

# The Dual Angiogenesis/Heparanase Inhibitor PG545 Suppresses Metastasis in Models of Melanoma, Breast and Lung Cancer - An Advantage Over Tyrosine Kinase Inhibitors?



Web – [www.progen-pharma.com](http://www.progen-pharma.com)  
 Contact – [keithd@progen-pharma.com](mailto:keithd@progen-pharma.com)  
 (Keith Dredge, Director of Preclinical Development)  
 PO Box 2403 Toowong Queensland 4066 Australia  
 T + 61 7 3842 3340

Keith Dredge<sup>1</sup>, Edward Hammond<sup>1</sup>, Paul Handley<sup>1</sup>, Thomas J Gonda<sup>2</sup>, Ian Bytheway<sup>1</sup>. <sup>1</sup>Progen Pharmaceuticals Ltd, Brisbane, Australia; <sup>2</sup>Diamantina Institute for Cancer, Immunology and Molecular Medicine, University of Queensland, Brisbane, QLD, Australia.

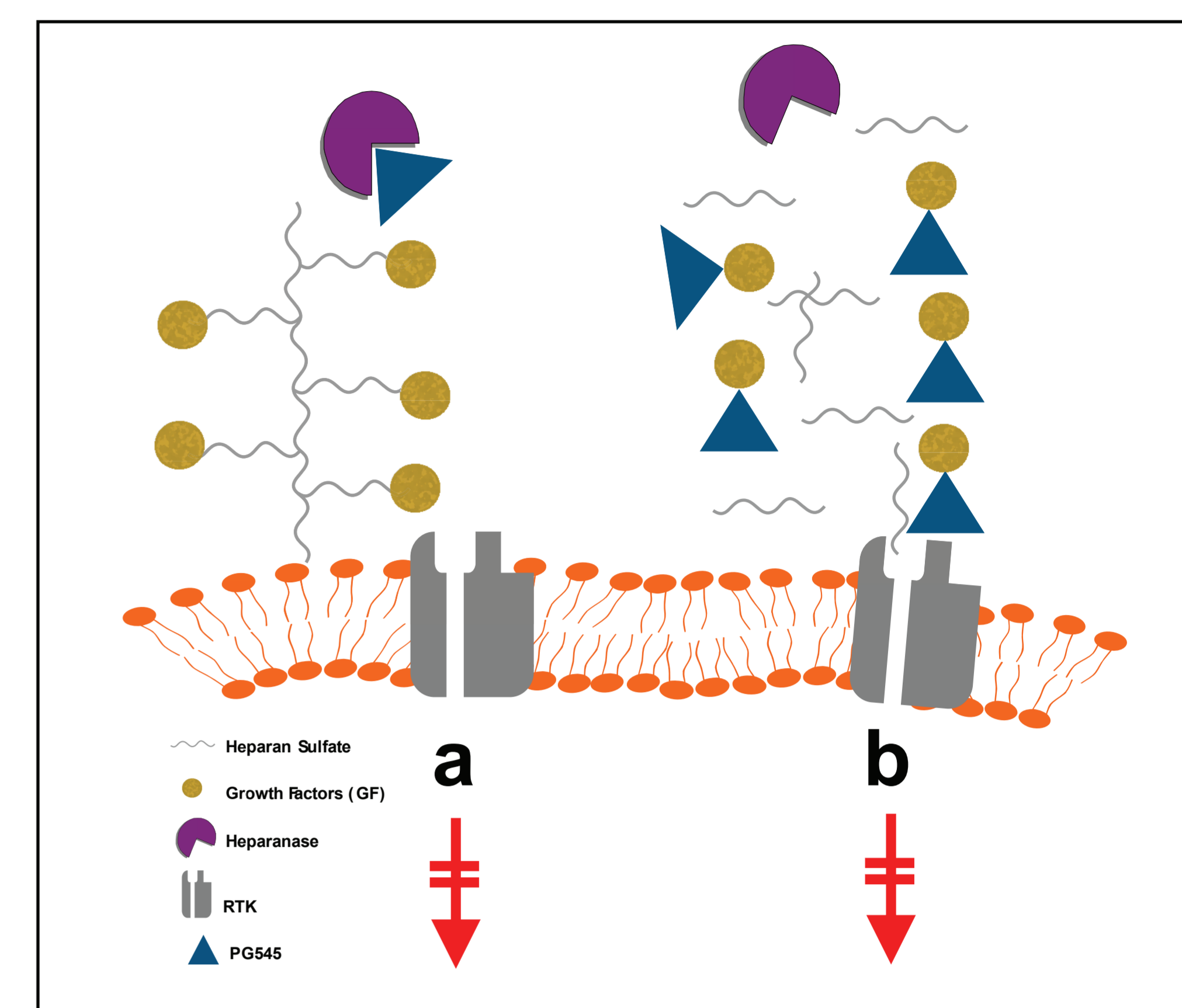
## Introduction

**PG545 is a dual angiogenesis and heparanase inhibitor that interferes with critical processes in cancer:**

- Heparanase Upregulation**  
PG545 potently inhibits the enzymatic activity of heparanase which in turn prevents cleavage of heparan sulfate (HS) chains and growth factor (GF) release [1].
- Local and Metastatic Spread**  
Heparanase is involved in the breakdown of extracellular matrices, a critical process involved in the metastatic process.
- Tumour Vascularisation**  
For many heparin-binding proteins such as FGF and VEGF, the cognate interaction between GF (ligand), HS chain and receptor (RTK) is critical to ensure efficient signaling through the bound receptor ultimately leading to endothelial cell signaling and angiogenesis. PG545 acts as a HS mimetic by complexing with the heparin-binding domain of certain GF (PG545 has a greater affinity to GF in comparison to HS) preventing the formation of the HS-GF-RTK ternary complex. Thus PG545 can limit the binding of exogenous GF (produced, for example, by tumour cells) to its respective RTK.

### Significance of the Tumour Microenvironment

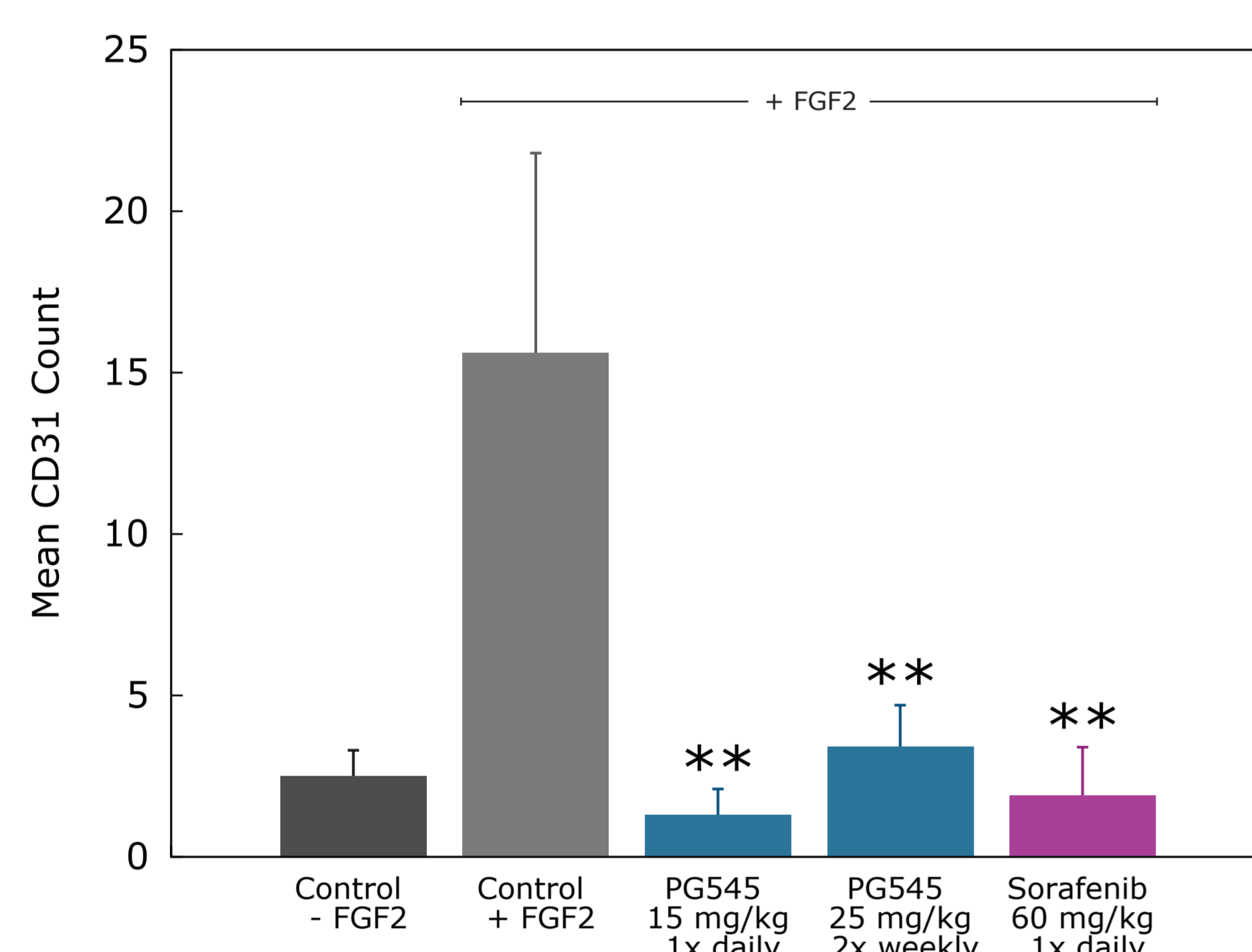
The preference of PG545 to bind to heparanase or GF is probably dictated by the local environment. The concentration of heparanase or the accessibility to GFs are likely to influence the relative binding to PG545 to each molecule. Therefore, PG545 inhibits tumour development via two independent, but not mutually exclusive, mechanisms which may impact on both tumour angiogenesis and metastasis (Figure 1).



**Figure 1**  
Schematic view of PG545 interacting with (a) heparanase to prevent cleavage and release of HS (b) GF to prevent receptor binding. Either process blocks intracellular signaling in tumour-associated endothelial cells.

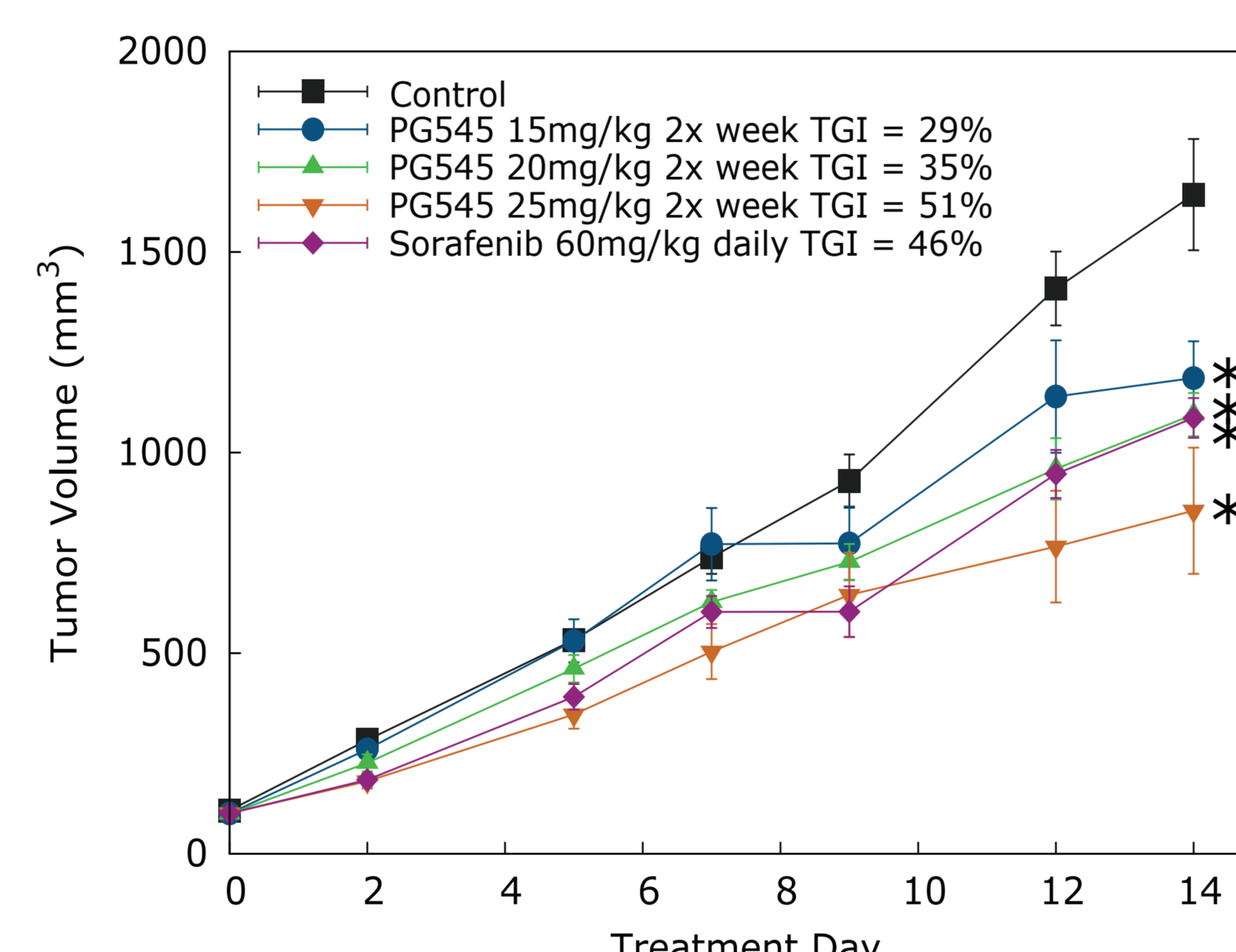
## PG545 Inhibits Angiogenesis *In vivo* and Solid Tumour Growth

### PG545 Inhibits Angiogenesis *in vivo*



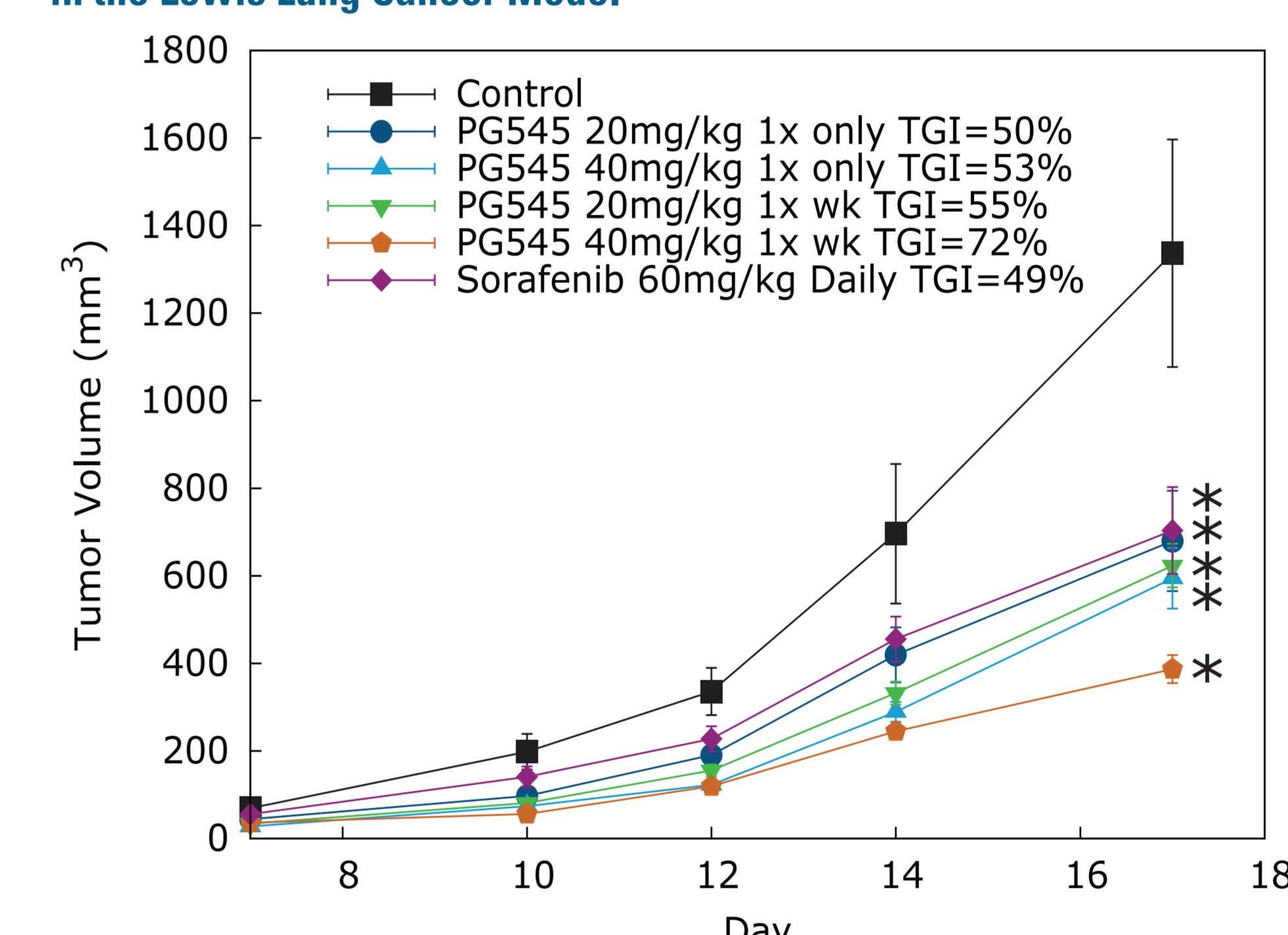
**Figure 2**  
PG545 inhibited angiogenesis in the AngioSponge™ model following both daily and twice-weekly doses similar to sorafenib. FvB mice (n = 10) received a subcutaneously implanted AngioSponge™ loaded with FGF-2 (for induction control) or unloaded (as control). Treatments were administered over a ten day period and efficacy was evaluated by counting CD31/PECAM-1 positive endothelial cells after immunohistochemical staining of blood vessels. \*\*= P<0.01 versus vehicle control & FGF-2 (one-way ANOVA followed by Holm-Sidak multiple comparison test).

### PG545 Shows Efficacy with Twice-Weekly Dosing in the Orthotopic 4T1 Breast Cancer Model



**Figure 3**  
PG545 dosed twice weekly was as efficacious as sorafenib dosed daily in the 4T1 breast cancer model. Balb/c mice (n=10) were injected orthotopically into the mammary fat pads with 5 x 10<sup>4</sup> 4T1 cells in 10 μL HBSS. The treatment of mice began 7 days after inoculation of the 4T1 cells when the average tumour volume was 102 mm<sup>3</sup>. \* = P<0.05 versus vehicle control (Kruskal-Wallis followed by Dunn's multiple comparison test).

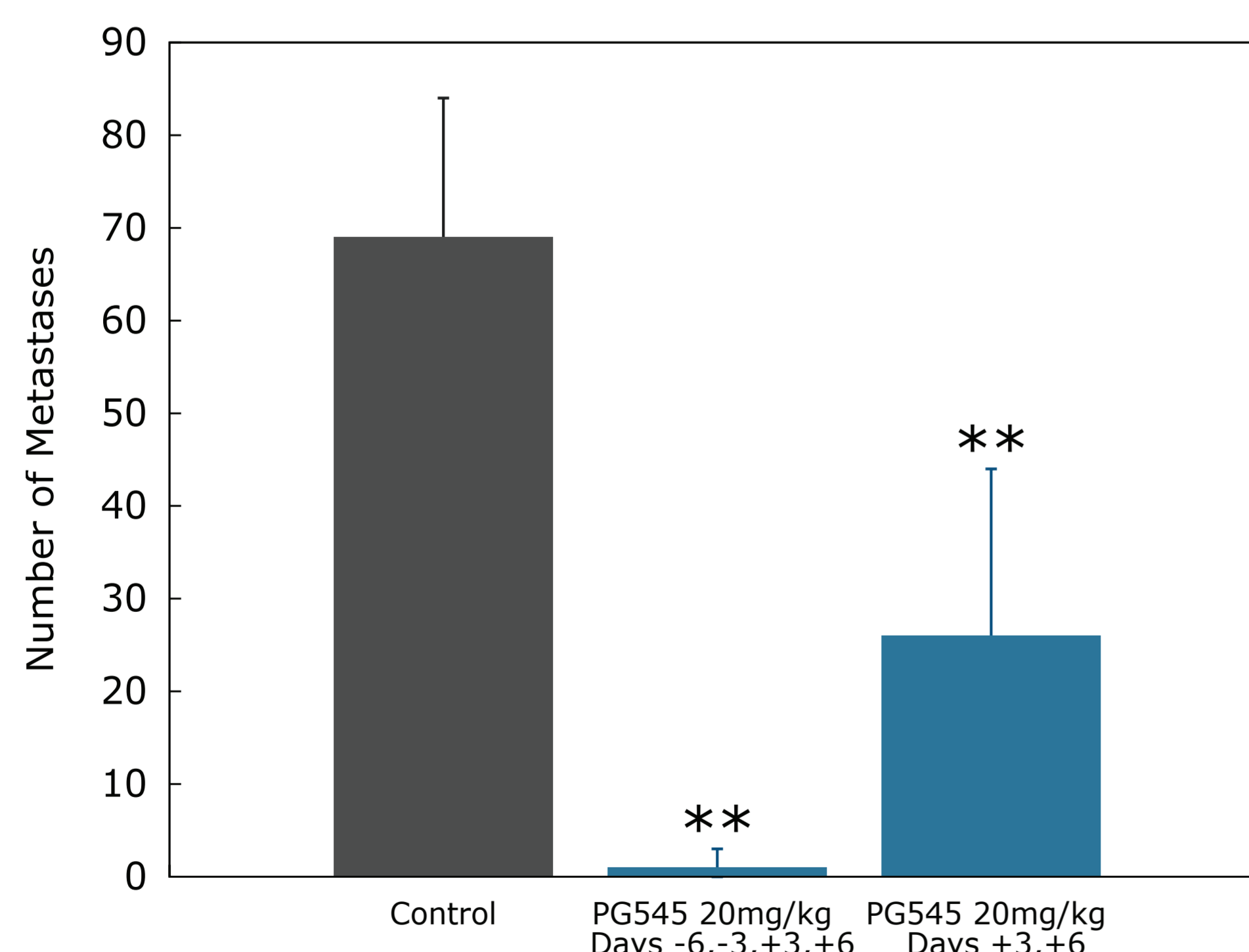
### PG545 Shows Efficacy with Single Treatment and Once-weekly Dosing in the Lewis Lung Cancer Model



**Figure 4**  
PG545 dosed once-only and once-weekly was as efficacious as sorafenib dosed daily in the Lewis Lung (LL2) carcinoma model. C57 mice (n=10) were injected subcutaneously with 2 x 10<sup>6</sup> LL2 cells in 10 μL HBSS. The treatment of mice began at day 0. All treatment groups were significant (P<0.05) versus vehicle control (one-way ANOVA followed by Holm-Sidak multiple comparisons test).

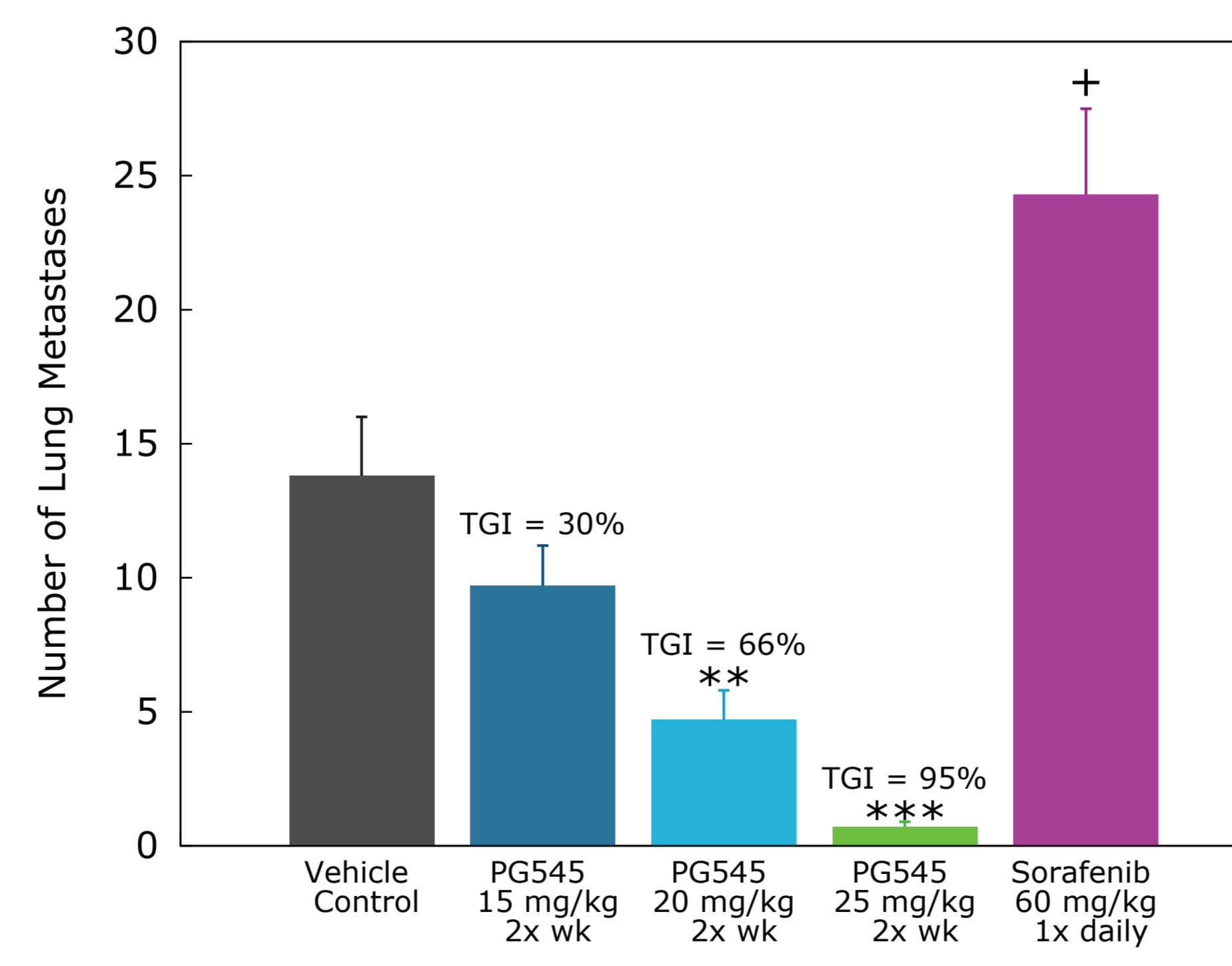
## PG545 Inhibits Experimental and Spontaneous Metastasis

### PG545 Inhibits Lung Metastasis in an Experimental B16 Melanoma Model



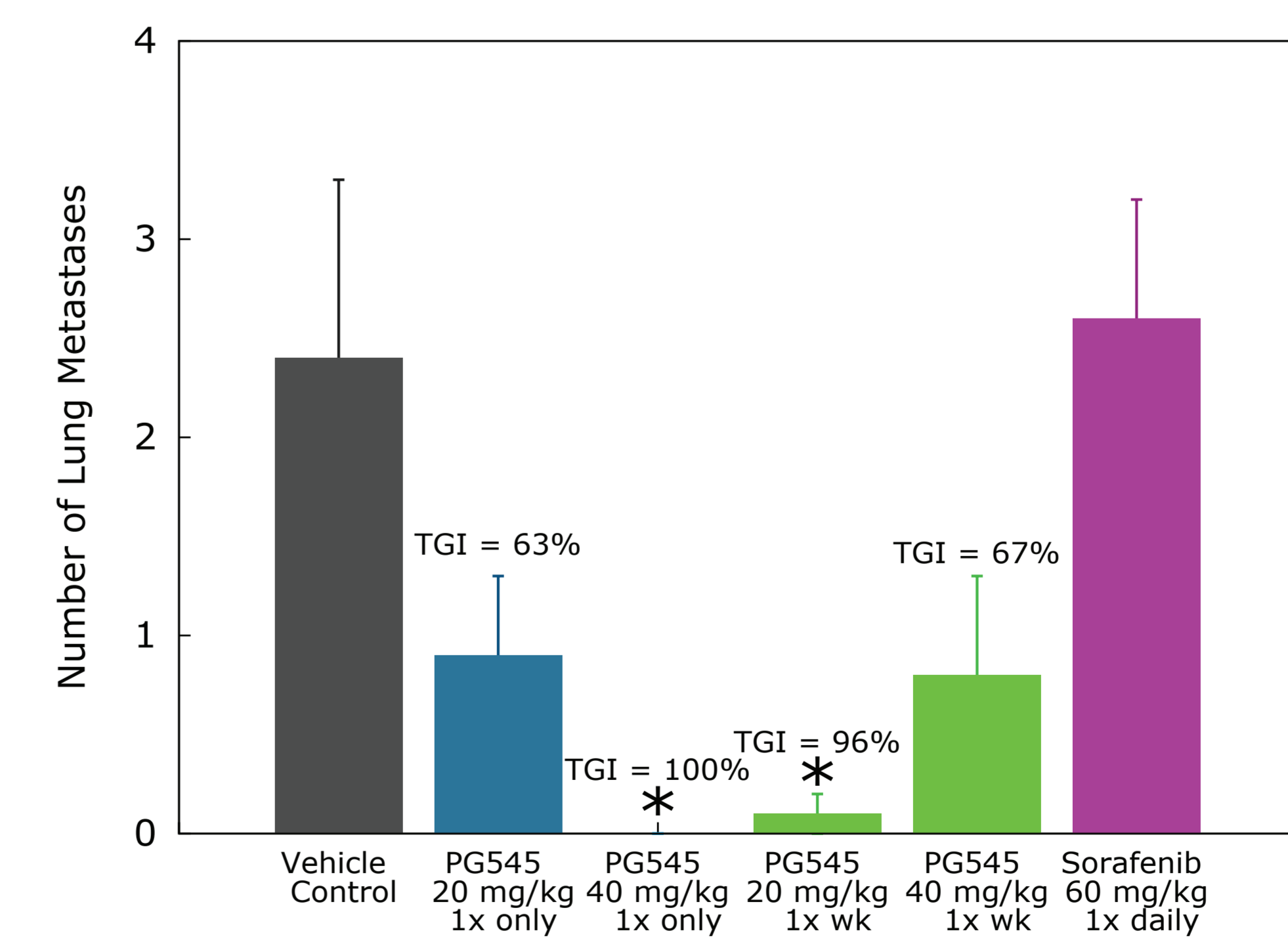
**Figure 5**  
PG545 was effective in inhibiting metastasis in the B16 melanoma metastasis model when administered twice-weekly before and/or after tumour cell inoculation. C57 mice (n=8) were injected intravenously via tail vein with 2 x 10<sup>6</sup> B16 cells on day 0. Treatment started either before injection of tumour cells (day -6,-3,+3,+6) or after tumour challenge (day +3,+6). Lungs were evaluated for metastatic nodules on day 12. \*\* = P<0.01 versus vehicle control (one-way ANOVA followed by Dunnett's post hoc test).

### PG545, but not Sorafenib, Inhibits Spontaneous Lung Metastasis in the 4T1 Orthotopic Breast Cancer Model



**Figure 6**  
PG545 dosed twice-weekly inhibited the spontaneous formation of metastasis in the 4T1 orthotopic breast cancer model while sorafenib promoted metastases. Balb/c mice (n=10) injected orthotopically into the mammary fat pads with 4T1 cells were assessed for spontaneous lung metastases at the end of the study. \*\* = P<0.01 and \*\*\* = P<0.001 versus vehicle control (one-way ANOVA followed by Holm-Sidak multiple comparisons test). + = P<0.05 versus vehicle control (one-way ANOVA followed by Holm-Sidak multiple comparisons test).

### PG545, but not Sorafenib, Inhibits Spontaneous Lung Metastasis in the Lewis Lung Cancer Model



**Figure 7**  
When dosed once-only or once-weekly PG545 inhibited the spontaneous formation of metastasis in the Lewis Lung (LL2) model while sorafenib did not. C57 mice (n=10) were injected subcutaneously with 2 x 10<sup>6</sup> LL2 cells in 10 μL HBSS. The treatment of mice began at day 0. Lungs were evaluated for metastases on day 20. \* = P<0.05 versus vehicle control (one-way ANOVA followed by Holm-Sidak multiple comparisons test).

## Conclusion

These *in vivo* data demonstrate that PG545 is an effective angiogenesis inhibitor with anti-metastatic activity. Bodyweight gain was not affected in the 4T1 and LL/2 models but some loss (8%) was noted in the AngioSponge™ and B16 models. These findings bode well for the continued development of PG545 with the results described here showing that this compound inhibits metastatic development, in contrast to recent findings for other angiogenesis inhibitors [2-3].

### Acknowledgements

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### References

1. Dredge *et al. Invest. New Drugs* 2010; In Press. 2. Pàez-Ribes *et al. Cancer Cell* 2009; 15: 220-23. 3. Ebos *et al. Cancer Cell* 2009; 15: 232-239.