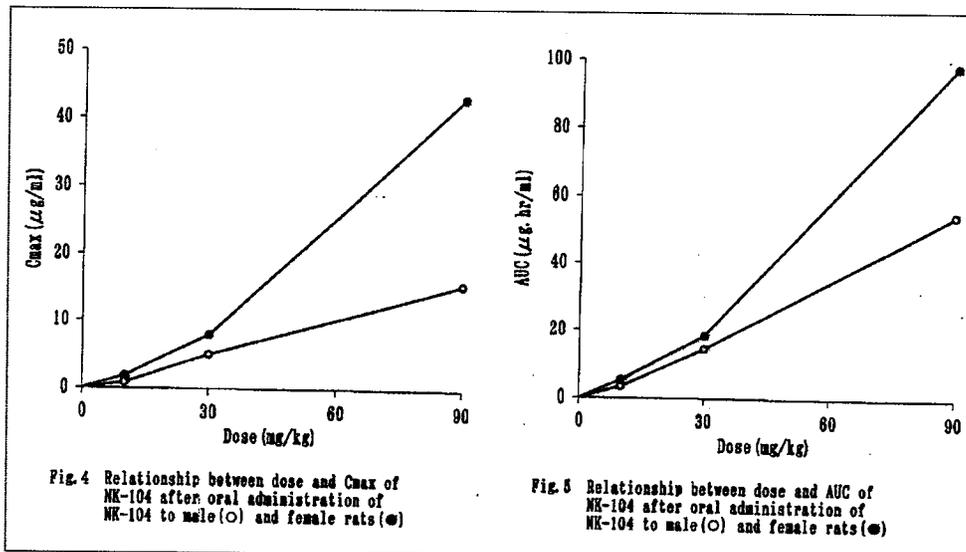


**AG25003 – Plasma drug level in rats (Crj:CD(SD)) in single oral dose toxicology study of NK-104**

Crj:CD (SD) rats (n=4 or 5/sex/time-point) were administered a single oral dose of 10, 30, and 90 mg/kg NK-104.  $C_{max}$  and AUC was significantly higher in females than males at the 90 mg/kg dose; females showed higher total exposure to NK-104 at every dose than did males.



(Sponsor, M4, AG25003, p12)

**Kimata H, Fujino H, Koide T, Yamada Y, Tsunenari Y, Yanagawa Y. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (1): Absorption, distribution, metabolism and excretion in rats; Xenobio Metabol Dispos 1998; 13: 484-498 [Kimata *et al.*, 1998]**

**Key study findings:**

- The authors also examined the pharmacokinetic parameters of unchanged NK-104 in rats. The lung and liver showed the longest half-life and heart and skeletal muscle showed the lowest half-life.
- Greater than 99% of radioactivity was excreted in feces within 72 hours after dosing, while less than 1% was excreted in urine.
- NK-104 was estimated to be a poor substrate for P450 enzymes in rats.

The authors examined the pharmacokinetic parameters of unchanged NK-104 in plasma, heart, lung, liver, kidney, and skeletal muscle in rats. Major site of distribution was to the liver, and liver  $C_{max}$  and AUC were approximately 19 and 32 times higher than plasma  $C_{max}$  and AUC, respectively. Heart tended to accumulate radioactivity up to 24 hours after radiolabeled pitavastatin administration, and these metabolites were determined to be  $\beta$ -oxidation products (mostly M-6,  $C_{max}$  ~1 hour, to a lesser extent M-3,  $C_{max}$  ~3 hours, and M-8 accumulated and became the predominant metabolite in heart much later,  $C_{max}$  ~24 hours). The authors also examined the pharmacokinetic parameters of unchanged NK-104 in rats. The lung and liver showed the longest half-life and heart and skeletal

muscle showed the lowest half-life. Greater than 99% of radioactivity was excreted in feces within 72 hours after dosing, while less than 1% was excreted in urine. NK-104 was estimated to be a poor substrate for P450 enzymes in rats.

**Table V A:** Concentration of unchanged NK-104 and its metabolites in plasma, liver, kidney, lung, heart and skeletal muscle after oral administration of <sup>14</sup>C-NK-104 to male rats (dose: 1 mg/kg)

Tissue	Time (hr)	Concentration (µg/g)										
		NK-104	Lactone	M-3	M-4	M-6	M-7	M-8	M-10	M-11	Unknown	Polar
Plasma	0.5	0.200±0.077	n.d.	0.011±0.005	0.001±0.001	0.013±0.012	n.d.	0.004±0.000	n.d.	n.d.	0.004±0.001	0.005±0.002
	1	0.079±0.029	n.d.	0.003±0.004	n.d.	0.005±0.004	n.d.	0.004±0.001	n.d.	n.d.	0.002±0.001	0.004±0.001
	3	0.033±0.013	n.d.	0.001±0.002	n.d.	0.006±0.007	n.d.	0.004±0.001	n.d.	n.d.	n.d.	0.005±0.001
	6	0.013±0.005	n.d.	n.d.	n.d.	0.003±0.002	n.d.	0.005±0.002	n.d.	n.d.	n.d.	0.007±0.001
	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.007±0.001	n.d.	n.d.	n.d.	0.006±0.001
Liver	0.5	3.812±1.545	0.055±0.030	0.029±0.012	0.014±0.012	0.022±0.019	0.004±0.002	0.009±0.004	0.009±0.008	0.009±0.006	0.114±0.057	0.796±0.330
	1	2.112±0.205	0.027±0.017	0.050±0.044	0.008±0.007	0.013±0.011	0.006±0.005	0.008±0.006	0.008±0.009	0.007±0.007	0.112±0.059	0.516±0.080
	3	0.794±0.210	0.008±0.003	0.006±0.002	0.002±0.002	0.004±0.004	0.002±0.001	0.011±0.002	0.003±0.002	0.003±0.002	0.031±0.013	0.304±0.002
	6	0.404±0.116	0.002±0.002	0.002±0.002	0.001±0.001	0.002±0.002	n.d.	0.009±0.005	0.004±0.004	0.001±0.000	0.009±0.004	0.197±0.027
	24	0.006±0.005	n.d.	n.d.	n.d.	n.d.	n.d.	0.005±0.002	0.002±0.002	0.001±0.000	0.002±0.001	0.064±0.013
Kidney	0.5	0.366±0.090	0.001±0.001	0.032±0.017	0.001±0.001	0.007±0.007	n.d.	0.002±0.000	n.d.	n.d.	0.012±0.004	0.069±0.011
	1	0.149±0.019	n.d.	0.015±0.004	n.d.	0.004±0.004	n.d.	n.d.	n.d.	n.d.	0.003±0.001	0.058±0.017
	3	0.048±0.008	n.d.	0.008±0.004	n.d.	0.004±0.005	n.d.	0.001±0.001	n.d.	n.d.	0.001±0.002	0.025±0.011
	6	0.032±0.006	n.d.	0.003±0.002	n.d.	0.003±0.002	n.d.	0.002±0.001	n.d.	n.d.	n.d.	0.018±0.005
	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.010±0.001	n.d.	n.d.	n.d.	0.008±0.001
Lung	0.5	0.131±0.027	n.d.	0.001±0.001	n.d.	0.003±0.003	n.d.	n.d.	n.d.	n.d.	n.d.	0.017±0.008
	1	0.034±0.030	n.d.	0.007±0.002								
	3	0.017±0.003	n.d.	0.004±0.000								
	6	0.011±0.004	n.d.	0.004±0.001								
	24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.004±0.001
Heart	0.5	0.101±0.021	n.d.	0.008±0.007	0.001±0.001	0.046±0.040	n.d.	0.001±0.001	n.d.	n.d.	0.001±0.001	0.012±0.004
	1	0.041±0.020	n.d.	0.003±0.002	n.d.	0.047±0.040	n.d.	0.001±0.000	n.d.	n.d.	n.d.	0.012±0.003
	3	0.007±0.003	n.d.	0.021±0.025	n.d.	0.029±0.034	n.d.	0.005±0.002	n.d.	n.d.	n.d.	0.008±0.005
	6	n.d.	n.d.	0.001±0.001	n.d.	0.022±0.013	n.d.	0.008±0.004	n.d.	n.d.	n.d.	0.006±0.001
	24	n.d.	n.d.	n.d.	n.d.	0.031±0.015	n.d.	0.038±0.001	n.d.	n.d.	0.007±0.002	0.006±0.002
Skeletal muscle	0.5	0.010±0.010	n.d.	n.d.	n.d.	0.003±0.002	n.d.	n.d.	n.d.	n.d.	n.d.	0.003±0.002
	1	0.011±0.003	n.d.	n.d.	n.d.	0.003±0.003	n.d.	n.d.	n.d.	n.d.	n.d.	0.004±0.001
	3	0.003±0.003	n.d.	n.d.	n.d.	0.004±0.005	n.d.	n.d.	n.d.	n.d.	n.d.	0.002±0.001
	6	n.d.	n.d.	n.d.	n.d.	0.002±0.002	n.d.	n.d.	n.d.	n.d.	n.d.	0.002±0.002
	24	n.d.	n.d.	n.d.	n.d.	0.006±0.003	n.d.	0.008±0.001	n.d.	n.d.	n.d.	0.003±0.001

Each value represents the mean±S.D. of three rats. n.d. : not detected

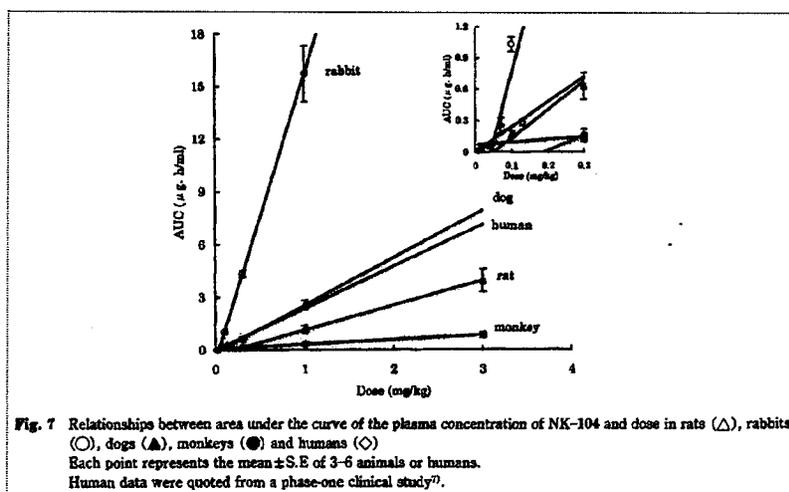
(Sponsor, M4, Kimata et al., p14-15)

**Fujino H, Kojima J, Yamada Y, Kanda H, Kimata H. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (4): Interspecies variation in laboratory animals and humans; Xenobio Metabol Dispos 1999; 14: 79-91 [Fujino et al., 1999a]**

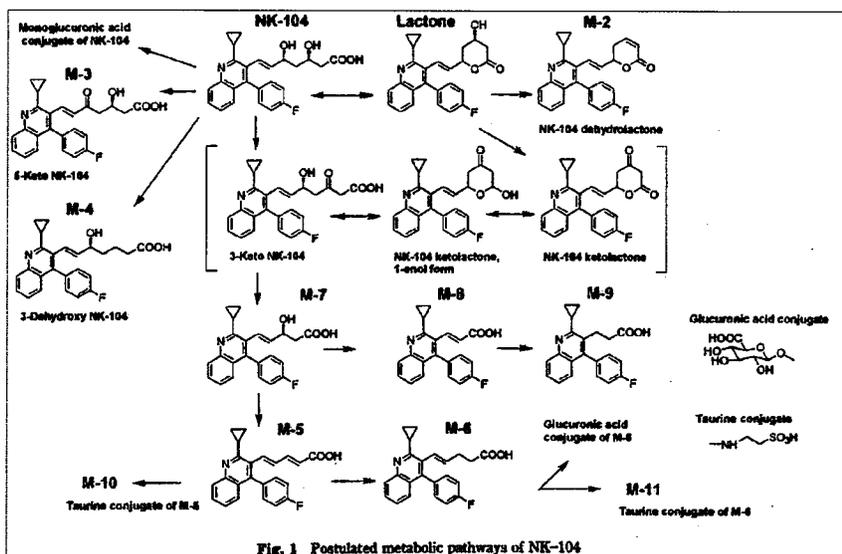
#### Key study findings:

- Exposure to NK-104 (AUC) as a function of dose was greatest in rabbits >>dogs ≥humans >rats >monkeys.
- NK-104 lactone was the primary metabolite in humans and dogs.
- Fecal excretion was the primary route of excretion in humans, rats, dogs, and monkeys; urinary excretion was the primary route of excretion in rabbits.
- NK-104 was determined to be a poor substrate for P450 enzymes, due to relatively low concentrations of oxidation products detected in human plasma.

Pharmacokinetic parameters were tested for humans, rats, rabbits, dogs, and monkeys after single intravenous administration and/or single oral administration of pharmacologic doses of NK-104 (~ 0.1 mg/kg i.v., rabbits and dogs; 0.3 mg/kg i.v., monkeys; 1 mg/kg i.v., rats; ~ 0.1 to 1 mg/kg p.o., rabbits and dogs; 0.3 to 3 mg/kg, rats and monkeys). Humans were administered 2 mg NK-104 p.o. daily for 5 days (the approved dose in Japan).



(Sponsor, M4, Fujino et al. 1999, p13)



(Sponsor, M4, Fujino et al. 1999, p5)

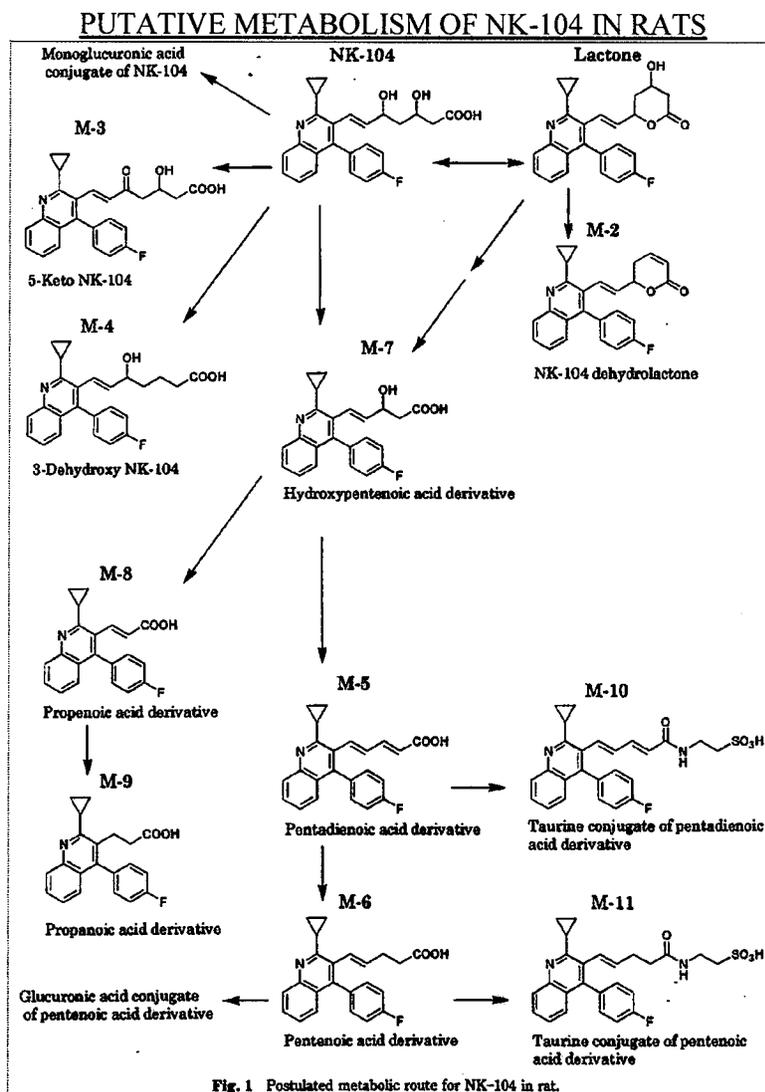
**Fujino H, Tsunenari Y, Koide T, Yonemitsu M, Yanagawa Y, Kimata H. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (2): Absorption, distribution, metabolism, excretion and accumulation following repeated oral administration of [<sup>14</sup>C]-NK-104 in rats; Xenobio Metabol Dispos 1998; 13: 499-507 [Fujino et al., 1998a]**

**Key study findings:**

- Of that amount accumulated in heart tissue, the predominant form was a pentenoic acid derivative (M-6) followed by a propenoic acid derivative (M-8).
- M-6 and M-8 were also the form that predominated in skeletal muscle.

[<sup>14</sup>C]-Labeled NK-104 was administered to rats (n=4) for 9 days at 1 mg/kg. NK-104 appeared to be more concentrated in the liver, kidney, and skeletal muscle. Excretion

was almost exclusively in feces. Parent NK-104 was the predominant form excreted. Of that amount accumulated in heart tissue, the predominant form was a pentenoic acid derivative (M-6) followed by a propenoic acid derivative (M-8). M-6 and M-8 were also the form that predominated in skeletal muscle. Steady state plasma concentrations were reached on Day 4, which were approximately 2-fold higher than plasma concentrations at  $C_{max}$  on Day 1.



(Sponsor, M4, Fujino et al. 1998, p4)

**RF9932 – Plasma concentration of NK-104 after repeated oral administration to pregnant rats**

NK-104 (3, 10, and 30 mg/kg) was administered to pregnant Crj:CD rats (2-4 animals/group) during the period of fetal organogenesis (Day 7-18 of pregnancy/segment II) and 1 mg/kg NK-104 was administered to Jla:Wistar rats (2-3 animals/group) during late pregnancy (Days 17-21/early segment III). Doses for the segment II study were

based on a teratogenicity study conducted earlier (see study RFG2508), where a NOEL was determined to be 10 mg/kg. Doses for the early segment III study were based on an earlier study (see study RFG2511). Plasma NK-104 and NK-104 lactone were quantitated by HPLC. Pharmacokinetics of NK-104 exposure were similar between non-pregnant rats, pregnant rats during the period of fetal organogenesis, and pregnant rats during the early segment III period from Day 17 to parturition.

**Table IV. Plasma concentrations and pharmacokinetic parameters of NK-104 after repeated oral administration of NK-104 to the pregnant during the period of fetal organogenesis**

Group No.	Plasma Concentration (µg/mL)					T <sub>max</sub> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>0-24</sub> (µg.hr/mL)
	Time after administration (hr)							
	0.5	1	2	6	24			
<b>Group: 1 (1 mg.kg)</b>								
1	0.10	0.11	0.14	0.07	0.06	2.0	0.14	1.79
2	0.08	0.08	0.04	0.04	0.04	0.5	0.08	1.00
3	0.08	0.04	0.06	0.03	0.04	0.5	0.08	0.91
4	0.11	0.12	0.09	0.05	0.04	1.0	0.12	1.28
Mean	0.09	0.09	0.08	0.05	0.05	1.0	0.11	1.25
S.D.	0.02	0.04	0.04	0.02	0.01	0.7	0.03	0.40
<b>Group: 2 (3 mg.kg)</b>								
1	0.18	0.14	0.12	0.10	0.09	0.5	0.18	2.41
2	1.33	0.74	0.39	0.14	0.05	0.5	1.33	4.19
3	0.20	0.26	0.30	0.08	0.09	2.0	0.30	2.74
4	0.31	0.29	0.24	0.17	0.13	0.5	0.31	4.01
Mean	0.51	0.36	0.26	0.12	0.09	0.9	0.53	3.33
S.D.	0.55	0.26	0.11	0.04	0.03	0.8	0.54	0.90
<b>Group: 3 (10 mg.kg)</b>								
1	2.07	1.69	1.05	0.52	0.18	0.5	2.07	12.27
2	-	-	1.49	0.75	0.11	2.0	1.49	12.97
Mean	2.07	1.69	1.27	0.64	0.15			
S.D.			0.31	0.16	0.05			
<b>Group: 3 (10 mg.kg) Non pregnant Animal</b>								
3	5.04	3.32	2.87	0.53	0.14	0.5	5.04	19.28
4	6.80	3.48	1.34	0.41	0.29	0.5	6.80	16.48
Mean	5.92	3.40	2.11	0.47	0.22	0.5	5.92	17.88
S.D.	1.24	0.11	1.08	0.08	0.11	0.0	1.24	1.98

-: No sample because missing of extraction of blood

(Sponsor, M4, RF9932, p19)

**Table V. Plasma concentrations and pharmacokinetic parameters of NK-104 after repeated oral administration of NK-104 to the pregnant rats during the late pregnancy**

Group No.	Plasma Concentration (µg/mL)					T <sub>max</sub> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>0-24</sub> (µg·hr/mL)
	Time after administration (hr)							
	0.5	1	2	6	24			
<b>Group: 4 (1 mg.kg)</b>								
1	0.05	0.04	0.05	0.03	0.08	24.0	0.08	1.23
2	0.09	0.06	n.d.	n.d.	0.05	0.5	0.09	0.54
3	n.d.	0.01	n.d.	n.d.	0.02	24.0	0.02	0.19
4	0.08	0.06	0.02	n.d.	0.04	0.5	0.08	0.50
Mean	0.06	0.04	0.02	0.01	0.05	12.3	0.07	0.61
S.D.	0.04	0.02	0.02	0.02	0.03	13.6	0.03	0.44
<b>Group: 5 (3 mg.kg)</b>								
1	1.51	0.80	0.54	0.18	0.45	0.5	1.51	8.74
2	0.09	0.09	0.05	n.d.	0.04	0.5	0.09	0.60
3	0.50	0.34	0.31	0.04	0.14	0.5	0.50	2.98
Mean	0.70	0.41	0.30	0.07	0.21	0.5	0.70	4.10
S.D.	0.73	0.36	0.25	0.09	0.21	0.0	0.73	4.18
<b>Group: 6 (10 mg.kg)</b>								
2	6.51	4.39	2.07	0.44	0.10	0.5	6.51	26.46
3	1.78	1.60	0.99	0.14	0.09	0.5	1.78	6.92
Mean	4.15	3.00	1.53	0.29	0.60	0.5	4.15	16.69
S.D.	3.34	1.97	0.76	0.21	0.71	0.0	3.34	13.82
<b>Group: 5 (3 mg.kg) Non pregnant Animal</b>								
4	0.98	0.51	0.44	0.10	0.02	0.5	0.98	3.25
<b>Group: 6 (10 mg.kg) Non pregnant Animal</b>								
1	2.35	1.99	1.38	0.44	0.24	0.5	2.35	13.12
4	2.93	2.03	1.51	0.31	0.12	0.5	2.93	11.25
Mean	2.64	2.01	1.45	0.38	0.18	0.5	2.64	12.19
S.D.	0.41	0.03	0.09	0.09	0.08	0.0	0.41	1.32

n.d.: Below limit of quantification (&lt; 0.01 µg/mL)

(Sponsor, M4, RF9932, p19)

**R98042 – Pharmacokinetic of [<sup>14</sup>C]-NK-104 in guinea pigs****Key study findings:**

- T<sub>½</sub> was extremely long in guinea pigs, at 38.3 hours
- Urinary excretion represented ~20% of total excretion in that model

Plasma concentrations and urinary/fecal excretion were studied in guinea pigs. Guinea pigs (n=4) were administered 1 mg/kg NK-104. Blood was collected at 0.25, 0.5, 1, 2, 3, 6, 9, 12, 24, 48, 72, 96, and 120 hours post-dose. Metabolic cages were utilized to collect 24-hour fecal and urine samples daily for 6 days.

**PHARMACOKINETICS IN PLASMA**

	Tmax (hr)	Cmax (µg eq./ml)	AUC(t) (µg eq. hr/ml)	AUC(Inf.) (µg eq. hr/ml)	T1/2 (hr)
NK-1	1.0	0.044	1.23	1.44	20.3

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**of exposure in rabbits**

**Key study findings:**

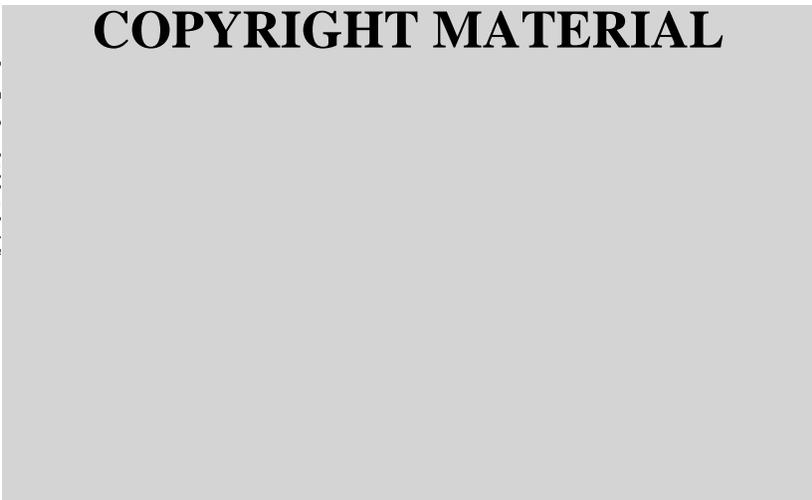
- Doe rabbits showed decreased food consumption and decreased weight at the high-dose of 1 mg/kg pitavastatin.
- Total cholesterol tended to decrease at 0.3 and 1.0 mg/kg pitavastatin, compared to controls that also experienced a decrease in total cholesterol during the administration period.

Kbl:JW(SPF) rabbits were administered NK-104 by oral gavage at 0.1, 0.3, and 1 mg/kg/day from Day 6 to Day 18 of pregnancy. On Day 20 of gestation, animals were anesthetized and euthanized. Ovaries and uterus were removed and corpora lutea and implantations were counted. The state of fetuses was recorded, and live fetuses were examined for anomalies and were weighed; placentas were examined. Blood samples were obtained for toxicokinetic analyses.

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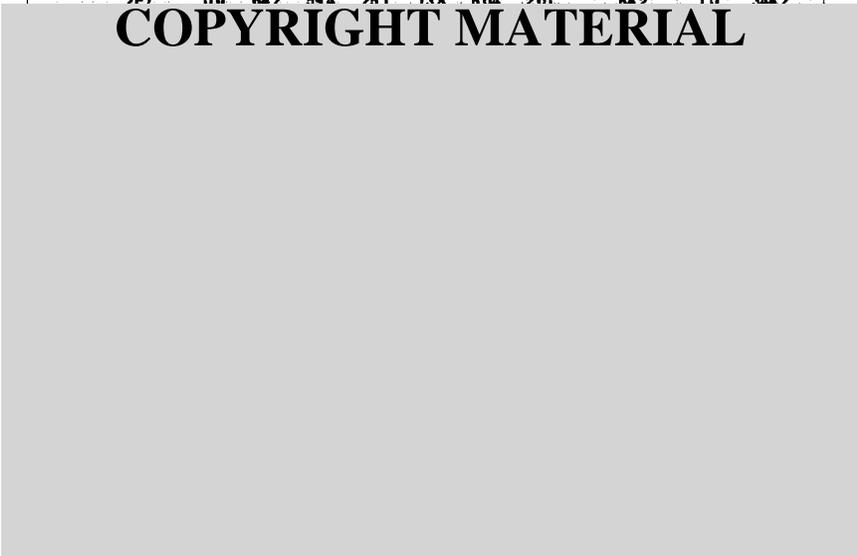
Gro
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8
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12



0.1	2F5	0.0	62.9	76.6	68.7	29.4	15.0	6.97	76.6	2.0	653.3
	2F6	0.0	36.3	45.5	36.6	14.8	5.83	1.26	45.5	2.0	327.8
	2F7	0.0	64.2	58.4	25.1	13.8	8.04	2.01	64.2	1.0	344.2

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SD	0.0	339	163	224	72	48	23.1	210	0.9	1133.6
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(Sponsor)

PHARMACOKINETICS OF PITAVASTATIN, DAY 18

Dose (mg/kg)	Animal No.	Plasma concentration of NK-104 (ng/mL)							Pharmacokinetic parameters		
		0	1	2	4	8	12	24	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hr)	AUC (ng·hr/mL)
0.1	2F1	1.40	58.8	50.3	25.6	12.2	5.89	0.74	58.8	1.0	310.5
	2F2	19.1	88.2	82.8	87.5	50.4	29.1	17.0	87.5	4.0	1018.9
	2F3	1.38	97.3	88.8	58.6	34.8	16.6	2.19	97.3	1.0	693.6
	2F4	4.46	92.8	65.7	38.1	24.9	18.1	6.31	92.8	1.0	584.1
	2F5	5.11	89.5	72.4	38.8	20.8	11.5	3.55	89.5	1.0	513.6
	2F6*	1.19	61.0	52.1	28.9	13.4	5.52	0.98	61.0	1.0	330.1
	2F7	5.65	79.1	78.3	54.4	33.8	12.7	3.50	79.1	1.0	619.
	2F8	4.42	60.2	57.7	40.2	27.4	12.2	4.81	60.2	1.0	505.6

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**R99052 – F**  
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Key study f

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- Parent NK  
glucuroni

Pharmacoki  
Male cynom  
mg/kg NK-

24 hours post-dose, plasma was prepared from whole blood. Urine and feces were collected after 24, 48, and 72 hours (cumulative in 24 hour increments) post-dose. M-13 and NK-104 parent were present in low amounts in urine (~0.2% excretion rate over 72 hours for each).

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**9L804 – Absorption, metabolism, and excretion after single oral and intravenous administration of NK-104 in Cynomolgus monkey**

**Key study findings:**

- In monkeys, NK-104 and metabolites were excreted predominantly in the feces (~78%)
- Urinary excretion was a minor route (~11%)
- Most radioactivity that was recovered in urine and feces was in the form of parent NK-104
- M-14 (aryldihydrodiol) was the predominant metabolite in feces (~13% of total radioactivity recovered) and in urine (47% of total radioactivity recovered)

[<sup>14</sup>C]-labeled NK-104 was administered to male cynomolgus monkeys (n=3) at 3 mg/kg as a single oral dose. One additional monkey was administered a single dose of 0.3 mg/kg radiolabeled NK-104 by i.v. All animals were analyzed for radioactive parent and metabolite levels in plasma, feces, and urine. Blood was collected 30 min, 1, 2, 4, 8, 12, 24 hours, 2, 3, 5 and 7 days for orally administered animals. Additional time points were added for i.v. administered animals at 2, 5, 10 and 20 minutes post-injection. Urine and feces were collected cumulatively for 24 hours for 7 days.

**PHARMACOKINETIC PARAMETERS**

Parameter	3 mg/kg, p.o.		0.3 mg/kg, i.v.	
	Blood	Plasma	Blood	Plasma
t <sub>max</sub> (hr)	3.0 ± 1.7	3.0 ± 1.7	N.A.	N.A.
C <sub>max</sub> (ng eq./mL)	410.4 ± 41.6	540.2 ± 74.2	N.A.	N.A.
AUC <sub>0-168</sub> (µg eq.·hr/mL)	14.7 ± 3.1	22.0 ± 5.2	1.39	1.91
AUC <sub>0-∞</sub> (µg eq.·hr/mL)	15.7 ± 2.9	23.4 ± 4.9	1.56	2.41
F. (%)	101.0 ± 18.6	97.1 ± 20.3	-	-
t <sub>1/2</sub> (λz) (hr)	46.4 ± 14.5	44.4 ± 11.3	49.0	85.6

Data of the p.o. group are expressed as the mean ± S.D. of three monkeys.  
N.A. : Not applicable

(Sponsor, M4, 9L804, p39)

**EXCRETION OF RADIOLABEL AFTER ADMINISTRATION  
OF NK-104 p.o AND i.v.**

Time (hr)	Cumulative excreted radioactivity (% of dose)					
	3 mg/kg, p.o.			0.3 mg/kg, i.v.		
	Urine	Feces	Total	Urine	Feces	Total
0~24	9.1 ± 2.5	4.3 ± 7.3	13.4 ± 9.8	10.1	0.1	10.1
0~48	10.1 ± 2.6	29.0 ± 22.3	39.0 ± 24.9	10.7	52.7	63.4
0~72	10.6 ± 2.6	57.5 ± 6.9	68.0 ± 9.5	11.0	70.2	81.2
0~96	10.8 ± 2.6	68.1 ± 5.4	78.9 ± 6.5	11.1	79.5	90.6
0~120	10.9 ± 2.6	73.9 ± 4.5	84.8 ± 4.2	11.2	86.0	97.2
0~144	11.0 ± 2.6	76.3 ± 4.3	87.3 ± 3.1	11.3	87.6	98.9
0~168	11.0 ± 2.6	77.5 ± 4.4	88.6 ± 2.8	11.3	88.3	99.6

Data of the p.o. group are expressed as the mean ± S.D. of three monkeys.

(Sponsor, M4, 9L804, p39)

#### 2.6.4.4 Distribution

The highest protein-binding was detected in humans >rabbits >rats >monkeys >dogs >mice. Distribution to lens is highest in dogs and mice. The plasma protein-binding ratio for pitavastatin lactone was determined to be 98.95-99.33% in human plasma. Pitavastatin crosses the blood-milk barrier.

Pitavastatin is concentrated in milk (up to 7.2-fold greater than maternal plasma concentration at 6 hours post administration. Pitavastatin (parent) was the primary form found in rat milk. 5-Ketopitavastatin was the primary metabolite observed in rat milk. Fetal pitavastatin concentrations were ≤36% of maternal plasma pitavastatin concentrations.

#### **RF9945 – Plasma concentration of NK-104 after single oral administration to mice**

##### **Key study finding:**

- NK-104 is distributed to bone marrow and plasma at roughly equal concentrations in mice

This study was carried out in order to determine how much NK-104 was found in the bone marrow versus the amount present in plasma.

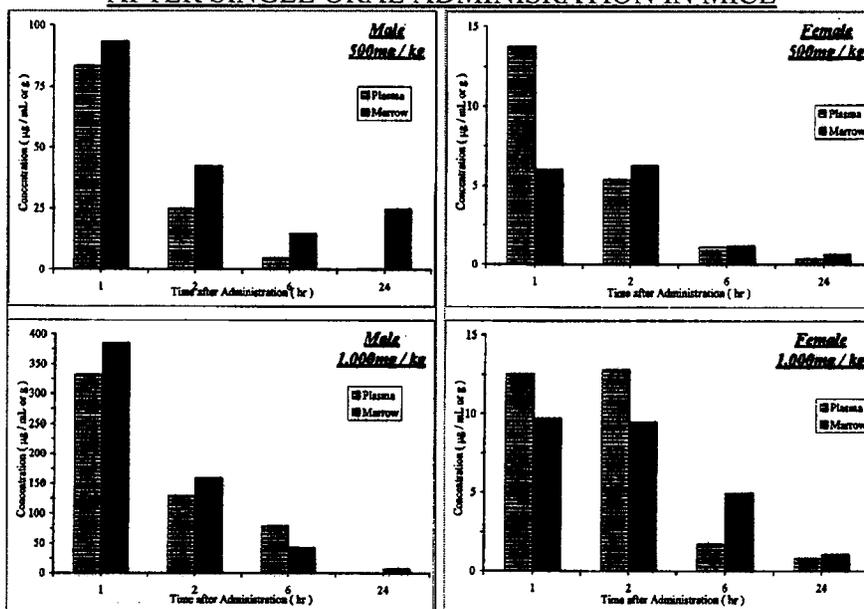
**STUDY GROUP ASSIGNMENTS**

Group No.	Dose (mg/kg)	Volume (mL/kg)	Animal Number (/group)	
			Male	Female
1-4	500	10	5	5
5-8	1000	20	5	5

Sampling point (time after administration)  
 Group 1 & 5: 1 hr, Group 2 & 6: 2 hr  
 Group 3 & 7: 6 hr, Group 4 & 8: 24 hr

(Sponsor, M4, RF9945, p5)

**CONCENTRATION OF NK-104 IN BONE MARROW VERSUS PLASMA AFTER SINGLE ORAL ADMINISTRATION IN MICE**



(Sponsor, M4, RF9945, p12-13)

**Fujino H, Yamada I, Kojima J, Hirano M, Matsumoto H, Yoneda M. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (5): *In vitro* metabolism and plasma protein binding in animals and human; Xenobio Metabol Dispos 1999; 14: 415-424**

**Key study findings:**

- NK-104 was strongly protein bound in all species studied
- The highest protein-binding was detected in humans >rabbits >rats >monkeys >dogs >mice
- Distribution to lens is highest in dogs and mice

**PLASMA PROTEIN BINDING IN RATS, MICE, RABBITS, DOGS, MONKEYS, AND HUMANS**

Animal	Test condition	Time (h)	Concentration (µg/ml)	Ratio of unbound (%)
Male rat	<i>in vitro</i>		0.1	0.8±0.0
			0.3	0.8±0.0
			1.0	0.7±0.0
	<i>ex vivo</i>	0.5	0.504	0.7±0.0
		1	0.361	0.8±0.1
		2	0.195	0.8±0.1
4*		0.121	0.9	
Female rat	<i>in vitro</i>		0.1	0.5±0.0
			0.3	0.6±0.0
			1.0	0.7±0.0
Mouse	<i>in vitro</i>		0.1	3.3±0.4
			0.3	3.2±0.1
			1.0	3.4±0.2
Rabbit	<i>in vitro</i>		0.1	0.6±0.1
			0.3	0.5±0.0
			1.0	0.5±0.0
Dog	<i>in vitro</i>		0.1	1.6±0.1
			0.3	1.7±0.1
			1.0	1.7±0.1
Monkey	<i>in vitro</i>		0.1	1.3±0.0
			0.3	1.3±0.0
			1.0	1.3±0.0
Human	<i>in vitro</i>		0.1	0.5±0.0
			0.3	0.4±0.0
			1.0	0.4±0.0
4% HSA**	<i>in vitro</i>	"	0.1	0.4
		*	0.3	0.4
		*	1.0	0.5
0.06% α <sub>1</sub> -AGP***	<i>in vitro</i>		0.1	5.7±0.1
			0.3	5.1±0.8
			1.0	5.5±0.7

Each value represents the mean±S.E.  
 \*: n=2  
 \*\*HSA : Human serum albumin  
 \*\*\*α<sub>1</sub>-AGP : Human acidic α<sub>1</sub>-glycoprotein

(Sponsor, M4, Fujino et al. 1999, p5)

None of the prototype drugs for high protein-binding in human plasma significantly affected the unbound ratio of NK-104.

**EFFECTS OF HIGHLY PROTEIN BOUND DRUGS ON PROTEIN  
BINDING OF NK-104 IN HUMAN PLASMA**

Interacting Drug	Concentration ( $\mu\text{g/ml}$ )	Unbound ratio of NK-104 (%)	Interacting Drug	Concentration ( $\mu\text{g/ml}$ )	Unbound ratio of NK-104 (%)
Warfarin	0	0.4 $\pm$ 0.0	Furosemide	0.0	0.3 $\pm$ 0.0
	3	0.4 $\pm$ 0.0		0.5	0.3 $\pm$ 0.0
	15	0.4 $\pm$ 0.0		2.5	0.4 $\pm$ 0.0
Diazepam	0	0.5 $\pm$ 0.0	Ibuprofen	0	0.4 $\pm$ 0.0
	15	0.5 $\pm$ 0.0		50	0.4 $\pm$ 0.0
	75	0.5 $\pm$ 0.0		250	0.4 $\pm$ 0.0
Digitoxin	0.0	0.3 $\pm$ 0.0	Nitrendipine	0.0	0.3 $\pm$ 0.0
	0.1	0.4 $\pm$ 0.0		0.1*	0.3 $\pm$ 0.0
	0.5	0.4 $\pm$ 0.0		0.5	0.3 $\pm$ 0.0
Phenylbutazone	0	0.5 $\pm$ 0.0	Glibenclamide	0	0.4 $\pm$ 0.1
	100	0.4 $\pm$ 0.0		100	0.5 $\pm$ 0.1
	500	0.6 $\pm$ 0.0		500*	0.6 $\pm$ 0.2
Phenytoin	0	0.3 $\pm$ 0.1			
	20	0.4 $\pm$ 0.0			
	100	0.4 $\pm$ 0.0			

Each value represents the mean  $\pm$  S.E. of four plasma samples.  
\* n=3  
Final plasma concentration of NK-104 was 0.3  $\mu\text{g/ml}$ .

(Sponsor, M4, Fujino et al. 1999, p6)

NK-104 increased the unbound ratio of protein-bound digitoxin when the concentration of NK-104 was above 0.1  $\mu\text{g/mL}$ . However, the effect was a small increase in unbound digitoxin of  $\sim$ 2.2% to  $\sim$ 2.7%, between 0.1  $\mu\text{g/mL}$  and 1.0  $\mu\text{g/mL}$  NK-104.

**EFFECTS OF NK-104 ON PROTEIN BINDING OF OTHER  
HIGHLY PROTEIN BOUND DRUGS IN HUMAN PLASMA**

Interacting Drug	NK-104 ( $\mu\text{g/ml}$ )	Unbound ratio of interacting drug (%)	Interacting Drug	NK-104 ( $\mu\text{g/ml}$ )	Unbound ratio of interacting drug (%)
Warfarin	0.0	0.8 $\pm$ 0.0	Propranolol	0.0	13.9 $\pm$ 1.4
	0.3	0.8 $\pm$ 0.1		0.3	13.6 $\pm$ 1.0
	1.0	0.8 $\pm$ 0.1		1.0	13.0 $\pm$ 1.2
Diazepam	0.0	1.5 $\pm$ 0.1	Nitrendipine	0.0	7.1 $\pm$ 0.2
	0.3	1.5 $\pm$ 0.2		0.3	6.7 $\pm$ 0.1
	1.0	1.5 $\pm$ 0.1		1.0	6.8 $\pm$ 0.1
Digitoxin	0.0	2.2 $\pm$ 0.1	Glibenclamide	0.0	1.0 $\pm$ 0.0
	0.3	2.6 $\pm$ 0.1*		0.3	1.2 $\pm$ 0.2
	1.0	2.7 $\pm$ 0.0**		1.0	1.1 $\pm$ 0.0
Digitoxin	0.0	2.4 $\pm$ 0.1			
	0.03	2.3 $\pm$ 0.0			
	0.1	2.2 $\pm$ 0.0			

Each value represents the mean  $\pm$  S.E. of four plasma samples.

(Sponsor, M4, Fujino et al. 1999, p6)

For formation of M-13, M-3, and M-8, female rat microsomes appeared to be the most similar to human microsomes of the species tested, although there was a bigger fraction of non-M-13, M-3, and M-8 metabolites (metabolites of unknown structure) in human microsomes than in female rat microsomes (4.3% versus 0.9%).

**METABOLITES IN MICROSOMAL FRACTIONS FROM RAT, DOG, RABBIT, GUINEA PIG, MONKEY AND HUMAN (AND HUMAN S9) AFTER 2 HOURS**

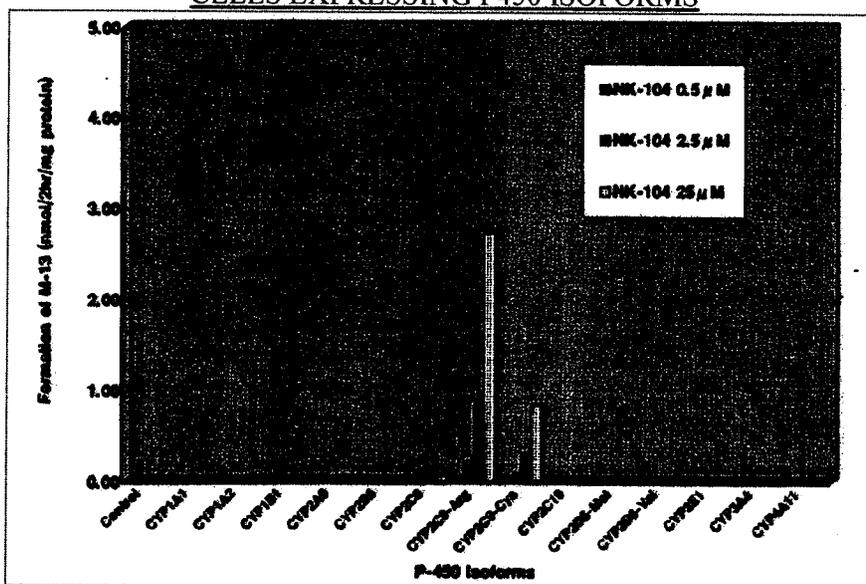
Species	% of composition				
	NK-104	M-13	M-3	M-8	Unknown
Male rat microsomes	83.4±0.8	9.5±0.8	4.3±0.2	0.7±0.2	2.1±0.3
Female rat microsomes	81.0±0.8	17.7±0.5	0.4±0.0	0.0	0.9±0.3
Dog microsomes	98.0±0.6	0.4±0.0	1.5±0.5	0.1±0.0	0.1±0.0
Rabbit microsomes	93.1±0.4	2.0±0.2	0.0	0.0	5.0±0.2
Guinea pig microsomes	89.8±0.5	2.7±0.2	0.0	0.0	7.5±0.5
Cynomolgus monkey microsomes	39.4±3.1	38.4±2.3	0.7±0.1	0.0	21.4±1.4
Human microsomes	78.8±1.2	16.3±0.8	0.5±0.1	0.0	4.3±0.6
Human S-9	90.5±0.8	7.5±0.8	0.1±0.0	0.0	2.0±0.2

Each value represents the Mean ± S.E. of four-five samples.

(Sponsor, M4, Fujino et al. 1999, p7)

CYP2C9 appeared to be the only P450 isoform that hydroxylated NK-104 to any extent.

**HYDROXYLATION OF NK-104 IN HUMAN LYMPHOBLASTOID CELLS EXPRESSING P450 ISOFORMS**



(Sponsor, M4, Fujino et al. 1999, p8)

**RI107017 – Calculation of the human plasma protein binding ratio of NK-104 lactone by ultracentrifugation method**

**Key Study Findings:**

- The plasma protein-binding ratio for pitavastatin lactone was determined to be 98.95-99.33% in human plasma in this *ex vivo* assay.

The human plasma protein to plasma binding ratio was determined using ultracentrifugation and equilibrium dialysis techniques. Plasma samples were obtained from 9 healthy male volunteers. Pitavastatin or pitavastatin lactones were added to

plasma at 300, 1000, and 3000 ng/mL. Lactone stability was measured to evaluate the loss of lactone over 24 hours, so that corrections for lactone hydrolysis could be applied over the course of the experiment (~3.2% over 4 hours at 4 °C. Pitavastatin lactone was measured in samples using column-switching HPLC with UV detection before and after ultracentrifugation. The apparent protein-binding ratio for pitavastatin lactone was very high (98.95 to 99.33%) in plasma, in this assay.

#### **ATR-148-100 – Mechanism of uptake of NK-104 by human liver**

##### **Key study findings:**

- Pitavastatin appears to be a substrate for OATP2 (LST-1)

To determine the uptake mechanism of NK-104 in human liver *Xenopus* oocytes expressing LST-1(OATP2, SLC21A6), a sodium independent organic anion transporter expressed only in the liver and uptake in human hepatocytes was examined. The  $K_m=5.53 \pm 1.7 \mu\text{M}$  for LST-1 was observed. A linear relationship between the amount and duration of uptake was observed for 45 minutes in the transfected oocytes. The  $K_m=2.99 \pm 0.79 \mu\text{M}$  was observed in human hepatocytes where linear uptake is seen from 20-100 sec.

#### **R101068 – Study on various transporters and NK-104**

##### **Key study findings:**

- Pitavastatin appeared to be a p-glycoprotein substrate at 1 hour, but not at 6 hours post-administration
- This may indicate a time-dependent saturation of P-gp.
- The 1 hour time-points were omitted from a publication of these data in the public domain

Sponsor evaluated the concentration of radioactive NK-104 and its metabolites in transgenic p-glycoprotein knockout mice versus their non-transgenic counterparts. The study indicated that p-glycoprotein may be a transporter for NK-104 or its major metabolites, because the tissue distribution was dissimilar at 1 hour, but similar between the mouse strains at 6 hours post-administration. Since there was higher concentrations in liver of -/- mice than +/- mice at 1 hour but the difference was absent at 6 hours, this may indicate a time-dependent saturation of P-gp or that the data simply are confounded.

#### CONCENTRATIONS OF NK-104 AND METABOLITES IN LIVERS OF MDR1a/b TRANSGENIC MICE AND THEIR NON-TRANSGENIC COUNTERPARTS

		Concentration (ug/ml or g)					
strain		NK-104	M-3	M-8	M-6	M-11	Unknown metabolites
Liver	1hr						
	mdr1a/b(+/+)	0.063 ± 0.072	0.095 ± 0.078	0.052 ± 0.072	0.073 ± 0.110	0.006 ± 0.011	0.033 ± 0.046
	mdr1a/b(-/-)	0.510 ± 0.307	0.079 ± 0.014	0.381 ± 0.189	0.319 ± 0.153	0.036 ± 0.062	0.003 ± 0.006
	6hr						
mdr1a/b(+/+)	0.441 ± 0.440	0.320 ± 0.194	0.015 ± 0.012	0.296 ± 0.237	0.121 ± 0.138	0.035 ± 0.037	
mdr1a/b(-/-)	0.403 ± 0.470	0.236 ± 0.095	0.060 ± 0.025	0.165 ± 0.058	0.093 ± 0.104	0.059 ± 0.103	

Data represents the mean and S.D. of three mice.

(Sponsor, M4, R101068, p22)

**KOW 025/003656 – [<sup>14</sup>C]-NK-104 quantitative tissue distribution in the pigmented rat after single oral administration**

**Key study findings:**

- Concentrations of radiolabel were similar in eye and skin of pigmented and non-pigmented rats

In liver of pigmented Lister Hooded rats, levels of [<sup>14</sup>C]-NK-104 were ~43 times higher than plasma at 1 hour post-dose. Only heart, liver, kidney and bone showed similar or higher radioactivity than plasma at 24 hours post-dose, with all other tissues showing less radioactivity.

**Tissue : plasma radioactivity concentration ratios following a single oral administration of <sup>14</sup>C-NK-104 (1 mg/kg) to male pigmented rats**

Tissue/organ	Rat no./Time of sacrifice					
	9M	2M	4M	3M	5M	6M
	1 hour	24 hours	72 hours	168 hours	336 hours	Day 21
Whole blood	0.67	0.62	0.75	ND	ND	ND
Eyes	ND	0.14	ND	ND	ND	ND
Heart	0.76	12.97	12.00	ND	ND	ND
Kidney	1.59	2.07	1.75	ND	ND	ND
Urinary bladder	ND	ND	ND	ND	ND	ND
Liver	43.36	6.52	6.25	NC	NC	ND
Bone	0.19	1.07	ND	ND	ND	ND
Skin (non-pigmented)	0.16	0.28	ND	ND	ND	ND
Skin (pigmented)	0.22	0.45	ND	ND	ND	ND

NC Not calculable (radioactivity detected in tissue, but not in plasma)  
 ND No radioactivity detected in tissue

(Sponsor, M4, KOW025-003656, p17)

**Fujino H, Morikawa S, Kanda H, Kimata H. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (3): Foeto-placental transfer and mammary excretion after oral administration in rats; Xeno Meta Disp 1998; 13: 508-515**

**Key study findings:**

- Pitavastatin crosses the blood-milk barrier.
- Pitavastatin is concentrated in milk (up to 7.2-fold greater than maternal plasma concentration at 6 hours post administration).
- Pitavastatin (parent) was the primary form found in rat milk
- 5-Ketopitavastatin was the primary metabolite observed in rat milk
- Fetal pitavastatin concentrations were ≤36% of maternal plasma pitavastatin concentrations

Pitavastatin was administered to pregnant rats on the 13<sup>th</sup> and 18<sup>th</sup> day of gestation and lactating female rats at 1 mg/kg and the distribution of [<sup>14</sup>C]pitavastatin was monitored in maternal and fetal tissues.

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Table II Tissue concentration (dpm/g)		on the 18th day of gestation			
Tissue		24 hr			
Dam	Placenta	0.011±0.003			
	Blood	0.008±0.001			
	Brain	0.003±0.000			
	Heart	n.d.			
	Eyes	0.004±0.001			
	Thyroid	n.d.			
	Spleen	0.005±0.001			
	Thymus	0.005±0.002			
	Heart	0.119±0.035			
	Lungs	0.007 <sup>#</sup>			
	Liver	0.046±0.003			
	Kidney	0.034±0.006			
	Adipose	0.008±0.002			
	Spleen	0.002±0.002			
	Pancreas	0.005±0.001			
	Abdominal fat	0.012±0.002			
	Skeletal muscle	0.016±0.004			
Skin	0.005±0.001				
Bone	n.d.				
Uterus	0.007±0.001				
Placenta	0.013±0.003				
Amnion	n.d.				
Ovary	0.008±0.001				
Mammary gland	0.094±0.036				
Fetus	Whole	0.008±0.002	0.011±0.001	0.006±0.000	0.004±0.001
	Blood	0.010±0.002	0.008±0.007	n.d.	n.d.
	Brain	0.005±0.000	0.006±0.001	0.003±0.001	n.d.
	Heart	0.016±0.001	0.020±0.004	0.013±0.005	0.012±0.004
	Lung	0.003±0.003	n.d.	n.d.	n.d.
	Liver	0.024±0.002	0.035±0.011	0.023±0.009	0.008±0.003
	Kidney	n.d.	n.d.	n.d.	n.d.

Each value represents the mean ± S.D. of three rats.  
n.d. : not detected, <sup>#</sup> : n=2

(Sponsor, M4, Fujino et al. 1998, p7)

**Table III Milk and plasma concentration of radioactivity and ratios of milk/plasma after oral administration of <sup>14</sup>C-NK-104 to**

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E:  
n.d.

	0.5	1	3	6	24	48
<b>NK-104</b>	0.082	0.120	0.238	0.340	n.d.	n.d.
<b>M-3</b>	0.001	0.003	0.024	0.036	n.d.	n.d.
<b>Unknown</b>	0.001	0.001	n.d.	0.002	n.d.	n.d.
<b>Polar</b>	n.d.	0.010	0.010	0.021	0.028	0.007

n.d. : not detected.

(Sponsor, M4, Fujino et al. 1998, p8)

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liver and kidney, at 2508, 21.9, and 2.7 times plasma concentrations, respectively.

Table 1 Concentration of radioactivity in tissues 4 hours after a single oral administration of  $^{14}\text{C}$ -NK-104 (3mg/kg) to male cynomolgus monkeys

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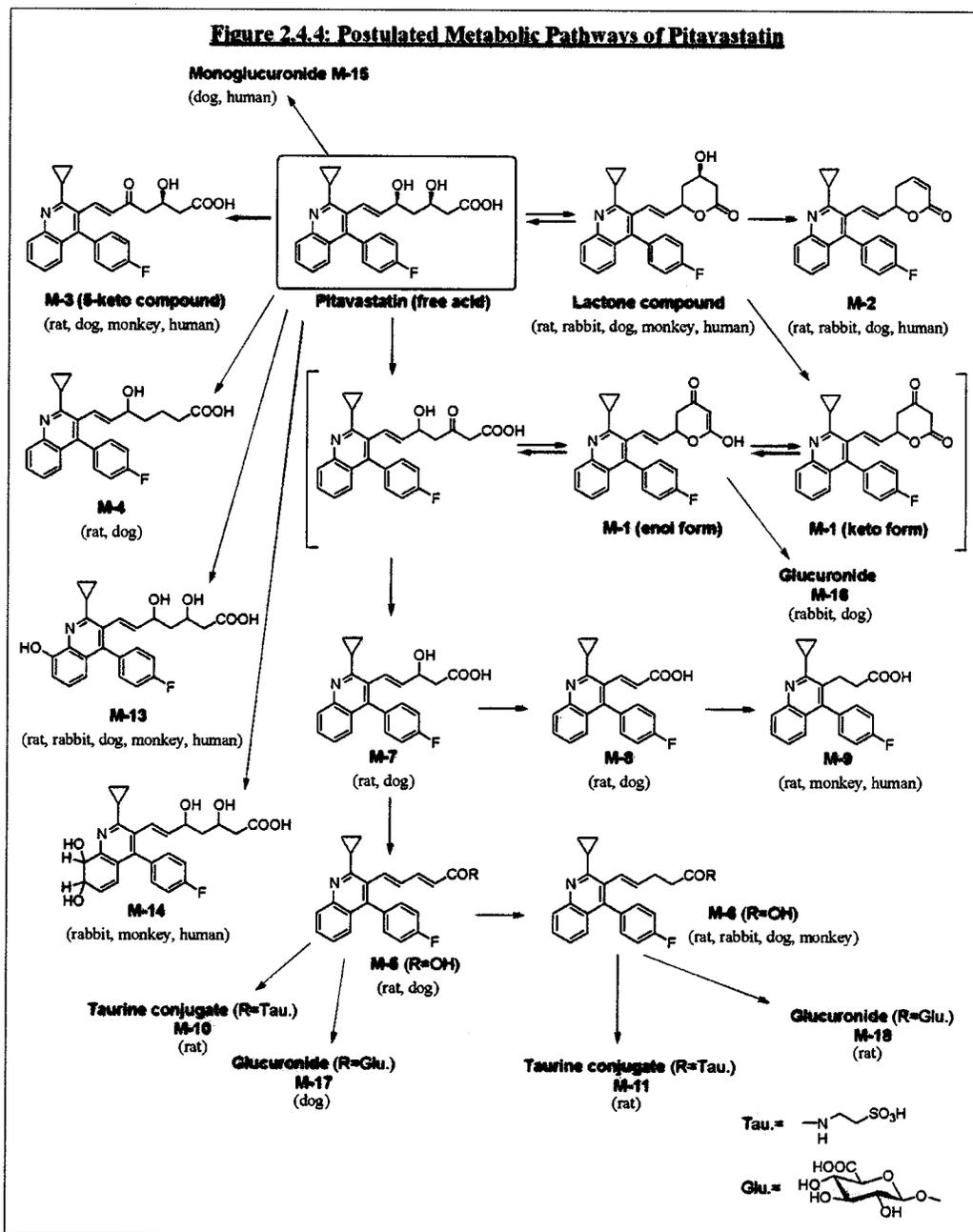
(Sponsor, M4, 9L805, p21)

Table 2 Concentration of radioactivity in tissues 48 hours after a single oral administration of  $^{14}\text{C}$ -NK-104 (3mg/kg) to male cynomolgus monkeys

Tissue \_\_\_\_\_ Concentration of radioactivity, ng equivalent of NK-104/g or mL (Tissue/plasma ratio)

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2.6.4.5 Metabolism:



(Sponsor, M2, Non-clinical overview)

CYP2C9 appeared to be the only human P450 isoform that hydroxylated NK-104 to any extent. Hydroxylation of NK-104 was greatest in monkey >>human >female rat >male rat >guinea pig >rabbit >dog. Except for rat and mouse, lactonization is the primary metabolite, which is formed by glucuronidation of parent NK-104 and subsequent hydrolysis to form the lactone. The lactone can be further hydrolyzed to reform the

parent NK-104 compound. CYP3A4 is not a major metabolic pathway for NK-104 in contrast to several other drugs in this class.

#### **AE-2544 – *In vitro* study of NK-104 metabolism**

##### **Key study findings:**

- CYP2C9 was the P450 enzyme that metabolized NK-104 to the greatest extent at 2.5  $\mu$ M NK-104.
- NK-104 lactone was metabolized by CYPs 1A1, 1A2, 2B6, 2C19, 2D6 and 3A4 to a low degree at a concentration of 0.5  $\mu$ M NK-104 lactone.
- CYP2C8 was the only P450 enzyme where NK-104 showed inhibition of activity.
- CYP2C8 was not inhibited by NK-104 lactone at the concentration tested.

A panel of human P450 enzymes was expressed in a lymphoblastoid cell line, from which microsomes were produced for the experiment. NK-104 and NK-104 lactone were not significantly metabolized by human P450 isoforms (see table).

#### **PITAVASTATIN AND PITAVASTATIN LACTONE METABOLISM BY A PANEL OF THE CYTOCHROMES P450**

P450 isoform	Remaining ratio (% of control)		
	NK-104 lactone 0.5 $\mu$ M	NK-104 0.5 $\mu$ M	NK-104 2.5 $\mu$ M
control*	100	100	100
CYP1A1	86.9	91.6	105
CYP1A2	80.5	99.8	104
CYP1B1	97.0	87.4	108
CYP2A6	104	113	104
CYP2B6	85.0	111	98.8
CYP2C8	99.5	102	111
CYP2C9-Arg	101	83.1	101
CYP2C9-Cys	107	104	100
CYP2C19	82.4	100	92.5
CYP2D6-Val	83.0	90.5	108
CYP2D6-Met	81.7	104	91.3
CYP2E1	97.2	99.4	117
CYP3A4	81.0	114	114

\*: using control microsomes is reductase or control (vector)

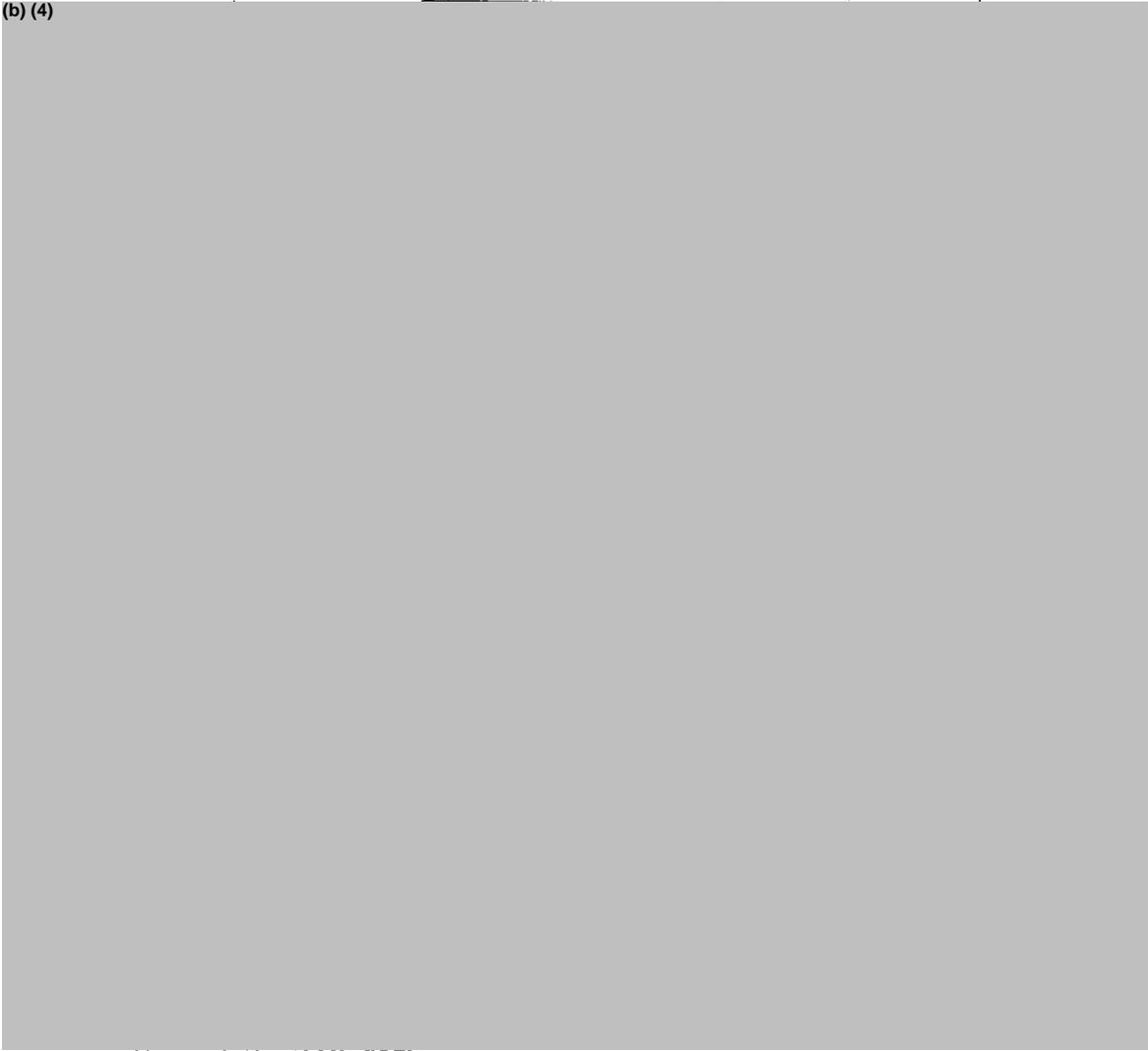
(Sponsor, M4, AE2544, p29)

Pitavastatin did not inhibit a panel of P450 enzymes, except CYP2C8, which was inhibited ~30% at 2.5  $\mu$ M NK-104, but was not inhibited by the NK-104 lactone at the concentration tested (0.025  $\mu$ M), but there may have been slight inhibition (~10%) of CYPs 2D6 and 2E1 at the same concentration.

PITAVASTATIN AND PITAVASTATIN LACTONE INHIBITION  
OF A PANEL OF THE CYTOCHROMES P450

Relative activity (% of control)

(b) (4)



Metabolism of NK-104 was observed in hepatic microsomes obtained from male cynomolgus monkeys, male and female Wistar rats, male beagles, male rabbits, and male guinea pigs. Significant quantities of conjugated products were not observed. In S9 fractions, there was not significant metabolism of NK-104.

**R101029 – *In vitro* studies on NK-104 using human metabolic enzyme system: A novel mechanism of lactonization by UDP-glucuronosyltransferase**

Lactonization, the major route of NK-104 metabolism, proceeds through a glucuronidated intermediate. The lactone is a cyclic ester and is subject to hydrolysis, which reforms pitavastatin. Therefore, the lactone appears to be interconvertible with parent, and the equilibrium likely depends on the rate of glucuronidation, which favor lactone formation,

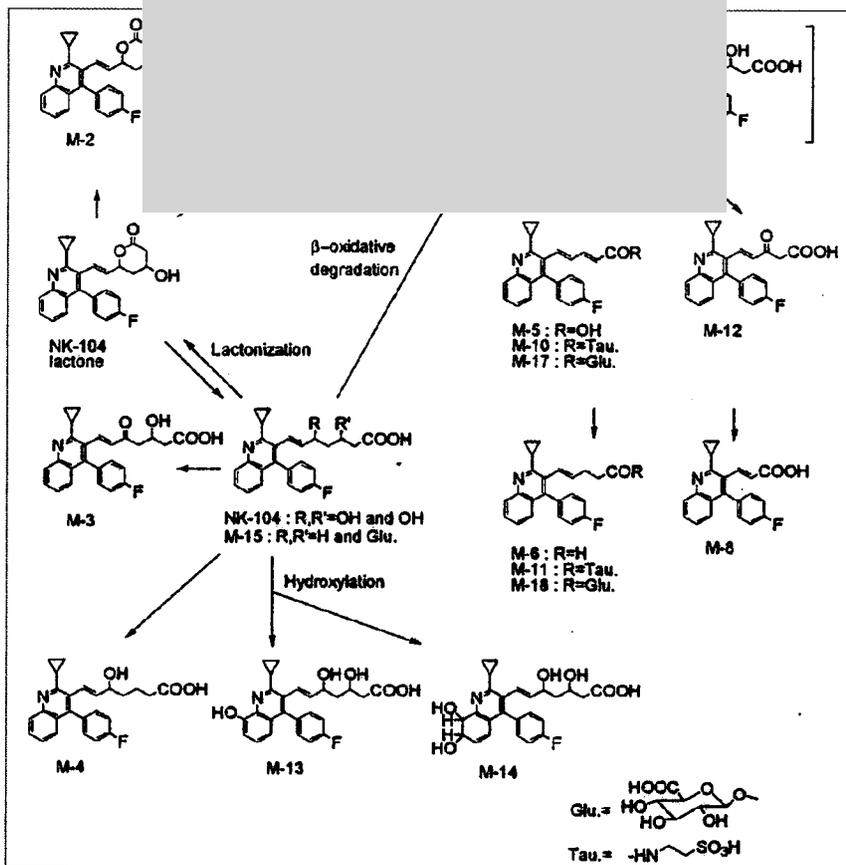
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drugs in this class.

Metabolites have been identified in rat, rabbit, monkey and dog. Four biotransformations have been observed: oxidation of the side chain, hydroxylation of the side chain, and conjugation with  $\beta$ -glucuronic acid and

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rat, rabbit, monkey  
ionization,  $\beta$ -  
and conjugation with



(Kojima et al., 1999)

**R97047 – An analytical investigation to determine the plasma and urinary drug concentrations in the pharmacokinetic study of NK-104 in elderly healthy volunteers (Second Report): Determination of NK-104 metabolites in non-elderly group**

NK-104 and NK-104 lactone were not detected in plasma of 22-24 year old volunteers at any time-point. This study was confounded.

**2.6.4.6 Excretion:**

**Table 2.4.4: Excretion of Radioactivity in Rats, Guinea pigs and Monkeys following Administration of [<sup>14</sup>C]-Pitavastatin**

Species	Route	Dose (mg/kg)	Excretion (% of dose)		Reference
			Urine	Faeces	
Rat: Male	p.o.	1	0.2	99.2	[Kimata <i>et al.</i> , 1998]
	i.v.	1	0.4	92.9	
Rat: Female	p.o.	1	2.2	97.0	
	i.v.	1	3.6	93.2	
Guinea pig	p.o.	1	12.7	87.7	[R98042]
Monkey	p.o.	3	11.0	77.5	[9L804]
	i.v.	0.3	11.3	88.3	

p.o.: *per os* (oral)

(Sponsor, M2.4, Nonclinical overview, p23)

**Table 2.4.5: Urinary and Faecal Cumulative Excretion after Oral Administration of 1 mg/kg of Pitavastatin**

Species	Urinary Excretion (% of dose)		Faecal Excretion (% of dose)	
	Pitavastatin	Pitavastatin Conjugate, Lactone and Lactone Conjugate	Pitavastatin	Pitavastatin Conjugate, Lactone and Lactone Conjugate
Rat	Negligible	< 5%	61.6%	< 5%
Rabbit	35.7%	< 5%	13.0%	< 5%
Dog	Negligible	< 5%	46.2%	< 5%
Monkey	0.1%	< 5%	5.2%	< 5%

(Sponsor, M2.4, Nonclinical overview, p24)

In rats, greater than 99% of radioactivity was excreted in feces within 72 hours after dosing, while less than 1% was excreted in urine. Fecal excretion was the primary route of excretion in humans, rats, dogs, and monkeys; urinary excretion was the primary route of excretion in rabbits. In monkeys, NK-104 and metabolites were excreted predominantly in the feces (~78%), while urinary excretion was a minor route (~11%). Most radioactivity that was recovered in urine and feces was in the form of parent NK-104. In monkeys, M-14 (aryldihydrodiol) was the predominant metabolite in feces (~13% of total radioactivity recovered) and in urine (47% of total radioactivity

recovered). Bile is the predominant pathway for NK-104 elimination after intravenous administration in dogs. NK-104 undergoes extensive enterohepatic circulation.

**R101066 – Pharmacokinetic study of NK-104 by use of EHBR – Biliary excretion study**

Male Sprague-Dawley or EHBR (Esai Hyperbilirubinemic Rat) rats were administered NK-104 by bile duct cannulations or a 1 mg/kg bolus by i.v. Since EHBR mice have a defect in the cMOAT protein, this transporter is not active in those mice. Excretion of NK-104 was not significantly different between these two strains of mice, which indicates that pitavastatin is not likely to be a substrate for cMOAT.

**R99047 – Biliary excretion of NK-104 after intravenous administration in dogs****Key study findings:**

- Bile is the predominant pathway for NK-104 elimination after intravenous administration in dogs.
- NK-104 undergoes extensive enterohepatic circulation.

After 48 hours, 98% was excreted into bile following IV dosing and 75% after oral dosing). Radioactive bile obtained after oral administration of drug was administered into the duodenum of recipient dogs with bile duct cannulations. About 72% of the dose was excreted in the bile after 48 h with almost no radioactivity in the urine indicating that NK-104 undergoes enterohepatic circulation.

**2.6.4.7 Pharmacokinetic drug interactions**

There were no changes in plasma protein-binding noted for two different concentrations of [<sup>14</sup>C]-NK-104 in the presence of increasing concentrations of bezafibrate, clofibrate, gemfibrozil, or ciprofibrate. NK-104 is an OATP1B1 substrate.

**H-TB-9731 – Enzyme-inhibitory study of NK-104 - Effects of NK-104 single administration on the drug-metabolizing enzyme system in female rats - Enzyme induction study of NK-104 –**

In rat liver microsomes, processed at 1 hour post-single oral administration of pitavastatin at 1, 3, and 10 mg/kg in rats, P450 activity and potential inhibition were evaluated. There was no inhibition noted for a variety of CYPs, NADPH-cytochrome P450 reductase, glucuronyl-transferase, or with cytochrome  $\beta_5$ . Liver weights were not different, but protein contents were lower (n.s.s.) in pitavastatin-administered animals compared to vehicle-only controls.

**H-TB-9607 – Effects of NK-104 on the drug-metabolizing enzyme system in rats -**

In rat liver microsomes, processed at 1 hour post-single oral administration of pitavastatin at 1, 3, and 10 mg/kg in rats, P450 activity and potential inhibition were evaluated. There was no inhibition noted for a variety of CYPs, NADPH-cytochrome P450 reductase, glucuronyl-transferase, or with cytochrome  $\beta_5$ . Neither liver weights nor protein contents were lower in pitavastatin-administered animals compared to vehicle-only controls.

**R101113 – *In vitro* drug interaction between NK-104 and fibrate drugs****Key study findings:**

- There were no changes in plasma protein-binding noted for two different concentrations of [<sup>14</sup>C]-NK-104 in the presence of increasing concentrations of bezafibrate, clofibrate, gemfibrozil, or ciprofibrate.

Plasma protein-binding of several fibrates by [<sup>14</sup>C]-NK-104 was determined. Potential effects on P450 metabolism and NK-104 concentrations by fibrates were also investigated using human liver microsomes (consisting of individual and pooled human microsomes, as well as baculovirus expression systems expressing human P450 enzymes.

The time-course for examining the effects of fibrates on [<sup>14</sup>C]-NK-104 appeared too short (60 minutes) to make a conclusion regarding inhibition of fibrates on metabolism of NK-104. Clofibrate data were confounded by the short time-course, and increasing concentrations of ciprofibrate appeared to inhibit the breakdown of parent NK-104 in a significant, dose-dependent manner.

While metabolism of NK-104 by human hepatic microsomes appeared to be slow compared to cerivastatin, there were similar trends in the presence of fibrates for NK-104 and cerivastatin. The significance of such an effect on NK-104 could only be determined by initiating a longer assay.

The breakdown of gemfibrozil appears to be short enough as to preclude much of an effect on NK-104 metabolism (but less so at higher concentrations), while bezafibrate, ciprofibrate and clofibrate (and high concentrations of gemfibrozil) appear to present for extended periods of time (>2 hrs) in human liver microsomes with little to no change in levels during that time. Cerivastatin and NK-104 appear not to affect gemfibrozil metabolism at concentrations tested (up to 10 µM), while fluvastatin inhibits gemfibrozil metabolism in a dose-dependent manner.

P450 isoforms that were identified as important for gemfibrozil metabolism were CYP 1A2, 2C9, and 2C19, but not 2C8, 2D6, or 3A4. P450 isoforms identified as important for cerivastatin were CYP 2C8 and 3A4, but not 1A2, 2C9, 2C19, and 2D6.

Antibodies to several cytochrome P450 isoforms were utilized to screen for inhibition of metabolism of NK-104. CYP2C9 and, to a lesser extent, 2C8 were identified as potential metabolisers of NK-104. Antibodies to CYP2C8 and 3A4 were most potent for inhibition of cerivastatin metabolism, albeit at a less efficient level of inhibition compared to NK-104; these results were corroborated by correlation studies between cerivastatin and P450 activities in several human microsomal preparations.

Fibrates appear to generally inhibit the two CYP enzymes associated with NK-104 metabolism, including gemfibrozil, ciprofibrate, bezafibrate and clofibrate for CYP2C9 (in order of highest to lowest inhibition potential) and gemfibrozil, bezafibrate,

ciprofibrate and clofibrate for CYP2C8 (in order of highest to lowest inhibition potential).

In conclusion, there appears to be potential for fibrates that inhibit CYP2C8 and 2C9 to affect metabolism of NK-104 by these two P450 isoforms, especially those that affect CYP2C9. There may also be reason for concern regarding potential effects on metabolism of gemfibrozil by NK-104 via CYP2C9, but less concern that NK-104 will affect gemfibrozil metabolism via CYP2C8, as gemfibrozil does not appear to be significantly metabolized by CYP2C8.

**R99035 – An *in vitro* study of drug metabolism of NK-104 Report No. 5: A study of drug-drug interaction mediated by CYP2C9**

CYP2C9 was identified in a prior study as the primary CYP isoform for clinical concern with NK-104. In the current study, the Sponsor compared the effects of tolbutamide on the hydroxylation of NK-104 and fluvastatin by CYP2C9, and of fluvastatin and NK-104 on metabolism of tolbutamide by CYP2C9.

NK-104 had little to no effect on the kinetics of tolbutamide hydroxylation by CYP2C9 at concentrations of NK-104 less than 25  $\mu\text{M}$ . In contrast, fluvastatin inhibited hydroxylation of tolbutamide by 2C9 with an apparent  $K_i$  of 1  $\mu\text{M}$ . Thus there appears to be only small potential for drug-drug interaction through CYP2C9.

**ATR-149-035 – Interaction with cyclosporine A in the uptake of NK-104 using LST-1 expressing *Xenopus* oocytes**

Cyclosporin A inhibited the LST-1 mediated uptake of radiolabeled NK-104 and pravastatin in *Xenopus* oocytes in a concentration dependent manner. The  $\text{IC}_{50}$  of cyclosporine against statin uptake was  $2.91 \pm 0.78 \mu\text{M}$  and  $1.21 \pm 0.16 \mu\text{M}$ , respectfully.

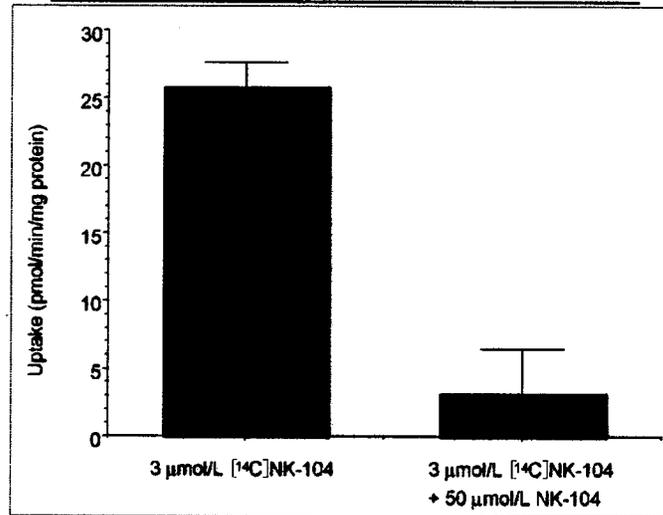
**FBM 06-T350 – Inhibition studies of concomitant drugs on hOATP1B1 uptake of [ $^{14}\text{C}$ ]-NK-104**

**Key study findings:**

- NK-104 is an OATP1B1 substrate, and atazanavir inhibited OAT1B1 with an  $\text{IC}_{50}$  of 2.1  $\mu\text{M}$ . Atazanavir, and perhaps other OATP1B1 substrates (except enalaprilat and nipradilol) coadministered with NK-104 may inhibit the uptake of NK-104 into human hepatocytes in a dose-dependent manner.

Human OATP1B1 was expressed in HEK293 cells. These cells were used to study the uptake of [ $^{14}\text{C}$ ]-labeled NK-104 and inhibition of [ $^{14}\text{C}$ ]-NK-104 uptake by unlabeled NK-104. OATP1B1 transported radiolabeled NK-104 into the cells, and this process was inhibited by unlabeled NK-104.

UPTAKE OF RADIOLABELED PITAVASTATIN  
BY CELLS EXPRESSING HUMAN OATP1B1



(Sponsor, M4, FBM 06-T350, p24)

**COPYRIGHT MATERIAL**

The  $K_i$  for  
presented  
in a com

data are  
1.7  $\mu\text{M}$ ,

**RI10202**

**or NK-**

**104 after single oral administration to rats**

The potential for drug-drug interaction with gemfibrozil and NK-104 was evaluated. There was an increase in exposure to NK-104 when coadministered with gemfibrozil ( $\uparrow 7\%$ ).

### 2.6.4.8 Other Pharmacokinetic Studies

#### **F-01 – Synthesis of [<sup>14</sup>C]-NK-104**

Synthesis was carried out by Amersham (UK). Batch CFQ6923: 98.5% chemical purity based on peak area normalization. 98.3% radiological purity by HPLC. Batch CFQ8596: 99.3% chemical purity based on peak area normalization. 98.6% radiological purity by HPLC.

#### **R95033 – Examination of NK-104 absorption site in rats**

Pitavastatin lactone was rapidly converted (probably to pitavastatin) in plasma ( $T_{1/2}$  was ~2 minutes). Pitavastatin was very stable in plasma and matrixes tested out to 240 minutes (plasma, gastric juice, duodenal juice, gastric homogenate, and duodenal homogenate). Pitavastatin lactone had a half-life of 2 minutes in plasma, and >240 minutes for the other preparations. Duodenum had the highest absorption of NK-104> ileum>colon>stomach.

#### **RT2001/2503 – Study on pharmacokinetics of NK-104 in rat models of liver dysfunction induced by carbon tetrachloride**

Liver damage induced by carbon tetrachloride caused exposure (AUC) for NK-104 to be 3-fold higher at 0.5 mL/kg to up to 10-fold higher at 3 mL/kg and NK-104 administered at 1/mg/kg. This indicates that liver damage will change the pharmacokinetics of NK-104 by leading to significantly higher total exposure to NK-104.

#### **RF9935 – Plasma concentration of NK-104 epimer and NK-104 enantiomer after single oral administration to mice**

Dosing was based on the maximum tolerated dose of the enantiomer, determined in a previous dose-range finding assay for the micronucleus assay for the enantiomer. The epimer was administered at the same doses (2.5 and 5 mg/kg). Blood samples were obtained by laparotomy at 1, 2, 6, and 24 hours after administration.

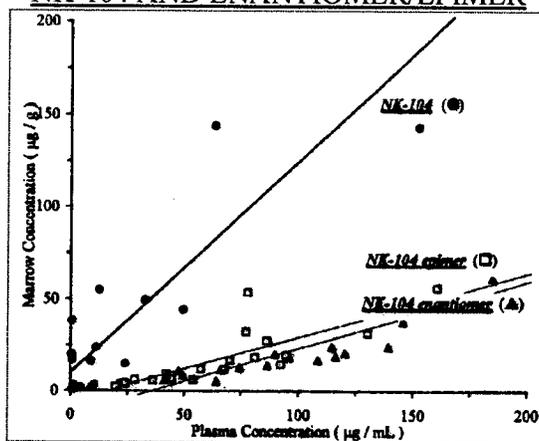
Group No.	Drug	Dose (mg/kg)	Concentration (mg/kg)	Volume (ml/kg)	Animal Number (/group)
1-4	Epimer	250	2.5	10	5
5-8	Epimer	500	5	10	5
9-12	Enantiomer	250	2.5	10	5
13-16	Enantiomer	500	5	10	5

Sampling point (time after administration)  
Group 1, 5, 9, 13: 1hr; Group 2, 6, 10, 14: 2 hr; Group 3, 7, 11, 15: 6 hr; Group 4, 8, 12, 16: 24 hr

(Sponsor, M4, RF9935, p6)

Femurs were removed and marrow was checked for exposure to NK-104 enantiomer and epimer.

**PLASMA VERSUS BONE CONCENTRATIONS OF  
NK-104 AND ENANTIOMER/EPIMER**



(Sponsor, M4, RF9935, p13)

**2.6.4.9 Discussion and Conclusions**

**SINGLE ORAL RADIOLABELED DOSE OF 32 mg**

Parameter	Radioactivity	NK-104	NK-104 lactone	8-OH-NK-104
C <sub>max</sub> (ng/ml)	1169	857.7	274.2	2.99
T <sub>max</sub> (hr)	0.5	0.5	0.75	1.25
AUC <sub>t</sub> (ng-hr/ml)	10268	2991	1818	12
λ <sub>z</sub> (hr <sup>-1</sup> )	0.0103	0.0486	0.0428	0.1782
t <sub>1/2</sub> (hr)	67.6	14.3	16.2	3.9
AUC (ng-hr/ml)	12299	3175	2074	16
CL/F (ml/min)	-	183	-	-
V <sub>z</sub> /F (litres)	-	226	-	-

(Sponsor, SNY 419/013926, p8)

**21 DAY REPEAT DOSE PHARMACOKINETICS IN HUMANS**

Name of company NEGMA LABORATOIRES Name of finished product		INDIVIDUAL STUDY TABLE		FOR NATIONAL AUTHORITY USE ONLY	
Name of active ingredient NK 104		Ref. :	Vol. :		
		Page : 4/9			
<b>RESULTS : NK 104</b>					
Parameters	Day 1	Day 8	Day 21	Anova	
	Dose 4 mg Mean ± SD	Dose 4 mg Mean ± SD	Dose 4 mg Mean ± SD	Food effect	Effect of repeated dose
C <sub>max</sub> (ng.ml <sup>-1</sup> )	67 ± 41	47 ± 25	55 ± 22	NS	NS
T <sub>max</sub> (h)	0.67 ± 0.26	1.2 ± 0.61	1.1 ± 0.66	NS	NS
AUC <sub>0-24</sub> (ng.h.ml <sup>-1</sup> )	107 ± 57	102 ± 47	153 ± 46	NS	S p = 0.0012
AUC <sub>0-inf</sub> (ng.h.ml <sup>-1</sup> )	117 ± 62	126 ± 57	ND	NS	ND
Lz (h <sup>-1</sup> )	0.31 ± 0.14	0.15 ± 0.075	0.12 ± 0.080	S p = 0.027	NS
T <sub>1/2</sub> (h)	2.6 ± 1.1	6.3 ± 4.3	8.9 ± 6.1	NS	NS

Cross-species Comparison of NK-104 (parent) Tissue Distribution (relative to plasma)									
Species	Time (hr)	Dose (mg/kg)	Plasma	Liver	Kidney	Lung	Heart	Skeletal muscle	Brain
Mice	1	1	1.0	3.3	1.7	ND	3.4	ND	0.3
Rats	1	1	1.0	27	1.9	0.4	0.5	0.1	ND

ND, not detected

Lens opacity was seen in dogs administered 1 mg/kg/day p.o. for 12 months, in dogs administered 3 mg/kg/day p.o. for 12 weeks, and in mice administered 75 mg/kg/day p.o. for 13 weeks. Distribution studies indicated preferential distribution to the dog lens and suggest a correlation between cataract formation/lens opacity and NK-104 concentration in the lens of dogs, and to lesser extent mice. Ocular distribution to the aqueous humor rather than the lens predominated in the monkey and rabbit. The extent of distribution to the dog lens suggests that the dog is more susceptible to NK-104 distribution and that it remains in this tissue to a greater extent than in monkey or rabbit.

<b>Cross-species Comparison of NK-104 Distribution to Lens Protein (<i>in vitro</i> and <i>in vivo</i>)</b>				
Species	K <sub>eye</sub>	K <sub>plasma</sub>	P <sub>eye,vitro</sub> *	P <sub>eye,vivo</sub> **
Mice	15.1	29	0.54	ND
Rats	21.4	130	0.17	0.09
Rabbits	10.9	187	0.06	0.06
Dogs	35.8	61	0.59	0.45
Monkeys	12.7	76	0.18	0.14
Humans	25.8	230	0.12	ND

\*P<sub>eye,vitro</sub> = (K<sub>eye</sub> + 1)/(K<sub>plasma</sub> + 1), in males

\*\*P<sub>eye,vivo</sub> = C<sub>eye</sub>/C<sub>max</sub>, post-mortem based on data not shown, in males  
ND indicates not detected

Pitavastatin lactone is a circulating metabolite in monkeys and humans, but is not detectable in rat plasma. NK-104 lactone appears to be a major metabolite in humans only.

<b>Limited Cross-species Comparison of NK-104 Lactone and 8-Hydroxy-NK-104 Concentrations in Plasma (after a single oral dose)</b>				
Species	Time (hr)	Dose (mg/kg)	NK-104 lactone (% of parent)	8-OH-NK-104 (% of parent)
Rats	0.5	1	ND	ND
Monkeys	1	3	1.5	ND
Dogs	C <sub>max</sub>	1	16	NS
Humans	C <sub>max</sub>	~0.5	32	0.3

ND, not detected

NS, not studied

<b>Cross-species Comparison of NK-104 Metabolites in Plasma (plasma concentration normalized (%) to parent NK-104 concentration, after a single oral dose)</b>												
Species	Time (hr)	Dose (mg/kg)	NK-104 lactone	5-ketone	M4	M-6	M-8	Un-known	Polar	M-13 (8-OH-NK-104)	M-14	
Rats	0.5	1	ND	6	0.5	7	2	2	2.5	ND	ND	
Monkeys	1	3	1.5	5	ND	0.9	ND	35	ND	ND	39	
Dogs	C <sub>max</sub>	1	16	NS	NS	NS	NS	NS	NS	NS	NS	
Humans	C <sub>max</sub>	~0.5	32	ND	ND	ND	ND	ND	ND	0.3	ND	

ND, not detected

Metabolism in the dog, rat, rabbit, and monkey includes primarily lactonization (except rat),  $\beta$ -oxidation of the side chain and hydroxylation of the quinolone ring and conjugation with  $\beta$ -glucuronic acid and taurine.

<b>Cross-species Comparison of NK-104 Metabolites in Microsomes (after 2 hours, normalized to % of parent NK-104 concentration)</b>				
<b>Species</b>	<b>5-ketone (M-3)</b>	<b>8-OH-NK- 104 (M-13)</b>	<b>Propenoic acid derivative (M-8)</b>	<b>Unknown</b>
<b>Rats</b>	5.0	11	0.8	2.5
<b>Dogs</b>	1.5	0.4	0.1	0.1
<b>Rabbits</b>	ND	2.1	ND	5.4
<b>Guinea Pigs</b>	ND	3.0	ND	8.4
<b>Monkeys</b>	1.8	97	ND	54.0
<b>Human</b>	0.6	21	ND	5.4

ND, not detected

<b>Cross-species Comparison of NK-104 Excretion</b>			
<b>Species</b>	<b>Dose (mg/kg)</b>	<b>Urinary excretion (% of dose)</b>	<b>Fecal excretion (% of dose)</b>
<b>Rats</b>	1	1.2	98.1
<b>Dogs</b>	*i.v.	≤40	60
<b>Guinea Pigs</b>	1	12.7	87.7
<b>Monkeys</b>	3	11.0	77.5
<b>Human</b>	0.5	15.1	78.6

\*study in bile-duct cannulated dogs by continuous i.v. infusion

In rats and dogs most NK-104 is excreted in the feces. Fecal excretion was 84% in rats after 24h and 99% by 72 h. Urinary excretion is minimal in rats (0.2%) and dogs (0.5%) but is the major excretory route in rabbits. In monkey fecal and urinary excretion of drug is minimal (5%) because of the extensive hepatic metabolism. Monkeys produce larger amounts of M-13 and other metabolites. Most of the NK-104 present in plasma and excreted in bile, urine and feces is the parent with smaller amounts present as the lactone or conjugates.

2.6.4.10 Tables and figures to include comparative TK summary

Cross-species comparisons:

**Table 2.4.3: Pharmacokinetics of Pitavastatin after Oral Administration**

Species	Dose (mg/kg)	T <sub>max</sub> (hours)	C <sub>max</sub> (ng/mL)	AUC (ng·h/mL)	t <sub>1/2</sub> (hours)	F* (%)
Rat	0.3	0.7	22 ± 2	140 ± 10	7.7	31
	1.0	0.9	231 ± 35	1170 ± 220	6.7	80
	3.0	0.5	911 ± 210	3950 ± 650	6.5	91
Rabbit	0.1	6.0	69 ± 5	1030 ± 70	4.2	64
	0.3	4.5	314 ± 33	4340 ± 190	4.7	89
	1.0	1.5	1184 ± 154	15670 ± 1550	3.9	97
Dog	0.1	1.9	29 ± 9	170 ± 30	4.0	58
	0.3	2.5	93 ± 24	630 ± 130	4.3	71
	1.0	0.6	724 ± 131	2570 ± 270	4.3	88
Monkey	0.3	2.7	17 ± 5	160 ± 60	4.5	31
	1.0	3.2	61 ± 11	310 ± 70	4.5	18
	3.0	2.2	165 ± 43	850 ± 160	3.7	17

\* F: Bioavailability; AUC: Area under the concentration-time curve; Results are expressed as mean ± SE

(Sponsor, M2.4, Nonclinical overview, p18)

**Human Pharmacokinetics at Clinical Doses**

Species	Dose (mg)	T <sub>max</sub> (hours)	C <sub>max</sub> (ng/mL)	AUC <sub>0-24</sub> (ng·h/mL)	t <sub>1/2</sub> (hours)	F (%)
Human	1	1.6	13 ± 4	(b) (4)	1.4	ND
	2	1.5	20 ± 8	50 ± 31	(b) (4)	ND
	4	1.1	55 ± 22	153 ± 46	(b) (4)	ND

(b) (4) values were determined after 21 days repeat oral daily administration to healthy adult Caucasian males

(b) (4) (b) (4)

(b) (4)

(b) (4)

**2.6.5 PHARMACOKINETICS TABULATED SUMMARY**  
(b) (4)

<b>2.6.5.6A Pharmacokinetics: Plasma Protein Binding</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1	
Reference: [Ujino <i>et al.</i> , 1999b]		Location in CTD:		Vol: * Section: *	
<b>Method:</b>	<p><i>In vitro</i>: Plasma samples were obtained from animals fasted overnight (n = 4). Mouse plasma was withdrawn from 15 animals, pooled and split into three aliquots. The human plasma was obtained from four healthy male volunteers. Pitavastatin was mixed with plasma at three different concentrations. An equilibrium dialysis system was utilised.</p> <p><i>Ex vivo</i>: Between two and five male Wistar rats were used per group. Pitavastatin was administered orally at 3 mg/kg. The animals were anaesthetised and sacrificed by exsanguinations at 0.5, 1, 2 and 4 hours after dosing. The plasma binding assay was conducted as described above. Pitavastatin determination was performed using HPLC-MS<sup>2</sup> and total radioactivity was measured using liquid scintillation counting.</p>				
<b>Results:</b>	<p>Pitavastatin was highly bound to plasma protein: the unbound fraction ratio was 0.7% to 0.8% in male rats, 0.5% to 0.7% in female rats, 3.2% to 3.4% in male mice, 1.6% to 1.7% in male dogs, 0.5% to 0.6% in male rabbits, 1.3% in male monkeys and 0.4% to 0.5% in humans, respectively. Binding was independent of sex in rats and of concentration in all species. The major pitavastatin binding protein was human serum albumin. Binding of pitavastatin was also high to human <math>\alpha_1</math>-acid glycoprotein.</p>				
<b>Plasma Unbound Fraction of Pitavastatin in Different Species</b>					
<b>Species / Gender</b>	<b>Test Condition</b>	<b>Time (hours)</b>	<b>Concentration (µg/mL)</b>	<b>Ratio of Unbound (Mean ± SE%)</b>	
Wistar male rats	<i>In vitro</i> (n = 4)	NA	1.0	0.8 ± 0.0, 0.8 ± 0.0, 0.7 ± 0.0	
	<i>Ex vivo</i> (n = 5)	0.5	0.504	0.7 ± 0.0	
	<i>Ex vivo</i> (n = 5)	1	0.361	0.8 ± 0.1	
	<i>Ex vivo</i> (n = 5)	2	0.195	0.8 ± 0.1	
	<i>Ex vivo</i> (n = 2)	4	0.121	0.9	
Wistar female rats	<i>In vitro</i> (n = 4)	NA	0.1, 0.3, 1.0	0.5 ± 0.0, 0.6 ± 0.0, 0.7 ± 0.0	
CD male mice	<i>In vitro</i> (n = 3)	NA	0.1, 0.3, 1.0	3.3 ± 0.4, 3.2 ± 0.1, 3.4 ± 0.2	
JW male rabbits	<i>In vitro</i> (n = 4)	NA	0.1, 0.3, 1.0	0.6 ± 0.1, 0.5 ± 0.0, 0.5 ± 0.0	
HRA male beagle dogs	<i>In vitro</i> (n = 4)	NA	0.1, 0.3, 1.0	1.6 ± 0.1, 1.7 ± 0.1, 1.7 ± 0.1	
Male cynomolgus monkeys	<i>In vitro</i> (n = 4)	NA	0.1, 0.3, 1.0	1.3 ± 0.0, 1.3 ± 0.0, 1.3 ± 0.0	
Male humans	<i>In vitro</i> (n = 4)	NA	0.1, 0.3, 1.0	0.5 ± 0.0, 0.4 ± 0.0, 0.4 ± 0.0	
4% Human serum albumin	<i>In vitro</i> (n = 2)	NA	0.1, 0.3, 1.0	0.4, 0.4, 0.5	
0.06% Human $\alpha_1$ -acid glycoprotein	<i>In vitro</i> (n = 4)	NA	0.1, 0.3, 1.0	5.7 ± 0.1, 5.1 ± 0.8, 5.5 ± 0.7	
<b>Additional Information:</b>					
*: Not applicable to an electronic submission. a: HPLC-MS as described in [Kojima <i>et al.</i> , 1999a], the Sponsor identified that the validation methods summarised in [Kojima <i>et al.</i> , 1999a] were [R92052], [R95077] and [R98018]					

<b>2.6.5.6B Pharmacokinetics: Plasma Protein Binding</b>		<b>Test Article: Lactone</b>		Page 1 of 1	
Report No.: [R1107017]		Location in CTD:		Vol: * Section: *	
<b>Method:</b>	<p>Lactone is partially hydrolysed to pitavastatin during ultracentrifugation, accordingly, the unbound plasma protein ratio of lactone was evaluated on the basis of total lactone and pitavastatin concentrations in the supernatant (unbound fraction). Plasma samples were obtained from nine healthy male volunteers fasted overnight; fresh plasma (stored at 0°C) was used to evaluate the protein binding ratio. An ultracentrifugation method conducted at 4 °C was utilised with plasma samples spiked with lactone at 300, 1000 and 3000 ng/ml. Concentrations of lactone and pitavastatin in samples were measured with HPLC-MS method (as described in several reports, including [R92052], [R95077], [R1105032]).</p>				
<b>Results:</b>	<p>The human plasma protein unbound ratio of lactone was 0.67% to 1.05% and was not dependent on concentration. The human plasma protein unbound ratio of pitavastatin, calculated under identical conditions, was about 0.2%.</p>				

<b>2.6.5.7A Pharmacokinetics: Study in Pregnant or Nursing Animals</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 4	
Reference: [Ujino <i>et al.</i> , 1998b]		Location in CTD:		Vol: * Section: *	
<b>Species:</b>	Wistar pregnant rats weighing approximately 222 g on Gestation Day 13 (GD13) and weighing approximately 260 g on Gestation Day 18 (GD18). Lactating rats weighing about 290 g were obtained in-house.				
<b>Feeding Condition:</b>	Pregnant animals were fasted for 16 hours prior to dosing and for 6 hours after dosing. Lactating animals were not fasted.				
<b>Dose Regimen:</b>	Single dose on GD13 and GD18 and lactating animals were given a single dose 14 days after delivery (Lactation Day 14 (LD14)).				
<b>Dose Groups:</b>	n = 3 per group with one animal per group used for whole body autoradiography.				
<b>Vehicle/Formulation</b>	Solution in 0.5% CMC sodium solution				
<b>Method of Administration</b>	Oral (gavage)				
<b>Radioisotope:</b>	<sup>14</sup> C-pitavastatin				
<b>Dose:</b>	1				
<b>Specific Activity:</b>	2.18 MBq/mg				
<b>Tissue/Organs:</b>	Whole body autoradiography; various tissues				
<b>Sampling Time:</b>	Whole body autoradiography 1, 6 and 24 hours after dosing on GD18; tissue distribution 0.5, 1, 6 and 24 hours after dosing on GD13 and GD18 (foetal tissues were only examined on GD18).				
<b>Assay:</b>	Liquid scintillation counting				
<b>Results:</b>	<p>Whole body autoradiography: High levels of radioactivity in liver and gastrointestinal contents at 1 and 6 hours after dosing. Trace levels of radioactivity in heart, kidney and lungs. Weak radioactivity in placenta, amniotic fluid and foetus. Although distribution of radioactivity in most tissues decreased at 24 hours after dosing, levels in the larger intestinal contents were relatively high with low levels in the heart, muscle and mammary gland. No radioactivity observed in the foetus at 24 hours after dosing.</p> <p>The results for tissue concentrations of radioactivity following administration on GD13 and GD18 are summarised on the following pages:</p> <p>Most of the tissues showed maximum concentrations at 0.5 to 1 hour after administration on GD13 and GD18. On GD13, the maternal plasma concentration reached a maximum at 0.5 hours thereafter declining with a t<sub>1/2</sub> of 5.2 hours and decreased to less than 3% of the C<sub>max</sub> at 24 hours. The concentration in the liver was highest with a C<sub>max</sub> about 34 times higher than that in the plasma. The concentration in the kidney was approximately five times higher than that in the plasma. The concentration in the foetus indicated limited placental transfer. The concentrations in the maternal heart and mammary gland at 24 hours were about 10 and six times that in the plasma, respectively. On GD18 the maternal plasma concentrations were similar to those on GD13. The concentration in the liver at 0.5 hours was about 24 times higher than that in the plasma. No marked difference was noted in tissue concentrations between GD13 and GD18. There was limited placental transfer of radioactivity on GD13 or GD18. The concentration in the foetus was less than maternal plasma levels at both time points. Although foetal liver had the highest concentration, its levels were less than 35 ng eq./g. The concentrations in the maternal heart and mammary gland at 24 hours were about 11 and nine times that in the plasma, respectively.</p>				

\*: Not applicable to an electronic submission  
ng eq./g: Nanogramme equivalents per gramme

2.6.5.7A Pharmacokinetics: Study in Pregnant or Nursing Animals		Test Article: Pitavastatin			Page 2 of 4
Reference: [Fujino <i>et al.</i> , 1998b] (continued)					
Tissue Concentration (Mean $\pm$ SD; ng eq./mL or g) of Radioactivity in Pregnant Rats after Oral Administration of [ <sup>14</sup> C]-Pitavastatin on GD13					
Tissue	Time of sampling after dosing				
	0.5 hours	1 hour	6 hours	24 hours	
Plasma	286 $\pm$ 15	203 $\pm$ 19	57 $\pm$ 13	8 $\pm$ 5	
Blood	167 $\pm$ 2	119 $\pm$ 11	32 $\pm$ 6	ND	
Brain	13 $\pm$ 2	10 $\pm$ 3	4 $\pm$ 1	ND	
Hypophysis	65 $\pm$ 7	52 $\pm$ 15	ND	ND	
Eye ball	12 $\pm$ 2	10 $\pm$ 2	3 $\pm$ 1	ND	
Thyroid	55 $\pm$ 6	46 $\pm$ 15	10 $\pm$ 9	ND	
Submaxillary gland	86 $\pm$ 12	62 $\pm$ 11	7 $\pm$ 1	2 $\pm$ 3	
Thymus	35 $\pm$ 2	31 $\pm$ 3	8 $\pm$ 1	ND	
Heart	189 $\pm$ 31	229 $\pm$ 58	87 $\pm$ 18	76 $\pm$ 57	
Lung	182 $\pm$ 16	130 $\pm$ 38	34 $\pm$ 2	5 $\pm$ 3	
Liver	9675 $\pm$ 304	5284 $\pm$ 1209	1273 $\pm$ 302	61 $\pm$ 50	
Kidney	1432 $\pm$ 95	840 $\pm$ 182	171 $\pm$ 80	22 $\pm$ 17	
Adrenal	127 $\pm$ 20	98 $\pm$ 29	29 $\pm$ 1	ND	
Spleen	57 $\pm$ 9	35 $\pm$ 1	12 $\pm$ 1	2 $\pm$ 2	
Pancreas	91 $\pm$ 10	62 $\pm$ 4	18 $\pm$ 2	3 $\pm$ 2	
Abdominal fat	44 $\pm$ 9	45 $\pm$ 9	24 $\pm$ 4	7 $\pm$ 4	
Skeletal muscle	36 $\pm$ 6	34 $\pm$ 7	11 $\pm$ 2	13 $\pm$ 13	
Skin	39 $\pm$ 5	42 $\pm$ 10	12 $\pm$ 2	ND	
Bone marrow	50 $\pm$ 4	35 $\pm$ 2	12 $\pm$ 3	ND	
Uterus	90 $\pm$ 4	91 $\pm$ 18	27 $\pm$ 2	4 $\pm$ 2	
Placenta	63 $\pm$ 3	64 $\pm$ 12	26 $\pm$ 3	7 $\pm$ 2	
Amniotic fluid	ND	ND	ND	ND	
Ovary	92 $\pm$ 10	80 $\pm$ 7	25 $\pm$ 4	6 $\pm$ 4	
Mammary gland	113 $\pm$ 10	102 $\pm$ 14	46 $\pm$ 13	46 $\pm$ 36	
Foetus (whole)	3 $\pm$ 3	5 $\pm$ 2	ND	ND	

2.6.5.7A Pharmacokinetics: Study in Pregnant or Nursing Animals		Test Article: Pitavastatin			Page 3 of 4
Reference: [Fujino <i>et al.</i> , 1998b] (continued)					
Tissue Concentration (Mean $\pm$ SD; ng eq./mL or g) of Radioactivity in Pregnant Rats after Oral Administration of [ <sup>14</sup> C]-Pitavastatin on GD18					
Tissue	Time of sampling after dosing				
	0.5 hours	1 hour	6 hours	24 hours	
Plasma	343 $\pm$ 55	237 $\pm$ 19	49 $\pm$ 9	11 $\pm$ 3	
Blood	222 $\pm$ 33	165 $\pm$ 12	32 $\pm$ 7	8 $\pm$ 1	
Brain	18 $\pm$ 3	11 $\pm$ 1	3 $\pm$ 2	3 $\pm$ 0	
Hypophysis	127 $\pm$ 47	71 $\pm$ 9	18 $\pm$ 3	ND	
Eye ball	13 $\pm$ 2	13 $\pm$ 1	4 $\pm$ 1	4 $\pm$ 1	
Thyroid	89 $\pm$ 37	57 $\pm$ 5	ND	ND	
Submaxillary gland	119 $\pm$ 49	68 $\pm$ 7	15 $\pm$ 3	5 $\pm$ 1	
Thymus	66 $\pm$ 10	49 $\pm$ 8	10 $\pm$ 2	5 $\pm$ 2	
Heart	209 $\pm$ 153	198 $\pm$ 4	45 $\pm$ 10	119 $\pm$ 35	
Lung	228 (n = 2)	135 $\pm$ 5	25 $\pm$ 3	7 (n = 2)	
Liver	8310 $\pm$ 1857	5418 $\pm$ 525	860 $\pm$ 226	46 $\pm$ 3	
Kidney	1227 $\pm$ 149	807 $\pm$ 106	116 $\pm$ 34	34 $\pm$ 6	
Adrenal	172 $\pm$ 68	97 $\pm$ 11	22 $\pm$ 5	8 $\pm$ 2	
Spleen	63 $\pm$ 6	41 $\pm$ 2	10 $\pm$ 2	2 $\pm$ 2	
Pancreas	96 $\pm$ 11	71 $\pm$ 8	15 $\pm$ 3	5 $\pm$ 1	
Abdominal fat	39 $\pm$ 11	57 $\pm$ 4	22 $\pm$ 5	12 $\pm$ 2	
Skeletal muscle	45 $\pm$ 2	40 $\pm$ 3	11 $\pm$ 1	16 $\pm$ 4	
Skin	43 $\pm$ 3	58 $\pm$ 9	13 $\pm$ 2	5 $\pm$ 1	
Bone marrow	77 $\pm$ 15	46 $\pm$ 6	12 $\pm$ 1	ND	
Uterus	97 $\pm$ 2	116 $\pm$ 17	25 $\pm$ 6	7 $\pm$ 1	
Placenta	78 $\pm$ 6	69 $\pm$ 1	23 $\pm$ 6	13 $\pm$ 3	
Amniotic fluid	ND	ND	1 $\pm$ 1	ND	
Ovary	115 $\pm$ 11	87 $\pm$ 7	22 $\pm$ 4	8 $\pm$ 1	
Mammary gland	139 $\pm$ 13	166 $\pm$ 17	44 $\pm$ 11	94 $\pm$ 36	

2.6.5.7A Pharmacokinetics: Study in Pregnant or Nursing Animals		Test Article: Pitavastatin				Page 4 of 4
Reference: [Pujino <i>et al.</i> , 1998b] (continued)						
<b>Tissue Concentration (Mean ± SD; ng eq./mL or g) of Radioactivity in Foetal Tissues after Oral Administration of [<sup>14</sup>C]-Pitavastatin on GD18 to Dams</b>						
Tissue	Time of sampling after dosing					
	0.5 hours	1 hour	6 hours	24 hours		
Foetus: Whole	8 ± 2	11 ± 1	6 ± 0	4 ± 1		
Blood	10 ± 2	8 ± 7	ND	ND		
Brain	5 ± 0	6 ± 1	3 ± 1	ND		
Heart	16 ± 1	20 ± 4	13 ± 5	12 ± 4		
Lung	3 ± 3	ND	ND	ND		
Liver	24 ± 2	35 ± 11	23 ± 9	8 ± 3		
Kidney	ND	ND	ND	ND		
<b>Results cont.:</b> The concentration of radioactivity in milk reached a C <sub>max</sub> at 6 hours after dosing, declined with a t <sub>1/2</sub> of 4.8 hours and was below the level of detection at 48 hours post-dosing. The plasma C <sub>max</sub> was achieved at 0.5 hours, declining with a t <sub>1/2</sub> of 7.5 hours and was below the level of detection at 72 hours. The ratios of milk to plasma increased with time reaching a maximum at 6 hours after dosing. Whole body autoradiography of pups at 2, 4, 6 and 24 hours after dosing of lactating rats showed trace levels of radioactivity in gastrointestinal contents of pups and low levels of radioactivity at 6 and 24 hours.						
<b>Milk and Plasma Concentration of Radioactivity and Ratios of Milk/Plasma after Oral Administration of [<sup>14</sup>C]-Pitavastatin to Lactating Rats (Mean ± SD)</b>						
Time (hours)	Milk Concentration (ng eq./g)	Plasma Concentration (ng eq./g)	Milk/Plasma Ratio			
0.5	75 ± 27	205 ± 43	0.38 ± 0.18			
1	182 ± 47	160 ± 52	1.17 ± 0.26			
3	325 ± 68	118 ± 36	2.85 ± 0.63			
6	450 ± 94	63 ± 18	7.23 ± 0.47			
24	34 ± 8	10 ± 2	3.30 ± 0.50			
48	ND	2 ± 2	NA			
72	ND	ND	NA			
<b>Concentration (ng or ng eq./g) of Unchanged Pitavastatin and Its Metabolites in Milk After Oral Administration of [<sup>14</sup>C]-Pitavastatin to Lactating Rats</b>						
Compound	Time after Dosing (hours)					
	0.5	1	3	6	24	48
Pitavastatin	82	120	238	340	ND	ND
5-ketone	1	3	24	36	ND	ND
Unknown	1	1	ND	2	ND	ND
Polar	ND	10	10	21	28	7
2.6.5.7B Pharmacokinetics: Study in Pregnant or Nursing Animals		Test Article: Pitavastatin				Page 1 of 1
Report No.: [RP9932]		Location in CTD:				
		Vol.: *   Section: *				
Species:	Crl:CD (Sprague Dawley) rats aged 11 weeks on Gestation Day 3 (GD3)					
Group Assignment:	Animals were allocated to groups on the basis of body weight to achieve similar mean body weights between groups.					
Dose Regimen:	Single daily dose from Gestation Day 7 (GD7) through to Gestation Day 17 (GD17) (11 days dosing) or single daily dose from GD17 through to Gestation Day 21 (GD21) (5 days dosing)					
Dose Groups:	Dosing from GD7 to GD17: Group 1: 1 mg/kg/day pitavastatin (n = 4 dams); Group 2: 3 mg/kg/day pitavastatin (n = 4 dams); Group 3: 10 mg/kg/day pitavastatin (n = 2 dams) Dosing from GD17 to GD21: Group 4: 1 mg/kg/day pitavastatin (n = 3 dams); Group 2: 3 mg/kg/day pitavastatin (n = 3 dams); Group 3: 10 mg/kg/day pitavastatin (n = 2 dams)					
Necropsy Day:	Dosing from GD7 to GD17: Necropsy on GD18; Dosing from GD17 to GD21: Necropsy on Gestation Day 22 (GD22)					
Number of Non-Pregnant Females:	Non-pregnant females were excluded from analysis. Dosing from GD7 to GD17: 2 animals at 10 mg/kg/day; Dosing from GD17 to GD21: 1, 1 and 2 animals in the 1, 3 and 10 mg/kg/day dose groups, respectively.					
Observations:	No treatment related clinical observations, changes in body weight or food consumption, macroscopic findings, changes in the number of implantations or foetal viability.					
Vehicle/Formulation	Suspension in 0.5% CMC sodium solution					
Method of Administration	Oral (gavage; 2 mL/kg)					
Analyte	Pitavastatin, lactone					
Assay	HPLC-MS <sup>a</sup>					
Time (h)	Dosing from GD7 to GD17: GD17, 0.5 to 24 hours after last dose; Dosing from GD17 to GD21: GD21, 0.5 to 24 hours after last dose					
Pitavastatin Dose (mg/kg)	Pitavastatin PK Parameters (Mean ± SD)					
	Dosing from GD7 to GD17: GD17 (last day of dosing)			Dosing from GD17 to GD21: GD21 (last day of dosing)		
	Pitavastatin			Pitavastatin		
	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hours)	AUC (ng* h/mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hours)	AUC (ng* h/mL)
1	110 ± 30	1.0 ± 0.7	1250 ± 400	70 ± 30	12.3 ± 13.6	610 ± 440
3	530 ± 540	0.9 ± 0.8	3330 ± 900	700 ± 730	0.5 ± 0.0	4100 ± 4180
10 (n = 2)	1490, 2070	0.5, 2.0	12270, 12970	1780, 6510	0.5, 0.5	6920, 26460
Additional Information:	Lactone was not detected in the plasma in animals (LLOQ 10 ng/mL).					

<sup>a</sup>: Not applicable to an electronic submission

a: HPLC-MS as described in [RP9708]

2.6.5.7C Pharmacokinetics: Study in Pregnant or Nursing Animals		Test Article: Pitavastatin		Page 1 of 1		
Report No.: [G252G]		Location in CTD:		Vol.: * Section: *		
Species:	Kb1: JW rabbits aged 19 to 20 weeks at the start of administration (females only)					
Group Assignment:	Plasma total cholesterol was measured during acclimatisation period and animals were distributed into groups in order to attain similar mean cholesterol levels between groups.					
Dose Groups:	Group 1: Control (n = 8); Group 2: 0.1 mg/kg pitavastatin (n = 8); Group 3: 0.3 mg/kg pitavastatin (n = 8); Group 4: 1 mg/kg pitavastatin (n = 12)					
Dose Regimen:	Single daily dose from Gestation Day 6 (GD6) through to GD18					
Necropsy Day and Number of Dams:	(H20): Control: 8/8; 0.1 mg/kg pitavastatin: 7/8; 0.3 mg/kg pitavastatin: 7/8; 1.0 mg/kg pitavastatin: 12/12					
Observations:	Decreased faeces were observed in one dam in the 0.1 mg/kg group and in six dams in the 1.0 mg/kg group. No treatment related effects on body weight, food consumption, gross pathology, number of corpora lutea, number of implantations, number of live foetuses, number of dead embryos, number of dead foetuses and number of external foetal abnormalities were observed.					
Vehicle/Formulation:	Suspension in 0.5% CMC sodium solution					
Method of Administration:	Oral (gavage; 1 ml/kg)					
Analyte / Assay:	Pitavastatin; lactone / HPLC-CS*					
Time (h):	Pre-dose (acclimatisation period); GD6, GD13 and GD18					
Dose (mg/kg)	Pitavastatin PK Parameters (Mean ± SD)					
	GD6 (Day 1 of dosing)			GD18 (Day 13 of dosing)		
	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hours)	AUC (ng·h/mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hours)	AUC (ng·h/mL)
Control	NA	NA	NA	NA	NA	NA
0.1	75 ± 21	1.9 ± 1.1	552 ± 227	81 ± 16	1.4 ± 1.1	607 ± 218
0.3	273 ± 55	1.3 ± 0.5	1544 ± 244	295 ± 60	1.1 ± 0.4	2045 ± 605
1.0	980 ± 210	1.4 ± 0.9	5197 ± 1134	1484 ± 836	2.0 ± 1.3	22538 ± 20500
Dose (mg/kg)	Lactone PK Parameters (Mean ± SD)					
	GD6 (Day 1 of dosing)			GD18 (Day 13 of dosing)		
	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hours)	AUC (ng·h/mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hours)	AUC (ng·h/mL)
Control	NA	NA	NA	NA	NA	NA
0.1	1 ± 0	3.6 ± 4.0	5 ± 6	2 ± 1	2.0 ± 1.0	13 ± 7
0.3	2 ± 1	1.7 ± 0.5	18 ± 9	7 ± 3	1.3 ± 0.5	60 ± 24
1.0	8 ± 1	1.5 ± 0.9	53 ± 14	33 ± 24	5.0 ± 4.6	630 ± 602

\*: Not applicable to an electronic submission; a: HPLC-CS as described in [RF2000V56]

2.6.5.8A Pharmacokinetics: Other Distribution Studies		Test Article: Pitavastatin		Page 1 of 1	
Report No.: [ATR-149-100]		Location in CTD:		Vol.: * Section: *	
Study Design:	<i>In vitro</i> study to examine the mechanism of uptake of pitavastatin by sodium-independent OATP1B1 in transgenic oocytes of <i>Xenopus laevis</i> and by human hepatocytes.				
Methods:	Oocytes of <i>Xenopus laevis</i> expressing human liver-specific sodium (Na)-independent OATP1B1 and human cryopreserved hepatocytes were utilised. [ <sup>14</sup> C]-pitavastatin was used (specific activity of 981 kBq/mg). The transgenic oocytes were prepared and the experiments were conducted in triplicate using oocytes from different frogs. Human cryopreserved hepatocytes were obtained commercially and hepatocytes from three different donors were used for each experiment.				
Results:	<p>The uptake of [<sup>14</sup>C]-pitavastatin (30 µmol/L) by transgenic oocytes was approximately twice that of the uptake of [<sup>14</sup>C]-pitavastatin by non-transgenic oocytes suggesting that pitavastatin is a substrate for OATP1B1. A linear relationship between the amount of uptake and the time of uptake was observed up to 45 minutes (oocytes were incubated for 10, 20, 30, 45 and 60 minutes). Uptake of pitavastatin mediated by OATP1B1 expressed in oocytes showed saturability complying with Michaelis-Menten formula (results expressed as mean ± SD; Km 5.53 ± 2.95 µmol/L; individual Km values of 2.65, 3.38, 8.55).</p> <p>The uptake of [<sup>14</sup>C]-pitavastatin (0.64 µmol/L) by human hepatocytes increased almost linearly from 20 to 100 seconds. Uptake of the positive control, [<sup>3</sup>H]-estradiol-17βD-glucuronide, also increased almost linearly from 30 to 90 seconds (data not shown). Saturability was also found in uptake of pitavastatin by human hepatocytes (results expressed as mean ± SD; Km 2.99 ± 1.37 µmol/L; individual Km values of 1.46, 3.42, 4.09). The Km value for [<sup>3</sup>H]-estradiol-17βD-glucuronide was Km 23.72 ± 12.83 µmol/L (results expressed as mean ± SD; individual Km values of 12.78, 20.53, 37.84).</p>				

Km: Michaelis constant  
[<sup>3</sup>H]: Hydrogen isotope 3

2.6.5.8B Pharmacokinetics: Other Distribution Studies		Test Article: Pitavastatin		Page 1 of 2	
Report No.: [R101068]		Location in CTD:		Vol.: * Section: *	
Study Design:	To examine the uptake of pitavastatin by P-glycoprotein (P-gp) in <i>mdrla/b</i> knockout mice.				
Species:	<i>mdrla/b</i> ( <i>mdrla/b</i> <sup>-/-</sup> ) knockout male mice; 8 weeks old and approximately 28 g at the time of use.				
Gender (M/F)/No. of Animals:	FVB/N ( <i>mdrla/b</i> <sup>+/+</sup> ) male mice were used as the control group and were 8 weeks old and approximately 28 g at the time of use.				
Feeding Condition:	3M per time point				
Vehicle/Formulation:	Fasted overnight (16 hours) before dosing and for 6 hours after dosing; water <i>ad libitum</i>				
Method of Administration:	Solution in physiological saline				
Dose (mg/kg):	i.v. (bolus)				
Radionuclide:	1: single dose				
Specific Activity:	[ <sup>14</sup> C]-pitavastatin				
Tissues/Organs:	981 kBq/mg				
Sampling Time:	Whole body autoradiography:				
	Tissue concentrations: Plasma, brain, heart, liver, kidney, adrenal and testis.				
	(i) Whole body autoradiography: 15 minutes, 1, 6 and 24 hours after dosing				
	(ii) Tissue concentrations: 1 and 6 hours after dosing				
	(iii) Metabolites in the liver; samples of the liver at 1 and 6 hours after dosing from (ii) were used to determine the concentrations of pitavastatin, 5-ketone, M-8, M-6 and M-11.				
Assay:	Liquid scintillation counting; HPLC-RLG				
Results:	Whole body autoradiography: 15 minutes after dosing the highest concentration of radioactivity was in the liver and GIT followed by the heart and kidney in both mouse strains. Distribution of radioactivity into other tissues was low. The radioactivity in the brain and testis was markedly low in the <i>mdrla/b</i> ( <i>mdrla/b</i> <sup>-/-</sup> ) mice. At 1 hour after dosing similar tissue distribution was observed with the radioactivity distributed mainly to liver, gall bladder and GIT. At 24 hours after dosing, the radioactivity markedly decreased and no accumulation was observed in any tissue. Distribution of radioactivity into the brain was negligible in both strains of mice. There were no obvious differences in tissue distribution of radioactivity between the two mouse strains indicating that pitavastatin was not a substrate for P-gp.				

HPLC-RLG: High performance liquid chromatography with radioluminography

2.6.5.8B Pharmacokinetics: Other Distribution Studies		Test Article: Pitavastatin				Page 2 of 2	
Report No.: [R101068] (continued)							
<b>Results cont.:</b>		Tissue concentrations: 1 and 6 hours after dosing. There were no obvious differences in tissue distribution of radioactivity between the two mouse strains indicating that pitavastatin was not a substrate for P-gp.					
<b>Tissue Concentration of Radioactivity in Both Mouse Strains 1 and 6 Hours after Intravenous Administration of [<sup>14</sup>C]-Pitavastatin</b>							
Mouse Strain	Tissue	Tissue Concentration (Mean ± SD; n = 3; ng eq./mL or g)					
		Time after Dosing					
		1 hour	Ratio (-/-):(+/+)	6 hours	Ratio (-/-):(+/+)		
Control (mdrla/b(+/+))	Plasma	117 ± 31	2.23	160 ± 123	1.14		
	Brain	40 ± 33	1.18	24 ± 7	1.32		
	Testis	62 ± 8	1.40	43 ± 14	1.42		
	Heart	399 ± 173	1.80	393 ± 240	1.92		
	Kidney	193 ± 165	1.11	127 ± 82	1.50		
	Adrenal	98 ± 19	1.36	160 ± 122	0.76		
	Liver	383 ± 279	3.76	1264 ± 676	0.88		
Test (mdrla/b(-/-))	Plasma	261 ± 162		182 ± 96			
	Brain	47 ± 22		31 ± 14			
	Testis	87 ± 19		61 ± 18			
	Heart	716 ± 313		756 ± 359			
	Kidney	215 ± 101		190 ± 138			
	Adrenal	133 ± 63		121 ± 36			
	Liver	1437 ± 483		1107 ± 644			
<b>Results cont.:</b>		Metabolites in the liver: There were no obvious differences between the two mouse strains indicating that neither pitavastatin nor its metabolites were substrates for P-gp.					
<b>Concentration of Pitavastatin and Its Metabolites in the Liver of Both Mouse Strains 1 and 6 Hours after Intravenous Administration of [<sup>14</sup>C]-Pitavastatin</b>							
Time (h)	Mouse Strain	Concentration (Mean ± SD; ng/g; n = 3)					
		Pitavastatin	5-ketone	M-8	M-6	M-11	Unknown
1	mdrla/b(+/+)	63 ± 72	95 ± 78	52 ± 72	73 ± 110	6 ± 11	33 ± 46
	mdrla/b(-/-)	510 ± 307	79 ± 14	361 ± 169	319 ± 153	36 ± 62	3 ± 6
6	mdrla/b(+/+)	441 ± 440	320 ± 194	15 ± 12	286 ± 237	121 ± 138	35 ± 37
	mdrla/b(-/-)	403 ± 470	236 ± 95	60 ± 25	165 ± 58	93 ± 104	59 ± 103
2.6.5.8C Pharmacokinetics: Other Distribution Studies		Test Article: Pitavastatin				Page 1 of 1	
Reference: [Kimata <i>et al.</i> ; 1998]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 364]; [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]		Location in CTD:		Vol.:		Section:	
<b>Species:</b>		Jia: Wistar rats and ACI/N Icos pigmented rats					
<b>Gender (M/F)/No. of Animals:</b>		Jia: Wistar rats 4M per group; ACI/N Icos pigmented rats 4M					
<b>Feeding Condition:</b>		Fasted overnight before dosing and for 6 hours after dosing					
<b>Vehicle/Formulation:</b>		Solution in 0.5% CMC sodium solution (oral) or physiological saline (i.v.)					
<b>Method of Administration:</b>		Oral (gavage) and i.v. (bolus injection)					
<b>Dose (mg/kg):</b>		1					
<b>Radionuclide:</b>		[ <sup>14</sup> C]-pitavastatin					
<b>Specific Activity:</b>		2.2 to 3.5 MBq/mg					
<b>Assay:</b>		Whole body autoradiography					
<b>Sampling Time:</b>		Oral: 0.5, 1, 6, 24 and 72 hours after dosing; i.v.: 2.5 minutes, 1, 6, 24 and 72 hours after dosing					
<b>Results:</b>		<p><b>Oral administration:</b> 0.5 hours: high levels of radioactivity in gastrointestinal contents and liver, followed by blood in portal vein and distribution into other tissues was very low. 1 hour: high levels of radioactivity in intestinal contents, liver and blood in portal vein. Low levels in lung, kidney, heart and skeletal muscle with trace amounts in other tissues. 6 hours: radioactivity in gastric contents markedly decreased but high levels in small and large intestine; high level in liver had decreased and trace amounts in other tissues also decreased except for heart and skeletal muscle. 24 hours: High levels of radioactivity in large intestine contents and trace amounts in liver, heart and skeletal muscle; below the detection limit in other tissues. 72 hours: no radioactivity detected (data not shown). Distribution of radioactivity similar in pigmented rats (data not shown) and no binding of pitavastatin to uveal pigment of eye.</p> <p><b>i.v. administration:</b> 2.5 minutes: high levels of radioactivity in liver and kidney, followed by heart, lung, intestinal wall and blood. Moderate levels in brown fat, Harder's gland, submaxillary gland, skeletal muscle and bone marrow. Very weak levels of radioactivity in brain, spinal cord and testis. 1 hour: highest levels of radioactivity in liver followed by small intestinal contents. Moderate levels in heart and kidney cortex, and low levels in brown fat, blood, skeletal muscle and Harder's gland. Radioactivity in lung, submaxillary gland, eye ball, brain and testis close to the limit of detection. 6 hours: radioactivity highest in small intestinal contents followed by liver. Radioactivity in the kidney, heart and lung decreased compared to 1 hour time point. No radioactivity in eye ball, brain or testis. 24 hours: Distribution of radioactivity was similar to that observed with oral administration. 72 hours: no radioactivity detected (data not shown).</p>					

2.6.5.9A Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin		Page 1 of 1
Reference: [Fujino <i>et al.</i> , 1999a]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]			Location in CTD: Vol.: * Section: *	
Species:	Wistar rats			
Gender (M/F)/No. of Animals:	5M – surgically prepared with common bile duct cannulae under ether anaesthesia			
Feeding Condition:	Fasted overnight before dosing			
Vehicle/Formulation:	Suspension in 0.5% CMC sodium solution			
Method of Administration:	Oral			
Dose (mg/kg):	1			
Assay:	HPLC-CS <sup>a</sup>			
Sampling Time:	The bile was continuously collected for 24 hours in the dark to prevent degradation of biliary metabolites.			
Analyte	Composition of Pitavastatin and Its Metabolites in Bile After Oral Administration to Rats			
	Composition (Mean ± SE; % of dose)			
	Unconjugated			Conjugated
Pitavastatin	32.27 ± 4.73			0.32 ± 0.20
Lactone	5.56 ± 1.29			0.10 ± 0.10
M-2	0.00			0.02 ± 0.02
5-ketone	7.08 ± 0.85			1.01 ± 0.35
M-4	0.12 ± 0.02			0.01 ± 0.00
M-5	0.00			0.34 ± 0.01
M-6	1.52 ± 0.38			11.22 ± 1.32
M-7	0.25 ± 0.02			0.00
M-8	0.07 ± 0.04			0.02 ± 0.02
M-9	0.00			0.00
M-10	NA			1.97 ± 0.21
M-11	NA			3.07 ± 0.49
Total recovery	64.96 ± 5.97			
Additional Information:				
*: Not applicable to an electronic submission				
a: HPLC-CS as described in [Kojima <i>et al.</i> , 1999a]; the Sponsor identified that the validation methods summarised in [Kojima <i>et al.</i> , 1999a] were [R92052], [R95077] and [R98018]				

2.6.5.9B Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin		Page 1 of 2								
Reference: [Kimata <i>et al.</i> , 1998] and Corrigenda published in [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]			Location in CTD: Vol.: * Section: *									
Species:	Jia:Wistar rats		Gender (M/F)/No. of Animals:	3M								
Feeding Condition:	Fasted overnight before dosing and for 6 hours after dosing											
Vehicle/Formulation:	Solution in 0.5% CMC sodium solution											
Method of Administration:	Oral (gavage)											
Dose (mg/kg):	1											
Radioisotope:	[ <sup>14</sup> C]-pitavastatin		Specific Activity:	2.2 to 3.5 MBq/mg								
Tissues/Organs:	Plasma, liver, kidney, lung, heart and skeletal muscle											
Sampling Time:	Oral: 0.5, 1, 3, 6 and 24 hours after dosing											
Assay:	HPLC-RLG											
Tissue	Time (hours)	Concentration of Pitavastatin and Its Metabolites in Plasma, Liver, Kidney, Lung, Heart and Skeletal Muscle after Oral Administration of [ <sup>14</sup> C]-Pitavastatin to Male Rats (Mean ± SD; ng/mL or g)										
		Pitavastatin	Lactone	5-ketone	M-4	M-6	M-7	M-8	M-10	M-11	Unknown	Polar
Plasma	0.5	200 ± 77	ND	11 ± 5	1 ± 1	13 ± 12	ND	4 ± 0	ND	ND	4 ± 1	5 ± 2
	1	79 ± 20	ND	3 ± 1	ND	5 ± 4	ND	4 ± 1	ND	ND	2 ± 1	4 ± 1
	3	33 ± 13	ND	1 ± 2	ND	6 ± 7	ND	4 ± 1	ND	ND	ND	5 ± 2
	6	13 ± 5	ND	ND	ND	3 ± 2	ND	5 ± 2	ND	ND	ND	7 ± 2
	24	ND	ND	ND	ND	ND	ND	7 ± 1	ND	ND	ND	6 ± 1
Liver	0.5	3812 ± 1545	55 ± 30	29 ± 12	14 ± 12	22 ± 19	4 ± 2	9 ± 4	9 ± 8	9 ± 6	114 ± 57	796 ± 330
	1	2112 ± 205	27 ± 17	50 ± 44	8 ± 7	13 ± 11	6 ± 5	8 ± 6	8 ± 9	7 ± 7	112 ± 59	516 ± 80
	3	794 ± 210	8 ± 3	6 ± 2	2 ± 2	4 ± 4	2 ± 1	11 ± 2	3 ± 2	3 ± 2	31 ± 13	304 ± 2
	6	404 ± 116	2 ± 2	2 ± 2	1 ± 1	2 ± 2	ND	6 ± 5	4 ± 4	1 ± 0	9 ± 4	197 ± 27
	24	6 ± 5	ND	ND	ND	ND	ND	5 ± 2	2 ± 2	1 ± 0	2 ± 1	64 ± 13
Kidney	0.5	366 ± 90	1 ± 1	32 ± 17	1 ± 1	7 ± 7	ND	2 ± 0	ND	ND	12 ± 4	69 ± 11
	1	149 ± 19	ND	15 ± 4	ND	4 ± 4	ND	ND	ND	ND	3 ± 1	59 ± 17
	3	48 ± 8	ND	8 ± 4	ND	4 ± 5	ND	1 ± 1	ND	ND	1 ± 2	25 ± 11
	6	32 ± 6	ND	3 ± 2	ND	3 ± 2	ND	2 ± 1	ND	ND	ND	17 ± 5
	24	ND	ND	ND	ND	3 ± 3	ND	10 ± 1	ND	ND	ND	8 ± 1

2.6.5.9B Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin		Page 2 of 2								
Reference: [Kimata <i>et al.</i> , 1998] and Corrigenda published in [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]			Location in CTD: Vol.: * Section: *									
Tissue	Time (hours)	Concentration of Pitavastatin and Its Metabolites in Plasma, Liver, Kidney, Lung, Heart and Skeletal Muscle after Oral Administration of [ <sup>14</sup> C]-Pitavastatin to Male Rats (Mean ± SD; ng/mL or g)										
		Pitavastatin	Lactone	5-ketone	M-4	M-6	M-7	M-8	M-10	M-11	Unknown	Polar
Lung	0.5	131 ± 27	ND	1 ± 1	ND	3 ± 3	ND	ND	ND	ND	ND	17 ± 9
	1	34 ± 30	ND	ND	ND	ND	ND	ND	ND	ND	ND	7 ± 2
	3	17 ± 3	ND	ND	ND	ND	ND	ND	ND	ND	1 ± 1	4 ± 1
	6	11 ± 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	4 ± 1
	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4 ± 1
Heart	0.5	101 ± 21	ND	8 ± 7	1 ± 1	46 ± 40	ND	1 ± 1	ND	ND	1 ± 1	11 ± 4
	1	41 ± 20	ND	3 ± 2	ND	47 ± 40	ND	1 ± 0	ND	ND	ND	12 ± 4
	3	7 ± 3	ND	21 ± 25	ND	29 ± 34	ND	5 ± 2	ND	ND	ND	8 ± 5
	6	ND	ND	1 ± 1	ND	22 ± 13	ND	8 ± 4	ND	ND	ND	6 ± 1
	24	ND	ND	ND	ND	31 ± 15	ND	38 ± 1	ND	ND	ND	7 ± 2
Skeletal muscle	0.5	19 ± 10	ND	ND	ND	3 ± 2	ND	ND	ND	ND	ND	3 ± 2
	1	11 ± 3	ND	ND	ND	3 ± 3	ND	ND	ND	ND	ND	4 ± 1
	3	3 ± 3	ND	ND	ND	4 ± 5	ND	ND	ND	ND	ND	1 ± 1
	6	ND	ND	ND	ND	2 ± 2	ND	ND	ND	ND	ND	2 ± 2
	24	ND	ND	ND	ND	5 ± 3	ND	8 ± 1	ND	ND	ND	3 ± 1

2.6.5.9C Pharmacokinetics: Metabolism <i>In Vivo</i> (Repeat Dose)				Test Article: Pitavastatin				Page 1 of 2	
Reference: [Ujino <i>et al.</i> , 1998a]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 364]				Location in CTD:				Vol: *	
Species: Wistar rats				Gender (M/F)/No. of Animals: 4M				Section: *	
Feeding Condition: Fed				Vehicle/Formulation: Solution in 0.5% CMC sodium solution					
Method of Administration: Oral (gavage)				Dose Regimen: 1 mg/kg daily for 9 days					
Radiolabeled / Specific Activity: <sup>14</sup> C-pitavastatin / 2.20 MBq/mg									
Tissues/Organs: Plasma, heart, skeletal muscle, urine and faeces									
Sampling Time: 0.5 and 24 hours after the 1 <sup>st</sup> dose, 24 hours after the 6 <sup>th</sup> dose and 0.5, 24 (1 day), 72 (3 days) and 144 hours (6 days) after the 9 <sup>th</sup> dose									
Assay: HPLC-RLG									
<b>Concentration of Pitavastatin and Its Metabolites in Plasma after Repeated Oral Administration of [<sup>14</sup>C]-Pitavastatin to Rats</b>									
Concentration (Mean ± SD; ng/mL or g)									
Number of Doses and Time after Dosing									
		Single Dose		6 <sup>th</sup> Dose		9 <sup>th</sup> Dose			
		0.5 hours	24 hours	24 hours	0.5 hours	24 hours	72 hours	144 hours	
Pitavastatin		34 ± 6	2 ± 3	8 ± 6	52 ± 16	5 ± 4	ND	ND	
5-ketone		ND	ND	ND	1 ± 1	ND	ND	ND	
M-6		2 ± 1	ND	5 ± 6	5 ± 4	4 ± 3	ND	ND	
M-8		ND	ND	5 ± 4	8 ± 4	5 ± 3	ND	ND	
M-10		ND	ND	ND	1 ± 1	ND	ND	ND	
Polar		3 ± 1	3 ± 1	7 ± 1	12 ± 4	9 ± 3	4 ± 1	ND	
<b>Concentration of Pitavastatin and Its Metabolites in Heart after Repeated Oral Administration of [<sup>14</sup>C]-Pitavastatin to Rats</b>									
Concentration (Mean ± SD; ng/mL or g)									
Number of Doses and Time after Dosing									
		Single Dose		6 <sup>th</sup> Dose		9 <sup>th</sup> Dose			
		0.5 hours	24 hours	24 hours	0.5 hours	24 hours	72 hours	144 hours	
Pitavastatin		13 ± 5	ND	ND	15 ± 7	ND	ND	ND	
M-6		23 ± 9	49 ± 32	69 ± 30	119 ± 48	82 ± 24	ND	ND	
M-8		ND	10 ± 2	37 ± 9	56 ± 10	38 ± 7	24 ± 6	8 ± 5	
M-9		ND	ND	1 ± 1	2 ± 2	1 ± 2	ND	ND	
Polar		2 ± 1	2 ± 1	4 ± 1	7 ± 2	4 ± 1	2 ± 0	1 ± 1	

2.6.5.9C Pharmacokinetics: Metabolism <i>In Vivo</i> (Repeat Dose)				Test Article: Pitavastatin				Page 2 of 2	
Reference: [Ujino <i>et al.</i> , 1998a]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 364] (continued)									
<b>Concentration of Pitavastatin and Its Metabolites in Skeletal Muscle after Repeated Oral Administration of [<sup>14</sup>C]-Pitavastatin to Rats</b>									
Concentration (Mean ± SD; ng/mL or g)									
Number of Doses and Time after Dosing									
		Single Dose		6 <sup>th</sup> Dose		9 <sup>th</sup> Dose			
		0.5 hours	24 hours	24 hours	0.5 hours	24 hours	72 hours	144 hours	
Pitavastatin		5 ± 2	ND	ND	7 ± 3	ND	ND	ND	
M-6		2 ± 2	7 ± 5	10 ± 3	13 ± 8	12 ± 5	ND	ND	
M-8		ND	ND	5 ± 1	6 ± 1	6 ± 1	2 ± 2	ND	
Polar		1 ± 0	ND	1 ± 0	2 ± 0	2 ± 1	1 ± 0	ND	
<b>Composition of Pitavastatin and Its Metabolites in Faeces and Urine after Repeated Oral Administration of [<sup>14</sup>C]-Pitavastatin to Rats</b>									
Composition (Mean ± SD; % radioactivity)									
		1 <sup>st</sup> dose		6 <sup>th</sup> dose		9 <sup>th</sup> dose			
Faeces	Pitavastatin		75.5 ± 2.1		75.5 ± 2.6		73.0 ± 1.7		
	Lactone		2.0 ± 0.3		1.1 ± 0.2		1.5 ± 0.2		
	5-ketone		1.8 ± 0.1		1.9 ± 0.1		1.9 ± 0.5		
	M-6		0.9 ± 0.8		2.8 ± 1.4		3.6 ± 1.5		
	M-7		0.2 ± 0.1		0.3 ± 0.1		0.3 ± 0.2		
	M-8		0.2 ± 0.1		0.2 ± 0.0		0.2 ± 0.1		
	M-10		1.0 ± 0.2		1.3 ± 0.1		1.2 ± 0.3		
	M-11		0.9 ± 0.1		0.8 ± 0.2		1.2 ± 0.4		
	Unknown		3.7 ± 0.6		3.6 ± 0.7		5.1 ± 1.7		
Urine	Pitavastatin		33.3 ± 10.0		5.4 ± 6.6		4.6 ± 3.5		
	M-10		0.9 ± 1.7		0.0		1.7 ± 2.5		
	M-11		0.0		5.0 ± 6.1		2.6 ± 4.0		
	Unknown		14.8 ± 6.9		34.1 ± 2.6		28.5 ± 8.0		
	Polar		50.7 ± 4.5		55.4 ± 9.3		62.7 ± 5.3		

2.6.5.9D Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)				Test Article: Pitavastatin				Page 1 of 1	
Report No.: [R98054]				Location in CTD:				Vol: * Section: *	
Species: Wistar rats				Beagle dogs					
Gender (M/F)/Number of Animals: 4M				4M					
Feeding Condition: Fasted				Fasted					
Vehicle/Formulation: Solution in 0.5% CMC sodium solution				Gelatine capsule					
Method of Administration: Oral (gavage)				Oral					
Dose (mg/kg): 3				1					
Sample (whole blood, plasma, serum etc.): Plasma (pre-dose and up to 6 hours post-dose)				Plasma (pre-dose and up to 8 hours post-dose)					
Analyte: Pitavastatin <sup>a</sup> , optical isomers (enantiomer [3S, 5R]; 5-epimer [3R, 5R]; 3-epimer [3S, 5S])				Pitavastatin <sup>a</sup> , optical isomers (enantiomer [3S, 5R]; 5-epimer [3R, 5R]; 3-epimer [3S, 5S])					
Assay: HPLC-CP				HPLC-CP					
<b>PK Parameters (Mean ± SE):</b>				<b>PK Parameters (Mean ± SE):</b>					
		Pitavastatin	Enantiomer	5-epimer	3-epimer	Pitavastatin	Enantiomer	5-epimer	3-epimer
C <sub>max</sub> (ng/mL)		439 ± 67	-	ND	ND	719 ± 285	ND	ND	ND
T <sub>max</sub> (hours)		0.5 to 2.0	-	NA	NA	0.5 to 4.0	NA	NA	NA
<b>Additional Information:</b>				<b>Additional Information:</b>					
Since the peak of the 3S, 5R optical isomer was influenced by an unknown metabolite this isomer was excluded. Pitavastatin at concentrations ≥ 70 ng/mL were observed in 3/4 animals at 6 hours post-dose. 3R, 5R and 3S, 5S isomers were not detected (LLOQ: 33.3 ng/mL).				Pitavastatin at concentrations 1/5 to 1/2 of the C <sub>max</sub> value was observed at 8 hours post-dose. 3S, 5R, 3R, 5R and 3S, 5S isomers were not detected (LLOQ: 10 ng/mL)					

a: 0.2 mL of 25 mol/L sodium hydroxide was added to 1 mL of dog plasma or 0.3 mL of rat plasma (diluted with water to make a total of 1 mL) and allowed to stand at room temperature for 30 minutes so that the lactone co-existing with pitavastatin was hydrolysed, the total concentration as pitavastatin was determined.  
 -: Analysis of enantiomer was not conducted

2.6.5.9E Pharmacokinetics: Metabolism <i>In Vivo</i> (Continuous infusion)		Test Article: Pitavastatin		Page 1 of 1
Reference: [Kojima <i>et al.</i> , 1999b]		Location in CTD:	Vol.: *	Section: *
Species:	Jla: Wistar rats; MJ:JW rabbits; beagle dog			
Gender (M/F)/Number of Animals:	9M rats; 6M rabbits; 1M dog			
Feeding Condition:	NA			
Vehicle/Formulation:	Solution in 4% (weight/volume (w/v)) trisodium citrate-saline (1:9)			
Animal Preparation:	Animals were anaesthetised and the inferior vena cava and bile duct cannulated with polyethylene tubing.			
Method of Administration:	Rat: continuous i.v. infusion at 0.5 mL/hour for 24 hours Rabbit: continuous i.v. infusion at 2 mL/hour for 8 hours Dog: continuous i.v. infusion at 8 mL/hour for 10.5 hours			
Dosing Solution (mg/mL):	7 to 7.5			
Sample (whole blood, plasma, serum etc.):	Bile: approximately 100 mL collected from the rats and dog and approximately 300 mL collected from the rabbits			
Analyte:	Pitavastatin and metabolites			
Assay:	Structural assignment of metabolites was made using LC-MS-MS and proton NMR analyses			
Results:	The predominant analyte in each species was unchanged pitavastatin. Eight metabolites were present in the rat, four metabolites in the rabbit and ten metabolites in the dog. These bile metabolites were purified and isolated using preparative HPLC and biotransformation pathways elucidated for pitavastatin. The pathways identified were: (i) lactonisation; (ii) $\beta$ -oxidative degradation of the side-chain; (iii) hydroxylation of the quinoline ring; (iv) conjugation with $\beta$ -glucuronic acid and taurine. $\beta$ -oxidative degradation of the side-chain with other HMG-CoA reductase inhibitors is necessary for epimerisation of the hydroxyl group which has an R-configuration. However, M-16, glucuronide of the ketolactone derivative, was obtained as a key metabolite suggesting another $\beta$ -oxidative pathway for the side-chain.			

**Pitavastatin and its Metabolites Isolated from Bile in Rats, Rabbits and Dogs**

Species	Pitavastatin	Lactone	M-2	5-ketone	M-3	M-5	M-6	M-8	M-10	M-11	M-12	$\beta$ -hydroxy pitavastatin	M-14	M-15	M-16	M-17	M-18
Rat	Present	Present	Present	Present	ND	ND	Present	ND	Present	Present	Present	Present	ND	ND	ND	ND	Present
Rabbit	Present	ND	Present	ND	ND	ND	Present	ND	ND	ND	ND	ND	Present	ND	Present	ND	ND
Dog	Present	Present	Present	Present	Present	Present	Present	Present	ND	ND	Present	ND	ND	Present	Present	Present	ND

2.6.5.9F Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin		Page 1 of 1
Reference: [Fujino <i>et al.</i> , 1999a]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]		Location in CTD:	Vol.: *	Section: *
Species:	JIRA-beagle dogs			
Gender (M/F)/No. of Animals:	4M			
Feeding Condition:	Fasted			
Vehicle/Formulation:	Suspension in 0.5% CMC sodium solution (oral) or solution in saline (i.v.)			
Method of Administration:	Oral or i.v. (bolus injection)			
Dose (mg/kg):	1 or 10 (oral) or 0.1 (i.v.)			
Tissues/Organs:	Plasma			
Sampling Time:	Not stated			
Assay:	HPLC-CS*			

**PK Parameters of Pitavastatin and its Metabolites in Plasma after Single Oral or Intravenous Administration to Dogs (Mean  $\pm$  SE)**

Dose Regimen	Analyte	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (hours)	AUC (ng <sup>2</sup> h/mL)	t <sub>1/2</sub> (hours)
0.1 mg/kg i.v.	Pitavastatin	-	-	398 $\pm$ 37	-
	Lactone	11 $\pm$ 2	1.0 $\pm$ 0.4	42 $\pm$ 6	-
	5-ketone	2 $\pm$ 1	0.5 $\pm$ 0.0	2 $\pm$ 1	-
	M-5	7 $\pm$ 2	1.9 $\pm$ 0.8	25 $\pm$ 8	-
1 mg/kg oral	Pitavastatin	526 $\pm$ 107	0.8 $\pm$ 0.2	1590 $\pm$ 270	-
	Lactone	73 $\pm$ 2	2.5 $\pm$ 0.5	440 $\pm$ 30	-
	5-ketone	9 $\pm$ 2	0.7 $\pm$ 0.2	20 $\pm$ 10	-
	M-5	36 $\pm$ 10	4.0 $\pm$ 0.0	170 $\pm$ 30	-
10 mg/kg oral	Pitavastatin	9509 $\pm$ 2488	1.3 $\pm$ 0.3	35480 $\pm$ 4320	5.6 $\pm$ 0.5
	Lactone	1013 $\pm$ 177	4.0 $\pm$ 1.4	14500 $\pm$ 1650	5.8 $\pm$ 1.0
	5-ketone	97 $\pm$ 21	1.0 $\pm$ 0.0	460 $\pm$ 140	-
	M-4	7 $\pm$ 1	3.5 $\pm$ 1.5	110 $\pm$ 40	-
	M-5	400 $\pm$ 57	6.0 $\pm$ 1.2	7570 $\pm$ 850	13.8 $\pm$ 0.9
	M-6	4 $\pm$ 1	2.5 $\pm$ 0.5	40 $\pm$ 30	-
M-8	40 $\pm$ 11	4.5 $\pm$ 1.3	1070 $\pm$ 320	28.4 $\pm$ 4.3	

\*: Not applicable to an electronic submission

a: HPLC-CS as described in [Kojima *et al.*, 1999a]; the Sponsor identified that the validation methods summarised in [Kojima *et al.*, 1999a] were [R92052], [R95077] and [R98018]

2.6.5.9G Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin		Page 1 of 4				
Report No.:	191.8041	Location in CTD:		Vol.:	*			
Species:	Cynomolgus monkeys		Gender (M/F)/Number of Animals:	3M (oral) and 1M (i.v.)				
Feeding Condition:	Fasted overnight before dosing and for 8 hours after dosing							
Vehicle/Formulation:	Suspension in 0.5% CMC sodium (oral) or solution in physiological saline (i.v.)							
Method of Administration:	Oral (gavage) or i.v.		Dose (mg/kg):	3 (oral; 2 mL/kg) or 0.3 (i.v.; 1 mL/kg)				
Tissues/Organs:	Blood and plasma		Radionuclide / Specific Activity:	<sup>14</sup> C-pitavastatin / 981 kBq/mg				
Sampling Time:	Up to 48 hours post-dosing		Assay:	HPLC-RI, HPLC-RLG				
<b>Pitavastatin and Its Metabolites in Plasma After a Single Oral Dose of [<sup>14</sup>C]-Pitavastatin (Mean ± SD; ng eq. Pitavastatin/mL)</b>								
Analyte	30 minutes	1 hour	2 hours	4 hours	8 hours	12 hours	24 hours	48 hours
Pitavastatin	160.0 ± 113.6	226.2 ± 90.1	125.2 ± 34.7	222.8 ± 82.8	112.3 ± 65.6	29.2 ± 6.9	8.3 ± 3.4	ND
Lactone	ND	3.5 ± 3.1	19.4 ± 13.3	12.5 ± 1.1	ND	ND	ND	ND
5-ketone	9.0 ± 5.8	11.4 ± 8.3	10.9 ± 9.6	14.2 ± 2.0	7.1 ± 6.2	ND	ND	ND
M-6	ND	2.0 ± 2.6	2.8 ± 2.9	3.7 ± 2.9	ND	ND	ND	ND
M-8	ND	ND	ND	ND	ND	ND	ND	ND
M-9	ND	ND	ND	1.5 ± 1.3	ND	ND	ND	ND
M-14	34.7 ± 21.7	88.8 ± 26.1	82.5 ± 14.2	67.2 ± 20.5	56.6 ± 10.4	21.0 ± 7.9	7.2 ± 9.7	34.2 ± 16.4
Others	11.7 ± 12.5	78.9 ± 7.6	139.2 ± 52.0	134.7 ± 37.5	180.1 ± 7.4	140.3 ± 60.1	104.4 ± 52.1	190.3 ± 101.0
Unextracted	7.4 ± 12.9	22.7 ± 8.1	46.7 ± 44.6	62.3 ± 18.3	33.3 ± 18.5	33.4 ± 10.2	18.2 ± 8.0	31.9 ± 15.8
<b>Pitavastatin and Its Metabolites in Plasma After a Single Intravenous Dose of [<sup>14</sup>C]-Pitavastatin (ng eq. Pitavastatin/mL)</b>								
Analyte	5 minutes	30 minutes	1 hour	2 hours	4 hours	8 hours		
Pitavastatin	1719.6	231.5	94.0	49.0	30.0	ND		
Lactone	3.7	ND	ND	ND	ND	ND		
5-Ketone	11.0	ND	ND	1.2	ND	ND		
M-6	ND	5.1	7.7	3.3	ND	ND		
M-8	ND	ND	ND	ND	ND	ND		
M-9	ND	ND	ND	ND	ND	ND		
M-14	ND	3.2	14.8	12.7	ND	ND		
Others	7.3	ND	ND	ND	ND	ND		
Unextracted	88.0	17.7	13.5	0.0	0.0	1.6		

\*: Not applicable to an electronic submission; HPLC-RI: High performance liquid chromatography with radioimaging (radioisotope detection)

2.6.5.9G Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin		Page 2 of 4	
Report No.:	191.8041 (continued)				
Species:	Cynomolgus monkeys				
Gender (M/F)/Number of Animals:	3M (oral) and 1M (i.v.)				
Feeding Condition:	Fasted overnight before dosing and for 8 hours after dosing				
Vehicle/Formulation:	Suspension in 0.5% CMC sodium (oral) or solution in physiological saline (i.v.)				
Method of Administration:	Oral gavage or i.v.				
Dose (mg/kg):	3 (oral; 2 mL/kg) or 0.3 (i.v.; 1 mL/kg)				
Tissues/Organs:	Urine				
Radionuclide:	<sup>14</sup> C-pitavastatin				
Specific Activity:	981 kBq/mg				
Sampling Time:	Up to 72 hours post-dosing				
Assay:	HPLC-RI, HPLC-RLG				
<b>Pitavastatin and Its Metabolites in Urine After a Single Oral Dose of [<sup>14</sup>C]-Pitavastatin (Mean ± SD, % of dose)</b>					
Analyte	0 to 24 hours	24 to 48 hours	48 to 72 hours	Total	
Pitavastatin	0.794 ± 0.277	0.062 ± 0.011	ND	0.864 ± 0.276	
8-hydroxy pitavastatin	0.057 ± 0.054	ND	ND	0.057 ± 0.054	
M-14	6.403 ± 1.734	0.687 ± 0.126	0.364 ± 0.072	7.455 ± 1.824	
Pitavastatin, 8-hydroxy pitavastatin and M-14	7.254	0.749	0.364	8.376	
Not recovered	0.140 ± 0.025	0.026 ± 0.005	0.016 ± 0.006	0.182 ± 0.021	
<b>Pitavastatin and Its Metabolites in Urine After a Single Intravenous Dose of [<sup>14</sup>C]-Pitavastatin (% of dose)</b>					
Analyte	0 to 24 hours	24 to 48 hours	48 to 72 hours	Total	
Pitavastatin	5.375	ND	ND	5.375	
8-hydroxy pitavastatin	ND	ND	ND	ND	
M-14	4.530	0.623	ND	5.153	
Pitavastatin, 8-hydroxy pitavastatin and M-14	9.905	0.623	0.000	10.528	
Not recovered	0.161	0.041	0.015	0.217	

2.6.3.9G Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin			Page 3 of 4
Report No.: [91.804] (continued)					
Species:	Cynomolgus monkeys		Gender (M/F)/Number of Animals:	3M(oral) and 1M(i.v.)	
Feeding Condition:	Fasted overnight before dosing and for 8 hours after dosing				
Vehicle/Formulation:	Suspension in 0.5% CMC sodium (oral) or solution in physiological saline (i.v.)				
Method of Administration:	Oral gavage or i.v.	Dose (mg/kg):		3 (oral; 2 mL/kg) or 0.3 (i.v.; 1 mL/kg)	
Tissues/Organs:	Faeces				
Radioisotope:	<sup>14</sup> C-pitavastatin				
Specific Activity:	981 kBq/mg				
Sampling Time:	Up to 72 hours post-dosing				
Assay:	HPLC-RI, HPLC-RLG				
Pitavastatin and Its Metabolites in Faeces After a Single Oral Dose of [ <sup>14</sup> C]-Pitavastatin (Mean ± SD, % of dose)					
Analyte	0-24 hours	24-48 hours	48-72 hours	Total	
Pitavastatin	1.271 ± 2.177	5.741 ± 3.358	6.856 ± 3.452	13.867 ± 2.341	
Lactone	ND	2.673 ± 1.796	3.301 ± 1.411	6.144 ± 0.719	
M-6	ND	ND	0.161 ± 0.190	0.259 ± 0.132	
8-hydroxy pitavastatin	ND	2.098 ± 1.216	4.068 ± 1.920	6.477 ± 0.668	
M-14	1.120 ± 1.888	3.103 ± 1.305	3.035 ± 1.871	7.258 ± 1.586	
Pitavastatin, lactone, M-6, 8-hydroxy pitavastatin and M-14	2.391	13.615	17.421	34.005	
Not recovered	0.533 ± 0.918	6.265 ± 2.138	5.248 ± 4.276	12.045 ± 2.727	
Pitavastatin and Its Metabolites in Faeces After a Single Intravenous Dose of [ <sup>14</sup> C]-Pitavastatin (% of dose)					
Analyte	0-24 hours	24-48 hours	48-72 hours	Total	
Pitavastatin	0.068	13.636	3.783	17.487	
Lactone	ND	3.422	0.911	4.333	
M-6	ND	0.526	0.228	0.754	
8-hydroxy pitavastatin	ND	7.792	2.365	10.157	
M-14	ND	5.581	1.539	7.140	
Pitavastatin, lactone, M-6, 8-hydroxy pitavastatin and M-14	0.068	30.957	8.846	39.871	
Not recovered	0.000	13.057	5.763	18.820	

2.6.3.9G Pharmacokinetics: Metabolism <i>In Vivo</i> (Single Dose)		Test Article: Pitavastatin			Page 4 of 4
Report No.: [91.803] (continued)					
Species:	Cynomolgus monkeys				
Gender (M/F)/Number of Animals:	2M				
Feeding Condition:	Fasted overnight before dosing and for 8 hours after dosing				
Vehicle/Formulation:	Suspension in 0.5% CMC sodium (oral) or solution in physiological saline (i.v.)				
Method of Administration:	Oral gavage or i.v.				
Dose (mg/kg):	3 (oral; 2 mL/kg) or 0.3 (i.v.; 1 mL/kg)				
Tissues/Organs:	Bile, collection of bile samples described in [91.805]				
Radioisotope:	<sup>14</sup> C-pitavastatin				
Specific Activity:	981 kBq/mg				
Sampling Time:	Up to 48 hours post-dosing				
Assay:	HPLC-RI, HPLC-RLG				
Pitavastatin and Its Metabolites in Bile After a Single Oral Dose of [ <sup>14</sup> C]-Pitavastatin (Mean, % of dose)					
Analyte	4 hours	48 hours			
Pitavastatin	4.953	0.422			
Lactone	6.683	0.339			
8-hydroxy pitavastatin	1.593	0.341			
M-14	2.270	0.745			
Pitavastatin, lactone, 8-hydroxy pitavastatin and M-14	15.499	1.847			
Recovery from HPLC (%)	>96.8	>99.6			

2.6.5.10A Pharmacokinetics: Metabolism <i>In Vitro</i>		Test Article: Pitavastatin			Page 1 of 1
Report No.: [R99007]					
Type of Study:		Location in CTD:		Vol: *	Section: *
In vitro study to assess the metabolism of [ <sup>14</sup> C]-pitavastatin in liver microsomes from rats (male and female), male guinea pigs, male rabbits, male beagle dogs, male cynomolgus monkeys and humans; in addition rat (male), monkey (male) and human S-9 was examined.					
Method: Pooled liver microsomes from humans (five males and five females) and pooled human S-9 (five males and five females) were used. All microsomes and S-9 were obtained commercially. [ <sup>14</sup> C]-pitavastatin was used at a final concentration of 0.5 and 2.5 μmol/L. A 2 hour incubation at 37°C was utilised and the incubations were terminated using methanol. Total radioactivity was measured using liquid scintillation counting and the measurement of the radioactivity of pitavastatin and its metabolites was conducted using HPLC-RLG.					
Results: [ <sup>14</sup> C]-pitavastatin was metabolised slightly in all species with the exception of monkey microsomes which markedly metabolised pitavastatin, primarily to 8-hydroxy pitavastatin and unknown metabolites. In the other species, pitavastatin was largely unchanged with 8-hydroxy pitavastatin as the predominant metabolite.					
Composition of Pitavastatin and Its Metabolites After a 2 Hour Incubation with Liver Microsomes and S-9 (Mean ± SE; n = 4-5; % of composition)					
Species	Pitavastatin	8-hydroxy pitavastatin	5-ketone	M-8	Unknown
Male Wistar rat microsomes	83.4 ± 0.8	9.5 ± 0.8	4.3 ± 0.2	0.7 ± 0.2	2.1 ± 0.3
Female Wistar rat microsomes	81.0 ± 0.8	17.7 ± 0.5	0.4 ± 0.0	0.0	0.9 ± 0.3
Male guinea pig microsomes	89.8 ± 0.5	2.7 ± 0.2	0.0	0.0	7.5 ± 0.5
Male rabbit microsomes	93.1 ± 0.4	2.0 ± 0.2	0.0	0.0	5.0 ± 0.2
Male beagle dog microsomes	98.0 ± 0.6	0.4 ± 0.0	1.5 ± 0.5	0.1 ± 0.0	0.1 ± 0.0
Male cynomolgus monkey microsomes	39.4 ± 3.1	38.4 ± 2.3	0.7 ± 0.1	0.0	21.4 ± 1.4
Human microsomes <sup>a</sup>	78.8 ± 1.2	16.3 ± 0.8	0.5 ± 0.1	0.0	4.3 ± 0.6
Human microsomes <sup>b</sup>	79.1 ± 1.1	15.4 ± 1.6	1.1 ± 0.0	0.0	4.4 ± 0.3
Male rat S-9	94.0 ± 0.5	0.5 ± 0.2	1.1 ± 0.0	0.1 ± 0.0	4.3 ± 0.5
Male cynomolgus monkey S-9	85.0 ± 1.3	11.5 ± 1.2	0.3 ± 0.0	0.0	3.3 ± 0.2
Human S-9 <sup>a</sup>	90.5 ± 0.8	7.5 ± 0.8	0.1 ± 0.0	0.0	2.0 ± 0.2
Human S-9 <sup>b</sup>	92.4 ± 0.6	6.0 ± 0.5	0.2 ± 0.0	0.0	1.4 ± 0.2
Additional Information:					

a: Two separate experiments were conducted

<b>2.6.5.10B Pharmacokinetics: Metabolism <i>In Vitro</i></b>		<b>Test Article: Pitavastatin</b>		Page 1 of 2
<b>Reference:</b> [Ujino <i>et al.</i> , 1999b]		<b>Location in CTD:</b>	<b>Vol: *</b>	<b>Section: *</b>
<p><b>Type of Study:</b> <i>In vitro</i> study to assess the metabolism of [<sup>14</sup>C]-pitavastatin in liver microsomes from rats (male and female), male guinea pigs, male rabbits, male beagle dogs, male cynomolgus monkeys and humans; in addition human S-9 was examined (results previously presented in [2.6.5.10A], [R99007]). The apparent K<sub>m</sub> and V<sub>max</sub> values of pitavastatin metabolism were determined.</p> <p><b>Method:</b> Pooled liver microsomes from humans and pooled human S-9 were used. All microsomes and human S-9 were obtained commercially. [<sup>14</sup>C]-pitavastatin was used at a final concentration of 2.5 μmol/L. A 2 hour incubation at 37°C was utilised and the incubations were terminated using methanol. The apparent K<sub>m</sub> and V<sub>max</sub> values were determined using [<sup>14</sup>C]-pitavastatin at 2.5 to 170 μmol/L (male and female rat microsomes), 0.5 to 50 μmol/L (monkey microsomes) and 1.0 to 100 μmol/L (human microsomes) and an incubation time of 30 minutes. Total radioactivity was measured using liquid scintillation counting and the measurement of the radioactivity of pitavastatin and its metabolites was conducted using HPLC-RLG.</p>				
<b>Kinetic Constants for the Metabolism of Pitavastatin to 8-hydroxy pitavastatin</b>				
<b>Microsomes</b>	<b>K<sub>m</sub> (μmol/L)</b>	<b>V<sub>max</sub> (pmol/min/mg protein)</b>	<b>V<sub>max</sub>/K<sub>m</sub> (μL/min/mg protein)</b>	
Male rat	323.3	319.4	0.99	
Female rat	83.1	173.1	2.08	
Monkey	13.6	82.4	6.06	
Human	45.1	77.4	1.79	
	(Individual values: 22.6, 32.9, 47.7, 77.0)	(Individual values: 48.7, 63.8, 64.0, 133.0)	(Individual values: 1.34, 1.73, 1.94, 2.15)	

<b>2.6.5.10B Pharmacokinetics: Metabolism <i>In Vitro</i></b>		<b>Test Article: Pitavastatin</b>		Page 2 of 2
<b>Reference:</b> [Ujino <i>et al.</i> , 1999b] (continued)				
<p><b>Type of Study:</b> <i>In vitro</i> study to assess the effects of CYP inhibitors on metabolism of [<sup>14</sup>C]-pitavastatin in human liver microsomes.</p> <p><b>Method:</b> [<sup>14</sup>C]-pitavastatin (2.5 μmol/L) was co-incubated with SKF-525A (300 μmol/L), α-naphthoflavone (5 μmol/L), ketoconazole (2 μmol/L), or sulphaphenazole (4 μmol/L) prior to assay. For immuno-inhibition experiments, human microsomes were incubated at room temperature for 20 minutes with 50 μL (0.5 mg eq. IgG) of rabbit/goat antiserum or control serum prior to assay. For the assay, a 2 hour incubation at 37°C was utilised and the incubations were terminated using methanol. The inhibitory effect was estimated from the ratio of 8-hydroxy pitavastatin formation in the presence and absence of inhibitor.</p> <p>Using recombinant human CYP expressing microsomes of lymphoblastic cell lines, the human CYP isoforms involved in the hydroxylation of pitavastatin to 8-hydroxy pitavastatin were investigated. At concentrations of 0.5, 2.5 and 25 μmol/L pitavastatin, the reaction mixtures (1 mg protein/ml) were incubated at 37°C for 2 hours with the NADPH regeneration system. Control microsomes (native AHH-1 cells) were also used.</p> <p>The inhibitory effect of pitavastatin (0.5 to 5 μmol/L) on CYP mediated metabolism was examined using [<sup>14</sup>C]-tolbutamide (40 to 800 μmol/L) co-incubated with CYP marker substrate prior to assay. Human liver microsomes were added to give a final concentration of 1 mg protein/mL and the reaction mixtures were incubated at 37°C for 1 hour. In the presence or absence of pitavastatin, CYP2C9 mediated 4-hydroxytolbutamide production was measured.</p> <p>Total radioactivity was measured using liquid scintillation counting and the measurement of the radioactivity of pitavastatin and its metabolites was conducted using HPLC-RLG. The measurement of the radioactivity of 4-hydroxytolbutamide was conducted using TLC-RLG.</p> <p><b>Results:</b> SKF-525A and sulphaphenazole had strong inhibitory effects on 8-hydroxy pitavastatin (approximately 25% to 30% as estimated from the graph); α-naphthoflavone and ketoconazole had no effect on the metabolism of pitavastatin to 8-hydroxy pitavastatin. In the immuno-inhibition studies, strong inhibition (approximately 30% to 35% as estimated from the graph) on the metabolism of pitavastatin to 8-hydroxy pitavastatin was observed with human anti-CYP2C and antiserum P-450 NADPH reductase.</p> <p>CYP2C9 (CYP2C9-Arg and CYP2C9-Cys) was principally responsible for the hydroxylation of pitavastatin by human microsomes with slight involvement of CYP2C8. In contrast, no 8-hydroxy pitavastatin was produced in the presence of CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C19, CYP2D6-val, CYP2D6-met, CYP2E1, CYP3A4 or CYP4A11. There was little difference in the production of 8-hydroxy pitavastatin between CYP2C9-Arg of wild type and CYP2C9-Cys variant type.</p> <p>CYP2C9-mediated tolbutamide 4-hydroxylation was not inhibited by pitavastatin up to a concentration of 5 μmol/L.</p>				
<b>Additional Information:</b>				
<p><b>TLC-RLG:</b> Thin layer chromatography with radioluminography</p> <p><b>mg eq IgG:</b> milligramme equivalents of immunoglobulin G</p> <p><b>NADPH:</b> β-nicotine adenine diphosphate (NADP), glucose-6-phosphate and glucose-6-phosphate dehydrogenase</p>				

<b>2.6.5.10C Pharmacokinetics: Metabolism <i>In Vitro</i></b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1
<b>Report No.:</b> [A]-2544		<b>Location in CTD:</b>	<b>Vol: *</b>	<b>Section: *</b>
<p><b>Type of Study:</b> <i>In vitro</i> study using microsomes from B-lymphoblastic cell lines expressing human CYP isoforms, to identify the human CYP isoforms involved in the metabolism of pitavastatin and lactone.</p> <p><b>Method:</b> Pitavastatin (0.5 and 2.5 μmol/L) and lactone (0.5 μmol/L) were incubated separately with CYP isoforms in the presence of a NADPH regeneration system at 37°C for 2 hours. The remaining ratios of pitavastatin, lactone and potential metabolites were analysed by LC-MS* to identify the human CYP isoforms responsible for metabolism.</p> <p><b>Results:</b> Pitavastatin and lactone underwent minimal metabolism by the 13 human CYP isoforms used. In addition, no metabolites (M-1 (H-2), M-2 (H-1), 5-ketone (H-5) or M-4 (H-6)) were detected. However, in the experiments using lactone, hydrolysis of the lactone to pitavastatin in the incubation mixtures was observed.</p>				
<b>Metabolism of Pitavastatin and Lactone <i>In Vitro</i> by Microsomes from B-Lymphoblastic Cell Lines Expressing cDNAs Encoding Human CYP</b>				
<b>CYP Isoform</b>	<b>Remaining Ratio (% of Control)</b>			
	<b>Lactone (0.5 μmol/L)</b>	<b>Pitavastatin (0.5 μmol/L)</b>	<b>Pitavastatin (2.5 μmol/L)</b>	
Control	100	100	100	
CYP1A1	86.9	91.6	105	
CYP1A2	80.5	99.8	104	
CYP1B1	97.0	87.4	108	
CYP2A6	104	113	104	
CYP2B6	85.0	111	98.8	
CYP2C8	99.5	102	111	
CYP2C9-Arg	101	83.1	101	
CYP2C9-Cys	107	104	100	
CYP2C19	82.4	100	92.5	
CYP2D6-Val	83.0	90.5	108	
CYP2D6-Met	81.7	104	91.3	
CYP2E1	97.2	99.4	117	
CYP3A4	81.0	114	114	

**Control:** Control (Vector) or reductase  
**cDNA:** Complementary deoxyribonucleic acid  
 a: LC-MS; Inertsil ODS-3 (4.6 mm ID x 150 mm); mobile phase A: 5 mmol/L ammonium acetate buffer (pH 4) and mobile phase B: acetonitrile; 0.8 mL/min flow rate; monitor ion pitavastatin 422.1; lactone 404.1, M-1 402.1, M-2 386.1, 5-ketone 420.1, M-6 406.1.

<b>2.6.5.10D Pharmacokinetics: Metabolism In Vitro</b>		Test Article: Pitavastatin		Page 1 of 1		
Report No.: [R101029]		Location in CTD:		Vol.: * Section: *		
Type of Study: <i>In vitro</i> study to assess the involvement of human UGT in the metabolism of pitavastatin to lactone, a major metabolite.						
Method: Pooled human liver microsomes, microsomes from baculovirus-transfected insect cells expressing human UGT (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2H7, 2H5 and control), human small intestine microsomes and human renal microsomes were purchased commercially. Pitavastatin and [ <sup>14</sup> C]-pitavastatin (specific activity of 2.2 MBq/mg) were utilised. [ <sup>14</sup> C]-pitavastatin was used at a concentration of 2.5 µmol/l, with the exception of enzyme kinetics which utilised concentrations of 1, 2.5, 5, 10, 20, 40 and 80 µmol/L and experiments with microsomes from baculovirus-transfected insect cells utilising concentrations of 2.5, 10 and 50 µmol/L.						
Results: 8-hydroxy pitavastatin was detected in the presence of a NADPH regeneration system (phase I) but not in the presence of UDP-glucuronic acid (UDPGA) (phase II). Lactone was produced in the reaction system containing human hepatic microsomes and UDPGA and was inhibited by UGT inhibitors demonstrating that lactone was formed from pitavastatin by UGT. The postulated metabolic pathway is formation by UGT of ester-type pitavastatin glucuronide conjugate, a metabolic intermediate, followed by elimination of the glucuronate moiety to form the lactone. UGT-mediated lactone formation was observed in human small intestine, renal and liver microsomes. UGT1A3 and UGT2B7 were involved along with other UGTs at higher pitavastatin concentrations.						
Composition of Radioactivity (Mean ± SE; %) as Pitavastatin, Lactone or Unknown Metabolites After Incubation of [ <sup>14</sup> C]-Pitavastatin (2.5 µmol/L) with Microsomes (n = 2 or 3) in the Presence of UDPGA						
Human Hepatic Microsomes			Human Renal Microsomes		Human Intestinal Microsomes	
Pitavastatin	Lactone	Unknown	Pitavastatin	Lactone	Unknown	Pitavastatin
88.6 ± 1.9	9.6 ± 1.5	1.8 ± 0.4	72.8 ± 0.5	22.1 ± 0.4	10.1 ± 0.4	96.0
						2.6
						1.5
Kinetic Constants for Metabolism of Pitavastatin after Incubation with Microsomes						
Tissue	Metabolites	K <sub>m</sub> (µmol/L)	V <sub>max</sub> (pmol/min/mg protein)	V <sub>max</sub> /K <sub>m</sub> (µL/min/mg protein)		
Liver	Lactone	49.4	124.6	2.52		
	8-hydroxy pitavastatin*	45.1	77.4	1.79		
Kidney	Lactone	48.8	115.6	2.37		
	8-hydroxy pitavastatin	Not calculable	Not calculable	Not calculable		

<b>2.6.5.12A Pharmacokinetics: Induction/Inhibition of Drug-Metabolising Enzymes</b>		Test Article: Pitavastatin		Page 1 of 1
Report No.: [AL-3544]		Location in CTD:		Vol.: * Section: *
Type of Study: <i>In vitro</i> study, using microsomes from B-lymphoblastic cell lines expressing human CYP isoforms, to assess the potential inhibitory effect of pitavastatin on the metabolic activity of human CYP isoforms.				
Method: Pitavastatin (0.025, 0.25 and 2.5 µmol/l) was used as an inhibitor and incubated with each CYP isoform in the presence of a NADPH regeneration system at 37°C for a designated time. After pre-treatment of the incubation sample, the concentration of metabolite produced was determined by fluorescence spectrophotometry or HPLC and the inhibitory effect of pitavastatin was examined by comparing the concentrations of metabolites with or without the addition of pitavastatin.				
Results: Pitavastatin had no inhibitory effect on the metabolic activity of the human CYP isoforms studied with the exception of CYP2C8. The relative activity (% of control) for CYP2C8 was 96.0%, 84.8% and 69.6% for 0.025, 0.25 and 2.5 µmol/L pitavastatin, respectively; for the positive control (SKF-525A, 300 µmol/L) the relative activity (% of control) was 22.3%.				
Inhibitory Effect of Pitavastatin on the Metabolism of Each Model Substrate in Microsomes from B-Lymphoblastic Cell Lines Expressing cDNAs Encoding Human CYP				
CYP isoform	Remaining Ratio (% of Control)			
	Pitavastatin (0.025 µmol/L)	Pitavastatin (0.25 µmol/L)	Pitavastatin (2.5 µmol/L)	Positive Control*
Reference (no test article)	100	100	100	
CYP1A1	107	108	106	61.7
CYP1A2	105	105	103	21.0
CYP1B1	103	105	98.0	26.6
CYP2A6	97.4	106	100	55.5
CYP2B6	102	113	107	14.4
CYP2C8	96.0	84.8	69.6	22.3
CYP2C9-Arg	98.8	92.2	95.4	52.3
CYP2C9-Cys	103	97.9	95.3	62.3
CYP2C19	101	99.4	101	15.9
CYP2D6-Val	105	99.3	101	0.0
CYP2D6-Met	91.9	97.9	96.7	0.741
CYP2E1	91.0	90.1	90.1	5.81
CYP3A4	99.1	101	100	9.02

Additional Information:  
 \*: Not applicable to an electronic submission  
 #: Positive controls: α-naphthoflavone (5 µmol/l) was utilised for CYP1A1, CYP1A2 and CYP1B1; diethylthiocarbamate (100 µmol/l) was utilised for CYP2E1; SKF 525A (300 µmol/l) was utilised for the other CYP isoforms.

<b>2.6.5.12B Pharmacokinetics: Induction/Inhibition of Drug-Metabolising Enzymes</b>		Test Article: Pitavastatin		Page 1 of 1	
Report No.: [11-TB-9731]		Location in CTD:		Vol.: * Section: *	
Species / Gender (M/F)/No. Animals:	Wistar rats / 5F	Feeding Condition: Fed			
Vehicle/Formulation:	Suspension in 0.5% CMC; physiological saline was used for positive control				
Method of Administration:	Pitavastatin: Oral (gavage); SKF-525A: Intraperitoneal				
Dose Regimen:	Pitavastatin: single dose at 0 (vehicle control), 1, 3 or 10 mg/kg; positive control was SKF-525A (SKF): single dose at 50 mg/kg				
Method:	Animals were sacrificed 1 hour after dosing and the livers removed, weighed and microsomes prepared. The liver weight ratio was determined. The microsomal protein, CYP and cytochrome b5 contents were determined and the activities of NADPH cytochrome c reductase, aniline hydroxylase, aminopyrine N-demethylase, 7-ethoxycoumarin O-deethylase and UGT determined.				
Results:	There were no significant treatment related differences between the control and pitavastatin dose groups, whereas liver/weight ratio, CYP, aminopyrine-N-demethylase and 7-ethoxycoumarin O-deethylase were significantly lower in the SKF-525A dose group compared to the control group.				
Effect of Pitavastatin 1 Hour after a Single Oral Dose on Rat Hepatic Drug-Metabolising Enzymes (Mean ± SD; % of control in parentheses)					
Parameter	Control	1 mg/kg/day Pitavastatin	3 mg/kg/day Pitavastatin	10 mg/kg/day Pitavastatin	50 mg/kg/day SKF
Liver weight ratio (g liver/100 g body weight)	4.164 ± 0.094 (100)	4.054 ± 0.170 (97.4)	3.989 ± 0.225 (95.8)	4.149 ± 0.321 (99.6)	3.823 ± 0.145 <sup>#</sup> (91.8)
CYP content (nmol/mg protein)	0.74 ± 0.06 (100)	0.72 ± 0.05 (97.8)	0.74 ± 0.04 (100.3)	0.75 ± 0.04 (101.4)	0.66 ± 0.05 <sup>#</sup> (89.2)
Cytochrome b5 content (nmol/mg protein)	0.38 ± 0.01 (100)	0.39 ± 0.02 (104.3)	0.37 ± 0.03 (98.9)	0.39 ± 0.03 (103.2)	0.39 ± 0.02 (103.2)
NADPH cytochrome c reductase (µmol/min/mg protein)	0.18 ± 0.01 (100)	0.19 ± 0.01 (105.7)	0.18 ± 0.01 (101.1)	0.17 ± 0.01 (94.3)	0.17 ± 0.01 (98.9)
Aniline hydroxylase (nmol/min/mg protein)	0.73 ± 0.06 (100)	0.82 ± 0.05 <sup>#</sup> (112.9)	0.78 ± 0.07 (106.9)	0.73 ± 0.04 (100.6)	0.67 ± 0.06 (92.3)
Aminopyrine N-demethylase (nmol/min/mg protein)	4.23 ± 0.52 (100)	4.17 ± 0.16 (98.4)	4.17 ± 0.24 (98.5)	3.99 ± 0.28 (94.4)	2.04 ± 0.15 <sup>###</sup> (48.3)
7-ethoxycoumarin O-deethylase (nmol/min/mg protein)	0.87 ± 0.04 (100)	0.89 ± 0.06 (102.3)	0.86 ± 0.06 (98.2)	0.81 ± 0.08 (92.7)	0.77 ± 0.11 <sup>#</sup> (87.9)
UGT (nmol/min/mg protein)	13.87 ± 1.59 (100)	14.00 ± 1.65 (100.9)	13.33 ± 1.55 (96.1)	13.13 ± 1.40 (94.7)	13.05 ± 0.94 (94.1)

#: p<0.05; ###: p<0.001 using Student's t-test

<b>2.6.5.12C Pharmacokinetics: Induction/Inhibition of Drug-Metabolising Enzymes</b>		<b>Test Article: Pitavastatin</b>		<b>Page 1 of 1</b>	
Report No.: [11-1B-9607]		Location in CTD: Vol: *		Section: *	
Species / Gender (M/F)/No. Animals:	Wistar rats / 5M				
Feeding Condition:	Fed				
Vehicle/Formulation:	Suspension in 0.3% CMC; distilled water for injection was used for positive control				
Dose Regimen:	Pitavastatin at 0 (vehicle control), 1, 3, 10 mg/kg daily for 7 days; positive control was phenobarbital (PB) at 80 mg/kg daily for 7 days				
Method:	Animals were sacrificed 24 hours after the 7 <sup>th</sup> dose and the livers removed, weighed and microsomes prepared. The liver weight ratio was determined. The microsomal protein, CYP and cytochrome b5 contents were determined and the activities of NADPH cytochrome c reductase, aniline hydroxylase, aminopyrine N-demethylase, 7-ethoxycoumarin O-deethylase and UGT were determined.				
Results:	There were no significant differences between the control and pitavastatin dose groups, whereas all parameters (except cytochrome b5 which was significantly lower than control) were significantly higher in the phenobarbital dose group compared to the control group. Pitavastatin did not affect rat liver drug-metabolising enzymes.				

<b>Effect of Pitavastatin Oral Administration for 7 Days on Rat Hepatic Drug-Metabolising Enzymes (Mean ± SD; % of control in parentheses)</b>					
Parameter	Control	1 mg/kg/day Pitavastatin	3 mg/kg/day Pitavastatin	10 mg/kg/day Pitavastatin	80 mg/kg/day PB
Liver weight ratio (g liver/100 g body weight)	4.482 ± 0.179 (100)	4.273 ± 0.155 (95.3)	4.385 ± 0.142 (97.8)	4.346 ± 0.150 (97.0)	5.396 ± 0.199 <sup>***</sup> (124.8)
Microsomal protein (mg/g liver)	13.3 ± 1.4 (100)	13.5 ± 1.1 (100.3)	13.1 ± 2.7 (97.0)	14.2 ± 1.6 (105.2)	21.5 ± 3.6 <sup>***</sup> (159.5)
CYP content (nmol/mg protein)	0.71 ± 0.12 (100)	0.70 ± 0.08 (98.3)	0.73 ± 0.06 (102.2)	0.68 ± 0.08 (94.7)	1.79 ± 0.25 <sup>***</sup> (250.4)
Cytochrome b5 (nmol/mg protein)	0.31 ± 0.03 (100)	0.29 ± 0.02 (93.5)	0.32 ± 0.02 (103.3)	0.29 ± 0.03 (94.8)	0.23 ± 0.05 <sup>***</sup> (73.9)
NADPH cytochrome c reductase (µmol/min/mg protein)	0.23 ± 0.04 (100)	0.26 ± 0.04 (110.3)	0.23 ± 0.02 (99.1)	0.25 ± 0.03 (106.9)	0.48 ± 0.08 <sup>***</sup> (206.0)
Aniline hydroxylase (µmol/min/mg protein)	0.82 ± 0.10 (100)	0.79 ± 0.11 (96.3)	0.74 ± 0.06 (90.5)	0.79 ± 0.09 (96.8)	1.35 ± 0.12 <sup>***</sup> (165.0)
Aminopyrine N-demethylase (nmol/min/mg protein)	7.25 ± 0.81 (100)	7.40 ± 0.55 (102.1)	7.16 ± 0.67 (98.8)	7.08 ± 0.49 (97.7)	10.71 ± 0.94 <sup>***</sup> (147.8)
7-ethoxycoumarin O-deethylase (µmol/min/mg protein)	1.01 ± 0.08 (100)	1.09 ± 0.08 (107.3)	1.04 ± 0.09 (102.4)	1.04 ± 0.07 (103.0)	2.03 ± 0.19 <sup>***</sup> (200.2)
UGT (nmol/min/mg protein)	5.11 ± 1.41 (100)	6.37 ± 0.91 (124.8)	5.26 ± 1.40 (103.0)	5.80 ± 1.41 (113.6)	12.82 ± 1.28 <sup>***</sup> (251.2)

\*: Not applicable to an electronic submission; \*\*\*: p<0.001 using Student's t-test

<b>2.6.5.13A Pharmacokinetics: Excretion (Single Dose)</b>		<b>Test Article: Pitavastatin</b>		<b>Page 1 of 1</b>	
Reference: [Kimata <i>et al.</i> , 1998]. Corrigenda published in [Xeno Metab Dispos 1999; 14: 36-4], [Xeno Metab Dispos 1999; 14: 41-2] and [Xeno Metab Dispos 2000; 15: 306]		Location in CTD: Vol: *		Section: *	
Species:	Jia; Wistar rats				
Gender (M/F)/No. of Animals:	Jia; Wistar rats 4M and 4F per group				
Feeding Condition:	Fasted overnight before dosing and for 6 hours after dosing				
Vehicle/Formulation:	Solution: 0.5% CMC sodium solution (oral) or physiological saline (i.v.)				
Method of Administration:	Oral (gavage) and i.v. (bolus injection)				
Dose (mg/kg):	1				
Radionuclide:	<sup>14</sup> C]-pitavastatin				
Specific Activity:	2.2 to 3.5 MBq/µg				
Assay:	Total radioactivity in urine and faeces				
Sampling Time:	24, 48, 72 and 96 hours after dosing				
Sex	<b>Excretion of Radioactivity (Mean ± SD, % of dose) at Various Time Points After Dosing</b>				
	Sample	24 hours	48 hours	72 hours	96 hours
Male	<b>Oral Administration</b>				
	Faeces	83.9 ± 8.0	97.1 ± 3.0	99.2 ± 2.5	-
	Urine	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	-
Female	Total	84.1 ± 8.0	97.2 ± 3.0	99.4 ± 2.4	-
	Faeces	66.1 ± 31.5	88.7 ± 10.9	96.0 ± 2.2	97.0 ± 2.1
	Urine	1.7 ± 0.4	2.1 ± 0.7	2.2 ± 0.7	2.2 ± 0.7
Male	Total	67.8 ± 31.4	90.8 ± 10.2	98.1 ± 1.8	99.2 ± 2.0
	<b>Intravenous Administration</b>				
	Faeces	36.5 ± 29.6	80.8 ± 13.0	88.5 ± 6.9	92.9 ± 2.2
Female	Urine	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2
	Total	36.8 ± 29.5	81.2 ± 12.9	89.0 ± 6.8	93.4 ± 2.2
	Faeces	64.3 ± 11.4	89.6 ± 3.1	92.7 ± 2.6	93.2 ± 2.7
Male	Urine	3.1 ± 0.2	3.3 ± 0.2	3.5 ± 0.4	3.6 ± 0.7
	Total	67.3 ± 11.3	92.9 ± 2.9	96.2 ± 2.8	96.9 ± 3.0
	<b>Additional information:</b> No marked difference between male and female rats.				

<b>2.6.5.13B Pharmacokinetics: Excretion (Repeat Dose)</b>		<b>Test Article:</b> Pitavastatin		Page 1 of 1	
Reference: [Ujino <i>et al.</i> , 1998a]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 364]		Location in CTD:		Vol.: * Section: *	
Species:	Wistar rats				
Gender (M/F)/No. of Animals:	4M				
Feeding Condition:	Fed				
Vehicle/Formulation:	Solution in 0.5% CMC sodium solution				
Method of Administration:	Oral (gavage)				
Dose Regimen:	1 mg/kg daily for 9 days				
Radioisotope:	<sup>14</sup> C-pitavastatin				
Specific Activity:	2.20 MBq/mg				
Tissues/Organs:	Urine and faeces				
Sampling Time:	Urine and faeces were collected every 24 hours after daily administration until the 9 <sup>th</sup> day and from the 10 <sup>th</sup> to 14 <sup>th</sup> day (144 hours after the 9 <sup>th</sup> dose) without dosing				
Assay:	Liquid scintillation counting				

Cumulative Excretion of Radioactivity in Urine and Faeces after Repeated Oral Administration of <sup>14</sup> C-Pitavastatin to Rats									
Sample	Excreted Radioactivity (Mean ± SD, % of cumulative dose) after Dosing								
	Number of Doses								
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>
Urine	0.03 ± 0.01	0.06 ± 0.04	0.08 ± 0.04	0.09 ± 0.04	0.10 ± 0.04	0.11 ± 0.04	0.12 ± 0.04	0.13 ± 0.05	0.14 ± 0.05
Faeces	7.88 ± 0.47	18.18 ± 0.51	28.72 ± 0.71	39.17 ± 0.90	49.98 ± 1.24	61.68 ± 1.27	73.46 ± 1.33	84.78 ± 1.56	96.87 ± 1.47
Total	7.91 ± 0.48	18.24 ± 0.50	28.80 ± 0.73	39.26 ± 0.92	50.08 ± 1.26	61.79 ± 1.28	73.58 ± 1.34	84.91 ± 1.57	97.00 ± 1.46

Cumulative Excretion of Radioactivity in Urine and Faeces after Repeated Oral Administration of <sup>14</sup> C-Pitavastatin to Rats					
Sample	Excreted Radioactivity (Mean ± SD, % of cumulative dose) after Dosing				
	Hours after 9 <sup>th</sup> dose				
	48 hours	72 hours	96 hours	120 hours	144 hours
Urine	0.14 ± 0.05	0.14 ± 0.05	0.14 ± 0.05	0.14 ± 0.05	0.14 ± 0.05
Faeces	97.68 ± 1.24	97.81 ± 1.19	97.87 ± 1.16	97.89 ± 1.16	97.91 ± 1.15
Total	97.82 ± 1.23	97.95 ± 1.19	98.01 ± 1.17	98.03 ± 1.16	98.05 ± 1.15

<b>2.6.5.13C Pharmacokinetics: Excretion (Single Dose)</b>		<b>Test Article:</b> Pitavastatin		Page 1 of 1	
Reference: [Ujino <i>et al.</i> , 1999a]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]		Location in CTD:		Vol.: * Section: *	
Species:	Wistar rats	TW rabbits	HRA-beagle dogs	Cynomolgus monkeys	
Gender (M/F)/No. of Animals:	4M	3M	4M	3M	
Feeding Condition:	Fasted	Fasted	Fasted	Fasted	
Vehicle/Formulation:	Suspension; 0.5% CMC <sup>a</sup>				
Method of Administration:	Oral	Oral	Oral	Oral	
Dose (mg/kg):	1	1	1	1	
Sample (whole blood, plasma, serum etc.):	Urine and faeces	Urine and faeces	Urine and faeces	Urine and faeces	
Analyte:	Pitavastatin, lactone and conjugates of both				
Assay:	HPLC-CS <sup>b</sup>	HPLC-CS <sup>b</sup>	HPLC-CS <sup>b</sup>	HPLC-CS <sup>b</sup>	

Species	Dose mg/kg	Urinary and Faecal Excretion after Oral Pitavastatin Administration (% of dose)							
		Urine				Faeces			
		Pitavastatin	Pitavastatin Conjugate	Lactone	Lactone Conjugate	Pitavastatin	Pitavastatin Conjugate	Lactone	Lactone Conjugate
Rat	1	Negligible	Less than 5% of the dose	Less than 5% of the dose	61.6%	Less than 5% of the dose	Less than 5% of the dose	Less than 5% of the dose	
Rabbit	1	35.7%	Less than 5% of the dose	Less than 5% of the dose	13.0%	Less than 5% of the dose	Less than 5% of the dose	Less than 5% of the dose	
Dog	1	Negligible	Less than 5% of the dose	Less than 5% of the dose	46.2%	Less than 5% of the dose	Less than 5% of the dose	Less than 5% of the dose	
Monkey	1	0.1%	Less than 5% of the dose	Less than 5% of the dose	5.2%	Less than 5% of the dose	Less than 5% of the dose	Less than 5% of the dose	

Excretion was nearly complete after the first 24 hours after administration (data not shown).  
i.v. administration: The following dose levels were used: rats (1 mg/kg), rabbits (0.1 mg/kg), dogs (0.1 mg/kg) and monkeys (0.3 mg/kg). Urinary and faecal excretion of pitavastatin, pitavastatin conjugate, lactone and lactone conjugates were similar to that observed following oral administration.

<sup>a</sup>: CMC sodium solution  
<sup>b</sup>: HPLC-CS as described in [Kojima *et al.*, 1999a]; the Sponsor identified that the validation methods summarised in [Kojima *et al.*, 1999a] were [R92052], [R95077] and [R98018]

<b>2.6.5.13D Pharmacokinetics: Excretion (Single Dose)</b>		<b>Test Article:</b> Pitavastatin		Page 1 of 1	
Report No.: [R98042]		Location in CTD:		Vol.: * Section: *	
Species:	Hartley guinea pigs				
Gender (M/F)/Number of Animals:	4M per group				
Feeding Condition:	Fasted overnight before dosing				
Vehicle/Formulation:	Suspension in 0.5% CMC sodium solution				
Method of Administration:	Oral (gavage)				
Dose (mg/kg):	1				
Radioisotope:	<sup>14</sup> C-pitavastatin				
Specific Activity:	2.2 MBq/mg				
Tissue/Organs:	Urine and faeces				
Sampling Time:	24, 48, 72, 96, 120 and 144 hours post-dosing				
Assay:	Liquid scintillation counting				
Results:					

Cumulative Urinary and Faecal Excretion of <sup>14</sup> C-Pitavastatin after Single Oral Dose Administration of 1 mg/kg to Male Guinea Pigs (Mean ± SE, % of cumulative dose)			
Time Post Dose (hours)	Urine	Faeces	Total
24	4.0 ± 0.5	23.1 ± 8.8	27.1 ± 8.9
48	6.4 ± 0.6	35.8 ± 16.5	42.1 ± 16.7
72	9.6 ± 0.7	53.0 ± 16.9	62.6 ± 16.2
96	11.6 ± 1.5	75.8 ± 9.5	87.4 ± 8.1
120	12.4 ± 1.8	85.0 ± 5.6	97.3 ± 3.9
144	12.7 ± 1.9	87.7 ± 4.3	100.4 ± 2.4

2.6.5.13E Pharmacokinetics: Excretion (Single Dose)		Test Article: Pitavastatin		Page 1 of 1
Reference: [Ujino <i>et al.</i> ; 1999a]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]		Location in CTD:		Vol.: * Section: *
Species / Gender (M/F)/No. Animals: HIRA-beagle dogs / 4M		Feeding Condition:	Fasted overnight before dosing   Assay: HPLC-CS*	
Vehicle/Formulation:		Suspension in 0.5% CMC sodium solution (oral) or solution in saline (i.v.)		
Method of Administration:		Oral or i.v. (bolus injection)   Dose (mg/kg): 1 or 10 (oral) or 0.1 (i.v.)		
Tissues/Organs:		Urine and faeces   Sampling Time: Urine and faeces were collected every 24 hours for 3 days after a single dose		
Urinary and Faecal Excretion Ratios of Pitavastatin and Its Metabolites after Single Oral or Intravenous Administration to Dogs (Mean ± SE, % of dose)				
Dose Regimen	Analyte	Urine	Faeces	Total
0.1 mg/kg i.v.	Pitavastatin	0.00 ± 0.00	31.13 ± 0.70	31.13
	Lactone	0.00 ± 0.00	0.31 ± 0.31	0.31
	M-5	0.00 ± 0.00	2.22 ± 1.28	2.22
	Total Recovery			33.65
1 mg/kg oral	Pitavastatin	0.18 ± 0.06	49.92 ± 5.69	50.10
	Lactone	0.00 ± 0.00	2.59 ± 0.34	2.59
	M-2	0.00 ± 0.00	0.03 ± 0.03	0.03
	5-ketone	0.00 ± 0.00	0.19 ± 0.05	0.19
	M-5	0.00 ± 0.00	1.73 ± 0.11	1.73
	M-8	0.00 ± 0.00	0.07 ± 0.04	0.07
Total Recovery				54.71
10 mg/kg oral	Pitavastatin	0.16 ± 0.05	38.71 ± 3.36	38.86
	Lactone	0.00 ± 0.00	2.28 ± 0.07	2.28
	M-2	0.00 ± 0.00	0.19 ± 0.02	0.19
	5-ketone	0.01 ± 0.01	0.25 ± 0.10	0.26
	M-4	0.00 ± 0.00	0.03 ± 0.03	0.03
	M-5	0.00 ± 0.00	1.76 ± 0.11	1.76
	M-6	0.00 ± 0.00	0.09 ± 0.03	0.09
	M-7	0.00 ± 0.00	0.01 ± 0.01	0.01
	M-8	0.00 ± 0.00	0.29 ± 0.06	0.29
Total Recovery				43.76

\*: Not applicable to an electronic submission; a: HPLC-CS as described in [Kojima *et al.*; 1999a]; the Sponsor identified that the validation methods summarised in [Kojima *et al.*; 1999a] were [R92052], [R95077] and [R98018].

2.6.5.13F Pharmacokinetics: Excretion (Single Dose)		Test Article: Pitavastatin		Page 1 of 2	
Report No.: [R99052]		Location in CTD:		Vol.: * Section: *	
Species:		Cynomolgus monkeys			
Gender (M/F)/Number of Animals:		4M per group			
Feeding Condition:		Fasted overnight before dosing			
Vehicle/Formulation:		Suspension in 0.5% CMC sodium solution			
Method of Administration:		Oral (gavage)			
Dose (mg/kg):		0.5 or 3 (3 ml/kg)			
Sample (whole blood, plasma, serum etc.):		Urine (0 to 72 hours daily after dosing); faeces (0 to 72 hours daily after dosing)			
Analyte:		Pitavastatin, 8-hydroxy pitavastatin*			
Assay:		I.C-APCI-MS			
Duration	Free Type or Conjugated Type	Urinary Excretion Ratio (Mean ± SE, %)			
		0.5 mg/kg Pitavastatin		3 mg/kg Pitavastatin	
		Pitavastatin	8-hydroxy pitavastatin	Pitavastatin	8-hydroxy pitavastatin
Day 1 (0 to 24 hours)	Free	0.15 ± 0.02	0.01 ± 0.01	0.11 ± 0.03	0.02 ± 0.00
	Conjugated	ND	0.13 ± 0.03	ND	0.14 ± 0.04
Day 2 (24 to 48 hours)	Free	0.06 ± 0.04	ND	0.03 ± 0.01	0.01 ± 0.00
	Conjugated	0.00 ± 0.00	0.04 ± 0.02	ND	0.01 ± 0.00
Day 3 (48 to 72 hours)	Free	0.02 ± 0.02	ND	0.01 ± 0.00	0.00 ± 0.00
	Conjugated	ND	0.02 ± 0.01	ND	0.01 ± 0.00
Cumulative Excretion Ratio (0 to 72 hours)	Free	0.21 ± 0.07	0.01 ± 0.01	0.15 ± 0.04	0.03 ± 0.00
	Conjugated	0.00 ± 0.00	0.18 ± 0.02	ND	0.17 ± 0.04
Additional Information:					

a: As described in [R99051]. The pitavastatin excretion ratio determined in this assay included lactone as the assay method used did not differentiate between pitavastatin and lactone.

2.6.5.13F Pharmacokinetics: Excretion (Single Dose)		Test Article: Pitavastatin		Page 2 of 2	
Report No.: [R99052] (continued)		Location in CTD:		Vol.: * Section: *	
Duration	Free Type or Conjugated Type	Faecal Excretion Ratio (Mean ± SE, %)			
		0.5 mg/kg Pitavastatin		3 mg/kg Pitavastatin	
		Pitavastatin	8-hydroxy pitavastatin	Pitavastatin	8-hydroxy pitavastatin
Day 1 (0 to 24 hours)	Free	3.75 ± 1.29	0.26 ± 0.19	6.98 ± 3.42	0.26 ± 0.10
	Conjugated	0.06 ± 0.04	0.25 ± 0.13	0.18 ± 0.17	0.22 ± 0.10
Day 2 (24 to 48 hours)	Free	3.01 ± 1.40	0.27 ± 0.08	9.12 ± 2.98	0.57 ± 0.09
	Conjugated	0.09 ± 0.09	0.18 ± 0.06	0.21 ± 0.10	0.27 ± 0.05
Day 3 (48 to 72 hours)	Free	1.88 ± 0.61	0.29 ± 0.12	2.67 ± 0.99	0.32 ± 0.10
	Conjugated	0.01 ± 0.01	0.14 ± 0.06	0.02 ± 0.02	0.08 ± 0.03
Cumulative Excretion Ratio (0 to 72 hours)	Free	8.63 ± 2.23	0.81 ± 0.23	18.77 ± 3.87	1.15 ± 0.09
	Conjugated	0.15 ± 0.12	0.56 ± 0.11	0.41 ± 0.19	0.57 ± 0.04
The pitavastatin excretion ratio determined in this assay included lactone as the assay method used did not differentiate between pitavastatin and lactone					

2.6.5.13G Pharmacokinetics: Excretion (Single Dose)		Test Article: Pitavastatin		Page 1 of 1		
Report No.: [9L804]		Location in CTD:		Vol.: * Section: *		
Species:	Cynomolgus monkeys					
Gender (M/F)/Number of Animals:	3M (oral) and 1M (i.v.)					
Feeding Condition:	Fasted overnight before dosing and for 8 hours after dosing					
Vehicle/Formulation:	Suspension in 0.5% CMC sodium solution (oral) or solution in physiological saline (i.v.)					
Method of Administration:	Oral (gavage) or i.v. (injection)					
Dose (mg/kg):	3 (oral; 2 mL/kg) or 0.3 (i.v.; 1 mL/kg)					
Tissue/Organs:	Urine and faeces (up to 168 hours post-dose)					
Radioisotope:	<sup>14</sup> C-pitavastatin					
Specific Activity:	981 kBq/mg					
Sampling Time:	Every 24 hours up to 7 days post-dosing					
Assay:	Liquid scintillation counting					
<b>Cumulative Urinary and Faecal Excretion of Radioactivity After a Single Oral or Intravenous Dose of [<sup>14</sup>C]-Pitavastatin</b>						
Time (hours)	Oral 3 mg/kg (Mean ± SD; % of dose)			Intravenous 0.3 mg/kg (% of dose)		
	Urine	Faeces	Total	Urine	Faeces	Total
0 to 24	9.1 ± 2.5	4.3 ± 7.3	13.4 ± 9.8	10.1	0.1	10.1
0 to 48	10.1 ± 2.6	29.0 ± 22.3	39.0 ± 24.9	10.7	52.7	63.4
0 to 72	10.6 ± 2.6	57.5 ± 6.9	68.0 ± 9.5	11.0	70.2	81.2
0 to 96	10.8 ± 2.6	68.1 ± 5.4	78.9 ± 6.5	11.1	79.5	90.6
0 to 120	10.9 ± 2.6	73.9 ± 4.5	84.8 ± 4.2	11.2	86.0	97.2
0 to 144	11.0 ± 2.6	76.3 ± 4.3	87.3 ± 3.1	11.3	87.6	98.9
0 to 168	11.0 ± 2.6	77.5 ± 4.4	88.6 ± 2.8	11.3	88.3	99.6

2.6.5.14A Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin		Page 1 of 1	
Reference: [Kimata <i>et al.</i> ; 1998]; Corrigenda published in [Xeno Metab Dispos 1999; 14: 364]; [Xeno Metab Dispos 1999; 14: 412] and [Xeno Metab Dispos 2000; 15: 306]		Location in CTD:		Vol.: * Section: *	
Species:	Jia-Wistar rats				
Gender (M/F)/No. of Animals:	4M per group				
Feeding Condition:	Fasted overnight before dosing and for 6 hours after dosing				
Vehicle/Formulation:	Solution in 0.5% CMC sodium solution (oral) or physiological saline (i.v.)				
Method of Administration:	Biliary Excretion: The bile ducts were cannulated with polyethylene tubes under anaesthesia. The animals were dosed after recovery from anaesthesia. Oral (gavage) and i.v. (bolus injection). Entero-hepatic circulation: The bile was collected from four orally dosed rats within 4 hours of dosing and the radioactive bile was injected (4 mL/kg) into the duodenum of other bile-cannulated rats.				
Dose (mg/kg):					
Radioisotope:	<sup>14</sup> C-pitavastatin				
Specific Activity:	2.2 to 3.5 MBq/mg				
Assay:	Total radioactivity in urine, faeces and bile				
Sampling Time:	Bile was collected 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24 and 24 to 48 hours after dosing; urine and faeces were collected 0 to 24 and 24 to 48 hours after dosing				
Sample	<b>Cumulative Excretion of Radioactivity (Mean; % of dose) at Various Time Points After Dosing</b>				
	2 hours	6 hours	24 hours	48 hours	
<b>Oral Administration</b>					
Bile	-	56	72	75	
Faeces	-	-	-	7.7	
Urine	-	-	-	Little	
<b>Intravenous Administration</b>					
Bile	72	93	-	99	
Faeces	-	-	-	Little	
Urine	-	-	-	Little	
Additional information:	When the radioactive bile obtained from donor rats after oral administration of [ <sup>14</sup> C]-pitavastatin was injected into the duodenum of recipient rats, the cumulative biliary, faecal and urinary excretion of radioactivity was 69.9%, 20.6% and 0.2%, respectively, of the dose within 48 hours. About 70% of the administered radioactivity has been excreted once again in the bile by 48 hours. Pitavastatin efficiently undergoes enterohepatic circulation.				

2.6.5.14B Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin		Page 1 of 2	
Report No.: [R101066]		Location in CTD:		Vol.: * Section: *	
Species:	EHHR/His rats <sup>a</sup> ; Sprague Dawley rats				
Gender (M/F)/No. of Animals:	4M per rat strain				
Feeding Condition:	Fasted for 16 hours before dosing				
Vehicle/Formulation:	Solution in physiological saline				
Method of Administration:	i.v. (bolus injection)				
Dose (mg/kg):	1				
Radioisotope:	<sup>14</sup> C-pitavastatin				
Specific Activity:	981 kBq/mg				
Assay:	Liquid scintillation counting; HPLC-RLG				
Sampling Time:	Bile duct cannulated animals; bile was collected for 0 to 1, 1 to 2, 2 to 3, 3 to 4 and 4 to 6 hours.				
Results:	There was no difference in the cumulative urinary and faecal excretion of radioactivity between the control (Sprague Dawley) rats and the EHHR rats. Pitavastatin is not a substrate of cMOAT <sup>a</sup> . The major radioactive compound in the bile was unchanged pitavastatin.				
Time (hours)	<b>Cumulative Urinary and Faecal Excretion of Radioactivity after Intravenous Administration of [<sup>14</sup>C]-Pitavastatin to EHHR and Sprague Dawley Rats (Mean ± SD, %)</b>				
	EHHR Rats		Sprague Dawley Rats		
1	45.8 ± 8.2		31.4 ± 4.2		
2	67.6 ± 9.8		53.2 ± 6.9		
3	76.7 ± 13.8		66.1 ± 6.7		
4	82.6 ± 13.0		76.6 ± 4.3		
6	89.5 ± 11.0		80.3 ± 3.6		

a: cMOAT is expressed in the bile duct lateral membrane plays a major role in the transport of anionic xenobiotics across the bile canalicular membrane. Eisai-hyperbilirubinemic rats (EHBR), defective in cMOAT, are mutants of Sprague Dawley (Sprague Dawley) rats.

2.6.5.14B Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin						Page 2 of 2
Report No.: [R101066] (continued)								
Cumulative Biliary Excretion of Pitavastatin and Its Metabolites after Intravenous Administration of [ <sup>14</sup> C]-Pitavastatin to EHBR and Sprague Dawley Rats (Mean ± SD, %)								
Rat Strain	Time	Pitavastatin	Lactone	M-11	M-6	M-6 glucuronide	5-ketone	
Sprague Dawley	0 to 1 hour	26.70 ± 4.82	0.91 ± 0.35	0.86 ± 0.39	0.04 ± 0.03	0.51 ± 0.26	0.61 ± 0.26	
	0 to 2 hours	42.12 ± 7.09	2.11 ± 0.79	2.26 ± 1.02	0.13 ± 0.04	2.15 ± 0.60	1.43 ± 0.62	
	0 to 3 hours	50.06 ± 7.34	2.83 ± 1.01	3.35 ± 1.52	0.21 ± 0.06	3.25 ± 0.97	2.11 ± 0.83	
	0 to 4 hours	55.65 ± 5.97	3.31 ± 1.06	4.53 ± 2.05	0.36 ± 0.08	4.59 ± 0.96	2.82 ± 1.01	
	0 to 6 hours	57.59 ± 5.60	3.47 ± 1.07	4.94 ± 2.21	0.40 ± 0.10	5.37 ± 1.08	3.17 ± 1.02	
EHBR	0 to 1 hours	41.42 ± 8.10	0.01 ± 0.02	0.97 ± 0.98	0.71 ± 1.28	0.30 ± 0.36	0.26 ± 0.37	
	0 to 2 hours	59.19 ± 11.34	0.01 ± 0.02	1.70 ± 0.84	1.44 ± 1.46	1.04 ± 0.74	0.47 ± 0.41	
	0 to 3 hours	66.68 ± 15.62	0.01 ± 0.02	1.96 ± 0.95	1.79 ± 1.51	1.91 ± 1.23	0.58 ± 0.44	
	0 to 4 hours	70.60 ± 15.39	0.01 ± 0.02	2.31 ± 0.99	2.21 ± 1.53	2.42 ± 1.47	0.70 ± 0.45	
	0 to 6 hours	74.12 ± 14.62	0.01 ± 0.02	2.88 ± 1.06	2.82 ± 1.61	3.28 ± 1.66	0.89 ± 0.47	

2.6.5.14C Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin		Page 1 of 5
Report No.: [R99047]		Location in CTD:		
		Vol.:	Section:	
Species:	Beagle dogs			
Gender (M/F/No. of Animals):	3M			
Animal Preparation:	A cannula was inserted into the bile duct approximately 1 cm to the liver side and a second cannula was inserted about 4 cm to the duodenum side. These cannulae were connected externally. Bile was collected after separation of the two cannulae by cutting. In addition a cannula for flash was inserted approximately 1 cm from the cystic duct to the common bile duct side. The animals were used at least 2 weeks after the cannulae were inserted.			
Feeding Condition:	Fasted overnight before dosing and for 8 hours after dosing			
Vehicle/Formulation:	Solution in physiological saline			
Method of Administration:	i.v.: on two occasions; (i) the bile duct cannula was connected so that the bile was perfused to the duodenum side (i.e. drug excreted in the bile is reabsorbed (enterohepatic circulation)); (ii) the bile duct cannula was disconnected so that bile was collected. A drug washout period of at least 1 week was used.			
Dose (mg/kg):	0.1			
Sample (whole blood, plasma, serum etc.):	Plasma and bile			
Analyte:	Pitavastatin and lactone			
Assay:	HPLC-MS			
Sampling Time:	Plasma: 0 (pre-dose) and at 2, 5, 10, 30 and 60 minutes, 2, 3, 4, 6, 8, 12 and 24 hours after dosing. Bile: 0 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, 50 to 60, 60 to 80, 80 to 100, 100 to 120, 120 to 150 and 150 to 180 minutes, 3 to 4, 4 to 6, 6 to 8, 8 to 12 and 12 to 24 hours after dosing			
Results:	When the bile cannulae were connected, mimicking enterohepatic circulation, the plasma pitavastatin concentration declined triexponentially with a $t_{1/2}$ for terminal elimination of 5.0 hours and an $AUC_{0-\infty}$ of 367 ng* $h/mL$ . The $AUC_{0-\infty}$ of lactone was 219 ng* $h/mL$ . When the bile cannulae were disconnected (bile collected), the plasma pitavastatin concentration declined triexponentially with a $t_{1/2}$ for terminal elimination of 3.1 hours and an $AUC_{0-\infty}$ of 368 ng* $h/mL$ . These two parameters decreased as compared to when the bile cannulae were connected (enterohepatic circulation). The $AUC_{0-\infty}$ of lactone was 121 ng* $h/mL$ . The contribution ratio ( $R_{EH}$ ) of pitavastatin to enterohepatic circulation was 1.48 and approximately 50% of pitavastatin was reabsorbed. In the bile by 24 hours after dosing approximately 56% of the dose was detected as pitavastatin, 4% as the lactone and total excretion reached about 60% in the bile.			

\*: Not applicable to an electronic submission; #: As described in [R99046], [R95077]

$R_{EH}$ : Contribution ratio of enterohepatic circulation

2.6.5.14C Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin		Page 2 of 5
Report No.: [R99047] (continued)				
Plasma Concentrations of Pitavastatin and Lactone after a Single Intravenous Dose of Pitavastatin to Chronic Bile-Duct Cannulated Dogs				
Condition	Time	Pitavastatin Concentration (Mean ± SD, ng/mL)	Lactone Concentration (Mean ± SD, ng/mL)	
Both cannulae connected (enterohepatic circulation)	0 minutes	0.0 ± 0.0	0.0 ± 0.0	
	2 minutes	927.4 ± 132.8	2.7 ± 2.8	
	5 minutes	511.7 ± 141.7	3.1 ± 3.0	
	10 minutes	330.4 ± 134.1	5.3 ± 1.4	
	30 minutes	176.8 ± 104.3	9.7 ± 3.3	
	60 minutes	89.9 ± 66.2	11.0 ± 3.6	
	2 hours	46.2 ± 34.1	12.8 ± 3.2	
	3 hours	30.8 ± 18.0	13.1 ± 1.8	
	4 hours	29.0 ± 17.0	13.3 ± 2.4	
	6 hours	20.5 ± 13.3	11.6 ± 1.7	
	8 hours	17.9 ± 13.9	10.4 ± 2.4	
	12 hours	11.6 ± 7.9	8.4 ± 2.1	
	24 hours	0.5 ± 0.8	0.9 ± 0.8	
	Cannulae disconnected externally (bile collection)	0 minutes	0.0 ± 0.0	0.0 ± 0.0
2 minutes		723.3 ± 128.3	2.7 ± 2.6	
5 minutes		437.3 ± 29.8	3.8 ± 4.0	
10 minutes		310.7 ± 80.4	6.9 ± 3.6	
30 minutes		151.5 ± 52.6	11.0 ± 4.2	
60 minutes		74.9 ± 37.8	11.5 ± 4.4	
2 hours		32.3 ± 20.2	11.9 ± 4.2	
3 hours		18.2 ± 11.3	11.4 ± 4.0	
4 hours		14.3 ± 10.4	11.1 ± 4.2	
6 hours		8.0 ± 5.2	8.7 ± 3.8	
8 hours		4.7 ± 2.8	6.0 ± 2.8	
12 hours		1.9 ± 1.8	3.5 ± 1.9	
24 hours		0.0 ± 0.0	0.0 ± 0.0	

The concentration below the LLOQ (1 ng/mL) expressed as zero

2.6.5.14C Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin		Page 3 of 5		
Report No.: [R99047] (continued)						
<b>Results:</b>						
<b>PK Parameters of Pitavastatin and Lactone after a Single Intravenous Dose of Pitavastatin to Chronic Bile-Duct Cannulated Dogs</b>						
Condition	Compound	Parameter	Mean ± SD			
Both cannulae connected (enterohepatic circulation)	Pitavastatin	A (ng/mL)	1244.3 ± 121.6			
		B (ng/mL)	401.0 ± 127.0			
		C (ng/mL)	52.3 ± 35.9			
		$\alpha$ (min <sup>-1</sup> )	0.4561 ± 0.0561			
		$\beta$ (min <sup>-1</sup> )	0.04172 ± 0.01267			
		$\gamma$ (min <sup>-1</sup> )	0.00237 ± 0.00045			
		$t_{1/2}$ (hours)	5.01 ± 1.02			
		AUC <sub>0-24</sub> (ng* <sup>h</sup> /mL)	567.1 ± 289.0			
		AUC <sub>0-14</sub> (ng* <sup>h</sup> /mL)	557.4 ± 288.0			
		CL <sub>p</sub> (mL/min/kg)	3.79 ± 2.55			
		V <sub>ss</sub> (mL/kg)	988.2 ± 711.6			
		MRT (hours)	4.25 ± 0.25			
		Lactone	Lactone	T <sub>max</sub> (minutes)	180.1 ± 59.5	
				C <sub>max</sub> (ng/mL)	13.7 ± 2.5	
				AUC <sub>0-24</sub> (ng* <sup>h</sup> /mL)	218.7 ± 32.9	
				AUC <sub>0-14</sub> (ng* <sup>h</sup> /mL)	186.0 ± 43.0	
MRT (hours)	12.10 ± 5.24					

2.6.5.14C Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin		Page 4 of 5		
Report No.: [R99047] (continued)						
<b>Results:</b>						
<b>PK Parameters of Pitavastatin and Lactone after a Single Intravenous Dose of Pitavastatin to Chronic Bile-Duct Cannulated Dogs</b>						
Condition	Compound	Parameter	Mean ± SD			
Cannulae disconnected externally (bile collection)	Pitavastatin	A (ng/mL)	825.4 ± 320.3			
		B (ng/mL)	274.8 ± 162.1			
		C (ng/mL)	25.7 ± 8.8			
		$\alpha$ (min <sup>-1</sup> )	0.2486 ± 0.1584			
		$\beta$ (min <sup>-1</sup> )	0.02738 ± 0.01509			
		$\gamma$ (min <sup>-1</sup> )	0.00384 ± 0.00091			
		$t_{1/2}$ (hours)	3.12 ± 0.73			
		AUC <sub>0-24</sub> (ng* <sup>h</sup> /mL)	368.1 ± 135.8			
		AUC <sub>0-14</sub> (ng* <sup>h</sup> /mL)	367.1 ± 134.9			
		CL <sub>p</sub> (mL/min/kg)	5.11 ± 2.38			
		V <sub>ss</sub> (mL/kg)	502.6 ± 87.8			
		MRT (hours)	1.844 ± 0.786			
		Lactone	Lactone	T <sub>max</sub> (minutes)	110.4 ± 113.3	
				C <sub>max</sub> (ng/mL)	12.6 ± 3.8	
				AUC <sub>0-24</sub> (ng* <sup>h</sup> /mL)	121.1 ± 55.1	
				AUC <sub>0-14</sub> (ng* <sup>h</sup> /mL)	118.2 ± 50.8	
MRT (hours)	7.29 ± 1.06					
Ratio of parameter from enterohepatic circulation to bile collection	Pitavastatin	AUC <sub>0-24</sub> (ng* <sup>h</sup> /mL)	1.48 ± 0.36			
		V <sub>ss</sub> (mL/kg)	1.71 ± 0.74			
	Lactone	AUC <sub>0-24</sub> (ng* <sup>h</sup> /mL)	2.11 ± 1.14			

AUC<sub>enterohepatic</sub>: Area under the concentration-time curve under enterohepatic circulation  
 AUC<sub>bile</sub>: Area under the concentration-time curve under bile collection  
 $t_{1/2}$  (enterohepatic): Terminal half life of drug under enterohepatic circulation  
 $t_{1/2}$  (bile): Terminal half life of drug under bile collection

2.6.5.14C Pharmacokinetics: Excretion into Bile		Test Article: Pitavastatin		Page 5 of 5
Report No.: [R99047] (continued)				
<b>Results:</b>				
<b>Biliary Excretion and PK Parameters of Pitavastatin and Lactone after a Single Intravenous Dose of Pitavastatin to Chronic Bile-Duct Cannulated Dogs (Mean ± SD, <math>\mu</math>g)</b>				
Condition	Time (minutes)	Biliary Excretion of Pitavastatin	Biliary Excretion of Lactone	
Cannulae disconnected externally (bile collection)	0 to 10	1.3 ± 2.2	0.0 ± 0.0	
	10 to 20	54.0 ± 83.9	0.8 ± 1.4	
	20 to 30	76.2 ± 99.5	2.3 ± 3.3	
	30 to 40	123.4 ± 65.7	4.6 ± 2.4	
	40 to 50	77.5 ± 31.5	3.4 ± 1.4	
	50 to 60	40.4 ± 4.2	1.8 ± 0.3	
	60 to 80	47.6 ± 17.8	2.6 ± 1.2	
	80 to 100	55.8 ± 32.4	3.5 ± 1.8	
	100 to 120	31.5 ± 25.6	2.6 ± 2.2	
	120 to 150	39.0 ± 26.7	3.9 ± 1.8	
	150 to 180	15.4 ± 12.3	1.7 ± 0.6	
	180 to 240	15.0 ± 9.0	2.6 ± 0.5	
	240 to 360	11.7 ± 5.5	3.2 ± 0.3	
	360 to 480	8.4 ± 4.4	2.7 ± 0.4	
	480 to 720	13.0 ± 7.8	3.8 ± 0.9	
720 to 1440	12.4 ± 7.1	3.7 ± 1.2		
X <sub>BIL</sub> ( $\mu$ g)	NA	624.5 ± 35.5	-	
Body Weight (kg)	NA	11.0 ± 0.68	-	
Dose (mg/body)	NA	1.12 ± 0.06	-	
Biliary Excretion Ratio (%)	NA	56.1 ± 6.2	4.2 ± 0.3	
AUC (ng* <sup>h</sup> /mL)	NA	367.1 ± 134.9	118.2 ± 50.8	
CL <sub>BIL</sub> (mL/min)	NA	32.53 ± 17.06	6.83 ± 2.71	

X<sub>BIL</sub>: Amount of drug excreted over a given duration  
 CL<sub>BIL</sub>: Bile clearance  
 Molecular weights of 440.449 and 403.452 were used for pitavastatin and lactone, respectively

<b>2.6.5.15A Pharmacokinetics: Drug-Drug Interactions</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1	
<b>Reference:</b> [Ujino <i>et al.</i> , 1999b]		<b>Location in CTD:</b>		<b>Vol.:</b> * <b>Section:</b> *	
<b>Method:</b>	The drug-drug interaction for protein binding in human plasma between pitavastatin and various highly protein bound drugs was investigated. The human plasma was obtained from four healthy male volunteers for each drug studied. Firstly, to examine the influence of competitive drugs on pitavastatin, pitavastatin was mixed with plasma to give a final concentration of 0.3 µg/ml. Concurrently, competitive drugs at therapeutic plasma concentrations were added. Secondly, to examine the influence of pitavastatin on competitive drugs, pitavastatin was mixed with plasma to give a final concentration of 0.3 and 1.0 µg/ml. Concurrently, competitive drugs at therapeutic plasma concentrations were added. The binding assay for plasma protein binding was carried out with the measurement of radioactivity of competitive drugs or pitavastatin after equilibrium dialysis.				
<b>Results:</b>	The binding of pitavastatin to proteins was unaffected by warfarin, diazepam, digitoxin, phenylbutazone, phenytoin, furosemide, ibuprofen, nitrendipine and glibenclamide. Similarly, pitavastatin had no effect on the binding of [ <sup>14</sup> C]-warfarin, [ <sup>14</sup> C]-diazepam, [ <sup>3</sup> H]-propranolol, [ <sup>3</sup> H]-nitrendipine and [ <sup>3</sup> H]-glibenclamide. Although the unbound fraction of [ <sup>3</sup> H]-digitoxin was slightly affected at high concentrations of pitavastatin, no significant interaction between digitoxin and pitavastatin was found at therapeutic concentrations of pitavastatin (0.03 to 0.1 µg/mL).				
<b>Influence of Different Concentrations of Several Highly Bound Drugs on Protein Binding of Pitavastatin in Human Plasma</b>					
<b>Interacting Drug</b>	<b>Concentration (µg/mL)</b>	<b>Ratio of Unbound Pitavastatin (Mean ± SE, %)</b>			
Warfarin	0, 3, 15	0.4 ± 0.0, 0.4 ± 0.0, 0.4 ± 0.0			
Diazepam	0, 15, 75	0.5 ± 0.0, 0.5 ± 0.0, 0.5 ± 0.0			
Digitoxin	0.0, 0.1, 0.5	0.3 ± 0.0, 0.4 ± 0.0, 0.4 ± 0.0			
Phenylbutazone	0, 100, 500	0.5 ± 0.0, 0.4 ± 0.0, 0.6 ± 0.0			
Phenytoin	0, 20, 100	0.3 ± 0.1, 0.4 ± 0.0, 0.4 ± 0.0			
Furosemide	0.0, 0.5, 2.5	0.3 ± 0.0, 0.3 ± 0.0, 0.4 ± 0.0			
Ibuprofen	0, 50, 250	0.4 ± 0.0, 0.4 ± 0.0, 0.4 ± 0.0			
Nitrendipine	0.0, 0.1 (n = 3), 0.5	0.3 ± 0.0, 0.3 ± 0.0, 0.3 ± 0.0			
Glibenclamide	0, 100, 500 (n = 3)	0.4 ± 0.1, 0.5 ± 0.1, 0.6 ± 0.2			
<b>Influence of Different Concentrations of Pitavastatin on Protein Binding of Several Highly Bound Drugs in Human Plasma</b>					
<b>Interacting Drug</b>	<b>Concentration Pitavastatin (µg/mL)</b>	<b>Ratio of Unbound Interacting Drug (Mean ± SE, %)</b>			
[ <sup>14</sup> C]-Warfarin (3 µg/mL)	0.0, 0.3, 1.0	0.8 ± 0.0, 0.8 ± 0.1, 0.8 ± 0.1			
[ <sup>14</sup> C]-Diazepam (15 µg/mL)	0.0, 0.3, 1.0	1.5 ± 0.1, 1.5 ± 0.2, 1.5 ± 0.1			
[ <sup>3</sup> H]-Digitoxin (0.1 µg/mL) <sup>a</sup>	0.0, 0.0, 0.03, 0.1, 0.3, 1.0	2.2 ± 0.1, 2.4 ± 0.1, 2.3 ± 0.0, 2.2 ± 0.0, 2.6 ± 0.1*, 2.7 ± 0.0**			
[ <sup>3</sup> H]-Propranolol (1 µg/mL)	0.0, 0.3, 1.0	13.9 ± 1.4, 13.6 ± 1.0, 13.0 ± 1.2			
[ <sup>3</sup> H]-Nitrendipine (0.1 µg/mL)	0.0, 0.3, 1.0	7.1 ± 0.2, 6.7 ± 0.1, 6.8 ± 0.1			
[ <sup>3</sup> H]-Glibenclamide (100 µg/mL)	0.0, 0.3, 1.0	1.0 ± 0.0, 1.2 ± 0.2, 1.1 ± 0.0			
*: Not applicable to an electronic submission; a: Repeated at lower pitavastatin concentrations; 0, 0.03, 0.1; *: p<0.05; **: p<0.01 vs. control using Dunnett's test					
<b>2.6.5.15B Pharmacokinetics: Drug-Drug Interactions</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1	
<b>Report No.:</b> [ATR-149-035]		<b>Location in CTD:</b>		<b>Vol.:</b> * <b>Section:</b> *	
<b>Study Design:</b>	<i>In vitro</i> study to examine the effect of cyclosporine A on the uptake of pitavastatin and pravastatin by Na-independent OATP1B1 in transgenic oocytes of <i>Xenopus laevis</i> .				
<b>Methods:</b>	Oocytes of <i>Xenopus laevis</i> expressing OATP1B1 were utilised. [ <sup>14</sup> C]-pitavastatin (specific activity of 981 kBq/mg) and [ <sup>14</sup> C]-pravastatin (specific activity of 1.19 MBq/mg) were used. The transgenic oocytes were prepared and the experiments were conducted in triplicate using oocytes from different frogs.				
<b>Results:</b>	Cyclosporine A concentration-dependently inhibited the OATP1B1 mediated uptake of [ <sup>14</sup> C]-pitavastatin and [ <sup>14</sup> C]-pravastatin. The IC <sub>50</sub> values (mean ± SD of three experiments) of cyclosporine A against [ <sup>14</sup> C]-pitavastatin and [ <sup>14</sup> C]-pravastatin uptake were 2.91 ± 1.35 and 1.21 ± 0.28 µmol/L, respectively.				
<b>2.6.5.15C Pharmacokinetics: Drug-Drug Interactions</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1	
<b>Report No.:</b> [FBM 06-T350]		<b>Location in CTD:</b>		<b>Vol.:</b> * <b>Section:</b> *	
<b>Study Design:</b>	<i>In vitro</i> study to examine the effect of concomitant drugs, such as atazanavir, enalaprilat and nibradilol, on the uptake of [ <sup>14</sup> C]-pitavastatin by HEK293 cells expressing OATP1B1				
<b>Methods:</b>	HEK293 cells expressing OATP1B1 and mock cells (HEK293 cells transfected with an empty vector) were cultured for 2 to 4 days prior to preparation of cell suspensions. The cell suspensions (HEK293 cells expressing OATP1B1 and mock cells) were seeded and cultured for a further 2 days. The culture plates were washed and then incubated for 2 minutes in the presence of 3 µmol/L [ <sup>14</sup> C]-pitavastatin. The reaction was terminated and the cells washed and lysed. The radioactivity in the lysate was measured using liquid scintillation counting. The protein concentration of the lysate was also determined.				
<b>Results:</b>	The inhibitory effects of atazanavir (0.05, 0.15, 0.5, 1.5, 5 µmol/L), enalaprilat (1, 3, 10, 30, 100 µmol/L) and nibradilol (1, 3, 10, 30, 100 µmol/L) on OATP1B1-mediated [ <sup>14</sup> C]-pitavastatin (3 µmol/L) uptake in HEK293 cells expressing OATP1B1 was measured. Rifampicin (1, 3, 10, 30, 100 µmol/L) was included as a reference compound. [ <sup>14</sup> C]-pitavastatin was taken up into HEK293 cells expressing OATP1B1 and, in the presence of pitavastatin, uptake of [ <sup>14</sup> C]-pitavastatin was reduced. Enalaprilat and nibradilol had no inhibitory effect on OATP1B1-mediated [ <sup>14</sup> C]-pitavastatin uptake (IC <sub>50</sub> values > 100 µmol/L). Atazanavir (IC <sub>50</sub> value of 2.10 µmol/L) and rifampicin (IC <sub>50</sub> values of 3.14 and 1.95 µmol/L) both demonstrated an inhibitory effect on OATP1B1-mediated [ <sup>14</sup> C]-pitavastatin uptake. Atazanavir showed competitive inhibition with a K <sub>i</sub> value of 1.70 µmol/L (Lineweaver-Burk analysis).				
<b>2.6.5.15D Pharmacokinetics: Drug-Drug Interactions</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1	
<b>Report No.:</b> [RI01113]		<b>Location in CTD:</b>		<b>Vol.:</b> * <b>Section:</b> *	
<b>Method:</b>	The potential drug interaction between pitavastatin and several fibrate drugs (gemfibrozil, bezafibrate, clofibrate and ciprofibrate) was evaluated <i>in vitro</i> (plasma protein binding and metabolic inhibition studies using microsomes).  Plasma samples to evaluate the influence of fibrates on plasma protein binding of pitavastatin were prepared from blood of healthy volunteers. [ <sup>14</sup> C]-pitavastatin (0.7 and 2.3 µmol/L), gemfibrozil (0, 40 and 200 µmol/L), bezafibrate (0, 15 and 75 µmol/L), clofibrate (0, 200 and 1000 µmol/L) and ciprofibrate (0, 100 and 500 µmol/L) were used with four samples per concentration.  Metabolic inhibition studies were conducted using pooled human liver microsomes, individual human liver microsomes and baculovirus, expressing human CYP (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and control), microsomes with an NADPH regeneration system. [ <sup>14</sup> C]-pitavastatin (1 µmol/L) or cerivastatin (1 µmol/L) were used concomitantly with fibrates (10 and 100 µmol/L or 10, 100, 300 and 1000 µmol/L for gemfibrozil). In addition, the metabolism of gemfibrozil in human hepatic microsomes in the presence of pitavastatin, cerivastatin and fluvastatin was evaluated; the HMG-CoA reductase inhibitors were used at concentrations of 1, 3 and 10 µmol/L.				
<b>Results:</b>	The binding of pitavastatin to plasma proteins was unaffected by gemfibrozil, bezafibrate, clofibrate and ciprofibrate.  The metabolic clearance of pitavastatin was decreased in a dose-dependent manner by the four different fibrates used. The metabolic clearance of pitavastatin in the absence of fibrates ranged from 3.1 to 4.3 µL/min/mg protein, however, for cerivastatin the metabolic clearance was 30.9 µL/min/mg protein. The metabolism of cerivastatin (high metabolic clearance) was completely inhibited by gemfibrozil. The metabolic clearance of pitavastatin was low and inhibition by fibrates is likely to result in only a small increase in plasma concentrations. Gemfibrozil is readily metabolised by human hepatic microsomes and this was inhibited by fluvastatin but not by pitavastatin or cerivastatin. Gemfibrozil inhibited the metabolic reactions of CYP2C8 and CYP2C9 involved in the metabolism of pitavastatin and CYP2C8 and CYP3A4 involved in the metabolism of cerivastatin.				

<b>2.6.5.15E Pharmacokinetics: Drug-Drug Interactions</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1
Report No.: [R99035]		<b>Location in CTD:</b>		
		<b>Vol.: *</b>		<b>Section:</b>
<b>Method:</b>	The potential drug interaction between pitavastatin and fluvastatin was evaluated <i>in vitro</i> by investigation of the effects of these drugs on the metabolism of tolbutamide, a model substrate for CYP2C9.			
	Metabolic inhibition studies were conducted using pooled human liver microsomes; the effect of tolbutamide (50 and 300 µmol/L) on the hydroxylation of pitavastatin (2.5 and 25 µmol/L) and the effect of pitavastatin (0.5 to 5 and 2.5 to 25 µmol/L) and fluvastatin (0.5 to 5 µmol/L) on the metabolism of tolbutamide (40 to 800 µmol/L) were studied.			
<b>Results:</b>	The formation of 8-hydroxy pitavastatin was inhibited to a small extent and is unlikely to result in a clinically significant drug interaction. Similarly, pitavastatin did not inhibit the hydroxylation of tolbutamide over a wide concentration range (0.5 to 25 µmol/L). However, fluvastatin, which is metabolised by CYP2C9, competitively inhibited the hydroxylation of tolbutamide in a concentration dependent manner with a Ki value of 1 µmol/L. Ki values could not be determined for pitavastatin as there was no evidence of tolbutamide hydroxylation.			
<b>Pitavastatin Concentration (as substrate for CYP2C9)</b>		<b>Inhibition of Pitavastatin Hydroxylation by CYP2C9 (Mean (n = 2), % of Control)</b>		
		<b>Control</b>	<b>Tolbutamide (50 µmol/L)</b>	<b>Tolbutamide (300 µmol/L)</b>
2.5 µmol/L		100	81.6	86.8
25 µmol/L		100	104.4	69.3

<b>2.6.5.15F Pharmacokinetics: Drug-Drug Interactions</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1
Report No.: [R102020]		<b>Location in CTD:</b>		
		<b>Vol.: *</b>		<b>Section:</b>
<b>Study Design:</b>	<i>In vivo</i> drug interaction study to evaluate the effects of gemfibrozil on plasma concentrations of pitavastatin or cerivastatin when administered concomitantly to rats.			
<b>Method:</b>	Six male SD rats per group fasted overnight before dosing. Single dose study, oral gavage Group 1 Pitavastatin (physiological saline) 1 mg/kg Group 2 Pitavastatin (physiological saline) 1 mg/kg with 30 mg/kg gemfibrozil concomitantly (0.5% CMC solution) Group 3 Cerivastatin (0.5% CMC solution) 1 mg/kg Group 4 Cerivastatin (0.5% CMC solution) 1 mg/kg with 30 mg/kg gemfibrozil concomitantly (0.5% CMC solution)			
	Blood samples were taken 0.5, 1, 1.5, 2, 4, 6, 8 and 10 hours after dosing and plasma prepared. Pitavastatin concentrations were determined using HPLC-MS and cerivastatin concentrations were determined using LC/MS/MS method.			
<b>Results:</b>	The PK parameters are presented below. The AUC for pitavastatin in the presence of gemfibrozil was 1.07 fold higher than the AUC after pitavastatin administration alone. However, the AUC for cerivastatin in the presence of gemfibrozil was 1.5 fold higher than the AUC after cerivastatin administration alone. Concomitant administration of pitavastatin and gemfibrozil is unlikely to cause a significant increase in pitavastatin plasma concentrations.			
<b>PK Parameters for Pitavastatin and Cerivastatin when given Concomitantly with Gemfibrozil (Mean ± SD)</b>				
<b>Dose Group</b>	<b>T<sub>max</sub> (h)</b>	<b>C<sub>max</sub> (ng/mL)</b>	<b>AUC<sub>0-10</sub> (ng·h/mL)</b>	
Pitavastatin 1 mg/kg	0.7 ± 0.3	100 ± 33	335 ± 71	
Pitavastatin 1 mg/kg and 30 mg/kg Gemfibrozil	1.0 ± 0.4	130 ± 49	359 ± 81	
Cerivastatin 1 mg/kg	0.9 ± 0.4	30 ± 10	103 ± 33	
Cerivastatin 1 mg/kg and 30 mg/kg Gemfibrozil	1.1 ± 0.6	54 ± 13	155 ± 42	

a: HPLC-MS; Cosmosil 5C<sub>18</sub> (4.6 mm ID × 150 mm) column; mobile phase 1: 0.2 mol/L ammonium acetate buffer (pH 4) / acetonitrile (1:1, v/v) and mobile phase 2: 0.2 mol/L acetic acid / acetonitrile (1:1, v/v); 1 mL/min flow rate; UV detection at 250 nm  
AUC<sub>0-10</sub>: Area under the concentration-time curve from zero to 10 hours post-dose

<b>2.6.5.16A Pharmacokinetics: Other</b>		<b>Test Article: Pitavastatin</b>		Page 1 of 1
Report No.: [R95033]		<b>Location in CTD:</b>		
		<b>Vol.: *</b>		<b>Section:</b>
<b>Type of Study:</b>	<i>In vitro</i> study to assess the stability of pitavastatin and lactone in various biological matrices of rats, to identify the absorption site(s) of pitavastatin and to establish a method to extract pitavastatin from gastrointestinal tissue. Pitavastatin and lactone concentrations were determined using HPLC-MS.			
<b>Stability of pitavastatin and lactone in various biological matrices:</b>	Pitavastatin was stable in plasma, gastrointestinal juice and tissue homogenates from the stomach and duodenum with t <sub>1/2</sub> > 24 hours when incubated at 37°C. Lactone had a t <sub>1/2</sub> of 1.3 minutes (37°C), 1.1 hours (0°C) and 7.2 hours (0°C) in the presence of 1 mol/L potassium dihydrogenphosphate in the ratio 2:5 with plasma and a t <sub>1/2</sub> of 5.3 and 4.8 hours at 37°C for tissue homogenates from the stomach and duodenum, respectively.			
<b>Extraction ratio of pitavastatin from rat intestinal homogenates:</b>	The mean extraction ratios were 101.9% and 98.6% for stomach and duodenum tissue homogenates, respectively.			
<b>Absorption of pitavastatin from rat gastrointestinal loops:</b>	Absorption was highest in the duodenum followed by the colon and ileum with the stomach having the lowest absorption of pitavastatin.			
<b>Loop</b>	<b>Amount found (Mean ± SD; % of Injection)</b>			
	<b>0.5 hours after injection (n = 4)</b>		<b>1.0 hours after injection (n = 5)</b>	
Remains	Stomach	85.4 ± 2.6	79.7 ± 4.3	
	Duodenum	16.9 ± 3.5	23.6 ± 14.9	
	Ileum	66.0 ± 6.5	67.9 ± 8.7	
	Colon	47.4 ± 11.0	36.3 ± 10.1	
Tissue	Stomach	6.0 ± 1.4	5.9 ± 0.8	
	Duodenum	17.2 ± 0.8	16.1 ± 5.6	
	Ileum	10.9 ± 1.6	10.8 ± 2.6	
	Colon	18.3 ± 4.0	12.1 ± 8.2	
Absorption <sup>b</sup>	Stomach	8.6 ± 2.2	14.4 ± 3.5	
	Duodenum	65.9 ± 4.1	60.3 ± 20.3	
	Ileum	23.1 ± 7.7	21.3 ± 6.6	
	Colon	34.4 ± 7.4	51.6 ± 14.9	

a: HPLC-MS as described in [R92052]

b: Absorption = (injected amount - remaining amount in loop - amount in tissue) / injected amount \* 100

<b>2.6.5.16B Pharmacokinetics: Other</b>		<b>Test Article:</b> Pitavastatin		Page 1 of 1	
<b>Report No.:</b> [R12001/2503]		<b>Location in CTD:</b>		<b>Section:</b> *	
		<b>Vol:</b> *		<b>Section:</b> *	
<p><b>Type of Study:</b> <i>In vivo</i> study to evaluate the PK of pitavastatin in a carbon tetrachloride (CCl<sub>4</sub>) induced model of liver dysfunction following a single oral dose of pitavastatin at 1 mg/kg and atorvastatin at 10 mg/kg in fasting rats. Atorvastatin was used as a comparator.</p> <p><b>Study Design:</b> The study design is given below. Pitavastatin or atorvastatin were administered 24 hours after administration of CCl<sub>4</sub> when the elevation of AST and ALT were highest. Animals were fasted for 16 hours prior to administration of pitavastatin or atorvastatin. Blood samples were taken at 0.5, 1, 2, 4, 8 and 24 hours after dosing. Pitavastatin was determined using the HPLC-CS<sup>a</sup> method.</p>					
<b>Dose Group (n = 5/group)</b>	<b>CCl<sub>4</sub> Dose (mL/kg; subcutaneous administration)</b>	<b>Pitavastatin (1 mg/kg, oral gavage)</b>	<b>Atorvastatin (10 mg/kg, oral gavage)</b>		
1	-	1	-		
2	0.5	1	-		
3	1.0	1	-		
4	2.0	1	-		
5	3.0	1	-		
6	-	-	10		
7	0.5	-	10		
8	1.0	-	10		
9	2.0	-	10		
10	3.0	-	10		
<p><b>Results:</b> C<sub>max</sub> and AUC increased in a dose-dependent manner with respect to increasing CCl<sub>4</sub> dose. In this rat model of liver dysfunction (grade 1; AST and ALT elevated 1.25 to 2.5 fold the upper limit of their normal levels) the AUCs of pitavastatin and atorvastatin versus those in CCl<sub>4</sub> untreated groups were increased 2.6 to 3.4 and 4.9 to 5.9 times, respectively. The increase in AUC was lower with pitavastatin than atorvastatin.</p> <p><b>a:</b> HPLC-CS as described in [R95077]</p> <p><b>AST:</b> Aspartate aminotransferase; also known as glutamic oxaloacetic transaminase (SGOT) or aspartate transaminase</p> <p><b>ALT:</b> Alanine aminotransferase; also known as glutamic-pyruvic transaminase (SGPT) or alanine transaminase</p>					

<b>2.6.5.16C Pharmacokinetics: Other</b>		<b>Test Article:</b> Pitavastatin		Page 1 of 1				
<b>Report No.:</b> [R19935]		<b>Location in CTD:</b>		<b>Section:</b> *				
		<b>Vol:</b> *		<b>Section:</b> *				
<p><b>Type of Study:</b> Single oral dose study in male Crj:CD-1 (ICR) mice to determine the concentrations of epimer and enantiomer in the plasma and bone marrow after administration of epimer or enantiomer at dose levels of 250 or 500 mg/kg.</p> <p><b>Study Design:</b> The epimer and enantiomer were suspended in 0.5% CMC sodium solution and animals were dosed by oral gavage (10 mL/kg). Each dose group comprised five animals. Blood samples were taken at 1, 2, 6 and 24 hours after dosing. The analytical target substance was pitavastatin (since optical isomers were not distinguished in this method, pitavastatin was used a general name for the epimer and enantiomer). The HPLC-CS<sup>a</sup> method was used.</p> <p><b>Results:</b> Approximately one-third of the drug was found in the bone marrow compared to that in the plasma. The ratio of concentration in bone marrow to that in plasma was equivalent for epimer and enantiomer. Stereospecific recognition of the side-chain in transfer to bone marrow was suggested.</p>								
<b>Time post-dose (hours)</b>	<b>Epimer Concentrations (Mean ± SD, ng/mL)</b>				<b>Enantiomer Concentrations (Mean ± SD, ng/mL)</b>			
	<b>Plasma</b>		<b>Bone Marrow</b>		<b>Plasma</b>		<b>Bone Marrow</b>	
	<b>250 mg/kg</b>	<b>500 mg/kg</b>	<b>250 mg/kg</b>	<b>500 mg/kg</b>	<b>250 mg/kg</b>	<b>500 mg/kg</b>	<b>250 mg/kg</b>	<b>500 mg/kg</b>
1	50700 ± 49600	109400 ± 35100	6200 ± 3100	32300 ± 15100	87000 ± 49300	101100 ± 36800	24000 ± 19800	17700 ± 6200
2	20000 ± 23600	71500 ± 16900	5600 ± 8500	14500 ± 5500	107000 ± 18100	154600 ± 42900	23000 ± 12200	45500 ± 27500
6	24700 ± 26200	29300 ± 10600	5000 ± 6100	4400 ± 1700	38400 ± 33600	72700 ± 17100	5300 ± 3500	13900 ± 3700
24	4500 ± 9300	48000 ± 23900	1000 ± 2200	16900 ± 20800	700 ± 900	38600 ± 21000	100 ± 100	5500 ± 2800
<b>a:</b> HPLC-CS as described in [R199810]								

## 2.6.6 TOXICOLOGY

## 2.6.6.1 Overall toxicology summary

Dose-limiting Toxicities in Repeat-dose Studies				
Species - duration	Duration	Toxicity	NOAEL (mg/kg/day)	Safety multiple at 4 mg HED
Mouse	3 mo	Forestomach hyperkeratosis	ND, <25 <b>(625.5 = AUC<sub>0-24</sub>)</b>	<4.1
	1 mo		ND, <50	<8.2
	1 mo rasH2		ND, <70	<11.5
Rat	6 mo	Forestomach hyperkeratosis	0.3	1.7
	3 mo		<10 <b>(8634 = AUC<sub>0-24</sub>)</b>	<56
	1 mo		2	11.3
	1 mo (non- <i>glp</i> )		3	17
	2 wk (iv)	Epididymes, nodules, sperm granuloma, cellular debris	1	5.6
Dog	12 mo	Lens opacity, not recovered	0.3 <b>(336 = AUC)</b>	2.8
	3 mo	Lung, foamy cells in alveolus, recovered	1	7
		Mammary gland, lipogranuloma, recovered	3	24.0
	2 wk	Mortality, cardiac congestion, hemorrhage in decedents; thymic congestion possibly related to multi-organ failure	5	40
2 wk (iv)	No toxicity observed	>2	No TK	
Monkey	6 mo	Lung, foamy cells in alveolus, recovered; Esophagus, mononuclear cell infiltration, recovered; Adrenal, mineralization between cortex/medulla, recovered	1 <b>(437.2 = AUC)</b>	2.9
	1 mo	Kidney, necrosis, regeneration; lacrimal gland, mononuclear cell infiltration; heart, mononuclear cell infiltration; spleen, atrophy of white pulp, pancreas, acinar cell atrophy; hepatocyte, granuloma; adrenal hyperplasia, decreased fat in cortex	<3 <b>(514.0 = AUC)</b>	<3.4

ND, not determined

Human AUC<sub>0-24</sub> at 4 mg/kg/day was 153 ng·hr/mL

AUC values for each species are in bold. Where absent, the exposures determined for the longest duration study were used. Extrapolation assumed linear, dose-proportional exposure.

### General toxicology:

#### Lens toxicities

Cataracts were seen in dogs in repeat dose studies at  $\geq 3$  mg/kg/day in a 13 week study and at  $\geq 1$  mg/kg/day in the 12 month study (2-fold human exposure based on AUC at 4 mg/day). This finding has been associated with statin treated dogs. Distribution studies indicate that the dog lens has a higher level of pitavastatin than other species with a rank order of distribution in the lens-to-plasma ratio of: dog (0.6) > mouse (0.54) > monkey (0.18)  $\approx$  rat (0.17) > rabbit (0.06). The lens-to-plasma ratio in humans is 0.05-0.17.