A Phase I Biological and Pharmacologic Study of the Heparanase Inhibitor PI-88 in Patients with Advanced Solid Tumors

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Abstract

Purpose: PI-88 is a mixture of highly sulfated oligosaccharides that inhibits heparanase, an extracellular matrix endoglycosidase, and the binding of angiogenic growth factors to heparan sulfate. This agent showed potent inhibition of placental blood vessel angiogenesis as well as growth inhibition in multiple xenograft models, thus forming the basis for this study.

Experimental Design: This study evaluated the toxicity and pharmacokinetics of PI-88 (80-315 mg) when administered s.c. daily for 4 consecutive days bimonthly (part 1) or weekly (part 2).

Results: Forty-two patients [median age, 53 years (range, 19-78 years); median performance status, 1] with a range of advanced solid tumors received a total of 232 courses. The maximum tolerated dose was 250 mg/d. Dose-limiting toxicity consisted of thrombocytopenia and pulmonary embolism. Other toxicity was generally mild and included prolongation of the activated partial thromboplastin time and injection site ecchymosis. The pharmacokinetics were linear with dose. Inpatient variability was low and interpatient variability was moderate. Both AUC and Cmax correlated with the percent increase in activated partial thromboplastin time, showing that this pharmacodynamic endpoint can be used as a surrogate for drug exposure. No association between PI-88 administration and vascular endothelial growth factor or basic fibroblast growth factor levels was observed. One patient with melanoma had a partial response, which was maintained for >50 months, and 9 patients had stable disease for >6 months.

Conclusion: The recommended dose of PI-88 administered for 4 consecutive days bimonthly or weekly is 250 mg/d. PI-88 was generally well tolerated. Evidence of efficacy in melanoma supports further evaluation of PI-88 in phase II trials.

Inhibition of angiogenesis has recently been shown to be an important therapeutic strategy for cancer patients (1). Numerous targets of cancer-induced angiogenesis exist, including enzymes involved in the breakdown of the extracellular matrix, such as matrix metalloproteinases and heparanase. Preclinical evidence suggests that heparanase is important to metastasis and angiogenesis and may therefore be an important therapeutic target (2-4). Elevated heparanase expression is associated with locoregional lymph node metastases or distant metastases in multiple solid tumors, including bladder, breast, esophagus, oral cavity, pancreas, stomach, and thyroid papillary carcinoma, and is associated with shorter survival in patients with pancreatic cancer (5-13).

PI-88 is a mixture of highly sulfated oligosaccharides derived from the yeast Pichia (Hanensa) holstii NRRL Y-2448 (14, 15). It was selected for development because it is a potent inhibitor of heparanase. PI-88 has shown antiangiogenic activity in vitro and in vivo, which is attributable to three distinct mechanisms: (a) inhibition of heparanase, an endoglycosidase that releases vascular endothelial growth factor (VEGF) and active complexes of fibroblast growth factor by cleaving heparan sulfate proteoglycans in blood vessel basement membranes and the extracellular matrix; (b) direct inhibition of heparan sulfate binding to the growth factors VEGF and fibroblast growth factor; and (c) stimulation of the release of tissue factor pathway inhibitor, an endogenous antiangiogenic protein (16-18).

Previously, PI-88 was tested in healthy volunteers by s.c. injection and in cancer patients by prolonged continuous infusion. First, a phase I trial was conducted in cancer patients evaluating PI-88 administered by prolonged i.v. infusion at doses between 0.57 and 2.28 mg/kg/d. Dose-limiting grade 3 thrombocytopenia occurred in two of six patients treated with 2.28 mg/kg/d as a 14-day infusion. Both patients developed anti-heparin platelet factor 4 (PF4) complex antibodies, suggesting that the thrombocytopenia was immune mediated. Only 2 of 14 patients developed prolongation of the activated...
partial thromboplastin time (APTT), a pharmacodynamic marker of PI-88 (19). Thus, because of the development of dose-limiting toxicity (DLT) in the absence of appreciable pharmacodynamic effects, an alternative dosing strategy was evaluated using a s.c. formulation. Next, a phase IA study evaluated s.c. administration of PI-88 in healthy volunteers. Doses of up to 160 mg were well tolerated. Mild injection site erythmosis was noted as well as dose-dependent prolongation of the APTT. The maximum increase in APTT occurred 1 to 2 hours after dosing, and the levels returned to baseline within 14 hours after dosing. Mean ± SD bioavailability was 96 ± 22% (20). These results supported the evaluation of PI-88 by s.c. administration along with premedication with dexamethasone to potentially ameliorate immune-mediated thrombocytopenia. A fixed dose was evaluated because at the time there were no data available to support dosing by weight or body surface area.

Based on evidence showing the role of heparanase in angiogenesis and metastasis, encouraging preclinical data, and the favorable safety and pharmacokinetic profiles of PI-88 in healthy volunteers, the present phase I dose escalation study was undertaken in patients with advanced solid tumors. The objectives of this study were to (a) characterize the toxicities of PI-88 when administered s.c. daily for 4 consecutive days bi-monthly or weekly, (b) determine the maximum tolerated dose (MTD) and recommended dose for subsequent phase II trials, (c) characterize the pharmacokinetic profile of PI-88 administered s.c. in this population, (d) seek preliminary evidence of antitumor activity in patients with advanced solid tumors, and (e) assess the effects of PI-88 on soluble biomarkers, including VEGF and basic fibroblast growth factor (bFGF).

**Patients and Methods**

**Patient selection.** Eligible patients included adults with pathologically confirmed solid malignancy that was refractory to standard therapy or for which no standard therapy existed. Other relevant eligibility criteria included the following: (a) age ≥18 years; (b) Eastern Cooperative Oncology Group performance status ≤2; (c) adequate hematopoietic function [absolute neutrophil count >1,500/μL, platelet count >100,000/μL, APTT within normal limits (20-34 seconds), prothrombin time <1.5 times the institutional upper limit of normal], adequate hepatic function (total bilirubin level <1.5 times the institutional upper limit of normal, aspartate aminotransferase and alanine aminotransferase concentrations ≤2.0 times the institutional upper limit of normal, unless due to hepatic metastases, in which case elevations ≤5.0 times the institutional upper limit of normal were permitted), and adequate renal function (calculated creatinine clearance >60 mL/min using the Cockcroft-Gault formula); (d) no concomitant use of aspirin, nonsteroidal anti-inflammatory medications (except selective cyclooxygenase-2 inhibitors), heparin, low molecular weight heparin, or warfarin; (e) no neoplasms within 2 weeks before enrollment; (f) no chemotherapy, investigational therapy, or hormonal therapy in the previous 4 weeks; and (g) no evidence of anti-heparin antibodies by the serotonin release assay (SRA). Patients with the following were excluded: (a) history of brain metastases, (b) history of platelet diseases or allergy to anticoagulants, (c) significant cardiovascular history (including myocardial infarction, stroke, or congestive heart failure within 3 months before enrolling in the study), (d) history of acute gastrointestinal bleeding within the past 2 years, or (e) concomitant illnesses of sufficient severity to limit participation or full compliance with the study guidelines. Female subjects of childbearing age were required to have a negative pregnancy test, and informed consent was obtained from all patients in compliance with federal and institutional guidelines.

**Drug administration.** Patients received PI-88 on one of two dosing schedules. Patients enrolled in part 1 of the trial were dosed bimonthly starting at 80 mg PI-88 daily for 4 consecutive days (i.e., days 1-4 and 15-18 of a 28-day cycle). The starting dose was one half of the maximum dose evaluated in healthy volunteers. In an attempt to observe and potentially limit immune-mediated thrombocytopenia, PI-88 was administered for a maximum of 4 consecutive days followed by a 10-day observation period, and patients received 10 mg dexamethasone p.o. on the evening before starting each 4-day PI-88 treatment period. PI-88 was administered as a s.c. injection into the stomach, thigh, or arm. The starting dose of PI-88 (80 mg/d) was increased 33% for each subsequent dose level and there was no inpatient dose escalation. Three patients were enrolled per cohort until DLT was experienced by one of the three patients in the cohort. DLT was defined as any of the following adverse events, which occurred during the first treatment course and were possibly related to treatment: (a) grade 3/4 nonhemato logic toxicity (excluding nausea and vomiting), (b) grade 3/4 nausea or vomiting despite aggressive antiemetic support, (c) grade 4 neutropenia or neutropenia complicated by fever, (d) grade 3 thrombocytopenia, (e) APTT more than three times the upper limit of the institutional normal, or (f) grade 3 injection site reaction. In addition, inability to receive >75% of the planned study drug dose for the treatment period or inability to begin the next course of treatment within 2 weeks of the last dose due to unresolved toxicity qualified as dose limiting. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. If DLT was experienced by one patient, the treatment cohort was expanded to include up to six patients. If no others experienced DLT, dose escalation continued. If another patient experienced DLT within that cohort (for a total of two of six patients), the dose level before that was defined as the MTD. A total of 12 patients were treated at the determined MTD before advancing to part 2 of the study.

Once the MTD was established in part 1 of the study, the observation period between periods of consecutive daily dosing was reduced from 10 to 3 days, and the safety and tolerability of PI-88 was reevaluated. Patients enrolled in part 2 of the trial were dosed weekly in the same manner, starting at one dose level below the MTD determined from part 1 of the trial (cohort 4 = 190 mg). Part 2 then followed the same dose escalation scheme as part 1 until MTD was determined for the weekly dosing regimen. Dose escalation in part 2 was stopped at the MTD for the bimonthly regimen (250 mg/d), as it was considered unlikely that the increased dose frequency would be associated with reduced toxicity. Patients were allowed to continue on treatment until criteria for study withdrawal were met. If a patient experienced a positive result on the functional serotonin release anti-heparin antibody test (SRA) at any time during his or her participation on the trial, treatment was discontinued.

PI-88 was provided by Progen Industries Ltd. (Darra, Queensland, Australia) in glass vials containing 400 mg sterile lyophilized powder. PI-88 was reconstituted to give a final concentration of 140 mg/mL solution for doses of 250 mg or a 350 mg/mL solution for doses of 250 to 315 mg. The total daily dose was then prepared by the hospital pharmacy for the patient’s 4 consecutive days of home treatment before dispensing the drug in 1.0 or 2.0 mL syringes.

**Pretreatment and follow-up studies.** Before each cycle, complete medical histories, physical examinations, current medication profiles, assessments of performance status, and routine laboratory studies were done. Routine laboratory assessments included a complete blood count, differential WBC count, electrolytes, blood urea nitrogen, serum creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, lactate dehydrogenase, alkaline phosphatase, total bilirubin, alanine aminotransferase, aspartate aminotransferase, prothrombin time/international normalized ratio, APTT, fibrinogen, and D-dimer. In addition, before entering the study and at study discontinuation, electrocardiogram, routine urinalysis, and pregnancy test, when
were centrifuged at 4°C and immediately placed on ice. Within 15 minutes of blood collection, samples were centrifuged at 1,950 g, 88 dose, day 28, and, for part 1 of the study only, days 5 and 19. Blood urine samples were collected on days 1 and 15 before administering PI-88 to control plasma samples from healthy volunteers. Typically, standards in the range 0.05 to 75.6 μg/mL were prepared and processed without dilution (0.05-0.35 μg/mL) or after dilution of the filtrate 1:6, 1:36, or 1:216 (0.3-2.1, 1.8-12.6, and 10.8-75.6 μg/mL, respectively). At these dilutions, the standards produce linear responses in the fluorescence quenching assay. The precision and accuracy of the PI-88 assay in plasma were determined to be within acceptable ranges for assay validation and cross-validation showed the robustness of the assay.

**Pharmacokinetic analysis.** The pharmacokinetic profile of PI-88 was analyzed using compartmental methods. The equation for this model is

\[
C_{\text{plasma}} = \frac{Dose \cdot k_{\text{abs}}}{V_d(k_{\text{abs}} - k_{\text{el}})} \cdot e^{-k_{\text{el}} \cdot t} - e^{-k_{\text{abs}} \cdot t},
\]

where \( V_d \) is the volume of distribution, \( k_{\text{abs}} \) is the absorption first-order rate constant, and \( k_{\text{el}} \) is the elimination first-order rate constant.

Data were fit to the model equation using WinNonlin software version 4.1 (Pharsight Corp., Mountain View, CA) with 1 / \( y^2 \) weighting. Pharmacokinetic variables were calculated in a model-dependent manner. Linear regression and correlation of patient characteristics with pharmacokinetic variables was done using Graph-Pad Prism version 4.02 (GraphPad Software, San Diego, CA).

**Measurement of systemic and urinary angiogenic growth factor levels.** To measure angiogenic growth factor levels, whole blood and urine samples were collected on days 1 and 15 before administering PI-88 dose, day 28, and, for part 1 of the study only, days 5 and 19. Blood samples were placed on ice and then centrifuged at 1,950 \( \times \) g for 10 minutes in a precooled centrifuge. Plasma and urine samples were stored at ~70°C before analysis. Plasma VEGF and urine bFGF were measured using reliable and validated sandwich immunoassays (R&D Systems, Minneapolis, MN). Each control and sample was run in triplicate according to the manufacturer’s specifications. Quality control samples containing known amounts of the substrate were run in parallel with other samples to determine the interday coefficient of variation. Only assays exhibiting <20% coefficient of variation were considered valid.

**Table 1. Patient characteristics**

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<tr>
<th>Characteristic</th>
<th>Value</th>
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<td>No. patients</td>
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<tr>
<td>Total no. assessable courses</td>
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<tr>
<td>No. courses/patients</td>
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<tr>
<td>Median</td>
<td>53</td>
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<tr>
<td>Range</td>
<td>19-78</td>
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</table>

**Table 2. Dose escalation scheme**

<table>
<thead>
<tr>
<th>Study part</th>
<th>Dose level (mg/d)</th>
<th>No. patients</th>
<th>No. courses</th>
<th>Patients with DLT in course 1</th>
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<tr>
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<td>106</td>
<td>6*</td>
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<tr>
<td></td>
<td>190</td>
<td>3</td>
<td>42</td>
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<tr>
<td></td>
<td>250</td>
<td>12</td>
<td>67</td>
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</tr>
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<td>2</td>
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<td>190</td>
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<td>5</td>
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<tr>
<td></td>
<td>250</td>
<td>6</td>
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</table>

*Three patients treated at this dose level did not complete 28 days of treatment due to the development of intercurrent illness.
Results

General. Forty-two patients received a total of 232 courses of PI-88 at dose levels between 80 and 315 mg. The median number of courses administered was 2 (range, 1-51). Two patients were still in treatment at the time of this report (July 25, 2005). Four patients were withdrawn from the study before completing 28 days of treatment due to the development of intercurrent illness. All patients were assessable for toxicity. Patient characteristics are listed in Table 1. Most patients had a good performance status (88% had Eastern Cooperative Oncology Group performance status, 0-1). A wide range of tumor types was represented in this study, although patients with melanoma constituted 45% (19 of 42). The number of patients treated and the number of cycles administered as a function of dose level are depicted in Table 2.

In part 1 of the study, 33 patients were treated at six dose levels. Two of six patients treated with 315 mg experienced DLT, including one patient with grade 3 thrombocytopenia and another with grade 4 pulmonary embolism in the setting of grade 2 thrombocytopenia. The 250 mg dose level was then expanded to include 12 patients. At this dose level, one patient experienced DLT, grade 3 thrombocytopenia. In part 2 of the study, three patients were treated at the 190 mg dose level and six were treated at the 250 mg level. No patients treated in part 2 experienced DLT. Thus, 250 mg administered for 4 consecutive days was established as the MTD for the weekly schedule as well as for the bimonthly regimen.

Toxicity. Thrombocytopenia occurred in 15 of 238 (6%) treatment courses and was generally mild to moderate. Two episodes of grade 3 thrombocytopenia were related to PI-88, whereas the third occurrence was attributed to the development of acute myelogenous leukemia in a patient with melanoma. In three patients, thrombocytopenia was associated with DLT, and the time course is depicted in Fig. 1. In all three of these patients, the platelet concentration decreased precipitously between days 15 and 16 of course 1, and PI-88 was discontinued on day 16. In one patient at the 315 mg/d dose level, the platelets decreased from 196,000 to 61,000 (69%) concomitant with a large, grade 4 pulmonary embolism, which was deemed to be possibly related to PI-88 and constituted DLT. Anticoagulation with a direct thrombin inhibitor was initiated, but the patient died in hospice care of rapidly progressive melanoma 10 days after the last dose of PI-88. In another patient treated at this dose level, the platelet concentration decreased from 176,000 to 48,000 (73%) without complications and the patient died 23 days after the last dose of PI-88 due to disease progression. The third patient with DLT was treated at the 250 mg/d dose level. In this patient, the platelets decreased from 217,000 to 70,000 (68%) between days 15 and 16 to a nadir of 28,000 on day 17, concomitant with a diagnosis of heart failure thought to be unrelated to PI-88. Four days later, the platelets increased to 110,000 and the patient died of complications from heart failure. No postmortem evaluation was done, but the cause of death was felt to be unrelated to PI-88.

Prolongation of the APTT was anticipated from preclinical studies and the results of the study in normal volunteers. Table 3 delineates the maximum observed APTT by dose level during course 1 of treatment. In part 1 of the study, the maximum prolongation of the APTT most commonly was observed 2 hours after s.c. injection (range, 1-8 hours). Twenty-four hours after PI-88 administration, the APTT returned to normal in 329 of 359 (92%) doses evaluated and to grade 1 in the remaining 30 (8%) doses. In part 2 of the study, at 24 hours after administration, the APTT had returned to normal in 31 of 42 (74%) doses and to grade 1 in 9 (21%) doses. The APTT remained elevated to grade 2/3 in two other dose administrations. No episodes of clinically significant bleeding related to PI-88 were observed, and no relationship was established between PI-88 dose and protime, fibrinogen, or D-dimer.

Table 3. Principal toxicities of PI-88

<table>
<thead>
<tr>
<th>Study part</th>
<th>Dose (mg/d)</th>
<th>Total no. cycles</th>
<th>Fatigue</th>
<th>Injection site ecchymosis</th>
<th>Thrombocytopenia</th>
<th>APTT*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grade 1/2</td>
<td>Grade 3/4</td>
<td>Grade 1/2</td>
<td>Grade 3/4</td>
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<td>80</td>
<td>54</td>
<td>3</td>
<td>0</td>
<td>25</td>
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<tr>
<td>106</td>
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<td>3</td>
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</tr>
<tr>
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</tr>
<tr>
<td>190</td>
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<td>0</td>
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<tr>
<td>250</td>
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<tr>
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<td>5</td>
<td>1</td>
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<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

*Maximum observed prolongation of APTT during course 1.

1 Grade 3 thrombocytopenia was attributed to the development of acute myelogenous leukemia in a patient with melanoma.
Other toxic effects occurring in at least 10% of cycles included injection site ecchymosis and fatigue (Table 3). Grade 1/2 ecchymosis was observed in 112 of 204 (55%) courses in part 1 of the study and 25 of 28 (89%) courses in part 2. Less than 10% of patients reported pain or itching at the injection sites. Grade 1/2 fatigue occurred in 27 of 204 (13%) courses in part 1 and 11 of 28 (39%) courses in part 2. Grade 3 fatigue was only reported in 2 courses after cycle 1.

**Anti-PI-88/PF4 IgG antibody production.** Twenty-nine patients treated on part 1 of the study were assessed for the development of anti-PI-88/PF4 antibodies during the first course of treatment. There were no anti-PI-88/PF4 IgG antibodies detected in patients by EIA at baseline. Among the 26 patients that did not develop thrombocytopenia during course 1, no detectable anti-PI-88/PF4 IgG antibodies developed. Among the three patients who developed thrombocytopenia, anti-PI-88/PF4 and anti-heparin/PF4 IgG antibodies were documented by EIA, suggesting an immune-mediated etiology. One of the three patients with uncomplicated thrombocytopenia developed a positive heparin-induced thrombocytopenia (HIT)-SRA with both unfractionated heparin (0.1 units/mL) and PI-88 (10–200 μg/mL). Interestingly, the patient treated with PI-88 315 mg/d who had grade 2 thrombocytopenia and a documented pulmonary embolism had a negative HIT-SRA.

**Antitumor activity.** Thirty-eight patients were assessable for antitumor activity. Of these, 10 (26%) patients experienced clinical benefit as defined by a partial response (PR) or stable disease (SD) for six treatment courses: 6 of 17 patients with melanoma, 2 of 5 patients with renal cell carcinoma, 1 patient with carcinoid, and 1 patient with adenoid cystic carcinoma. Four patients received >1 year of therapy. One patient, a 47-year-old man with metastatic melanoma, had a PR (see Fig. 2) and has received treatment with PI-88 for >50 months. Additional details regarding patients who experienced clinical benefit are presented in Table 4.

**Pharmacokinetic studies.** Shown in Fig. 3 is the pharmacokinetic profile of s.c. dosed PI-88. The profile is described by a one-compartment model with first-order elimination and first-order absorption from an extravascular site. Goodness-of-fit correlations >0.900 were observed in 92% (73 of 79) of the plasma concentration versus time curves analyzed. The resulting model-dependent variables from both days 1 and 15 of dosing at the six dose levels are shown in Table 5. Pharmacokinetic analysis shows that PI-88 plasma concentration is linear with respect to dose, and this is reflected in both AUC and $C_{\text{max}}$ (Fig. 4A and B).

**Pharmacokinetic/pharmacodynamic relationships.** Because in vitro plasma assays showed that PI-88 results in linear,

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**Table 4.** Patients with PR or SD for ≥6 months (≥6 × 28-day treatment periods)

<table>
<thead>
<tr>
<th>Study part</th>
<th>PI-88 (mg/d)</th>
<th>Tumor*</th>
<th>Response</th>
<th>Time on study (mo)</th>
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<tbody>
<tr>
<td>1</td>
<td>80</td>
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<td>PR</td>
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<td>Melanoma</td>
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<td></td>
<td>Renal cell</td>
<td>SD</td>
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<tr>
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<td></td>
<td>Melanoma</td>
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<td>Melanoma</td>
<td>SD</td>
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<td>Melanoma</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Renal cell</td>
<td>SD</td>
<td>7</td>
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*All patients included in this table had progressed on a prior chemotherapy regimen before enrolling in the study, except the patient with melanoma treated at the 190 mg dose level and the patient with adenoid cystic carcinoma.

‡Patients continued on an extension trial.
dose-dependent prolongation of the APTT, the effect of PI-88 on APTT was measured in patients in conjunction with pharmacokinetic sampling to determine if this simple clinical test could be used as a surrogate to PI-88 plasma drug analysis. Comparisons of both weight-corrected (based on the analysis below showing correlation between patient weight and both AUC and $C_{\text{max}}$) drug exposure (AUC) and peak exposure ($C_{\text{max}}$) were correlated to the percent increase in APTT and the maximum APTT (Max APTT), and the results are shown in Fig. 5A and B. These results reveal that the percent increase in APTT and Max APTT had a linear correlation with AUC ($r^2 = 0.481$ and 0.413). $C_{\text{max}}$ showed a better correlation with both percent increase in APTT ($r^2 = 0.727$) and Max APTT ($r^2 = 0.592$). This correlation also held in the absence of correcting the AUC and $C_{\text{max}}$ for weight. More complicated nonlinear relationships did not yield better correlations.

**Correlation of patient characteristics with PI-88 pharmacokinetic variables.** Patient age, weight, and creatinine clearance were independently analyzed for correlation to PI-88 pharmacokinetic variables. The correlation of dose-corrected AUC ($AUC/Dose$) and $C_{\text{max}}$ ($C_{\text{max}}/Dose$) and CL/F were independently analyzed with respect to patient age, weight, and creatinine clearance, and the corresponding P values are shown in Table 6. As shown in Fig. 6A to C, patient weight correlated to

### Table 5. Pharmacokinetic variables of PI-88 on days 1 and 15

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Day (n)</th>
<th>AUC (µg h/mL)</th>
<th>$C_{\text{max}}$ (µg/mL)</th>
<th>$k_{\text{el}}$ (h$^{-1}$)</th>
<th>$t_{1/2}$ (h)</th>
<th>$V_d/F$ (L)</th>
<th>CL/F (L/h)</th>
<th>$T_{\text{max}}$ (h)</th>
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<tr>
<td>80</td>
<td>1 (3)</td>
<td>27.8 ± 18.0</td>
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<td>20.9 ± 13.2</td>
<td>4.41 ± 3.74</td>
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<tr>
<td></td>
<td>15 (3)</td>
<td>26.2 ± 16.0</td>
<td>4.2 ± 2.7</td>
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<td>3.55 ± 0.38</td>
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<td>1 (6)</td>
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<td>3.67 ± 1.94</td>
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<td>3.22 ± 0.62</td>
<td>1.6 ± 0.7</td>
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<tr>
<td></td>
<td>15 (3)</td>
<td>42.0 ± 14.8</td>
<td>6.9 ± 3.7</td>
<td>0.222 ± 0.122</td>
<td>3.69 ± 1.58</td>
<td>14.7 ± 9.3</td>
<td>2.73 ± 0.88</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>140</td>
<td>1 (3)</td>
<td>46.0 ± 13.2</td>
<td>6.7 ± 4.1</td>
<td>0.183 ± 0.042</td>
<td>3.92 ± 0.85</td>
<td>18.8 ± 8.6</td>
<td>3.20 ± 0.85</td>
<td>1.7 ± 0.8</td>
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<tr>
<td></td>
<td>15 (3)</td>
<td>51.7 ± 16.6</td>
<td>7.3 ± 4.2</td>
<td>0.189 ± 0.032</td>
<td>3.74 ± 0.66</td>
<td>16.0 ± 6.9</td>
<td>2.88 ± 0.81</td>
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<td>190</td>
<td>1 (3)</td>
<td>80.4 ± 20.7</td>
<td>8.5 ± 1.9</td>
<td>0.137 ± 0.015</td>
<td>5.09 ± 0.53</td>
<td>18.0 ± 3.7</td>
<td>2.46 ± 0.57</td>
<td>1.6 ± 0.4</td>
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<td>15 (3)</td>
<td>87.5 ± 26.7</td>
<td>7.7 ± 1.8</td>
<td>0.117 ± 0.003</td>
<td>5.93 ± 0.17</td>
<td>19.6 ± 6.1</td>
<td>2.31 ± 0.66</td>
<td>2.3 ± 0.6</td>
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<tr>
<td>250</td>
<td>1 (10)</td>
<td>127.2 ± 50.6</td>
<td>9.4 ± 4.0</td>
<td>0.085 ± 0.039</td>
<td>7.59 ± 4.45</td>
<td>19.4 ± 9.4</td>
<td>1.70 ± 0.71</td>
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<td>15 (10)</td>
<td>145.7 ± 44.1</td>
<td>10.2 ± 2.2</td>
<td>0.091 ± 0.028</td>
<td>8.54 ± 3.60</td>
<td>21.4 ± 6.7</td>
<td>1.86 ± 0.56</td>
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<tr>
<td>315</td>
<td>1 (5)</td>
<td>127.0 ± 22.8</td>
<td>12.5 ± 3.3</td>
<td>0.166 ± 0.031</td>
<td>4.28 ± 0.74</td>
<td>15.8 ± 4.6</td>
<td>2.55 ± 0.46</td>
<td>3.1 ± 1.2</td>
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<tr>
<td></td>
<td>15 (4)</td>
<td>117.5 ± 65.5</td>
<td>10.2 ± 5.8</td>
<td>0.188 ± 0.118</td>
<td>4.34 ± 2.88</td>
<td>16.5 ± 10.3</td>
<td>2.72 ± 1.52</td>
<td>3.3 ± 2.2</td>
</tr>
</tbody>
</table>

**NOTE:** Pharmacokinetic variables were calculated in a model-dependent manner as described in Materials and Methods. Mean ± SD of individual patient variables.
all three pharmacokinetic variables, and creatinine clearance correlated with $C_{\text{max}}$. The coefficients of determination ($r^2$) for CL/F, AUC/Dose, and $C_{\text{max}}$/Dose versus patient weight were 0.1864, 0.1761, and 0.7365, respectively, for linear regression analysis. No correlation was observed between the occurrence of DLT and AUC or $C_{\text{max}}$.

**Growth factor concentrations.** The effect of PI-88 administration on plasma concentrations of VEGF and bFGF and urine concentrations of bFGF was examined. No distinct relationship between PI-88 dose and changes in growth factor levels was observed. When patients with a PR or SD for ≥6 months were compared with patients with disease progression within 6 months of treatment initiation, no significant differences were found in mean plasma VEGF, plasma bFGF, and urine bFGF concentrations at baseline or at days 15 and 28 of course 1 (Table 7). Within each group of patients, there was no significant change in growth factor levels during course 1.

**Discussion**

This phase I study was designed to evaluate the feasibility, safety, and pharmacokinetics of PI-88 when administered s.c. for 4 consecutive days either bimonthly or weekly. On both schedules, PI-88 was well tolerated at the MTD and recommended phase II dose level of 250 mg/d. DLT consisted of grade 3 thrombocytopenia in one patient and grade 4 pulmonary embolism in another. The weekly regimen is recommended for further development based on the hypothesis that the antiangiogenic mechanism of PI-88 supports a dosing schedule that leads to more sustained concentrations over time.
Although thrombocytopenia was a DLT in this study, no bleeding complications were observed, whereas grade 2/3 thrombocytopenia was associated with the development of antibodies to PF4 by EIA in all patients with thrombocytopenia and DLT. HIT is a serious, immunologic, prothrombotic disorder mediated by complexes of PF4, anti-PF4 IgG, and heparin. Thrombosis occurs in 35% to 75% of patients with HIT (24). The diagnosis of rapid-onset HIT requires the following: (a) thrombocytopenia, defined as a ≥50% decrease in platelet count; (b) an abrupt decrease in platelet count within 24 hours of starting heparin in patients recently exposed to heparin; and (c) positive EIA and SRA for HIT antibodies (i.e., anti-PF4/heparin antibodies). PI-88 is structurally similar to heparin and, like heparin, binds PF4. Development of antibodies to PI-88/PF4 could theoretically give a HIT-like immune disorder.

The three patients who developed grade 2/3 thrombocytopenia associated with DLT possessed many features of HIT, including a decrease in the platelet count of ≥50% and a time course consistent with rapid-onset HIT. Only one patient had a positive SRA result, whereas in the other two patients the diagnosis was suspected based on clinical features. One patient with grade 2/3 thrombocytopenia and a large pulmonary embolism may have had HIT-like immune-mediated thrombocytopenia with thrombosis, assuming that the sensitivity of the standard SRA for detecting anti-PI-88/PF4 is not 100%. Alternatively, the patient may have had anti-PI-88/PF4 antibodies that did not result in platelet activation and thrombocytopenia; rather, the thrombocytopenia may have been secondary to the pulmonary embolism itself (24).

This study shows that PI-88 can cause immune-mediated thrombocytopenia with or without thrombosis, similar to heparin. The extent to which dexamethasone reduced the risk of developing PI-88-induced immune-mediated thrombocytopenia is unknown, as patients were not randomly assigned to receive dexamethasone versus placebo. However, patients who did continue on study for multiple cycles and were allowed to discontinue dexamethasone did not develop HIT.

PI-88 treatment results in prolongation of the APTT that was found to be linearly correlated to the AUC and $C_{\text{max}}$. PI-88 was not associated with clinically significant bleeding. We suspect no correlation between PI-88 dose and APTT was found in the initial phase I trial because insufficient doses were evaluated in that trial (19). Prior studies have shown that PI-88 prolongs the APTT by potentiating heparin cofactor II–mediated inhibition of thrombin activity. PI-88 has no effect on the prothrombin time (14, 18).

In this study, we explored the effects of PI-88 on circulating angiogenic growth factors, because this agent is thought to mediate its effect, in part, through the inhibition of VEGF and bFGF release from the extracellular matrix (25). However, no significant effect of PI-88 administration on angiogenic growth factor levels was observed, nor was clinical benefit associated with baseline growth factor levels or changes in these levels.

![Fig. 6. Correlation of PI-88 pharmacokinetic variables (AUC/Dose, $C_{\text{max}}$/Dose, and CL/F) with patient weight. A, the $r^2$ for CL/F versus weight was 0.1864. B, the $r^2$ for AUC/Dose versus weight is 0.1761. C, the $r^2$ for $C_{\text{max}}$/Dose versus weight is 0.7365.](image-url)
Interpreting systemic growth factor levels is difficult because of
the multiple sites of production and storage of these growth
factors and their complex cross-regulation (26). Although PI-88
may have effectively reduced the release of growth factors from
heparan sulfate proteoglycans by inhibiting heparanase, this
may not be manifested as a change in systemic growth factors
due to a potential increase in growth factor release from other
components of the extracellular matrix (such as fibronectin,
fibrin, and thrombospondin), an increase in release of stored
VEGF from activated platelets, or a compensatory increase in
VEGF production by leukocytes, megakaryocytes, or the
neoplasm itself. These results are consistent with studies of
other agents, such as imatinib and endostatin, and do not rule
out a biological effect of the compound (27–29). Preclinical
studies are ongoing to assess whether heparanase expression
before therapy may be used to preselect patients or to predict
clinical benefit, but these studies are not yet validated. We
recommend that future efficacy studies collect baseline tumor
specimens to determine whether heparanase expression corre-
lates with clinical benefit.

The pharmacokinetics of s.c. dosed PI-88 are best described
using a one-compartment model with first-order absorption
and distribution. This is similar to what has been shown with
low molecular weight heparins. The PI-88 dose was linear with
respect to AUC and $C_{\text{max}}$. The data showed low intra-
individual variability but considerably higher interpatient variability.
These data suggest that target dosing for individuals would be
best achieved by individual assessment of plasma levels or by
using percent increase in APTT as a surrogate. The linear
relationship between percent increase in APTT and PI-88 AUC
and $C_{\text{max}}$ levels could prove useful in optimizing patient
dosing based on this relatively simple clinical test as opposed
to the more complicated analysis for PI-88 drug concentra-
tions. Further, the correlations between patient weight and
pharmacokinetic variables suggest that part of the interpatient
variability could be corrected for by dosing by body weight
with this agent.

Antitumor activity was shown in this study. One patient
with metastatic melanoma had an objective response that was
maintained for >50 months. Five additional patients with
melanoma, most of whom had a low disease burden, had SD
for 7 to >38 months. These favorable outcomes suggest that
PI-88 has growth-inhibitory activity in patients with melano-
ma, although randomized studies are needed to confirm this.
Prolonged disease stability may be related to the preclinical
observation that the rate of metastasis of melanoma was
dependent on the activity of heparanase (2). Prolonged disease
stabilization with PI-88 treatment is also consistent with
preclinical data showing that heparanase expression is
increased in human metastatic melanoma and that PI-88
decreases tumor growth and invasion in a mouse model of
multistage cancer in which heparanase is up-regulated (30).
A phase II clinical trial evaluating the efficacy of PI-88 in
patients with metastatic melanoma is ongoing.

The results of this phase I and pharmacokinetic study show
that PI-88 is generally well tolerated but occasionally results in
thrombocytopenia associated with anti-PF4 antibodies and
HIT. The recommended dose of PI-88 administered daily for
4 days every week is 250 mg. Given the prolonged disease
stabilization observed in patients with melanoma and the
single PR, further randomized studies of this agent in
melanoma are warranted. Additionally, future studies should
incorporate preclinically validated biological assays that relate
to the heparanase-inhibiting capacity of PI-88.

| Table 7. Mean angiogenic growth factor levels during course 1 in patients with disease progression within 6 months of initiation of treatment and in patients with prolonged disease stabilization |
|-----------------|-----------------|-----------------|
| Growth factor   | Day             | Mean (95% confidence interval) growth factor levels (pg/mL) |
|                 |                 | Subjects with disease progression in <6 courses | Subjects with SD ≥ 6 courses or a PR |
| Plasma VEGF     | 1               | 53.1 (20.8-85.8) | 84.8 (13.6-156.0) |
|                 | 15              | 58.5 (15.9-101.1)| 56.6 (40.0 to 153.2)|
|                 | 28              | 80.9 (25.8-135.9)| 95.2 (88.8 to 279.3)|
| Plasma bFGF     | 1               | 26.1 (18.0-34.2) | 23.7 (11.2-36.1) |
|                 | 15              | 24.3 (17.6-31.0) | 19.8 (7.4-32.2) |
|                 | 28              | 21.2 (14.4-28.0) | 16.4 (9.0-25.8) |
| Urine bFGF      | 1               | 3.69 (1.6 to 9.0) | 0.5 (0.3 to 1.3) |
|                 | 15              | 11.0 (5.4 to 27.5) | 1.7 (1.7 to 5.2) |
|                 | 28              | 6.1 (1.1 to 12.6) | 5.8 (1.1 to 12.6) |

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name-1 gene expression in thyroid papillary carcinomas:


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Michele Basche, Daniel L. Gustafson, Scott N. Holden, et al.


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