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PPT Presentation Open Access

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A Novel M-phase Inhibitor DEA-1496

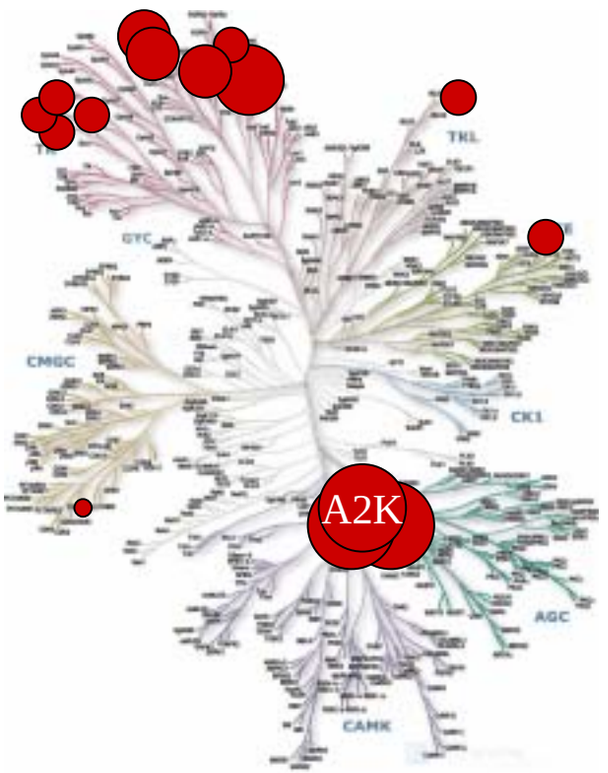
Drug Discovery Program of Aurora Kinase Inhibitor

To find a new M-phase inhibitor which can…

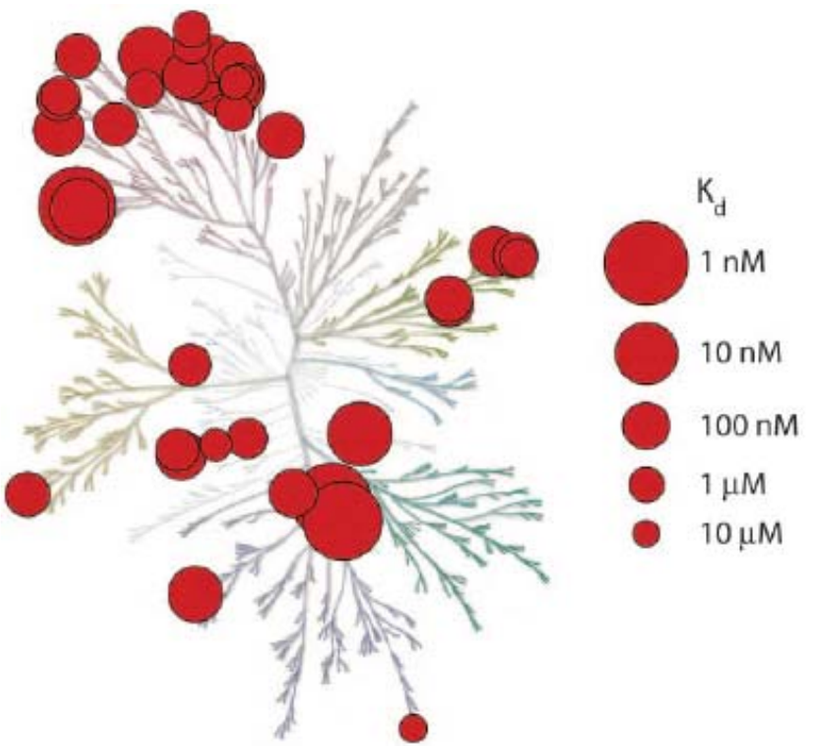
- **inhibit aurora kinases with comparable activity to that of others.**
- **be differentiated from other M-phase inhibitors on the basis of cell culture assays and *in vivo* anti-tumor efficacy.**
- **cooperate to kill tumor cells with established chemotherapeutic agents including Taxol.**

DEA-1496 shows higher selectivity of kinase inhibition than that of VX-680

Both compounds inhibit A2K with similar Ki (2nmol/L; in house) and also active against A1K, FLT3, Ret and TrkA



DEA-1496 (profiling performed by Upstate Biotechnology)



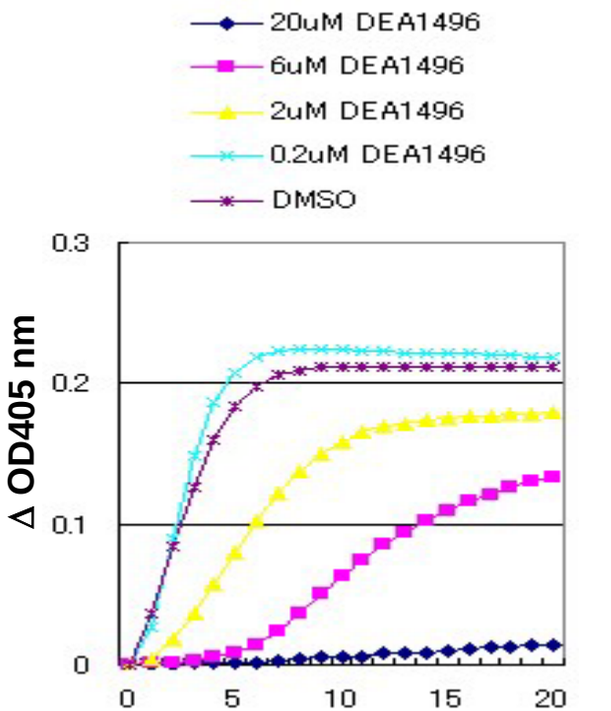
VX-680 (profiling performed by Ambit; PNAS 102:11011 '05)

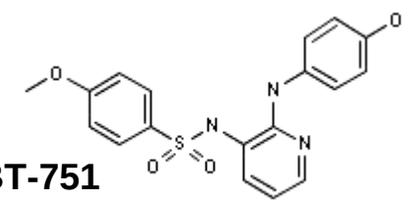
Summary of Pharmacological Profile of DEA-1496

compound	*in vitro* A2K inhibition	*in vivo* tumor growth inhibition			*in vitro* growth inhibition	
	Ki	HCT-116		PC-3	tumor cell growth inhibition spectrum	combination with Taxol
	nM	ED50 (mpk)	30mpk %inh.	30mpk %inh.	*in vitro* XTT	*in vitro* XTT
DEA-1496	2	15 (p.o.)	82 (p.o.)	95 (p.o.)	29 responders / 29	synergistic
VX-680	2	10.7 (i.v.)	79 (50mpk i.v.)	12 (i.v.)	10 responders / 29	antagonistic

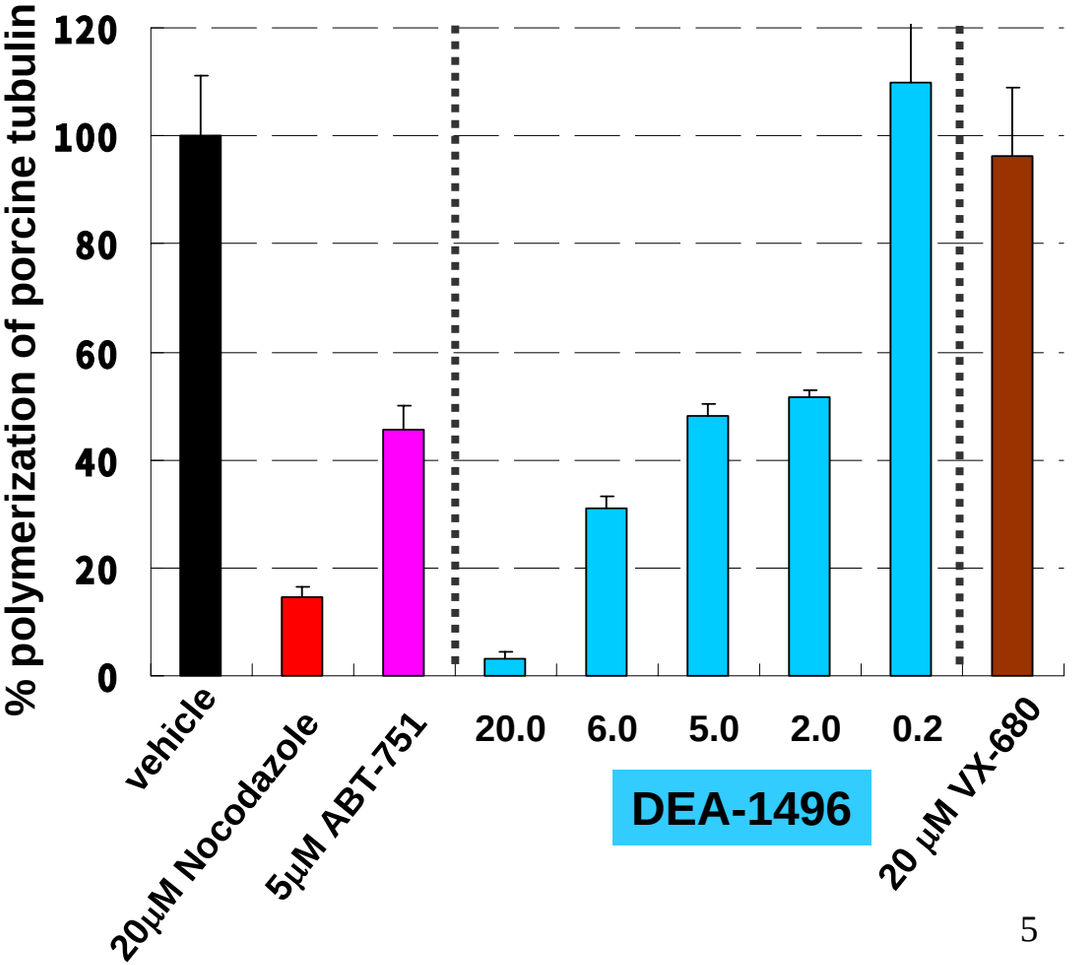
Inhibition of tubulin polymerization by DEA-1496 in a cell-free turbidity assay

DEA-1496 inhibits tubulin polymerization *in vitro* as potent as ABT-751

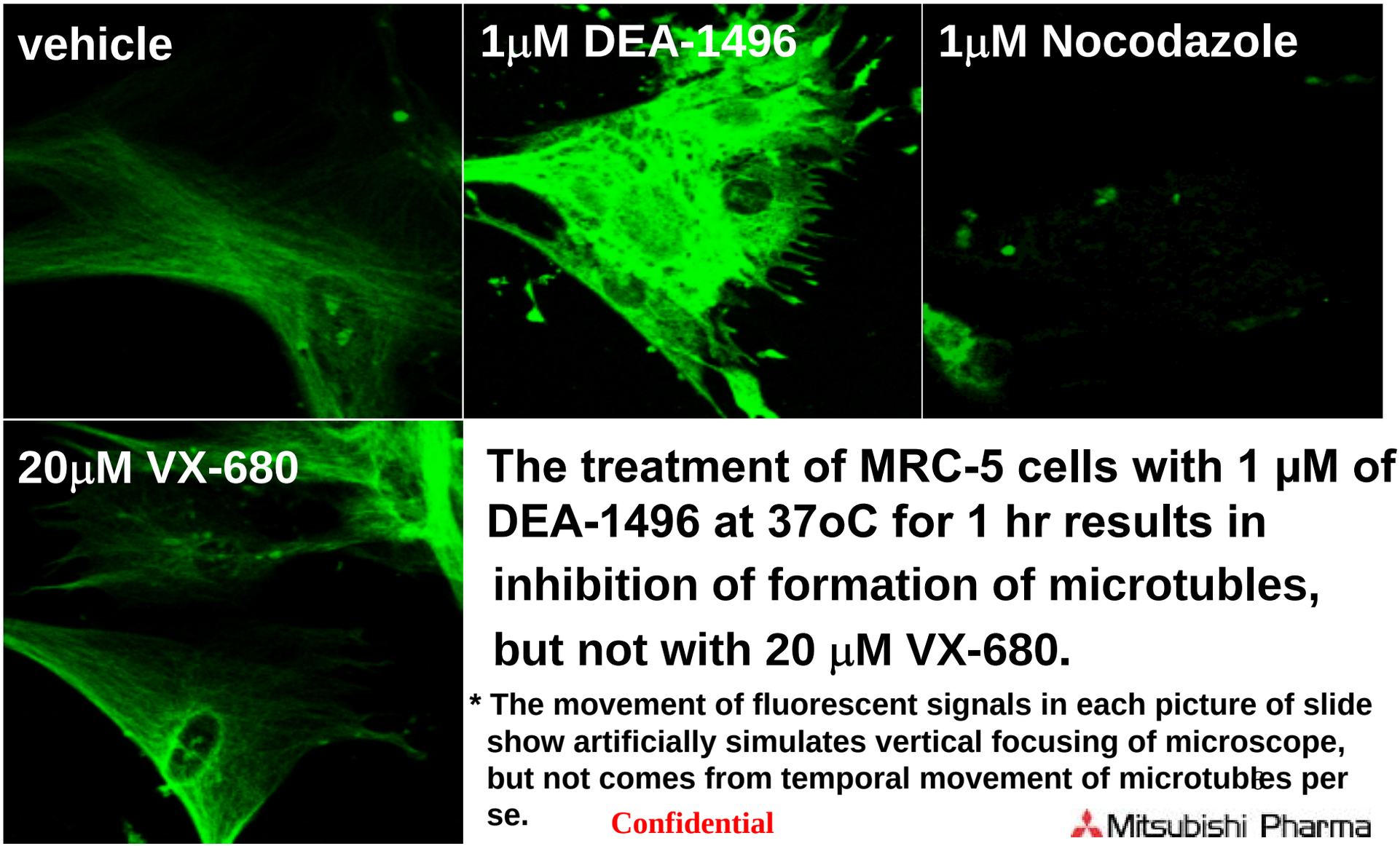




ABT-751
(P2; Abbott/Eisai)



Inhibition of microtuble formation in intact MRC-5 cells by DEA-1496



The treatment of MRC-5 cells with 1 μM of DEA-1496 at 37oC for 1 hr results in inhibition of formation of microtubles, but not with 20 μM VX-680.

*** The movement of fluorescent signals in each picture of slide show artificially simulates vertical focusing of microscope, but not comes from temporal movement of microtubles per se.**

DEA-1496…can still be differentiated from other M-phase inhibitors

Comparison of GI_{50} (nmol/L; 72hrs) against a panel of human tumor cell lines

cell line origin	HCT116	SW620 Colorectal	SW480	Caki-1 Kidney	MDA-MB-453 Breast	kim-1 Liver	Alex
VX680	15	407	5932	166	15950	8907	10176
AZD-1152	16	6374	2000	5701	3879	5747	7705
PHA-680632	77	3494	2399	646	321	6572	13682
DEA-1496	7	8	9	2	15	34	17
ABT-751	65	283	191	205	319	353	405

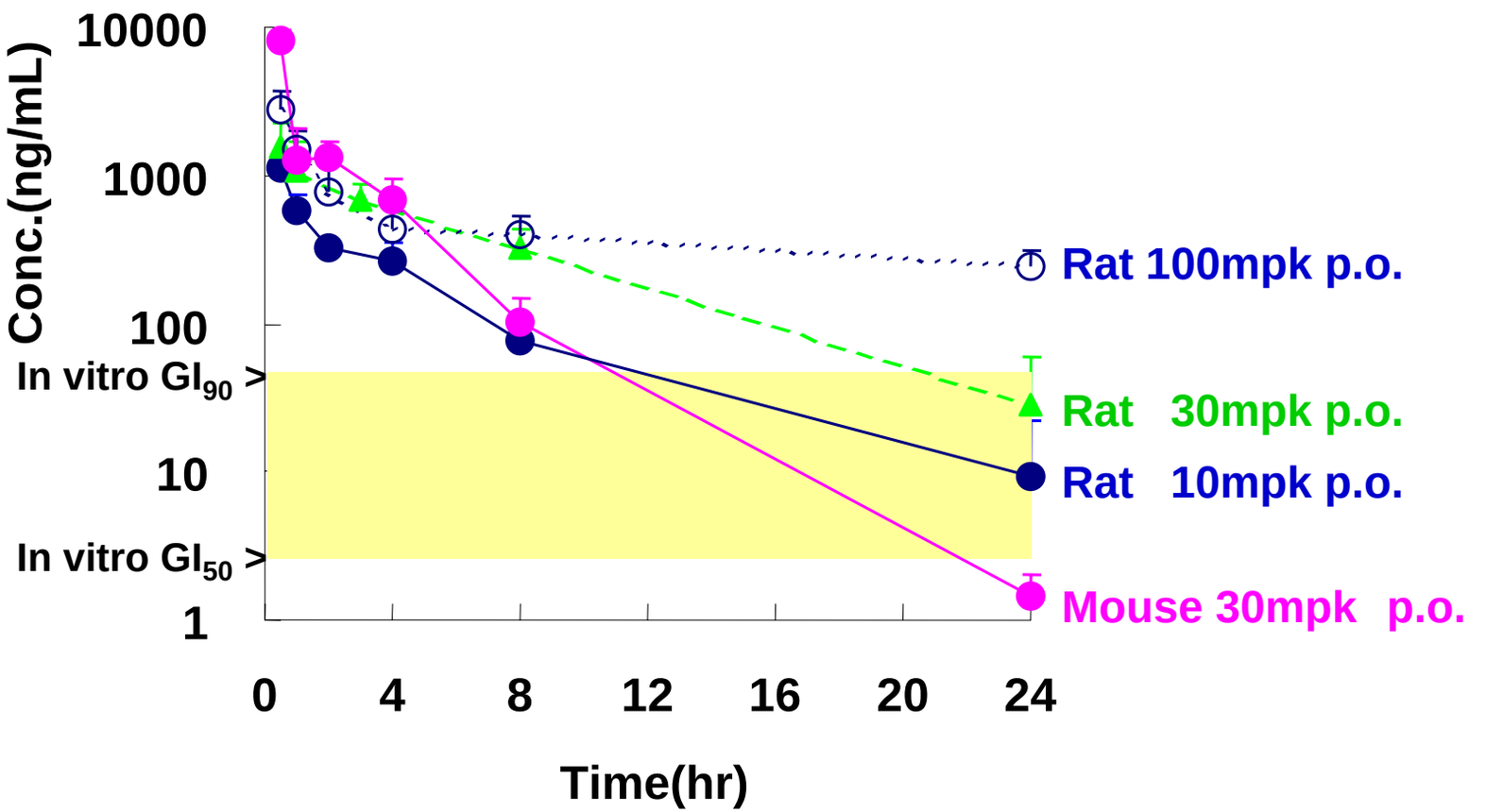
cell line origin	PC3	DU145 Prostate	T47D	U937	HL60 Leukemia	K562	MV4;11
VX680	6471	2637	930	25	12	21	9
AZD-1152	9711	11910	5411	4162	1	7814	<6.4
PHA-680632	7574	1693	711	476	138	5978	66
DEA-1496	4	13	9	6	7	62	1
ABT-751	225	351	320	125	327	340	237

cell line origin	PK8	PANC-1 Pancreas	HPAC	H1650	H1666 Lung	H1975	C6 Glioma
VX680	5950	23550	5866	>20000	4871	6683	34
AZD-1152	5800	16890	6448	6912	4850	6100	<6.4
PHA-680632	2580	38200	10319	9338	1182	3449	507
DEA-1496	8	8	44	173	54	16	9
ABT-751	208	801	477	578	1116	346	884

Preliminary TOX

		DEA-1496	VX-680
Ames		Negative	Positive
hERG at 10 μmole/L		30.0% Inh.	35.1% Inh.
Receptor Binding at 10 μmole/L		Adenosine A2a (52% Inh.) Dopamine transporter (83% Inh.) Opiate (77% Inh.)	α2-Adrenergic (50% Inh.) Dopamine D1 (55% Inh.) Dopamine transporter (55% Inh.) Na channel (61% Inh.)
Rat 4d	po daily	Dose: 10, 30, 100 mpk	
		Death = 100 mpk GI tox., BM suppression, and histopathologic changes in skin, liver, thymus, testes, etc. Reversible at 30 mpk	
	bolus iv daily	Dose: 10, 30, 60 mpk	Dose: 30, 100, 300 mpk
		No death up to 60 mpk Generally, milder than those observed in the po study above	Death = 300 mpk GI tox., BM suppression, and histopathologic changes in skin, liver, thymus, etc.
Human CFU-GM colony formation		Inh. potency: Paclitaxel > DEA-1496 ~ VX680	

In vivo PK Profiles of DEA-1496



DMPK Profiles of DEA-1496

Solubility (μg/mL)	at pH7.0 at pH4.0	2.6 > 180
Caco-2 permeablity		High
Protein binding (mixture of human serum albumin and α1AGP)		95.4%
Metabolic stablity (liver microsomes) (mL/min/mg protein)	mouse rat human	0.12 0.11 0.11
Rat BA		54.9%
CYP inhibition (recombinant human CYP) (IC50; μmole/L)	1A2 2D6 3A4 2C9 2C19	2.0 >50.0 4.5 4.9 4.5
CYP mRNA induction (concentrations giving 2-fold mRNAs compared to vehicle-treated human hepatocytes; μmole/L)	1A1 1A2 3A4 2B6	0.4 0.6 1.6 >5.0
CYP isoforms involved in metabolism	human rat	1A2 > 3A4 3A2 ≧ 2C11
Transcellular transport assay of MDR1-tranfected cells		Transported by P-gp

Summary of DEA-1496 as of May, ’06 (1)

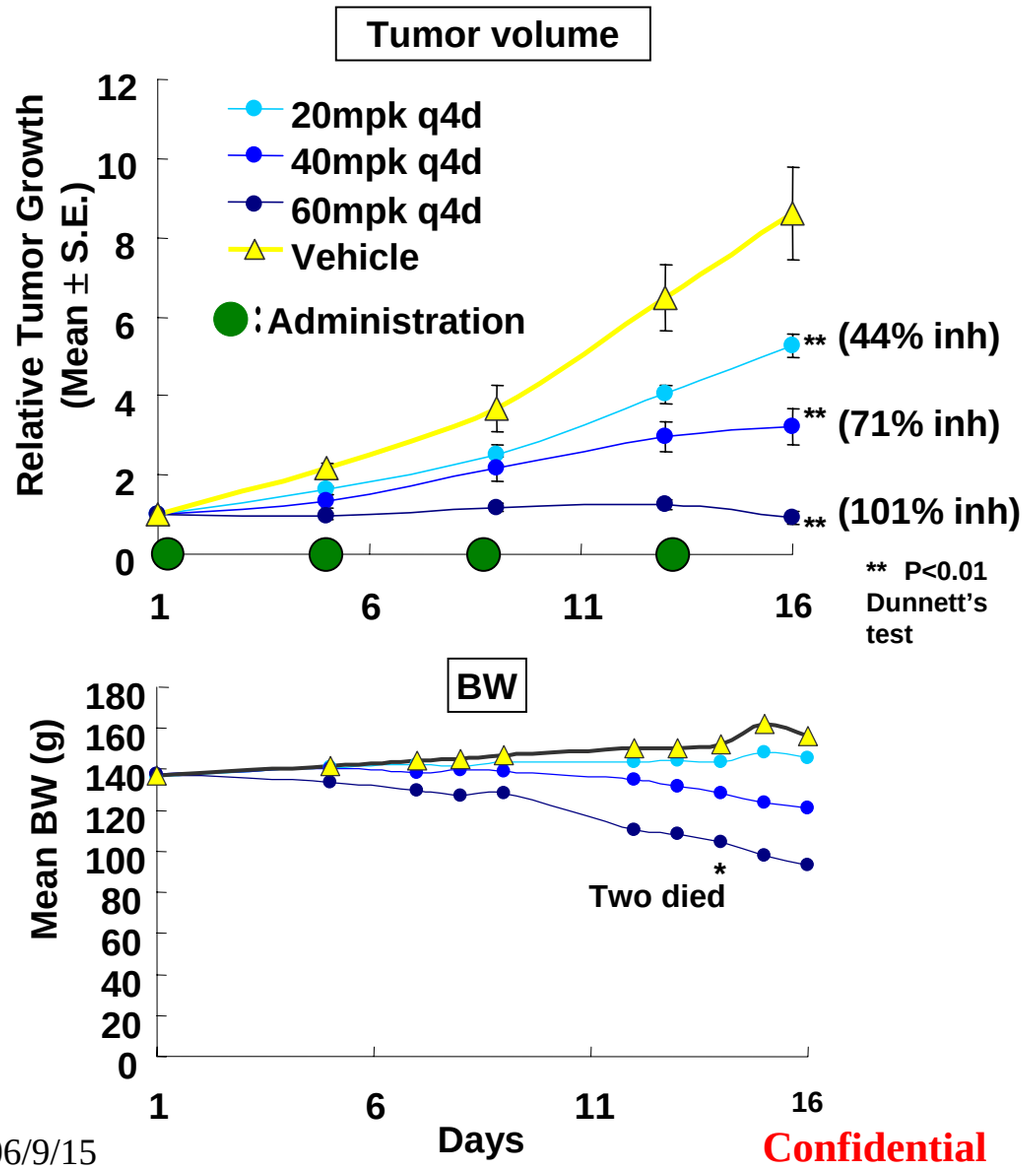
- **DEA-1496 is a novel M-phase inhibitor with a unique dual mechanism of action;**
  - inhibition of Aurora kinases,
  - inhibition of microtuble polymerization.
- **DEA-1496 could be differentiated from VX-680.**
  - broader spectrum of inhibition of growth of tumor cell lines *in vitro* than that of the competitors.
  - *in vivo* anti-tumor efficacy against tumor cell line resistant to VX-680 (PC-3) and to Taxol (CT-26).
- **DEA-1496 exerts synergistic on inhibition of *in vitro* tumor cell proliferation with Taxol.**

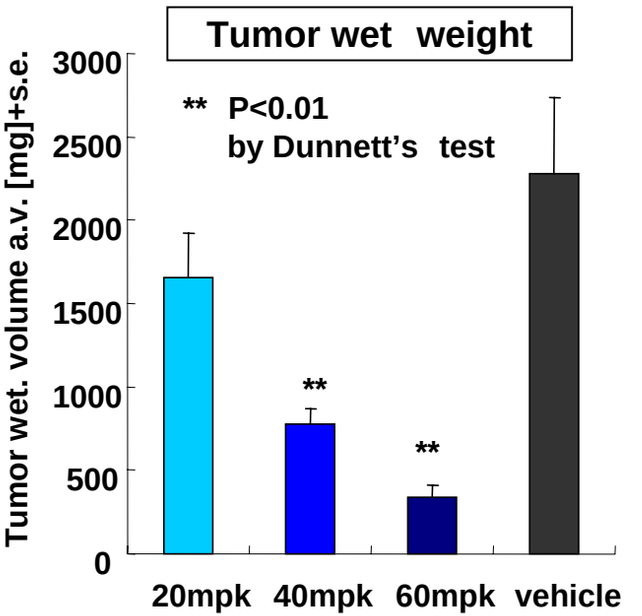
Summary of DEA-1496 as of May, ’06 (2)

- **Preliminary Rat 4-day TOX studies revealed;**
  - Comparable TOX profile to that of VX-680.
  - GI toxicity and BM suppression as possible major DLT.
- **In cultured human hepatocytes, DEA-1496 significantly induced CYP1A2, which was in parallel suspected to metabolize the compound most efficiently in human.**
  - Failure of achievement of effective drug concentration in patients’ blood is concerned.
  - However, as the CYP induction was reversible in cultured hapatocytes after 48 hrs, intermittent dosing should be effective to avoid such undesirable phenomenon.
- **Since the intermittent dosing of DEA-1496 could exert significant *in vivo* anti-tumor activity in nude rats, a rat 2w non-GLP TOX study with the similar dosing regimen is currently being conducted.**

An update (2006.9.8)

Intermittent oral administrations of DEA-1496 significantly inhibited growth of HCT-116 *in vivo*



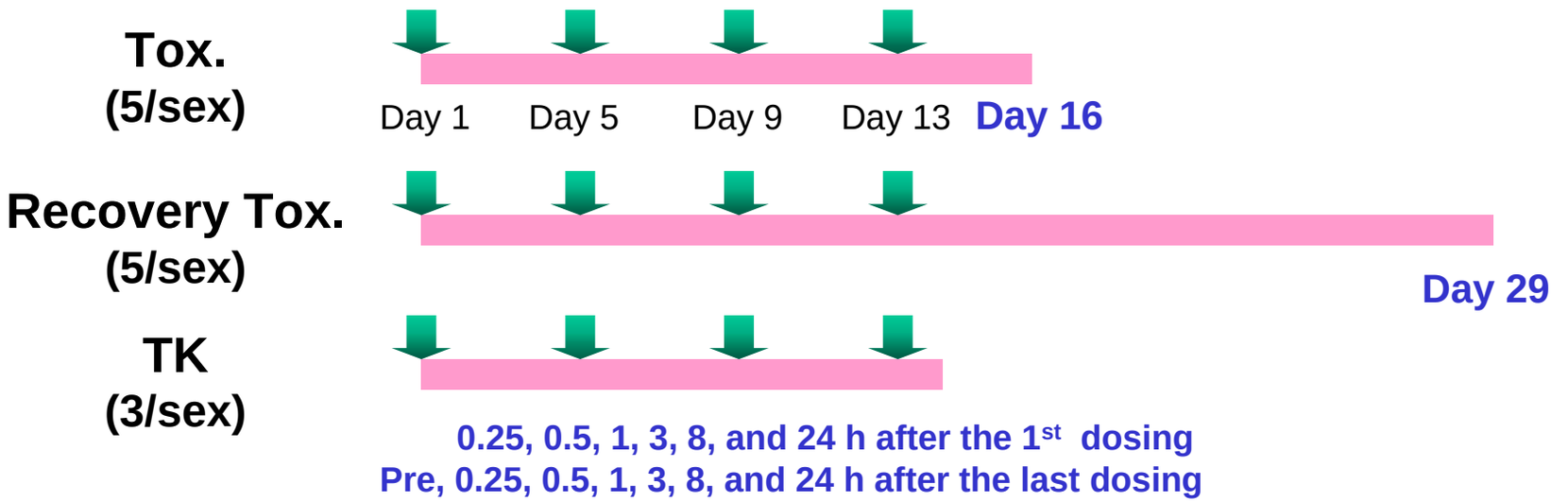


Study no.06C8410

DEA-1496 non-GLP Rat 2-week toxicity study

Study No.: 6R217

DEA-1496: 0, 20, 40, 60 mg/kg/day, 1on/3off x 4-cycle (po)



Summary

Mortality: 40 mg/kg/day (1 male) and 60 mg/kg/day (2 males and 2 females)

NOAEL: <20 mg/kg/day

Diarrhea, loss of fur, BW decrease, myelosuppression, gastrointestinal toxicity (atrophy, mitosis, karyomegary, etc.), atrophy in lymphoid organs, testicular toxicity were observed. After a 2-week recovery period, testicular toxicity, myelosuppression, and loss of fur were not disappeared, however, there seemed to be with reversibility.

Rat TK study of DEA-1496 (draft)

Dose (mg/kg/day)		20		40		60	
Sex		Male	Female	Male	Female	Male	Female
Day 1	Cmax (ng/mL)	1481 ± 379	1739 ± 549	1757 ± 141	2918 ± 874	2110 ± 579	3429 ± 1484
	Tmax (h)	0.6 ± 0.4	0.7 ± 0.3	0.3 ± 0.1	0.7 ± 0.3	1.5 ± 1.3	3.0 ± 0.0
	AUC 0-24h (ng*h/mL)	7280 ± 2835	9132 ± 1803	14018 ± 3288	17864 ± 2559	17030 ± 5308	29066 ± 7986
Day 13	Cmax (ng/mL)	1245 ± 305	2326 ± 413	2647 ± 402	3225 ± 754	3079 ± 1466	5581 ± 641
	Tmax (h)	0.7 ± 0.3	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	2.1 ± 1.6	0.6 ± 0.4
	AUC 0-24h (ng*h/mL)	7004 ± 302	10181 ± 891	17948 ± 3090	17470 ± 2352	22823 ± 3454	27960 ± 5338

Rat 2-week toxicity study of DEA-1496 (draft)

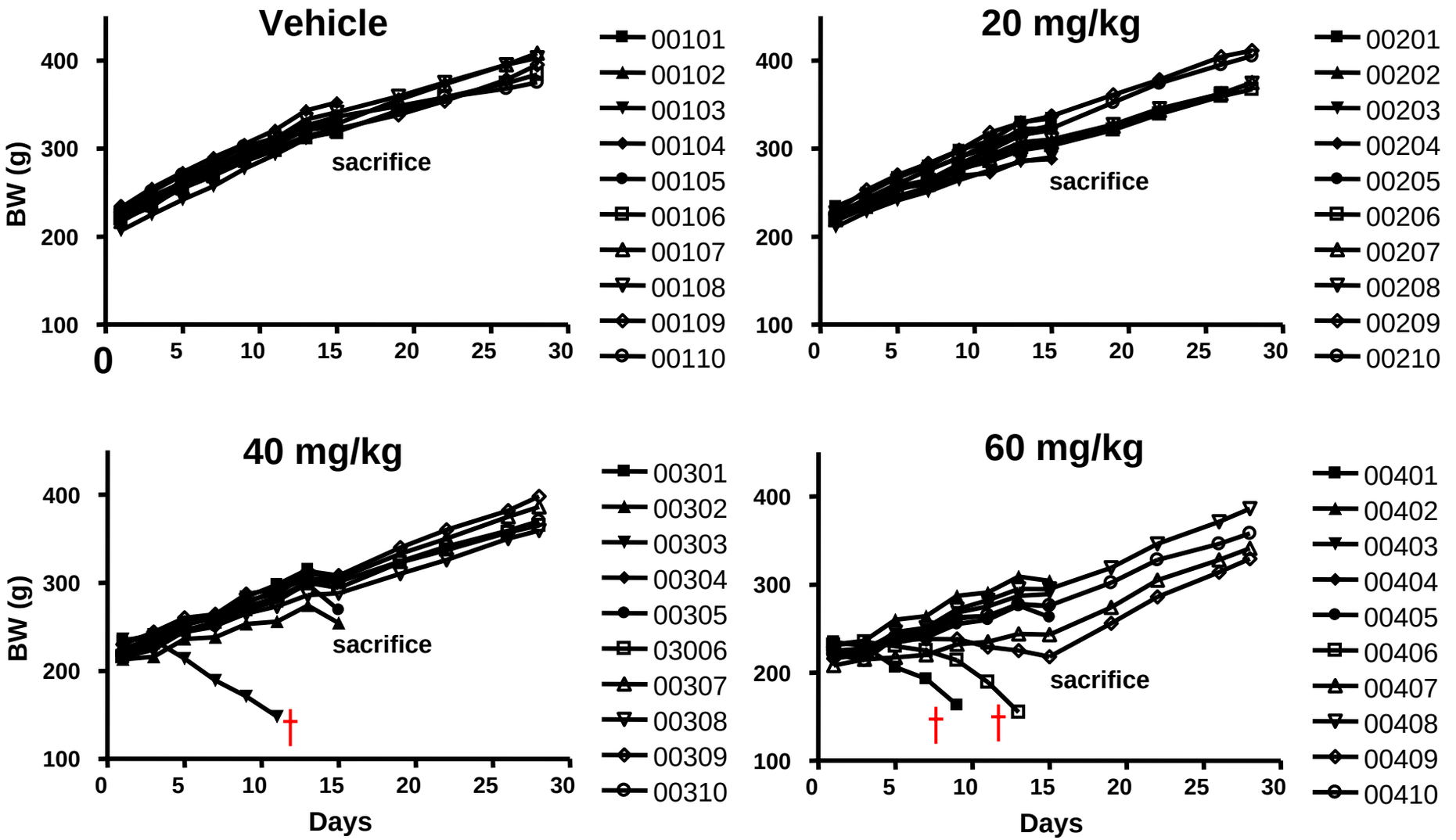
Dose (mg/kg/day)	20	40	60
Mortality	0/20	1/20	4/20
Clinical signs		Decrease in locomotor activity; loss of fur; diarrhea.	
			Hypothermia; abdominal distention; loose stool.
BW	Suppression / decrease.		
FC	Decrease.		
Hematology	↓ Reticulocyte, monocyte, and eosinophil.		
		↓ WBC, neutrophil, lymphocyte, RBC, Hb, and Ht.	
			↓ Platelet.
Blood chemistry		↑ Inorganic phosphorus.	
			↓ T-chol, PL, Alb, and A/G.
Histopathology (Scheduled sacrifice animals)	Atrophy in thymus; changes of extramedullary hematopoiesis in spleen, changes (atrophy, degenerative spermatogenic cells, decrease of sperm, etc.) in testes and epididymides, changes in skin.		
		Atrophy in lymph node; decrease of hematopoiesis in bone marrow; gastrointestinal toxicity (single cell death, atrophy, mitosis, karyomegary, etc.); foreign body giant cell granuloma in subcutis.	
			Extramedullary hematopoiesis in liver; hypertrophy or mitosis in adrenals; single cell death in corneal epithelium.

Rat recovery toxicity study of DEA-1496 (draft)

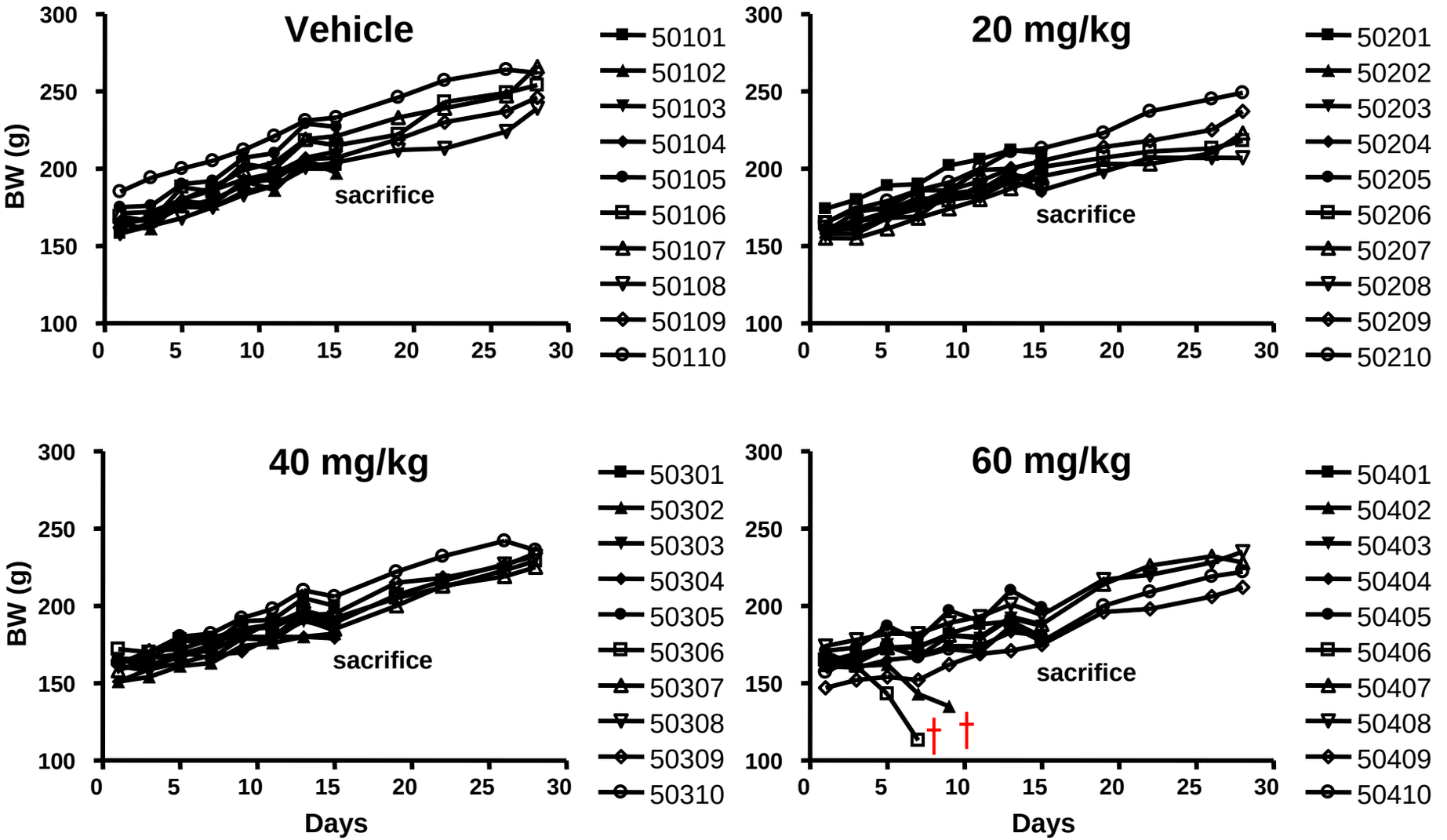
Dose (mg/kg/day)	20	40	60
Mortality	0/10	0/10	0/8
Clinical signs		Loss of fur.	
BW	Suppression.		
Hematology		↓ Monocyte; ↑ Reticulocyte and MCV.	
Histopathology (Scheduled sacrifice animals)	Increase of extramedullary hematopoiesis in spleen; atrophy of seminiferous tubule and hyperplasia of Leydig cells in testes; decrease of sperm and degenerative spermatogenic cells in epididymides; changes in skin.		
		Decrease of hematopoiesis in bone marrow.	
			Foreign body giant cell granuloma in subcutis.

	2w wash-out	
BM suppression	→	BM suppression
Loss of fur	→	Loss of fur
Testicular toxicity	→	Testicular toxicity
Suppression of BW gain		…were still observed,
Diarrhea, GI toxicities		but seemed to be
Atrophy in lymphoid organs		with reversibility

BW of individual animals (Male)



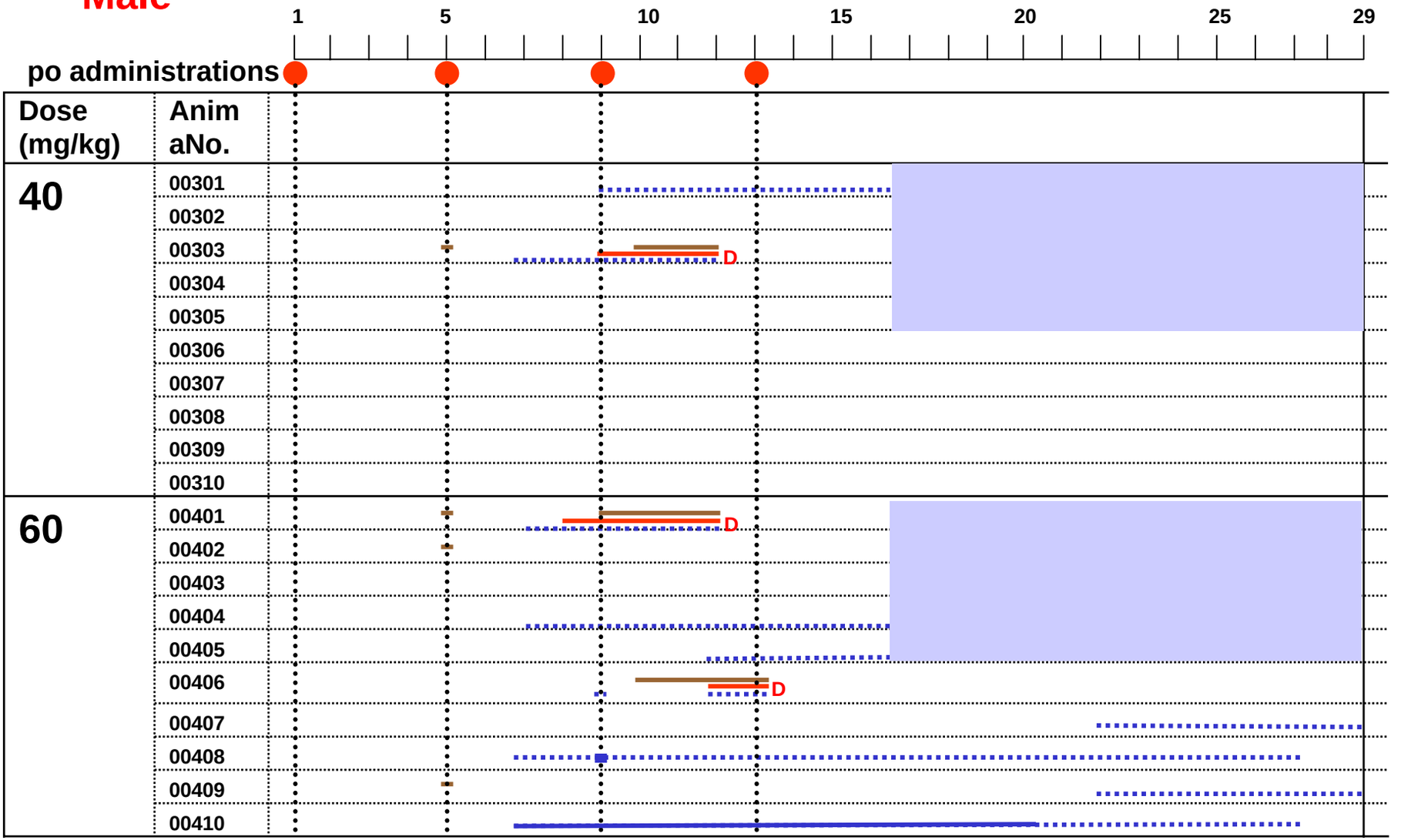
BW of individual animals (Female)



Study No.: 6R217

Observed symptoms

Male



Dirty fur of abdomen, Diarrhea, Soft feces, Abdominal enlargement

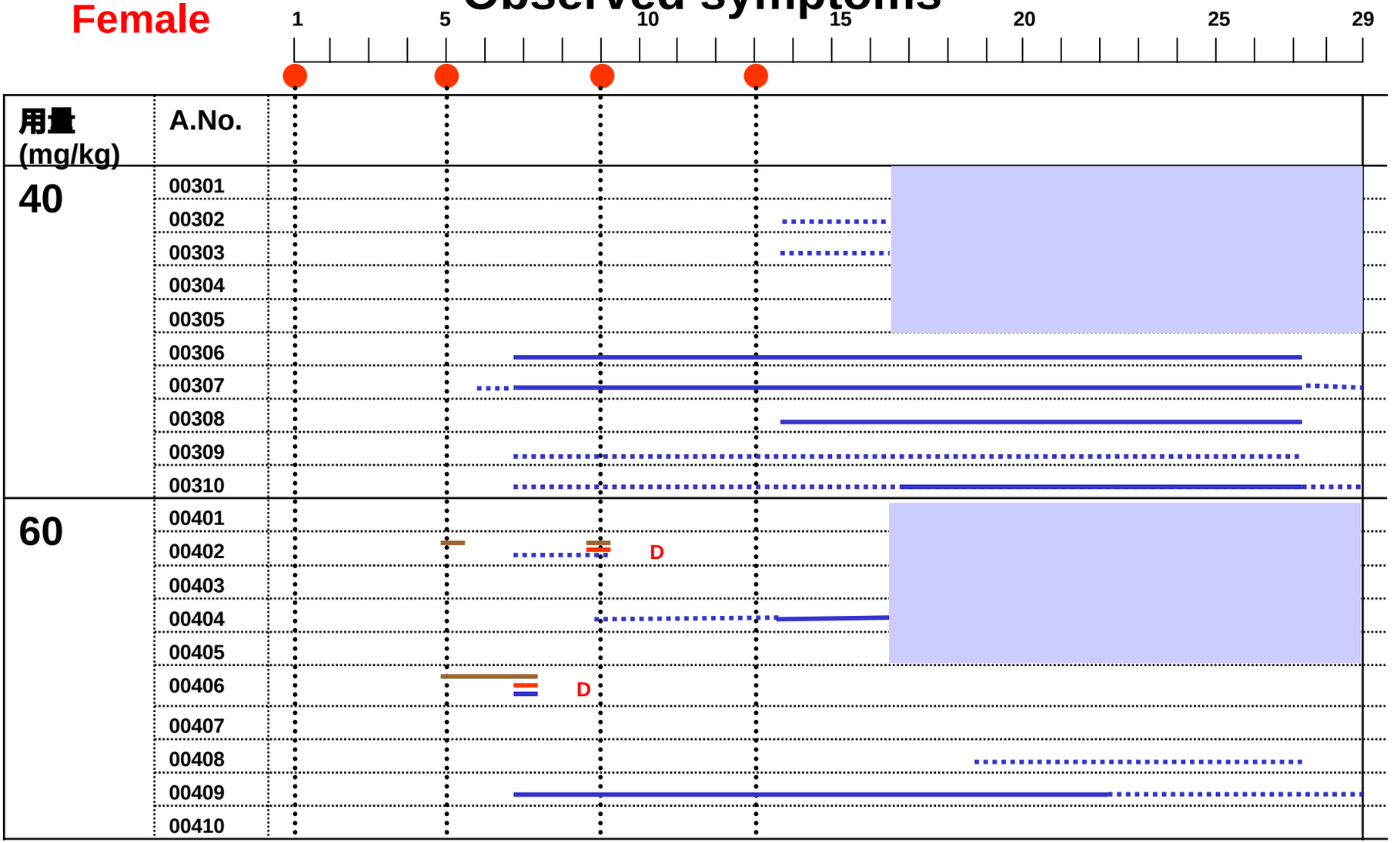
Decrease of spontaneous activity and body temperature, Dirty fur around nose

Abnormal fur Alopecia D: dead

Study No.: 6R217

Observed symptoms

Female



Dirty fur of abdomen, Diarrhea, Soft feces, Abdominal enlargement

Decrease of spontaneous activity and body temperature, Dirty fur around nose

Abnormal fur Alopecia D: dead

Needed to be done before IND application

Tox.

- ☐ Mutagenesity
  - ☑ Ames
  - ◧ Micronucleus
  - ◧ Chromosomal abnormality
- ☑ hERG
- ☑ Receptor binding
- ☐ CV Tox.
  - ◧ Dog ECG
- ☐ Neuronal Tox.
  - ◧
- ☐ Preliminary Tox.
  - ☑ Rat2W
  - ◧ Dog2W
- ☐ GLP tox.
  - ◧ Acute (2 species)
  - ◧ Subacute (2w, 2sp.)
  - ◧ TK validation (2sp.)

PK

- ☑ Solubility
- ☑ Permeability
- ☑ Protein binding
- ☑ Metabolic stability in microsomes
- ☐ PK & BA
  - ☑ Rat
  - ◧ Dog w/ or w/o feeding
- ☑ CYP
  - ☑ Inhibition
  - ☑ Induction
  - ☑ Isoforms involved in its metabolism
- ☑ P-gp
- ☑ Metabolites
  - ☑ Differences among human and others
  - ☑ Estimated main metabolites

Pharmacology

- ☑ Enzyme inhibition
- ☑ Cell line
- ☑ in vivo efficacy
- ☐ in vitro and in vivo differentiation from ;
  - ☑ Chemos
  - ☑ other aurora inh.
  - ◧ other tubulin inh.

CMC

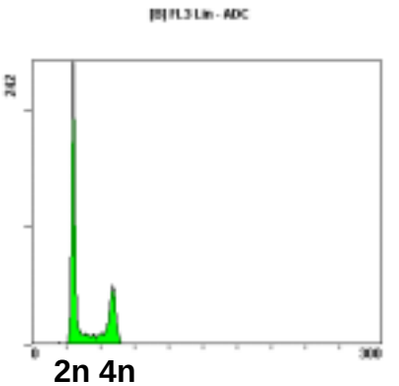
- ☑ ~10g synthesis (5 steps) from commercially available materials without using chromato-graph
- ◧ Scale up for GLP studies
- ◧ Formulation studies (good reproducibility of crystallization with HCl salt-form)

Plans in 2006

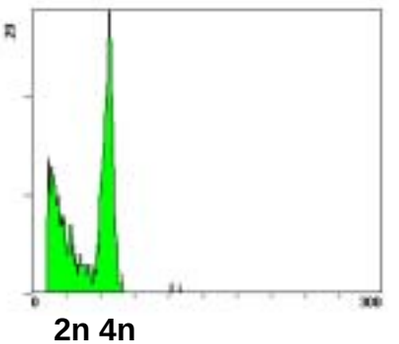
- **Pharmacology**
  - **Further characterization *in vivo* anti-tumor efficacy by using nude rats to confirm the superiority to other chemotherapeutic agents.**
  - **Further studies of mechanisms of action.**

Cell cycle effect

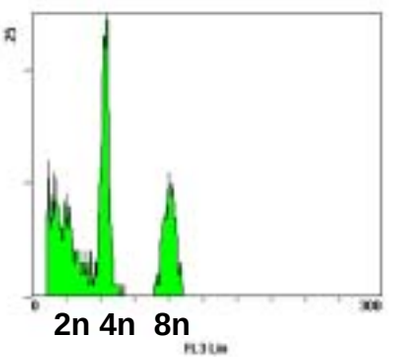
HCT-116 + cpds
↓ 24hr
PI staining, FCM



DMSO



200 nmole/L
Taxol



200 nmole/L
DEA-1496

The cell cycle of HCT-116 (p53wt) was arrested at M-phase by the treatment with DEA-1496 with concomitant decrease of phospho-Histone H3 (not shown).

DEA-1496 has a broad spectrum of inhibitory activity to *in vitro* tumor cell growth

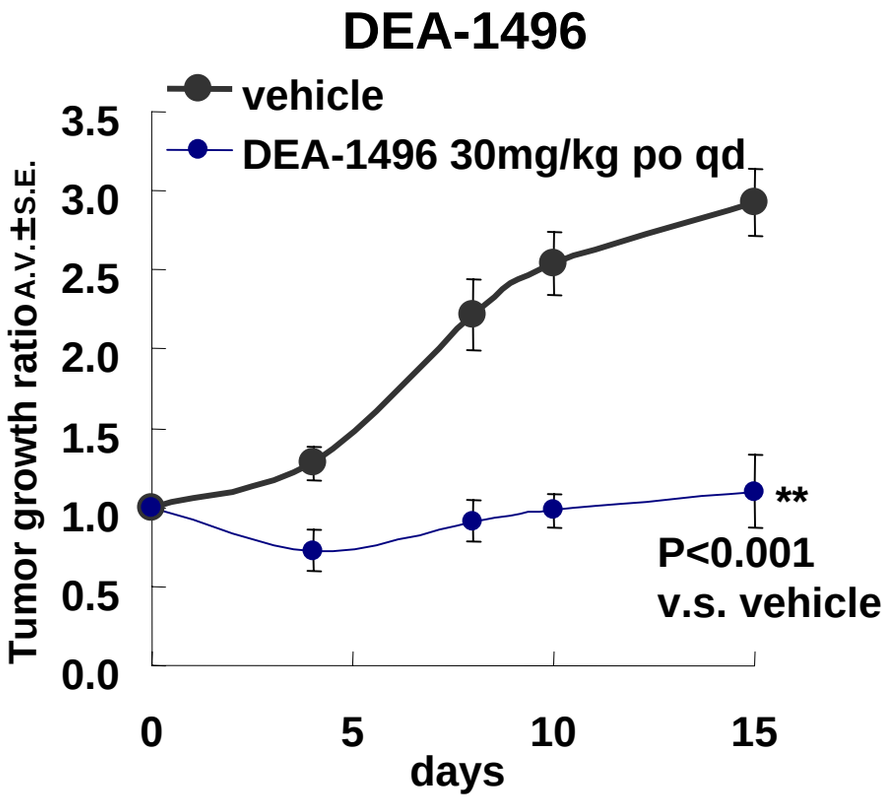
Comparison of GI_{50} (nmol/L; 72hrs) of Aurora kinase inhibitors against a panel of human tumor cell lines

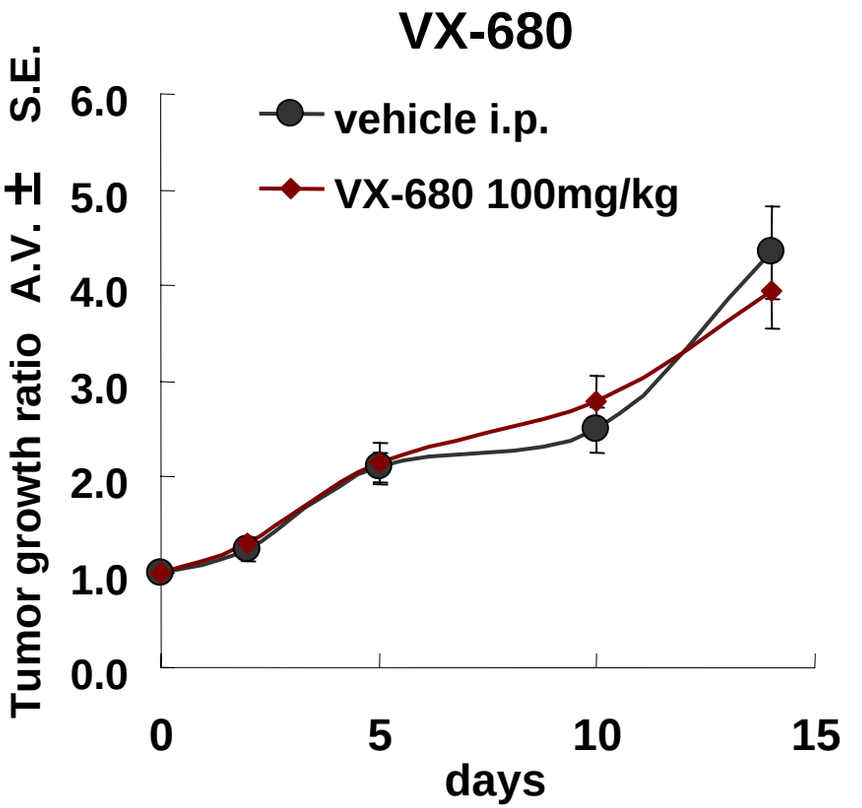
cell line origin	HCT116	SW620	SW480	DLD-1	Caki-1	HCC1937	MDA-MB-435	MDA-MB-453	A431
	colorectal				kidney	Breast			vulva
VX680	15	407	5932	1800	166	16530	5924	15950	>20000
AZD-1152	16	6374	2000	11120	5701	6451	4615	3879	4717
PHA-680632	77	3494	2399	15600	646	5537	3329	321	4377
DEA-1496	7	8	9	7	2	14	<6.4	15	9

cell line origin	PC3	DU145	T47D	U937	HL60	K562	MOLT4	MV4;11	SKOV3	A2780
	prostate			leukemia					ovarian	
VX680	6471	2637	930	25	12	21	619	9	10008	16
AZD-1152	9711	11910	5411	4162	1	7814	1044	<6.4	6124	<6.4
PHA-680632	7574	1693	711	476	138	5978	353	66	2459	87
DEA-1496	4	13	9	6	7	62	9	1	3	6

cell line origin	PK8	PANC-1	HPAC	BxPC3	H1650	H1666	H1975	KLN205	Calu-6	A549
	pancreatic							NSCLC		
VX680	5950	>20000	5866	9565	>20000	4871	6683	3325	13650	3267
AZD-1152	5800	16890	6448	34	6912	4850	6100	5718	8311	2479
PHA-680632	2580	>20000	10319	758	9338	1182	3449	>20000	17390	2448
DEA-1496	8	9	44	6	173	54	16	7	129	44

Inhibition of *in vivo* tumor growth of PC-3 resistant to VX-680, on nude mice



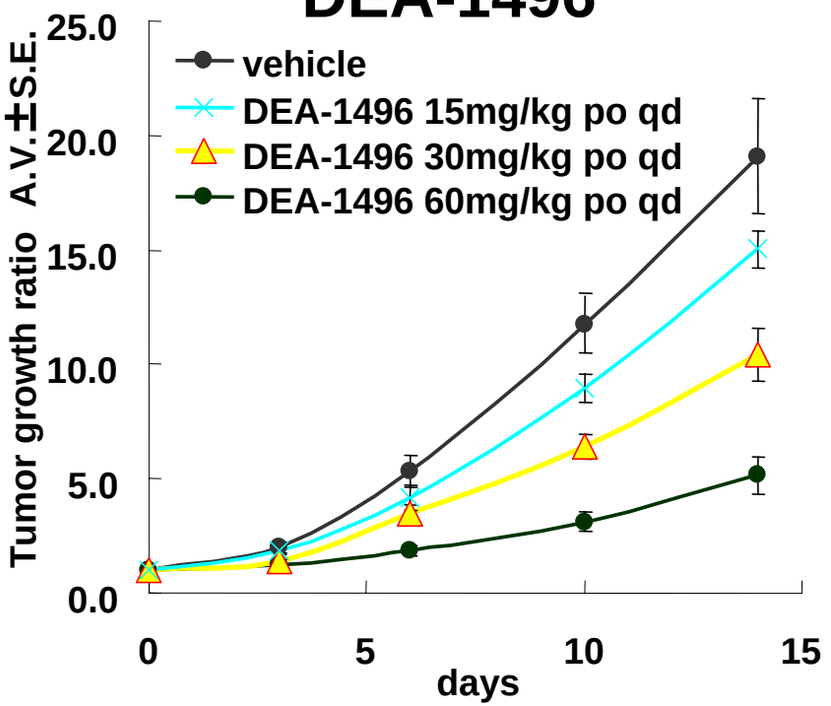


30 mg/kg, p.o.	95.0%inh.

100 mg/kg, i.p.	12.1%inh.

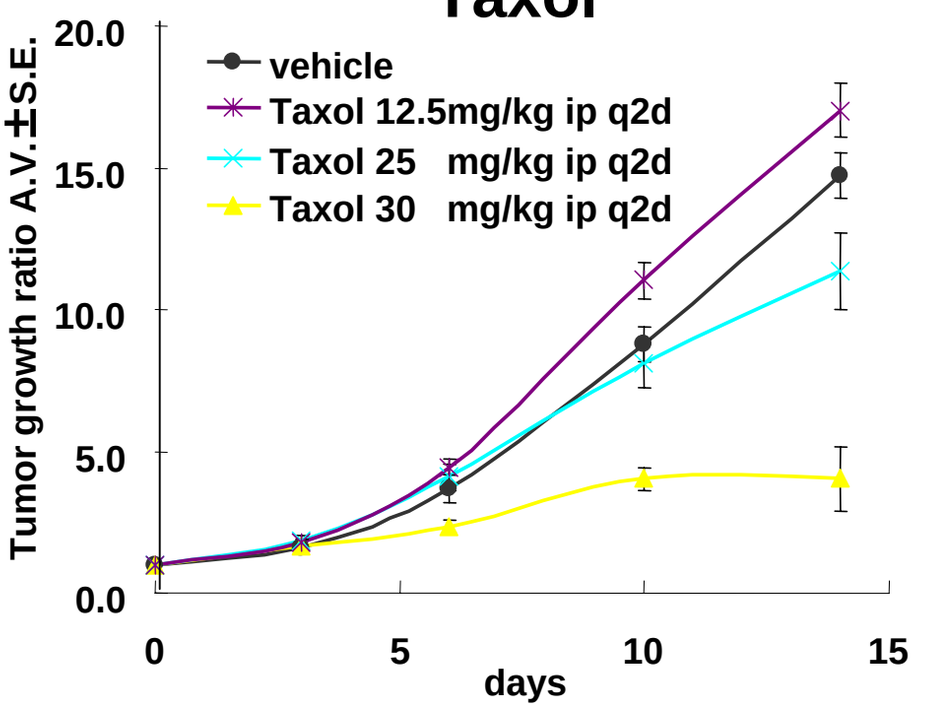
Inhibition of in vivo tumor growth of CT-26 (a mouse colon cancer cell line; SC) on Balb/c mice

DEA-1496



Dosage [mg/kg, po]	% inh.
15	22.5
30	47.8
60 (MTD)	77.1

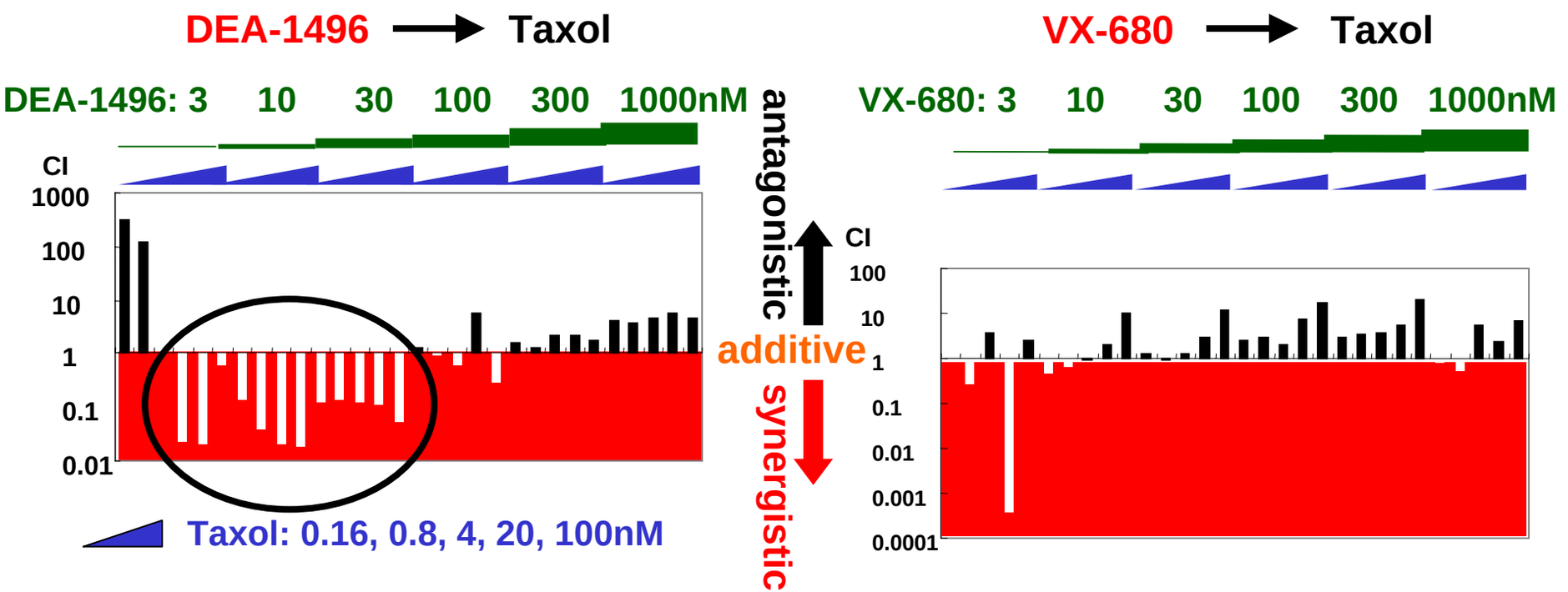
Taxol



Dosage [mg/kg, ip]	% inh.
12.5(q2d)	22.5
25(q2d; MTD)	47.8
30(q2d)	Lethal (3/7)

Synergistic inhibition of *in vitro* tumor cell growth by DEA-1496 and Taxol

Only the sequential treatment with DEA-1496 followed by Taxol elicited synergistic inhibition of the growth of HCT-116 for 3 days



Combination Index (CI) Plot

Induction of expression of human CYP1A2 by DEA-1496 is reversible

Intermittent dosing may be effective to avoid induction of CYP1A2 which metabolized DEA-1496 itself most efficiently

Exposure cultured human hepatocytes to each conc of drugs for 48hrs

↓

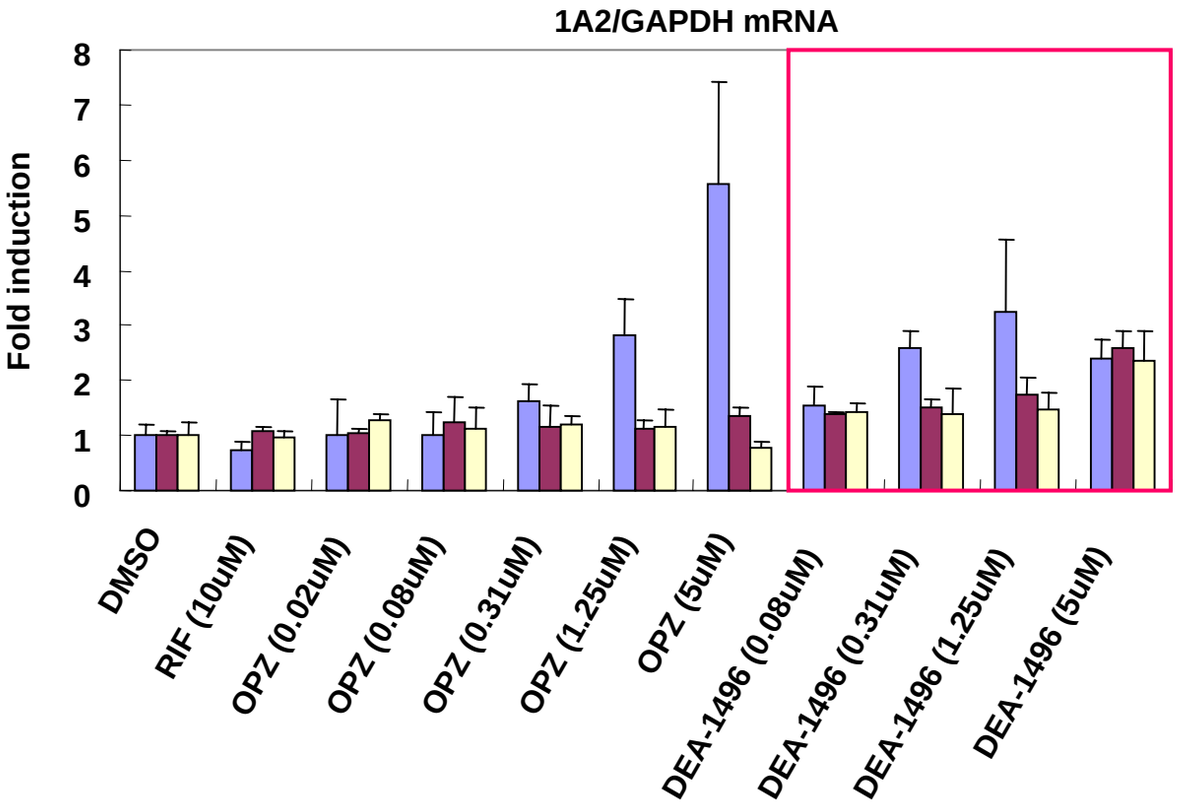
Wash out

↓

Further incubation for 0hr, 24hrs, or 48hrs

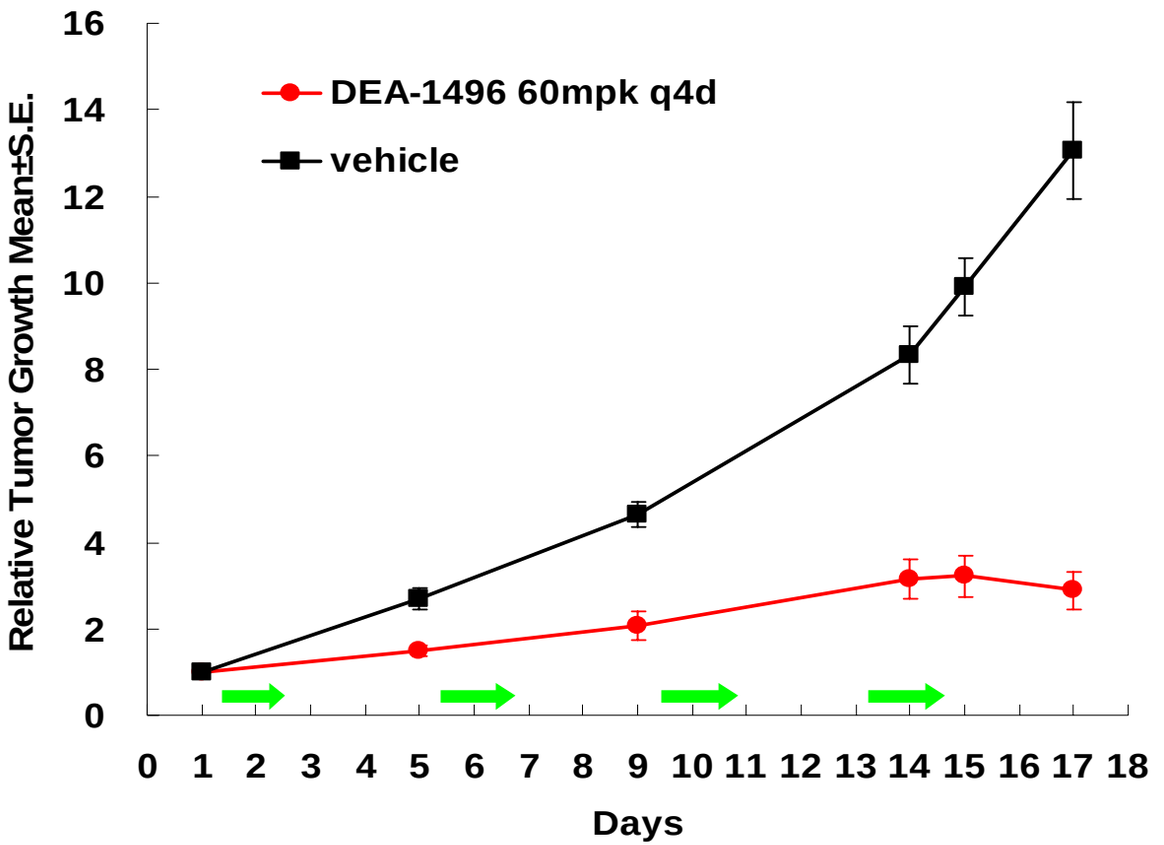
↓

mRNA sampling



Inhibition of *in vivo* growth of HCT-116 Sc xenografts on nude rats

As intermittent oral administrations of DEA-1496 significantly inhibited growth of HCT-116 *in vivo*, Rat 2w TOX is currently being conducted on a similar dosing and schedule



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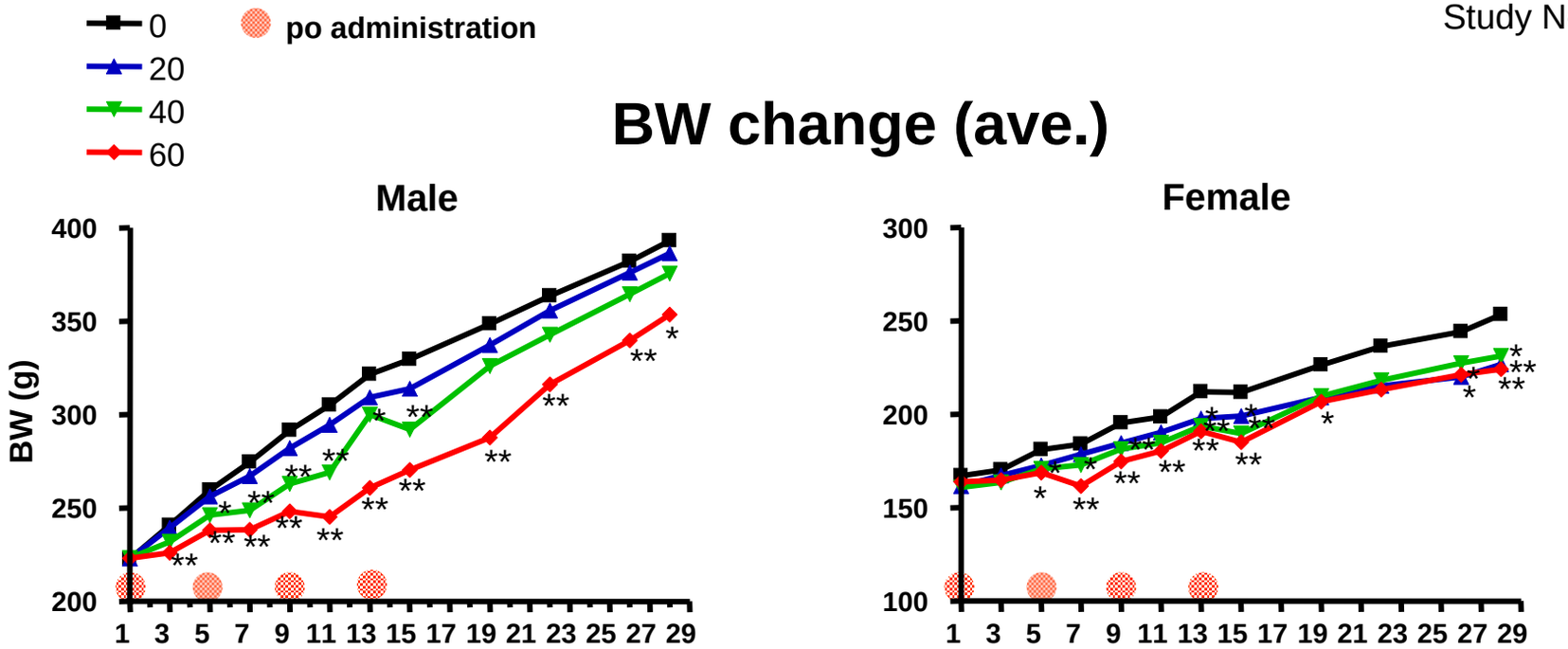
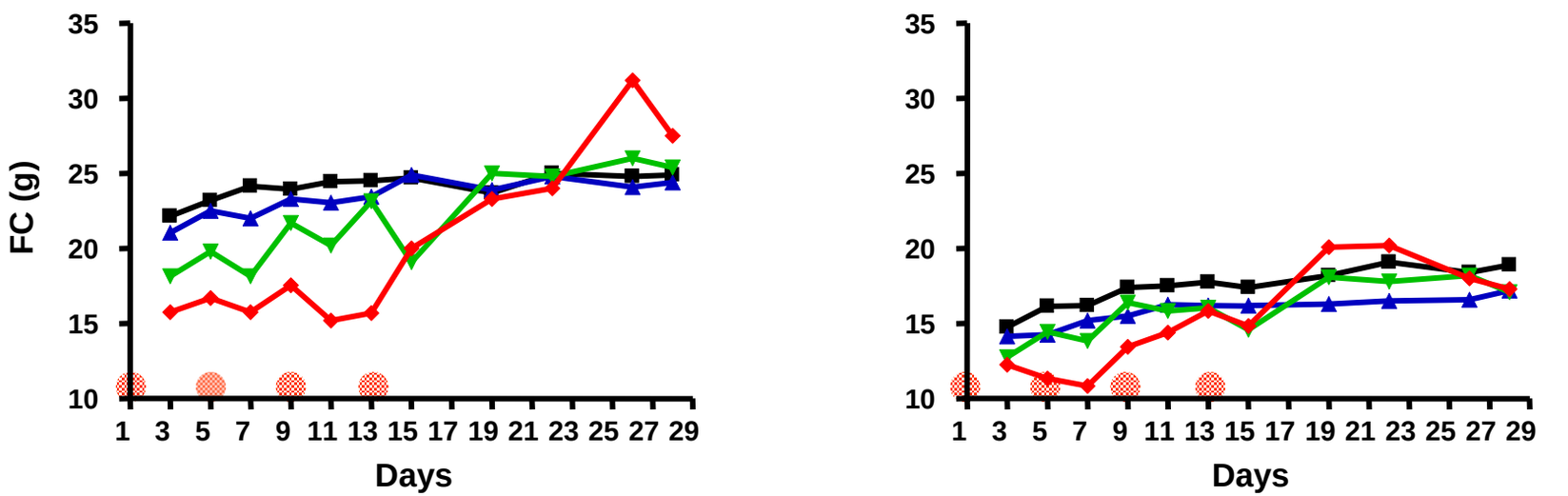
BW change (ave.)



Food intake (ave)



Thank you!