Supplementary Figure S6

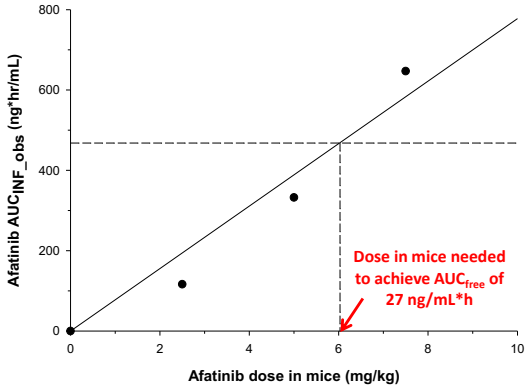
A

Drug	Human dose	Human plasma AUC_{total} (ng*hr/mL)	% free drug in human plasma	Human plasma AUC_{free} (ng*hr/mL)	% free drug in mouse plasma	Total AUC in mouse plasma to achieve human AUC_{free}	Nude mouse dose to match HED
Afatinib	40 mg daily (PO)	324	8.21	27	5.77	468	6 mg/kg daily (PO)

B

	Mouse	Human
Fraction bound (±) SD	94.23 ± 0.63	91.79 ± 0.46

C



D

	Afatinib plasma concentrations (ng/mL) ± SEM at various timepoints (h)					
Dose	0.5 h	1 h	3 h	6 h	9 h	15 h
2.5 mg/kg	5.68 ± 1.56	6.34 ± 1.91	12.49 ± 2.13	15.34 ± 7.54	5.07 ± 1.10	0.89 ± 0.16
5 mg/kg	15.74 ± 0.97	17.34 ± 4.07	49.87 ± 4.63	46.52 ± 8.94	7.93 ± 0.52	1.20 ± 0.60
7.5 mg/kg	36.56 ± 5.66	71.12 ± 2.86	118.18 ± 10.89	61.03 ± 3.87	12.15 ± 7.89	1.23 ± 0.12

E

Dose	C_{max} (ng/mL)	T_{max} (h)	Half-life (h)	AUC_{inf_obs} (ng/mL*h)
2.5 mg/kg	15.34	6	2.21	116.32
5 mg/kg	49.87	3	1.46	332.55
7.5 mg/kg	118.18	3	1.63	647.00

Supplementary Figure S6. Estimation of human equivalent dose of afatinib in nude mice. A) Afatinib protein binding and plasma pharmacokinetic analysis in humans and mice. **B)** Fraction of afatinib bound to plasma proteins following micro-equilibrium dialysis with NIH-III mouse plasma and human plasma. **C)** Relationship between total plasma AUC for afatinib against dose in NIH-III mice based on values presented in B. **D)** Afatinib plasma pharmacokinetic analysis in mice. Nude mice dosed (p.o.) at 2.5 mg/kg, 5 mg/kg or 7.5 mg/kg and plasma harvested at timepoints mentioned below (N=3 mice per timepoint). **E)** Afatinib mouse plasma pharmacokinetic parameters based on data in D. Pharmacokinetic parameters estimated using non-compartmental analysis on Phoenix Winnollin.