SKI-606 (Bosutinib) Blocks Prostate Cancer Invasion, Growth, and Metastasis \textit{In vitro} and \textit{In vivo} through Regulation of Genes Involved in Cancer Growth and Skeletal Metastasis

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Abstract

In the current study, we have examined the efficacy of a Src/Abl kinase inhibitor SKI-606 (Bosutinib) for its effect on prostate cancer growth and skeletal metastasis. Treatment of highly invasive human prostate cancer cells PC-3 and DU-145 with different doses of SKI-606 decreased Src activation, cell proliferation, migration, and invasion as determined by Matrigel Boyden chamber invasion assay. For \textit{in vivo} studies, PC-3 cells were inoculated through s.c. or i.t. route into male BALB/c nu/nu or Fox Chase severe combined immunodeficient mice, respectively. Experimental animals treated with SKI-606 developed tumors of a significantly smaller volume and a significant decrease (50%) in experimental skeletal lesion area. A marked increase (32%) in bone volume to tumor volume ratio was also seen by micro-computed tomography analysis of tibias from control and experimental groups of animals. Western blot analysis showed the ability of SKI-606 to significantly decrease the phosphorylation of signaling molecules (AKT, mitogen-activated protein kinase, focal adhesion kinase) and the expression of tumor progression-associated genes uPAR, MMP-2, MMP-9, N-cadherin, fibronectin, BMP-2 (bone morphogenetic protein 2), BMP-6 (bone morphogenetic protein 6), IL-8 (interleukin 8), and TGF-β (transforming growth factor β) in prostate cancer cells. SKI-606 is currently in clinical trials for breast cancer and chronic myelogenous leukemia. Results from these studies provide convincing evidence for evaluating its efficacy in prostate cancer patients. \textit{Mol Cancer Ther}; 9(5); 1147–57. ©2010 AACR.

Introduction

Prostate cancer is one of the leading malignancies in men that continue to result in high rate of morbidity and mortality associated with the development of skeletal metastasis (1). Of several factors implicated in prostate cancer progression, Src is of particular significance because it acts in several key intracellular signaling pathways (2, 3).

Src is a nonreceptor protein tyrosine kinase belonging to the Src family of kinases (SFK; ref. 3). Elevated levels and activity of c-Src occur in several human cancers because of extragenic alterations resulting in the constitutive activation of its kinase domain (2). Src activity is also implicated in ligand independent activation of the androgen receptor pathway by growth factors and cytokines in prostate tumor cells (4). Increased levels of SFKs occur in prostate tumors and in a variety of prostate cancer cell lines (5). Src also promotes cytoskeletal organization and osteoclastic bone resorption important in bone turnover (6). Targeted knock out of Src in mice results in osteopetrosis, whereas bone-specific expression of Src in these mice rescues normal osteoclast function (7). Because Src plays a pivotal role in tumor progression and bone turnover, its signaling is of particular significance in the development and progression of skeletal metastases associated with several cancers (8).

In recent years, several studies have evaluated the effect of Src inhibition on tumor growth and metastasis in mouse models (2). The Src inhibitor 4-amino-5-(4-chlorophenyl)-7-(1-butyl)pyrazolo[3,4-alpha]pyrimidine decreased tumor growth and metastasis in a xenograft model of pancreatic cancer (9). Other small molecule Src inhibitors are in clinical development (10–12). Among these, the multitkine inhibitor dasatinib has shown efficacy in several preclinical models, including chronic myelogenous leukemia (CML) and pancreatic and prostate cancer, and is approved for treating imatinib resistant or intolerant CML (2, 12–14). Another small molecule Src inhibitor, SKI-606 (Bosutinib), was initially shown to inhibit CML and colon tumor growth in nude mice (10, 15–17). In studies carried out by us and others (18, 19), SKI-606 inhibited human breast cancer cell proliferation, invasion and migration \textit{in vitro}. SKI-606 was also effective in reducing primary tumor growth and tumor metastases to lungs, liver, and spleen in an orthotopic breast tumor model. (18). The Src inhibitor AZD0530 (saracatenib)

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has also shown efficacy in several tumor models (11, 20). Currently, several clinical trials are evaluating dasatinib, bosutinib, and AZD0530 in patients with solid tumors. In this study, we describe SKI-606 activity against prostate tumor xenografts and skeletal metastases in vivo.

Materials and Methods

Cell culture. Human prostate cancer cell line PC-3 and DU-145 cells were obtained from the American Type Culture Collection. Cells were incubated with SKI-606 or its analog compound 24 with half maximal inhibitory
concentration of 1.4 μmol/L in the Src enzyme assay compared with 3.5 nmol/L of SKI-606 (21).

Treatment and transfection of siRNA. Human prostate cancer cells PC-3 and DU-145 were plated in 60-mm Petri dish at a concentration of 3.5 × 10^5 per dish and grown overnight in RPMI containing 10% fetal bovine serum (FBS). The following day, cells were washed with sterile 1× PBS twice and transfected with 125 nmol/L of Src siRNA–targeting c-Src, nontargeting scrambled siRNA as negative control (Santa Cruz Biotechnology),

Figure 2. Continued. These cells were treated with two different doses of SKI-606 (0.1 and 1 μmol/L) containing 2% FBS, and migrating cells were photographed at different time points (C). Percent wound healing was recorded at different time points, and percentage of wound healing with respect to T₀ was calculated using the equation described in Materials and Methods. PC-3 and DU-145 cells were plated onto soft agar for anchorage independent growth in the presence of vehicle alone or SKI-606 (0.1 and 1 μmol/L). Number of colonies was counted as described in Materials and Methods (D). Results are presented as the mean ± SE of two different experiments in duplicate from control and experimental cells. *, P < 0.05 (significant differences from the control).
and transfection reagent only using Lipofectamine (Invitrogen) in Opti-MEM I Reduced Serum Media (Invitrogen). After 4 hours, media were replaced with RPMI containing 10% FBS and incubated at 37°C. The cells were harvested at 48 hours for further analysis.

**Western blotting assay.** PC-3 and DU-145 cells were plated in 100-mm Petri dishes (1 × 10⁶) and then serum starved overnight. The following day the cells were treated with vehicle alone (dimethyl sulfoxide), SKI-606, its inactive analog, or transfected with Src siRNA or scrambled siRNA in the presence of 2% FBS for different periods. (10). Equal amounts of protein were loaded on SDS-PAGE, transferred to nitrocellulose, and probed with antibody to Src (Millipore), PY418 Src (Abcam), and actin (Chemicon), and Alexia Fluor (Invitrogen) or IRR Dy (Rockland Immunochemicals) as second antibodies. Blots were probed with and analyzed with an Odyssey Imager.

**Cell proliferation invasion, wounding, and colony formation assay.** PC-3 and DU-145 cells were plated in duplicate at a density of 50,000 cells per well in 2 mL of culture media in six-well plates. Cells were grown in culture medium with 10% FBS with vehicle alone or SKI-606. The effect of two different doses of SKI-606 (0.1 and 1 μmol/L) or 125 nmol/L Src siRNA or scrambled siRNA (Santa Cruz Biotechnology) was evaluated.

The invasive capacity of prostate cancer cells PC-3 and DU-145 were examined using two-compartment Boyden chamber Matrigel invasion assay (Costar Transwell, Corning Corporation) as described previously (22). Invasive capacity of 5 × 10⁴ cells was examined following treatment with or without SKI-606 (0.1 and 1 μmol/L) or after transfection with 125 nmol/L Src siRNA or scrambled siRNA for 24 hours.

PC-3 and DU-145 cells were grown in six-well plates in the presence of 10% FBS. Once the cells formed a monolayer, the wound was done manually with a sterile 1,000 μL pipette tip in the center of each well (18). Cells treated with vehicle alone, inactive analog, or 0.1 and 1 μmol/L of SKI-606 were grown in the presence of 2% FBS. At different time points the migrating cells were photographed, selected for analysis, and quantified using Image Pro-Plus software and calculated as percentage wound healing using the equation % wound healing = [1 - (wound area at Tₓ) / wound area at T₀], wherein Tₓ is the respective time point and T₀ is the time immediately after wounding. These experiments were repeated twice in duplicates.

For colony formation assay of PC-3 and DU-145, 3 × 10³ cells were seeded in triplicate onto six-well Petri dishes in the presence of 4 mL of culture medium containing 0.33% agar solution at 37°C. Medium was changed every 48 hours, and the number of colonies were scored as >100 cells after 14 days of plating (23).

**Reverse transcriptase-PCR (RT-PCR) analysis.** Total cellular RNA from control and SKI-606 (1 μmol/L) treated PC-3 cells were extracted after 48 hours using TRizol (Invitrogen) according to the manufacturer’s protocol. Two micrograms of total RNA was used for reverse transcription and amplification. The DNA was amplified under the following conditions: initial denaturation at 95°C for 5 minutes (30 cycles), denaturation at 95°C for 30 seconds, annealing temperature of the respective primer for 45 seconds, elongation at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The following primer sequences were used for amplification of respective genes: 5′-ATAGGTGACAGCCTAAGAGATG-3′ and 5′-CATCGAGTAGATCAGTGAGAAT-3′ for p53, 5′-ATACCTTTGTCGGCGTGTG-3′ and 5′-TGATGAGAACGCAGCAGATC-3′ for MMP-2, 5′-CCATGCCTGCCTCTTGTAG-3′ and 5′-CCAGCGAGAACACTACAC-3′ for MMP-9, 5′-ATGAAGGAGACCATGATCTTCT-3′ and 5′-CTGCCTGACTTCCATGAC-3′ for N-cadherin, 5′-TCCAGTGTCAGTTGGACTCAAAG-3′ and 5′-ATGATATGATACACACCA-3′ for fibronectin, 5′-CCACATGAGAAGATCTGTGG-3′ and 5′-ACTTGTTGACTCAG(CCT-3′ and 5′-CAGCTTACTTGCTGAGC3′ for BMP-2 (bone morphogenetic protein 2), 5′-GCACAGAGACTCTGTGGCTGG3′ and 5′-CAGCTTACTGCTTGAGG3′ for BMP-6 (bone

![Figure 3. Effect of SKI-606 on PC-3 morphology and inhibition of tyrosine phosphorylation of signaling molecules in vitro.](image-url)
morphogenetic protein 6), 5′ATGACTTTCAAGCTGCGT3′ and 5′CCTCTTCAAAAACTTCTCCACACC3′ for IL-8 (interleukin 8), 5′GTACCTGAACCCGTGTTGCT3′ and 5′TACAGCTGCCGACCGAGCA3′ for TGF-β (transforming growth factor β), 5′ACCCACCTCTAAGGCCATCT3′ and 5′CAGCACGTACACAGCCCTAA3′ for E-cadherin, 5′CTTCTGTTCTTTTGTGCT3′ and 5′AGAAAGATGGAGTGGGAAC3′ for TIMP-1 (tissue inhibitor of metalloprotease 1), 5′CTTCCCTTCTAGGCGAC3′ and 5′AAGACCTGAAGGAATCCACC3′ for TIMP-2 (tissue inhibitor of metalloprotease 2), and 5′GAGGGGGCATTCCACAGTCTTCTG3′ and 5′CCCTTCATTGCACACTACTGCT3′ for GAPDH (glyceraldehyde-3-phosphate dehydrogenase). The gels were quantified by using the Scion Software (Scion Corporation) for the densitometry.

Animal protocols. Four- to five-week-old male BALB/c nu/nu mice were obtained from National Cancer Institute Research Resources. PC-3 cells were grown in RPMI + 10% FBS medium, washed with Hanks’ balanced buffer, trypsinized, and centrifuged at 1,500 rpm for 5 minutes. An anesthetic cocktail of ketamine (50 mg/kg), xylazine (5 mg/kg), and acepromazine (1 mg/kg) was injected i.m. of the mice, and 2.0 × 10⁶ cells per mouse were inoculated in 100 μL of saline (20% Matrigel) using a 26-gauge needle s.c. into the right flank of the anesthetized mice. Animals were randomized and divided into a control
group and an experimental group, which were treated with SKI-606 (150 mg/kg; daily by oral gavage) for 10 weeks from day 3 post–tumor cell inoculation. Animals were monitored at weekly intervals. Tumor volume was determined according to this formula: tumor volume = shorter diameter × longer diameter / 2. Results were presented as the mean of tumor volumes recorded from both groups.

In other studies, 6-week-old male Fox Chase severe combined immunodeficient (SCID) mice were obtained from Charles River. Following the administration of anesthetic cocktail as described above, PC-3 cells were inoculated at 2 × 10⁵ cells per mouse in 40 μL saline with a 27-gauge needle into the left tibia using a drilling motion. Animals were treated with vehicle alone or SKI-606 (150 mg/kg), respectively, daily by oral gavage for 4 weeks starting from day 3 post–tumor cell inoculation. The mice were monitored weekly for tumor burden. On weeks 3 and 4, digital radiography of hind limbs of all animals was done using a Faxitron X-ray machine (Faxitron X-ray Corp.) to monitor the development of skeletal lesions. On week 4, the mice were euthanized, and the left tibias were collected and fixed in 10% buffered formalin solution for 24 hours. The X-ray scoring method is described as follows: no lesions, minor changes, small lesions, significant lesions (minor peripheral margin breaks, 1% to 10% of bone surface disrupted), and significant lesions (major peripheral margin breaks, >10% of bone surface broken) rating 0 to 4, respectively (24). The whole tibia (trabecular and cortical) of four different animals from each group were analyzed by micro-computed tomography with a SkyScan 1072 scanner and associated analysis software (SkyScan). Image acquisition was done at 45 kV with a 0.9 rotation between frames. During scanning, the tibias were enclosed in a tightly fitted plastic wrap to prevent movement and dehydration. All the experimental animal protocols were in accordance with the McGill University Animal Care Committee guidelines.

Statistical analysis. Results were analyzed as the mean ± SE, and comparisons of the experimental data were analyzed by an independent two-sample t test at P < 0.05 level of significance.

Results

Effect of SKI-606 on Src kinase activity in prostate cancer cells. SKI-606 has been reported to reduce Src activation in several cell lines at submicromolar concentrations. Phosphorylation of the “autophosphorylation site” Y418 is typically used as the measure of Src activation. We evaluated the effect of 2-hour treatment with SKI-606 on Y418 phosphorylation in PC-3 and DU-145 cells. As shown in Fig. 1, Y418 phosphorylation was diminished by submicromolar amounts of SKI-606 as reported in other studies. An analog of SKI-606, compound 24 from Boschelli et al. (21), which is 1/50 as active against Src as SKI-606 did not affect Y418 phosphorylation at these concentrations.
Effect of SKI-606 on prostate cancer cells proliferation, invasion, and migration. Src activity promotes cell migration and invasiveness, and SKI-606 reduces migration and invasion of breast tumor cells. We therefore examined the effect of SKI-606 on these characteristics in the two invasive human prostate cancer cell lines PC-3 and DU-145. Treatment of both PC-3 and DU-145 cells with 1.0 μmol/L SKI-606 also had a significant effect in inhibiting cell proliferation when incubation was prolonged beyond the 3-day period commonly used in 96-well proliferation assays (Fig. 2A). In addition, the invasive capacity of PC-3 and DU-145 cells as determined by Boyden chamber Matrigel invasion assay was reduced by SKI-606 treatment in a dose-dependent manner (Fig. 2B). These anti-invasive effects were independent of the anti-proliferative effects shown in Fig. 2A because the total number of cells in the upper and lower part of Boyden chambers remained constant over the period of the measurement.

The effect SKI-606 on cell migration was then examined by a wound healing assay. In these studies, PC-3 and DU-145 cells were treated with vehicle alone or different concentrations of SKI-606, followed by evaluation of cell migration capacity at various time points. SKI-606 decreased cell migration at all times compared with vehicle-treated control cells. These effects were quite pronounced at 48 hours as shown in representative photomicrographs (Fig. 2C). Cpd 24 had no effect on prostate cancer cells proliferation, invasion, and migration at these concentrations (data not shown). In a caspase activation assay with cells growing on tissue culture plates, 1.0 μmol/L SKI-606 had no effect on PC-3 and DU-145 cell apoptosis (data not shown). PC-3 and DU-145 cell colony formation in soft agar was examined following treatment with vehicle alone or SKI-606 for 14 days. In both these prostate cancer cell lines, a dose-dependent decrease in colony formation onto soft agar was seen following treatment with SKI-606 (Fig. 2D).

Knockdown of Src by siRNA treatment (Fig. 3A) led to inhibition of the invasive capacity of PC-3 cells as compared to control cells treated with vehicle alone or transfected with scrambled siRNA (Fig. 3A). RT-PCR measurements also showed knockdown of Src mRNA similar to the reduction in Src protein levels (data not shown). The decrease in PC-3 cells invasiveness was similar to that seen in cells treated with SKI-606. These data suggest that interfering with Src kinase expression or activation inhibits prostate cancer cell invasion. However, siRNA treatment did not markedly inhibit tumor cell proliferation and migration. These results may reflect incomplete knockdown and the presence of other SFKs or indicate that SKI-606 inhibits PC-3 cell migration and proliferation by inhibiting other kinases.

SKI-606 affects downstream intracellular signaling pathways in vitro. PC-3 cells were treated with SKI-606, which resulted in a change from a spindle-like to an epithelial shape with cluster formation as shown in representative photomicrographs (Fig. 3B). Associated with the morphologic changes described above in Fig. 3B were alterations in signaling pathways in which Src plays a role. Treatment of PC-3 cells with SKI-606 led to inhibition of AKT, mitogen-activated protein kinase (MAPK), and FAK activation as compared with control cells treated with vehicle alone. No significant change in the levels of expression of total AKT, MAPK, or FAK was seen in cells treated with SKI-606 (Fig. 3C).

Effect of SKI-606 on PC-3 tumor growth in vivo. We next examined the effect of SKI-606 administration on primary tumor growth in a s.c. PC-3 tumor xenograft model. Animals were treated once daily with vehicle alone or SKI-606 at 150 mg/kg through oral gavage, a dose we showed to be effective in breast tumor models...
Figure 6. Effect of SKI-606 on the expression of genes associated with prostate cancer progression. PC-3 cells were treated with vehicle alone as control or with 1 μmol/L of SKI-606 for 48 hours, and total cellular RNA was isolated with TRIzol. RNA from control and treatment groups was analyzed for the expression of genes involved in tumor progression. Changes in the mRNA expression of the representative genes were determined by plotting the relative ratio against GAPDH, which was used as loading control. Results are presented as the mean ± SE of two different experiments in duplicate from control and experimental cells. *, $P < 0.05$ (significant differences from the control).
IL-8, and TGF-

significant inhibition in the expression of BMP-2, BMP-6, and fibronectin, proteins involved in cell-cell and cell-

reduced expression of uPAR, MMP-2, MMP-9, N-cadherin, E-cadherin, TIMP-1, and TIMP-2, which attenuate the

degradation (Fig. 6). Furthermore, SKI-606 treatment resulted in a significant (80%) decrease in tumor volume in xenograft-bearing mice treated with SKI-606. Whether or not all of the in vivo effects of SKI-606 treatment can be ascribed to inhibition of Src or SFKs, SKI-606 treatment does reduce Src activation. In addition, SKI-606 treatment reduced pMAPK, pFAK, and pAKT levels, which control proliferative, migratory, and survival functions. Others have shown that phosphorylation of focal adhesion complex--signaling molecules such as FAK and p130cas, which directly interact with Src, is significantly inhibited by SKI-606 (16, 18, 19). Inhibition of MAPK T202/204 phosphorylation by SKI-606, which is most likely more indirect, has been reported by others to be transient in MDA-MB-468 cells (19). Modest inhibition of AKT phosphorylation on Ser473 by SKI-606 in MDA-MDA-231 cells was reported by us (18) but is not always observed (19, 27). Interestingly, recent studies using biopsy samples from patients with breast cancer link Src activity to AKT activation and bone metastasis (28).

Breast cancer cells with constitutively active Src preferentially metastasize to bone, whereas cells with minimum kinase activity developed fewer skeletal metastases (29). The ability of SKI-606 to retard tumor growth and lytic activity in the bone might thus reflect its capacity to inhibit Src but, in any case, is of particular significance because limited therapeutic modalities are currently available for this common complication associated with malignancies like breast and prostate cancer. Because primary tumor growth, skeletal tumor growth, and lytic activity were reduced by SKI-606 treatment, it is possible that SKI-606 affects tumor cell and osteoclast function. Further mechanistic studies are necessary to determine whether the effects of SKI-606 on tumor growth and skeletal metastasis are associated with only Src inhibition or whether additional inhibitory activities of SKI-606 are also involved. The results of our siRNA knockdown experiments highlight the difficulty of determining this in an experimentally unambiguous manner because complete knockdown is rarely achieved and other Src family kinases are present.

The ability of SKI-606 to inhibit a number of genes promoting tumor metastases as shown in this report allows us to postulate that SKI-606 treatment will result in not only a decrease in tumor growth but also reduced tumor cell extravasation, extracellular matrix degradation, and development of distant metastases. The panel of genes selected for monitoring change in expression was based on their well-described roles in prostate cancer and skeletal metastases. Examination of clinical biopsy samples and serum

Effect of SKI-606 on PC-3 experimental skeletal metastasis in vivo. We next examined the effect of SKI-606 treatment on i.t. PC-3 tumors. Animals treated once daily with SKI-606 at 150 μg/kg by oral gavage exhibited reduced total skeletal lesion area (~50%) compared with the vehicle-treated control group at week 4 post-

tumor cell inoculation (Fig. 4B). At the end of these studies, the tibia were removed and subjected to micro-

computed tomography. Results from micro–computed tomography clearly showed the ability of SKI-606 to reduce skeletal lesions, leading to an increase in bone volume–to–tumor volume ratio in experimental animals (Fig. 5). In all in vivo studies presented in Figs. 4 and 5, treatment with SKI-606 had no significant effect on animal weight (data not shown). Collectively, analysis of skeletal lesions by these different methods (X-ray, micro–computed tomography) consistently showed the ability of SKI-606 to markedly reduce tumor growth and lytic areas in the bone.

Effect of SKI-606 on prostate cancer–associated gene expression. We then examined the ability of SKI-606 to alter the expression of various well-described genes known to play important roles in prostate cancer growth and metastases. The data in Fig. 6 show the results of RT-PCR analysis of RNA from PC-3 cells treated with 1 μmol/L SKI-606 for 24 and 48 hours. SKI-606 treatment reduced expression of uPAR, MMP-2, MMP-9, N-cadherin, and fibronectin, proteins involved in cell-cell and cell-

matrix interactions and extracellular matrix degradation (Fig. 6). Furthermore, SKI-606 treatment resulted in a significant inhibition in the expression of BMP-2, BMP-6, IL-8, and TGF-β, all of which play roles in the development and progression of skeletal metastases. Interestingly, SKI-606 treatment also induced the expression of E-cadherin, TIMP-1, and TIMP-2, which attenuate the activities of proteases in involved in extracellular matrix degradation (Fig. 6).

Discussion

SFKs play critical roles in the progression of various malignancies because of their ability to interact with several substrates that are differentially expressed during the multistep process of tumor progression (2). Because of this, several small molecule Src inhibitors are in early clinical development (10–12). Of particular interest is the ability of these inhibitors to block organ-specific metastases (13, 18, 25).

Here, we have extended earlier studies on the multi-

kinase inhibitor SKI-606, originally developed as an Src in-
hibitor, to its effects on prostate tumor cells because Src signaling is implicated in androgen-dependent prostate cancer proliferation, progression to androgen independence, and appearance of skeletal metastases (4, 26). The androgen-independent PC-3 prostate cancer cells typify late-stage disease and provide an experimental model of skeletal metastasis. Treatment of these prostate cancer cells with SKI-606 changed cell morphology and reduced cell proliferation, migration, colony formation, and invasion, much as previously reported in other tumor cells (16, 18). In agreement with these in vitro studies, SKI-606 treatment resulted in a significant (80%) decrease in tumor volume in xenograft-bearing mice treated with SKI-606. Whether or not all of the in vivo effects of SKI-606 treatment can be ascribed to inhibition of Src or SFKs, SKI-606 treatment does reduce Src activation. In addition, SKI-606 treatment reduced pMAPK, pFAK, and pAKT levels, which control proliferative, migratory, and survival functions. Others have shown that phosphorylation of focal adhesion complex–signaling molecules such as FAK and p130cas, which directly interact with Src, is significantly inhibited by SKI-606 (16, 18, 19). Inhibition of MAPK T202/204 phosphorylation by SKI-606, which is most likely more indirect, has been reported by others to be transient in MDA-MB-468 cells (19). Modest inhibition of AKT phosphorylation on Ser473 by SKI-606 in MDA-MDA-231 cells was reported by us (18) but is not always observed (19, 27). Interestingly, recent studies using biopsy samples from patients with breast cancer link Src activity to AKT activation and bone metastasis (28).

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has shown increased production of urokinase type plasminogen activator, uPAR, MMP-2, and MMP-9 in late prostate cancer patients (30, 31). A shift from E-cadherin to N-cadherin expression suggests the occurrence of an epithelial to mesenchymal transition but is also associated with prostate cancer progression (32). Indeed, the altered cell morphology observed following treatment with SKI-606 was associated with induction of E-cadherin and reduced expression of N-cadherin. The outcome of upregulation of the TIMPs by SKI-606 treatment is less predictable. TIMP-1 is considered a poor prognosis factor in some settings; in others, it is associated with survival, whereas TIMP-2 has antiangiogenic activity (33–35). TIMPs balance the activity of proteases in cooperation with additional factors within the tumor microenvironment but may also promote tumor metastases (36). Another study reports that decreasing the expression of TIMPs by gene transfer or by chemical agents results in decreased tumor metastases (37). Repression of several other genes, such as BMP-2, BMP-6, IL-8, and TGF-β, by SKI-606 is most likely beneficial. These genes have been shown to specifically promote the development of skeletal metastasis, an effect that can be reversed following down regulation of these genes (38–42). Overall results from these studies will lead to the identification of a panel of genes, change in the levels of expression of which can also be used as a marker for response to therapy with SFKs inhibitors in different cancers.

The activity of dasatinib and saracatinib (AZD0530) has been evaluated in prostate tumor cells in vitro and in orthotopic and lytic bone models (14). Both of these Src inhibitors affected proliferation, migration, and invasion in vitro, much as reported here for bosutinib. Both compounds caused significant inhibition of tumor growth in orthotopic models with PC3-MM2 GL tumors (dasatinib) and DU-145 tumors (saracatinib). Separate studies showed that dasatinib treatment reduced lymph node metastasis and also resulted in normalization of bone density in an i.t. bone lysis model (43, 44). The agreement among these studies and the results reported here that different classes of Src inhibitors result in anti-tumor activity in a prostate tumor model setting provides a strong rationale for using Src inhibitors in a prostate cancer patients.

SKI-606 (bosutinib) is in phase III clinical trials in patients with CML and phase II breast cancer in combination with hormone ablation therapy. Its ability to elicit significant antitumor effects in additional common malignancies provides compelling rationale to broaden the scope of these studies to other cancers. In summary, results presented in the current study have broadened the scope of the use of Src kinase inhibitors such as SKI-606 in prostate cancer patients, alone or in combination with other therapeutic agents, to achieve anti tumor and antimetastatic effects and help decrease cancer associated morbidity and mortality.

Disclosure of Potential Conflicts of Interest

Frank Boschelli is an employee at Wyeth (now Pfizer), which is pursuing clinical development of bosutinib.

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