Inhibition of Platelet-derived Growth Factor-mediated Proliferation of Osteosarcoma Cells by the Novel Tyrosine Kinase Inhibitor STI571


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ABSTRACT

Purpose: Osteosarcoma is an aggressive primary bone cancer characterized by expression of platelet-derived growth factor (PDGF) and its cognate receptor. Coexpression of the growth factor and receptor suggests their role in autocrine or paracrine growth mechanisms. It has been reported previously that STI571 has specific activity in inhibiting select tyrosine kinase receptors, including PDGF and c-Kit. Osteosarcomas express low levels of c-Kit but abundant levels of PDGF receptor (PDGFR).

Experimental Design: To investigate the potential of STI571 as therapy for osteosarcoma, we studied its effects on PDGF-mediated cell growth in vitro and in an in vivo mouse model.

Results: PDGF acted as a potent mitogen in a dosedependent manner in two osteosarcoma cell lines. STI571 (1.0 μM) inhibited both PDGFR-α and PDGFR-β phosphorylation and the downstream phosphorylation targets extracellular signal-regulated kinase and Akt. STI571 also inhibited PDGF-mediated growth and induced apoptosis in vitro as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and terminal deoxynucleotidyl transferase-mediated nick end labeling staining. To study the effect of STI571 alone or in combination with Taxol in an in vivo model, an osteosarcoma cell line (KRIIB) was transplanted into the tibia of athymic nude mice. Mice were treated with STI571 (50 mg/kg p.o. q M-F), Taxol (8 mg/kg i.p. weekly), or STI571 plus Taxol for 6 weeks. There was no significant difference in tumor size between treatment and control mice. Aberrant signaling pathways downstream of the PDGFR in the v-Ki-ras oncogene-transformed KRIIB cell line may in part explain this finding.

Conclusions: Our data demonstrate that STI571 inhibits PDGF-mediated growth and leads to apoptosis of osteosarcoma cells in vitro by selective inhibition of the PDGFR tyrosine kinase. The effectiveness of STI571 in our studies suggests targeting of PDGFRs as a novel treatment for osteosarcoma.

INTRODUCTION

Osteosarcoma is the most common primary malignant bone tumor in children. Clinically evident metastatic disease is present in 10–20% of patients at diagnosis. Despite advancements in multimodality treatment, 5-year survival rates are approximately 40–50% (1). In recent years, increasing attention has been directed at determining the role of growth factors in the pathogenesis of human tumors, including osteosarcoma. There is growing evidence that osteosarcoma and other tumors are dependent on growth factors that act by autocrine or paracrine mechanisms (2). Selective inhibition of these growth factor-receptor signaling loops may be a new treatment strategy for managing osteosarcoma as well as other types of cancer.

PDGF3 is a potent mitogen for cells of mesenchymal origin. PDGF plays a central role in regulating cell proliferation, chemotaxis, and survival in normal cells as well as in various disease states such as cancer, atherosclerosis, and fibrotic disease (3). The PDGF family is composed of dimeric isoforms (PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC, and PDGF-DD), which exert their cellular effects by differentially binding to two receptor tyrosine kinases (3–5). PDGFR-α and PDGFR-β have molecular masses of ~170 and 180 kDa, respectively. Each receptor contains five extracellular immunoglobulin-like domains and an intracellular tyrosine kinase domain. The structure of PDGFRs is similar to colony-stimulating factor-1 receptor and c-Kit. PDGFR-α binds to all PDGF isoforms except PDGF-DD, whereas PDGFR-β binds PDGF-BB and PDGF-DD homodimers preferentially.

By definition, autocrine growth is suggested to occur in cells expressing both ligand and its cognate receptor, and osteosarcomas meet this criterion. Coexpression of PDGF and PDGFRs has been observed in various human tumors including meningiomas, melanomas, neuroendocrine tumors, ovarian cancer, pancreatic cancer, gastric cancer, lung cancer, and prostate cancer. It is suggested that PDGFRs are overexpressed in PDGF-dependent tumors, that the PDGF/PDGFR system is activated by ligand and receptor upregulation, and that PDGFRs are frequently activated by v-Ki-ras oncogenes. STI571 inhibits autocrine and paracrine growth mechanisms (6, 7). STI571 is a small molecule with high specificity for the 210-kDa PDGFR-α and PDGFR-β kinases, and this specificity is required for its antiproliferative activity in vitro and in vivo (8).

3 The abbreviations used are: PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; Erk, extracellular signal-regulated kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; PI3K, phosphatidylinositol 3'-kinase.
cancer (6). Several studies have confirmed the expression of PDGF and PDGFR in osteosarcoma cell lines (7) as well as in human osteosarcoma samples. A recent retrospective immunohistochemical study of human osteosarcoma samples demonstrated PDGF and PDGFR expression in approximately 86% and 81% of samples, respectively (8). Another study found PDGF-AA and PDGFR-α expressed in 34% and 27% of osteosarcomas, respectively (9).

At the same time PDGF promotes cell growth, it may exert a protective effect on cells by inhibiting apoptosis. In fibroblasts and neurons, activation of the PI3K pathway by binding of PDGF to its receptor leads to phosphorylation of the serine/threonine kinase Akt (10, 11). Akt activation leads to phosphorylation of the BCL-2 family member BAD, which in turn inhibits BAD’s interaction with the BCL family member BCL-XL, a normal activator of the apoptotic pathway in some cells (12, 13).

The importance of growth factors in mediating tumor growth in vivo is underscored by studies demonstrating inhibition of osteosarcoma xenografts in nude mice by the compound suramin (7). Suramin appears to act in a nonselective manner, interfering with the binding of several growth factors to their receptors (14). In recent years, antagonists specific for PDGF and PDGFR have been developed and used to investigate the role of PDGF stimulation in various disease states (15). More recently compounds have been developed that inhibit PDGFR kinases. STI571 (Gleevec, imatinib mesylate, and also formerly known as CGP57148B; Novartis Pharmaceuticals) was developed as an ATP competitive inhibitor of ABL tyrosine kinase (16). At concentrations required for inhibition of Bcr-Abl, STI571 also inhibits other tyrosine kinase receptors, including PDGFR and c-Kit (17). The effectiveness of this drug in blocking Bcr-Abl and c-Kit tyrosine kinases has led to Food and Drug Administration approval for the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia and c-Kit-positive gastrointestinal stromal tumors. STI571 has also been used to study the role of PDGFR signaling in vivo for various tumors including dermatofibrosarcoma protuberans and chronic myelomonocytic leukemia (18, 19). The purpose of this study was to determine whether blocking PDGFR activation by STI571 would reduce osteosarcoma proliferation in vitro or tumor growth in vivo.

MATERIALS AND METHODS

Cell Culture. TE-85 osteosarcoma cell lines were kindly provided by Dr. Z. Zhou (University of Texas M. D. Anderson Cancer Center, Houston, TX). MNNG-HOS (Cl #5) and Saos-2 cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cells were grown in 10% FCS and MEM made complete by the addition of glutamine, HEPES, streptomycin, nonessential amino acids, and multivitamins. For experiments in which growth arrest of cells was required, cell lines were grown to 30% confluence in the presence of FCS and then grown in MEM without FCS for 36–48 h.

Western Blot Analysis. Cells were incubated with or without STI571 (kindly provided by Novartis Pharmaceuticals) for 2 h and then stimulated with PDGF for 10 min. After PDGF stimulation, cells were washed with ice-cold PBS solution and lysed in 0.2 ml of lysis buffer (Cell Signaling Technologies, Beverly, MA) at 4°C for 30 min. Lysates were cleared by a 10-min centrifugation at 10,000 × g, and protein determination was carried out according to the method of Bradford. Samples were subjected to 7.5% PAGE analysis after they were boiled for 5 min in sample buffer containing SDS. The separated proteins were transferred to an Immobilon-P membrane (Millipore, Bedford, MA) and then blocked for 1 h in TBST (1 × Tris-buffered saline + 0.1% Tween 20) containing 5% nonfat milk. The membrane was then incubated with primary antibody in TBST overnight at 4°C. Primary antibodies for PDGFR-α, PDGFR-β, activated PDGFR-α, activated PDGFR-β, and c-Kit (sc-338, sc-339, sc-12910, sc-12909, and c-19, respectively) were obtained from Santa Cruz Bionotechnology (Santa Cruz, CA). Primary antibodies to activated Akt and Erk (9277L and 9101S, respectively) were obtained from Cell Signaling Technologies. Equal loading of protein samples was confirmed by incubating membranes with a primary antibody specific for actin (Santa Cruz Bionotechnology). Membranes were washed and then incubated for 1 h at room temperature with secondary antibody. Bound antibody was detected using enhanced chemiluminescence reagent (ECL; Amersham Pharmacia, Piscataway, NJ).

Proliferation Experiments. Cells were harvested in 75-cm² flasks, washed twice with PBS, and transferred in complete MEM plus 10% FCS into 96-well plates at a seeding density of 1 × 10³ cells/well. Each treatment group was plated in replicates of 12. After 48 h, cell growth was arrested. Thirty-six h after growth arrest, PDGF-AA or PDGF-BB was added (R&D Systems, Minneapolis, MN) to untreated cells or cells pretreated with STI571 for 2 h.

Proliferation Assay. Proliferation was assessed on day 0 (treatment day) through day 5. Proliferation was assayed by the MTT dye technique, which relies on the metabolic reduction of 1 × 10³ cells/well. Each treatment group was plated in replicates of 12. After 48 h, cell growth was arrested. Thirty-six h after growth arrest, PDGF-AA or PDGF-BB was added (R&D Systems, Minneapolis, MN) to untreated cells or cells pretreated with STI571 for 2 h.

Proliferation Assay. Proliferation was assessed on day 0 (treatment day) through day 5. Proliferation was assayed by the MTT dye technique, which relies on the metabolic reduction of the tetrazolium salt MTT by living cells. MTT (0.8 mg/ml) was added to each well, the cells were incubated at 37°C for 2 h, and then DMSO was added. Fifteen min after the addition of DMSO, the absorbance was determined by an automated dual-wavelength spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT) against a reagent blank at a test wavelength of 570 nm and a reference of 630 nm.

In Situ TUNEL Assay. The TUNEL assay was performed using a commercial kit according to the manufacturer’s protocol (Promega, Madison, WI). Cells in chamber slides were fixed in 4% paraformaldehyde in PBS for 20 min and then permeabilized by incubation with 0.5% Triton X-100 in PBS for 5 min at room temperature. After being rinsed twice with PBS for 5 min, the slides were incubated with terminal deoxynucleotidyl transferase buffer for 10 min. Terminal deoxynucleotidyl transferase and buffer were then added to the tissue sections and incubated in a humid atmosphere at 37°C for 1 h. The reaction was terminated by immersing the slides in 2 × SSC for 15 min at room temperature. The slides were washed three times with PBS for 5 min and then counterstained with Hoechst stain. Immunofluorescence microscopy was performed using a 10× objective (Zeiss Plan-Neofluar) on an epifluorescence microscope equipped with narrow bandpass excitation filters mounted on a filter wheel (Ludl Electronic Products, Hawthorne, NY) to select for green fluorescence. Images were captured using a
cooled charge-coupled device camera (Photometrics, Tucson, AZ) and SmartCapture software (Digital Scientific, Cambridge, United Kingdom) on a Macintosh computer. Images were further processed using Adobe PhotoShop software (Adobe Systems, Mountain View, CA). Quantitation of TUNEL was determined by conventional 3,3'-diaminobenzidine staining. Results are presented as mean percentage ± SD of apoptotic cells from the total number of cells counted in 5 fields/chamber. Statistical analysis was performed using a two-sample t test. P < 0.05 was considered statistically significant.

Intratibial Implantation of KRIB Cells in a Nude Mouse Model. Female athymic nude mice were purchased from the animal production area of the National Cancer Institute-Frederick Cancer Research Facility (Frederick, MD) and maintained in specific pathogen-free barrier animal facilities approved by the American Association for Accreditation of Laboratory Animal Care. They were used for experiments at 9 weeks of age.

Cultured KRIB cells (80% confluence) were harvested for intratibial injection by a brief exposure to 0.25% trypsin and 0.02% EDTA. Trypsinization was stopped after 2 min with medium containing 10% FBS, and the cells were washed once in serum-free medium and resuspended in HBSS. Only suspensions consisting of single cells with >90% viability were used for the injections. The cells were injected (5 × 10⁴ cells/mouse) into the right tibia of nude mice anesthetized with Nembutal (50 mg/kg i.p.). To evaluate for bone lesions, the mice were anesthetized weekly beginning 3 weeks after tumor cell injection; tumor growth was monitored by radiographic imaging with a Faxitron MX-20 X-ray unit (Wheeling, IL), and the images were captured digitally. The experiment was ended when there was marked tibia bone destruction with a soft tissue extension of the tumor in the control group.

Therapy of Osteosarcoma Cells Growing in the Tibia of Nude Mice. Three days after intratibial injection of KRIB cells into the nude mice, they were randomized into the following treatment groups (n = 5): (a) oral administration of STI571 (50 mg/kg) five times/week; (b) i.p. injection of Taxol [8 mg/kg (200 μl) weekly]; and (c) a combination of the above STI571 and Taxol doses. Control mice received oral vehicle solution and i.p. HBSS. Mice were killed by exposure to carbon dioxide, and the body weight was determined. The right tibia was harvested and processed. Histopathology confirmed the nature of the disease.

RESULTS

STI571 Inhibits Phosphorylation of PDGFR-α and PDGFR-β in Osteosarcoma Cells. Expression of PDGFRs on five osteosarcoma cell lines was determined by Western blotting with primary antibodies specific for either PDGFR-α or
PDGFR-β. PDGFR-α levels were high in MG-63, KRIIB, and MNNG-HOS cells and low in TE-85 and Saos-2 cells (Fig. 1). PDGFR-β was observed in MG-63 and TE-85 cells but was undetectable in MNNG-HOS and Saos-2 cells (Fig. 1). Extremely low levels of PDGFR-β were present on KRIIB cells (Fig. 1). The presence of PDGFR-β on KRIIB cells was confirmed by finding that PDGFR-β was phosphorylated after stimulation with PDGF-BB ligand (Fig. 5). Only cell lines that expressed both PDGFRs were studied further. TE-85, KRIIB, and MG-63 cells expressed undetectable levels of c-Kit (data not shown).

The PDGF isoform PDGF-BB was used to study PDGF phosphorylation because of its ability to stimulate all three receptor isoforms (PDGFR-α, PDGFR-αβ, and PDGFR-ββ). Different concentrations of PDGF-BB (0.2–50 ng/ml) were used to stimulate the TE-85 osteosarcoma cells (Fig. 2). Receptor phosphorylation was determined by Western blotting with primary antibodies specific for epitopes corresponding to the short amino acid sequences containing phosphorylated Tyr-720 and Tyr-1021 of PDGFR-α and PDGFR-β, respectively. A dose of 10 ng/ml PDGF-BB resulted in maximally stimulated PDGFR-α and PDGFR-β after 10 min (Fig. 2). The decrease in phosphorylation observed at higher concentrations of ligand may have been due to receptor internalization. STI571 inhibited PDGF-BB phosphorylation of both PDGFR-α and PDGFR-β when added to TE-85 cells 2 h before stimulation. Inhibition of receptor phosphorylation by STI571 was dose dependent, with >50% inhibition at 1.0 μM and complete inhibition at 10.0 μM.
The same results were obtained for MG-63 cells (data not shown).

**STI571 Effects on Downstream Signaling of Akt and Erk.** PDGF isoforms exert their cellular effects by activating downstream effector molecules after binding to their cognate receptors. Activation of PI3K results in activation of mitogen-activated protein kinase and Akt, pathways that are implicated in the stimulation of cell growth and antiapoptosis, respectively. Near complete inhibition of Erk and Akt activation occurred in TE-85 cells treated with 1.0 μM STI571 before stimulation with PDGF-BB (Fig. 4). The same results were obtained for MG-63 cells (data not shown). PDGF-BB stimulation resulted in phosphorylation of PDGFR-α and PDGFR-β in the KRB cell line, implying normally functioning receptors (Fig. 5). However, there was no observed difference in either Erk or Akt phosphorylation before or after stimulation (Fig. 5). Furthermore, STI571 had no effect on inhibition of Erk or Akt activation in this cell line (Fig. 5). These findings demonstrate that STI571
inhibits PDGF-mediated activation of the PDGFR and downstream effector molecules in both MG-63 and TE-85 cells. They also suggest that the inability of STI571 to affect downstream effector molecules after stimulation with PDGF may in part be due to an aberration in the signal transduction pathway downstream of the receptor, resulting in constitutive activation of Akt and Erk in the absence of PDGF.

**STI571 Inhibits PDGF-AA- and PDGF-BB-mediated Cell Growth in Vitro.** To investigate whether PDGF contributes to the growth of osteosarcoma cells, the effects of STI571 on cell proliferation were studied in vitro. When stimulated by PDGF-AA, growth-arrested MG-63 and TE-85 cells began to proliferate. The proliferative effect was observed 1 day after stimulation. The maximum extent of proliferation (i.e., efficacy) on day 5 did not differ significantly between the two cell lines and was ~0.4 absorbance unit. The proliferative effect was dose dependent, with the greatest effect occurring with the highest dose of PDGF-AA studied (10 ng/ml; Fig. 6A). STI571 (1.0 and 10.0 μM) significantly inhibited PDGF-AA-mediated proliferation in MG-63 cells compared with the control (cells stimulated with PDGF-AA only; Fig. 6B). Similar findings were observed in TE-85 cells, although STI571 doses as low as 0.1 μM were also effective, possibly due to less PDGFR-α expression on these cells compared with MG-63 cells (data not shown). PDGF-BB also induced proliferation in growth-arrested MG-63 and TE-85 cells. The proliferative effect was rapid in TE-85 cells (day 1; data not shown) but was delayed until day 4 in MG-63 cells (Fig. 6C). The maximum extent of proliferation on day 5 was ~0.4 absorbance unit for TE-85 cells and 0.5 absorbance unit for MG-63 cells. STI571 (1.0 and 10.0 μM) significantly inhibited PDGF-BB-mediated proliferation in the MG-63 cell lines (Fig. 6D) and the TE-85 cell line (data not shown). A significant increase in proliferation of KRIB cells was not observed after stimulation with either PDGF-AA or PDGF-BB (data not shown). This observation is consistent with our findings that downstream signaling to Akt and Erk by PDGFRs is not affected by PDGF-BB or STI571 treatment.

**STI571 Induces Apoptosis in Osteosarcoma Cells.** In MG-63 and TE-85 cells, stimulation with PDGF-BB activates Akt. Activation of Akt leads to phosphorylation of the BCL-2 family member BAD that can suppress its apoptotic effect in some cell types (12). Therefore, inhibition of PDGF-mediated growth by STI571 could in part have been due to induction of apoptosis. The effect of STI571 treatment on apoptosis of MG-63 and TE-85 cells in vitro was assessed on days 1, 3, and 5 by TUNEL staining. Only a small fraction of growth-arrested MG-63 and TE-85 cells underwent apoptosis after 5 days. STI571 treatment resulted in more apoptotic cells at each successive time point studied, with 20–25% of cells undergoing apoptosis by day 5 (Table 1). Significantly fewer cells underwent apoptosis in both cell lines when stimulated with PDGF-BB after STI571 treatment. This finding is not unexpected because we have demonstrated that 1.0 μM STI571 did not completely inhibit Akt phosphorylation after stimulation with 10 ng/ml PDGF-BB. PDGF-BB alone had no effect on TE-85 cells. Similarly, PDGF-BB did not have an effect on MG-63 cells on day 1 or day 3. The small number of apoptotic MG-63 cells on day 5 is within the normal range of cells undergoing apoptosis at any point in time.

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<th>Apoptotic cells (%)</th>
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<tr>
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*P < 0.05, which is considered statistically significant for any treatment group sample when matched to control (no treatment).

**Effect of STI571 on the Growth of the KRIB Human Osteosarcoma in Vivo.** An animal model of osteosarcoma has been established using the KRIB osteosarcoma cell line. When KRIB cells are orthotopically transplanted into the tibia of congenitally athymic nude mice, bone tumors that are histologically and radiographically similar to human primary osteosarcomas develop within 4 weeks (20). To study the effect of STI571 on osteosarcoma growth in vivo, KRIB cells were orthotopically transplanted into the tibia of 20 nude mice by intraosseous injection. Three days after transplant, mice were randomized to a control arm (untreated) and one of three treatment arms (Taxol, STI571, or Taxol plus STI571). Our data suggest that STI571 may induce apoptosis by blocking the antiproliferative effect of PDGF. Therefore, one treatment arm (STI571 plus Taxol) was studied to determine whether treatment with STI571 could sensitize osteosarcoma cells to the cytotoxic effects of Taxol. Mice in all treatment groups including the control arm developed tumors by week 2 as assessed radiographically. The tumors were radiographically indistinguishable from primary human osteosarcoma, including the development of periosteal reaction with new bone formation. There was no observed difference in tumor size at week 2, 4, or 6 in any treatment arm when compared with those in the control mice (Fig. 7).

**DISCUSSION**

Our in vitro studies demonstrate that PDGF-BB acts as a potent mitogen for MG-63 and TE-85 cell lines. PDGF-BB levels as low as 0.2–2.0 ng/ml resulted in marked proliferation of these cells compared with untreated controls. The levels of PDGF activity in sera from mice and humans are comparable [1–2 ng/ml (21, 22)]. An earlier study examined the proliferative effect of PDGF-AB on a single osteosarcoma xenograft referred to as L-II OSM, which expressed both PDGFRα and PDGFRβ. The group did not detect any increase in tumor volume after 14 days when tumor-bearing mice were given PDGF-AB continuously by an i.p. mini-osmotic pump (23). However, by virtue of the PDGFR ligand used in the study, the lack of effect can only...
be attributed to stimulation of PDGFR-α and PDGFR-β dimers. It is also possible that, in vivo, lower levels of PDGF are sufficient to satisfy the PDGFRs or that a redundancy in growth factor loops exists. Redundancies in growth factor loops in osteosarcoma cells have been described previously (7). To the best of our knowledge, the effects of selective PDGFR antagonists on osteosarcoma cell lines have not been characterized.

Although many different PDGFR antagonists exist, we chose to investigate the effect of STI571 on PDGF-mediated growth in osteosarcoma. STI571 is a selective inhibitor of the Abl and Bcr-Abl tyrosine kinases and of the tyrosine kinase receptor for stem cell factor, c-Kit, and PDGFR. PDGFR-α and PDGFR-β phosphorylation was inhibited in a dose-dependent manner by STI571 in both MG-63 and TE-85 cells. Greater than 50% inhibition of PDGFR phosphorylation in MG-63 and TE-85 cells was observed after treatment with 1 μM STI571. This finding is in accordance with prior studies showing that pretreatment of Swiss 3T3 cells with STI571 caused a dose-dependent inhibition of PDGFR phosphorylation with an IC₅₀ value of approximately 0.1 μM (24). Proliferation was also decreased with an IC₅₀ of approximately 1 μM, comparable with the concentration required to inhibit the growth of Philadelphia chromosome-positive hematopoietic cells in vitro (25).

A reduction in osteosarcoma cellular proliferation observed in STI571-treated cells was in part due to apoptosis. PDGF not only acts as a potent mitogen but also actively prevents some cells from dying (26). MG-63 and TE-85 cells treated with 1 μM STI571 underwent apoptosis. This effect was observed 1 day after treatment in MG-63 and TE-85 cells and was time dependent, with up to 25% of the cells undergoing apoptosis by day 5. The percentage of apoptotic cells was significantly lower when cells were treated with both PDGF and STI571. This finding suggests that PDGF may inhibit STI571-induced apoptosis. Our finding that 1 μM STI571 was not sufficient to completely inhibit PDGFR phosphorylation may explain why a smaller fraction of these cells underwent apoptosis as compared with cells treated with STI571 alone.

The effectiveness of STI571 in vitro led us to search for an in vivo mouse model of osteosarcoma. Samid et al. (27) established and characterized the v-Ki-ras-transformed human osteosarcoma cell line KRB1. What makes this cell line especially useful as an in vivo model is that when orthotopically implanted into the tibia of athymic mice, interosseous tumors develop that are histologically and radiographically indistinguishable from human primary bone osteosarcomas. Moreover, consistent with the clinical course of human osteosarcoma, these cells subsequently metastasize to the lungs (20).

Three days after intratibial injection of KRB1 cells, mice were randomized to a control arm and one of three treatment arms (Taxol, STI571, or STI571 plus Taxol). After 6 weeks, there was no significant difference in tumor size in any treatment group compared with control mice. In vitro studies demonstrate that PDGFR-α and PDGFR-β on KRB1 cells were phosphorylated after stimulation with PDGF-BB. STI571 was able to inhibit this phosphorylation. This suggests that the PDGFRs on KRB1 cells are functional. However, the downstream effector moleculesAkt and Erk appeared to be constitutively activated in KRB1 cells. Neither PDGF-BB nor STI571 had an effect on the baseline level of activation. The lack of effect of either PDGF or STI571 on the KRB1 cells may be explained by the method used to immortalize and transform this cell line. KRB1 cells have been transformed with the v-Ki-ras oncogene. Ras is intimately involved in cross-talk between different signaling pathways. Ras has been shown to interact with PI3K, activate the Rho and Rac family of GTP-binding proteins, and activate mitogen-activated protein kinase through activation of Raf-1 (28–32). Therefore, uncontrolled expression of the ras oncogene in KRB1 cells may lead to aberrant activation of effector molecules downstream of the PDGFR. It would then stand to reason that stimulation or inhibition of PDGFR would have no effect on cell proliferation or apoptosis in the KRB1 cell line.

We have orthotopically implanted TE-85 and MG-63 cells into the tibia of nude mice. At 8 weeks, there was radiographic evidence of intraosseous tumor formation in mice implanted with TE-85 cells, but not in those implanted with MG-63 cells. At 12 weeks, TE-85 tumors appeared stable with little interim growth. The slow growth rate of this tumor currently limits in...
vivo studies. We plan to recycle the tumor through the tibia to generate a more aggressive cell line with growth characteristics similar to those of KIRIB.

In conclusion, our study has shown that the novel tyrosine kinase inhibitor STI571 may inhibit PDGF-mediated proliferation of osteosarcoma cells. The effectiveness of STI571 in vitro suggests that targeting PDGFs may lead to a novel treatment in osteosarcoma. Animal models of human osteosarcoma are limited, and therefore the need to develop additional animal models to study the pathophysiology of this cancer and the effect of STI571 in vivo is paramount.

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REFERENCES
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