Supplementary Figures

Figure S1: Trend in regional gefitinib PK/PD in U87vIII brain tumors following a single dose of 150 mg/kg gefitinib orally.

A

B
**Figure S2:** Trend in regional gefitinib PK/PD in U87VIII brain tumors following a single dose of 50 mg/kg gefitinib orally.

A

B.
**Figure S3:** Intratumoral immunohistochemical analysis of MPI and MTI in U87vIII brain tumors following single doses of 50 mg/kg gefitinib orally.

A

![Graph A: Microvessel pericyte index (MPI)](image)

B

![Graph B: Microvessel Transporter (Pgp) index (MTI)](image)

C

![Image C](image)

D

![Image D](image)

E

![Image E](image)
Figure S4: Intratumoral PD modeling of gefitinib in athymic mice bearing intracerebral U87vIII tumors following either 50 mg/kg or 150 mg/kg single oral doses.

A

B
E

Predicted_Medium MPI_High dose
△ Observed_Medium MPI_High dose

F

Predicted_High MPI_High dose
△ Observed_High MPI_High dose
**Figure S5**: Effect of gefitinib on pERK and pAKT protein levels in U87vIII glioma cells as a function of drug concentration.
Figure S6: Diagnostic plots (model-predicted vs observed gefitinib concentrations and model-predicted gefitinib concentrations vs residuals) for brain tumor PK model.

A.
C.

Tumor PK_High MPI

Observed gefitinib brain tumor conc (ng/g)

Predicted gefitinib brain tumor conc (ng/g)

Residual

Predicted gefitinib brain tumor conc (ng/g)
Figure S7: Diagnostic plots (model-predicted vs observed fraction of baseline pERK and model-predicted fraction of baseline pERK vs residuals) for tumor PD model.

A.
Supplementary Figure Legends

**Figure S1:** Trend in regional gefitinib PK/PD in U87vIII brain tumors following a single dose of 150 mg/kg gefitinib orally. **A.** Gefitinib brain tumor concentrations (mean ± SD, n=3) in peripheral (R1) and central (R4) regions; note 2 different bar graphs to adequately scale data, and **B.** Gefitinib PD response based on pERK (mean fraction of baseline ± SD) in the two most contrasting regions; R1=peripheral and R4=central.

**Figure S2:** Trend in regional gefitinib PK/PD in U87VIII brain tumors following a single dose of 50 mg/kg gefitinib orally. **A.** Gefitinib brain tumor concentrations (mean ± SD, n=3) in peripheral (R1) and central (R4) regions; note 2 different bar graphs to adequately scale, and **B.** Gefitinib PD response based on pERK (mean fraction of baseline ± SD) in the two most contrasting regions; R1=peripheral and R4=central.

**Figure S3:** Intratumoral immunohistochemical analysis of MPI and MTI in U87vIII brain tumors following single doses of 50 mg/kg gefitinib orally. **A.** Regional variability in MPI values (mean ± SD from each tumor region n=21) increased from tumor periphery to center (p < 0.05), **B.** Regional variability in MTI values (mean ± SD from each tumor region n=8) increased from tumor periphery to center (p < 0.05), **C-E.** Representative images of PgP transporter expression (PgP staining with mouse anti-P-glycoprotein (clone C219) antibody), MVD (CD31 staining) and overlaid image (MTI) at tumor periphery, and **F-H.** at tumor center, respectively, **I.** Relationship between regional MTI and MPI (spearman correlation coefficient r=0.6, p = 0.012.)

**Figure S4:** Intratumoral PD modeling of gefitinib in athymic mice bearing intracerebral U87vIII tumors following either 50 mg/kg or 150 mg/kg single oral doses. The model predicted (---, 50 mg/kg;—, 150 mg/kg) and observed (Mean ± SD) (●, 50 mg/kg; ▲, 150 mg/kg) fractional
inhibition of pERK are presented in A, D. Low, B, E. Medium and C, F. High MPI tumor groups.

**Figure S5**: Effect of gefitinib on pERK and pAKT protein levels in U87vIII glioma cells as a function of drug concentration. Semiquantitative measurement of phospho-ERK1/2, total ERK 1/2, phospho-Akt and total Akt protein levels were measured by western blot assays following 1 hr gefitinib exposures at various concentrations. Both markers showed a dose-dependent inhibition in phosphorylation (when normalized to their respective total proteins) yet pERK showed a greater dose-response range than pAkt. The results are presented as mean ± SD from three independent experiments and expressed as fraction of baseline protein expression in untreated cells, which is given a value of 1.

**Figure S6**: Diagnostic plots (model-predicted vs observed gefitinib concentrations and model-predicted gefitinib concentrations vs residuals) for brain tumor PK model. A. Low, B. Medium and C. High MPI groups. The correlation-coefficient ($R^2$) values for the predicted vs observed gefitinib brain tumor concentrations for the low, medium and high MPI groups were 0.76, 0.74 and 0.72 respectively.

**Figure S7**: Diagnostic plots (model-predicted vs observed fraction of baseline pERK and model-predicted fraction of baseline pERK vs residuals) for tumor PD model. A. Low and B. High dose groups. The correlation-coefficient ($R^2$) values for the predicted vs observed fraction of baseline pERK for the low and high dose groups were 0.20 and 0.62, respectively.