Drugs of the Future 2008, 33(1): 21-26

CCC: 0377-8282/2008
DOI: 10.1358/dof.2008.033.01.1165464

PI-88

Heparanase Inhibitor
Antiangiogenic Agent
Oncolytic

Highly sulfonated oligosaccharide (phosphomannan) isolated from *Pichia holstii* NRRL Y-2448, obtained as a randomly, but reproducibly, sulfated mixture with an average sulfation of three sulfates per mannose residue

CAS: 185077-23-0

EN: 247949

Abstract

PI-88 (phosphomannopentaose sulfate) is a mixture of sulfated oligosaccharides prepared by hydrolysis of the extracellular phosphomannan produced from the yeast *Pichia holstii*. It is the only heparanase inhibitor to date that has undergone clinical trials, both as a single agent and in combination with chemotherapy. The development of PI-88 has been especially exciting as it shows the most promise in two fields of oncology, melanoma and hepatocellular carcinoma, where treatment options are very limited indeed. It has been granted orphan drug status for the treatment of advanced melanoma and as adjuvant treatment following hepatocellular carcinoma resection. Toxicities seen to date include injection-site discomfort, thrombocytopenia, which may be immune-related, and thrombosis. PI-88 on the whole is well tolerated. Different schedules of s.c. PI-88 are currently under investigation, with good patient compliance as the drug is selfadministered at home. PI-88 has also been investigated in patients with non-small cell lung cancer (NSCLC), prostate cancer and multiple myeloma. Analogues of PI-88 are being developed which may confer improved efficacy and pharmacokinetic profiles compared to the parent drug.

Preparation

PI-88 is prepared by extensive sulfation of oligosaccharide phosphate fractions obtained from acid-catalyzed hydrolysis of the extracellular phosphomannan produced by of *Pichia holstii* NRRL Y-2448 (1). The composition and the structures of the different PI-88 fractions have been determined by capillary electrophoresis, gel chromatography and nuclear magnetic resonance (2-4). PI-88 consists of at least 7 components and their generalized structures are shown in Figure 1. The major components are the penta- and tetrasaccharide phosphates, which constitute 90% of the drug. Individual components of PI-88 have also been artificially synthesized (5). Analogues of PI-88 have been developed with introduction of specific lipophilic hydrophobic/aromatic group(s) at the reducing end of the oligosaccharide chain. Preclinical testing of single oligosaccharides of PI-88 analogues has shown that these may confer an improved pharmacokinetic profile and efficacy, measured as endothelial cell proliferation and endothelial tube formation (6).

Background

Heparan sulfate proteoglycan is a major component of the basement membrane and the extracellular matrix. It consists of a protein core with multiple complex glycosaminoglycan heparain sulfate (HS) side-chains. Heparanase is an endo-β-D-glucuronidase that cleaves these HS side-chains, thereby physically disrupting the basement membrane and assisting tumor cell invasion (7). Growth factors stored in the extracellular matrix, including vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGFs), are also released, which promotes angiogenesis and therefore tumor growth and metastasis. Furthermore, HS side-chains regulate functions of proteins with clusters of positively charged amino acids by binding to them to alter their activities and concentrations.

Heparanase is the predominant HS-degrading enzyme found in human cancer tissues. Increased heparanase activity correlates with poor prognosis in patients with pancreatic and cervical cancer (8, 9). Various compounds have been developed targeting heparanase as a strategy for the treatment of cancer, including small-molecule inhibitors (10), sugar inhibitors (PI-88), protein inhibitors (11) and neutralizing antibodies (12).

In addition to its antitumor effects, PI-88 has a wide pharmacodynamic spectrum, with activity against infections and benign pathophysiological processes. PI-88 is active against herpes simplex virus type 1 (HSV-1) (13,

Edwina N. Scott*, Anne L. Thomas. Department of Cancer Studies and Molecular Medicine, University of Leicester, Osborne Building, Leicester Royal Infirmary, Leicester LE1 5WW, U.K. *Correspondence: es92@le.ac.uk

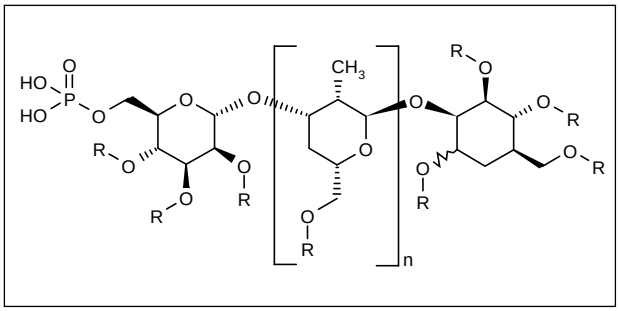

Fig. 1. Structure of the oligophosphosulfomannan components of PI-88. R = SO_3^-, Na^+ or H; n = 0-4.

14), *Plasmodium falciparum* (15), dengue and encephalitic flaviviruses (16). PI-88 also reduced proteinuria in passive Heymann nephritis (17) and restenosis postangioplasty (18) in animal models. This review, however, will concentrate on the development of PI-88 as an anticancer agent.

Preclinical Pharmacology

PI-88 has multiple mechanisms of action. It inhibited heparanase and increased cell-surface HS levels in pancreatic cancer cells *in vitro* (19). PI-88 also prevented HS side-chains from binding to angiogenic growth factors, especially VEGF in a rodent model of pancreatic cancer (20) and FGF-1, as demonstrated by surface plasmon resonance (21). An endogenous antiangiogenic protein, tissue factor pathway inhibitor (TFPI), is released after administration of PI-88 in primates (3). TFPI is a Kunitz-type protease inhibitor that inhibits the formation of the tissue factor/factor VIIa complex in a factor Xa-dependent manner (22). The anticoagulant properties of PI-88 are mediated by heparin cofactor II by inhibition of thrombin generation (3).

Pharmacokinetics and Metabolism

The pharmacokinetic profile of PI-88 was investigated in healthy male volunteers following s.c. and i.v. administration at doses up to 160 mg (23). PI-88 plasma levels were measured as prolongation of activated partial thromboplastin time (APTT), as the latter is known to reflect drug concentration. Peak plasma concentrations (C_{max}) were reached (t_{max}) at 2 h in a dose-dependent manner. Similarly, systemic drug exposure, measured as area under the curve (AUC), also increased with dose. Bioavailability based on AUC values was 96% and the mean elimination half-life was 2.4 h after i.v. administration.

The pharmacokinetic profile of s.c. PI-88 in patients with advanced solid tumors was investigated using two different schedules and the data are summarized in Table I. The first schedule was bimonthly, with drug administration on days 1-4 and 15-19 of a 28-day cycle, while the second schedule was weekly, with 4 consecutive days of drug administration every week on a 28-day cycle (24). The pharmacokinetic profile was best described by a one-compartment model with first-order elimination and first-order absorption from an extravascular site. PI-88 plasma concentrations were linear to dose, as measured by AUC and C_{max}. Intrapatient variability was low and interpatient variability was moderate. Patient weight but not age correlated significantly with AUC, C_{max} and total body clearance (CL/F). Creatinine clearance also correlated significantly with C_{max} values. There was no drug accumulation with chronic dosing. At 24 h after PI-88 administration, APTT levels for 92% and 74%, respectively, of doses given on the bimonthly and the weekly schedules returned to normal. The weekly schedule was therefore recommended for further study, as it maintained a more sustained drug concentration over time.

Safety

The main serious clinical toxicity of PI-88 is thrombocytopenia, which may be associated with anti-heparin platelet factor 4 (PF4) complex antibodies and thrombosis. Thrombocytopenia did not occur in preclinical trials of PI-88 in monkeys and rodents (3, 20, 25). Dexamethasone administered orally at 20 mg the evening before and on the day of PI-88 injection on the bimonthly schedule prevented thrombocytopenia in 18 patients with advanced solid malignancies (26), but 17 of these patients developed elevations in serum glucose, reaching grade 3/4 in 5 courses of treatment. Dexamethasone administered at a lower dose of 10 mg orally the evening before drug administration (24) did not prevent thrombocytopenia.

A review in 2007 of 402 patients who had been administered PI-88 to date showed 17 cases (4.2%) of CTC (Common Toxicity Criteria) grade 2, 3 or 4 immune-mediated thrombocytopenia, occurring most frequently in the first 8-19 days of treatment (27). In the remaining cases with later onset of immune-mediated thrombocytopenia, 4 of 5 patients were receiving combination treatment with docetaxel.

All other toxicities of PI-88 were generally mild, with the commonest being discomfort at the administration site, but this did not influence patient compliance. PI-88 also caused gastrointestinal symptoms, fatigue, headache and hot flushes.

Clinical Studies

The clinical trials of PI-88 are summarized in Table II. The efficacy data for PI-88 have been especially encouraging in patients with advanced melanoma and as adjuvant treatment for patients following hepatocellular carcinoma resection. PI-88 was granted orphan drug status in May 2004 for the treatment of advanced melanoma and it was granted fast track status by the U.S. FDA (Food and Drug Administration) for the adjuvant treatment of hepatocellular carcinoma and received orphan drug status for this indication in Europe in September 2007.

PI-88 was initially administered i.v. as a 2-h infusion, increasing in dose and duration up to 14 days continu-

Table I: Day 1 pharmacokinetic profile of PI-88 in patients with advanced solid tumors (24) (values are mean ± SD).

PI-88 dose	AUC (μg.h/ml)	C_{max} (μg/ml)	$t_{1/2}$ (h)	Vd/F (l)	CL/F (l/h)	t_{max} (h)
80 mg (n=3)	27.8 ± 18.0	4.1 ± 3.3	3.65 ± 1.12	20.9 ± 13.2	4.41 ± 3.74	1.6 ± 0.6
106 mg (n=6)	34.0 ± 6.4	5.6 ± 2.9	3.67 ± 1.94	17.2 ± 10.0	3.22 ± 0.62	1.6 ± 0.7
140 mg (n=3)	46.0 ± 13.2	6.7 ± 4.1	3.92 ± 0.85	18.8 ± 8.6	3.20 ± 0.85	1.7 ± 0.8
190 mg (n=3)	80.4 ± 20.7	8.5 ± 1.9	5.09 ± 0.53	18.0 ± 3.7	2.46 ± 0.57	1.9 ± 0.4
250 mg (n=10)	127.2 ± 50.6	9.4 ± 4.0	7.59 ± 4.45	19.4 ± 9.4	1.7 ± 0.71	2.0 ± 1.0
315 mg (n=5)	127.0 ± 22.8	12.5 ± 3.3	4.28 ± 0.74	15.8 ± 4.6	2.55 ± 0.46	3.1 ± 1.2

SD, standard deviation; AUC, area under the concentration-time curve; C_{max}, peak plasma concentration; $t_{1/2}$, half-life; Vd/F, apparent volume of distribution; CL/F, apparent total body clearance; t_{max}, median time to reach peak plasma concentration.

ously, in a phase I study in 14 patients with advanced solid malignancies (28). Dose-limiting toxicities (DLTs) occurred at 2.28 mg/kg/day for 14 days, with grade 3 immune-mediated thrombocytopenia, as evidenced by the presence of antibodies to PF4, in 4 patients. Thrombocytopenia did not result in clinically significant complications and resolved with cessation of PI-88. Prolongation in APTT, however, was not observed and in view of the lack of pharmacodynamic effects and toxicities, PI-88 administration was changed to the s.c. route.

PI-88 administered s.c. was investigated in a phase I study on bimonthly or weekly schedules in 42 patients with advanced solid malignancies (24). The maximum tolerated dose (MTD) for both schedules was 250 mg, with DLTs of thrombocytopenia and pulmonary embolism. There were, however, no episodes of clinically significant bleeding. The development of anti-PI-88/PF4 IgG antibodies in cycle 1 occurred only in the 3 patients who experienced thrombocytopenia. Overall, toxicities were mainly mild, being CTC grades 1/2, and included thrombocytopenia (6%), echymosis at injection sites (55% and 89% of cycles with bimonthly and weekly schedules, respectively) and fatigue (13% and 39% of cycles with bimonthly and weekly schedules, respectively). VEGF and FGF were excluded as pharmacodynamic markers, as plasma and urinary levels did not correlate with PI-88 administration or efficacy. Prothrombin time, fibrinogen and D-dimers were also not related to drug levels. Although efficacy was not an endpoint of this study, 26% of patients experienced stable disease or a partial response (PR) for 6 treatment cycles. This included 6 of 17 patients with advanced melanoma, with 1 patient achieving PR and receiving study medication for > 50 months. These encouraging data led to further studies of PI-88 in patients with metastatic melanoma, both as a single agent and in combination with dacarbazine.

A phase II study of PI-88 administered at 250 mg/day s.c. for 4 consecutive days every week on a 28-day cycle was carried out in 44 patients with advanced melanoma (27). Drug-related serious adverse events (SAEs) included thrombocytopenia (9%), with antibodies to PF4 detected in 3 of these 4 patients, hemorrhagic cerebral metastases (5%), vascular thrombosis or cardiac ischemia (4%), transaminitis (2%), pancreatitis (2%), myalgia (2%) and hypoesthesia (2%). All patients recovered from their SAEs, except for 1 who died due to hemorrhagic cerebral metastasis. All AEs noted were of CTC grades 1/2 only, except for one report of hot flush. The commonest AE was injection-site reaction (64%), but there was complete patient compliance. Other AEs reported included fatigue (32%), constipation (9%), diarrhea (7%), nausea (30%), alopecia (14%), fever/hot flush (16%) and headache (14%). A PR or stable disease was achieved as best response in 16% of patients. Median time to progression and overall survival were 1.7 and 9 months, respectively. The authors concluded that the efficacy of PI-88 was similar to standard chemotherapy, but an alternative daily dosing regimen may improve its efficacy.

Daily continuous administration of PI-88 at doses of 140, 190 and 250 mg has been investigated in a phase I study in patients with metastatic melanoma (29). The MTD was established at 250 mg and a second cohort of patients was recruited for administration of daily PI-88 at 140 or 190 mg with dacarbazine at 1000 mg/m^2 every 21 days. All DLTs in both cohorts were grade 3/4 immune-mediated thrombocytopenia, with one report of cerebral venous sinus thrombosis. PI-88 did not increase dacarbazine toxicity. TFPI levels correlated with drug administration but not with PI-88 dose. PI-88 administered alone did not result in radiological efficacy, but 3 of 9 patients experienced a PR in the combination treatment group. A phase II study is now ongoing with PI-88 at 190 mg/day continuously with dacarbazine 1000 mg/m^2 ever 3 weeks *versus* dacarbazine alone in patients with metastatic melanoma (30, 31).

PI-88 administered for 4 consecutive days every week on a 28-day cycle was investigated as adjuvant treatment in patients following hepatocellular carcinoma resection (32). In this phase II study, 172 patients were randomized to one of three arms: standard of care, PI-88 160 mg/day or PI-88 250 mg/day for 9 treatment cycles and follow-up of 12 weeks. At study completion, 63% of patients in the 160-mg dose group were disease-free compared to 50% of controls and 41% of patients in the 250-mg dose group. The disease-free survival for patients in the 160-mg group was 48 weeks compared to 27 weeks in the control group, with a trend for significance (p = 0.09, HR 1.7, 30). Four SAEs were reported as possibly related to PI-88 and included intracerebral hemorrhage, gum bleeding, tumor recurrence and subsequent rupture. In the 250-mg group,

Table II: Summary of clinical trials of PI-88.

Ref.	Dose and schedule	Patients	Endpoint	Toxicities
28	0.57 mg/kg i.v. for 2 h, escalating to 2.28 mg/kg/day for 14 days	Advanced solid malignancies (n=14)	MTD and DLT; MTD was 2.28 mg/kg/day for 14 days	DLT was CTC grade 3 thrombocytopenia, but no clinical complications were observed
26	80-250 mg/day s.c. on days 1-4 and 15-19 on a 28-day cycle	Advanced solid malignancies (n=18)	Pharmacokinetics, MTD, DLT and toxicities; MTD not reached	No DLT; dexamethasone 20 mg the day before and on the day of treatment caused hyperglycemia that was grade 1/2 in 31 courses and grade 3/4 in 5 courses
36	Not specified	Multiple myeloma refractory to standard treatment	39% clinical response, defined as stable or decrease in paraprotein levels	Not specified
23	80-160 mg s.c. or 160 mg i.v. over 2 h as single doses	Healthy male volunteers (n=9)	Pharmacokinetic profile	Not specified
34	106-315 mg/day s.c. on days 1-4, 8-11 and 15-19 of a 28-day cycle with docetaxel 30 mg/m^2 on days 1, 8 and 15 of a 28-day cycle	Advanced solid malignancies (n=3)	Pharmacokinetics, MTD, DLT and toxicities	No DLT to date and recruitment ongoing; no significant drug toxicity seen
24	80-315 mg/day s.c. on days 1-4 and 15-18 of a 28-day cycle or 190-250 mg/day s.c. on days 1-4 every week on a 28-day cycle	Advanced solid malignancies (n=42)	Pharmacokinetics, MTD, DLT, pharmacodynamic markers and toxicities; MTD 250 mg/day for both schedules	DLTs of thrombocytopenia and pulmonary embolism; common mild toxicities include fatigue, bruising at injection site
27	250 mg/day s.c. on days 1-4 every week on a 28-day cycle	Advanced melanoma (n=44)	Progression-free survival, overall survival, response rate and time to progression	SAEs of thrombocytopenia, bleeding and thrombosis; common AEs include fatigue, bruising at injection site, fever and gastrointestinal events
29	140-250 mg/day s.c. continuously alone or with dacarbazine 1000 mg/m^2 every 3 weeks	Metastatic melanoma (n=19)	MTD, DLT, pharmacodynamic markers, efficacy and toxicities; MTD PI-88 190 mg/day s.c. continuously with dacarbazine 1000 mg/m^2 every 3 weeks	DLT was CTC grade 3/4 immune-mediated thrombocytopenia and thrombosis; 3 of 9 patients in combination arm had partial response
35	250 mg/day s.c. on days 1-4, 8-11 and 15-19 of a 28-day cycle with docetaxel 30 mg/m^2 on days 1, 8 and 15 of a 28-day cycle *versus* docetaxel alone	Advanced non-small cell lung cancer as second-line treatment	Progression-free survival, time to progression, overall survival, response rate and quality of life; PI-88 did not improve any of these parameters compared to docetaxel alone	SAEs related to PI-88 included thrombocytopenia and thrombosis
32	160 or 250 mg/day s.c. on days 1-4 every week on a 28-day cycle	Adjuvant treatment for hepatocellular carcinoma	Efficacy and toxicity; 160 mg improved disease-free survival, with a trend for significance	SAEs included bleeding, tumor recurrence and tumor rupture
37	Variable s.c. dose on days 1-4 every week or daily with docetaxel 75 mg/m^2 every 21 days and prednisolone 5 mg twice daily	Androgen-independent prostate cancer, recruiting	Prostate-specific antigen response rate, radiological response rate, progression-free survival, overall survival, toxicity, quality of life, pharmacodynamic markers	Study ongoing
33	160 mg/day s.c. on days 1-4 every week on a 28-day cycle	Adjuvant treatment for hepatocellular carcinoma	Primary endpoint of disease-free survival	Recruitment commenced December 2007
30, 31	190 mg/day s.c. continuously with dacarbazine 1000 mg/m^2 every 21 days	Advanced melanoma, recruiting	Efficacy	Study ongoing

DLT, dose-limiting toxicity; MTD, maximum tolerated dose; CTC, Common Toxicity Criteria; SAE, serious adverse event.

24% of patients did not complete study treatment, and although this may have affected drug efficacy assessment, it was felt that the 160-mg dose level should be carried through alone into a phase III trial. This phase III study, which started in December 2007, aims to recruit 600 patients (33).

PI-88 was investigated in combination with docetaxel in a phase I study in patients with advanced solid malignancies (34), and then in a phase II trial in NSCLC patients who had failed first-line platinum chemotherapy. Results of the lung cancer study were disappointing, with no improvement in progression-free rate, time to progression, response rate, overall survival or quality of life compared to docetaxel alone (35). PI-88 has also been investigated in patients with advanced multiple myeloma (36) and a phase II study of PI-88 in combination with docetaxel and prednisolone is ongoing in patients with androgen-independent prostate cancer (37).

Source

Progen Pharmaceuticals, Ltd. (AU).

References

1. Ferro, V., Fewings, K., Palerma, M.C., Li, C. *Large-scale preparation of the oligosaccharide phosphate fraction of Pilchia holstii NRRL Y-2448 phosphomannan for use in the manufacture of PI-88*. Carbohydr Res 2001, 332(2): 183-9.

2. Yu, G., Gunay, N.S., Linhardt, R.J. et al. *Preparation and anticoagulant activity of the phosphosulfomannan PI-88*. Eur J Med Chem 2002, 37(10): 783-91.

3. Demir, M., Iqbal, O., Hoppensteadt, D.A. et al. *Anticoagulant and antiprotease profiles of a novel natural heparinomimetic mannopentaose phosphate sulfphate (PI-88)*. Clin Appl Thromb Hemost 2001, 7(2): 131-40.

4. Ferro, V., Li, C., Fewings, K., Palermo, M., Linhardt, R., Toida, T. *Determination of the composition of the oligosaccharide phosphate fraction of Pichia (Hansenula) holstii NRRL Y-2448 phosphomannan by capillary electrophoresis and HPLC*. Carbohydr Res 2002, 337(2): 139-46.

5. Fairweather, J.K., Karoli, T., Ferro, V. *The synthesis of phosphorylated disaccharide components of the extracellular phosphomannan of Pichia (Hansenula) holstii NRRL Y-2448*. Bioorg Med Chem 2004, 12(23): 6063-75.

6. Ferro, V., Dredge, K., Liu, L. et al. *PI-88 and novel heparin sulfphate mimetics inhibit angiogenesis*. Semin Thromb Hemost 2007, 33(5): 557-68.

7. McKenzie, E.A. *Heparanase: A target for drug discovery in cancer and inflammation*. Br J Pharmacol 2007, 151(1): 1-14.

8. Quiros, R.M., Rao, G., Plate, J. et al. *Elevated serum heparanase-1 levels in patients with pancreatic carcinoma are associated with poor survival*. Cancer 2006, 106(3): 532-40.

9. Shinyo, Y., Kodama, J., Hongo, A., Yoshinouchi, M., Hiramatsu, Y. *Heparanase expression is an independent prognostic factor in patients with invasive cervical cancer*. Ann Oncol 2003, 14(10): 1505-10.

10. Pan, W., Maio, H.-Q., Xu, Y.-J. et al. *1-[4-(1H-Benzoimidazol-2-yl)-phenyl]-3-[4-(1H-benzoimidazol-2-yl)-phenyl]-urea derivatives as small molecule heparanase inhibitors*. Bioorg Med Chem Lett 2006, 16(2): 409-12.

11. Marchetti, D., Liu, S., Spohn, W.C., Carson, D.D. *Heparanase and a synthetic peptide of heparin sulfphate-interacting protein recognize common sites on cell surface and extracellular matrix heparin sulphate*. J Biol Chem 1997, 272(25): 15891-7.

12. He, X., Brenchley, P.E., Jayson, G.C., Hampson, L., Davies, J., Hampson, I.N. *Hypoxia increases heparanase-dependent tumor cell invasion, which can be inhibited by antiheparanase antibodies*. Cancer Res 2004, 64(11): 3928-33.

13. Ekblad, M., Adamiak, B., Bergefall, K. et al. *Molecular basis for resistance of herpes simplex virus type 1 mutants to the sulfphated oligosaccharide inhibitor PI-88*. Virology 2007, 367(2): 244-52.

14. Nyberg, K., Ekblad, M., Bergström, T., Freeman, C., Parish, C.R., Ferrro, V., Trybala, E. *The low molecular weight heparin sulfate-mimetic, PI-88, inhibits cell-to-cell spread of herpes simplex virus*. Antiviral Res 2004, 63(1): 15-24.

15. Adams, Y., Freeman, C., Schwartz-Albiez, R., Ferro, V., Parish, C.R., Andrews, K.T. *Inhibition of Plasmodium falciparum growth in vitro and adhesion to chondroitin-4-sulfate by the heparin sulfate mimetic PI-88 and other sulphated oligosaccharides*. Antimicrob Agents Chemother 2006, 50(8): 2850-2.

16. Lee, E., Pavy, M., Young, N., Freeman, C., Lobigs, M. *Antiviral effect of the heparin sulfphate mimetic, PI-88, against dengue and encephalitic flaviviruses*. Antiviral Res 2006, 69(1): 31-8.

17. Levidiotis, V., Freeman, C., Punler, M. et al. *A synthetic heparanase inhibitor reduces proteinuria in passive Heymann nephritis*. J Am Soc Nephrol 2004, 15(11): 2882-92.

18. Francis, D.J., Parish, C.R., McGarry, M. et al. *Blockade of vascular smooth muscle cell proliferation and intimal thickening after balloon injury by the sulfphated oligosaccharide PI-88: Phosphomannopentaose sulfphate directly binds FGF-2, blocks cellular signalling, and inhibits proliferation*. Circ Res 2003, 92(8): e70-7.

19. Xu, X., Rao, G., Quiros, R.M. et al. *In vivo and in vitro degradation of heparin sulfate (HS) proteoglycan by HPR1 in pancreatic adenocarcinoma*. J Biol Chem 2007, 282(4): 2363-73.

20. Joyce, J.A., Freeman, C., Meyer-Morse, N., Parish, C.J., Hanahan, D. *A functional heparin sulfate mimetic implicates both heparanase and heparain sulfate in tumor angiogenesis and invasion in a mouse model of multistage cancer*. Oncogene 2005, 24(25): 4037-51.

21. Cochran, S., Caiping, L., Fairweather, J.K., Kett, W.C., Coombe, D.R., Ferro, V. *Probing the interactions of phosphosulfomannans with angiogenic growth factors by surface plasmon resonance*. J Med Chem 2003, 46(21): 4601-8.

22. Tobu, M., Ma, Q., Iqbal, O., Schultz, C., Jeske, W., Hoppensteadt, D.A., Fareed, J. *Comparative tissue factor pathway inhibitor release potential of heparins*. Clin Appl Thromb Hemost 2005, 11(1): 37-47.

23. Creese, B.R., Rolan, P., Hussein, Z., Mills, R., Ribbons, R., Nguyen, T. *Pharmacokinetic studies of PI-88, a novel anti-angiogenic heparanase inhibitor*. Proc Am Soc Clin Oncol (ASCO) 2003, 22: Abst 937.

24. Basche, M., Gustafson, D.L., Holden, S.N. et al. *A phase I biological and pharmacologic study of the heparanase inhibitor PI-88 I patients with advanced solid tumors*. Clin Cancer Res 2006, 12(8): 5471-80.

25. Parish, C.R., Freeman, C., Brown, K.J., Francis, D.J., Cowden, W.B. *Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity*. Cancer Res 1999, 59(14): 3433-41.

26. Holden, S., Basche, M., O'Bryant, C. et al. *A phase I study of the heparanase inhibitor of PI-88 given subcutaneously in patients with advanced solid malignancies*. Eur J Cancer [14th EORTC-NCI-AACR Symp Mol Targets Cancer Ther (Nov 19-22, Frankfurt) 2002] 2002, 38(Suppl. 7): Abst 234.

27. Lewis, K.D., Robinson, W.A., Millward, M.J. et al. *A phase II study of the heparanase inhibitor PI-88 in patients with advanced melanoma*. Invest New Drugs 2007, Epub ahead of print.

28. Rosenthal, M.A., Rischin, D., McArthur, G. et al. *Treatment with the novel anti-angiogenic agent is associated with immune-mediated thrombocytopenia*. Ann Oncol 2002, 13(5): 770-6.

29. Millward, M., Hamilton, A., Thomson, D., Gautam, A., Wilson, E. *Final results of a phase I study of daily PI-88 as a single agent and in combination with dacarbazine in patients with metastatic melanoma*. J Clin Oncol [43rd Annu Meet Am Soc Clin Oncol (ASCO) (June 1-5, Chicago) 2007] 2007, 25(18, Suppl.): Abst 8532.

30. *Trial of PI-88 with dacarbazine in patients with metastatic melanoma (NCT00130442)*. ClinicalTrials.gov Web site, December 20, 2007.

31. *PI-88 and/or dacarbazine in treating patients with metastatic melanoma that cannot be removed by surgery (NCT00128648)*. ClinicalTrials.gov Web site, December 20, 2007.

32. *Progen's phase 2 liver cancer trial exceeds efficacy objective*. Progen Pharmaceuticals Limited Press Release (and Clinical Appendix), April 15, 2007.

33. *Phase III study of PI-88 in post-resection hepatocellular carcinoma (PATHWAY) (NCT00568308)*. ClinicalTrials.gov Web site, December 20, 2007.

34. Holden, S.N., Basche, M., Gore, L. et al. *A phase I study of the heparanase inhibitor PI-88 and weekly docetaxel in patients with advanced solid malignancies*. Proc Am Soc Clin Oncol (ASCO) 2003, 22: Abst 899.

35. *Progen announces PI-88 phase 2 lung cancer results*. Progen Pharmaceuticals Limited Press Release (and Clinical Appendix), September 24, 2007.

36. Progen Pharmaceuticals Web site [Pipeline >> Clinical Trials], Accessed December 20, 2007.

37. *Pilot efficacy study of PI-88 with docetaxel to treat prostate cancer (NCT00268593)*. ClinicalTrials.gov Web site, December 20, 2007.