Emerging Role of Platelet-Derived Growth Factor Receptor-β Inhibition in Radioimmunotherapy of Experimental Pancreatic Cancer

Janina Baranowska-Kortylewicz,1 Michio Abe,2 Jessica Nearman,1 and Charles A. Enke1

Abstract

Purpose: Thus far, the therapy of pancreatic cancer remains an insurmountable challenge. Not a solitary therapeutic modality in the battery of available therapeutic options is capable to cure or, at the very least, stop the progression of this disease in any meaningful way. The purpose of reported here studies was to implement a multimodality approach to radioimmunotherapy of pancreatic cancer and, ultimately, to develop a course of therapy with the clinical value.

Experimental Design: Animal model was NCr-­nu/nu mouse bearing s.c. xenografts of SW1990 pancreatic adenocarcinoma. Radioimmunotherapy based on 131I-­CC49, a TAG-72-­targeting monoclonal antibody, was augmented with imatinib, a potent inhibitor of platelet-­derived growth factor receptor-β. The postulated interactions between these two modalities depended on the imatinib-­induced drop in the tumor interstitial fluid pressure and the subsequent increase of 131I-­CC49 uptake into the tumor, resulting in improved tumor responses to radioimmunotherapy.

Results: Biodistribution studies revealed a 50% improvement in the tumor uptake of 131I-­CC49 in mice treated with imatinib. Tumor development was practically arrested for ~3 weeks in response to the treatment composed of 131I-­CC49 and imatinib with tumor quadrupling time (TQ) of 40.8 days. 131I-­CC49 alone and imatinib alone also delayed the tumor growth to TQ of 30.2 and 31.2 days, respectively. Unanticipated was the significant response of SW1990 to a brief treatment with imatinib given i.p. at 100 mg/kg b.i.d. for 3 days. Xenografts in control mice receiving injection of PBS had TQ of 23 days.

Conclusions: The inclusion of imatinib in the radioimmunotherapy regimen is beneficial and it does not produce any overt side effects. The improved responses of pancreatic cancer xenografts to the multimodality treatment comprising radioimmunotherapy and platelet-­derived growth factor receptor-β inhibition suggest that this approach to therapy of pancreatic cancer may also be successful in patients.

The annual incidence of pancreatic cancer in the United States is almost equivalent to the annual mortality, with the 2006 estimates at 33,730 and 32,300 cases, respectively (1). Similarly bleak statistics of ~40,000 deaths yearly are reported in Europe (2). Ever increasing efforts are concentrated on the development of adjuvant therapies intended to eradicate residual microscopic disease and thereby to improve survival following curative resection. However, only 10% to 15% of patients are eligible for the surgical intervention. The remaining >85% of patients present at diagnosis with locally advanced or metastatic disease. In this group of patients, chemotherapy provides symptom relief for some but its effect on patient survival is unremarkable, and thus far, various combination chemotherapy regimens have not revealed clear evidence of being superior to any of the single-agent treatments (3–5). The fundamental cause of the chemotherapy failure has its roots in the vast number of molecular anomalies involved in the pancreatic cancer development and invasion. It is well understood that a single-agent therapy is unlikely to alter the outcome of this disease.

Pancreatic cancer cells have a broad array of cellular defects promoting their uncontrolled growth. The introduction of clinically important platelet-­derived growth factor receptor (PDGFR) inhibitors, such as imatinib mesylate (previously STI571, Glivec, or Gleevec in the Unites States), has drawn a lot of attention to the use of PDGF signaling pathways for therapeutic purposes (6–9). PDGF receptor-β (PDGFR-β) regulates cell proliferation and migration. It is also implicated in...
in the development and growth of solid tumors principally through the activation of endothelial cells (10, 11).

Recently, the PDGFR-β signaling through stromal cells has been shown to regulate tumor interstitial fluid pressures (Pif; refs. 6, 12–15) and thereby to influence tumor uptake of anticancer drugs (12, 15). Retarded tumor growth has also been observed after inhibition of the PDGFR-β signaling in two experimental models of the autocrine PDGF growth stimulation: dermofibrosarcoma protuberans (16, 17) and glioblastoma multiforme (10, 11, 18, 19). More recently, clinically important therapeutic effects of PDGFR-β signaling inhibition with imatinib have been reported in dermofibrosarcoma protuberans and glioma patients (20, 21).

Imatinib is a tyrosine kinase inhibitor that blocks the kinase activity of c-Abl, PDGFR-β, and c-Kit tyrosine kinases, and it is approved for the treatment of Philadelphia chromosome – positive chronic myelogenous leukemia (7, 22) and gastrointestinal stromal tumors (23). This drug is also undergoing clinical testing with mixed results in other tumors (24–26), including pancreatic cancer, as a single or multimodality regimen. Relapsed small cell lung cancer treated with imatinib fails to respond in spite of selecting only patients with the c-Kit-expressing small cell lung cancer (8, 25). Several unsuccessful studies of imatinib in androgen-independent prostate cancer revealed additional complications in the use of PDGFR-β inhibitors and initiated a vigorous discussion on the role of the circulating PDGF ligands (27, 28). Although consensus was not reached about the reasons for the failure of imatinib in prostate cancer, the discussants agreed on the complexities of the signal inhibition in the PDGFR-β pathway.

The expression of c-Kit (29) and PDGFR-β (9) in a large number of pancreatic cancer biopsies was met with cautious optimism about the role of imatinib in therapy of this disease (30). In vivo studies in various mouse models suggested that imatinib may be useful in the treatment of pancreatic cancer (31, 32). The in vitro evaluation of imatinib in several human pancreatic adenocarcinoma cell lines produced far less enthusiasm about the potential role of imatinib in pancreatic cancer management (33, 34). In the face of these problems, schemes that incorporate imatinib into multimodality regimens to treat pancreatic cancer are regarded as a more realistic approach. Benefits of the multimodality treatments were recently reported in several mouse models of human pancreatic cancer (31, 32).

The mechanism of PDGFR-β inhibition with imatinib on the radiosensitivty of cancer cells is not fully understood and seems to depend greatly on the chosen model (15, 18, 35, 36). Studies in athymic mice bearing xenografts of LS174T colorectal adenocarcinoma implicated decreased Pif and ensuing enhancements of tumor oxygenation in response to PDGFR-β inhibition as the cause of improved xenograft responses to imatinib and radiation (15). Based on the results of a combination therapy comprising imatinib and radiation in two glioma tumor models in mice, the mechanism of the apparent synergy was ascribed to the inhibition of one of the involved kinases, which caused the tumor growth arrest in the G0-G1 phase (36). The reduction of Rad51 expression in response to imatinib was suggested as the principal origin of radiosensitization observed in glioma cells in vitro (35). This finding goes with the observation that oncogenic activation of the c-Abl tyrosine kinase is responsible for the elevated Rad51 level in leukemia and lymphoma cells (37). Improved responses of tumor to the unleashed sources of radiation were attributed to higher levels of tumor uptake brought about by the imatinib-dependent decrease of the Pif of the tumor (15).

Radioimmunotherapy alone is effective in lymphoma but its application to solid tumors will require a combined modality approach even when the targeted, tumor-associated antigen is expressed in ostensibly sufficient quantities. In a high-dose radioimmunotherapy trial with 99mTc-labeled CC49 monoclonal antibody (mAb), only two of four patients with the diagnosis of pancreatic cancer had measurable uptake of radioimmuno therapy in the tumor (38). The heterogeneity of the tumor uptake resulted in the radiation absorbed dose estimates between 180 and 950 cGy in one patient and 200 cGy in the other. There were no objective responses. Similarly, disheartening results were also reported for 131I-labeled mAb (39–41), prompting calls for a multimodality approach to radioimmunotherapy (42–45). Recently, paclitaxel administered after radioimmunotherapy in patients with breast and prostate cancers resulted in a modest enhancement in the tumor uptake and a synergy between radioimmunotherapy and paclitaxel. It is noteworthy that no increase in the normal tissue uptake was observed (45).

Collectively, publications reviewed above suggest that monochemotherapy approaches to treat pancreatic cancer are not promising and that interrupting only one signaling pathway could in fact exacerbate the problem. Within the existing limits, the prospects for success of radioimmunotherapy in pancreatic cancer are just as grim. In studies presented here, we show that disruption of the PDGFR-β signaling with imatinib combined with radioimmunotherapy can significantly improve pancreatic tumor responses in a mouse model.

### Materials and Methods

#### Reagents.

Imatinib was generously provided by Novartis Pharma AG (Basel, Switzerland). Sodium iodide-131 was purchased from Perkin-Elmer Life and Analytical Sciences, Inc. (Boston, MA). 131I radionuclide had a specific activity of >5 Ci/mg (>185 GBq/mg) and was supplied as an aqueous solution in 0.1 mol/L NaOH (pH 12–14). CC49 mAbs were radioiodinated using the Iodogen method (46). All radioiodinations were done on site. 131I-131I-labeled CC49 monoclonal antibody (mAb) was purified on a 2-mL Zeba Desalt Spin column (Pierce Biotechnology, Rockford, IL). The average specific activity of 131I-CC49 in the therapeutic dose was 8.1 ± 0.5 µCi/µg (300 ± 18 kBq/µg) before administration. Radiolabeled antibodies were diluted in 0.01 mol/L phosphate buffer (pH 7.2) and 0.01 mL of 131I (10 mCi; 370 MBq). The mixture was incubated at room temperature for 10 to 15 min. The reaction progress was measured using instant TLC with a methanol/water [1:4 (v/v)] as the elution system. 131I-CC49 was purified on a 2-mL Zeba Desalt Spin column (Pierce Biotechnology, Rockford, IL). The average specific activity of 131I-CC49 in the therapeutic dose was 8.1 ± 0.5 µCi/µg (300 ± 18 kBq/µg). Before administration, radiolabeled antibodies were diluted in 0.01 mol/L phosphate buffer (pH 7.2) at 25°C, 0.0027 mol/L potassium chloride, and 0.137 mol/L sodium chloride (PBS) containing 0.1% mouse serum (Sigma-Aldrich, St. Louis, MO) and added 0.1 mL of 10 mg/mL CC49 in PBS (pH 7.2) and 0.01 mL of 131I (10 mCi; 370 MBq). The mixture was incubated at room temperature for 10 to 15 min. The reaction process was measured using instant TLC with a methanol/water [1:4 (v/v)] as the elution system. 131I-CC49 was purified on a 2-mL Zeba Desalt Spin column (Pierce Biotechnology, Rockford, IL). The average specific activity of 131I-CC49 in the therapeutic dose was 8.1 ± 0.5 µCi/µg (300 ± 18 kBq/µg). Before administration, radiolabeled antibodies were diluted in 0.01 mol/L phosphate buffer (pH 7.2) at 25°C, 0.0027 mol/L potassium chloride, and 0.137 mol/L sodium chloride (PBS) containing 0.1% mouse serum (Sigma-Aldrich) to yield the injection dose volume of 0.2 mL per mouse. Triplicate standards equivalent to 0.001 mL of the injected dose were prepared in 0.1 mL PBS/0.1% mouse serum and counted in a gamma counter. Standards were stored at 0°C to 4°C and recounted with the necropsy samples at the conclusion of short-term therapy studies to allow calculations of the decay-corrected retention of radioactivity in tumor and tissues. The same method of radiolabeling was followed for doses used in the
biodistribution studies using 1 mCi (37 MBq) 131I per 1 mg CC49 to produce 131I-CC49 with the specific activities of 0.8 ± 0.02 μCi/μg (30 ± 0.7 Bq/μg).

**Tumor model.** The SW1990 cell line derived from spleen metastasis of a grade 2 human pancreatic ductal adenocarcinoma (47) was purchased from the American Type Culture Collection (Manassas, VA). Cells were grown in Leibovitz’s L-15 medium with 300 mg/L l-glutamine supplemented with 10% fetal bovine serum at 37°C without CO2. Four- to 6-week-old female athymic NCr-nu/mu mice (National Cancer Institute-Frederick, Frederick, MD) were allowed to acclimate for no less than 5 days. All protocols involving animals were approved by the Institutional Animal Care and Use Committee. Mice were housed in microisolator cages with free access to sterilized standard rodent diet and water. SW1990 cells in 0.2 mL of serum-free medium were implanted s.c. at 5 × 10^6 per mouse. One week later, identification transponders were implanted, also s.c. Body weights and tumor sizes were monitored twice weekly, and approximately 5 to 7 weeks after the cell implant, mice were randomized for therapy and biodistribution studies.

**In vitro cell growth assay.** SW1990 cells were seeded in 96-well plates at a density of 3,000 per well in either full growth medium (Leibovitz’s L-15 medium with 2 mmol/L l-glutamine supplemented with 10% fetal bovine serum) or serum-depleted growth medium containing 0.1% bovine albumin. After 24 h, culture medium was replaced with appropriate fresh medium containing 0, 0.1, 1.0, or 5.0 μmol/L imatinib and cells were allowed to grow for 24 or 48 h (n = 6 wells per concentration and per time point). One plate containing cells grown in serum-depleted medium was given fresh medium containing 10 ng/mL PDGF-BB in addition to 0, 0.1, 1.0, or 5.0 μmol/L imatinib. Subsequently, a colorimetric assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI) was used to measure the metabolic activity of cells. The fractional growth of untreated control cells was set to 1 or 100%.

**Cell cycle.** Cells were grown as described above with and without the addition of imatinib. The growth medium containing detached cells was combined with cells harvested from the monolayer with trypsin-EDTA and centrifuged at 1,000 × g for 10 min. A single cell suspension was prepared in PBS containing 0.1% bovine serum albumin. Cells were washed twice and the final suspension was prepared at approximately 1 × 10^6 to 2 × 10^6 cells/mL. Aliquot (1 mL) of this cell suspension was transferred into a 15 mL polypropylene conical tube, and 3 mL of ice-cold absolute ethanol were added to fix cells. Cells were stored in 70% ethanol at −20°C until ready for staining and flow cytometric analysis. Before the analysis, cells were rehydrated by washing twice in PBS and stained with propidium iodide with a simultaneous RNase treatment using the Telford reagent [50 μg/mL propidium iodide, 1 mmol/L EDTA, 0.1% Triton-X (v/v), 26.8 μg/mL DNA-free RNase A in PBS]. Samples were kept at 4°C until analyzed by flow cytometry, typically within 60 min of the Telford reagent addition. Cells were analyzed using a FACStar® Flow Cytometer and CellQuest Acquisition Software (Becton Dickinson, San Jose, CA) in the University of Nebraska Medical Center Cell Analysis Facility (Omaha, NE). Percentage of cells within G0-G1, S, and G2-M phases of the cell cycle were determined by the analysis of list mode data files with ModFit LT software (Verity Software House, Topsham, ME).

**In vitro radiosensitization assay.** SW1990 cells were treated as described above for the cell growth assay. The cells were irradiated at two radiation doses: 1 and 6 Gy at 1.9 Gy/min in the Mark I 68A research irradiator (6,000 Ci cesium-137 source; J.L. Shepherd and Associates, San Fernando, CA). Subsequently, cells were allowed to grow for 48 h before the cell proliferation was determined using the colorimetric kit. The proliferating fraction of cells irradiated in the absence of any additional treatment were set to 1 or 100%.

**Drug treatment.** The average weight of mice on day 0 was 21.5 ± 2.5 g. Saturated solution of potassium iodide was added to drinking water to a final concentration of −0.1% 3 days before 131I-CC49 injections (day −2). A lottery was conducted to split mice into four groups (Table 1) as follows: (group 1) NT group, control mice receiving i.p. injections of PBS (b.i.d.) on days −2, −1, and 0; (group 2) imatinib group, mice receiving imatinib as the i.p. doses of 100 mg/kg/d in PBS on days −2, −1, and 0; and (group 3) 131I-CC49 group, mice receiving i.p. injections of PBS (b.i.d.) on days −2, −1, and 0; and (group 4) 131I-CC49 plus imatinib group, mice receiving imatinib as the i.p. doses of 100 mg/kg/d in PBS on days −2, −1, and 0. On day 0, mice in groups 3 and 4 received via a tail vein i.v. doses of 131I-CC49 2 h after the last dose of imatinib.

**Biodistribution.** On day 0, 2 h after the last i.p. dose of imatinib, mice received an i.v. injection of 131I-CC49 via a tail vein. An average injected dose was 12.3 ± 0.4 μCi/mouse (0.46 ± 0.015 MBq/mouse). Mice were killed 120 h after 131I-CC49 injection using compressed CO2. Tissues and tumors were briefly rinsed in physiologic saline and patted dry, and wet weights were determined. Radioactivity was measured in a gamma counter. The data were analyzed in terms of the percentage injected dose per gram tissue (% ID/g).

**Therapy.** Radiolabeled 131I-CC49 was given i.v. via a tail vein on day 0. The average injected dose for therapy was 279.1 ± 9.4 μCi/mouse (10.3 ± 0.3 MBq/mouse). Mice were observed daily. Body weights and tumor dimensions were measured two to three times weekly. Tumor volume was approximated to the volume of ellipsoid. Therapy studies with the larger initial tumor sizes were terminated after 14 days and a necropsy was done. The therapy in mice with the average initial tumor size of <100 mm^3 was terminated after 6 weeks. The average tumor size on day 2 was 83 ± 40 mm^3 (median, 77 mm^3); maximum, 158 mm^3; minimum, 32 mm^3) and 566 ± 293 mm^3 (median, 480 mm^3; maximum, 1380 mm^3; minimum, 214 mm^3) in the group with small initial tumor volumes and in the group with large initial tumor volumes, respectively. The final necropsy included the determination of the radioactivity retention in tumor, blood, and heart. Sections of harvested tumors were snap frozen in liquid nitrogen and stored at −80°C until ready for ELISA and Western blotting analyses.

Table 1. Chronology of treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Day −2</th>
<th>Day −1</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>PBS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Biodistribution</td>
<td>Necropsy</td>
</tr>
<tr>
<td>131I-CC49 + PBS</td>
<td>131I-CC49</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>No</td>
<td>Biodistribution</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Imatinib</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Yes</td>
<td>Biodistribution</td>
</tr>
<tr>
<td>131I-CC49 + imatinib</td>
<td>131I-CC49</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>No</td>
<td>Biodistribution</td>
</tr>
</tbody>
</table>
Statistical analyses. In all summary statistics, Student's t test was used to compare the averages. Pharmacokinetics and whole body clearance curve fitting were done using SAAM II version 1.2 (SAAM Institute, University of Washington, Seattle, WA). Kaplan-Meier survival analyses were done using SigmaPlot 9.0 and SigmaStat 3.10 (Systat Software, Inc., Point Richmond, CA).

Results and Discussion

In vitro studies. SW1990 cell line is derived from splenic metastases of pancreatic adenocarcinoma of ductal origin (47). Levels of PDGFR-β are low in SW1990 cells grown in vitro but the inhibition with imatinib reduces the levels of phosphorylated PDGFR-β in a dose-dependent manner, indicating that the receptor is functional (Fig. 1A). Cytotoxicity of imatinib in the in vitro culture is also dose dependent. The concentration needed to inhibit growth of SW1990 cells by 50% (IC50) in full medium containing 10% fetal bovine serum was estimated at 23.0 ± 1.1 μmol/L. Cells grown in the absence of fetal bovine serum were more sensitive with IC50 of 7.0 ± 2.0 μmol/L. PDGF-BB (10 ng/mL) added to the medium of serum-depleted cells restored some of the chemoresistance and increased the IC50 value to 11.8 ± 1.6 μmol/L (Fig. 1B). These values are in line with the concentrations reported for several human pancreatic cancer cell lines. Li et al. (33) reported IC50 of imatinib in the range of 17 to 31.5 μmol/L for Colo-357, MiaPaCa, Aspc-1, BxPc-3, Capan-1, and T3M4 cell lines. They also observed a significant dependence on the serum concentration. When the same cells were grown under the low serum conditions, the IC50 values were reduced to the 9 to 20 μmol/L range. It is evident that these, as well as our IC50 values measured for SW1990 cells, are very much out of range of concentrations typically observed in plasma of patients treated with imatinib (7). However, the concentration of imatinib sufficient to inhibit 50% of PDGFR-β phosphorylation in SW1990 cells is at the ~1 μmol/L level even in the presence of 10% fetal bovine serum.

The buildup of SW1990 cells in a G2 phase in response to the imatinib treatment implied a likelihood of enhanced radiosensitivity (Fig. 1C). However, in all proliferation assays conducted at 24, 48, and 72 h after irradiation of the imatinib-treated cells, imatinib showed no measurable effect on the radiosensitivity of SW1990 cells. This observation is analogous to our results in LS174T colorectal adenocarcinoma cells (15) and in neuroblastoma cells (48). However, this is in contrast to the radiation enhancement ratios of ~1.5 reported for two glioma cells (35).

Tumor model, antibodies, and biodistribution. After s.c. implantation into athymic NCr-nu/nu mice, SW1990 cells produce slow-growing tumors that exhibit characteristics of a grade 2 pancreatic adenocarcinoma with solid tumor masses supported by large stromal component with some evidence of...
mucin production. The immunohistochemistry of SW1990 xenografts using CC49 mAb reveals a significant expression of TAG-72 antigen (Fig. 1D). CC49 binds to a unique disaccharide sialyl-Tn (sialic acid $\alpha_2,6\text{GalNAc}$) present on tumor-associated mucin TAG-72 in the majority of human adenocarcinomas (49). Nearly 70% of pancreatic cancer biopsies show the expression of TAG-72 antigen, but the number of immunoreactive cells varies from case to case (50–52). A similar heterogeneity in antigen expression was also shown with B72.3. This antibody recognizes TAG-72 as well and it reacted with 35% of the pancreatic tumor cells (49). mAb CC49 was initially developed by Colcher et al. (53) using a membrane-enriched fraction of human metastatic mammary carcinoma tissue as an immunogen and was selected for further clinical studies because of its higher affinity and more rapid plasma clearance compared with the earlier variants. The radiolabeling of CC49 under the conditions described above produces fully immunoreactive product in 85% radiochemical yield after purification. Typical autoradiograph of $\text{^{131}I}\text{CC49}$ analyzed using the gradient 4% to 20% SDS-PAGE gel run under nonreducing (−SH) and reducing (+SH) conditions is shown in Fig. 2A.

The uptake of $\text{^{131}I}\text{CC49}$ in SW1990 xenografts is lower than the values typically observed in LS174T tumors, a xenograft model used most often in preclinical evaluation of various forms of radioimmunotherapy that is directed to TAG-72 (15, 54–56). Only ~6% of the injected dose remains associated with 1 g of SW1990 tumor 120 h after administration. The inclusion of imatinib increased $\text{^{131}I}\text{CC49}$ levels in tumor by ~50% (Table 2). It should be noted that the levels of a nonspecific IgG labeled with $\text{^{131}I}$ in SW1990 xenografts were measured at 0.21 ± 0.06% ID/g and 0.31 ± 0.14% ID/g at 24 and 48 h, respectively. The radioactivity in blood of mice treated with $\text{^{131}I}\text{CC49}$ plus imatinib persisted at slightly elevated levels and was measured at 7.29% ± 2.04% ID/g blood in STI571-treated mice compared with 5.68% ± 2.28% ID/g blood in PBS-treated mice ($P = 0.1972$). This is in line with the imatinib-induced changes in the $\text{^{131}I}\text{CC49}$ distribution observed previously in LS174T xenografted mice during the biodistribution and imaging studies (15). Imatinib increased $\text{^{131}I}\text{CC49}$ uptake in LS174T tumors by >140%, and this

Table 2. Tissue and tumor distribution of $\text{^{131}I}\text{CC49}$ in athymic mice bearing s.c. xenografts of human pancreatic adenocarcinoma SW1990 (average ± SD) 120 h after i.v. administration of $\text{^{131}I}\text{CC49}$

<table>
<thead>
<tr>
<th>Tissue</th>
<th>PBS</th>
<th>Imatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>5.68 (2.28)</td>
<td>7.29 (2.04)</td>
</tr>
<tr>
<td>Liver</td>
<td>1.31 (0.58)</td>
<td>1.64 (0.58)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.16 (0.48)</td>
<td>1.61 (0.48)</td>
</tr>
<tr>
<td>Heart</td>
<td>1.29 (0.48)</td>
<td>1.56 (0.48)</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.37 (0.89)</td>
<td>2.92 (0.71)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.08 (0.38)</td>
<td>1.42 (0.28)</td>
</tr>
<tr>
<td>SW1990</td>
<td>6.06 (1.76)</td>
<td>9.03 (1.59)</td>
</tr>
</tbody>
</table>

*P value at 0.05 level of significance.
and mice, which were receiving i.p. injections on day 0. Mice in the experimental group, 131ICC49 + imatinib, received i.p. doses of imatinib as described above and 131ICC49 injections of PBS; imatinib-treated mice receiving i.p. doses of groups included untreated mice (NT) that received sham i.p. injections of PBS- and imatinib-treated mice (Fig. 2B). The autoradiogram shows the presence of a single radioactive band obtained from PBS- and imatinib-treated mice (Fig. 2B). The direct comparison of tumor sizes after necropsy revealed consistency in the data fit into a first-order rate excretion model, and whole body half-lives were calculated with a correction for 131I decay (Table 4). The 131I half-life in 131ICC49 + imatinib–treated mice was 96.3 h compared with 76.2 h in the 131ICC49 + PBS group. This delay can be largely attributed to the higher tumor retention of 131ICC49 in imatinib-treated mice. These differences in whole body elimination were confirmed by the outcome of therapy studies. Higher levels of 131I retained in tumor translated into a more significant tumor growth delay. To verify that there were no apparent differences in the metabolic/catabolic processing of 131ICC49, the identity of 131I-labeled species as 131ICC49 was confirmed by the SDS-PAGE analyses of tumor lysates obtained from PBS- and imatinib-treated mice (Fig. 2B). The outcome of radioimmunotherapy is shown in Fig. 2C and D. Tumor doubling times were estimated from the tumor growth curves (Table 3; Fig. 2C). 131ICC49 + imatinib–treated tumors doubled their volume within 26.1 days. This is twice the time required for the tumor doubling in imatinib-treated (13.0 days) or 131ICC49- treated (12.9 days) mice, and it is also nearly thrice longer compared with the growth rate of tumors in untreated control mice measured at 9.05 days. Equivalent results were obtained when Kaplan-Meier survival analyses were applied to the tumor quadrupling data (Table 3; Fig. 2D). Multiple comparisons were made using the Holm-Sidak method at the overall significance level of 0.05. Differences between the 131ICC49 + imatinib treatment and either imatinib only or 131ICC49 only are less pronounced when the probability of quadrupling tumor volume is analyzed. This is because the growth of 131ICC49 + imatinib–treated tumors was virtually arrested by the PDGF-β inhibition with imatinib and the ensuing reduction of the PIF of the tumor from 5.25 mm Hg in PBS-treated mice to 2.35 mm Hg in imatinib-treated mice, a >55% reduction (15). The changes in PIF in SW1990 xenografts are less significant—that is, PIF in SW1990 xenografts in control mice was measured at 4.64 ± 0.54 mm Hg compared with PIF in imatinib mice of 3.20 ± 0.30 mm Hg, a change of ~30%. These trends in the PIF drop in response to imatinib are in line with 131ICC49 levels in tumors.

### Table 3. Tumor growth variables in the therapy study commenced when the average volume of SW1990 xenografts was <100 mm³

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>Imatinib</th>
<th>131ICC49 + PBS</th>
<th>131ICC49 + imatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor doubling time, * days (SE)</td>
<td>9.05 (0.05)</td>
<td>13.03 (0.27)</td>
<td>12.86 (0.19)</td>
<td>26.06 (1.47)</td>
</tr>
<tr>
<td>Tumor quadrupling time, † days (SE)</td>
<td>23.00 (2.17)</td>
<td>31.20 (1.83)</td>
<td>30.20 (3.73)</td>
<td>40.78 (0.98)</td>
</tr>
</tbody>
</table>

*Exponential growth curve fit. P values for the Tukey-Kramer multiple comparisons test are as follows: 131ICC49 + imatinib versus NT, P < 0.001; 131ICC49 + imatinib versus imatinib, P < 0.001; 131ICC49 + imatinib versus 131ICC49 + PBS, P < 0.001; imatinib versus NT, P < 0.01; imatinib versus 131ICC49 + PBS, P > 0.05; and 131ICC49 + PBS versus NT, P < 0.001.

† Kaplan-Meier analyses. P values for all pairwise multiple comparison procedures using the Holm-Sidak method for the probability of growth curves (Table 3; Fig. 2C). 131ICC49 + imatinib–treated tumors doubled their volume within 26.1 days. This is twice the time required for the tumor doubling in imatinib-treated (13.0 days) or 131ICC49- treated (12.9 days) mice, and it is also nearly thrice longer compared with the growth rate of tumors in untreated control mice measured at 9.05 days. Equivalent results were obtained when Kaplan-Meier survival analyses were applied to the tumor quadrupling data (Table 3; Fig. 2D). Multiple comparisons were made using the Holm-Sidak method at the overall significance level of 0.05. Differences between the 131ICC49 + imatinib treatment and either imatinib only or 131ICC49 only are less pronounced when the probability of quadrupling tumor volume is analyzed. This is because the growth of 131ICC49 + imatinib–treated tumors was virtually arrested by the PDGF-β inhibition with imatinib and the ensuing reduction of the PIF of the tumor from 5.25 mm Hg in PBS-treated mice to 2.35 mm Hg in imatinib-treated mice, a >55% reduction (15). The changes in PIF in SW1990 xenografts are less significant—that is, PIF in SW1990 xenografts in control mice was measured at 4.64 ± 0.54 mm Hg compared with PIF in imatinib mice of 3.20 ± 0.30 mm Hg, a change of ~30%. These trends in the PIF drop in response to imatinib are in line with 131ICC49 levels in tumors.

### Radioimmunotherapy

Mice were divided into four groups. There were three control groups to assure that effects of each treatment on tumor growth were assessed separately. Control groups included untreated mice (NT) that received sham i.p. injections of PBS; imatinib-treated mice receiving i.p. doses of 100 mg/kg/d imatinib in PBS (b.i.d.) on days −2, −1, and 0; and 131ICC49-treated mice, which were receiving i.p. injections of PBS (b.i.d.) on days −2, −1, and 0 followed by 131ICC49 on day 0. Mice in the experimental group, 131ICC49 + imatinib, received i.p. doses of imatinib as described above and 131ICC49 on day 0. 131ICC49 was injected i.v. via a tail vein 2 h after the last i.p. dose of imatinib or PBS. The injected doses were quite consistent and the average dose was 279 ± 9 MBq/mouse (10.3 ± 0.3 MBq/mouse). Therapy studies in mice with larger initial tumor volumes were terminated after 14 days, and studies in mice with the average initial tumor volume of <100 mm³ were terminated after 6 weeks. The whole body clearance of radioactivity was measured daily for 5 days after the administration of 131ICC49. The data were fitted into a first-order rate excretion model, and whole body half-lives were calculated with a correction for 131I decay (Table 4). The 131I half-life in 131ICC49 + imatinib–treated mice was 96.3 h compared with 76.2 h in the 131ICC49 + PBS group. This delay can be largely attributed to the higher tumor retention of 131ICC49 in imatinib-treated mice. These differences in whole body elimination were confirmed by the outcome of therapy studies. Higher levels of 131I retained in tumor translated into a more significant tumor growth delay. To verify that there were no apparent differences in the metabolic/catabolic processing of 131ICC49, the identity of 131I-labeled species as 131ICC49 was confirmed by the SDS-PAGE analyses of tumor lysates obtained from PBS- and imatinib-treated mice (Fig. 2B). The autoradiogram shows the presence of a single radioactive band corresponding to 131ICC49. Comparable amounts of radioactivity were precipitable by trichloroacetic acid from plasma, indicating that characteristics of circulating radioactivity were also independent of the treatment. Concerns of hematologic consequences of a longer 131I half-life in mice treated with imatinib proved to be unfounded. Hemoglobin was determined at 13.40 ± 0.40 g/dL and 13.54 ± 0.37 g/dL in 131ICC49 + PBS–treated and 131ICC49 + imatinib–treated mice, respectively. Hematocrit levels were also virtually identical.

The outcome of radioimmunotherapy is shown in Fig. 2C and D. Tumor doubling times were estimated from the tumor growth curves (Table 3; Fig. 2C). 131ICC49 + imatinib–treated tumors doubled their volume within 26.1 days. This is twice the time required for the tumor doubling in imatinib-treated (13.0 days) or 131ICC49- treated (12.9 days) mice, and it is also nearly thrice longer compared with the growth rate of tumors in untreated control mice measured at 9.05 days. Equivalent results were obtained when Kaplan-Meier survival analyses were applied to the tumor quadrupling data (Table 3; Fig. 2D). Multiple comparisons were made using the Holm-Sidak method at the overall significance level of 0.05. Differences between the 131ICC49 + imatinib treatment and either imatinib only or 131ICC49 only are less pronounced when the probability of quadrupling tumor volume is analyzed. This is because the growth of 131ICC49 + imatinib–treated tumors was virtually arrested for ~3 weeks and the exponential growth pattern resumed ~4 weeks after treatment (Fig. 2C). Still, the tumor doubling time was 10 days longer for the combination treatment compared with either of the two single modality treatments.

The direct comparison of tumor sizes after necropsy revealed that indeed there are significant and treatment-dependent differences in the tumor response. In the therapy scheme commenced when the average initial volume of xenografts was 566 ± 293 mm³ (median, 480 mm³). 131ICC49 + imatinib treatment effectively arrested the tumor growth (in this model, tumor weight ≈ 0.9 × tumor volume in mm³ / 1,000). The weight of exiripated tumors in this group was 0.55 g compared with 1.06 ± 0.15 g in the 131ICC49 + PBS group and 1.08 ± 0.19 g in the STI571 group (Table 4). There were also significant differences in the amount of 131I retained by tumors, all in the form of virtually intact 131ICC49 (Fig. 2B), as well as in the levels of the circulating 131I (Table 4). Fourteen days after radioimmunotherapy administration, SW1990 xenografts in mice treated with imatinib stored ~55% more 131ICC49 than SW1990 xenografts in mice
receiving sham injections of PBS. The estimated radiation doses to tumor are 2,200 cGy/g for 131ICC4 plus imatinib–treated tumors and 1,400 cGy/gram for 131ICC4 + PBS–treated tumors, indicating a clear dosimetric advantage for the combined modality treatment. The dose assessment was done using conventional dosimetry methods that assume uniform radionuclide distribution within the tumor. The computational model used was adopted from Govindan et al. (57).

In the SW1990 model, unlike in the similar studies of the LS174T tumor model (15), imatinib treatment alone produced significant responses of SW1990 xenografts to imatinib (Table 4; Fig. 2C). It would seem that interactions between radioimmunotherapy and imatinib are additive, not synergistic. SW1990 cells grown in vitro have low levels of functional PDGFR-β (Fig. 1A), and conceivably, these were sufficient to influence tumor responses. Immunohistochemistry in this instance is not very helpful. Some tumor sections show weak staining for the PDGFR-β expression only in the stromal compartment, whereas some sections indicated low levels of PDGFR-β expression in tumor cells as well (data not shown).

The objective of this study was to take advantage of the PIF of the diminished tumor in response to the inhibition of PDGFR-β with imatinib and by this means to improve radioimmunotherapy of pancreatic cancer. A similar approach in a mouse model of human colorectal cancer (15), human anaplastic thyroid carcinoma (12, 58), and in a syngeneic colon adenocarcinoma model in rats (12) showed considerable positive influence of the PDGFR-β inhibition with imatinib on the outcome of therapy. The hypothesized source of this improvement was the reduction of the PIF of the tumor in response to the antagonist. Immunohistochemical studies confirmed that the primary target was stromal PDGFR-β, given that all detectable PDGFR-β was localized exclusively in the mesenchymal cells. With this in mind, we have undertaken imatinib-augmented radioimmunotherapy studies in SW1990 xenografts derived from a grade 2 human pancreatic adenocarcinoma. Immunohistochemistry of PDGFR-β in the SW1990 tumor model was ambiguous but the receptor seemed to be confined primarily to the stromal component. In vitro studies resolved this issue, showing that SW1990 tumor cells have low but functional levels of PDGFR-β responsive to imatinib. PIF measured in SW1990 xenografts in mice treated with imatinib was significantly lower than PIF in mice receiving sham treatment with PBS, and predictably, the tumor uptake of radiolabeled antibodies and the responses of SW1990 xenografts to radioimmunotherapy were enhanced in the imatinib-treated mice. Absorbed radiation doses were ~60% higher when compared with tumors in mice receiving 131ICC49 with PBS in place of imatinib. The unforeseen result was a significant growth delay of SW1990 xenografts in mice treated only with imatinib. Most of the successful single modality therapies with imatinib in various cancer models required 5 to 8 weeks of b.i.d. dosing of imatinib (31, 32, 59). In our study, the growth delay was apparent after only 3 days of dosing with imatinib at 100 mg/kg b.i.d. The ensuing investigation into the origins of this tumor response to imatinib led us to the discovery of an unprecedented aspect of the tumor cell-stromal cell interactions in this model of pancreatic adenocarcinoma.

The observed responses of pancreatic cancer xenografts to the combined modality treatment comprising radioimmunotherapy and PDGFR-β inhibition give hope that this approach to therapy of pancreatic cancer can be successful in patients. The differences in the tumor uptake are less than in other tumor models reported to date; still, the lack of any hematologic side effects opens up the possibility that these differences can be amplified using a fractionated approach to the radioimmunotherapy delivery.

**Acknowledgments**

We thank Drs. Yu Kimura, Kotaro Inoue, and Małgorzta Rybak for their assistance in some of the in vivo studies; Dr. Z. Paul Kortylewicz for his insightful comments as well as his help with radiochemistry aspects of these studies; University of Nebraska Medical Center Cell Analysis Facility under the direction of Dr. Charles A. Kuszynski for providing unparalleled services; Linda M. Willkie (University of Nebraska Medical Center Cell Analysis Facility) for her professional help and advice; and Drs. Kristian Pietras and Arne Ostman for the PIF determinations in SW1990 xenografts.

---

Table 4. Tumor and blood variables 14 d after the treatment in the therapy study commenced when the average volume of SW1990 xenografts was >500 mm³

<table>
<thead>
<tr>
<th>Treatment</th>
<th>131ICC49 + PBS</th>
<th>131ICC49 + imatinib</th>
<th>Imatinib</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor weight, grams (SE)</td>
<td>1.06 (0.15)</td>
<td>0.55 (0.08)</td>
<td>1.08 (0.19)</td>
<td>0.0190*</td>
</tr>
<tr>
<td>% ID/g in tumor (SD)</td>
<td>5.00 (1.46)</td>
<td>7.73 (3.29)</td>
<td>—</td>
<td>0.048</td>
</tr>
<tr>
<td>% ID/g in blood (SD)</td>
<td>0.96 (0.20)</td>
<td>1.71 (0.30)</td>
<td>—</td>
<td>0.019</td>
</tr>
<tr>
<td>% ID/g in heart (SD)</td>
<td>0.27 (0.12)</td>
<td>0.40 (0.16)</td>
<td>—</td>
<td>0.30</td>
</tr>
<tr>
<td>Whole body clearance</td>
<td>76.2 (2.5)</td>
<td>96.3 (2.7)</td>
<td>—</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Whole body half-life, hours (SE)</td>
<td>98.32 (1.33) × e⁻⁰.⁰⁰⁹⁹ₚ</td>
<td>98.40 (1.21) × e⁻⁰.⁰⁰⁷₂ₜ</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Imatinib versus 131ICC4 + imatinib.
131ICC49 versus 131ICC4 + imatinib.
1Imatinib versus 131ICC49.
All % ID/g values are corrected for decay.

---

3 In preparation.
Emerging Role of Platelet-Derived Growth Factor Receptor-β Inhibition in Radioimmunotherapy of Experimental Pancreatic Cancer

Janina Baranowska-Kortylewicz, Michio Abe, Jessica Nearman, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/13/1/299

Cited articles  This article cites 58 articles, 30 of which you can access for free at: http://clincancerres.aacrjournals.org/content/13/1/299.full.html#ref-list-1

Citing articles  This article has been cited by 2 HighWire-hosted articles. Access the articles at: /content/13/1/299.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.