

The Preclinical Development of PG545, a New Heparan Sulfate Mimetic with the Potential to Simultaneously Block Tumour Angiogenesis and Metastasis



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Summary

PG545 is a heparan sulfate mimetic that inhibits two key processes in tumour progression - angiogenesis and metastasis. The compound was designed to potently inhibit the activity of heparanase, the only enzyme which cleaves heparan sulfate, a process strongly implicated in cell dissemination associated with tumour metastasis. Anti-angiogenic properties were confirmed by growth factor binding, proliferation and *in vitro* and *in vivo* angiogenesis studies. PG545 significantly slowed tumour progression in multiple orthotopic and solid tumour models. Its pharmacokinetic profile in mice supported once- or twice-weekly dosing and therapeutically relevant concentrations of the drug were observed in the tumour when dosed according to the twice-weekly schedule. In combination with standard-of-care agents, PG545 enhanced their anti-tumour efficacy in a variety of models. Potent anti-metastatic activity of PG545 has been confirmed in several models: Lewis Lung Carcinoma (LLC), HT-29 and the 4T1 breast cancer model. In contrast, other angiogenesis inhibitors such as sorafenib displayed limited utility under the same conditions. Taken together, the dual inhibitory activity of PG545 on angiogenesis and metastasis highlights the potential utility of this new therapeutic approach as it enters Phase I clinical trials for advanced cancer.

Table 1. Inhibitory Effects of PG545 on Heparanase Activity, Growth Factors, Angiogenesis & Solid Tumour Models

	Result (\pm SD)
Heparanase Activity (K_i)	
Heparanase	6.1 ± 2.0 nM
BIAcore Binding Activity (K_d)	
FGF1	8 ± 4 nM
FGF2	390 ± 80 nM
FGF7	25 ± 24 nM
VEGF	29 ± 2 nM
Anti-angiogenic Activity (IC_{50})	
FGF1-induced HUVEC Prolif	1.2 ± 0.2 μ M
FGF2-induced HUVEC Prolif	0.7 ± 0.3 μ M
VEGF-induced HUVEC Prolif	0.5 ± 0.3 μ M
FGF2-induced dHMVEC Prolif	0.7 ± 0.1 μ M
VEGF-induced dHMVEC Prolif	0.8 ± 0.1 μ M
HUVEC Tube Formation	1.1 ± 0.4 μ M
dHMVEC Tube Formation	2.0 ± 0.5 μ M
Angiogenesis - Rat Aortic Assay	1.1 ± 0.2 μ M
Angiogenesis - AngioSponge (% \downarrow)	78%
Angiogenesis - In LLC Tumour (% \downarrow)	31%
Cytotoxicity (LC_{50})	
HUVEC Cytotoxicity	78 ± 32 μ M
B16 Cytotoxicity	>200 μ M
HepG2 Cytotoxicity	>100 μ M
<i>In vivo</i> Anti-Tumour Activity (%TGI)*	
MDA-MB231 (breast)	77%
4T1 (breast)	67%
LLC (lung)	72%
PC3 (prostate)	83%
HepG2 (liver)	55%
Hep 3B (liver)	55%
Cal27 (head and neck)	57%
A2780 (ovarian)	48%
HT-29 (colon, intrasplenic)	72%
HT-29 (colon, subcutaneous)	65%

Pharmacokinetic Data using ³H-PG545

C_{max} in Blood	29 μ g/mL
C_{max} in Tumour	36 μ g/g
C_{max} in Tissue	39-106 μ g/mL

*Results expressed as % tumour growth inhibition (%TGI) following 1x or 2x weekly PG545 treatment between 20-40 mg/kg

Conclusion

PG545 induces potent anti-angiogenic activity *in vivo* together with broad acting preclinical anti-tumour and anti-metastatic efficacy with a pharmacokinetic profile to support less frequent dosing compared with other HS mimetics. Phase I study of the safety and tolerability of PG545 in patients with advanced solid tumours commenced in late 2010 (www.clinicaltrials.gov).

Anti-Metastatic Activity in Lewis Lung Carcinoma

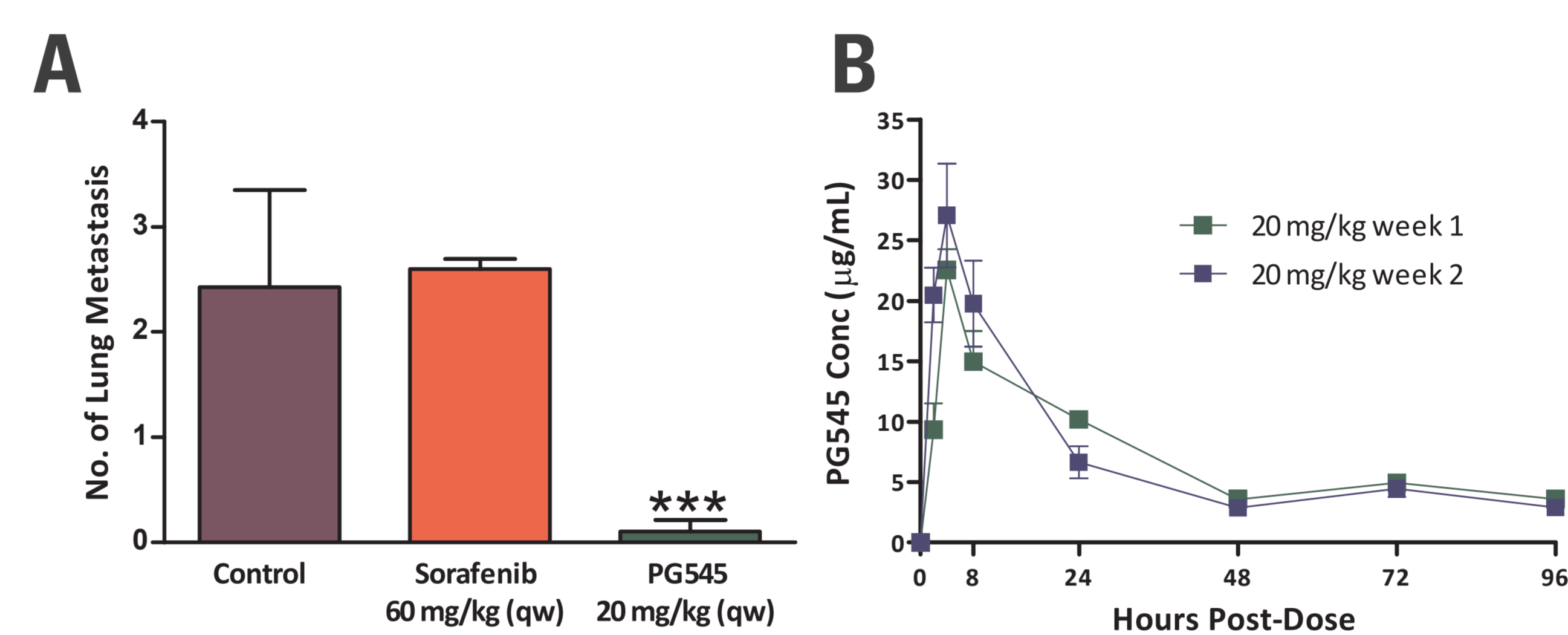


Figure 1

PG545 inhibits spontaneous metastasis from a subcutaneous site to the lung in the Lewis Lung Carcinoma model (LLC). A, PG545 dosed at 20 mg/kg once weekly significantly inhibited the incidence of lung metastases. Treatment with the tyrosine kinase inhibitor sorafenib failed to inhibit metastasis in this model. Data are presented as mean number of lung metastases with standard errors. ***= $P < 0.001$ versus vehicle control (one-way ANOVA followed by Holm-Sidak test or Dunnett's test). B, PG545 plasma concentration versus time curves following a once-weekly dose of PG545 in week 1 and week 2.

Anti-Metastatic Activity in HT-29 Colon Carcinoma

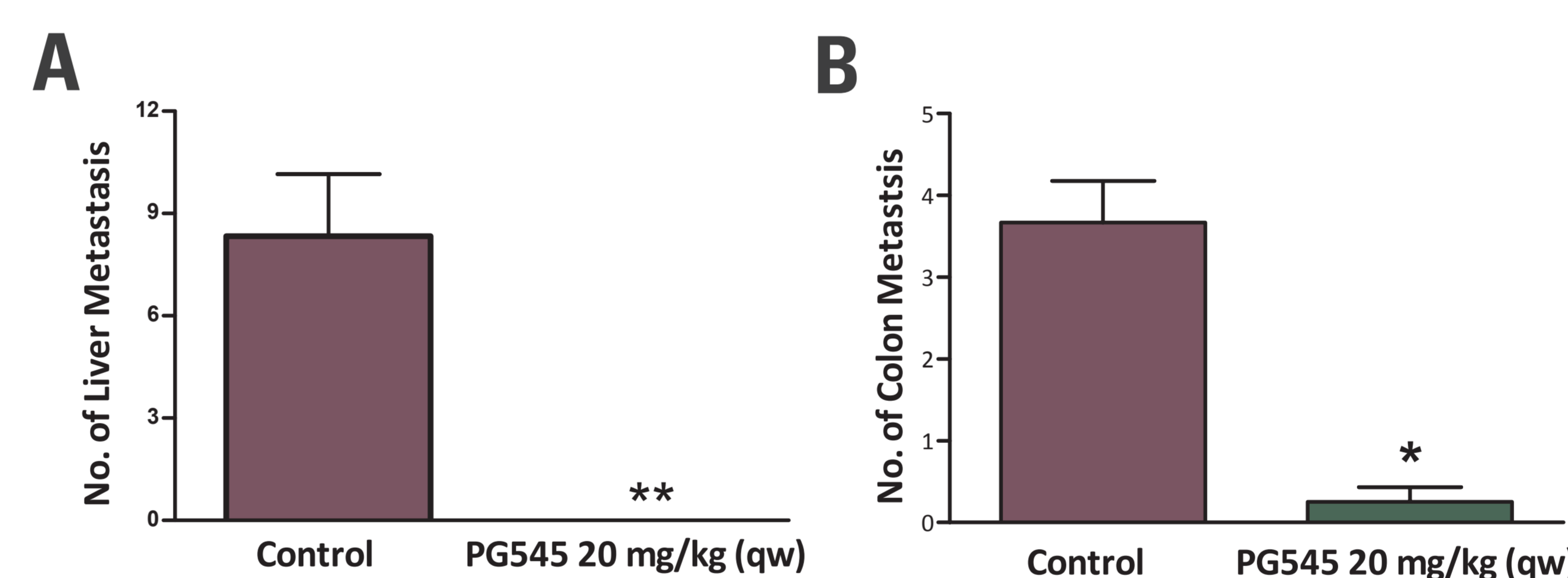


Figure 2

PG545 significantly inhibits metastases in the spontaneous metastasis model of HT-29 (colon). A, PG545 treatment at 20 mg/kg once weekly over 8 weeks completely prevented metastasis to the liver in the HT-29 model. B, PG545 treatment once weekly over 8 weeks significantly reduced metastasis to the colon in the HT-29 model. Data are presented as mean number of liver or colon metastases with standard errors. *= $P < 0.05$, **= $P < 0.01$ versus vehicle control (t-test).

Anti-Metastatic Activity in 4T1 Breast Carcinoma

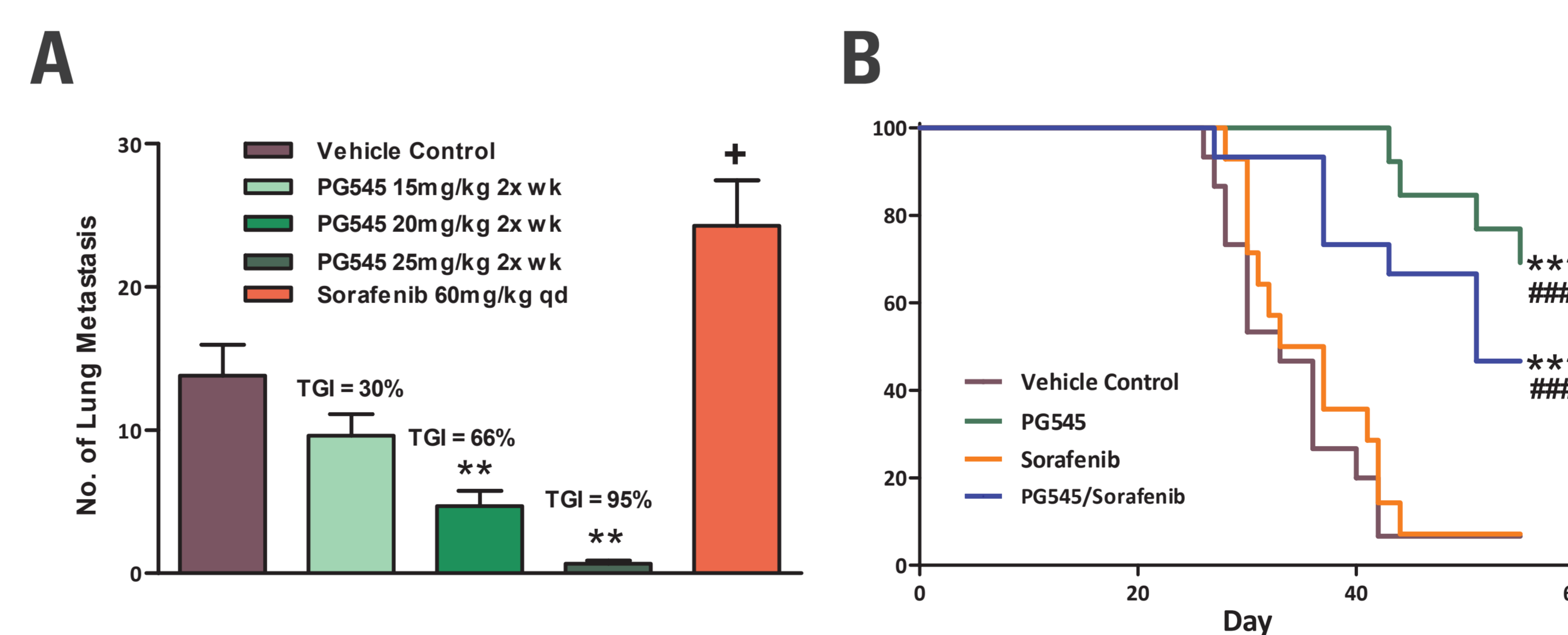


Figure 3

PG545 significantly inhibits metastasis which correlates with overall survival in the 4T1 breast carcinoma model. A, PG545 significantly inhibits primary tumour growth (See Table 1 for %TGI) and spontaneous metastasis to the lung in the 4T1 breast carcinoma model. **= $P < 0.01$ versus control following a non-parametric Dunn's Multiple Comparison Test. B, In a survival setting in which the primary tumour is resected, PG545 significantly enhances overall survival compared to control and compared with an approved angiogenesis inhibitor sorafenib. ***= $P < 0.001$ versus vehicle control, ###= $P < 0.001$ versus sorafenib. Statistics were performed using Fisher's Exact test, Log Rank Analysis and a Cox Stratified model.

Combination of PG545 with Approved Anti-Cancer Drugs

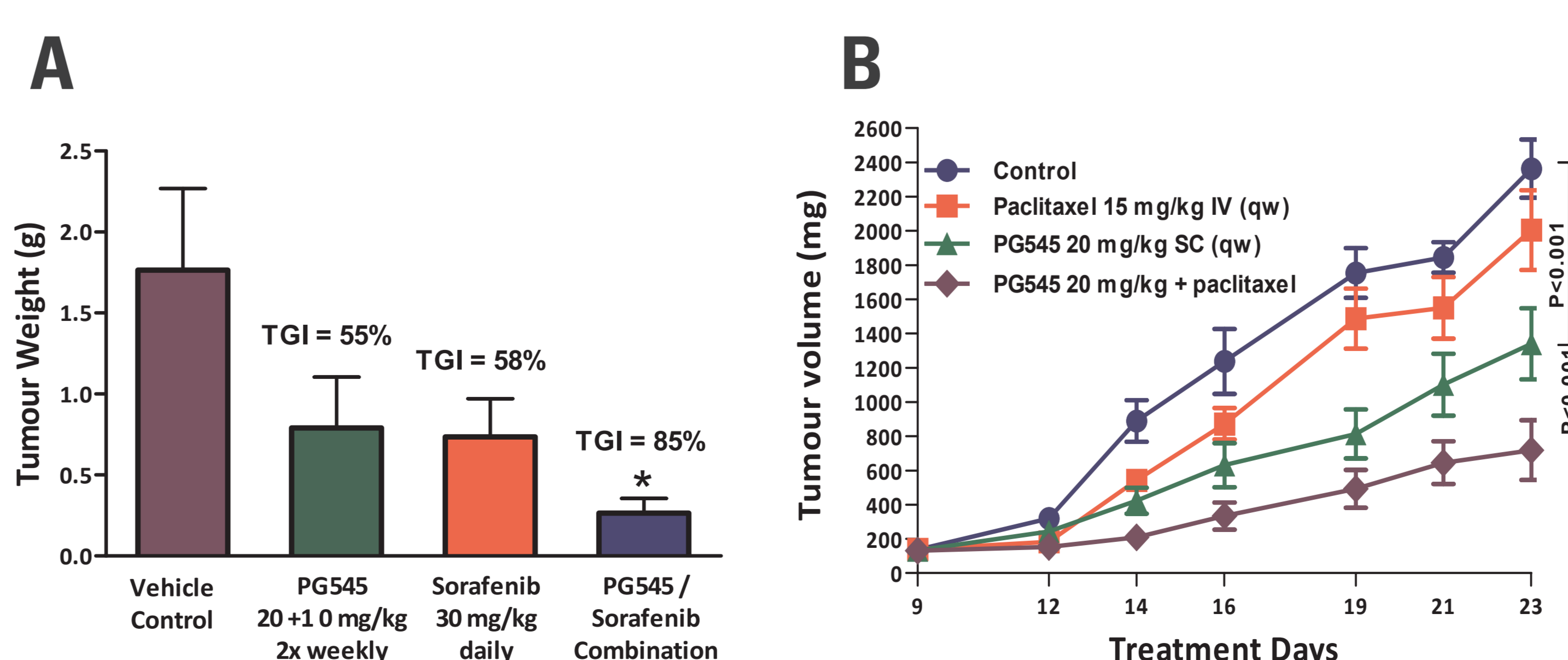


Figure 4

The anti-tumour activity of PG545 can be enhanced by addition of anti-cancer agents. A, efficacy of PG545 alone (20, then 10 mg/kg, 2xqw) and in combination with sorafenib (60 mg/kg, qd) in the Hep3b liver carcinoma model. B, efficacy of PG545 alone (20 mg/kg, qw) and in combination with paclitaxel (15 mg/kg, qw) in A2780 ovarian carcinoma model. *= $P < 0.05$ versus vehicle control (ANOVA followed by Holm-Sidak method).