

Activity of the Bcr-Abl Kinase Inhibitor PD180970 against Clinically Relevant Bcr-Abl Isoforms That Cause Resistance to Imatinib Mesylate (Gleevec, STI571)¹

Paul La Rosée, Amie S. Corbin, Eric P. Stoffregen, Michael W. Deininger, and Brian J. Druker²

Oregon Health and Sciences University Cancer Institute, Division of Hematology and Medical Oncology, Portland, Oregon 97239 [P. L. R., A. S. C., E. P. S., M. W. D., B. J. D.], and III. Medizinische Universitätsklinik, Fakultät für klinische Medizin Mannheim der Universität Heidelberg, 68305 Mannheim, Germany [P. L. R.]

Abstract

Imatinib mesylate, a selective inhibitor of the Abl tyrosine kinase, is effective as a single-agent therapy for chronic myelogenous leukemia. However, resistance has been reported, particularly in patients with advanced-stage disease. Mutations within the Abl kinase domain are a major cause of resistance, demonstrating that Bcr-Abl remains a critical drug target. Recently, a novel pyrido[2,3-d]pyrimidine derivative, PD180970, has been shown to potently inhibit Bcr-Abl and induce apoptosis in Bcr-Abl-expressing leukemic cells. We analyzed the inhibitory activity of PD180970 against Abl kinase domain mutations and cells expressing clinically relevant mutations. Our data indicate that PD180970 is active against several Bcr-Abl mutations that are resistant to imatinib and support the notion that developing additional Abl kinase inhibitors would be useful as a treatment strategy for chronic myelogenous leukemia.

Introduction

CML³ is a clonal hematopoietic stem cell disorder. It is characterized by excess proliferation of myeloid progenitors that retain the capacity for differentiation during the stable or chronic phase of the disease. After 4–6 years, the disease inevitably transforms into a fatal acute leukemia, referred to as blast crisis. Multiple lines of evidence have established the deregulated Bcr-Abl tyrosine kinase, the product of the Philadelphia chromosome, as the causative oncogene of CML (1). The introduction of imatinib mesylate (Gleevec, STI571), a 2-phenylaminopyrimidine and selective inhibitor of the Bcr-Abl tyrosine kinase, has demonstrated that a precise understanding of the molecular pathogenesis of a malignancy can lead to the development of a safe and effective therapy (2, 3). Nearly all patients with early-stage CML achieve a complete hematological remission, and in 40–70% of these patients, the Philadelphia chromosome is no longer detected using standard cytogenetic assays (2, 4, 5). Although 60% of patients in blast crisis respond to imatinib, the majority relapse despite continued therapy (3, 6).

In an effort to elucidate possible mechanisms of resistance, we performed biochemical analyses of various mutant isoforms of the isolated Abl kinase domain that were presumed to be essential for drug binding (7, 8). In line with predictions made from the crystal

structure of the Abl kinase in complex with imatinib (9, 10), we found that mutations at position T315 and Y253 are capable of disrupting the binding of imatinib. These predictions were subsequently verified by the finding that these and other mutations of the Abl kinase domain are present in clinical samples of resistant or relapsed patients (11–20). The reported detection frequency of such mutations ranges between 30% and 90%. In these reports, the most frequently mutated residues are amino acids 253, 255, 315, and 351 and account for approximately 60% of all reported mutations. The crystal structure of imatinib with the Abl kinase demonstrates that imatinib captures a specific inactive conformation of the Abl kinase (9, 10). Predictions from this structure suggest that some mutations may disrupt the unique conformation of the Abl kinase domain required for imatinib binding. In contrast, other Abl kinase domain mutants found in patients interfere with imatinib binding to Abl because they interrupt points of direct contact.

Given that the Bcr-Abl kinase is reactivated in relapsed patients, there has been extensive interest in identifying other Abl kinase inhibitors capable of inhibiting some or all of these mutant Abl kinases. One series of compounds of the pyrido[2,3-d]pyrimidine family, originally developed as Src family inhibitors (21), has also been found to have potent Abl inhibitory activity (22, 23). For example, PD180970 potently inhibited autophosphorylation of p210^{Bcr-Abl} and induced apoptosis of K562 cells, a CML blast crisis cell line. Interestingly, the crystal structure of a compound related to PD180970 with the Abl kinase has shown that it binds to a conformation of Abl in which the a-loop resembles that of an active kinase (9). These data suggest that compounds of this class may be capable of inhibiting some of the mutated Abl kinases found in relapsed patients, particularly those in which the Abl kinase is in an active configuration. This hypothesis was tested using isolated kinase domains and cell lines expressing Bcr-Abl with various mutations identified in relapsed patients. In particular, all of the commonly mutated residues were tested along with additional, representative mutations in the phosphate-binding domain and a-loop.

Materials and Methods

Site-directed Mutagenesis and Generation of Vectors. Mutagenesis for the generation of purified GST-Abl kinase was performed as described previously (8). Briefly, PCR with primers containing appropriate point mutations was used to amplify the Abl kinase domain consisting of c-Abl amino acids 220–498 subcloned into the *Bam*HI site of pGEX KG (Amersham Pharmacia). The presence of the mutations was confirmed by sequencing.

To introduce the mutations into full-length p210^{Bcr-Abl}, we performed PCR-based site-directed mutagenesis of the *Aat*II/*Kpn*I Abl kinase fragment subcloned into pGEM7 (Promega). This fragment was subcloned into full-length p210^{Bcr-Abl} (pGEM5-p210^{Bcr-Abl}), and the final product was subcloned into the *Eco*RI site of the mammalian expression vector pSR α .

Kinase Assays. Kinase assays of GST fusion proteins (wild-type and mutant Abl kinase) were performed as described previously (8). Quantitation

Received 9/17/02; accepted 10/31/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ B. J. D. is supported by grants from the National Cancer Institute, The Leukemia and Lymphoma Society, Burroughs Wellcome Fund, T. J. Martell Foundation, and the Doris Duke Charitable Foundation. P. L. R. is recipient of a postdoctoral grant of the Deutsche Krebshilfe, Dr. Mildred Scheel Stiftung, Germany. B. J. D. is consultant for Novartis Pharmaceuticals Inc.

² To whom requests for reprints should be addressed, at Oregon Health and Sciences University Cancer Institute, L592, 3181 Sam Jackson Park Road, Portland, OR 97239. Phone: (503) 494-5596; Fax: (503) 494-3366; E-mail: drukerb@ohsu.edu.

³ The abbreviations used are: CML, chronic myelogenous leukemia; GST, glutathione S-transferase; IL-3, interleukin 3; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; a-loop, activation loop; p-loop, ATP-binding loop.

of the Abl autophosphorylation signal intensity was performed with a PhosphorImager (Molecular Dynamics), and means from two to three independent experiments were calculated to determine the IC_{50} values. Abl immunoblots to demonstrate equal protein loading were performed with α -Abl Ab-2 (Oncogene) as described previously (8).

Infection of Murine Hematopoietic Cells. Bosc23 cells (24) were maintained in DMEM supplemented with 10% fetal bovine serum, 1 unit/ml penicillin, and 1 μ g/ml streptomycin. For the production of wild-type and mutant p210^{Bcr-Abl} as well as control retrovirus, Bosc23 cells were transiently transfected with pSR α -p210^{Bcr-Abl} or pSR α control plasmid, and the viral supernatant was harvested at 48 h after transfection. Ba/F3 cells were incubated with filtered retroviral supernatants containing 8 mg/ml polybrene and 15% WEHI conditioned media as the source of IL-3. Subsequent selection for stable transfectants was performed by maintaining cells in RPMI 1640 supplemented with 10% serum, 1 unit/ml penicillin, 1 μ g/ml streptomycin, and 0.75 mg/ml G418. Stably expressing cells were then further selected by removal of IL-3. After confirmation of Bcr-Abl expression by immunoblot analysis, cells were used in proliferation assays.

Proliferation Assays. Tetrazolium-based proliferation assays (MTT assay) were performed as described previously (25). Cells were plated in triplicate at 5×10^3 cells/well in 96-well microtiter plates. MTT uptake (absorbance at 570 nm) was assayed daily to ensure exponential growth of the untreated cells. The mean was calculated for each concentration of the drug. Means and SDs were generated from three to four independent experiments and reported as the percentage absorbance of control. Cell proliferation curves were derived from these data, and best fit curves were generated in Microsoft Excel. Results from day 3 were used to calculate the IC_{50} .

Drug Preparation. PD180970 (21) was kindly provided by Pfizer Global Research and Development (Ann Arbor, MI). The crystalline compound was dissolved as a 10 mM stock solution in 100% DMSO (Sigma), and aliquots were kept at $-20^{\circ}C$. Experiments were performed with serial dilutions of the 10 mM stock. Controls in biochemical and cellular assays were performed with solvent vector (DMSO; control).

Immunoblotting. Detection of Bcr-Abl expression was performed using standard Western blotting procedures. Equal amounts of cells were analyzed by immunoblotting with anti-ABL (8E9; kindly provided by Jean Y. J. Wang, University of California San Diego, La Jolla, CA).

Results and Discussion

Mutations within the a-loop Are Sensitive to PD180970. The a-loop of the Abl kinase begins at amino acid 381 with the highly conserved DFG motif, and beginning at this motif, the structures of Abl bound to imatinib and the PD180970-related compound diverge significantly (9). This region of the Abl kinase can adopt a closed or inactive conformation and an open or active conformation. Imatinib forces Abl into the closed or inactive conformation and is incapable of binding to the open, active configuration. In contrast, the PD180970-related compound is capable of binding to Abl regardless of its conformation. Therefore, mutations that stabilize the kinase in an active conformation are predicted to interfere with imatinib binding. One such mutation, observed in relapsed patients, is at histidine 396 (His³⁹⁶), which is very close to tyrosine 393, a key residue in regulating kinase activation (26). Replacement of H³⁹⁶ with proline (H396P) is predicted to favor an open, active conformation of the a-loop. Subsequently, binding of imatinib would be prevented. In contrast, because the PD180970-related compound has been shown to bind the kinase independent of the a-loop conformation, it may not be affected by kinase activation-inducing mutations (26). Consistent with these predictions, GST-Abl-H396P is as sensitive to PD180970 as wild-type Abl. This is in contrast to the significant increase in the IC_{50} observed for this mutation to imatinib (Fig. 1; Table 1). This mutation was also evaluated using reconstituted, growth factor-independent Ba/F3 cells with mutated p210^{Bcr-Abl-H396P}. MTT assays with imatinib and PD180970 were performed, and IC_{50} values were determined after 3 days of continuous drug exposure. IL-3-dependent Ba/F3 cells containing vector only (Ba/F3-pSR α) were used to detect

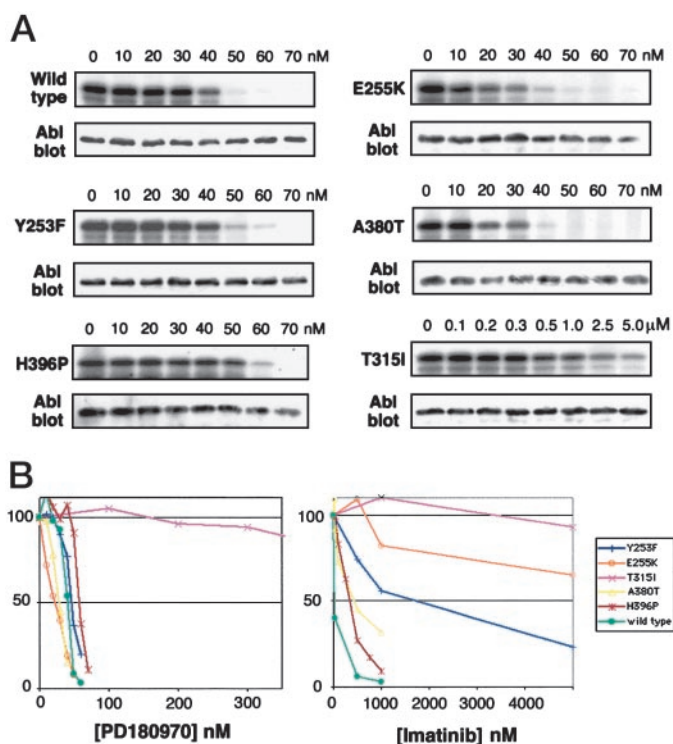


Fig. 1. Kinase assays of wild-type and Abl kinase domain mutations in the presence of PD180970. **A**, autophosphorylation of the Abl kinase domain was analyzed for wild-type, E255K, Y253F, A380T, H396P, and T315I in the presence of escalating doses of PD180970. Representative kinase assay gels are shown. Abl blots demonstrate equal protein loading. **B**, representative dose-response curves for PD180970 and imatinib kinase assays. Mutations E255K, A380T, H396P, and Y253F show intermediate or complete insensitivity to imatinib [data were reported previously in part (8, 12), for imatinib only] but retain full sensitivity to PD180970. T315I is equally insensitive to both inhibitors.

nonspecific cytotoxicity. Ba/F3-p210-H396P cells reveal a sensitivity profile to PD180970 that is very similar to Ba/F3-p210 wild-type cells, whereas the activity of imatinib differs significantly, indicating intermediate resistance induced by mutation H396P (Fig. 2; Table 1).

To further evaluate this concept of differential drug sensitivity due to the differences in target recognition governed by the a-loop topology, we substituted the alanine at amino acid 380, adjacent to the DFG motif, with threonine (A380T). Although not a mutation found in patient samples, it is adjacent to V379I, a recently described mutation in a chronic-phase patient who failed to obtain a cytogenetic response to imatinib (20). The IC_{50} value of PD180970 for GST-Abl-A380T is 43 nM, which is almost identical to that observed for wild-type Abl (Fig. 1; Table 1). In contrast, A380T has a 14-fold higher IC_{50} for imatinib. Proliferation assays using Ba/F3-p210-A380T cells in the presence of PD180970 showed an even lower IC_{50} value compared with the wild-type Bcr-Abl-expressing control cells (Fig. 2; Table 1), whereas the dose-response curve in the imatinib assay shows a shift to the right, with an 8-fold increase of the IC_{50} . Thus, consistent with predictions from the crystal structure, PD180970 is capable of inhibiting mutations, such as H396P or A380T, that force Abl to adopt the open, active configuration, whereas imatinib cannot inhibit these mutations.

Imatinib-resistant Mutations within the ATP-binding Loop (p-loop) Are Sensitive to PD180970. The ATP binding domain is defined by a glycine-rich sequence (GXGXXG) that spans amino acids 249–254. The nonconserved residues, 250, 252, and 253, and the adjacent residue 255 have all been identified as mutated in patient samples, with 253 and 255 being among the most common. Because both imatinib and PD180970 function as competitive inhibitors of

Table 1 IC_{50} values (in nM) of two alternative Abl kinase inhibitors against various Abl kinase mutants derived from biochemical (kinase assay) and cell based (MTT-assay) assays

	Imatinib mesylate		PD180970	
	Kinase assay	MTT assay	Kinase assay	MTT assay
Vector only		>10,000		710
Wild-type	25	300	45	25
Q252H	ND	2,800	ND	130
Y253F	1,800	3,040	48	120
E255K	>5,000	4,400	33	140
T315I	>5,000	>10,000	3,200	840
M351T	ND	930	ND	45
A380T	340	2,450	43	5
H396P	340	4,200	48	15

ATP binding, there are extensive contacts between these compounds and this domain. However, all of the interactions between imatinib, PD180970, and this domain of the Abl kinase are van der Waals interactions. Due to its glycine-rich content, this domain has considerable flexibility, thus allowing these compounds to induce a conformation favorable to their binding. Whereas the p-loop of the Abl kinase undergoes massive distortion as part of the induced fit with imatinib, the PD180970-related compound binds to this region without major clashes (9).

Tyrosine 253 interacts with both imatinib and PD180970 through van der Waals interactions. Mutation of this residue to phenylalanine, as observed in relapsed patients, confers intermediate resistance to imatinib in biochemical (8, 19) as well as cell-based assays using reconstituted Ba/F3-p210-Y253F cells (Ref. 20; Table 1). Despite the interaction between this residue and the PD180970 compound, the IC_{50} in the presence of PD180970 remains unchanged in biochemical assays (Fig. 1; Table 1). The results generated in cell-based assays reveal a 5-fold increase of IC_{50} for PD180970 (120 nM versus 25 nM). The differential sensitivity profile of Y253F in a biochemical assay compared with a cellular environment was even more significant with imatinib (3040 nM versus 300 nM) and has been attributed to the complex intracellular regulation of the Abl kinase that would ultimately influence the binding affinity of small molecule inhibitors (19). However, it is clear that PD180970 is much more potent against Y253F as compared with imatinib.

Mutations at position 255 have been reported by several groups and lead to replacement of glycine by either lysine (E255K) or valine (E255V). Amino acid 255 is located within the nucleotide-binding region of the kinase domain adjacent to Val²⁵⁶, a predicted contact point for both imatinib and the PD180970-related compound (9, 10). We reported a significant increase of the IC_{50} value of E255K for imatinib to $>5.0 \mu\text{M}$ in kinase assays, thereby classifying this Abl mutant as highly resistant (12). Analysis of the inhibitory potential of PD180970 against E255K in kinase assays revealed a sensitivity profile that is very similar to wild-type Abl with an IC_{50} value of 33 nM. Similar to Y253F, results obtained in the cell-based assays differ slightly from the findings in the biochemical analysis, with a 5-fold increased IC_{50} relative to wild-type Bcr-Abl, but confirm the sensitivity of E255K to PD180970. Cell line proliferation data obtained for Q252H, another clinically relevant representative of p-loop mutations, are very similar to those for Y253F and E255K (Fig. 2).

These data indicate that PD180970 has significantly more activity against p-loop mutants than imatinib. Presumably, the insensitivity of these mutations to imatinib is due to the inability of the mutated p-loop to adopt the conformation required for imatinib binding. In contrast, because PD180970 can bind to the p-loop without inducing a major conformational change, it is assumed that these mutations have much less effect on PD180970 binding to Abl and that, as a consequence, these Abl kinase domain mutations remain sensitive to PD180970.

T315I Is Resistant to Both Imatinib and PD180970. A common feature of the crystal structures of imatinib and the PD180970-related compound with the Abl kinase is that threonine 315 contacts both compounds. With imatinib, this residue forms a hydrogen bond, and with the PD180970-related compound, it forms a van der Waals interaction (9). We demonstrated previously that mutations at this residue result in complete insensitivity of the Abl kinase to imatinib (7, 8). A mutation of this residue to isoleucine (T315I) was subsequently the first and is now known to be one of the most common mutations identified in patients who relapse after responding to imatinib (11, 12, 15, 16, 18, 20). Therefore, we were interested in the ability of PD180970 to inhibit this mutant. As illustrated in Fig. 1, T315I induces a shift of the dose-response curve to the right, resulting in a dramatic increase of the IC_{50} value as compared with wild-type Abl. This mirrors the findings for imatinib (Fig. 1B) and demonstrates the likely importance of this contact point for drug binding with PD180970 as well. Steric inhibition of drug binding by the T315I mutation is also possible; however, only crystallographic studies will be able to determine the exact target-drug interaction at this mutated residue.

This mutation was also evaluated using IL-3-independent Ba/F3 cells expressing mutated p210^{Bcr-Abl-T315I}. As shown in Fig. 2, Ba/F3-p210-T315I cells are completely insensitive to imatinib at doses up to $10 \mu\text{M}$, with a dose-response curve resembling that of control cells (Ba/F3-pSR α). In accordance with the findings in the kinase assay, similar results were also obtained with PD180970. Specifically, the only growth inhibition that was observed occurred at doses greater than 250 nM, with equal inhibition of control cells. The most likely explanation for the difference in cytotoxicity of imatinib and PD180970 against control cells has to do with the different spectrum of kinases inhibited by these compounds. Because PD180970 inhibits the growth of control cells and cells expressing p210-315I at similar doses, this is consistent with this Abl mutation being insensitive to this compound.

These data indicate that PD180970 would not be predicted to be useful in a patient who relapses as a consequence of a point mutation at amino acid 315. Whether other inhibitors related to PD180970 can inhibit T315I is unknown. However, our data point out the difficulty in generating one compound that can inhibit wild-type Abl kinase and all of the mutated forms. Another strategy would be to down-regulate Bcr-Abl expression, *i.e.*, using Hsp90 inhibitors (27, 28). Alternatively, inhibition of signaling pathways downstream of Bcr-Abl could be useful, for example, targeting Ras (29), mitogen-activated protein kinase (30), or phosphatidylinositol 3'-kinase (31).

M351T Is Also Inhibited by PD180970. A substitution of threonine for methionine at amino acid 351 (M351T) is another frequent mutation of the Abl kinase that causes imatinib resistance in CML patients (12, 16, 20). It is not part of the ATP binding p-loop, nor is it within the a-loop. It appears, however, to have a close topologic

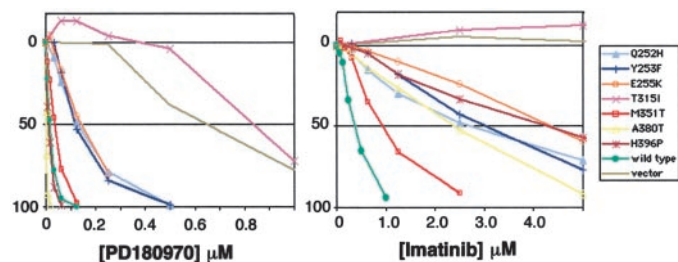


Fig. 2. Ba/F3 and Ba/F3-p210 dose-response curves generated by MTT proliferation assays. Data from day 3 are plotted as the concentration of inhibitor versus inhibition of proliferation. Each data point represents a mean across three to four independent experiments. Data from single experiments were created by means of quadruplicate analysis.

relation to the base of the a-loop, which may explain its ability to influence binding of imatinib. Our previously reported biochemical data revealed a >80% reduction of the isolated Abl kinase domain activity, which made it technically impossible to perform biochemical assays (12). Therefore, we focused our studies on analyzing drug sensitivity of this Abl kinase isoform to cell line proliferation assays. As illustrated in Fig. 2A, the inhibitory activity of PD180970 is not impaired in cells expressing M351T, whereas, consistent with previously reported data (20), Ba/F3-p210 cells expressing this mutation have intermediate sensitivity to imatinib (Fig. 2B; Table 1).

Implications of These Findings. The most common mutations in relapsed patients are Y253F, E255K, T315I, and M351T, accounting for approximately 60% or more of all mutations that were found to be associated with imatinib resistance. Some of these mutations have intermediate sensitivity to imatinib and may be at least partially sensitive to higher dose imatinib therapy. However, given that 5 μM is about the maximum level of imatinib that can be achieved *in vivo*, treatment with an inhibitor with more potency against these mutations would likely result in more meaningful responses. The fact that our study showed favorable activity of PD180970 against Y253F, E255K, M351T, Q252H, and H396P points to the encouraging notion that many of the common mutations that are associated with imatinib resistance could be combated by an alternative Bcr-Abl-specific inhibitor.

As additional Abl inhibitors become available, molecular genotyping would be extremely useful in selecting the appropriate inhibitor for relapsed patients. Furthermore, recent reports demonstrate that in some cases, Abl kinase mutations are detectable before treatment with imatinib. Therapy with imatinib, under such circumstances, would select for the resistant clones. In this scenario, one could imagine performing molecular genotyping before the initiation of therapy and, theoretically, treating with a mixture of Abl inhibitors to target all mutations present, analogous to what is currently performed for HIV therapy. In this manner, it might be conceivable to prevent the emergence of resistance with front-line therapy.

PD180970 was initially developed as an inhibitor of src kinases and was subsequently shown to inhibit the Abl kinase. Although imatinib does not inhibit Src family members, it does inhibit c-kit. This activity of imatinib has been exploited in the treatment of gastrointestinal stromal tumors. Interestingly, imatinib does not inhibit a mutation in the a-loop of c-kit that is the causative molecular abnormality of the majority of cases of systemic mastocytosis (32, 33). This and similar mutations may also be responsible for resistance to imatinib observed in some cases of gastrointestinal stromal tumor (34). Presumably, resistance to imatinib by mutation of residues in the a-loop of c-kit occurs through similar mechanisms of resistance of Abl a-loop mutations, that is, the inability of imatinib to bind to the active conformation of these kinases. Recently, PD180970 and related compounds were also shown to inhibit wild-type c-kit (23). Given the data for a PD180970 compound binding to the active configuration of the Abl kinase (9) and our data demonstrating that a-loop mutations are sensitive to PD180970, it is likely that a-loop mutations of c-kit will be sensitive to PD180970. Experiments to test this hypothesis are in progress.

The present study demonstrates favorable activity of PD180970 against many of the clinically relevant mutations in CML patients. Wisniewski *et al.* (23) reported preclinical data investigating several new small molecule inhibitors closely related to PD180970, two of which were even more potent Abl inhibitors. However, as we have seen, these compounds have some nonspecific toxicity against cells that do not express Bcr-Abl, presumably due to the profile of kinases inhibited by these compounds, including Src family members. Although there are some data suggesting that Src family members may

be downstream signaling intermediates for Bcr-Abl (35), treatment of Bcr-Abl-expressing cells with PPI, a Src inhibitor, has minimal effects on Bcr-Abl-mediated proliferation (22). *In vivo* testing of PD180970 and related compounds revealed no dose-limiting toxicity. However, due to the relative insolubility of the compounds, no clear dose response could be seen in a mouse xenograft tumor model (36). Whether an inhibitor with the profile of PD180970 will have improved efficacy or more severe side effects than imatinib will require evaluation in animal models and human clinical trials. Obviously, these compounds will have to be formulated into soluble compounds with favorable pharmacokinetic properties before this testing can be adequately performed. Regardless of this, our data support the notion that developing additional Abl kinase inhibitors would be useful as a treatment strategy for CML.

Acknowledgments

We are grateful to Kara Johnson and the Druker laboratory for expert technical advice and helpful discussions and to Sarah Anderson and Chris Koontz for administrative assistance. We also thank Dr. Alan Kraker (Pfizer Global Research and Development, Ann Arbor, MI) for providing PD180970 and for critically reading of the manuscript.

References

1. Deininger, M. W., Goldman, J. M., and Melo, J. V. The molecular biology of chronic myeloid leukemia. *Blood*, 96: 3343–3356, 2000.
2. Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., Lydon, N. B., Kantarjian, H., Capdeville, R., Ohno-Jones, S., and Sawyers, C. L. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.*, 344: 1031–1037, 2001.
3. Druker, B. J., Sawyers, C. L., Kantarjian, H., Resta, D. J., Reese, S. F., Ford, J. M., Capdeville, R., and Talpaz, M. 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, 2001.
4. Druker, B. J., for the IRIS (International Randomized IFN *versus* STI571) Study Group. STI571 (Gleevec/Glivec, imatinib) *versus* interferon (IFN) + cytarabine as initial therapy for patients with CML: results of a randomized study. *Proc. Am. Soc. Clin. Oncol.* 21: 1a, 2002.
5. Kantarjian, H., Sawyers, C., Hochhaus, A., Guilhot, F., Schiffer, C., Gambacorti-Passerini, C., Niederwieser, D., Resta, D., Capdeville, R., Zoellner, U., Talpaz, M., and Druker, B. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N. Engl. J. Med.*, 346: 645–652, 2002.
6. Sawyers, C. L., Hochhaus, A., Feldman, E., Goldman, J. M., Miller, C. B., Ottmann, O. G., Schiffer, C. A., Talpaz, M., Guilhot, F., Deininger, M. W., Fischer, T., O'Brien, S. G., Stone, R. M., Gambacorti-Passerini, C. B., Russell, N. H., Reiffers, J. J., Shea, T. C., Chapuis, B., Coutre, S., Tura, S., Morra, E., Larson, R. A., Saven, A., Peschel, C., Gratwohl, A., Mandelli, F., Ben Am, M., Gathmann, I., Capdeville, R., Paquette, R. L., and Druker, B. J. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a Phase II study. *Blood*, 99: 3530–3539, 2002.
7. Corbin, A. S., Toledo, L. M., Lydon, N. B., Buchdunger, E., Kuriyan, J., and Druker, B. J. Analysis of the structural basis of specificity of inhibition of the Abl kinase by STI571. *Blood* 96: 470a, 2000.
8. Corbin, A. S., Buchdunger, E., Pascal, F., and Druker, B. J. Analysis of the structural basis of specificity of inhibition of the Abl kinase by STI571. *J. Biol. Chem.*, 277: 32214–32219, 2002.
9. Nagar, B., Bornmann, W. G., Pellicena, P., Schindler, T., Veach, D. R., Miller, W. T., Clarkson, B., and Kuriyan, J. 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, 2002.
10. Schindler, T., Bornmann, W., Pellicena, P., Miller, W. T., Clarkson, B., and Kuriyan, J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science (Wash. DC)*, 289: 1938–1942, 2000.
11. Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N., and Sawyers, C. L. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science (Wash. DC)*, 293: 876–880, 2001.
12. Kreil, S., Muller, M. C., Lahaye, T., La Rosée, P., Corbin, A. S., Schoch, C., Cross, N. C. P., Berger, U., Rieder, H., Druker, B. J., Gschaidmeier, H., Hehlmann, R., and Hochhaus, A. Molecular and chromosomal mechanisms of resistance in CML patients after STI571 (Gleevec) therapy. *Blood*, 98: 435a, 2001.
13. Hochhaus, A., Kreil, S., Corbin, A., La Rosée, P., Lahaye, T., Berger, U., Cross, N. C., Linkesch, W., Druker, B. J., and Hehlmann, R. Roots of clinical resistance to STI-571 cancer therapy. *Science (Wash. DC)*, 293: 2163, 2001.
14. Barthe, C., Cony-Makhoul, P., Melo, J. V., and Mahon, J. R. Roots of clinical resistance to STI-571 cancer therapy. *Science (Wash. DC)*, 293: 2163, 2001.
15. von Bubnoff, N., Schneller, F., Peschel, C., and Duyster, J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet*, 359: 487–491, 2002.

16. Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K., Herrmann, R., Lynch, K. P., and Hughes, T. P. 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*: 3472–3475, 2002.
17. Roche-Lestienne, C., Soenen-Cornu, V., Grardel-Duflos, N., Lai, J. L., Philippe, N., Facon, T., Fenaux, P., and Preudhomme, C. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood*, *100*: 1014–1018, 2002.
18. Hofmann, W. K., Jones, L. C., Lemp, N. A., de Vos, S., Gschaidmeier, H., Hoelzer, D., Ottmann, O. G., and Koeffler, H. P. Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. *Blood*, *99*: 1860–1862, 2002.
19. Roumiantsev, S., Shah, N. P., Gorre, M. E., Nicoll, J., Brasher, B. B., Sawyers, C. L., and van Etten, R. A. Clinical resistance to the kinase inhibitor STI-571 in chronic myeloid leukemia by mutation of Tyr-253 in the Abl kinase domain P-loop. *Proc. Natl. Acad. Sci. USA*, *99*: 10700–10705, 2002.
20. Shah, N., Nicoll, J., Nagar, B., Gorre, M., Paquette, R., Kuriyan, J., and Sawyers, C. 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*: 117–125, 2002.
21. Kraker, A. J., Hartl, B. G., Amar, A. M., Barvian, M. R., Showalter, H. D., and Moore, C. W. Biochemical and cellular effects of c-Src kinase-selective pyrido[2, 3-d]pyrimidine tyrosine kinase inhibitors. *Biochem. Pharmacol.*, *60*: 885–898, 2000.
22. Dorsey, J. F., Jove, R., Kraker, A. J., and Wu, J. The pyrido[2, 3-d]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells. *Cancer Res.*, *60*: 3127–3131, 2000.
23. Wisniewski, D., Lambek, C. L., Liu, C., Strife, A., Veach, D. R., Nagar, B., Young, M. A., Schindler, T., Bornmann, W. G., Bertino, J. R., Kuriyan, J., and Clarkson, B. Characterization of potent inhibitors of the Bcr-Abl and the c-kit receptor tyrosine kinases. *Cancer Res.*, *62*: 4244–4255, 2002.
24. Pear, W. S., Nolan, G. P., Scott, M. L., and Baltimore, D. Production of high-titer helper-free retroviruses by transient transfection. *Proc. Natl. Acad. Sci. USA*, *90*: 8392–8396, 1993.
25. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, *65*: 55–63, 1983.
26. Dorey, K., Engen, J. R., Kretzschmar, J., Wilm, M., Neubauer, G., Schindler, T., and Superti-Furga, G. Phosphorylation and structure-based functional studies reveal a positive and a negative role for the activation loop of the c-Abl tyrosine kinase. *Oncogene*, *20*: 8075–8084, 2001.
27. Gorre, M. E., Ellwood-Yen, K., Chiosis, G., Rosen, N., and Sawyers, C. L. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. *Blood*, *100*: 3041–3044, 2002.
28. Nimmanapalli, R., O'Bryan, E., and Bhalla, K. Geldanamycin and its analogue 17-allylamino-17-demethoxygeldanamycin lowers Bcr-Abl levels and induces apoptosis and differentiation of Bcr-Abl-positive human leukemic blasts. *Cancer Res.*, *61*: 1799–1804, 2001.
29. Hoover, R. R., Mahon, F. X., Melo, J. V., and Daley, G. Q. Overcoming STI571 resistance with the farnesyl transferase inhibitor SCH66336. *Blood*, *100*: 1068–1071, 2002.
30. Yu, C., Krystal, G., Varticovski, L., McKinstry, R., Rahmani, M., Dent, P., and Grant, S. Pharmacologic mitogen-activated protein/extracellular signal-regulated kinase kinase/mitogen-activated protein kinase inhibitors interact synergistically with STI571 to induce apoptosis in Bcr/Abl-expressing human leukemia cells. *Cancer Res.*, *62*: 188–199, 2002.
31. Klejman, A., Rushen, L., Morrione, A., Slupianek, A., and Skorski, T. Phosphatidylinositol-3 kinase inhibitors enhance the anti-leukemia effect of STI571. *Oncogene*, *21*: 5868–5876, 2002.
32. Ma, Y., Zeng, S., Metcalfe, D. D., Akin, C., Dimitrijevic, S., Butterfield, J. H., McMahon, G., and Longley, B. J. The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. *Blood*, *99*: 1741–1744, 2002.
33. Heinrich, M. C., Blanke, C. D., Druker, B. J., and Corless, C. L. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J. Clin. Oncol.*, *20*: 1692–1703, 2002.
34. Demetri, G. D., von Mehren, M., Blanke, C. D., Van den Abbeele, A. D., Eisenberg, B., Roberts, P. J., Heinrich, M. C., Tuveson, D. A., Singer, S., Janicek, M., Fletcher, J. A., Silverman, S. G., Silberman, S. L., Capdeville, R., Kiese, B., Peng, B., Dimitrijevic, S., Druker, B. J., Corless, C., Fletcher, C. D., and Joensuu, H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.*, *347*: 472–480, 2002.
35. Danhauser-Riedl, S., Warmuth, M., Druker, B. J., Emmerich, B., and Hallek, M. Activation of Src kinases p53/56lyn and p59hck by p210bcr/abl in myeloid cells. *Cancer Res.*, *56*: 3589–3596, 1996.
36. Kraker, A. J., Roberts, B. J., Vincent, P. W., Patmore, S. J., and Elliott, W. L. *In vivo* antitumor activity of selective c-src tyrosine kinase (TK) inhibitors. *Proc. Am. Assoc. Cancer Res.*, *40*: 808, 1999.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Activity of the Bcr-Abl Kinase Inhibitor PD180970 against Clinically Relevant Bcr-Abl Isoforms That Cause Resistance to Imatinib Mesylate (Gleevec, STI571)

Paul La Rosée, Amie S. Corbin, Eric P. Stoffregen, et al.

Cancer Res 2002;62:7149-7153.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/62/24/7149>

Cited articles This article cites 33 articles, 20 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/62/24/7149.full#ref-list-1>

Citing articles This article has been cited by 47 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/62/24/7149.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/62/24/7149>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.