INTRODUCTION
STI571 is a tyrosine kinase inhibitor, which was found as a specific inhibitor of BCR/ABL, the major causative tyrosine kinase of chronic myelogenous leukemia (CML). However, it was also a powerful and selective inhibitor of platelet-derived growth factor receptor (PDGF-R) and c-Kit. Based on encouraging preclinical results, STI571 was approved by the Food and Drug Administration in May 2001 for the treatment of CML that is refractory to interferon therapy and in February 2002 for the treatment of metastatic gastrointestinal stromal tumors. Further STI571 studies for various other malignancies, such as glioblastoma and small cell lung carcinoma, are underway. Regarding osteosarcoma, limited studies have been published about the involvement and role of PDGF-R and c-Kit. The purpose of this study was to examine the expression and possible role of PDGF-R and c-Kit in a series of human osteosarcoma tumor samples and to investigate the effects of STI571 on the growth of patient-derived osteosarcoma cultures.

MATERIALS AND METHODS
Immunohistochemistry. Ninety-eight osteosarcoma surgical specimens were obtained from patients who had biopsy, definitive surgery, recurrence, or metastases. Core biopsies from defined morphologic areas of paraffin-embedded tumor samples were assembled on a tissue microarray and analyzed by immunohistochemistry with the anti-PDGFA, anti-PDGF-Ra, and anti-c-Kit antibodies (Santa Cruz Biotechnology, Santa Cruz, CA).

Cytotoxicity Assay. Cell lines were established from primary osteosarcoma specimens from six different patients. Cells were grown in RPMI 1640 medium supplemented with 10% FCS in 96-well culture plates (1 X 10^3 cells/well) and allowed to adhere overnight. Cells were then serum starved for 24 h and incubated for 30 min with the indicated concentrations of STI571 (Novartis Pharma AG, Basel, Switzerland) before stimulation with 10 ng/ml PDGF (Invitrogen Life Technologies, Carlsbad, CA) for 48 h. Cell growth was measured by the colorimetric XTT reduction assay.

Immunoprecipitation and Immunoblotting. Six cell lines used in the previous study were grown to 70% confluency in DMEM containing 20% FCS. Cells were serum starved for 24 h and pretreated for 90 min with the indicated concentrations of STI571 before stimulation with 100 ng/ml PDGF for 10 min. Whole-cell lysates were collected for protein content and immunoblotted with anti-PDGF-Ra antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), anti-PDGF-Rß antibodies (Upstate Biotechnology, Lake Placid, NY), anti-phospho-Akt antibodies, anti-phospho-MAPK antibodies, anti-Akt antibodies, or anti-MAPK antibodies (Cell Signaling Technology, Beverly, MA). Also, immunoprecipitation was performed with anti-PDGF-Ra antibodies or anti-PDGF-Rß antibodies, followed by immunoblotting with anti-phosphotyrosine antibodies (Upstate Biotechnology, Lake Placid, NY).

Statistical Analysis. Survival analysis was performed by Kaplan-Meier analysis and statistical significance evaluated by log-rank test. Statistical analysis for cell viability used one-way ANOVA and Scheff’s F-test for multiple comparisons. P < 0.05 was considered to be significant.

RESULTS
Expression of PDGF-R and C-Kit in Osteosarcoma. PDGFA, PDGF-Ra, and c-Kit expression was detected in 86.2%, 81.8%, and 16.3% of osteosarcoma specimens, respectively. Coexpression of PDGFA and PDGF-Ra was 77.4%. Higher frequencies of PDGFA and PDGF-Ra expression were found in metastatic and recurrent samples. At the time of initial biopsy or definitive surgery, there was significant correlation between PDGFA or PDGF-Ra expression and decreased event-free survival (p<0.05)(Fig. 1).

Molecular Effects on PDGF Signaling Cascade by STI571. Of six osteosarcoma cell lines, two cell lines expressed detectable both PDGF-Ra and PDGF-Rß, two expressed only PDGF-Rß, and two expressed neither PDGF-Ra nor PDGF-Rß was phosphorylated in response to PDGF stimulation and dephosphorylated by 1–10 µM STI571 in all PDGF-R positive cell lines. Similarly, Akt, one of PDGF downstream signaling molecules, was phosphorylated by PDGF, and 1-10 µM STI571 inhibited this phosphorylation. In contrast, MAP kinase, another PDGF downstream molecule, was constitutively activated regardless of PDGF stimulation and STI treatment in three of four PDGF-R positive cell lines and one of two PDGF-R negative cell lines.

Antiproliferative Activity of STI571 on Osteosarcoma Cells. STI571 inhibited viability of PDGF-R positive cell lines at the concentration up to 10 µM. Concentration at 50µM appeared to have non-specific effects on viability. IC50 values for PDGF-R positive cell lines were 5.6 - 9.5 µM, which were significantly lower than those for PDGF-R negative cell lines (P < 0.01). There was no significant difference of IC50 values between both PDGF-Ra and B positive cell lines and only PDGF-Rß positive cell lines.

DISCUSSION
Our immunohistochemical findings indicate highly frequent expression of PDGFA and PDGF-Ra in osteosarcoma and its correlation of inferior event-free survival, suggesting that PDGF pathway should be a potential prognostic factor and could be a potential molecular target by specific inhibitors. Our in vitro study demonstrates that STI571, which is a potent inhibitor of PDGF-R, blocks the PDGF-mediated intracellular signal transduction via inhibition of Akt phosphorylation and inhibits osteosarcoma cell growth. On the other hand, constitutive activation of MAPK was not affected by STI571 in four out of six osteosarcoma cell lines, whereas STI571 has been reported to inhibit both Akt and MAPK phosphorylation in CML. Further STI571 studies for various other malignancies, such as glioblastoma and small cell lung carcinoma, are underway.