PG545, a dual heparanase and angiogenesis inhibitor, induces potent anti-tumour and anti-metastatic efficacy in preclinical models

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BACKGROUND: PG545 is a heparan sulfate (HS) mimetic that inhibits tumour angiogenesis by sequestering angiogenic growth factors in the extracellular matrix (ECM), thus limiting subsequent binding to receptors. Importantly, PG545 also inhibits heparanase, the only endoglycosidase which cleaves HS chains in the ECM. The aim of the study was to assess PG545 in various solid tumour and metastasis models.

METHODS: The anti-angiogenic, anti-tumour and anti-metastatic properties of PG545 were assessed using in vivo angiogenesis, solid tumour and metastasis models. Pharmacokinetic (PK) data were also generated in tumour-bearing mice to gain an understanding of optimal dosing schedules and regimens.

RESULTS: PG545 was shown to inhibit angiogenesis in vivo and induce anti-tumour or anti-metastatic effects in murine models of breast, prostate, liver, lung, colon, head and neck cancers and melanoma. Enhanced anti-tumour activity was also noted when used in combination with sorafenib in a liver cancer model. PK data revealed that the half-life of PG545 was relatively long, with pharmacologically relevant concentrations of radiolabeled PG545 observed in liver tumours.

CONCLUSION: PG545 is a new anti-angiogenic clinical candidate for cancer therapy. The anti-metastatic property of PG545, likely due to the inhibition of heparanase, may prove to be a critical attribute as the compound enters phase I clinical trials.

Keywords: heparan sulfate; angiogenesis; heparanase; anti-tumour; anti-metastatic

Tumour angiogenesis has become a prominent drug target for cancer therapy (Carmeliet, 2005) and agents designed to target the vascular endothelial growth factor (VEGF) family and the fibroblast growth factor (FGF) family, along with their corresponding receptor tyrosine kinases, have received considerable attention (Sparano et al., 2004; Folkman, 2007). Anti-angiogenic therapies directed against these targets have resulted in promising and well-validated therapeutics, with both biologicals and small-molecule drugs that target VEGF/VEGFR already approved for a number of cancers. Nonetheless, most cancer deaths are due to the development of metastases (Eccles and Welch, 2007) and angiogenesis inhibitors do not necessarily affect this process. Indeed, recent pre-clinical studies have raised some concerns about the impact of such agents and metastatic disease (Ebos et al., 2009; Paez-Ribes et al., 2009; Steeg et al., 2009).

Cleavage of HS by the endo-β-glucuronidase heparanase (HPSE) is strongly implicated in cell dissemination associated with tumour metastasis (Barash et al., 2010). Endo-β-glucuronidase heparanase is also involved in angiogenesis, where it releases sequestered HS-binding angiogenic growth factors (GFs) from the extracellular matrix (ECM) (Nakajima et al., 1988; Vlodavsky et al., 1996; Zetter, 1998; Goldshmidt et al., 2002). It also induces VEGF expression (Zetser et al., 2006). The upregulation of HPSE in many tumours has been linked to poor prognosis (Chen et al., 2008; Mikami et al., 2008; Rivera et al., 2008; Zheng et al., 2009). The use of heparin, heparin derivatives, sulfated oligosaccharides and suramin and its analogues to inhibit HPSE activity have shown promise as anti-tumour or anti-metastatic agents (Vlodavsky et al., 1994; Marchetti et al., 2003; Basche et al., 2006; Norrby, 2006). The clinical utility of a mixture of sulfated oligosaccharides (PI-88) with dual heparanase and angiogenic inhibitory activity has been previously demonstrated in a phase II clinical trial for hepatocellular carcinoma (HCC) (Liu et al., 2009). The limited utility of current angiogenesis inhibitors and the critical need to address the metastatic component of cancer together provide a strong rationale to develop new HPSE inhibitors typified by aglycon-functionalised sulfated oligosaccharides (Karoli et al., 2005; Johnstone et al., 2010).
We report here the pharmacological profile of PG545, a synthetic, fully sulfated HS mimetic. We have previously demonstrated that PG545 is a potent heparanase inhibitor ($K_i = 6$ nM) and a potent angiogenesis inhibitor ($\leq 1\ \mu M$) using endothelial cell assays in vitro and the ex vivo rat aortic ring assay. Moreover, PG545 was found to have mild anti-coagulant properties using the activated partial thromboplastin time test and the Heptest compared with PI-88 or earlier PG500 series compounds (Dredge et al., 2010). In contrast to other HS mimetics which are mixtures, PG545 is a single molecular entity. PG545 induces potent anti-angiogenic activity in vivo together with broad acting pre-clinical anti-tumour and anti-metastatic efficacy with a pharmacokinetic (PK) profile to support less frequent dosing compared with other HS mimetics. An open-label, single centre phase I study of the safety and tolerability of PG545 in patients with advanced solid tumours commenced in late 2010 (http://www.clinicaltrials.gov).

MATERIALS AND METHODS

Compound synthesis and preparation

PG545 is a fully sulfated tetrasaccharide functionalised with a cholesteryl aglycon (Figure 1) designed at Progen Pharmaceuticals Ltd (Brisbane, QLD, Australia). PG545 and radiolabeled PG545, the latter being synthesised from $[1,2,3$-$\text{H}]$-cholesterol (GE Healthcare Australia Pty. Ltd., Rydalmere, NSW, Australia), were manufactured at Progen Pharmaceuticals Ltd and PharmaSynth (Brisbane, Australia). The tyrosine kinase inhibitor (TKI) sorafenib is an inhibitor of Raf-1 and other kinases involved in neovascularisation and tumour progression (Wilhelm et al., 2004) indicated for renal cell carcinoma and HCC. It was approved by US FDA in 2007 for patients with unresectable HCC and is the first approved systemic drug therapy for liver cancer. Sorafenib was sourced from Bayer Health Care (Leverkusen, Germany). PG545 was dissolved using cell culture medium for in vitro or ex vivo experiments and phosphate buffered saline (PBS), pH 7.2 for in vivo studies.

Cell lines and reagents

LL/2, Hep3b2.1-7 and HT-29 tumour cell lines were obtained from the American Type Culture Collection and working stocks did not exceed four passages. MDA-MB-231, PC3, Cal27 and HepG2 were from laboratory stocks at the Diamantina Institute, Brisbane, Australia. Identity was verified by STR analysis conducted by Cell Line Services Ltd (Brisbane, QLD, Australia). PG545 and radiolabeled PG545, the latter being synthesised from $[1,2,3$-$\text{H}]$-PG545 or plasma PG545 concentrations, were collected at 2, 4, 8, 24, 48, 72 and 96 h post treatment and levels of PG545 were determined using an LC–MS/MS method that was validated for assay sensitivity, run-time, carryover and specificity.

Figure 1 The structure of PG545.

Animals

Athymic (nude), SCID, FvB, BALB/c and C57Bl6 mice were obtained from the Animal Resources Centre (Perth, Australia). All animal studies were approved by the University of Queensland or the University of Adelaide Animal Ethics Committees. During the studies, the care and use of animals was conducted in accordance with the guidelines outlined by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 (National Health and Medical Research Council). For the HT-29 study, the protocol was approved by the Institutional Animal Care and Use Committee and the study was performed at the AAALAC certified animal facility in Pharma Legacy (Shanghai, China).

In vivo angiogenesis

Angiogenesis in vivo was evaluated using the AngioSponge model in which subcutaneously implanted, agarose-captured FGF2 gelfoam sterile sponges (Pfizer, West Ryde, Australia) induces vascularisation over a 10-day period responsive to antiangiogenesis inhibition as determined by the number of vessels with identifiable lumen that stained positive for CD31/PECAM-1, an antigen present specifically on endothelial cells (McCarty et al., 2002). PG545 was administered s.c. on the flank distal to the AngioSponge implant. This method was also used to examine five medium-sized primary tumours from each group of Lewis Lung Carcinoma (LL/2)-bearing mice.

Tumour models and drug administration

In the orthotopic Hep3b2.1-7 human liver cancer model, treatment of mice ($n = 10$) began 14 days after inoculation of $2.5 \times 10^6$ cells. Mice received PG545 (or $[3$-$\text{H}]$-PG545 for the efficacy/PK study) using either a once- or twice-weekly dosing schedule. To initiate xenografts, MDA-MB-231, PC3, Cal27 and HepG2 cells were injected s.c. into nude mice ($n = 8$) at 4, 5, 2 and $2 \times 10^5$ cells $\text{m}^{-1}$, respectively. PG545 was injected s.c. in a volume of $100–200\ \mu\text{l}$. Plasma PG545 concentrations, were collected at 12, 24, 48 and 96 h post treatment and levels of PG545 were determined using an LC–MS/MS method that was validated for assay sensitivity, run-time, carryover and specificity.

PK sampling and analysis

Levels of $[3$-$\text{H}]$-PG545 determined in blood and tumour samples collected at 12, 24, 48 and 96 h post treatment and levels of PG545 in plasma samples (LC–MS/MS method) collected at 2, 4, 8, 24, 48, 72 and 96 h post treatment were determined as outlined in Supplementary Methods. The PK parameters, $c_{\text{max}}$ (maximum plasma concentration), $T_{\text{max}}$ (time of maximum plasma concentration), $k_{\text{elim}}$ (terminal elimination rate constant), $t_1/2$ (half-life) and AUC (area under the concentration vs time curve) were derived from the blood $[3$-$\text{H}]$-PG545 or plasma PG545 concentration vs time data using model independent methods. Analysis of
the tumour [3H]-PG545 concentration vs time data required use of a more physiologically based PK model such that tumour concentrations were estimated from the tumour mass being perfused with blood containing [3H]-PG545 and where the blood [3H]-PG545 concentration changed over time.

**Statistics**

For multiple comparisons, a one-way analysis of variance (ANOVA) followed by a post hoc Holm-Sidak test or a Dunnett’s test was used, provided the data passed a normality test or the equal variance test. For comparison between a control and a treatment group, an unpaired t-test was used. A P-value < 0.05 was considered significant.

**RESULTS**

**PG545 inhibits angiogenesis in vivo**

In the AngioSponge model, PG545 was administered either daily or twice-weekly. Significant inhibition of CD31 staining was observed (Figure 2A). As the duration of the AngioSponge model is 10 days in total, once-weekly treatment was not examined in this model. However, the effect of once-weekly dosing with PG545 on tumour angiogenesis was determined in tumour-bearing mice (LL/2 model). Assessment of CD31-positively stained vessels with identifiable lumen in the primary (subcutaneous) tumours of mice confirmed that once-weekly treatment with PG545 significantly inhibited angiogenesis (Figure 2B).

**PK profile of radiolabeled PG545 indicates a long half-life in blood with evidence that efficacious doses penetrate solid tumours**

PG545 significantly reduced solid tumour growth in the orthotopic liver cancer model Hep3b2.1-7 under two different regimens: a twice-weekly s.c. administration schedule at 20 mg kg\(^{-1}\) (Figure 3A) or using 20 mg kg\(^{-1}\) as a loading dose followed by a twice-weekly 10 mg kg\(^{-1}\) maintenance dose (Figure 3B). For compounds with long half-lives, a loading dose is often used to quickly increase the plasma concentration of a drug to the desired steady state levels (Paw, 2006). These dosing schedules resulted in body weight loss of 9% in the 20 mg kg\(^{-1}\) twice-weekly group and 4% in the 20 mg kg\(^{-1}\) followed by twice-weekly 10 mg kg\(^{-1}\) group (data not shown). The data generated in separate experiments using 20 mg kg\(^{-1}\) twice-weekly or as a loading dose (followed by 10 mg kg\(^{-1}\) as a maintenance dose) exhibited a similar efficacy profile (Tumour Growth inhibition (TGI) values of 52% versus 55%). In combination, twice-weekly dosing with PG545 and sorafenib (30 mg kg\(^{-1}\), qd) led to a statistically significant reduction in tumour weight compared with control (TGI = 85%). Once-weekly dosing with PG545 at 20 mg kg\(^{-1}\) followed by 10 mg kg\(^{-1}\) increased the TGI to 63% compared to the twice-weekly regimen but the enhanced anti-tumour effects observed in combination with sorafenib were not improved with once-weekly dosing (data not shown).

The PK profile of PG545 in tumour-bearing animals was determined using 20 mg kg\(^{-1}\) [3H]-PG545 administered s.c. every 96 h for a total of three doses. The concentration of PG545 was assessed in both blood and tumour samples. The PK profile in blood indicated that the mean elimination half-life (\(t_\text{e}\)) of [3H]-PG545 was > 50 h for mice administered up to three s.c. bolus doses at 96 h intervals (Table 1). Visual inspection of the mean blood [3H]-PG545 concentrations vs time curve data showed that the mean blood \(C_{\text{max}}\) values were in the range 25.9 – 29.0 \(\mu\)g ml\(^{-1}\) and the corresponding mean \(T_{\text{max}}\) values were in the range 2 – 6 h (Figure 3C).

**Figure 2** PG545 inhibits angiogenesis in vivo. (A) PG545 treatment was shown to possess anti-angiogenic activity in the AngioSponge model following evaluation of CD31/PECAM-1 positive endothelial cells after inmunohistochemical staining of blood vessels. *P < 0.05 vs vehicle control without FGF-2; **P < 0.01 vs vehicle control and FGF-2. (B) Treatment with PG545 at 20 and 40 mg kg\(^{-1}\) once-weekly (s.c.) resulted in a significant (P < 0.001) decrease in CD31 endothelial staining in tumours by 30 and 22%, respectively.

Visual inspection of the mean tumour [3H]-PG545 concentration vs time curve data showed that the tumour \(C_{\text{max}}\) values were in the range 36.6 – 45.1 \(\mu\)g g\(^{-1}\) and that the corresponding mean \(T_{\text{max}}\) values in the tumour tissue were 12 h (one dose), 48 h (two doses) and > 96 h (three doses) (Figure 3D). Using a one-compartment PK model, the estimated mean rate constant for uptake of [3H]-PG545 from the bloodstream into the tumour (\(k_{\text{tu}}\)), derived from the mean blood and tumour concentration vs time data, was in the range 12.8 – 16.5 h. The corresponding estimated mean \(k_{\text{tu}}\) for return of [3H]-PG545 from the tumour into the bloodstream (\(k_{\text{bt}}\)), derived from the mean blood and tumour concentration vs time data, was in the range 15.7 – 16.3 h (Supplementary data, Supplementary Table S1). The \(C_{\text{max}}\) values in liver and kidney reached 106 and 39 \(\mu\)g g\(^{-1}\), respectively, following a single dose (data not shown). This equates to a tumour:organ ratio of 0.4 and a tumour:organ ratio of 1.3, 12 h after a single dose, suggesting good distribution into tumour tissue compared with other organs.

As a comparison, gefitinib (Iressa, Astra Zeneca, London, UK) has a tumour:organ ratio of 0.15 or 0.18 and a tumour:organ ratio of 0.35 or 0.56, 2 h or 8 h, respectively, after a single dose in LoVo tumour xenografts (McKillop et al, 2005).
PG545 inhibits tumour progression in a variety of xenograft models

Further investigation of once- or twice-weekly s.c. dosing schedules was performed in a variety of xenograft or syngeneic cancer models (Figure 4). Tumour growth inhibition in the MDA-MB-231 model (Figure 4A) was maximal at 20 mg kg\(^{-1}\) once-weekly (77%) or 20 mg kg\(^{-1}\) twice-weekly (71%) with 30 mg kg\(^{-1}\) groups inhibiting tumour growth by 69% (30 mg kg\(^{-1}\), once-weekly) or 59% (30 mg kg\(^{-1}\), twice-weekly). Body weight loss at the end of the study was 4% for 20 mg kg\(^{-1}\) once-weekly, 6% for 20 mg kg\(^{-1}\) twice-weekly, 11% for 30 mg kg\(^{-1}\) once-weekly and 10% for 30 mg kg\(^{-1}\) twice-weekly (data not shown). Employing the 20 mg kg\(^{-1}\) dose only in subsequent models, the differences noted between once- and twice-weekly schedules were not significant in the PC3 or HepG2 models. For example, in the PC3 model, once-weekly dosing led to a TGI of 74% while twice-weekly dosing resulted in a TGI of 83% (Figure 4B). Although body weight loss at 20 mg kg\(^{-1}\) once-weekly was 6%, the twice-weekly regimen was 16% (data not shown). However, in the HepG2 model (Figure 4C), the once-weekly (TGI = 55%), but not twice-weekly (TGI = 45%) schedule, significantly inhibited tumour growth to a similar extent as 20 mg kg\(^{-1}\) once-weekly treatment in the Cal27 model which produced a TGI of 57% (Figure 4D). In both models, 20 mg kg\(^{-1}\) once-weekly (and twice-weekly in the HepG2 model) led to body weight loss between 2% and 4% (data not shown). PG545 also significantly reduced primary tumour growth in the HT-29 model (Supplementary Figure S1a). The concentration of PG545 that is lethal to 50% of cells (LC\(_{50}\)) using HUV (78 μM), B16 (> 200 μM) or HepG2 cells (> 100 μM), indicated that PG545 is not cytotoxic in vitro (data not shown).

PG545 induces potent anti-metastatic effects in experimental and spontaneous models of metastasis

Twice-weekly injections of PG545 significantly inhibited the formation of metastases in the B16F0 experimental lung metastasis model, when administered either before or on the day of tumour cell inoculation (Figure 5A). When administered as a once-weekly schedule, PG545 also significantly inhibited the occurrence of spontaneously arising liver metastases (Figure 5B; Supplementary Figure S1b) and colon metastases (Figure 5C) in the HT-29 colon model. In terms of body weight loss, the nadir was 8% in the B16 model and 12% in the HT-29 model (data not shown).

The Lewis Lung Carcinoma model (LL/2) provided another model to investigate the effect of PG545 on spontaneous metastasis. PG545 was administered at weekly intervals or in some cases, as a single injection from the day of tumour inoculation. The body weight nadir during the study constituted a loss of 5% (data not shown). In addition to significant inhibition of primary tumour growth (Figure 6A), PG545 significantly reduced the number of lung metastases, with complete abrogation...
demonstrate the half-life estimation of [3H]-PG545 and PG545 by LC/MS/MS may include VEGF and FGFs and which PG545 was one, as potent inhibitors of heparanase, GFs following phenotypic resistance to VEGFR2 blockade (Casanovas et al., 2010). Clinical studies have typically noted a poorer prognosis in patients with high expression of heparanase, presumably due to its increased expression of FGF family members in late-stage tumours (data not shown). The absence of bruising in PG545-treated nude mice illustrates the mild anti-coagulant properties of PG545 (Dredge et al., 2010). Although high doses of PG545 increased activated partial thromboplastin time (which was reversible) in toxicology studies, it is not clear whether this is due to a depletion of clotting factors or a mild anti-coagulant effect (data not shown).

**DISCUSSION**

Heparanase-mediated degradation of HS is involved in tumour angiogenesis and metastasis, making it a major target for cancer research (Hammond et al., 2006; McKenzie, 2007; Barash et al., 2010). Clinical studies have typically noted a poorer prognosis in patients with high expression of heparanase, presumably due to its association with an aggressive, metastatic phenotype (Chen et al., 2008; Mikami et al., 2008; Rivera et al., 2008; Zheng et al., 2009). The pivotal role of VEGF and its receptors in promoting angiogenesis is also well established, and both antibody and small-molecule inhibitors of VEGF signaling have shown clinical utility. Despite no specific FGF inhibitors being clinically available, their relevance as a cancer therapy has become increasingly clear due to the increased expression of FGF family members in late-stage tumours following phenotypic resistance to VEGFR2 blockade (Casanova et al., 2005). We previously identified a series of HS mimetics, of which PG545 was one, as potent inhibitors of heparanase, GFs including VEGF and FGFRs and ex vivo angiogenesis (Dredge et al., 2010). Herein, we extend those findings to the pre-clinical arena to demonstrate the in vivo potency of PG545 in angiogenesis, solid tumour and metastasis models and provide initial data on the pharmacokinetics associated with the compound.

The AngioSponge model highlighted that daily and twice-weekly dosing with PG545 induced a potent anti-angiogenic effect in vivo, as measured by CD31 expression. In the LL/2 model, decreases in CD31 expression were statistically significant using once-weekly dosing. Unfortunately, as only five representative medium-sized tumours from each group were available, any dose-dependent correlation between reduction in CD31 staining and inhibition of solid tumour growth is not apparent. Overall, the data confirmed that a once- or twice-weekly dosing schedule is anti-angiogenic in vivo and this schedule is less frequent compared with those of other heparin-derived products or HS mimetics (Gandhi and Mancera, 2010). The body weight loss in mice may be species-specific because PG545 is not cytotoxic in vitro or in vivo (vacuolation was most common histopathology finding) and did not lead to significant body weight loss in other species during toxicity studies (data not shown). The ability of PG545 to induce biological change within the tumour for removal of a metabolite to range m from 0.2 to 0.4, 0.6 to 1.0, and 2 to 4 times the value of the drug was observed. The PK model found each of the estimated parameter values was internally consistent following administration of one, two or three doses (Supplementary data; Supplementary Table S1). However, visual inspection of the graph for tumour-bearing mice administered three doses of [3H]-PG545, with each dose separated by a 96-h interval, showed that the tumour concentration vs time curve for the third dose is different from that for the preceding two doses. The reason for this is unclear though it is possible that PG545 could have induced biological change within the tumour in the single dose 40 mg kg⁻¹ group and 96% inhibition in the 20 mg kg⁻¹ once-weekly group (Figure 6B). However, it is not clear why 40 mg kg⁻¹ once-weekly failed to reach significance but interestingly sorafenib demonstrated no effect on metastasis in this model. Plasma concentration vs time curves indicated that efficacious exposure levels should be between 20 and 70 μg ml⁻¹ (Figure 6C) and PK analysis revealed the apparent half-life estimation of [3H]-PG545 and PG545 by LC/MS/MS may include VEGF and FGFs and which PG545 was one, as potent inhibitors of heparanase, GFs following phenotypic resistance to VEGFR2 blockade (Casanovas et al., 2010). Clinical studies have typically noted a poorer prognosis in patients with high expression of heparanase, presumably due to its increased expression of FGF family members in late-stage tumours (data not shown). The absence of bruising in PG545-treated nude mice illustrates the mild anti-coagulant properties of PG545 (Dredge et al., 2010). Although high doses of PG545 increased activated partial thromboplastin time (which was reversible) in toxicology studies, it is not clear whether this is due to a depletion of clotting factors or a mild anti-coagulant effect (data not shown).
vascularity – such an assumption would not necessarily be applicable to the one-compartment PK model.

Tumour growth in the Hep3b2.1-7 model was significantly reduced following the once- and twice-weekly dosing schedule for PG545 or using a loading and maintenance dose approach, to an extent similar to that seen following treatment with sorafenib, a TKI approved for HCC. The combination of PG545 and sorafenib produced a TGI up to 85%, thus illustrating some potential utility for combination modalities using PG545. Taken together with the PK profile, the efficacy data indicated that once-weekly dosing may provide an optimal dosing frequency for PG545. This was further confirmed using a variety of subcutaneously implanted xenograft models.
or syngeneic models of prostate, breast, liver and head and neck cancer in the mouse.

We next tested the anti-metastatic activity of PG545 in three murine models of metastasis. In the B16 melanoma model, short-term therapy with PG545 was initiated either before or immediately after i.v. tumour inoculation of B16 cells and confirmed that both schedules significantly inhibited the development of lung metastases. In the spontaneously metastasising HT-29 colon model, complete abrogation of liver metastases was achieved using a weekly regimen of 20 mg kg\(^{-1}\) PG545 over 8 weeks (a dose in week 6 was omitted due to body weight concerns). In the spontaneously metastasising Lewis Lung Carcinoma model, PG545 significantly reduced the number of metastases and PK data estimated the efficacious plasma concentration range starts at ~20 mg l\(^{-1}\). This potent anti-metastatic effect of PG545 may differentiate it from other angiogenesis inhibitors, which have been associated with an acceleration of metastasis under certain circumstances (Ebos et al., 2009; Paez-Ribes et al., 2009). It is hypothesised that a metastatic ‘conditioning’ effect may occur via multiple mechanisms following treatment with angiogenesis inhibitors (Ebos et al., 2009; Paez-Ribes et al., 2009) which posed the question – are there any alternative anti-angiogenic agents that might not evoke this malignant behaviour? (Loges et al., 2009).

Thus, further elucidation of the contrasting mechanisms of action and possible differences in efficacy between HS mimetics and TKI/biologics is warranted.

In conclusion, we have clearly demonstrated the anti-tumour and anti-metastatic properties of PG545, which to our knowledge is the first HS mimic that is a single molecular entity to emerge as a clinical candidate. In addition to its anti-angiogenic properties, it is the anti-heparanase/anti-metastatic activity that may differentiate PG545 from other anti-angiogenic agents. Particularly in light of recent preclinical findings relating primarily to anti-VEGF therapies, this property may become a critical attribute as PG545 progresses towards the clinic.

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REFERENCES

Barash U, Cohen-Kaplan V, Dowek I, Sanderson RD, Ian N, Vlodavsky I (2010) Proteoglycans in health and disease: new concepts for heparanase function in tumor progression and metastasis. FEBS J 277(19): 3890 – 3903

Basche M, Gustafson DL, Holden SW, O’Bryant CL, Gore L, Witta S, Schultz MK, Morrow M, Levin A, Creese BR, Kangas M, Roberts K, Nguyen T, Davis K, Addison RS, Moore JC, Eckhardt SG (2006) A phase I biological and pharmacologic study of the heparanase inhibitor PI-88 in patients with advanced solid tumors. Clin Cancer Res 12: 5471 – 5480

Carmellit P (2005) Angiogenesis in life, disease and medicine. Nature 438: 932 – 936

Casanovas O, Hicklin DJ, Bergers G, Hanahan D (2005) Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell 8: 299 – 309

Chen G, Dang YW, Luo DZ, Feng ZB, Tang XL (2008) Expression of heparanase in hepatocellular carcinoma has prognostic significance: a tissue microarray study. Oncol Res 17: 183 – 189

Dredge K, Hammond E, Davis K, Li CP, Liu L, Johnstone K, Handley P, Wimmer N, Gonda TJ, Gumat A, Ferro V, Bytheway I (2010) The PG500 series: novel heparan sulfates mimetics as potent angiogenesis and heparanase inhibitors for cancer therapy. Invest New Drugs 28: 276 – 283

Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS (2009) Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 15: 232 – 239

Eccles SA, Welch DR (2007) Metastasis: recent discoveries and novel treatment strategies. Lancer 369(9574): 1742 – 1757

Folkman J (2007) Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov 6: 273 – 286

Gandhi NS, Mancera RL (2010) Heparin/heparan sulphate-based drugs. Drug Discov Today 15(23–24): 1058 – 1069

Goldshmidt O, Zcharia E, Abramovitch R, Metzger S, Aingorn H, Friedmann Y, Schirmacher V, Mistreni E, Vlodavsky I (2002) Cell surface expression and secretion of heparanase markedly promote tumor angiogenesis and metastasis. Proc Natl Acad Sci USA 99: 10031 – 10036

Hammond E, Bytheway I, Ferro V (2006) Heparanase as a target for anticancer therapeutics: new developments and future prospects. In New Developments in Therapeutic Glycomics, Delehedde M (ed), pp 251 – 282. Research Signpost: Kerala

Johnstone KD, Karoli T, Liu L, Dredge K, Copeman E, Li CP, Davis K, Hammond E, Bytheway I, Kostewicz E, Chiu FCK, Shackleford DM, Charman SA, Charman WN, Harenberg J, Gonda TJ, Ferro V (2010) Synthesis and biological evaluation of polysulfated oligosaccharide glycosides as inhibitors of angiogenesis and tumor growth. J Med Chem 53(4): 1686 – 1699

McEllip D, Partridge EA, Kemp JV, Spence MP, Kendrew J, Barnett S, Wood PG, Giles PB, Patterson AB, Bichat F, Guibaud N, Stephens TC (2005) Tumor penetration of gefitinib (Iressa), an epidermal growth factor receptor tyrosine kinase inhibitor. Mol Cancer Ther 4(9): 641 – 649

Karoli T, Liu L, Fairweather JK, Hammond E, Li CP, Cochran S, Bergefall K, Trybala E, Addisson RS, Ferro V (2005) Synthesis, biological activity, and preliminary pharmacokinetic evaluation of analogues of a phosphosulfomannan angiogenesis inhibitor (PI-88). J Med Chem 48: 8229 – 8236

Liu CJ, Lee PH, Lin DY, Wu CC, Jeng LB, Lin PW, Mok KT, Lee WC, Yeh HZ, Ho MC, Yang SS, Lee CG, Yu MC, Hu RH, Peng CY, Lai KL, Chang SS, Chen PJ (2009) Heparanase inhibitor PI-88 as adjuvant therapy for hepatocellular carcinoma after curative resection: a randomized phase II trial for safety and optimal dosage. J Hepatol 50: 958 – 968

Table 2  Model independent pharmacokinetic analysis of PG545 in the plasma of tumour-bearing mice (Lewis Lung Carcinoma)
Loges S, Mazzone M, Hohensinner P, Carmeliet P (2009) Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited. Cancer Cell 15(3): 167 – 170
Marchetti D, Reiland J, Erwin B, Roy M (2003) Inhibition of heparanase activity and heparanase-induced angiogenesis by suramin analogues. Int J Cancer 104: 167 – 174
McCartney MF, Baker CH, Bucana CD, Fidler JJ (2002) Quantitative and qualitative in vivo angiogenesis assay. Int J Oncol 21: 5 – 10
McKenzie EA (2007) Heparanase: a target for drug discovery in cancer and inflammation. Br J Pharmacol 151: 1 – 14
Mikami S, Oya M, Shimoda M, Mizuno R, Ishida M, Kosaka T, Mukai M, Nakajima M, Okada Y (2008) Expression of heparanase in renal cell carcinomas: implications for tumor invasion and prognosis. Clin Cancer Res 14: 6055 – 6061
Nakajima M, Irimura T, Nicolson GL (1988) Heparanases and tumor metastasis. J Cell Biochem 36: 157 – 167
Norrby K (2006) Low-molecular-weight heparins and angiogenesis. APMIS 114: 79 – 102
Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O (2009) Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 15: 220 – 231
Paw HGW (2006) Loading dose. In Handbook of Drugs in Intensive Care: An A-Z Guide, pp 190. 3rd Ed. HGW Paw and G Park (editors). Cambridge University Press, Cambridge, UK
Rivera RS, Nagatsuha H, Sitar GH, Gunduz M, Tsujiigawa H, Han PP, Katase N, Tamamura R, Ng KH, Naomi Y, Nakajima M, Nagai N (2008) Heparanase and vascular endothelial growth factor expression in the progression of oral mucosal melanoma. Oncol Rep 19: 657 – 661
Sparano JA, Gray R, Giontonio B, O’Dwyer P, Comis RL (2004) Evaluating antiangiogenesis agents in the clinic: the Eastern Cooperative Oncology Group Portfolio of Clinical Trials. Clin Cancer Res 10: 1206 – 1211
Steege PS, Anderson RL, Bar-Eli M, Chambers AF, Eccles SA, Hunter K, Itoh K, Kang Y, Matrisian LM, Sleeman JP, Theodorescu D, Thompson EW, Welch DR (2009) Preclinical drug development must consider the impact on metastasis. Clin Cancer Res 15: 4529
Vlodavsky I, Miao HQ, Modalon B, Danagher P, Ron D (1996) Involvement of heparan sulfate and related molecules in sequestration and growth promoting activity of fibroblast growth factor. Cancer Metastasis Rev 15: 177 – 186
Vlodavsky I, Mohsen M, Lider O, Svahn CM, Ekre HP, Vigoda M, Ishai-Michaeli R, Perez T (1994) Inhibition of tumor metastasis by heparanase inhibiting species of heparin. Invasion Metastasis 14: 290 – 302
Wilhelm SM, Carter G, Tang L, Wilkie D, McNabola A, Rong H, Chen G, Zhang X, Vincent P, McHugh M, Cao Y, Shiuath J, Gawlak S, Eveleigh D, Rowlley B, Liu L, Adnane L, Lynch M, Aucilair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA (2004) BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64: 7099 – 7109
Zetter B, Bashenlo Y, Edovitsky E, Levy-Adam F, Vlodavsky I, Ilan N (2006) Heparanase induces vascular endothelial growth factor expression: correlation with p38 phosphorylation levels and Src activation. Cancer Res 66: 1455 – 1463
Zetter BR (1998) Angiogenesis and tumor metastasis. Annu Rev Med 49: 407 – 424
Zheng LD, Tong QS, Tang ST, Du ZY, Liu Y, Jiang GS, Cai JB (2009) Expression and clinical significance of heparanase in neuroblastoma. World J Pediatr 5: 206 – 210