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

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ABSTRACT

Constitutive tyrosine kinase activity of Bcr-Abl promotes proliferation and survival of chronic myelogenous leukemia (CML) cells. Inhibition of Bcr-Abl tyrosine kinase activity or signaling proteins activated by Bcr-Abl in CML cells blocks proliferation and causes apoptotic cell death. The selective Abl kinase inhibitor, STI-571 (marketed as Gleevec), is toxic to CML cells in culture, causes regression of CML tumors in nude mice, and is currently used to treat CML patients. Here we describe a p.o. active, dual Src/Abl kinase inhibitor with potent antiproliferative activity against CML cells in culture. This 4-anilino-3-quinolinecarbonitrile (SKI-606) ablates tyrosine phosphorylation of Bcr-Abl in CML cells and of v-Abl expressed in fibroblasts. SKI-606 inhibits phosphorylation of cellular proteins, including STAT5, at concentrations that inhibit proliferation in CML cells. Phosphorylation of the autoactivation site of the Src family kinases Lyn and/or Hck is also reduced by treatment with SKI-606. Once daily oral administration of this compound at 100 mg/kg for 5 days causes complete regression of large K562 xenografts in nude mice.

INTRODUCTION

The Philadelphia chromosome t(9;22) is a diagnostic cytogenetic marker for CML (for reviews, see Refs. 1–4). This reciprocal translocation fuses the ABL locus on chromosome 9 with the BCR locus on chromosome 22, resulting in expression of the constitutively active protein tyrosine kinase Bcr-Abl. CML usually presents in its chronic phase, when patients have elevated levels of WBCs of myeloid lineage. The disease then progresses to an accelerated phase and blast crisis, characterized by an increasing burden of leukemic cells with accumulated genetic damage. CML patients have been treated with hydroxyurea, IFN-α, alone or in combination with cytotoxic agents, or with stem cell transplants from allogeneic donors (5, 6).

Bcr-Abl transforms myeloid cells in culture and induces leukemia in mice, whereas tyrosine kinase-deficient Bcr-Abl does not confer cytokine independence (7). An inhibitor of Bcr-Abl tyrosine kinase, STI-571, has antiproliferative and proapoptotic activity against CML cells in culture and causes regression of CML xenografts in nude mice (8–10). Recent clinical studies indicate that STI-571 is an effective therapeutic agent for the treatment of chronic phase CML patients, producing relatively mild side effects (11, 12). Patients with more advanced forms of the disease have a less durable response to STI-571 (13, 14). Mitogen-activated protein/extracellular signal-regulated kinase (MEK) and phosphatidylinositol 3-kinase (PI3K) signal transduction molecules including G proteins, are effective and survival of chronic myelogenous leukemia (CML) cells. Inhibition of H-ras signaling reduces transforming activity of Bcr-Abl expressed in fibroblasts or hematopoietic cells (17, 18). Myeloid cells expressing Bcr-Abl undergo apoptotic cell death when dominant negative Ras is expressed, and peptides that disrupt Grb2-Sos complexes also inhibit proliferation of CML cells from blast crisis patients (19). Farnesyltransferase inhibitors, which probably act on a variety of signal transduction molecules including G proteins, are effective antitumor agents against myeloid cells transformed by Bcr-Abl (20, 21). Mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitors act synergistically with STI-571 in CML lines, and antisense or dominant c-Raf inhibits Bcr-Abl-dependent survival (22–24). Inhibition of nuclear factor κB activation by a superrepressing form of IkBα is antitumorigenic in myeloid cells expressing Bcr-Abl, but it is not proapoptotic (25). In addition, Bcr-Abl associates with PI3K and up-regulates its kinase activity, whereas wortmannin, an inhibitor of PI3K, inhibits proliferation of CML cells (26, 27). STAT5 signaling, which is constitutively active in CML cells, is down-regulated by STI-571, and dominant negative STAT5 is toxic to CML cells (28–31). Simultaneous inhibition of any two of the Ras, STAT5, and PI3K pathways leads to a synergistic enhancement of apoptosis in CML cells (32). STAT5 signaling is coupled to Bcr-Abl by CrkL, which is phosphorylated on tyrosine in Bcr-Abl-positive cells (33, 34). Inhibition of CrkL signaling by antisense inhibits the growth of CML cells (35). c-Jun NH2-terminal kinase is also activated in myeloid cells expressing Bcr-Abl, and dominant negative c-Jun NH2-terminal kinase inhibits Bcr-Abl transformation of fibroblasts (36). In addition, Bcr-Abl expression activates both Lyn and Hck kinases, two Src family kinases expressed in myeloid cells, and Hck activated by Bcr-Abl phosphorylates STAT5 (37, 38). Hck also phosphorylates Bcr on Tyr373, a Grb2 docking site important for efficient promotion of leukemia in vivo (39), and dominant negative Hck inhibits proliferation of CML cells (40). Recent work demonstrates that the adaptor protein Gab2 associates with Grb2 when Y177 is phosphorylated. Mutations in Bcr-Abl (Y177F), Grb2, or Gab2 that block formation of this complex impair signaling through both PI3K and Ras and diminish the leukemogenic activity of Bcr-Abl (41, 42). These results indicate that numerous signal transduction molecules besides Bcr-Abl might be useful points for therapeutic intervention in CML. This observation is especially pertinent in view of the prevalence of Bcr-Abl mutations in STI-571-resistant patients (43, 44).

We recently reported the identification and characterization of a 4-anilino-3-quinolinecarbonitrile inhibitor of Src kinase activity, SKI-606. This compound inhibits Src in an enzyme assay with an IC50 of 1.2 nM, inhibits anchorage-independent growth of Src-transformed fibroblasts with an IC50 of 100 nM, and inhibits Src-dependent protein tyrosine phosphorylation at comparable or lower concentrations (45). SKI-606 inhibits other Src family kinases but does not inhibit growth factor receptor tyrosine kinases such as platelet-derived growth factor receptor, insulin-like growth factor I receptor, epidermal growth factor receptor, fibroblast growth factor receptor, and serine-threonine phosphorylation suggesting that Src kinase activity is not critical for the growth and survival of CML cells (46, 47). SKI-606 is cytotoxic to Bcr-Abl-positive myeloid cells at concentrations that do not inhibit Src kinase activity, and it constitutes an active, orally bioavailable drug when administered to C57BL/6 mice expressing Bcr-Abl (48). 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.
kinases such as Akt and Cdk4 (45). We report here that SKI-606 is an Abl tyrosine kinase inhibitor with potent antiproliferative activity against CML cells in culture. Similar observations with structurally unrelated Src inhibitors have been described recently (46, 47). We also show that once daily oral administration of SKI-606 over a 5-day period eradicates K562 CML tumor xenografts in nude mice.

MATERIALS AND METHODS

Cell Culture. Abl-MLV, Rat 2, MEG-01, and K562 cell lines were obtained from the American Type Culture Collection. The KU812 line was obtained from the European Collection of Cell Cultures. K562 and KU812 cell lines were maintained in RPMI 1640 supplemented with 10% FCS, glutamine, and nonessential amino acids (Life Technologies, Inc.). Rat 2 and Abl-MLV-transformed Rat 2 cells were maintained in high-glucose DMEM supplemented with 10% FCS. For proliferation assays, cells were exposed to compound for 72 h, and relative proliferation was measured with either 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfonyl)-2H-tetrazolium (Promega) or Cell-Glo (Promega). Anchorage-independent proliferation of Abl-MLV-transformed fibroblasts was measured in 96-well ultra-low binding plates (Costar) treated with Sigrmacle (Sigma) to block residual cell attachment. Data analysis for IC50 calculations was performed with the LSW Data Analysis Package plug-in for Excel (Microsoft).

For biochemical assays, cells were incubated with compound or DMSO alone for 4 h. Cells were prepared for FACS analysis as described in the CycleTest Plus DNA reagent kit (Becton Dickinson) and analyzed on a fluorescence-activated cell sorter flow cytometer.

Antibodies. Antibody Ab-3 (Oncogene Sciences) was used to immunoprecipitate v-Abl and Bcr-Abl and to detect Abl proteins on Western blots. Antibody to phosphorylated Abl (Y245) was obtained from Cell Signaling. STAT5, Lyn (H-6), and phosphoHck (Y411) antibodies were purchased from Santa Cruz Biotechnology. PhosphoSTAT5 (Y694/Y699; mouse mononclonal antibody 8-5-2) and phosphotyrosine antibody 4G10 were purchased from Upstate Biotechnology. PARP, PARP antibodies, and apoptotically induced HL-60 cell extract were obtained from Boimol Research Laboratories. Actin antibodies were purchased from Chemicon. Horseradish peroxidase-conjugated secondary antibodies were obtained from Amersham.

Extracts and Immunoprecipitations. Denatured whole cell extracts were prepared from equal cell numbers in urea lysis buffer [50 mM Tris-HCl (pH 7.5; room temperature)], 7 M urea, 0.1% SDS, and 0.5 mM EDTA. Cells were collected by centrifugation, washed twice with ice-cold PBS, and resuspended in the urea lysis buffer. Extracts were sonicated to shear DNA. Samples were mixed with Laemmli sample buffer and loaded without heating onto SDS-polyacrylamide gels. Native extracts were prepared in RIPA buffer [50 mM Tris-HCl (pH 7.5; room temperature) and 0.15 M NaCl] with 1% Triton X-100, 0.5% sodium deoxycholate, 0.5 mM EDTA, and 0.5 mM Na3VO4 supplemented with protease inhibitor cocktail (Calbiochem). Protein concentrations of native extracts were determined with the Bio-Rad DC assay. Immunoprecipitations were performed in RIPA buffer for 2 h at 4°C by rocking with antibody, protein G beads (Pierce), and extract. Beads were washed three times with RIPA buffer and once with 50 mM Tris-HCl (pH 7.5; room temperature)/10% glycerol before addition of Laemmli gel sample buffer and incubation at 99°C for 5 min.

Abl Kinase Assay. Bacterially expressed Abl kinase was obtained from New England Biolabs. Kinase assays were performed in a DELFIA solid phase europium-based detection assay format (Perkin-Elmer). The peptide was as described in Dorsey et al. (46). Biotinylated peptide (2 μM) was bound to streptavidin-coated microtitration plates (Perkin-Elmer CC11-205) for 1.5 h in 1 mg/ml ovalbumin in PBS. The plates were washed for 1 h with PBS/0.1% Tween 80, followed by a PBS wash. The kinase reaction was incubated for 1 h at 30°C. Abl kinase (10 units, NEB P6050L) was mixed with 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 80 μM EGTA, 100 μM ATP, 0.5 mM Na3VO4, 1% DMSO, 1 mM HEPES (pH 7), and 200 μg/ml ovalbumin. The reaction was stopped with EDTA at a final concentration of 50 mM. The DELFIA wash protocol suggested by the manufacturer (Perkin-Elmer) was modified by extending wash times to reduce background. The reaction was monitored with Eu-labeled phosphotyrosine antibody (Perkin-Elmer AD0040) and DELFIA enhancement solution (Perkin-Elmer 1244-105) according to manufacturer’s specifications.

In Vivo Studies. All animal studies were conducted under an approved Institutional Animal Care and Use Committee protocol. K562 cells were suspended to 50 million cells/ml in Matrigel (1 volume of cells with 1 volume of cold Matrigel). Nude female mice 6–7 weeks of age (Charles River, Wilmington, MA) were given injections of 0.2 ml of this suspension. Tumors were staged for 10 days, at which time they entered growth phase. At this time, compound was administered by oral gavage in a 0.2-ml suspension with 0.5% methocel/0.4% Tween 80.

Compound Synthesis. SKI-606 and STI-571 were prepared according to published methods (45, 48).

RESULTS

SKI-606 Is a Potent Inhibitor of CML Cell Proliferation and Survival. The antiproliferative activity of the Src kinase inhibitor SKI-606 (Fig. IA) against three different Bcr-Abl-positive leukemia cell lines (KU812, K562, and MEG-01) is shown in Fig. 1B. SKI-606 inhibited the proliferation of all three cell lines, with IC50s ranging from 5 nM in the KU812 line to 20 nM for the K562 and MEG-01 cell lines. In our hands, STI-571 inhibited proliferation of these cell lines.
with IC_{50} of 88 nm (KU812), 210 nm (K562), and 180 nm (MEG-01), in agreement with previously published results (10, 49). Inhibition of proliferation by SKI-606 is associated with cell cycle arrest and cell death as measured by FACS analysis (Fig. 1C) or by visual inspection of cells (data not shown). Treatment with SKI-606 at 100 nm for 24 h (KU812) or 48 h (K562; data not shown) resulted in a reduction of S and G_{2}-M phase cells and an increase of cells with a DNA content of less than 2N. Treatment with SKI-606 at 100 nm also led to PARP degradation after 48 h, as was observed with 1 mM STI-571 (Fig. 1D).

The potent antiproliferative activity of SKI-606 against CML lines was not a general property for leukemia cell lines. Molt-4, HL-60, Ramos, and other leukemia cell lines were unaffected by SKI-606 at concentrations less than 1 mM (data not shown).

**SKI-606 Blocks Bcr-Abl Phosphorylation.** The antiproliferative activity of SKI-606 was mirrored by a general decrease in protein tyrosine phosphorylation in whole cell extracts of the KU812 and the K562 lines (Fig. 2A). The pattern of inhibition of protein tyrosine phosphorylation was similar to that observed with STI-571, but higher concentrations of STI-571 than SKI-606 were required (Fig. 2B). The similarity of the pattern of inhibition suggested that these two compounds shared a common mechanism of inhibition. Furthermore, among those proteins with reduced phosphorylation was a high molecular weight protein that comigrated with Bcr-Abl. Therefore, Bcr-Abl was immunoprecipitated, and blots of the immunoprecipitates were probed with antibodies to phosphotyrosine and Abl (Fig. 2C). Low concentrations of SKI-606 not only reduced the phosphorylation of the protein (Fig. 2C, top) but also caused an increase in the total amount of immunoprecipitable Bcr-Abl (Fig. 2C, bottom). Phosphorylation of Bcr-Abl was also measured in whole cell extracts with antibody to activated Abl protein (α-pY245 Abl). SKI-606 inhibited Bcr-Abl phosphorylation on this residue and increased Bcr-Abl levels slightly, as did STI-571 (Fig. 2D). Higher concentrations of STI-571 than SKI-606 were required to ablate Bcr-Abl phosphorylation.

**SKI-606 Is an Abl Kinase Inhibitor.** Because these results suggested that SKI-606 might be an Abl kinase inhibitor, we tested SKI-606 in an Abl enzymatic assay. SKI-606 inhibited bacterially expressed Abl kinase activity with an IC_{50} of 1 nm (data not shown). We also examined Abl-MLV-transformed Rat 2 fibroblasts treated with SKI-606. Incubation of these cells with 0.5 mM SKI-606 for 4 h caused morphological reversion (Fig. 3A), suggesting that SKI-606 inhibited v-Abl. To obtain a quantitative evaluation of the activity of SKI-606 against Abl in cells, we measured inhibition of anchorage-independent proliferation because this type of growth should be dependent on v-Abl activity. As shown in Fig. 3B, SKI-606 inhibited the growth of the Abl-MLV-transformed fibroblasts, with an IC_{50} of 90 nm (n = 4). A similar value was observed with STI-571 under these conditions (90 nm; n = 2). STI-571 was reported to inhibit mast cells expressing v-Abl with an IC_{50} of 110 nm, in agreement with our results with anchorage-independent growth of Abl-MLV-transformed fibroblasts (50). Tyrosine phosphorylation was reduced in whole cell extracts of Abl-MLV-transformed fibroblasts treated with SKI-606 or STI-571 in a manner consistent with their antiproliferative activity (Fig. 3C). However, in contrast to our observations with the CML cells, incubation of Abl-MLV-transformed Rat 2 fibroblasts with comparable concentrations of SKI-606 and STI-571 resulted in quantitatively similar reductions in tyrosine phosphorylation of cellular proteins. Together, these experiments indicate that SKI-606 is an Abl kinase inhibitor.

We compared the relative activities of SKI-606 and STI-571 against v-Abl in cells more extensively in a dose-response experiment (Fig. 3, D and E). The IC_{50} for inhibition of v-Abl phosphorylation by SKI-606 was estimated to be 25–50 nm, and that for STI-571 was estimated to be 50–100 nm. Others have reported the IC_{50} for inhibition of v-Abl phosphorylation by STI-571 to be 100–300 nm, in reasonable agreement with our results (50). Thus, any distinction between the two compounds as inhibitors of v-Abl phosphorylation is small relative to the quantitatively distinct antiproliferative and Bcr-Abl phosphorylation-inhibitory activities observed in CML cells.

We then compared the dose response of Bcr-Abl and v-Abl in immunoprecipitates from extracts of KU812 cells and v-Abl-transformed fibroblasts. Tyrosine phosphorylation of Bcr-Abl was nearly ablated between 25 and 50 nm SKI-606 (Fig. 4A), whereas v-Abl phosphorylation in the immunoprecipitates did not decrease to this extent until 200 nm SKI-606 was present (Fig. 4B). These results indicated that tyrosine phosphorylation of v-Abl was less sensitive to SKI-606 than that of Bcr-Abl.
Effects of SKI-606 on STAT5 Phosphorylation. Tyrosine phosphorylation of STAT5 in Bcr-Abl-transformed cells is inhibited by treatment with STI-571 (51). We therefore performed a dose-response experiment using KU812 and K562 cells to examine the ability of SKI-606 to inhibit phosphorylation of STAT5. As shown in Fig. 5, tyrosine phosphorylation of STAT5 was ablated in KU812 cells by 10 nM SKI-606 and in K562 cells by 25 nM SKI-606. Thus, inhibition of STAT5 phosphorylation corresponded to the antiproliferative activity of SKI-606 in these two cell lines. STI-571 inhibited STAT5 tyrosine phosphorylation with IC₅₀ of about 100 and 200 nM in the KU812 and K562 cell lines, respectively (data not shown), also in agreement with the antiproliferative activity of STI-571. These results suggest that STAT5 tyrosine phosphorylation is a good downstream indicator of the proliferative and survival capacity of CML cells.

Inhibition of Lyn/Hck Phosphorylation by SKI-606. The Src family kinases Lyn and Hck have been implicated in downstream signaling from Bcr-Abl (37, 39, 40). We thus determined whether SKI-606 affects Lyn or Hck phosphorylation at the autoactivation sites, Tyr⁹⁷⁷ or Tyr⁴¹¹, respectively, in whole cell extracts from KU812 cells. KU812 cells express both of these Src family kinases, but Hck is less readily detectable. Blots of these samples were probed with antibody to Hck phosphorylated on its activation site, Tyr⁴¹¹ (this antibody cross-reacts with Lyn phosphorylated on Tyr⁹⁷⁷). A doublet that comigrates with Lyn was observed, along with another unidentified band with a slightly faster electrophoretic mobility than Lyn. We observed a reduction in Lyn/Hck phosphorylation after treatment with 500 nM SKI-606 for 4 h (Fig. 6A). However, exposure of the KU812 cells to 50 and 500 nM SKI-606 for 24 h (Fig. 6B) caused a more marked decrease in Lyn/Hck phosphorylation relative to total Lyn and actin levels. We also examined Lyn phosphorylation in immunoprecipitates of Lyn from K562 cells and observed the same pattern of inhibition for samples after exposure to SKI-606 for 20 h (data not shown). Reduced phosphorylation of the Tyr⁹⁷⁷ of Lyn would diminish Lyn kinase activity, but our data do not shed any light on the physiological relevance of Lyn inhibition.

In Vivo Activity of SKI-606 against K562 Xenografts. We have shown previously that SKI-606 is active against Src-transformed fibroblast xenografts in nude mice when administered i.p. and against HT29 xenografts when administered either i.p. or p.o. (45).
Src/Abl INHIBITOR CAUSES CML XENOGRAFT REGRESSION

Therefore tested SKI-606 for oral activity against K562 xenografts. Tumors were staged to 200–300 mg in nude mice, and SKI-606 was administered p.o. for 10 days at 75 mg/kg twice a day (Fig. 7A). Treatment via this regimen resulted in complete regression of the tumor for up to 64 days. We also examined the antitumor activity of SKI-606 administered once a day p.o. for 5 days at various doses (Fig. 7B). Animals dosed at 150 mg/kg remained tumor free, whereas at the lower doses, some relapse occurred over a 40-day period. At the lowest dose examined (50 mg/kg, once a day, Fig. 7B), administration for 5 days caused tumor regression, but the tumors reappeared in half of the animals. Table 1 shows the durability of tumor regression as a function of dose for the study shown in Fig. 7B. We also found that SKI-606 administered once a day at 100 mg/kg for 5 days was sufficient to eradicate large tumors (800–900 mg) for at least 40 days (Fig. 7C). No overt toxicity was observed at the highest doses in this model (150 mg/kg once daily or 75 mg/kg twice daily), even with more prolonged dosing periods. Thus, SKI-606 has excellent activity in this CML xenograft model.

DISCUSSION

SKI-606, originally identified as a Src inhibitor, is shown here to be a potent antiproliferative and proapoptotic agent against CML cells in culture. The apoptotic activity of SKI-606 against CML cells in culture is mirrored by its activity in vivo against CML cells. K562 tumors regress in nude mice when SKI-606 is administered p.o. once a day. The Abl-inhibitory activity of SKI-606 is likely a major contributor to the antiproliferative activity of SKI-606 against CML cells. Tyrosine phosphorylation of Bcr-Abl is eliminated at concentrations of SKI-606 greater than 100 nM, which alone should be sufficient to inhibit the proliferation and survival of Bcr-Abl-dependent myeloid cells. SKI-606 and STI-571 have comparable activity against v-Abl expressed in fibroblasts, whereas SKI-606 is an order of magnitude more potent than STI-571 as an inhibitor of CML cell proliferation and Bcr-Abl phosphorylation.

Tyrosine phosphorylation of p210Hck-Ab occurs on multiple residues (52). Phosphorylation of at least one of these sites, Tyr177 in the Bcr portion of the chimera, can be catalyzed by Hck, and both Hck and Lyn are activated by expression of Bcr-Abl (37, 39). It is interesting that tyrosine phosphorylation of Bcr-Abl is ablated by sufficiently high concentrations of SKI-606 and that similar results have been obtained by ourselves and others for STI-571, which does not inhibit Src (53). These results suggest that inhibition of Bcr-Abl kinase activity reduces Tyr177 phosphorylation. A dual Abl/Src family kinase inhibitor such as SKI-606 might also directly inhibit Lyn- or Hck-catalyzed phosphorylation of Tyr177, in addition to indirectly inhibiting those kinases by reducing Bcr-Abl activity. If so, inhibition of Src family kinases might contribute to the activity of SKI-606 against CML cells. This speculation is the subject of further investigations.

In CML cells, diminution of STAT5 tyrosine phosphorylation closely parallels the antiproliferative activity of both SKI-606 and
STI-571. These results are consistent with the findings from other laboratories that describe STAT5 as an important signal transduction molecule downstream of Bcr-Abl, although STAT5-deficient bone marrow cells can be efficiently transformed to a leukemogenic state by Bcr-Abl (29, 31, 32, 54, 55). STI571 is constitutively activated in cells transformed by Bcr-Abl, as are Janus kinases (30, 56, 57). However, Janus kinases, which can themselves activate STAT5, are not uniformly phosphorylated in CML cells (58). Recent reports describe STAT5 as a direct target ofSrc in K562 cells and of Hck in myeloid cells transformed by Bcr-Abl (59, 60), whereas Lyn phosphorylates STAT5 in erythropoietin-stimulated myeloid cells (61). Multiple mechanisms for STAT5 activation may therefore exist in Bcr-Abl-transformed cells, which has also been suggested for Ras activation by Bcr-Abl (62–64). Thus, an appropriate kinase inhibitor could have multiple points of intervention against STAT5 activation.

Because overall tyrosine phosphorylation of cellular proteins is also diminished in accordance with the antiproliferative activity of SKI-606, other proteins might also be used as indicators of compound activity. In particular, CrkL has been used as a marker in clinical samples, and both CrkL and Gab2 were used to show Abl inhibition in cell lines (43, 46).

Although the selective Abl kinase inhibitor STI-571 is efficacious and well tolerated by most patients in chronic-stage CML, patients in accelerated and blast crisis stages of the disease tend to be less responsive. Consequently, there is a need for alternative agents that are effective in late-stage disease. Simultaneous inhibition of two or more signal transduction targets important in CML by inhibitors such as SKI-606 could offer significant therapeutic advantages for patients refractory to STI-571 treatment.

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