

Executive Summary

PCI-27483

A potent small molecule inhibitor of the Factor VIIa/Tissue Factor complex in the treatment of Cancer

Product Class:	1st in class, small molecule
Mechanism of Action:	Active site, reversible inhibitor of Factor VIIa/tissue factor complex
Stage of Development:	Phase II
Clinical Status:	Ascending single-dose study of PCI-27483 in healthy volunteers has been completed Multi-center Phase II clinical trial in pancreatic cancer patients will commence in calendar Q4'2009 Subcutaneous delivery Efficacy in murine tumor models demonstrated Efficacy in baboon anti-thrombosis model demonstrated IND #78,433
Focus of Therapeutic Indications:	Treatment of cancer patients with tumors that overexpress tissue factor (e.g., pancreatic, colon, lung, breast tumors).
Other potential indications:	Antithrombotic therapy (venous or arterial) e.g., venous thromboembolism (VTE), including deep vein thrombosis and pulmonary embolism, acute coronary syndromes, anticoagulation during dialysis and catheter clearance.

Differentiating Features

- High resolution X-ray structures solved which enabled structure based design
- Inhibitors offer high potency and selectivity for FVIIa/TF complex
- Anti-cancer activity observed in animal models
- Projected half-life in humans greater than that of low-molecular weight heparins
- No major metabolites observed; no CYP inhibition
- Balanced renal and hepatobiliary excretion rates
- Scalable synthetic process established, cost of good expected to be less than that of low-molecular weight heparins
- High-load solution formulation developed for subcutaneous dosing
- Solid patent position



I. Background

Factor VII is a serine protease that becomes activated (FVIIa) by binding to tissue factor (TF). The FVIIa/TF complex triggers the extrinsic coagulation cascade that leads to the formation of a blood clot. Tissue factor is expressed in many cells such as fibroblasts and keratinocytes, but is absent from vascular cells that come in contact with circulating FVII in the blood.

Anticoagulation: This FVIIa program was acquired from Celera Genomics in early 2006 after a major medicinal chemistry lead optimization effort and safety evaluation. **The program was focused on the treatment and/or prevention of thrombotic events arising from the activity of the FVIIa/TF complex.** Preclinical models of thrombosis in several species have indicated that a selective inhibitor of the factor VIIa/Tissue Factor (FVIIa/TF) complex may have a greater therapeutic/safety index than inhibition of other coagulation factors. Pharmacyclics has shown that the lead clinical development compound, PCI-27483, exhibits a tightly controlled anticoagulation effect that is dose dependent in animal models as well as in a Phase I clinical trial.

Oncology: Pharmacyclics is also evaluating the lead clinical development compound, **CI-27483 as a potential anti-tumor agent.** Academic studies in experimental metastases have established the concept that tumor cell TF can facilitate metastatic tumor spread. Moreover, overexpression of TF in fibrosarcoma, pancreatic cancer cells and melanoma cells appear to enhance tumor growth. Knock-down models of TF in colon cancer cells conversely inhibit tumor expansion., PCI-27483 has been shown to significantly inhibit tumor growth in both syngeneic and xenograft tumor models.

II. Mechanism of Action

Anticoagulation: TF (tissue factor) is a membrane-bound protein not normally found in circulating plasma. Damage to the integrity of the vascular system or activation of monocytes or endothelial cells results in exposure of membrane-bound TF to the blood. Subsequently, the TF exposed to the blood system will bind to small amounts of activated Factor FVII (FVIIa) already circulating in the blood system to form the TF–FVIIa complex. This event initiates the coagulation cascade by further activating the zymogen form of Factor VII as well as converting downstream coagulation factors such as Factor IX and Factor X (FX) into Factor IXa and Factor Xa (FXa) respectively. Inhibition of FVIIa holds the potential of interrupting both the intrinsic (Factor IX to Factor IXa) and the extrinsic (Factor X to Factor Xa) coagulation pathways.

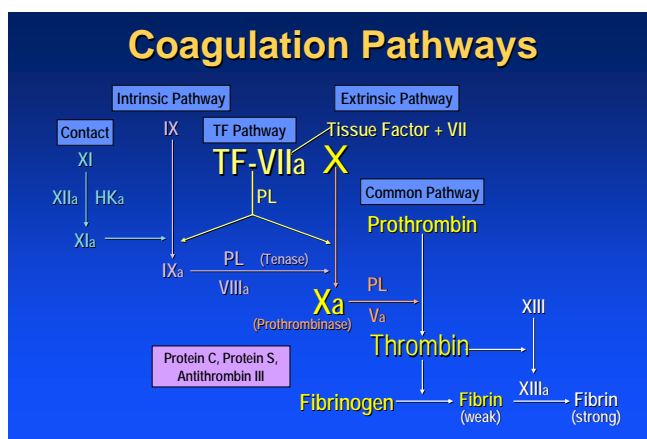


Figure 1. Factor VII's Role in the Coagulation Pathway¹

Oncology: Proteolytic hydrolysis of membrane-bound, G-protein-coupled protease activated receptors (PAR2) by the TF:FVIIa complex induces intracellular signaling pathways that lead to the activation of mitogen-activated protein (MAP) kinases.^{2,3} Activation of PAR2 on the surface of breast cancer cells by FVIIa triggers ERK1/2 and p38 MAP kinase phosphorylation and a downstream increase in the mRNA and protein levels of vascular endothelial growth factor (VEGF).⁴ FVIIa-mediated activation of PAR-2 results in an up-regulation of interleukin-8 (IL-8) expression in breast cancer cells and an increase in cell migration and invasion.⁵ IL-8 has been shown to stimulate the invasion, chemotaxis, and metastasis of many tumor cell types and to promote vascularization by enhancing endothelial cell proliferation.^{6,7,8,9} Cell signaling through PAR-2 but not PAR-1 promotes the development of mammary adenocarcinomas in mice.¹⁰

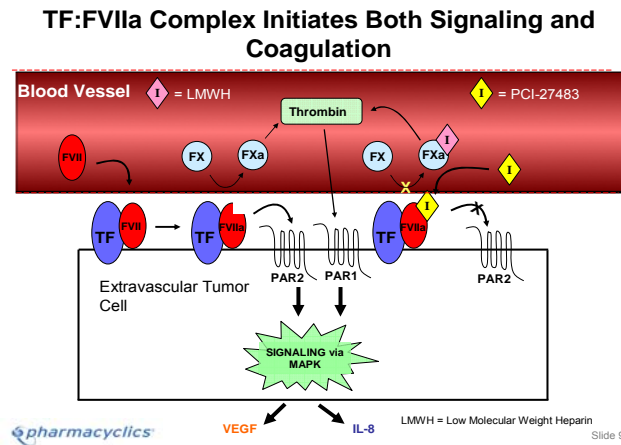


Figure 2. Factor VII's Additional Role in Tumor Signalling

Up-regulation of TF expression is observed in several human malignancies, including pancreatic cancer, melanoma, and breast cancer (Figure).^{11,12,13}

	Breast	Colon	Lung, NSC	Prostate	Ovary	Pancreas
1	1	0	0	0	3	
1	1	1	2	0	0	
1	2	1	0	2	2	
0	3	0	2	0	3	
0	0	2	0	0	1	
0	0	0	0	0	1	
0	0	1	1	0	0	
0	2	2	3	2	2	
1	0	2	4	0	3	
2	0	1	4	0	3	
Normal epithelium	0	0	0	1	0	0
Overexpression Δ+1 (% tumors)	50	30	70	50	20	80
Overexpression Δ+2 (% tumors)	10	10	30	30	20	60

N=10

0	1	2	3	4
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Figure 3. Tissue Factor Expression in Various Tumor Types as Determined by Immunohistochemistry Staining

FVII was detected in 12 of 13 human pancreatic carcinomas using immunohistochemical staining (unpublished results). Staining was present primarily in malignant cells with no staining of the normal surrounding fibrous tissue. The leading margins of tumor invasion often exhibited high levels of staining (Figure). In colorectal cancer cells, increased TF expression has been linked to the activation of the *K-ras* oncogene and loss of the *p53* tumor suppressor gene.¹⁴ Immunohistochemical studies have shown that many tumors historically associated with an increased incidence of venous thromboembolism overexpress TF.^{13,15} Overexpression of TF correlates with a worsened prognosis and poor histological grade in pancreatic cancer, colorectal cancer, and glioblastoma.^{16,17,18}

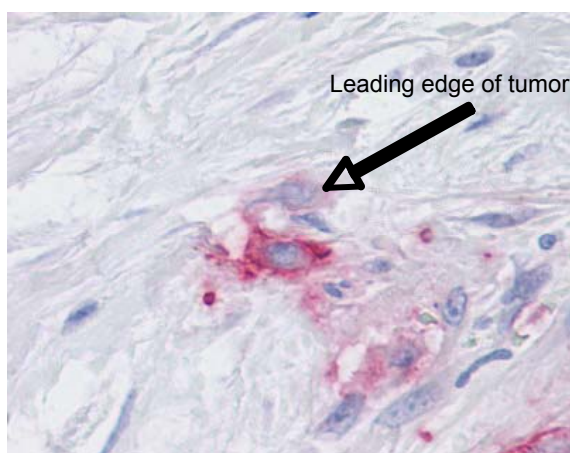


Figure 4. Immunohistochemical FVIIa Staining (magenta) of Primary Pancreatic Carcinoma Cells at the Leading Margin of Invasion (60X)

III. Preclinical Pharmacology

A. Biochemical Assays

PCI-27483 was evaluated in vitro for its **ability to inhibit purified human factor VIIa and other enzymes involved in hemostasis**. The K_i values of PCI-27483 in these assays are listed in Table 1.

Table 1. Selective Inhibition of FVIIa by PCI-27483 Compared with Other Enzymes Involved in Hemostasis

Assay	Parameter	Concentration (μM)	Selectivity
Factor VIIa	K_i	$0.00162 \pm 0.00045^{(a)}$	1
Factor Xa	K_i	5.71 ± 1.55	3525
Thrombin	K_i	>150.0	>92535
Factor IXa	K_i	1.10 ± 0.37	678
Factor XIa	K_i	1.68 ± 0.43	1035
Kallikrein-P	K_i	0.20 ± 0.06	120

^a Mean \pm SD

B. Ex vivo Assays of Coagulation

PCI-27483 was evaluated ex vivo in human plasma for its anticoagulation activity measured as an increase in prothrombin time (PT). PCI-27483 doubled PT at a concentration of $2.06 \pm 0.50 \mu\text{M}$ ($1.23 \pm 0.30 \mu\text{g/mL}$) (Table 2). No increase of activate partial thromboplastin time was observed at a concentration of $10 \mu\text{M}$ ($5.96 \mu\text{g/mL}$).

Table 2. Activity of PCI-27483 in Coagulation Assays with Human Plasma

Assay	Endpoint	Concentration (μM)	Selectivity
Prothrombin time	Doubling of clotting tme	2.06 ± 0.50	1
Activated partial thromboplastin time	Doubling of clotting tme	>10	>6

PCI-27483-mediated increases in PT were evaluated ex vivo using plasma from mice, rats, rabbits, dogs, and monkeys (Table 3). The concentration of PCI-27483 resulting in doubling of PT was generally higher in laboratory animals than in humans. The species rank order in sensitivity to PT increases was: mouse $<$ rat $<$ dog $<$ rabbit $<$ monkey \approx human.

Table 3. PCI-27483-Mediated Increase in Prothrombin Time (PT) in Blood from Different Species

Species	PT Doubling Concentration		Animal to Human Ratio for PT Doubling Concentration
	(μ M)	(μ g/mL)	
Mouse	29.1	17.4	16.17
Rat	24.2	14.4	13.44
Rabbit	4.46	2.66	2.47
Dog	10.31	6.14	5.73
Monkey	2.15	1.28	1.19
Human	2.06	1.23	N/A

C. Antithrombotic Effects in Baboons

The antithrombotic effects of subcutaneous PCI-27483 administration were determined in a baboon model of arterial thrombosis at dosages of 1, 2, and 4 mg/kg. Increasing subcutaneous doses of PCI-27483 progressively inhibited thrombus formation (Figure). Fibrin accumulation was similarly reduced by increasing doses of PCI-27483 (Figure). At the 4 mg/kg dose level of PCI-27483, maximum prothrombin times were generally 2 times pre-dosing values (Figure). At a dose of 4 mg/kg, PCI-27483 appeared to have similar anti-coagulation effects as Lovenox, a low-molecular weight heparin, dosed at 2 mg/kg.

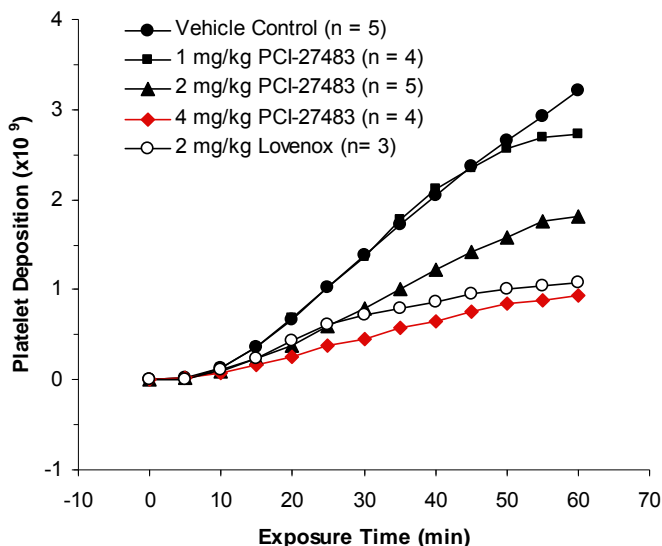


Figure 5. Dose-Dependent Inhibition of Platelet Deposition in Baboon AV Shunt Containing Tissue Factor Graft by PCI-27483 or Lovenox

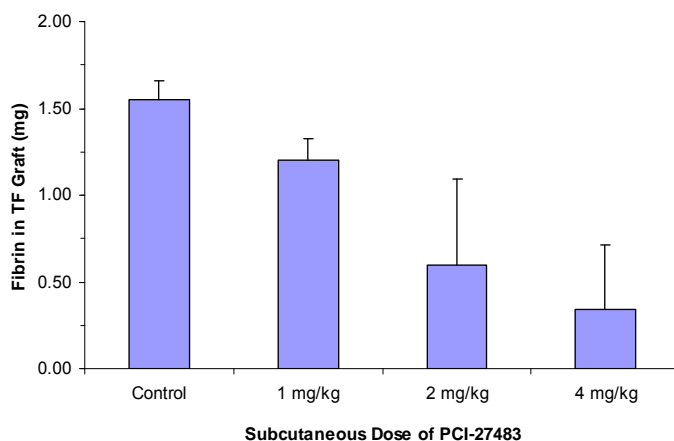


Figure 6. Dose-Dependent Inhibition of Fibrin Accumulation in Baboon AV Shunt Containing Tissue Factor Graft (error bars = SD)

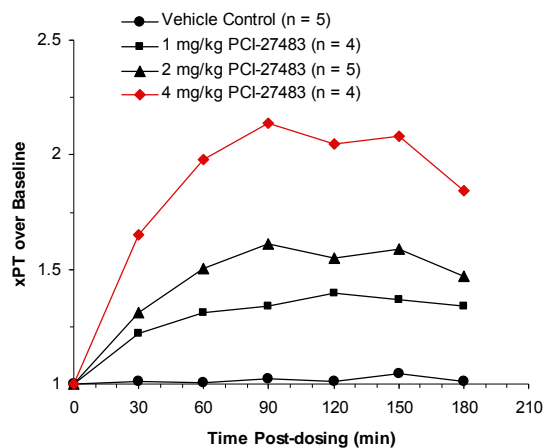


Figure 7. Dose-Dependent Increase in Prothrombin Time among Baboons Dosed with PCI-27483 Subcutaneously

D. Pharmacology of Anti-Tumor Effects

Intracellular Signaling In Vitro

- FVIIa stimulated secretion of IL-8 from MDA-MB-231 breast cancer cells is inhibited by PCI-27483

Pharmacology/Efficacy models

- Metastasis:
 - B16F10 melanoma lung metastasis model
 - Significant inhibition of lung colonization at 50 and 100 mg/kg PCI-27483

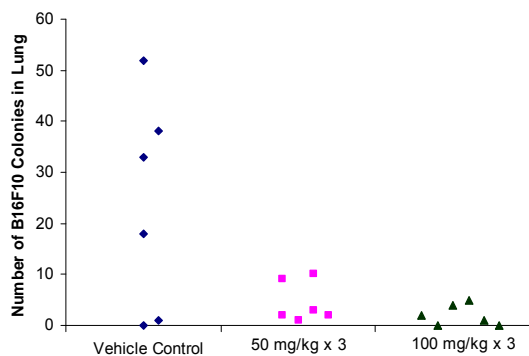


Figure 8. Lung Colonization by B16F10 Melanoma Cells in Mice Treated Subcutaneously with Vehicle or Formulated PCI-27483

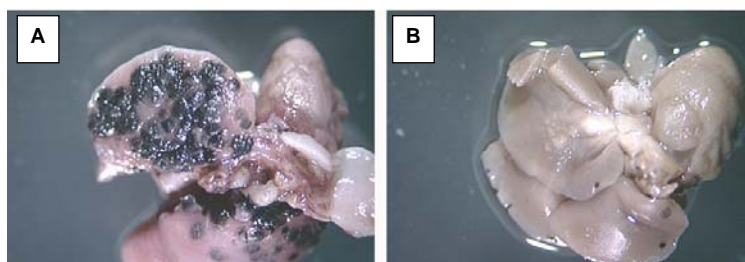


Figure 9. Gross Observation of Lung Colonization by B16F10 Melanoma Cells in Mice Treated with Vehicle or FVIIa Inhibitor

- Syngeneic:
 - Tumor growth models with Lewis lung carcinoma and B16F10 melanoma cells
 - Significant tumor inhibition seen at 15 to 60 mg/kg/dose given twice daily
 - ED100 = 60 mg/kg/dose (bid) with melanoma tumors (see Figure); ↑ PT = 2X
 - ED100 = 45 mg/kg/dose (bid) with LLC tumors (see Figure)

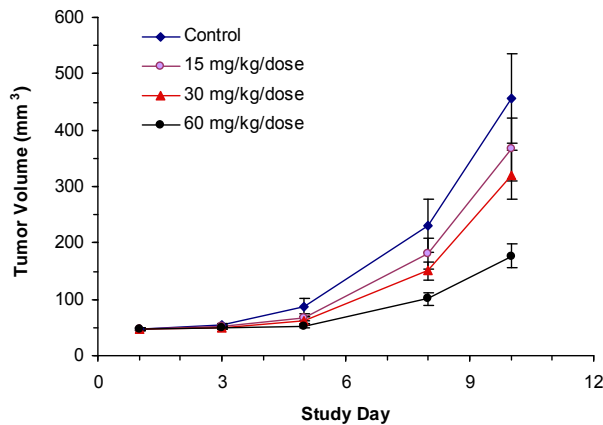


Figure 10. Tumor Volume Change of B16F10 Melanoma Tumors in Mice Administered PCI-27483

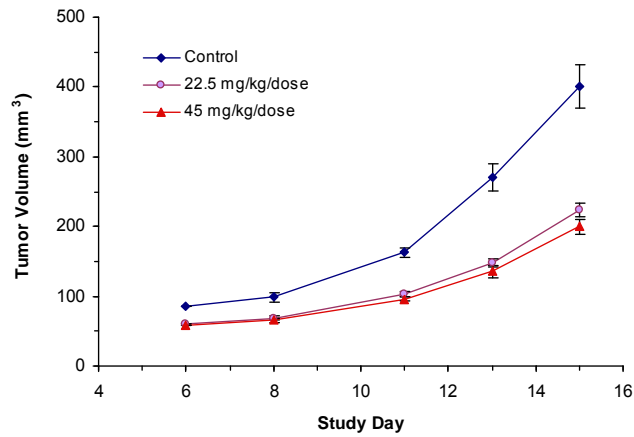


Figure 11. Effect of PCI-27483 on Growth of Lewis Lung Carcinoma Tumors in C57BL/6 Mice

- Xenograft:
 - Positive response in HCT-116 human colorectal tumor model
 - Positive response in BxPC3 human pancreatic tumor model as single agent and greatly enhances tumor inhibition effect of gemcitabine (see Figure)
 - ED100 = 75 to 90 mg/kg/dose (bid); ↑ PT = 2.5X to 3X

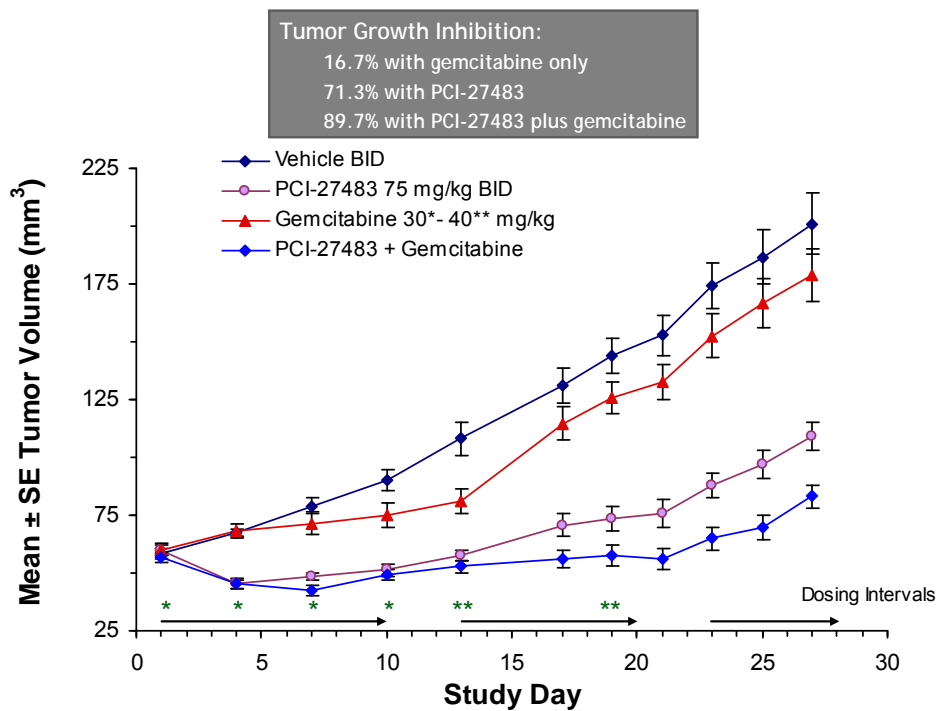


Figure 12. Inhibition of Tumor Growth by PCI-27483 in BxPC3 Pancreatic Carcinoma Xenograft Model with and without Gemcitabine

III. Preclinical PK/PD and Safety

- In vitro, PCI-27483 is a 16-fold more potent inhibitor of FVIIa in human plasma than in mouse plasma, accounting for the relatively high dose levels required to achieve prothrombin time and anti-tumor effects in mice.
- Treatment-related PT increases in monkeys significantly reversed 24 hours post-dosing
- NOAEL in rats: 30 mg/kg/day
- NOAEL in monkeys: 3 mg/kg/day
- Principal toxicologic effects associated with exaggerated pharmacodynamic response
- Safety data support doses up to 3.0 mg/kg/day in pancreatic cancer patients

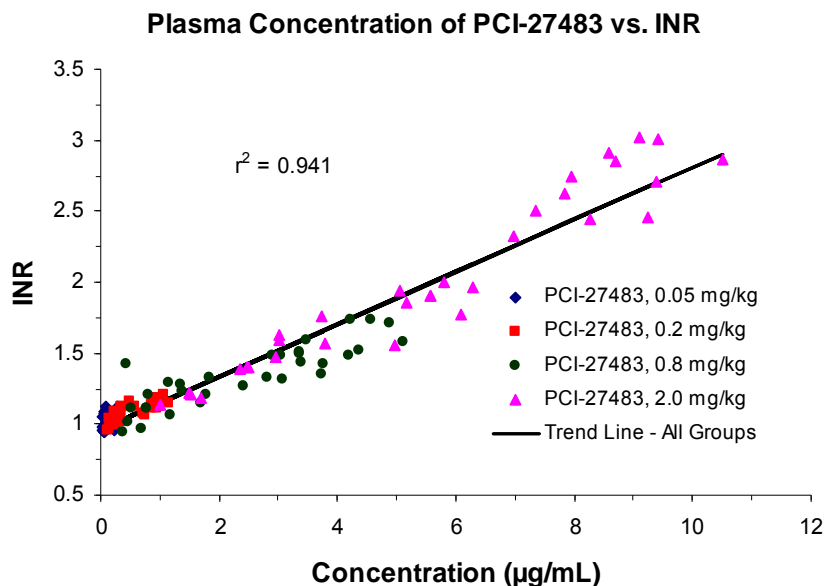
IV. Formulation

- Solution formulation for subcutaneous injection – 120 mg/mL
- Excellent stability

V. Clinical Studies

Healthy Volunteer Study

The primary objective of this ascending dose Phase I study was to assess the pharmacodynamic and pharmacokinetic profiles of PCI-27483 following a single, subcutaneous injection. In addition, the safety and tolerability of PCI-27483 was evaluated. The International Normalized Ratio (INR) of prothrombin time was used to measure pharmacodynamic effect at dose levels of 0.05, 0.20, 0.80 and 2.0 mg/kg. A mean peak INR of 2.7 was achieved without adverse effects at the highest dose level administered. There were no clinically significant changes in hematology and serum chemistry parameters. INR responses correlated with plasma concentrations of PCI-27483 (Figure). The half-life of PCI-27483 was 10 to 12 hours, which compares favorably to the single-dose half-life of the low



molecular weight heparins Lovenox (4.5 hours) and Fragmin (3 to 5 hours)

Figure 13. Correlation between Plasma Concentrations of PCI-27483 and INR Response in Healthy Volunteers



Phase II Study In Pancreatic Cancer Patients

This study will evaluate PCI-27483 for its potential to prevent tumor progression, increase overall survival, and improve quality of life in pancreatic patients. The starting dose for this study will be one expected to double prothrombin time at steady state. In the initial phase of the study, ascending repeated doses of PCI-27483 will be evaluated. In a subsequent phase of the study, patients will be randomized into groups to receive gemcitabine or gemcitabine plus PCI-27483. Twice daily dosing with PCI-27483 will proceed for up to 12 weeks. Additional endpoints will include INR response, level of circulating tissue factor, and frequency of venous thrombotic complications. We plan to initiate this Phase II trial in Q4'2009.

VI. Marketing

For 2008, the estimated new cases and deaths from pancreatic cancer in the United States¹⁹ are 37,680 and 34,290, respectively (worldwide estimates ~250,000 patients). Patients diagnosed with pancreatic cancer have a 5-year survival rate of less than 5%, making pancreatic cancer one of the most deadly forms of cancer. Currently, gemcitabine (Gemzar®) is approved as a single-agent for the 1st-line treatment of locally advanced or metastatic pancreatic cancer. The anti-metabolite, 5-FU or capecitabine (Xeloda®) is widely used for palliative therapy of pancreatic cancer, typically, prior to gemcitabine therapy or in combination with gemcitabine. Erlotinib (Tarceva®) is approved for use in combination with gemcitabine for treatment of locally advanced, unresectable, or metastatic pancreatic cancer.

Worldwide, adenocarcinoma of the stomach is the fourth most common cancer, with approximately 900,000 new cases diagnosed each year.²⁰ Tissue factor was found to be overexpressed expressed in 42% of intestinal-type gastric cancers²¹ In the United States and most of the Western world, the 5-year survival rate ranges from 5 to 15%. Chemotherapy is often used as an adjunct to surgical resection of stomach cancer. Standard chemotherapy for gastric cancer is 5-FU coupled with leucovorin.

Venous thrombosis (i.e., blood clot) is the second leading cause of death in patients with cancer.²² The number of people with pancreatic cancer who develop a venous thrombosis can range from 17% to 57%.¹³ This is higher than any other type of cancer.

The anticoagulation market is extremely large. The profilactic venous thromboembolism market alone (which includes annual diagnosed events of deep vein thrombosis and pulmonary embolisms) is approximately 1.4 million events in the G8 major markets.

VII. Patents

PCI-27483 (as a compound, in pharmaceutical compositions and in uses for treating a variety of diseases) is covered by US patent applications (issued and pending) and PCT National Phase patent applications in 14 other jurisdictions, including Europe, Canada, Japan, China, India, South Korea, Australia and Brazil. The projected expiration of this coverage is through Dec 2023 (without including patent term extensions in the various territories).

VIII. Partnering

Pharmacyclics seeks a global or regional partner to develop and commercialize PCI-27483, first-in-class, small molecule therapeutic for the treatment of cancer and related thromboses. We believe that Pharmacyclics is competitively positioned for this Factor VIIa inhibitor.

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