Abstract#: A18

The Dual Angiogenesis/Heparanase Inhibitor PG545, but not the Tyrosine Kinase Inhibitor Sorafenib, Inhibits Spontaneous Metastasis in Models of Breast and Lung Cancer

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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.

- Tumor 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 tumor cells) to its respective RTK.

Significance of the Tumor Microenvironment

The preference of PG545 to bind to heparanase or GF (particularly FGF-2) is probably dictated by the local environment. The concentration of heparanase or the accessibility to GF are likely to influence the relative binding to PG545 to each molecule. Therefore, PG545 inhibits tumor development via two independent, but not mutually exclusive, mechanisms which may impact on both tumor angiogenesis and metastasis *(Figure 1)*.

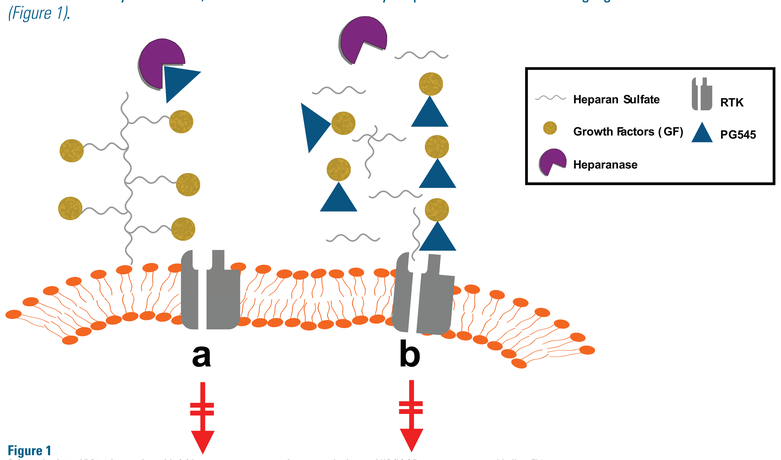

Figure 1
Schematic view of PG545 interacting with (a) heparanase to prevent cleavage and release of HS (b) GF to prevent receptor binding. Either process blocks intracellular signaling in tumor-associated endothelial cells.

Summary of Results

***In vivo* Angiogenesis Assay**
- PG545 (daily or twice-weekly) and sorafenib (daily) significantly reduced CD31 staining in the AngioSponge™ model *(Figure 2)*.

B16 Melanoma Metastasis Model
- PG545 inhibited metastasis when administered twice-weekly before and/or after tumor cell inoculation *(Figure 3)*.

T41 Breast Cancer Model
- Twice-weekly treatment with 25 mg/kg PG545, but not daily treatment with 60 mg/kg sorafenib, significantly inhibited solid tumor growth in the T41 model *(Figure 4a)*.
- PG545 significantly inhibited spontaneous lung metastases in a dose-dependent manner, whereas sorafenib significantly increased the number of metastases *(Figure 4b)*.

Lewis Lung Carcinoma (LL/2) Model
- Once-weekly treatment with PG545 at 20 mg/kg significantly reduced solid tumor growth to a similar extent as sorafenib while the 40 mg/kg dose appeared more efficacious than sorafenib *(Figure 5a)*.
- A significant reduction in spontaneous lung metastases was again associated with PG545 following either a single injection or once-weekly treatment while sorafenib had no appreciable effect on metastases *(Figure 5b)*.

PG545 was well tolerated with no significant loss in bodyweight observed

PG545 Inhibits Angiogenesis *in vivo*

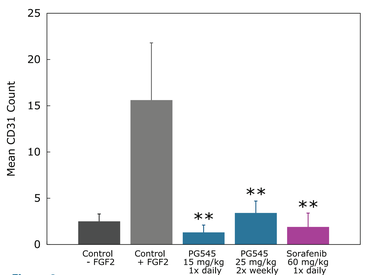

Figure 2
PG545 inhibited angiogenesis in the AngioSponge™ model at both daily and twice-weekly doses similar to sorafenib. FvB mice (n = 10) received a subcutaneously implanted AngioSponge™ loaded with FGF-2 (for induction control) or unloaded (as control). Treatments were administered over a ten day 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 Inhibits Metastasis in an Experimental B16 Melanoma Model

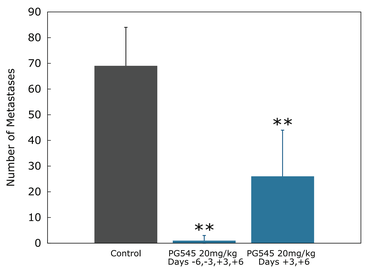

Figure 3
PG545 was effective in inhibiting metastasis in the B16 melanoma metastasis model when administered twice-weekly before and/or after tumor cell inoculation. C57 mice (n=8) were injected intravenously via tail vein with 2 x 10^5 B16 cells on day 0. Treatment started either before injection of tumor cells (day -6,-3, +3, +6) or after tumor 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 Shows Efficacy with Twice-Weekly Dosing in an Orthotopic Breast Cancer Model

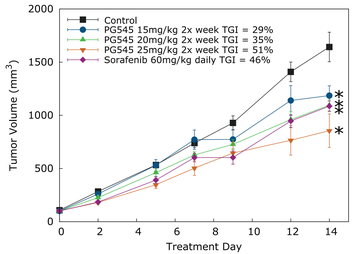

Figure 4a
PG545 dosed twice weekly was as efficacious as sorafenib dosed daily in the T41 breast cancer model. Balb/c mice (n=10) were injected orthotopically into the mammary fat pads with 5 x 10^5 T41 cells in 10 µL HBSS. The treatment of mice began 7 days after inoculation of the T41 cells when the average tumor volume was 102 mm^3. *=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 a Lung Cancer Xenograft Model

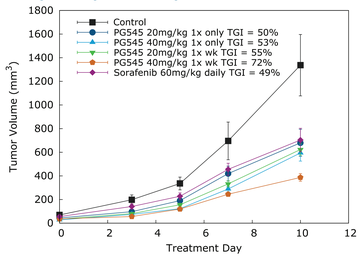

Figure 5a
PG545 dosed once-only and once-weekly was as efficacious as sorafenib dosed daily in the Lewis Lung (LL2) carcinoma model. C57 mice (n=10) were injected subcutaneously with 2 x 10^6 LL/2 cells in 10 µL HBSS. The treatment of mice began 7 days after inoculation of the LL/2 cells when the average tumor volume was 50 mm^3. All treatment groups were significant (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. These findings bode well for the continued development of PG545 with the results described here suggesting that this compound inhibits metastatic development, in contrast to recent findings for other angiogenesis inhibitors [2-3].

PG545, but not Sorafenib, Inhibits Spontaneous Metastasis in the T41 Orthotopic Breast Cancer Model

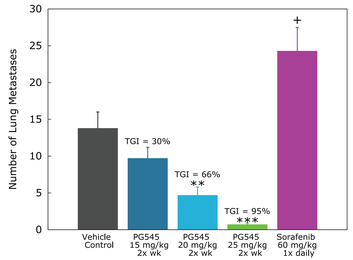

Figure 4b
PG545 dosed twice-weekly inhibited the spontaneous formation of metastasis in the T41 orthotopic breast cancer model while sorafenib promoted metastases. Balb/c mice (n=10) injected orthotopically into the mammary fat pads with T41 cells were assessed for spontaneous lung metastases at the 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 Metastasis in a Lung Cancer Xenograft Model

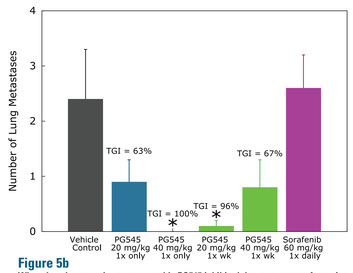

Figure 5b
When dosed once-only or once-weekly PG545 inhibited the spontaneous formation of metastasis in the Lewis Lung (LL2) model while sorafenib did not. C57 mice (n=10) were injected subcutaneously with 2 x 10^6 LL/2 cells in 10 µL HBSS. The treatment of mice began 7 days after inoculation of the LL/2 cells when the average tumor volume was 50 mm^3. Lungs were evaluated for metastases on day 20. *=P<0.05 versus vehicle control (one-way ANOVA followed by Holm-Sidak multiple comparisons test).

References

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

Acknowledgements

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