hirabayashi K, Maekawa T (2005) Anti-tumor activity of a novel Bcr-Abl/Lyn dual inhibitor, NS-187, in murine CNS Ph+ leukemia models. Blood 106(11 Suppl):148a