

Abstract #150

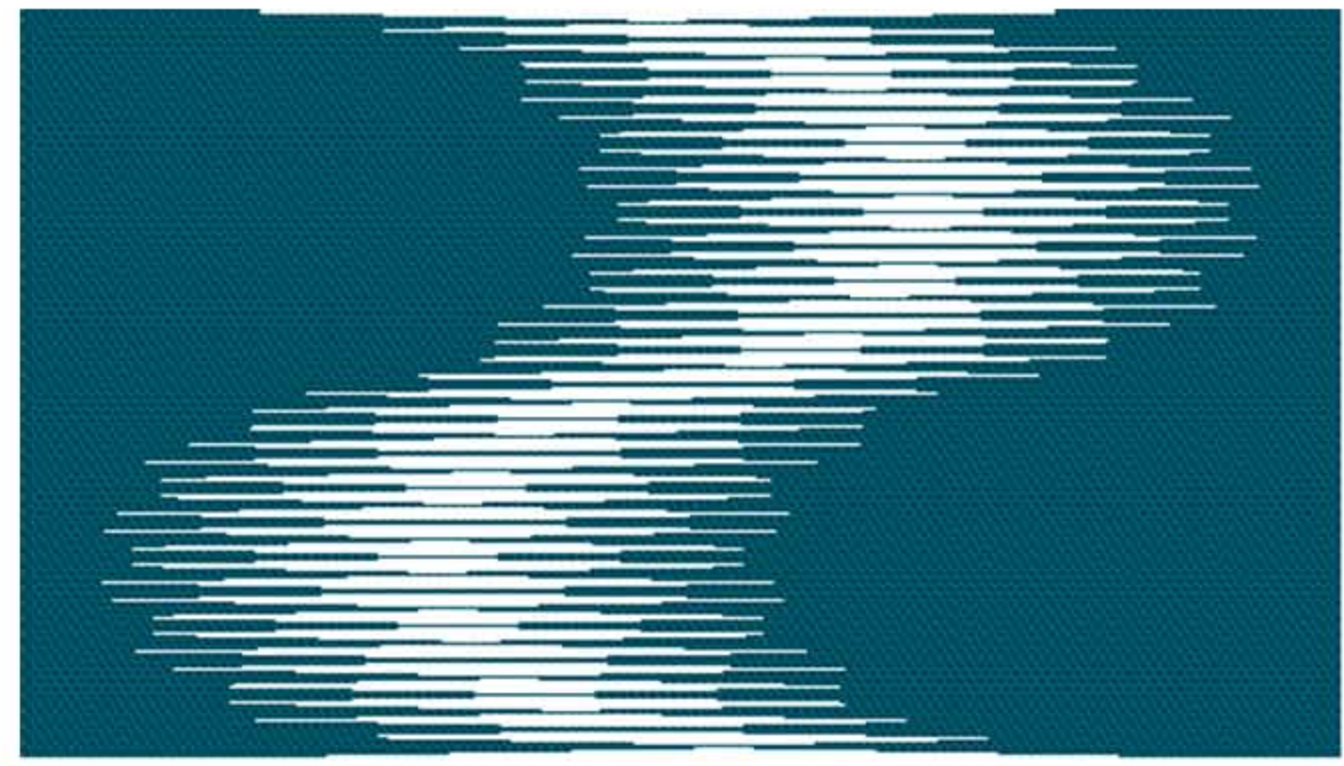
Heat shock proteins

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MPC-3100: A non-natural product Hsp90 inhibitor with anti-tumor activity in preclinical models

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Abstract

Background: The molecular chaperone Hsp90 stabilizes many proteins subserving tumor cell proliferation and survival. We have developed purine-based inhibitors that show good activity in *in vitro* binding and cellular assays, as well as *in vivo* animal models.

Materials and methods: The Her2-Luciferase reporter consists of the kinase domain of the Hsp90 client protein Her2 fused to Luciferase. Cells or mice were treated as indicated, and cells or tissues processed for Luciferase activity. HSP70 mRNA was extracted from mouse liver and measured by quantitative RT-PCR. Female nu/nu athymic mice were used as hosts for subcutaneously implanted tumor cells. At a median tumor volume of approximately 100 mm³, dosing was initiated using a 200 mg/kg p.o. qd, 5-days-on/2-days-off schedule for three cycles.

Results: Her2-Luciferase inhibition precedes loss of cell viability, consistent with target-mediated cell killing. Mouse pharmacokinetic studies show that compounds in this class are retained in tumor xenografts at high levels 48 h post dosing, while plasma levels are almost undetectable, and rat pharmacokinetic studies indicate good oral bioavailability of selected compounds (data not shown). As a means to predict *in vivo* activity of compounds, mice were given a single dose of 100 mg/kg p.o. and then sacrificed 6 h later, at which time quantitative RT-PCR was used to determine the level of HSP70 induction, a marker of Hsp90 inhibition. MPC-3100 showed a superior pharmacokinetic and pharmacodynamic profile and was taken into pre-clinical anti-tumor models. In HT-29 tumor-bearing mice, growth inhibition relative to vehicle-treated animals was significant at the end of dosing (68%, p<0.01), as well as nine days later (65%, p<0.05). After this point, xenograft growth resumed at a rate similar to vehicle-treated animals. Animals showed no weight loss and tolerated the dosing regimen well. MPC-3100 was also tested in a NCI-N87 HER2⁺ gastric colon carcinoma xenograft model, and at the end of dosing, the MPC-3100-treated cohort showed tumor shrinkage (44% regression, p<0.0001) with no significant weight loss. In comparison, tumors in 5-FU-treated mice (100 mg/kg i.p. weekly for three doses) did not shrink but grew slowly (91% tumor growth inhibition), and these mice experienced significant weight loss.

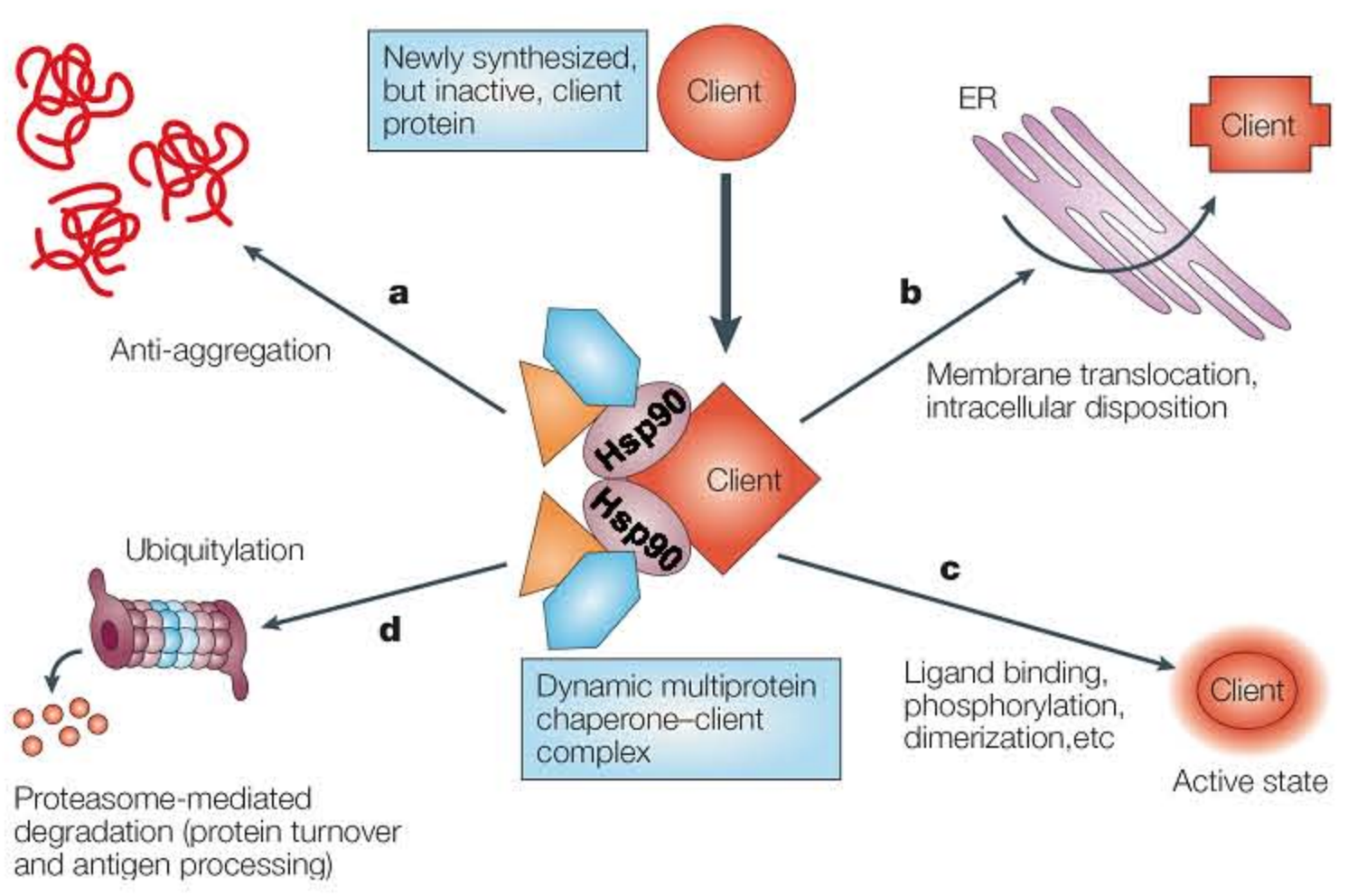
Conclusions: The orally bioavailable Hsp90 inhibitor MPC-3100 demonstrates anti-tumor activity using dosing regimens that are well tolerated.

Background

Hsp90 functions to stabilize proteins. Many signaling proteins and mutant oncogenes are unstable and require Hsp90. Hsp90 client proteins play roles in supporting all of the acquired capabilities or hallmarks of cancers¹.

Hsp90 Function²

- Hsp90 functions to stabilize proteins
 - Many signaling proteins and mutant oncogenes are unstable and require Hsp90



Methods (additional)

The Her2-Luciferase construct was constructed by joining sequences encoding the kinase domain (aa 641-975) of Her2 to those encoding firefly Luciferase^{3,4}. The Her2-Luciferase construct was introduced into HCT-116 tumor cells to create Her2-Luciferase/HCT-116 cells. After compound exposure, cell lysates were assayed for Her2-Luciferase activity.

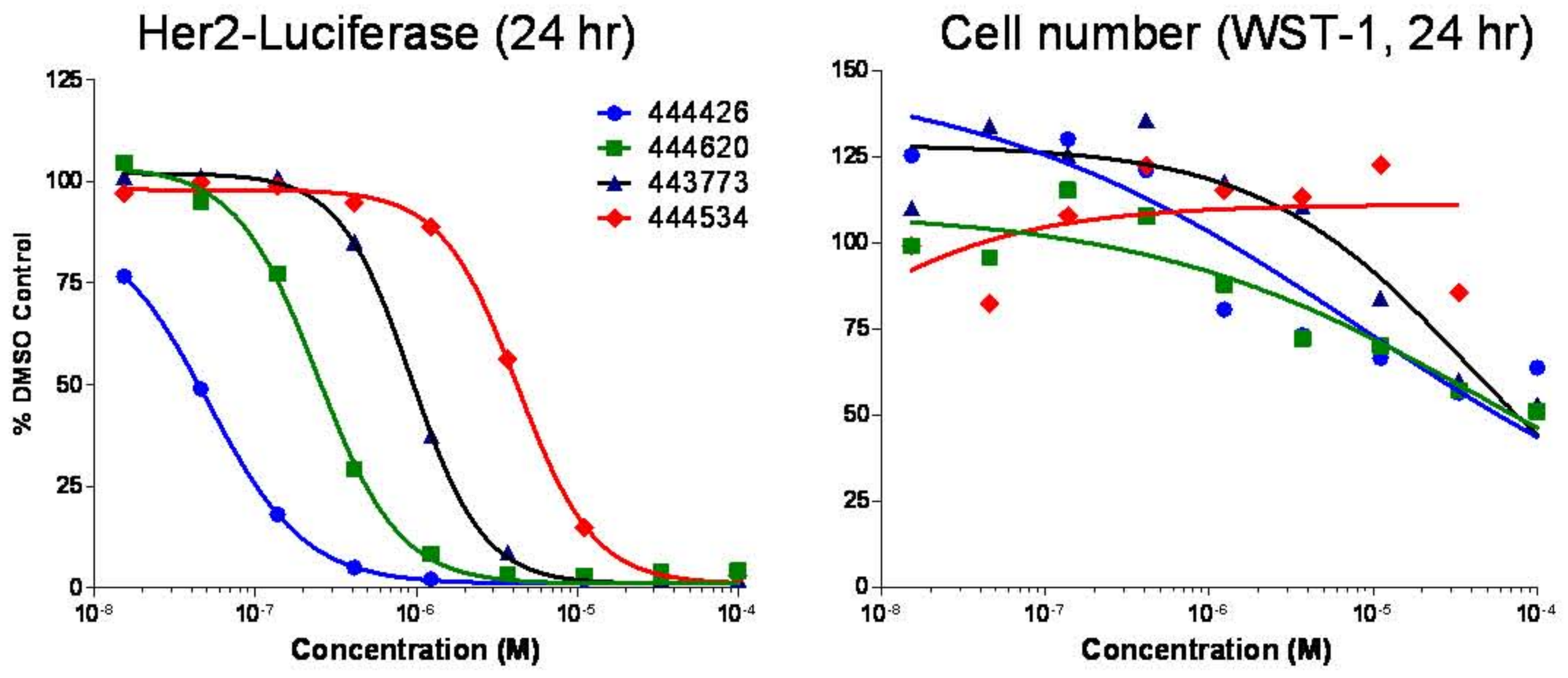
Her2-Luciferase Reporter

Her2-Luciferase Reporter Construct



The Her2-Luciferase fusion construct encodes a protein designed to be a cell-based reporter for inhibition of Hsp90 activity. HCT-116 tumor cells bearing the Her2-Luciferase reporter (Her2-Luciferase/HCT-116) provide a mechanism-based cellular assay to evaluate compounds for Hsp90 inhibitory activity.

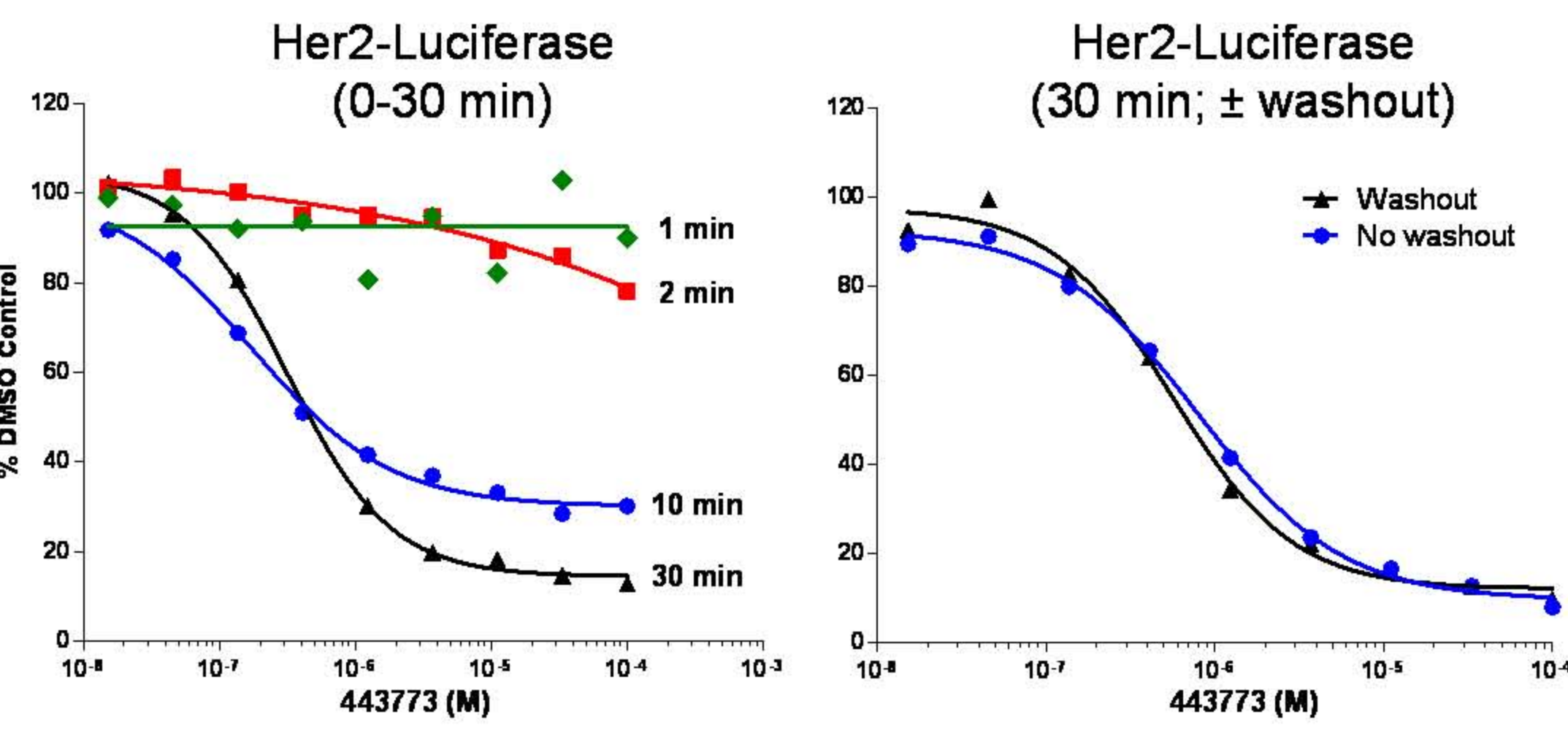
Her2-Luciferase Assay



Her2-Luciferase/HCT-116 cells were exposed to compounds for 24 hours, and then assayed for Her2-Luciferase (left panel) or cell number (WST-1, right panel). Her2-Luciferase inhibition is complete at 24 hours, while effect on cell number is not. Her2-Luciferase inhibition at 24 hours correlates with cell growth inhibition (GI₅₀) at 72 hours (table).

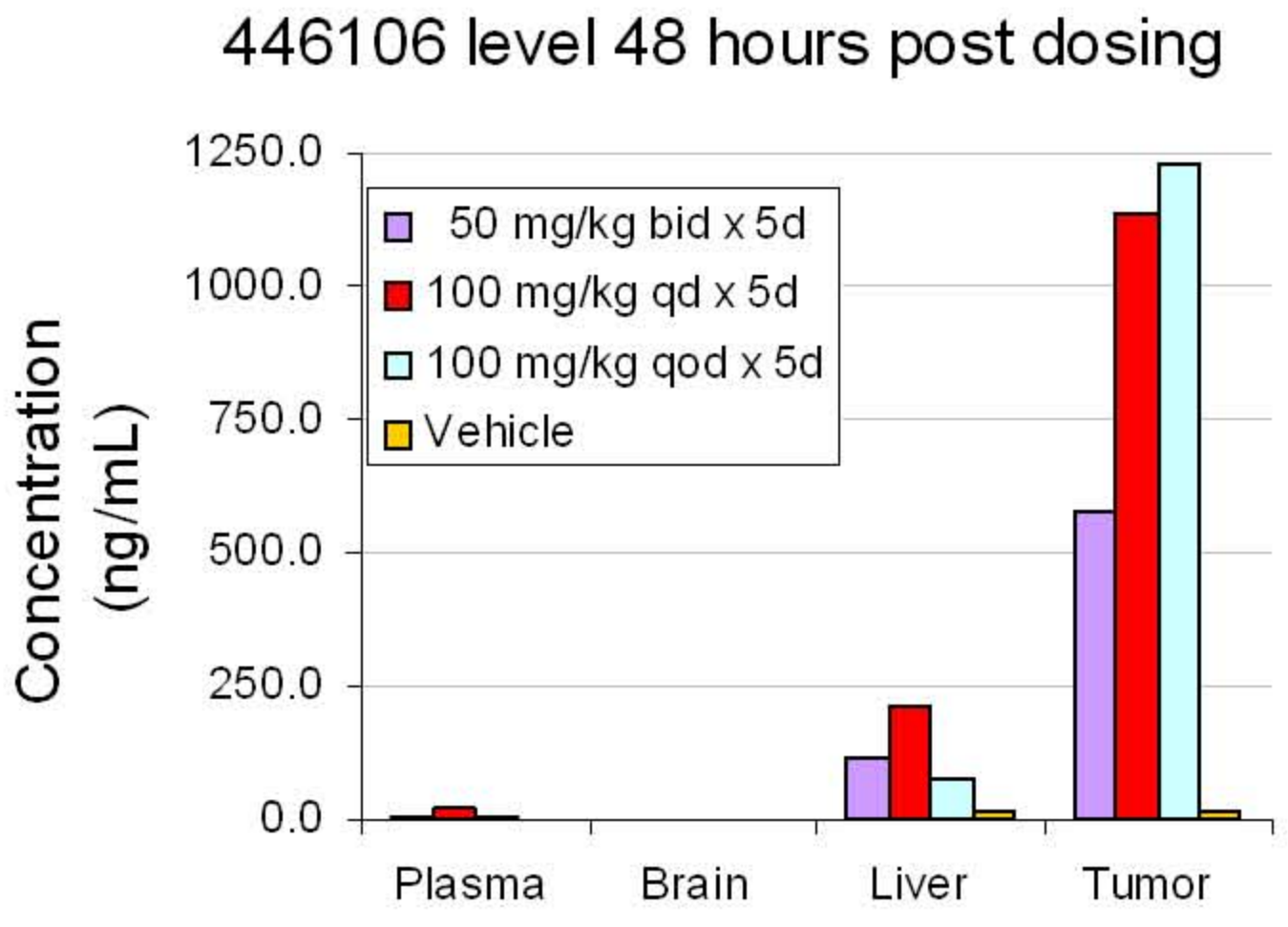
Compound	Binding	EC ₅₀ (μM)	Her2-Luc GI ₅₀ (72 hr)
444426	0.035	0.05	0.36
444620	0.12	0.24	1.6
443773	0.44	1.0	6.5
444534	1.8	4.3	26.7

Her2-Luciferase Assay Timecourse



Her2-Luciferase/HCT-116 cells were exposed to 443773 for 0-30 minutes (left panel), and inhibition of Her2-Luciferase activity was complete after 30 minutes, well before effects on cell number manifest (8-12 hours). Washing cells prior to assay (right panel) had no effect, consistent with a lack of direct Luciferase inhibition.

Tissue and Tumor Compound Levels



Mice bearing HCT-116 tumor xenografts were dosed with 446106 according to the indicated regimen for 5 days, and then sacrificed 48 hours after receiving the last dose. 446106 levels are highest in tumor compared to plasma, brain, and liver.

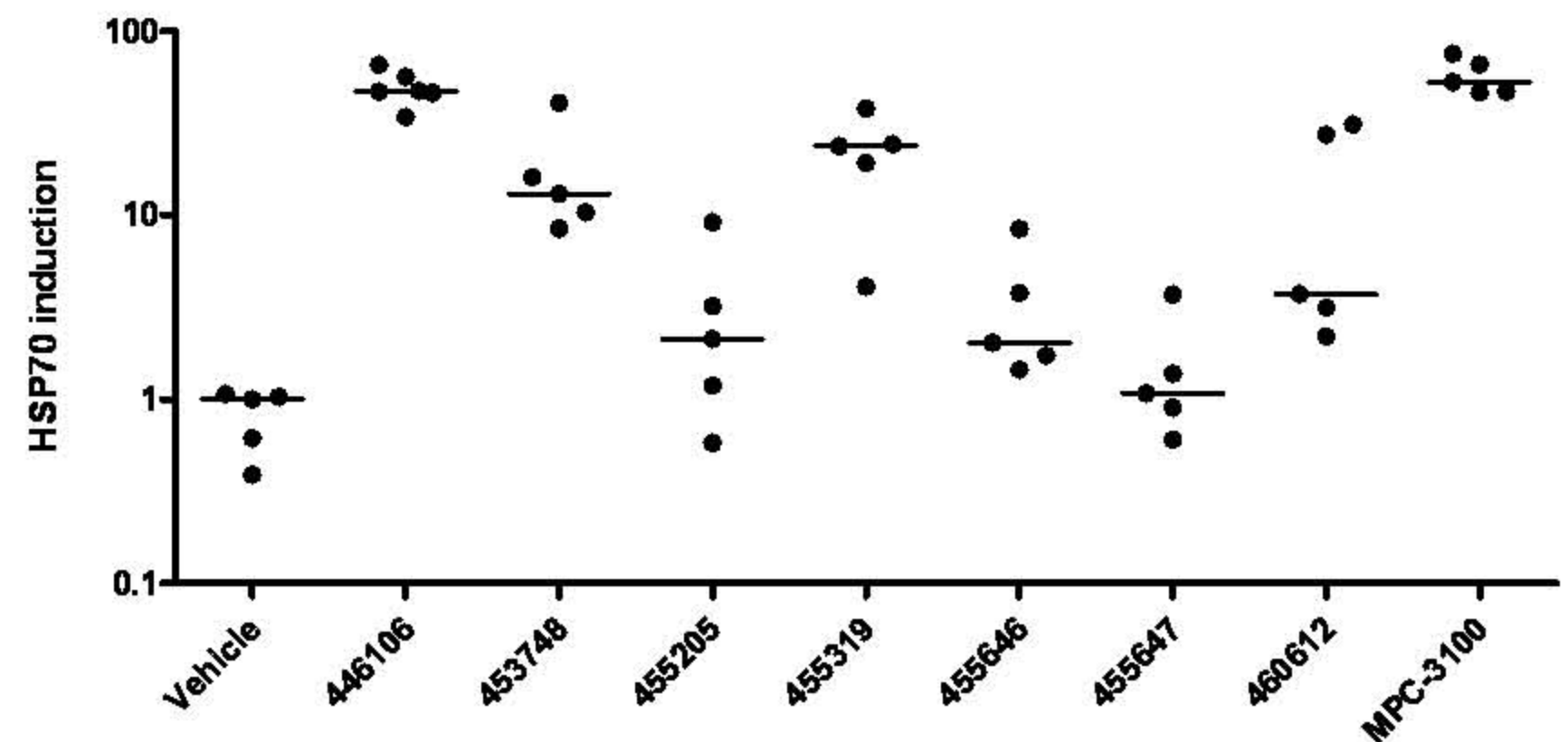
Pharmacokinetic and Pharmacodynamic Characterization

Maximizing Oral Bioavailability

Compound	C _{max} (μg/mL)	AUC (hr*μg/mL)	Bioavailability (oral, %)
MPC-3100	13.1	81.8	100
455646	11.6	40.4	69
446106	10.9	63.9	100
480812	9.3	32.6	54
455206	8.5	38.0	100
455319	8.4	50.3	76
453748	7.8	25.1	100
455647	7.5	18.2	89

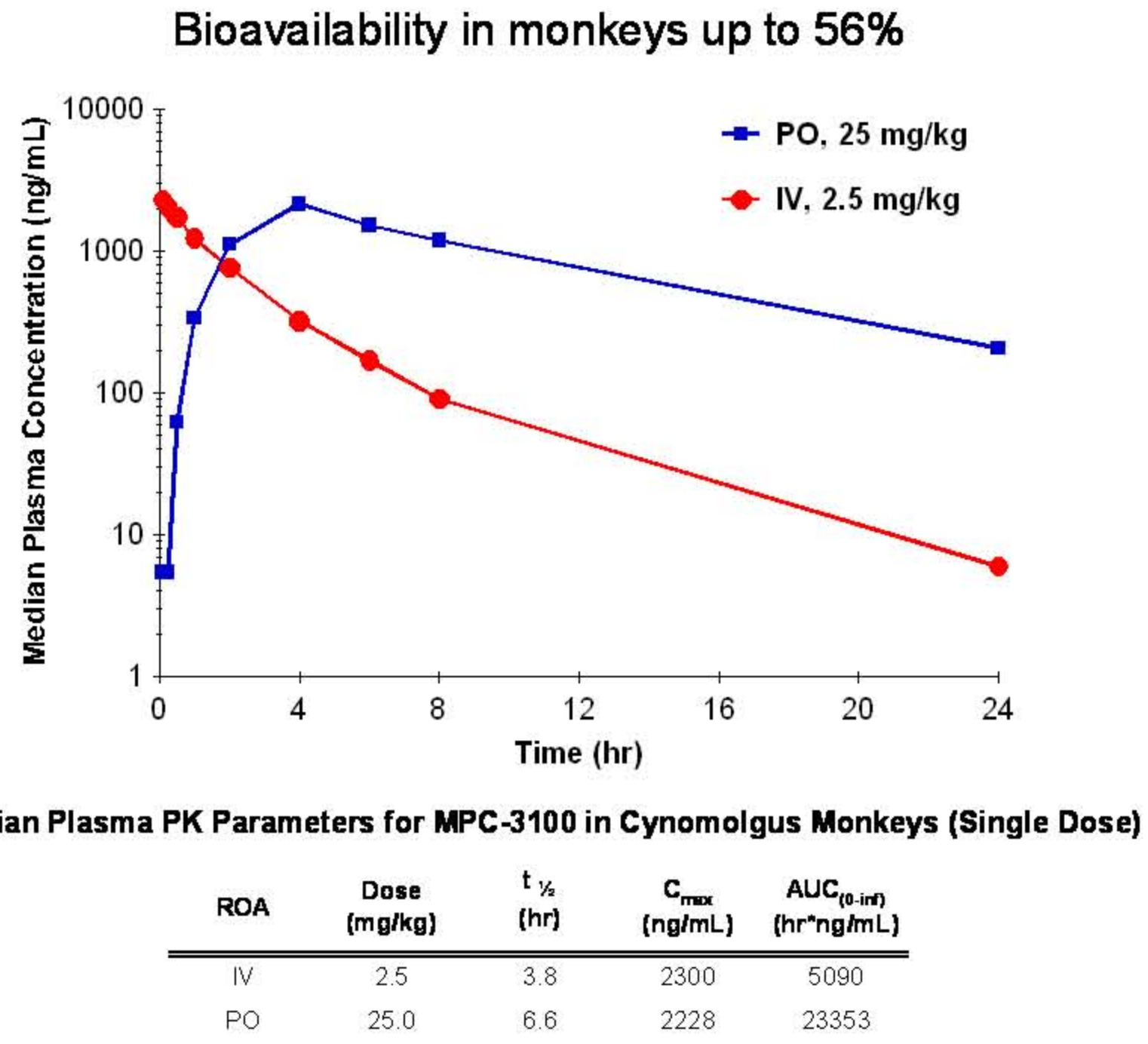
Mice were dosed at 100 mg/kg p.o. for determination of oral bioavailability, and MPC-3100 was shown to have the best PK properties in mice.

Maximizing Pharmacodynamic Properties



Mice were dosed at 100 mg/kg p.o. for determination of pharmacodynamic effects using HSP70 mRNA induction in liver, and MPC-3100 was shown to have the best PD properties in mice.

Oral Bioavailability

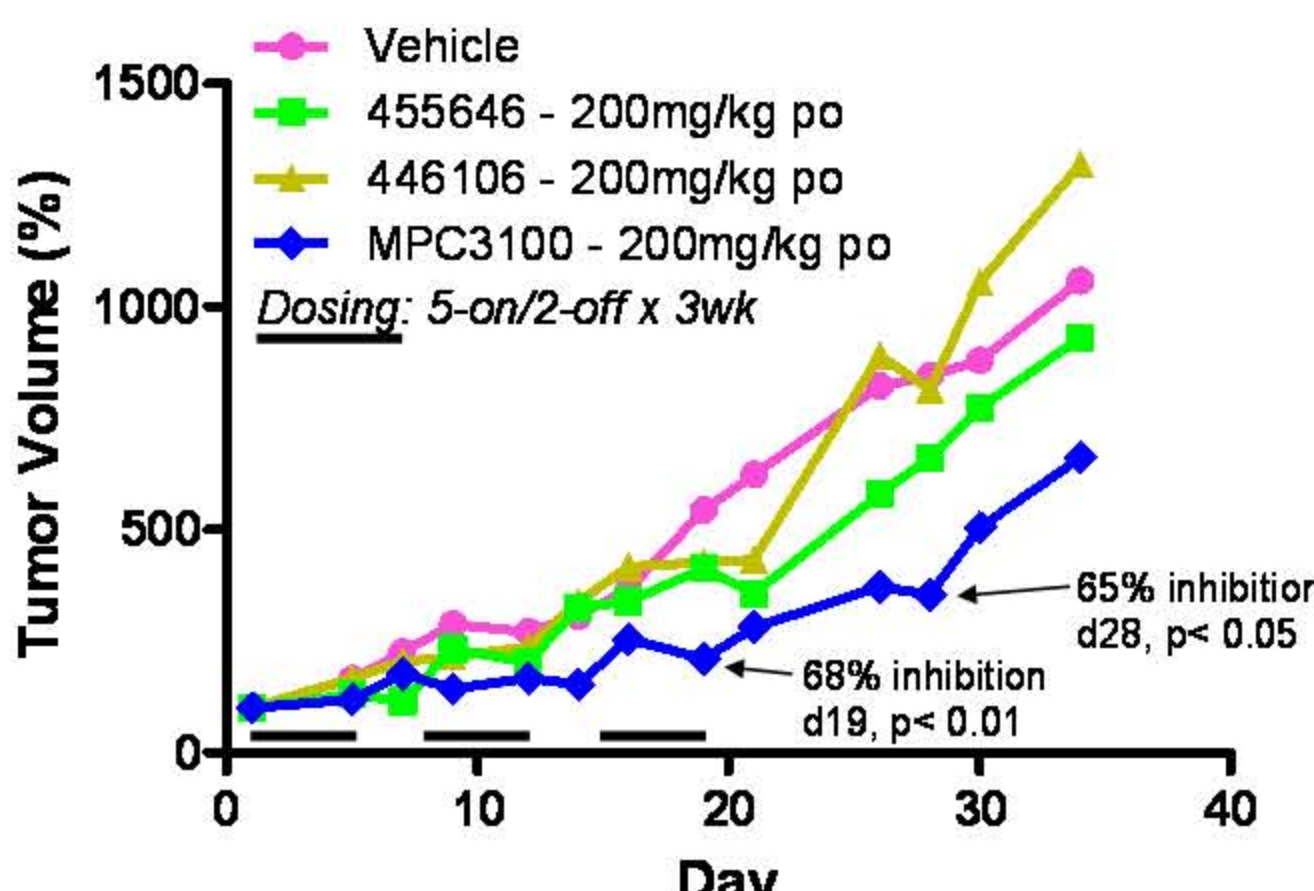


Median Plasma PK Parameters for MPC-3100 in Cynomolgus Monkeys (Single Dose)

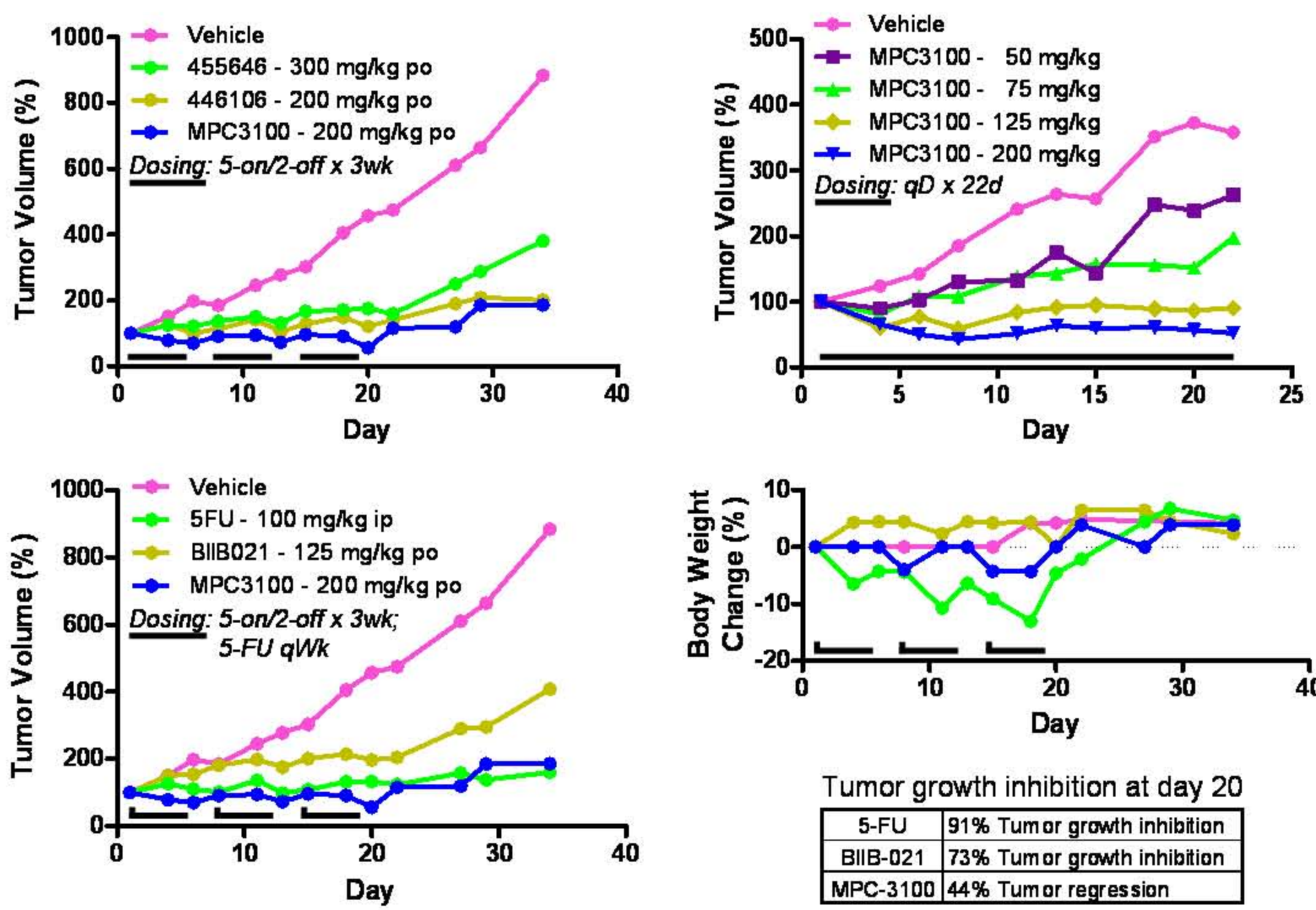
Route	Dose (mg/kg)	t _{1/2} (hr)	C _{max} (ng/mL)	AUC ₀₋₂₄ (hr*ng/mL)
IV	2.5	3.8	2300	5000
PO	25.0	6.6	2228	23553

Animal Models

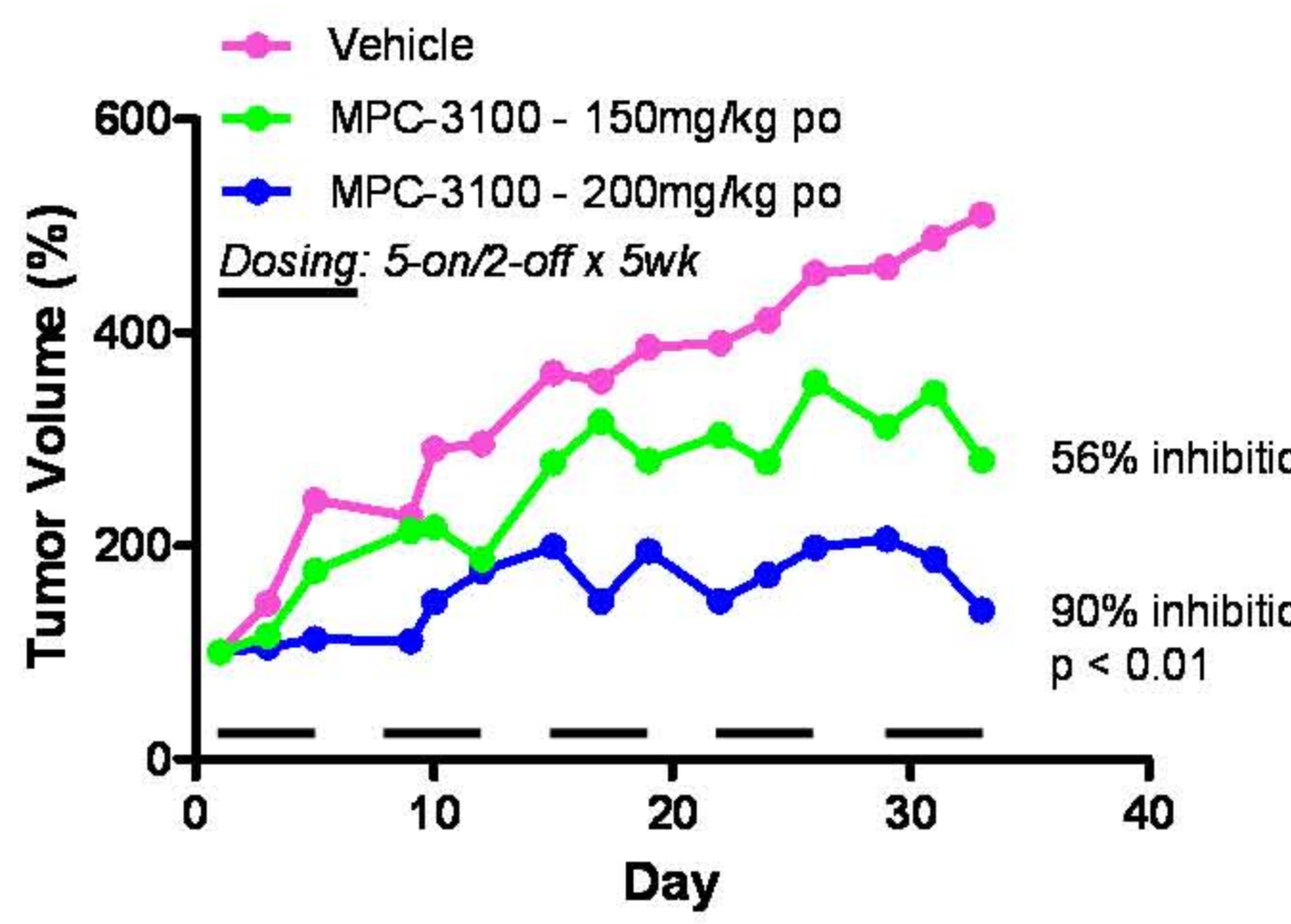
HT-29 Colon Cancer Xenograft



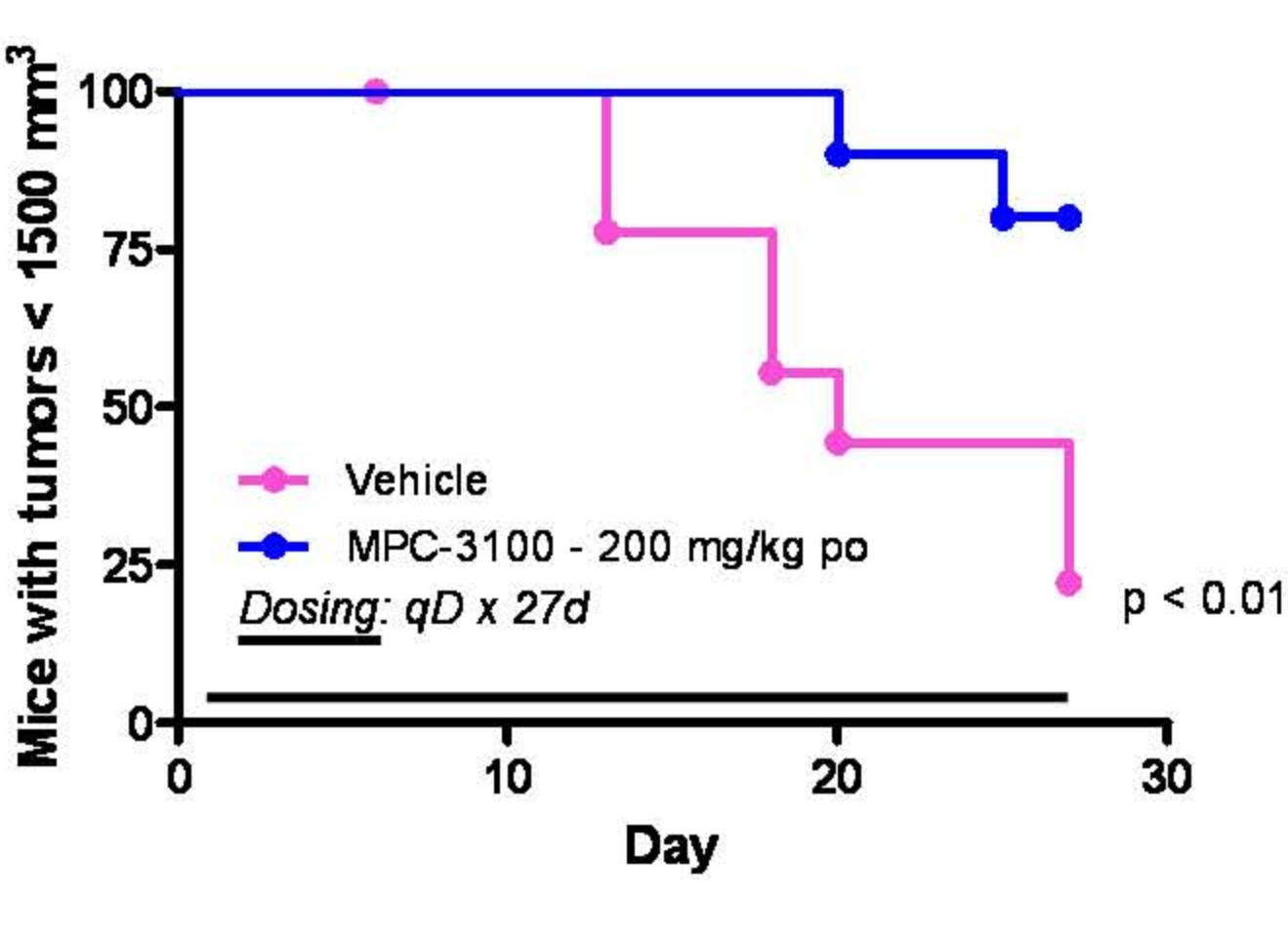
NCI-N87 HER2⁺ Gastric Cancer Xenograft



DU-145 Prostate Cancer Xenograft



NCI-H69 SCLC Xenograft



Summary

In Vitro Properties of MPC-3100

- MW ~550
- Hsp90 binding assay: IC₅₀ = 0.14 μM
- Cellular Her2 client protein degradation: IC₅₀ = 0.06 μM
- Cellular cytotoxicity: GI₅₀ = 0.15 – 1.5 μM

Tissue	Cell Line	G ₅₀ (μM)
Colon	HT-29	0.42
	SW-480*	1.5
	HCT-116	0.54
Prostate	LNCaP*	0.51
	PC-3*	0.97
Lung	DU145	0.53
	NCI-H69	0.15
	A549*	0.77
Ovarian	OVCAR8	0.49
Gastric	NCI-N87	0.20
Breast	BT-474	0.55
Leukemia	MV-4-11	0.25
Cervical carcinoma	HeLa*	0.53
Glioma	U-87 MG*	1.1

* 5-day assay

Conclusions

- Her2-Luciferase inhibition provides a useful measure of Hsp90 inhibitory activity
- MPC-3100 is a potent, orally bioavailable, synthetic Hsp90 inhibitor
- MPC-3100 has anti-tumor activity in all tested animal xenograft models
- No weight loss or toxicity is apparent at efficacious doses

References

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- Whitesell L and Lindquist SL (2005). Hsp90 and the chaperoning of cancer. Nat Rev Cancer 5(10):761-772
- Xu W, Mimnaugh E, Rosser MFN, Nicchitta C, Marcu M, Yarden Y, and Neckers L (2001). Sensitivity of mature ErbB2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. J Biol Chem. 276(5):3702-8.
- Tikhomirov O and Carpenter G (2003). Identification of ErbB-2 kinase domain motifs required for geldanamycin-induced degradation. Cancer Res. 63(1):39-43.