Effect of Platelet-Derived Growth Factor Receptor-\(\beta\) Inhibition with STI571 on Radioimmunotherapy

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Abstract

Whereas radioimmunotherapy of hematologic malignancies has evolved into a viable treatment option, the responses of solid tumors to radioimmunotherapy are discouraging. The likely cause of this problem is the interstitial hypertension inherent to all solid tumors. Remarkable improvements in tumor responses to radioimmunotherapy were discovered after the inclusion of STI571 in the therapy regimen. A combination of the tumor stroma–reactive STI571, a potent platelet-derived growth factor receptor-\(\beta\) (PDGFr-\(\beta\)) antagonist, and the tumor-seeking radiolabeled antibody B72.3 yielded long-lasting arrest of the human colorectal adenocarcinoma LS174T grown as s.c. xenografts in athymic mice. The interaction of STI571 with the stromal PDGFr-\(\beta\) reduced tumor interstitial fluid pressure (\(P_{IF}\)) by >50% and in so doing improved the uptake of B72.3. The attenuation of \(P_{IF}\) also had a positive effect on the homogeneity of antibody distribution. These effects were dose-dependent and under optimized dosing conditions allowed for a 2.45 times increase in the tumor uptake of B72.3 as determined in the biodistribution studies. Single-photon emission computed tomography imaging studies substantiated these results and indicated that the homogeneity of the radioisotope distribution was also much improved when compared with the control mice. The increased uptake of radioimmunotherapy into the tumor resulted in >400% increase in the tumor absorbed radiation doses in STI571 + radioimmunotherapy–treated mice compared with PBS + radioimmunotherapy–treated mice. The improved antibody uptake in response to the attenuation of tumor \(P_{IF}\) was identified as the primary reason for the growth arrest of the STI571 + radioimmunotherapy–treated tumors. Two related causes were also identified: (a) the improved homogeneity of monoclonal antibody distribution in tumor and (b) the increased tumor radiosensitivity resulting from the improved tumor oxygenation. (Cancer Res 2005; 65(17): 7824-31)

Introduction

Radioimmunotherapy, i.e., using monoclonal antibodies (mAb) coupled to radioactive isotopes as tumoricidal agents, has gained a prominent place in the treatment of lymphoma. Radioimmunotherapy allows for a selective recognition and killing of malignant cells while sparing normal tissues. Impressive responses to radioimmunotherapy have been observed in non-Hodgkin's lymphomas, even in a chemotherapy-relapsed and -refractory disease (1–3). Two agents (\(^{90}\)Y-ibritumomab tiuxetan and \(^{131}\)I-tositumomab) have already been approved by the U.S. Food and Drug Administration (FDA). High response rates and durable remissions in various subtypes of B-cell non-Hodgkin's lymphomas confirmed a single-agent efficacy of radioimmunotherapy in this disease (2, 3) and prompted several combination therapy clinical trials to further improve the outcome.

By contrast, solid tumors have proven thus far resistant to mAb-based radiotherapies (4–6). Efficient delivery of radioimmunotherapy to solid tumors encounters numerous physical barriers. Compromised tumor vasculature, slow diffusion and convection rates of large mAb molecules through the interstitial spaces, and high intratumoral pressures hinder mAb influx into tumors. Thus, mAb do not penetrate tumors uniformly. Instead, they tend to accumulate in the periphery of the tumor and in the perivascular zones (7–10). To reach all clonogenic tumor cells, mAb must cross the tumor endothelium and its underlying basement membrane, and filter through the tumor stroma and parenchyma. Notwithstanding the fact that tumor vessels are abnormally leaky to macromolecules, the extravasation of mAb into the tumor mass is inefficient (9–11). In clinical studies, tumor deposits at levels as low as 0.001% to 0.01% of the injected dose of radiolabeled antibody per gram of tumor are commonplace. Estimates of absorbed radiation doses range from 100 to 3,000 cGy and indicate that the majority of mAb fails to extravasate at the tumor site (see, e.g., refs. 5, 12, 13). To date, advances in radioimmunotherapy to treat solid tumors are lackluster. Various efforts to improve the mAb accretion in solid tumors and consequently to improve the efficacy of radioimmunotherapy have been instigated (4, 11, 14–18).

High interstitial fluid pressure (\(P_{IF}\)) is a property displayed by many solid tumors. \(P_{IF}\) creates a formidable physiologic barrier to tumor uptake of drugs from circulation and is largely responsible for the inefficient uptake of radioimmunotherapy (10, 19–21). Several recent studies have identified platelet-derived growth factor (PDGF) as a critical regulator of \(P_{IF}\) (10, 19–21). PDGF regulation of \(P_{IF}\) in normal loose connective tissue and solid tumors (22–26). PDGF regulation of \(P_{IF}\) in loose connective tissue was first shown by Rodt et al. in a rat model of anaphylaxis-induced attenuation of \(P_{IF}\). Local injections of PDGF-BB normalized \(P_{IF}\), implying that stromal cells actively control \(P_{IF}\) (25). In a similar model, the activation of phosphatidylinositol 3'-kinase through the PDGF-BB interaction with PDGF receptor-\(\beta\) (PDGFr-\(\beta\)) was found to be critical for the control of \(P_{IF}\) in the loose connective tissue (26).
A novel mechanism for therapeutic synergy between PDGFr-β antagonists and chemotherapeutic agents has been proposed by Pietras et al. (22) based on their observation that STI571, a potent PDGFr-β inhibitor, significantly reduces tumor PGF and augments accretion of Taxol in s.c. tumors in mice. Subsequent studies in tumor models having PDGFr-β expression restricted to stromal cells confirmed that the reduction in tumor PGF after treatment with PDGF inhibitors results in improvements in the tumor uptake of chemotherapeutic drugs (23, 24). The attenuation of tumor growth arrest at doses of these doses, severe radiotoxicity was readily evident with a lethal CC49 labeled with either 131I or 90Y, have shown any therapeutic efficacy in clinical trials (23, 24). The attenuation of tumor with PDGF inhibitors results in improvements in the tumor uptake of chemotherapeutic drugs (23, 24). The attenuation of tumor growth arrest at doses of these doses, severe radiotoxicity was readily evident with a lethal CC49 labeled with either 131I or 90Y, have shown any therapeutic efficacy in clinical trials (23, 24).

For this reason, a combination STI571-radioimmunotherapy emerged as a regimen that may well allow accumulation of therapeutically sufficient radiation doses in solid tumors. STI571 (Gleevec, imatinib mesylate) is a tyrosine kinase inhibitor that blocks tyrosine kinases of abl, c-kit, and PDGFr receptors. STI571 has been approved by the FDA in 2001 for the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors, where it acts by inhibition of bcr-abl and mutated c-kit, respectively (27). It is noteworthy that edema and fluid retention are the most common side effects after the prolonged STI571 treatment in CML patients. Although the mechanism for these problems remains to be fully characterized, one obvious process seems via the inhibition of PDGFr-β (28, 29).

The efficacy of the STI571-radioimmunotherapy regimen was evaluated in a human colorectal adenocarcinoma LS174T xenografted in athymic mice. LS174T tumors grown as s.c. xenografts express mucin-like tumor-associated glycoprotein-72 (TAG-72), to which mAb B72.3 was developed (30, 31). The treatment of LS174T-bearing mice with 131I-B72.3 produces some therapeutic efficacy in clinical trials (5, 33).

Materials and Methods

Animal and tumor models. Four- to 6-week-old athymic female mice (NCr nu/nu), average weight 18 g, were purchased from the National Cancer Institute Animal Program. Fox Chase severe combined immunodeficient mice of a similar age and weight were purchased from M&B (Ry, Denmark). Mice were housed in a fully accredited by Association for Assessment and Accreditation of Lab Animal Care Animal Facilities. Mice were acclimated for 5 to 7 days after arrival before any experiments. All procedures described here were approved by the local Institutional Animal Care and Use Committee. Mice had a free access to food and water and were kept on a 12-hour light cycle. Potassium iodide-supplemented water was provided for 3 days before and 4 days after any treatment with radiiodinated antibodies.

S.c. tumors were produced in these mice ~10 days after the s.c. injection of $5 \times 10^5$ LS174T human colorectal adenocarcinoma cells in 0.2 mL MEM (Invitrogen, Carlsbad, CA). The cells were obtained from subconfluent monolayers grown in the MEM supplemented with 10% fetal bovine serum (FBS).

Reagents. Mouse monoclonal antibody B72.3 was produced by the University of Nebraska Medical Center Monoclonal Antibody Facility. It was purified from mice ascites by protein-G affinity chromatography. B72.3 recognizes a high molecular weight glycoprotein complex designated as a TAG-72 and shows reactivity with over 85% of adenocarcinomas with minimal reactivity to normal tissues (31). Goat anti-PDGFr-β receptor antibody P-20 (Santa Cruz Biotechnology, Santa Cruz, CA), monoclonal antiphosphotyrosine antibody PY20 (Transduction Laboratories, Lexington, KY) were used as recommended by the suppliers. STI571 (Gleevec, imatinib mesylate) was generously provided by Novartis Pharma AG (Basel, Switzerland). The stannyl precursor of the nitromidazole-based radiodinated hypoxia tracer l-(ethyl-14C)iodobenzamide)-2-nitromidazole was prepared on site. The radiodiodination was also done on site. Sodium 125I and 131I were purchased from PerkinElmer Life and Analytical Sciences, Inc. (Boston, MA). 131I radionucleide had a specific activity of ~17 Ci (629 GBq)/mg and was provided in a 10$^{-5}$ mol/L NaOH (pH 8.11) (without reductants) at a concentration of 100 mCi/mL (3.7 GBq/mL). 131I radionucleide had a specific activity of >5 Ci (>185 GBq)/mg and was provided in 0.1 mol/L NaOH (pH 12-14). Antibodies were radioiodinated using the iodogen method (34) as follows. 131I radiolabeling was done in a glass test tube coated with 0.05 mg of iodogen. One hundred microliters of 1 mg/mL solution of B72.3 in PBS (pH 7.2) was provided in 0.1 mol/L phosphate buffer, 0.0027 mol/L potassium chloride, and 0.137 mol/L sodium chloride (pH 7.2) at 25°C and 0.1 mL of 125I (1.0 mCi; 37 MBq) were transferred into the iodogen-coated tube. The mixture was incubated at room temperature for 10 to 20 minutes. The reaction progress was measured using instant TLC with methane/water (1:4, v/v) as the elution system. 125I-B72.3 was purified on an Econo-Pac 10DG gel filtration column (Bio-Rad Laboratories, Hercules, CA) eluted with PBS. For therapy studies, B72.3 was labeled with 131I as follows: into a glass test tube coated with 0.1 mg of iodogen was added 0.1 mL of 10 mg/mL B72.3 in PBS (pH 7.2) and 0.01 mL of 131I (10 mCi, 370 MBq). The mixture was incubated at room temperature for 20 minutes. The reaction progress was measured using instant TLC with methane/water (1:4, v/v) as the elution system. 131I-B72.3 was also purified on a 10DG gel filtration column eluted with PBS. Before administration radiolabeled antibodies were diluted with PBS containing 0.1% mouse serum to yield the injection dose volume of 0.2 mL per mouse.

In vitro cell growth assay. LS174T cells were seeded in 96-well plates at a density of 3,000 cells per well in either full growth medium (EMEM with 2 mmol/L L-glutamine and Earle's balanced salt solution adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mmol/L nonessential amino acids, and 1.0 mmol/L sodium pyruvate, supplemented with 10% FBS) or serum-depleted growth medium containing 0.1% bovine albumin. After 24 hours of growth, the culture medium was replaced with fresh medium containing 0.1, 1.0, or 5.0 mmol/L STI571 and the cells were allowed to grow for 48 hours before the cell proliferation was determined using the colorimetric kit. The proliferating fraction of cells irradiated in the absence of any additional treatment was set to 1 (or 100%).

Immunoblotting. LS174T cells and porcine aortic endothelial cells, positive control (35) expressing both PDGFr and PDGFr-β (American Type Culture Collection, Manassas, VA), were seeded in 60-mm dishes (1.5 $\times 10^5$ cells per dish) and starved overnight in cell culture medium containing antibiotics and 0.1% bovine serum albumin. Next, cells were stimulated or not with 100 ng/mL PDGF-BB for 7 minutes at 37°C. All subsequent treatments were as described previously (22–24, 36, 37).

Immunohistochemistry. The expression of PDGFr-β was determined in deparaffinized, formalin-fixed sections from untreated LS174T xenografts as described previously for KAT-4 (23).

Effect of STI571 on in vivo phosphorylation of platelet-derived growth factor receptor-β. Two methods were employed to determine the levels of PDGFr-β phosphorylation in the STI571-treated LS174T tumors.
The first method used was as described by Pietras et al. (23) without any modifications. Quantification of blotted protein band intensities was done on a CCD camera (Fujifilm Sverige AB, Stockholm, Sweden). The intensity of the phosphotyrosine signal was divided by the intensity of the receptor signal to yield relative phosphotyrosine values. The average relative phosphotyrosine value of PBS-treated tumors was set to 1. The second method used the PathScan phospho-PDGFr-β sandwich ELISA kit (Cell Signaling Technology, Inc., Beverly, MA). After the protein content in tumor lysates was determined, aliquots were prepared containing 0.6 mg total protein and the volume of each sample was adjusted to 0.1 mL with PBS. From this point on, the protocol provided with the ELISA kit was followed without any modifications.

**Measurement of the tumor P}_{IF}.** Tumor \( P_{IF} \) was measured by the wick-in-needle technique, as described previously (22, 23). STI571 was given by gavage BID for a total dose of 100 mg/kg/day. Radiotracer \( ^{125}\text{I-B72.3} \) (10 mcCi/mouse) was injected i.v. via a tail vein (PBS control group, \( n = 8 \); STI571 group, \( n = 11 \)). All mice were killed 120 hours after \( ^{125}\text{I-B72.3} \) administration, tumors were removed and the amount of radioactivity in tumor, blood, and selected tissues was measured. The tissue and tumor uptake are expressed as percent injected dose per gram tumor.

**Biodistribution.** Mice were treated with either PBS or STI571 for 7 consecutive days as indicated in Table 1 for a total dose of 100 mg \( \times \) kg \(^{-1} \) \( \times \) day \(^{-1} \) in 200 \( \mu \)L of PBS (\( n = 5 \)) for four consecutive days before the measurement of the tumor \( P_{IF} \). Control mice were given PBS (\( n = 6 \)). The last administration of STI571 preceded the measurement of the tumor \( P_{IF} \) by 1 to 2 hours.

**Radiation therapy.** Mice were randomized into four groups: (a) no treatment (\( n = 10 \)), (b) \( ^{131}\text{I-B72.3} \) only (\( n = 10 \)), (c) STI571 only (\( n = 12 \)), and (d) \( ^{131}\text{I-B72.3} \) plus STI571 (\( n = 12 \)). Body weight and tumor sizes were measured thrice a week, and tumor volumes calculated according to the following formula:

\[
\text{volume} = \frac{\pi}{6} \times \text{longer diameter} \times (\text{shorter diameter})^2.
\]

STI571 was given as indicated in Table 1 and \( ^{131}\text{I-B72.3} \) (0.25 mCi) was injected i.v. via a tail vein (PBS control group, \( n = 8 \); STI571 group, \( n = 11 \)). All mice were killed 120 hours after \( ^{125}\text{I-B72.3} \) administration, tumors were removed and the amount of radioactivity in tumor, blood, and selected tissues was measured. The tissue and tumor uptake are expressed as percent injected dose per gram tumor.

**Imaging studies.** Four mice with size-matched tumors (1.9-2.1 g) were selected from groups treated with either STI571 (\( n = 2 \)) or PBS (\( n = 2 \), control), as shown in Table 1. \( ^{131}\text{I-B72.3} \) was injected i.v. and the imaging commenced 24 hours after the administration of the radiotracer. Images were acquired using a dedicated Animal Single-Photon Emission Computed Tomography Imaging System (Gamma Medica Instruments, Northridge, CA). The images were reconstructed using LumaGEM version 5.107 software with the Butterworth bandpass post-reconstruction filtering. To obtain quantitative evaluation of the uptake, the total counts in the region of interest drawn around the perimeter of the tumor were measured and divided by the number of pixels for each tumor at each time point and the tumor-specific uptake was obtained after subtraction of the background counts.

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**Table 1.** Dosing schedules for biodistribution and radioimmunotherapy studies

<table>
<thead>
<tr>
<th>STI Group</th>
<th>PBS Group</th>
<th>Days</th>
<th>LS174T implant</th>
<th>LS174T left flank</th>
<th>LS174T right flank</th>
<th>STI</th>
<th>( ^{131}\text{I-B72.3} )</th>
<th>necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>( ^{131}\text{I-B72.3} )</td>
<td>PBS</td>
<td>-2</td>
<td>0</td>
<td>3</td>
<td>10 or 28</td>
<td>120</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 1.** Characterization of PDGFr-β expressions and its interactions with STI571 in LS174T cells in vitro and in vivo. A, immunoblot of human adenocarcinoma LS174T cells and porcine aortic endothelial cells expressing the PDGFr-α and PDGFr-β (α)-PAE, control). Cells were left untreated or were stimulated with PDGF-BB and following sequential immunoprecipitation (IP) of PDGF receptors, immunoblotting to detect activated PDGFr was done using anti-phosphotyrosine antibodies. B, survival of LS174T cells in the presence of low concentrations of STI571. Cells were treated for 24 or 48 hours with STI571 and subsequently the metabolic/proliferative activities measured. The surviving fraction of the untreated control cells is one. C, survival of LS174T cells grown in the presence of various STI571 concentrations and irradiated with 1 and 6 Gy. D, immunohistochemical staining using antibodies against PDGFr-β or nonspecific rabbit IgG as a control in 5-μm sections of formalin-fixed LS174T tumors.
Statistical analyses. Statistical analyses for in vitro studies were done using the two-sided, unpaired Student’s t test at the significance level of ≤0.05. Error bars in figures represent SE. Kaplan-Meier survival analyses were done using MedCalc Software ver. 7.4.4.0 (Mariakerke, Belgium). To assess differences in tumor growth between treatment groups the generalized estimating equations were used. The log-rank test for trend analyses of tumor growth in the irradiated mice was done using the GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA).

Results and Discussion
LS174T cells do not express PDGF receptors in vitro, as judged by 125I-PDGF-BB binding assays or by immunoblotting with anti-phosphotyrosine antibodies after stimulation with PDGF-BB (Fig. 1A). LS174T cells grown in vitro as a monolayer are effectively unresponsive to STI571. Figure 1B shows the surviving fraction of LS174T at pharmacologic threshold levels of STI571 (i.e., up to 5 μmol/L after 24 and 48 hours of exposure to STI571). Based on these in vitro data, the cytotoxicity of STI571 in xenografted tumors was not anticipated. Next, the possibility that STI571 can influence radiosensitivity was taken into consideration. To experimentally eliminate/confirm this possibility, LS174T cells were grown as a monolayer and irradiated at a rate of 1.95 Gy/min, for a total radiation dose of 1 or 6 Gy, in the absence or presence of different concentrations of STI571 (Fig. 1C). LS174T cells did not exhibit any particularly unusual sensitivity to radiation in the presence of STI571. Treatment with STI571 in vitro neither enhances nor inhibits radiation-induced cell death in LS174T; that is, the external beam irradiation of in vitro grown cells in the presence of various concentrations of STI571 had only additive effects. As expected, the 6-Gy dose produced about 45% cell kill, whereas a sublethal dose of 1 Gy retarded the cell growth by 2% to 5%. The effect of combined treatment with 131I-labeled antibodies and STI571 in vitro was also tested. Two monoclonal antibodies 131I-anti-CEA (LS174T express carcinoembryonic antigen) and 125I-B72.3 were used. Responses of in vitro grown LS174T cells to 131I-labeled antibodies were not influenced by STI571.

LS174T cells grown as s.c. xenografts in athymic mice develop tumors rich in connective tissue (Fig. 1D, left). The presence of PDGFr-β in deparaffinized, formalin-fixed 5-μm sections of LS174T tumors was confirmed using polyclonal rabbit antibody 958 directed against PDGFr-β. Nonspecific rabbit IgG was used as a control (Fig. 1D, right). Goat anti-rabbit mAb conjugated to biotin were used to amplify the signal, which was subsequently developed using a 3,3’-diaminobenzidine staining kit. PDGFr-β was detected only in tumor stroma.

The premise of the STI571-radioimmunotherapy approach rests on the STI571-induced changes in tumor $P_{IF}$. The effect of STI571 on $P_{IF}$ in s.c. LS174T xenografts was therefore measured using the wick-in-needle technique. Mice carrying LS174T tumors were divided into tumor size–matched groups and either treated with vehicle or with oral doses of 50 mg/kg BID STI571, for four consecutive days. The mean tumor $P_{IF}$ in the vehicle-treated group ($n = 6$) was found to be $5.3 \pm 0.4$ mm Hg, whereas the mean tumor $P_{IF}$ of the STI571-treated group ($n = 5$) was significantly

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**Figure 2.** Response of LS174T tumors to various treatments with STI571. A, changes in the tumor interstitial fluid pressure $P_{IF}$. Tumor-bearing mice received vehicle ($n = 6$) or STI571 ($n = 6$) for four consecutive days before the measurement of tumor $P_{IF}$. B, effect of STI571 on the production of phospho-PDGFr-β in LS174T tumors grown as s.c. xenografts in athymic mice treated with either PBS (control) or STI571 ($n = 15-18$). C, arrest of LS174T tumor growth in response to the combination radioimmunotherapy and STI571 treatment. Mice were treated as outlined in Table 1. Data is plotted as a relative tumor growth normalized to the tumor size on day 3 when the first dose of STI571 was given. D, Kaplan-Meier analysis of the response of LS174T xenografts to the external beam radiotherapy in mice treated with oral doses of either PBS or STI571. E, single-photon emission computed tomography images acquired 72 hours after administration of $^{125}$I-B72.3 in LS174T-bearing mice treated with PBS (control) or STI571 as shown in Table 1. The PBS-treated tumor had an early onset of ulceration typical of LS174T tumors of this size. The pooling of the blood in the area of the ulcer is clearly noticeable in images shown in Fig. 2E and in panels 16, 19, and 22 of Fig. 4A (NT).
reduced by 55% to 2.4 ± 0.9 mm Hg (Fig. 2A; P < 0.001). The attenuation of Pfr, through the STI571 inhibition of PDGFr-β in these tumors, corroborates the use of this tumor model to measure the effect of STI571 on radioimmunotherapy in solid tumors. Further evidence on the involvement of STI571 in PDGFr-β-mediated improvement of radioimmunotherapy came from ELISA and blotting studies of lysates prepared from STI571-treated tumors compared with PBS-treated tumors. The ELISA results indicate ~40% reduction in levels of phospho-PDGFr-β (Fig. 2B). The protein bands analysed done after the Western blotting indicate that the average phosphorylation per PDGFr-β is reduced by at least 35% (data not shown). The results from both methods are virtually identical within the experimental error.

The tumor uptake and biodistribution of radiolabeled B72.3 mAb was measured in LS174T tumor-bearing mice after either PBS or STI571 administration (Table 2). The duration of the STI571 effect was also evaluated. The most effective scheme proved to be the fractionated dosing of STI571 over a period of 7 to 10 days, with two oral doses daily. The treatment with eight 50 mg/kg BID doses of STI571 yielded >2.4 times greater uptake of 125I-B72.3 in LS174T xenografts (Table 2) compared with LS174T xenografts in mice treated with PBS (P < 0.0001).

The therapeutic consequences of STI571-mediated increase in tumor B72.3 mAb uptake were subsequently investigated (Fig. 2C). LS174T tumors were implanted s.c. and allowed to grow for 10 days. Mice were randomized into four groups: (a) no treatment (n = 10), (b) 131I-B72.3 only (n = 10), (c) STI571 only (n = 12), and (d) 131I-B72.3 plus STI571 (n = 12). Body weight and tumor sizes were measured thrice a week, and tumor volumes calculated. Data is plotted as a relative tumor growth normalized to the tumor size on day −3 when the first dose of STI571 was given (Fig. 2C). On the day of 131I-B72.3 administration (day 0), the average tumor size in all groups was 270 ± 70 mm3. The STI571 seven-day dosing scheme was used as shown in Table 1. In as little as 1 week after the 0.25-mCi (9.25 MBq) dose of 131I-B72.3, the advantage of the combined treatment was apparent. Tumor sizes in mice treated with a combination STI571 + radioimmunotherapy were <50% of the untreated tumors (P = 0.009). During this same time, radioimmunotherapy alone produced ~10% decrease in tumor volume, whereas STI571 alone had no measurable effect. The generalized estimating equations were used to assess differences in tumor growth between treatment groups. The change in quadrupling time (Tq) was calculated on day 10 for the PBS and STI571 alone controls (termination date due to the excessive tumor burden >2,500 mm3) and on day 28 after 131I-B72.3 administration for the rest of mice (details are in Table 3). Supplementation of 131I-B72.3 radioimmunotherapy with STI571 improved overall antitumor effects by about 220% compared with radioimmunotherapy alone (P = 0.008) and confirmed that the inhibition of PDGFr-β signaling in tumor stroma and subsequent reduction in tumor Pfr enhances the therapeutic effects of radioimmunotherapy in solid tumors.

In vitro studies did not indicate any effects of STI571 on intrinsic radiosensitivity of LS174T tumor cells (Fig. 1C). To explore if STI571 interactions with tumor stroma altered radiosensitivity of in vivo grown tumors and thereby contributed to the therapeutic synergy, the effects of STI571 treatment on sensitivity to external beam radiation was analyzed. LS174T cells were implanted s.c., 5 × 10⁶ cells per mouse, and allowed to develop tumors >400 mm³ (average tumor volume = 710 ± 330 mm³). Treated mice received total of six oral doses of STI571 at 50 mg/kg body weight/dose for 3 days before irradiation. Control mice were given sham oral doses of PBS. Twenty-four hours after the last dose of STI571, mice were placed in a lead rig that shields the whole body and allows irradiation of tumors only. The external beam irradiations were carried out at a rate of 1.95 Gy/min for the total of 6 Gy. Mice were censored for Kaplan-Meier analyses when the tumor size tripled or exceeded 2,500 mm³. Figure 2D shows the summary of these results. There are no differences in the tumor growth in untreated control mice (n = 8) and STI571-treated control mice (n = 8, P = 0.3297). However, there is a difference between tumor responses in irradiated control mice (n = 8) compared with mice treated with STI571 and radiation (n = 9, P = 0.0553). Moreover, the log-rank test for trend indicates a statistically significant delay in tumor growth with P = 0.0075 (P values were obtained in the Mantel-Haenszel log-rank test by the GraphPad Software). Median survival times to tripling of tumor size were 6.5, 7, and 8 days and not determined (i.e., not reached) for untreated controls, STI571 controls, irradiated controls, and STI571 + irradiation, respectively. This increase of the median survival of the STI571 + external beam–treated mice, along with the log-rank trend analyses, hinted that additional factors such as improved tumor perfusion and the ensuing improvements in tumor oxygenation may have also contributed to the therapeutic synergy between STI571 and

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### Table 2. Biodistribution of 125I-B72.3 in LS174T-bearing mice treated with STI571 or PBS according to the schedule shown in Table 1

<table>
<thead>
<tr>
<th></th>
<th>PBS 120 h (n = 8), average (SD)</th>
<th>STI571 120 h (n = 11), average (SD)</th>
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</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.38 (0.42)</td>
<td>4.45 (0.34)</td>
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<tr>
<td>Liver</td>
<td>0.79 (0.26)</td>
<td>1.58 (0.14)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.70 (0.19)</td>
<td>1.20 (0.11)</td>
</tr>
<tr>
<td>Heart</td>
<td>0.29 (0.08)</td>
<td>0.93 (0.08)</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.66 (0.22)</td>
<td>2.03 (0.16)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.46 (0.09)</td>
<td>0.91 (0.08)</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.15 (0.04)</td>
<td>0.48 (0.04)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.17 (0.08)</td>
<td>0.38 (0.04)</td>
</tr>
<tr>
<td>Bone</td>
<td>0.19 (0.06)</td>
<td>0.58 (0.06)</td>
</tr>
<tr>
<td>Skin</td>
<td>0.42 (0.14)</td>
<td>1.23 (0.12)</td>
</tr>
<tr>
<td>Tumor</td>
<td>9.43 (2.53)</td>
<td>23.18 (2.48)</td>
</tr>
</tbody>
</table>

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### Table 3. Effect of radioimmunotherapy and combination radioimmunotherapy + STI571 on doubling times of LS174T xenografts in athymic mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Td (d), average (SD)</th>
<th>Tumor growth delay</th>
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</thead>
<tbody>
<tr>
<td>No treatment (n = 6)</td>
<td>7.74* (1.34)</td>
<td>1</td>
</tr>
<tr>
<td>STI571 (n = 10)</td>
<td>7.75* (1.20)</td>
<td>1</td>
</tr>
<tr>
<td>131I-B72.3 (n = 6)</td>
<td>18.95* (2.98)</td>
<td>2.4</td>
</tr>
<tr>
<td>STI571 plus 131I-B72.3 (n = 9)</td>
<td>40.63* (8.43)</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*Day 10.
†Day 28.

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radioimmunotherapy. Indeed, the enhanced $^{131}$I-B72.3 uptake into the STI571-treated tumors proved to be only one of the factors responsible for improvements in tumor responses to radioimmunotherapy when combined with the STI571 treatment. Two additional causes both related to the STI571-induced attenuation of $P_{IF}$ were identified: (a) improved homogeneity of mAb distribution in tumor and (b) increased tumor radiosensitivity in response to improved tumor oxygenation. These aspects were further characterized by analyzing in more detail the radioimmunoconjugate distribution within tumors and by analyzing tumor hypoxia.

The spatial and temporal distribution of radioimmunoconjugates in tumors after STI571 treatment was analyzed in imaging studies. Mice with size-matched tumors (1.9-2.1 g) were selected from groups treated for 10 days with either STI571 ($n=2$) or PBS ($n=2$, control), as shown in Table 1. $^{125}$I-B72.3 was injected i.v. and the imaging commenced 24 hours after the administration of the radioactive tracer. The greatly improved homogeneity of $^{125}$I-B72.3 in STI571-treated mice is apparent in images shown in Fig. 2E. Temporal images of the STI571-treated mouse are shown in Fig. 3. On average 8,400 counts per pixel were observed in tumors of the STI571-treated mice compared with 3,700 counts per pixel in the PBS-treated mice 48 hours after injection. This amounts to >220% greater uptake of the radioimmunoconjugate in the STI571-treated tumors. At 72 hours postinjection, these differences are even more pronounced with an average of 7,100 and 2,100 counts per pixel observed in STI571- and PBS-treated tumors, respectively. Noticeable gains in the retention of radioactivity are also evident in STI571-treated tumors compared with PBS controls; that is, the efflux of radioactivity from tumors in PBS-treated mice amounts to ~40%/d whereas <15%/d is lost from the tumors of STI571-treated mice. This translates into a significant increase in radiation doses deposited during 24 hours, from 2.6 Gy/MBq in PBS controls to 10.2 Gy/MBq in STI571-treated mice for a 2-g tumor.

It is unquestionable that the retention of $^{125}$I-B72.3 and the homogeneity of its distribution in tumors treated with STI571 are significantly improved compared with PBS-treated control mice. The remarkable contrast between the homogeneity of tumor uptake in the STI571-treated compared with PBS-treated tumors is best apparent in the sagittal images shown in Fig. 4A. The quantitative evaluation of counts in a 6 x 6 pixels regions of interest, located in the core of each tumor, confirms the gross evaluation of the images (Fig. 4B). At 72 hours after administration, the enhancement of the radioimmunotherapy uptake in the STI571-treated tumors is >300%.

Effects of STI571 on tumor hypoxia were measured using a nitroimidazole-based radiiodinated hypoxia tracer, $1\text{-}[\text{ethyl-}\{3\text{-}[^{125}\text{I}]\text{iodobenzamide}\}\text{]-2\text{-nitroimidazole}\}$. Mice bearing large LS174T tumors ($n=9$; average tumor size, 0.8 g; range, 0.4-1.4 g) were treated with two, four, and six oral doses of STI571. Control mice received six oral doses of the vehicle (PBS). Forty-eight hours after the last dose of STI571, an i.v. dose of 0.01 mCi/mouse (0.37 MBq/mouse) $1\text{-}[\text{ethyl-}\{3\text{-}[^{125}\text{I}]\text{iodobenzamide}\}\text{]-2\text{-nitroimidazole}\}$ was given. Mice were killed 2.5 hours later. Necropsy was done and the radioactive content of several tissues, blood, and tumors was determined. The evaluation of these data is complicated by the enhanced extravasation of the tracer in response to the STI571 treatment. An ~35% increase in the uptake of the hypoxia marker was observed after only two doses of STI571 compared with control mice treated with PBS. This increase in uptake is most certainly in response to the decreasing tumor $P_{IF}$ (22, 24). However, as the number of STI571 doses increased, the amount of the hypoxia marker uptake in the tumor decreased (Fig. 4C), indicating increased tumor oxygenation. It can be concluded with a reasonable certainty that this reduction of hypoxia in response to treatment with STI571 has a profoundly positive effect on the tumor responses to radioimmunotherapy.

Figure 3. Single-photon emission computed tomography images of athymic mice bearing S.C. LS174T xenografts acquired with the LumaGEM scintillation camera. Mice treated with STI571 as indicated in Table 1 and their images acquired 24, 48, and 72 hours after the administration of $^{125}$I-B72.3.
In conclusion, STI571, an inhibitor of the PDGF receptor tyrosine kinase, improves the anticancer effects of radioimmunotherapy with 131I-B72.3 antibodies in solid tumors. STI571 alone does not influence the growth of LS174T tumors. The improved responses to combination radioimmunotherapy and STI571 are the result of lowered tumor's PIF brought about by the inhibition of PDGFr-β localized in the tumor stroma. The ensuing increased uptake of radioimmunotherapy into the tumor provides radiation dose deposits on the order of 400% greater in the STI571 + radioimmunotherapy mice than in PBS-treated mice. The synergy between STI571 and radioimmunotherapy is further aided by the improved homogeneity of radioimmunotherapy distribution in tumors and by significantly reduced tumor hypoxic fraction. This latter effect produces indirect radiosensitization of the tumor cells.

Although it cannot formally be excluded that inhibition of c-kit or abl participate in these effects, the existing biological understanding suggests that inhibition of PDGFr-β is the prime molecular mechanism for the observed effects of STI571. It should also be noted that antiangiogenic effects of PDGF inhibitors, through targeting of PDGF receptors on endothelial cells or pericytes, have been described (39–42). To what extent these effects contribute to the antibody uptake and hypoxia, and to the therapeutic synergy, merits further studies. Findings presented here should encourage further experimental and clinical studies on the effects of STI571 on radioimmunotherapy and other radiation-based therapies.

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Effect of Platelet-Derived Growth Factor Receptor-β Inhibition with STI571 on Radioimmunotherapy

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