indicating no preferential retention in any tissue, with an apparent half life of about 12 to 20 hours.

Tissue distribution of radioactivity after a single oral dosing of [^{14}C]- rosuvastatin of 5 mg/kg in male rats (mean ± standard deviation, n = 3)

Tissue	Concentration of radioactivity (ng eq./g)						
Time after dosing (h)	0.25	1.5	4	8	24	48	120
Plasma	38.6 ± 12.0	79.4 ± 14.7	68.1 ± 8.1	44.8 ± 15.3	28.9 ± 12.7	10.9 ± 3.3	ND
Blood	24.8 ± 5.7	48.5 ± 12.2	47.7 ± 9.6	29.6 ± 8.7	ND	ND	ND
Adrenal gland	ND	65.7 ± 11.2	49.4 ± 11.2	ND	ND	ND	ND
Epididymis	ND	19.0 ± 1.7	22.4 ± 5.6	12.4 ± 3.2	ND	ND	ND
Fat	ND	41.5 ± 6.3	69.5 ± 14.4	27.7 ± 4.5	15.7 ± 1.7	ND	ND
Fat (brown)	33.7 ± 6.6	65.5 ± 8.3	57.2 ± 19.5	30.0 ± 4.3	25.2 ± 9.0	ND	ND
Harderian gland	21.0 ± 3.8	45.8 ± 6.6	49.2 ± 17.0	21.1 ± 5.5	ND	ND	ND
Heart	17.5 ± 2.5	31.5 ± 1.6	27.7 ± 7.3	16.6 ± 4.3	9.0 ± 1.9	ND	ND
Kidney	42.6 ± 9.1	89.5 ± 10.5	84.4 ± 13.9	49.3 ± 8.5	19.3 ± 4.3	9.5 ± 2.7	ND
Liver	965 ± 302	844 ± 139	722 ± 100	477 ± 71.8	239 ± 67.4	89.3 ± 13.1	19.8 ± 6.0
Lung	16.5 ± 1.9	31.7 ± 2.7	31.6 ± 8.8	16.7 ± 2.8	ND	ND	ND
Mandibular gland	14.1 ± 0.6	27.0 ± 1.5	23.2 ± 3.9	12.0 ± 3.5	ND	ND	ND
Mesenteric lymph node	64.5 ± 27.0	1042 ± 387	355 ± 189	74.4 ± 8.6	14.8 ± 7.6	ND	ND
Pancreas	26.1 ± 7.8	34.3 ± 6.3	26.4 ± 5.3	18.5 ± 11.1	ND	ND	ND
Prostate gland	ND	20.3 ± 0.8	18.2 ± 2.4	ND	ND	ND	ND
Skeletal muscle	ND	15.8 ± 5.1	14.1 ± 3.8	ND	ND	ND	ND
Skin	11.8 ± 0.8	30.1 ± 5.0	34.8 ± 7.6	23.0 ± 8.4	10.0 ± 1.3	ND	ND
Spleen	11.3 ± 1.8	18.0 ± 1.4	14.4 ± 5.3	8.8 ± 3.0	7.0 ± 2.5	ND	ND
Testis	ND	10.4 ± 1.1	11.3 ± 4.8	ND	ND	ND	ND
Thymus	ND	14.5 ± 1.7	ND	ND	ND	ND	ND
Aorta, Bone marrow, Cerebellum, Cerebrum, Eyeball, Spinal cord, Pituitary gland, Thyroid gland: - ND at all time points							

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Tissue distribution of radioactivity after 14-day oral dosing of [^{14}C]- rosuvastatin of 5 mg/kg in male rats (mean ± standard deviation, n = 3)

Tissue	Concentration of radioactivity (ng eq./g)						
Time after dosing (h)	0.25	1.5	4	8	24	48	120
Plasma	89.1 ± 22.5	154 ± 39.6	110 ± 23.3	106 ± 12.5	66.0 ± 12.8	20.9 ± 7.1	9.0 ± 3.0
Blood	69.9 ± 14.7	113 ± 26.4	90.4 ± 6.0	79.6 ± 7.0	63.1 ± 10.9	35.2 ± 1.8	31.9 ± 5.0
Adrenal gland	54.7 ± 21.4	100 ± 13.7	63.9 ± 6.3	61.7 ± 13.5	ND	ND	ND
Aorta	ND	73.9 ± 34.9	ND	ND	ND	ND	ND
Bone marrow	ND	34.6 ± 7.1	ND	ND	ND	ND	ND
Epididymis	12.5 ± 2.3	33.1 ± 11.9	26.7 ± 6.2	26.9 ± 3.9	11.9 ± 1.4	4.9 ± 0.7	ND
Eyeball	ND	11.1 ± 2.8	ND	8.4 ± 0.9	ND	ND	ND
Fat	21.1 ± 5.2	56.4 ± 11.3	57.5 ± 9.3	61.7 ± 3.6	17.8 ± 1.4	ND	ND
Fat (brown)	36.6 ± 4.3	140 ± 40.0	75.1 ± 12.9	68.0 ± 10.2	27.7 ± 4.0	11.8 ± 1.7	ND
Harderian gland	29.7 ± 9.6	81.9 ± 23.9	53.6 ± 8.0	45.5 ± 1.9	22.9 ± 5.8	ND	ND
Heart	38.0 ± 5.8	73.8 ± 21.1	50.2 ± 9.1	42.6 ± 6.2	26.0 ± 3.7	12.1 ± 3.0	ND
Kidney	87.8 ± 9.3	209 ± 53.9	133 ± 8.3	115 ± 17.1	60.8 ± 8.9	27.4 ± 3.5	20.2 ± 3.9
Liver	1532 ± 261	2138 ± 330	1324 ± 302	1227 ± 91.6	655 ± 204	219 ± 59.5	125 ± 29.0
Lung	32.0 ± 5.3	69.4 ± 16.6	48.6 ± 8.1	46.4 ± 7.1	23.3 ± 7.6	ND	ND
Mandibular gland	26.6 ± 2.2	50.8 ± 11.7	33.1 ± 4.3	25.7 ± 2.3	13.8 ± 2.1	ND	ND
Mesenteric lymph node	133 ± 76.7	1254 ± 762	867 ± 3.5	193 ± 167	29.7 ± 15.8	ND	ND
Pancreas	41.1 ± 13.3	61.3 ± 11.3	37.6 ± 4.2	37.7 ± 1.9	12.2 ± 2.5	ND	ND
Prostate gland	17.2 ± 3.8	34.3 ± 7.4	25.2 ± 6.7	19.9 ± 4.4	ND	ND	ND
Skeletal muscle	15.3 ± 4.0	25.5 ± 7.1	18.2 ± 0.8	17.8 ± 1.8	9.0 ± 1.1	ND	ND
Skin	25.4 ± 5.5	58.9 ± 20.3	44.8 ± 9.0	41.3 ± 4.5	19.9 ± 1.5	10.4 ± 0.5	ND
Spleen	21.9 ± 3.6	37.8 ± 9.1	27.2 ± 2.0	23.3 ± 0.8	13.0 ± 2.0	ND	ND
Testis	ND	20.8 ± 7.4	14.6 ± 3.4	15.4 ± 2.0	7.1 ± 0.4	ND	ND
Thymus	12.0 ± 3.1	25.7 ± 8.5	17.5 ± 2.7	14.0 ± 1.6	ND	ND	ND
Cerebellum, Cerebrum, Spinal cord, Pituitary gland, Thyroid gland: - ND at all time points							

Plasma protein binding was reversible, independent of concentration, and was principally to serum albumin. Results from *in vitro* experiments, using equilibrium dialysis were 78.4%, 95.8%, 85.5%, 82.0% and 88.5% in male mouse, rat, dog, monkey and human respectively, and 92.5% in female rabbit.

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Plasma Protein Binding in Mouse, Rat, Rabbit, Dog, Cynomolgus Monkey and Human. Values represent means across the tested concentration range (%, ± standard deviation in brackets with two determinations at each concentration)

Study	Sex	Mouse	Rat	Rabbit	Dog	Monkey	Human
S-4522-F-06-N (Exp 6)	m	N/A	96.1	N/A	92.2	N/A	96.2
D4522 KPJ036	m	64.6 (± 8.47)	95.3 (± 0.68)	86.2 (± 0.75)	77.9 (± 1.14)	86.6 (± 0.63)	86.4 (± 0.54)
	f	74.5 (± 5.69)	95.9 (± 1.02)	87.8 (± 0.37)	78.7 (± 0.59)	81.3 (± 0.96)	84.8 (± 0.64)
D4522 KPJ062	m	78.4 (± 4.93)	95.8 (± 0.69)	92.5 (± 0.64)	85.5 (± 0.40)	82.0 (± 1.73)	88.5 (± 0.99)

N/A Not available

Consistent with the findings of quantitative tissue distribution, a low distribution of rosuvastatin was found in fetus in rats (3% or 20% of maternal plasma concentration in fetal tissue or amniotic fluid, respectively) following a single oral dose of 25 mg/kg. Relative higher distribution in fetal tissue (25% of maternal plasma concentration) was observed in 1/4 fetus in rabbits following a single oral dose of 1 mg/kg. However, in the lactating rat, at equivalent timepoints, rosuvastatin was found in milk at concentrations up to 3 times those in plasma.

Enzyme induction

Samples of liver were taken from mice after dosing for 1 year in the oncogenicity study and from rats and dogs at the end of the one month toxicity studies. These were examined for total cytochrome P450 content and drug metabolizing capacity. In the mouse, dosed orally at 10, 60 and 200 mg/kg/day, small changes in the measured enzyme activities were observed. In the rat, dosed orally at 15, 50 and 150 mg/kg/day, there were no obvious changes in drug metabolizing enzymes. In the dog, dosed orally at 10, 30 and 90 mg/kg/day, slight changes in activities were noted, but these changes were small and not dose related or statistically significant. Therefore, rosuvastatin does not produce significant liver enzyme induction.

Metabolism:

In all species, following oral dosing, unchanged rosuvastatin was the major drug-related substance in the excreta. Metabolism was a minor pathway for drug clearance. Many minor metabolites were observed and some were identified. In mouse, rat, rabbit and dog, most of the metabolic changes took place in the heptenoic acid side chain, principally by β-oxidation. There was also evidence of demethylation of the sulfonamide of β-oxidation products in mouse and rabbit. In monkey and man, demethylation of the sulfonamide of parent was a major metabolic route, with β-oxidation less evident or not detected. In general none of the metabolites represented as much as 10% of the dose.

Rosuvastatin was the most abundant drug-related material in the circulation of rats and dogs at early timepoints post-dose, though the proportion declined with time after dosing. In the Cynomolgus monkey unchanged rosuvastatin was a minor component of the circulating radioactivity at all times (rosuvastatin lactone was the principal identified, circulating drug-related component).

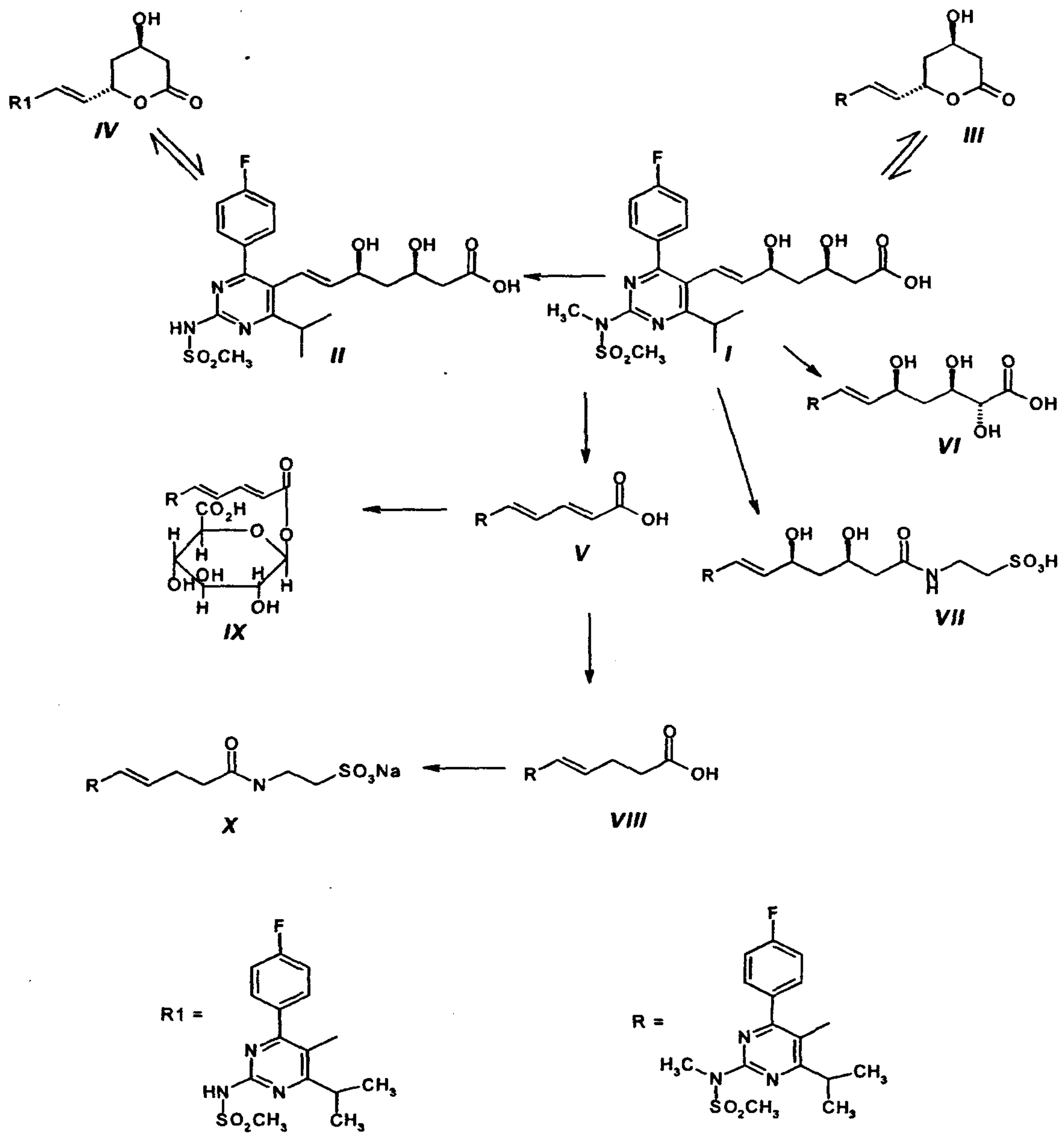
Metabolism of rosuvastatin in rat and dog was a minor pathway for clearance with parent compound predominant in liver and bile. Minor metabolites such as the pentenoic acid derivative were detected but found to have only weak enzyme inhibitory activity. Only desmethyl rosuvastatin has demonstrated significant inhibitory activity against HMG-CoA reductase or affinity for the cloned human catalytic domain of the enzyme, and its activity is from 2- to 7-fold less than rosuvastatin in these systems. In addition, in the rat, the peak of inhibition against HMG-CoA reductase in liver after oral dosing coincided with the peak of plasma rosuvastatin levels. The evidence suggests that rosuvastatin is the primary pharmacodynamic species in rat and dog.

Semi-quantitative comparison of metabolism in different species

	Moiety	Mouse	Rat		Rabbit	Dog		Monkey		Man	
		excreta	excreta	plasma	excreta	excreta	plasma	excreta	plasma	excreta	plasma
I	Rosuvastatin	✓✓✓	✓✓✓	✓✓	✓✓✓	✓✓✓	✓✓	✓✓✓	✓	✓✓✓	✓✓✓
II	Desmethyl rosuvastatin							✓	✓	✓	✓
III	Rosuvastatin .	✓	✓	✓	✓			✓	✓✓	✓	✓
IV	Desmethyl rosuvastatin								✓✓		
V	Rosuvastatin pentadieneoic acid	✓				✓	✓✓				
VI	Rosuvastatin triol					✓(bile)		✓	✓✓		
VII	Rosuvastatin taurine conjugate		✓(bile)	✓							
VIII	Rosuvastatin pentenoic acid	✓	✓(bile)	✓	✓						
IX	Rosuvastatin pentadienoic acid glucuronide					✓(bile)					
X	Rosuvastatin pentenoic acid taurine conjugate		✓(bile)								

✓✓✓ Major substance present (50% or more of dose in excreta or 50% or more of radioactivity in plasma at approximate C_{max})
✓✓ Substance present at 10% or more of dose in excreta or 10% or more of radioactivity in plasma at approximate C_{max}
✓ Minor substance (<10%; >1% of dose in excreta or <10%; >1% or more of radioactivity in plasma at approximate C_{max})
✓(bile) Substance identified in bile but not quantified
Data from single doses in all species

Proposed pathways for the established routes of metabolism of rosuvastatin



Quantitative Species Comparison of Metabolite Profiles in Excreta

Species (Study Reference)	Dose Route	Dose (mg/kg)	Sex	Metabolite	% of Dose excreted in -		
Mouse D4522 KMM030	Oral	10			Urine (0-72)	Feces (0-96)	
			m	ZD4522	3.3	73	
				ZD4522 Pentenoic acid	0.4	3.7	
				Desmethyl Pentenoic acid	0.9	1.9	
				ZD4522 Pentadienoic acid	0.1	1.9	
				ZD4522	0.9	0.9	
				Other metabolites	1.6	6.5	
			f	ZD4522	1.7	74	
				ZD4522 Pentenoic acid	0.2	5.0	
				Desmethyl Pentenoic acid	0.7	3.3	
				ZD4522 Pentadienoic acid	-	1.7	
				ZD4522	0.2	1.7	
				Other metabolites	1.2	6.7	
Rat S-4522-F-06-N, 7	Oral	5			Urine (0-24)	Feces (0-24)	Bile (0-24)
			m	ZD4522	0.1	80	36
				ZD4522	-	0.9	0.2
				ZD4522 Pentenoic acid	-	0.4	0.1
				ZD4522 Taurine conjugate	-	0.2	1.1
				Other metabolites	-	11	2.9
Rabbit D4522 KMB031	Oral	1			Urine (0-168)	Feces (0-168)	
			f	ZD4522	25	48	
				ZD4522 5S-Lactone	-	1.9	
				ZD4522 Pentenoic acid	0.8	2.3	
				Desmethyl Pentenoic acid	2.5	-	
				Other metabolites	1.6	2.7	
Dog S-4522-B-42-N					Urine (0-96)	Feces (0-96)	
	Oral	5	m	ZD4522	1.4	82	
				ZD4522 Pentadienoic acid	ND	3.6	
				Other metabolites	0.6	8.3	
	IV	5	m	ZD4522	5.7	66	
				ZD4522 Pentadienoic acid	-	5.4	
				Other metabolites	1.3	9.6	
Dog S-4522-B-44-N							Bile (0-72)
	Oral	5	m	ZD4522			38
				ZD4522 Pentadienoic acid			2.1
				Other metabolites			16
	IV	5	m	ZD4522			67
				ZD4522 Pentadienoic acid			1.2
				Other metabolites			13
Monkey D4522 KKP011	Oral	10			Urine (0-168)	Feces (0-168)	
			m	ZD4522	1.0	75	
				ZD4522 triol	0.5	3.5	
				Desmethyl ZD4522	1.7	3.5	
				Other metabolites	1.8	8.7	

ND Not Detected; Figures in parentheses following excreta names are the collection period in hours

Excretion:
The major excretory pathway was feces in mouse, rat, monkey, and dog, mostly as unchanged parent compound. The rate of excretion was rapid, with over 80% of dose excreted within 24 hours post dose, and over 99% within 96 hours. In humans, 100% of a 20-mg dose of [^{14}C]-rosuvastatin was recovered in excreta over a period up to 10 days post-dose: 90% in feces and 10% in urine. Approximately 71% of the dose was recovered in feces, and 9% in urine, within the first 72-h post-dose. Therefore, the fecal route is also the most important route of elimination in humans.

[^{14}C]-Rosuvastatin excretion balance

					% Dose excreted					
Species strain	Dose route	Sex (number)	Dose (mg/kg)	Duration of collection (h)	Urine	Feces	Bile	Carcass (incl. gut contents)	Cagewash	Total recovery
Mouse CD-1	oral	m (3)	10	0-168	3.1	86.1			4.0	94.1
		f (3)			1.6	88.4			2.4	95.9
Rat	oral	m (3)	5	0-168	0.4	98.0				98.3
Sprague-Dawley SPF		m (3)	5	0-48	0.5	41.0	55.1	2.3		98.9
		m (3)	5 for 14 days	168 after 14th dose	0.5	99.0				99.5
	oral	m (3) donor	5	0-48	0.4	42.3	-	6.9		99.2
		m (3) recipient			<0.1	21.0	19.1	9.5		
Rabbit New Zealand White	oral	f (3)	1	0-168	19.2	54.3		3.3	10.2	87.0
Dog	oral	m (3)	5	0-96	1.9	94.1			0.8	96.8
Beagle	iv	m (3)			7.0	80.9			1.0	88.9
	oral	m (3)	5	0-72	13.5	17.6	60.4		1.0	93.0
	iv	m (3)			9.5	3.3	81.3		0.6	94.6
Cynomolgus monkey	oral	m (4)	10	0-168	4.1	90.1			1.2	95.3

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The following safety pharmacokinetic and ADME studies were submitted in this NDA:

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Mouse $B_6C_3F_1$	S-4522-F-15-L (TK) Oral [redacted]	20, 60, 200 13 weeks Lot no R39001 Suspension in 5% aqueous gum arabic	RIA S-4522-F-15-L Non-compliant	3/sex/timepoint/group Samples taken after day 1 and 89 of dosing at pre-dose and timepoints of 0.5, 1.5, 4, 8 and 12 h post-dose Exposure decreased on multiple dosing Greater than proportional increase in exposure with increasing dose Possible sex difference
Mouse $B_6C_3F_1$	TCM1088 (TK also reported as D4522 KPM010) Oral [redacted]	10, 60, 200 107 weeks 00518I98 03516E98 60414K99 64725E99 62413K99 70888E00 Suspension in 5% aqueous gum arabic	[redacted] D4522 KPV018 Compliant	3/sex/timepoint/group Samples taken after 52 weeks at pre-dose and timepoints of 0.5, 1.5, 4, 8, 12 and 24 h post-dose High inter-individual variability Greater than proportional increase in exposure with increasing dose No sex difference
Mouse B_6C_3F1	D4522 KMM050 (ADME, liver samples from TCM1088) Oral [redacted]	0, 10, 60, 200 107 weeks 00518I98 03516E98 60414K99 64725E99 62413K99 70888E00 Suspension in 5% aqueous gum arabic	Measurement of P450 enzyme changes in liver N/A Compliant	Up to 6 livers/sex pooled at each dose level Samples taken after 52 weeks Small changes in drug metabolising enzymes were considered of no biological significance
Mouse CD-1	D4522 KMM030 (ADME) Oral [redacted]	10 Single dose [^{14}C]-ZD4522 batch 1R5 Solution in 5% aqueous gum arabic	[redacted] radioactivity detection N/A Compliant	3 males and 3 females Excreta and cage debris collected over 24 h periods up to 168 h post-dose Most of dose recovered in feces in 48 h Approx. 95% of dose recovered; 87% of dose in feces with 2.5% in urine after 168 h <0.1% in carcass at 168 h ZD4522 was the major excretory moiety in urine and feces (total approx 76% of dose) Up to 10 minor metabolites detected in excreta at >1% of dose (largest at 4.1% of dose) No sex difference in excretion balance or metabolism
Rat Jcl:SD	S-4522-F-13-L (TK) Oral [redacted]	2, 6, 20 6 months Lot no R39001 Suspension in 5% aqueous gum arabic	RIA S-4522-B-22-N-A Non-compliant	4/sex/3 timepoints/group Samples taken at day 1, 91 and 182 of dosing at pre-dose and timepoints of 0.5, 1.5, 4, 8 and 24 h post-dose t_{max} 0.5h Greater than proportional increase in exposure with increasing dose above 6 mg/kg No sex difference Exposure increases on multiple dosing
Rat CDF (F-344)	S-4522-F-16-L (TK) Oral [redacted]	6, 20, 60 13 weeks Lot no R39001 Suspension in 5% aqueous gum arabic	RIA S-4522-B-22-N-A Non-compliant	4/sex/2 timepoints/group Samples taken at day 1 and 90 of dosing at pre-dose and timepoints of 0.5, 1.5, 4, 8 and 24 h post-dose t_{max} 0.5h Greater than proportional increase in exposure with increasing dose No sex difference Exposure increases on multiple dosing, more markedly at highest dose

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Rat Crl: CD	TCR2852 (TK, also reported as D4522 KPR009) Oral	2, 20, 60, 80 104 weeks 00518I98 03516E98 60414K99 64725E99 62413K99 70888E00 Suspension in 5% aqueous gum arabic	D4522 KPV018 + D4522 KPV038 Compliant	3/sex/timepoint/group Samples taken at day 1, after 1 month and 1 year at pre-dose and timepoints of 0.5, 1.5, 4, 8, 12 and 24 h post-dose t_{max} 0.5h to 1.5h High variability Greater than proportional increase in exposure with increasing dose No apparent accumulation No apparent sex difference
Rat SD, SPF	S-4522-F-04-N (Exp 1) (ADME) Oral and IV	1, 5, 25 oral 5 IV Single dose ^{14}C-S-4522 Lot no 91-717-67-1, S-4522 Lot no 52 Aqueous soln	N/A Non-compliant	3 males/group at 1, 5, 25 mg/kg oral fed, 5 mg/kg oral fasted, and 5 mg/kg IV; 3 females at 5 mg/kg oral fed Blood taken serially from each rat at 5, 10, 30 min, 1, 1.5, 2, 4, 6, 8 and 24 hour post-dose oral, and 2, 5, 10, 30 min, 1, 1.5, 2, 4, 6, 8 and 24 hour post-dose IV Only total radioactivity measured Absorption more rapid, and exposure greater in fasted animals Greater than proportional increase in exposure with increasing dose No sex difference
Rat SD, SPF	S-4522-F-04-N (Exp 2) (ADME) Oral	5 Single Dose ^{14}C-S-4522 Lot no 91-717-67-1 Aqueous soln	N/A Non-compliant	3 males dosed fed Urine and feces collected every 24 h period up to 168 h 0.4% of dose in urine over 168 h 98% of dose in feces over 168 h, most in first 24 h ND in carcass at 168 h
Rat SD, SPF	S-4522-F-04-N (Exp 3) (ADME) Oral	5 Single Dose ^{14}C-S-4522 Lot no 91-717-67-1 Aqueous soln	N/A Non-compliant	3 male bile duct cannulated rats Bile collected 0-2, 2-4, 4-6, 6-8, 8-24 and 24-48 h post-dose. Urine and feces collected 0-24 and 24-48 h 55% in bile, 0.5% in urine, 41% in feces, 1.8% in gastro-intestinal contents and 0.5% in carcass up to 48 h post-dose
Rat SD, SPF	S-4522-F-05-N (Exp 4) (ADME) Oral	5 Single Dose ^{14}C-S-4522 Lot no 92-227-75-1 + 92-330-77-1 Aqueous soln	N/A Non-compliant	3 pairs of male bile duct cannulated rats. First, donor rat, given dose and bile output is re-directed to duodenum of recipient rat Bile collected up to 48 h post-dose from recipient rat. Urine and feces collected up to 48 h in both rats of each pair 19% of dose given to donor rats appeared in bile of recipient rats
Rat SD, SPF	S-4522-F-05-N (Exp 5) (ADME) Oral	5 Single Dose ^{14}C-S-4522 Lot no 92-227-75-1 + 92-330-77-1 Aqueous soln	N/A Non-compliant	3 male rats at each of 0.25, 1.5, 4, 8, 24, 48 and 120 h post-dose Series of tissues taken from each rat at each timepoint and radioactivity content measured Distribution above plasma concentrations only in liver and mesenteric lymph node (the latter at only early timepoints post-dose) Maximum concentrations in liver at 15 min; other tissues at 1.5-4 h Liver concentrations generally ≥10x any other tissue
Rat Jcl:SD	S-4522-B-45-N (ADME) Oral	5 Single Dose ^{14}C-S-4522 Lot no 91-717-92-3 Aqueous soln	N/A Non-compliant	1 male per timepoint WBA performed at timepoints of 0.25, 1.5, 4 and 24 h post-dose Radioactivity detected only in bile, blood, liver, gut contents Liver radioactivity highest at 15 min At 24 hours radioactivity only detected in gut.

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Rat Hooded Lister Hsd:CD	D4522 KMR013 (ADME) Oral	10 Single Dose [^{14}C]-ZD4522 batch 1R2, ZD4522 batch ADM00518198 Aqueous soln	Imaging) N/A Compliant	1 male per timepoint performed at timepoints of 1, 6, 24, 72, 168 and 336 h post-dose Highest concentrations of radioactivity in gut contents Liver and kidney were the only tissues with detectable radioactivity All concentrations much reduced from peak at 24 hours after dose and not detectable at 72 h Radioactivity not detected in the melanin-containing tissues on skin and eye
Rat SD	D4522 KMR033 (ADME) Oral	25 Single Dose [^{14}C]-ZD4522 batch 1R5, ZD4522 batch A87002 Solution in 5% aqueous gum arabic	N/A Compliant	5 time mated female rats, dosed at day 16 of gestation Samples of maternal plasma and tissue, and fetal tissue taken at 30 mins post-dose On average, fetal tissue concentrations of radioactivity were approximately 3% of maternal plasma concentrations Transfer of drug related material was demonstrated, but at levels comparable to the impurity in the dose, detected at dose formulation Transfer of <u>relevant</u> drug related material could not be concluded
Rat SD	D4522 KMR049 (ADME) Oral	0, 25 Single Dose ZD4522 batch A87002 Solution in 5% aqueous gum arabic	D4522 KPV018 D4522 KPV045 Compliant	4 time mated female rats, dosed at day 16 of gestation; a further 4 dosed with dose vehicle only Samples of maternal plasma and fetal tissue taken at 30 mins post-dose On average, fetal tissue concentrations of ZD4522 free acid were approximately 1.5% of maternal plasma concentrations Transfer of ZD4522 to rat fetus was demonstrated
Rat Alpk: AP$_f$SD	D4522 KMR052 (ADME) Oral AstraZeneca	38.4 Single Dose [^{14}C]-ZD4522 batch no 1R10 ZD4522 batch ADM64957J99 Suspension in 5% aqueous gum arabic	D4522 KPV047 Compliant	6 females given single dose at 13 days post-partum Milk and plasma samples taken from 3 animals at each of 0.5, 2, 4, 6, 8, 12 and 24 h post-dose Total radioactivity measured in plasma and milk, and ZD4522 concentration in milk ZD4522 was present in milk at all timepoints Radioactivity in milk was 1 to 3 times that in plasma at the same timepoint
Rat SD, SPF	S-4522-F-06-N (Exp 7) (ADME) Oral	5 Single Dose ^{14}C-S-4522 Lot no 91-717-67-1 + 92-227-75-1 + 92-330-77-1 Aqueous soln	Imaging S-4522-F-04-N, (Pre 2) Non-compliant	3 males dosed to provide bile Urine and feces from S-4522-F-04-N (Exp 2) Plasma and liver from S-4522-F-05-N (Exp 5) Parent compound principal component in excreta and at early timepoints in plasma and liver Several metabolites detected S-4522 5S lactone, S-4522 taurine conjugate and pentenoic acid metabolite of S-4522 tentatively identified by co-chromatography with authentic compounds
Rat SD, SPF	S-4522-F-07-N (Exp 8 parts 1, 2, 3) (ADME) Oral	5 14 days ^{14}C-S-4522 Lot no 92-330-77-1 Aqueous soln	N/A Non-compliant	3 males/group dosed fed Group 1, blood and plasma taken serially at 1.5 hours post-dose on odd dosing days and 0.25, 1.5 and 4 h post-dose after 14th dose. Group 2, blood and plasma taken serially at 5, 15, 30 min, 1, 1.5, 2, 4, 8, 24, 48, 72, 96, 120 h after 14th dose. Group 3, urine and feces collected over 24 h following each dose, and every 24 h to 168 h following 14th dose Only total radioactivity measured in blood and plasma Steady state by dose 5 Accumulation approx 2-fold Cumulative excretion similar to single dose 99% of dose in feces; 0.5 % in urine by 168h after 14th dose <0.1% in carcass at 168h after 14th dose
Rat SD, SPF	S-4522-F-07-N (Exp 8 Part 4) (ADME) Oral	5 14 days ^{14}C-S-4522 Lot no 92-330-77-1 Aqueous soln	N/A Non-compliant	1 male sacrificed at each of 0.25, 1.5, 4 and 24 h post-dose At 0.25 h, highest in gastro-intestinal content, bile and bile duct, lower in liver and portal blood and low in kidney and blood; undetectable in other tissues At 1.5 and 4 h, highest as above, lower in liver and low in urine, blood and kidney; undetectable in other tissues At 24 h, highest in intestinal content, low in bile and bile duct, liver, gastric contents and kidney

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Rat SD, SPF	S-4522-F-08-N (Exp 8 part 5) (ADME) Oral	5 14 days ^{14}C-S-4522 Lot no 92-330-77-1 Aqueous soln	N/A Non-compliant	3 males sacrificed at each of 24 h after the 6th and 10th doses, then 0.25, 1.5, 4, 8, 24, 48 and 120 h after 14th dose Series of tissues taken from each rat, at each timepoint, and radioactivity content measured Distribution pattern similar to that following a single dose Accumulation in each tissue was 2 to 3 times the levels found following a single dose Steady state occurred by 6 doses in some tissues while, in others, steady state was not demonstrated
Rat SD, SPF	S-4522-F-08-N (Exp 8 part 6) (ADME) Oral	5 14 days ^{14}C-S-4522 Lot no 92-330-77-1 Aqueous soln	Imaging S-4522-F-04-N, (Pre 2) Non-compliant	Plasma harvested from 3 males sacrificed at each of 0.25, 1.5 and 4 h after the 14th dose 0-24 h urine and feces from S-4522-F-07-N (Exp 8 part 1, 2, 3) and liver at 0.25, 1.5, 8, 24 and 48 h after 14th dose from study S-4522-F-08-N (Exp 8 part 5) Parent compound major component in excreta (approx. 90% of radioactivity in feces) Similar composition of drug related species from that following a single dose (S-4522-F-06-N (Exp 7) Parent concentration comparable in plasma, but 1.5 to 2 times higher in liver, than that following a single dose M1 and pentenoic acid metabolite increased, in both plasma and liver, compared to that following a single dose
Rat Jcl:SD	C-HIRAI K-002-D IV	200 Single Dose S-4522 batch no. not available Aqueous soln	Various methods N/A Non-compliant	40 male bile duct cannulated rats dosed and bile collected over a the period 0 to 4 h post-dose Main metabolite isolated and identified was the taurine conjugate of the pentenoic acid derivative Others, tentatively identified were taurine conjugate of ZD4522, taurine conjugate of pentadienoic acid derivative, 3-hydroxy pentenoic acid derivative and the pentenoic acid derivative The proportion of each metabolite, relative to dose, was not established
Rat Jcl : SD	S-4522-B-17-L (P450 induction) Oral	0, 15, 50, 150 1 month S-4522 Lot no 52 Suspension in 5% aqueous gum arabic	Multiple methods N/A Non-compliant	No effect on drug metabolising enzymes at any dose
Rat Jcl : SD	S-4522-B-18-N (ADME) Direct into gut loop	0.5 Single Dose [^{14}C]-S-4522 Lot no not available S-4522 Lot no TO-38-154-1 Aqueous soln	N/A Non-compliant	3 male rats/timepoint/absorption site Absorption occurred throughout the gastrointestinal tract (including the stomach) but was slow from all sites Approximately 81% of S-4522 remained after incubation in gastric juice for 2 h at 37°C. Approximately 95% of S-4522 remained after incubation in washings of the intestinal lumen
Rabbit Kbl : JW	S-4522-B-52-N (TK) Oral	5, 10 with and without 40 mg/kg/day mevalonic acid 14 days S-4522 Lot no R39001 Suspension in 5% aqueous gum arabic	This study Non-compliant	5 males per group Plasma samples taken at 0.25, 0.5, 2, 6 and 24 h post-dose on days 0, 6 and 13 t_{max} 0.25-0.5 h Approximate 2-fold accumulation Approximate proportional increase in C_{max} with dose 2 marked outliers on day 13 for animals dose ZD4522 alone In general, no effect of mevalonic acid on ZD4522 plasma concentration
Rabbit New Zealand White	D4522 KMB032 (ADME) Oral	1 Single Dose [^{14}C]-ZD4522 Batch 1R5 Solution in 5% aqueous gum arabic	Validation this study Compliant	4 time mated female rabbits, dosed at day 18 of gestation Samples of maternal plasma and tissue, and fetal tissue taken at 30 mins post-dose At 30 min post-dose, ZD4522 accounts for 39% of circulating radioactivity Transfer of radioactivity to fetus was only demonstrated in 2 of the 4 animals, but at levels comparable to the impurity in the dose, detected at dose formulation Placental transfer of drug related material to rabbit fetus could not be confirmed

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Rabbit New Zealand White	D4522 KMB048 (ADME) Oral	0, 1 Single Dose ZD4522 Batch A87002 Solution in 5% aqueous gum arabic	D4522 KPV046 Compliant	4 time mated female rabbits, dosed at day 18 of gestation Samples of maternal plasma and tissue, and fetal tissue taken at 30 mins post dose Although measurable levels of ZD4522 were found in maternal plasma of all rabbits, there were no detectable levels in fetus Placental transfer of ZD4522 to rabbit fetus could not be confirmed
Rabbit New Zealand White	D4522 KMB031 (ADME) Oral	1 Single Dose [^{14}C]-ZD4522 Batch 1R5 Solution in 5% aqueous gum arabic	radioactivity detection N/A Compliant	3 female rabbits were dosed, and urine, feces and cage wash collected for each 24 h period up to 168 h post-dose 87% of dose recovered over entire period, 54.3 and 19.2% in feces and urine respectively, with 3.3% remaining in tissues 76.5% of the dose was recovered in the first 72 h post-dose ZD4522 was the principal component in excreta, accounting for 72% of administered dose Up to 5 metabolites found, none accounting for more than 3.5% of the dose. The pentenoic acid metabolite, and 5S-lactone, accounted for 3.1 and 1.9% of the dose respectively
Dog Beagle	S-4522-B-20-L (TK) Oral	10, 30, 90 1 month Lot no 54 Encapsulated powder/lactose	This study Non-compliant	3 individuals/sex/group (5/sex/group at the top dose) Plasma samples taken from each individual at pre-dose then at 1, 2, 4, 6 and 24 h post-dose, on days 0 and 29 of the study Significant variability No accumulation Slight changes in markers of P450 metabolism, but no dose response
Dog Beagle	S-4522-B-21-L (TK) Oral	7.5, 15, 30 3 months Lot no 55 Encapsulated powder/lactose	Registration no S-4522-010 (S-4522-B-04-N-C) Non-compliant	3 individuals/ sex/ group (6/sex/group at the top dose) Plasma samples taken from each individual at pre-dose then at 1, 2, 4, 6 and 24 h post-dose, on days 0, 41 and 82 of the study High variability No accumulation No apparent sex difference in kinetics 5S-lactone ~ 50% of ZD4522
Dog Beagle	S-4522-B-33-L (TK) Oral	1, 2, 4 3 months Lot no 55 Gelatin capsules/lactose	RIA S-4522-B-22-N-C Non-compliant	3 individuals/sex/group Plasma samples taken from each individual at pre-dose then at 1, 2, 4, 6 and 24 h post-dose, on days 0, 43 and 82 of the study High variability Approximate linear increase in C_{max} with dose No accumulation No sex difference
Dog Beagle	S-4522-B-37-L (TK) Oral	1, 4 6 months Lot no 55 Encapsulated powder/lactose	RIA S-4522-B-22-N-C Non-compliant	3 individuals/sex/group Plasma samples taken from each individual at pre-dose then at 1, 2, 4, 6 and 24 h post-dose, on days 0, 89 and 179 of the study t_{max} approx 2h No sex difference No accumulation Increase in exposure slightly greater than in proportion with dose
Dog Beagle	S-4522-B-46-L (TK) Oral	1, 3, 6 12 months Lot no R39001 Encapsulated powder/lactose	RIA S-4522-B-22-N-C Non-compliant	4 individuals/sex/group (7/sex/group at the top dose) Plasma samples taken from each individual at pre-dose then at 1, 2, 4, 6 and 24 h post-dose, on days 0, 184 and 363 of the study T_{max} typically 2 h No sex differences Approximately dose proportional if 2 outliers at 6 mg/kg excluded Increasing concentrations with time with no steady state observed

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Dog Alderley Park Beagle	TAD1018 (TK) IV (slow bolus) AstraZeneca	1, 5, 12 14 to 15 days Lot no A91002 Aqueous soln	D4522 KPV018 Compliant	3 individuals/sex/group Plasma samples taken from each individual at 5, 15 and 30 min, then at 1, 3, 6, 12 and 24 h post-dose, on day 14 of the study Slightly greater than dose-proportional increase in exposure No sex difference
Dog Alderley Park Beagle	TAD1148 (TK) Oral AstraZeneca	12 29 days Lot no ADM63638D99 (containing 1.39% lactone) Encapsulated powder/lactose	D4522 KPV061 D4522 KPV028 Compliant	3 individuals/sex/group Plasma samples taken from each individual at 0.5, 1, 2, 4, 6, 8, 12 and 24 h post-dose, on days 1 and 28 of the study High variability of plasma concentrations of both ZD4522 and its 5S-lactone Lactone AUC(0-24) was approximately 30 to 50% of that of parent compound No trend towards accumulation of either compound No apparent sex difference for either compound
Dog Beagle	D4522 KPD008 (ADME) Oral	5 Single dose, cross-over study Amorphous lot no ADM00518l98 Crystalline lot no ADM01870l98 Encapsulated powder	D4522 KPV018 Compliant	14 males per group, crossed over between amorphous and crystalline forms of ZD4522, with 2 week washout between treatment periods Plasma samples were taken at pre-dose then at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 30, 48, 72, 96 and 120 h post-dose from each animal in each period Bioequivalence could not be tested because of a significant sequence effect within the study; the reason was not identified Pharmacokinetics of the amorphous drug were comparable to those found in S-4522-B-46-L Pharmacokinetics of the amorphous drug were similar to those determined in S-4522-B-43-N
Dog Beagle	S-4522-B-43-N (ADME) Oral and IV	5 Single doses [^{14}C]-S-4522 lot no not available Oral dose in capsules, IV dose as aqueous soln	Imaging S-4522-F-04-N, (Pre 2) Non-compliant	6 male dogs were dosed, 3 by the oral, and 3 by the intravenous route Plasma samples were taken at 5, 10, 20 and 30 min, then 1, 2, 4, 6, 8 and 24 h post-dose following the oral dose, and the same following an intravenous dose, except for the addition of a 2 min timepoint Bioavailability of ZD4522 was measured as 23.4% AUC of circulating radioactivity 6.6 times that of parent compound following an oral dose, indicating circulating metabolites Only 1 metabolite was identified in plasma; the ZD4522 pentadienoic acid AUC of ZD4522 pentadienoic acid was approximately 4 times that of parent compound following an oral dose
Dog Beagle	S-4522-B-42-N (ADME) Oral and IV	5 Single doses [^{14}C]-S-4522 lot no 91-717-92-3 Oral dose in capsules, IV dose as aqueous soln	Imaging S-4522-F-04-N, (Pre 2) Non-compliant	6 male dogs were dosed, 3 by the oral, and 3 by the intravenous route Urine was collected over 0-6, 6-24, 24-48, 48-72 and 72-96 h post-dose, and feces for each 24 h period up to 96 h Following oral dosing, 96.8% was recovered (94.2% within 48 h) 94.1% in feces and 1.9% in urine Following an IV dose, 88.9% was recovered (86.9 within 48 h) 80.9% in feces and 7.0% in urine Parent compound accounts for 82.2 (oral) and 65.9% of dose (IV) in feces, and 1.4 (oral) and 5.7% of dose (IV) in urine. ZD4522 pentadienoic acid and 5S-lactone were identified in excreta among several other metabolites
Dog Beagle	S-4522-B-44-N (ADME) Oral and IV	5 Single doses [^{14}C]-S-4522 lot no 91-717-92-3 and 94-116-83-1 Oral dose in capsules, IV dose as aqueous soln	Imaging S-4522-F-04-N, (Pre 2) Non-compliant	8 male dogs were dosed, 5 by the oral, and 3 by the intravenous route. Two of the orally dosed dogs were excluded from analysis because of vomiting Bile was collected over 0-2, 2-4, 4-6, 6-8, 8-24, 24-48, 48-72 h post-dose, urine was collected over 0-6, 6-24, 24-48, 48-72 h post-dose, and feces for each 24 h period up to 72 h Following oral dosing, 93.0% was recovered, 60.4, 13.5 and 17.6% in bile, urine and feces respectively Following an IV dose, 94.6% was recovered, 81.3, 9.5 and 3.3% in bile, urine and feces respectively Parent compound represented 37.7% of the dose in bile and 22.5% in excreta, and 66.8% of the dose in bile and 10% of the dose in excreta, following oral and IV doses respectively The pentadienoic acid metabolite was identified in bile among several other metabolites

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Dog Beagle	S-4522-B-48-N (ADME) Oral	50 Single Dose Lot no 20802 20% S-4522 in capsules	Various methods N/A Non-compliant	2 male dogs were dosed Bile was collected from 5 - 20 h post-dose Several metabolites identified, including - 5-O-N-acetyl-glucosamide derivative Triol derivative Dehydrolactone derivative and its glucuronide Propenoic acid derivative Pentadienoic acid derivative Quantification (as % of dose) was not determined
Dog Beagle	C4-02-02 (ADME) Oral	7.5, 15, 30 3 months See S-3422-B-21-L for compound and formulation	Various methods N/A Non-compliant	18 male dogs from study S-4522-B-21-L (6 per group) Bile was collected 24 hours after final dose Two metabolites identified - Pentadienoic acid derivative Glucuronide of pentadienoic acid derivative Quantification (as % of dose) was not determined
Monkey Cynomolgus (Macaca fascicularis)	S-4522-F-10.B1-N (TK) Oral	10, 30 30 days Lot no 56 Suspension in 5% aqueous gum arabic	RIA S-4522-B-22-N-C Non-compliant	1/sex/group Plasma samples taken from each individual at pre-dose then 0.5, 1, 2, 4, 6, and 24 h post-dose, on days 0 and 28 of the study T_{max} variable No accumulation No gender difference
Monkey Cynomolgus (Macaca fascicularis)	S-4522-F-14-L (TK) Oral	10, 30 6 months Lot no R39001 Suspension in 5% aqueous gum arabic	RIA S-4522-B-22-N-C Non-compliant	3/sex/group Plasma samples taken from each individual at pre-dose then at 1, 2, 4, 6, and 24 h post-dose, on days 0, 90 and 181 of the study High variability t_{max} 2 - 6 hours About 2-fold accumulation Steady state not clearly observed No obvious sex difference
Monkey Cyno-molgus	D4522 KKP011 (ADME) Oral	10 Single Dose [^{14}C]-ZD4522 batch no 1R2 ZD4522 batch no ADM00518198 Solution in 5% aqueous gum arabic	D4522 KPV018 Compliant	4 males were dosed Plasma samples taken from each individual pre-dose and at 0.5, 1, 2, 4, 6 and 24 h post-dose Urine and feces were collected daily for 7 days t_{max} of ZD4522 0.5 - 2 h; t_{max} of radioactivity was 1 h Terminal half life of ZD4522 was 3.7 to 9.7 h, and of radioactivity 25.0 to 34.1 h Parent compound represented approximately 7% of radioactivity at 0.5 h and 2% at 24 h post-dose 95.3% of dose was recovered (91.3% in 72 h) with 90.1% in feces and 4.1% in urine Parent compound in excreta accounted for 74.8% of the dose Triol derivative and 5S-lactone were identified, with tentative identification of desmethyl ZD4522 and corresponding lactones ZD4522 5S-lactone exceeded the concentration of parent compound in plasma

Species/ Strain	Study Reference (Study type)/ Route/Site of Conduct	Dose (mg/kg/day)/ Duration/Batch/ Formulation	Assay type/ Validation/ GLP status	Salient Findings
Monkey Cynomolgus	D4522 KKP064 (ADME) Oral	10, 30 10 days ZD4522 batch no ADM70888E00 Suspension in 5% aqueous gum arabic	D4522 KPV056 Compliant	4 males were dosed (same animals at each dose level) Plasma samples taken from each individual pre-dose and at 0.25, 0.5, 1, 2, 4, 8 and 24 h post-dose after the first and final dose at each dose level Samples were assayed for ZD4522 and desmethyl ZD4522 Systemic exposure of desmethyl ZD4522 was 63 - 80% of that of ZD4522 Plasma concentrations of desmethyl ZD4522 declined in parallel with ZD4522, suggesting the metabolite elimination was formation rate limited Both compounds accumulated on multiple dosing by up to 2-fold Extent of exposure of each was slightly less than dose proportional
Dog Beagle Monkey Cynomolgus	C-HIRAI K-002-C (ADME) Oral	Dog ~900 mg Monkey 10, 30 Dog in 2 doses 2 h apart. Monkey see S-4522-F-10.B1-N Batch no. dosed to dog not available Dog - in capsules	Various methods N/A Non-compliant	Dog bile collected 0 to 24 h post-dose Monkey collection times unspecified Triol derivative identified in bile of both species Quantification (as % of dose) was not determined
Monkey Cynomolgus Human	D4522 KMN023 Oral AstraZeneca	Monkey 10 Human 20 mg Single Doses See D4522 KKP011 for details of monkey See 4522IL/0003 for details of human	Various methods N/A Compliant	Monkey urine samples taken from D4522 KKP011 following a single dose of 10 mg/kg of [^{14}C]-ZD4522 Human urine and feces samples taken 4522IL/0003 following a single dose of 20 mg [^{14}C]-ZD4522 to human volunteers A peak from the urine of both species was isolated and exhibited the same chromatographic properties. Comparison of these peaks with an authentic standard, using /NMR showed the peak to be N-desmethyl ZD4522 There was no evidence of the presence of the 5R epimer of ZD4522 in human excreta from 4522IL/0003
Rat SD, SPF Dog Human	S-4522-F-06-N, 6 (ADME) Oral + In vitro	5 Single Dose ^{14}C-S-4522 Lot no 92-330-77-1 Aqueous soln	N/A Non-compliant	3 male rats dosed and plasma for ex vivo plasma protein binding taken at 0.25, 4 and 8 h post-dose Plasma from rat, dog and man taken for in vitro protein binding In vitro binding of 96.1, 92.2 and 96.2% in rat, dog and man Ex vivo binding in rat 92.3 to 96.9% over 0.25 to 8 h post-dose Principal binding protein in humans was serum albumin
Mouse CD1 Rat SD Rabbit NZ White Dog Beagle Monkey Cynomolgus Human	D4522 KPJ036 In vitro	N/A N/A ZD4522 Lot no ADM03515H98 Methanol soln	Equilibrium dialysis + D4522 KPV007 D4522 KPV018 D4522 KPV041 Compliant	Plasma from more than 1 male and 1 female of each species Binding of 64.6, 95.3, 86.2, 77.9, 86.6 and 86.4% in male mouse, rat, rabbit, dog, monkey and human Possible sex difference in monkey (female 81.3%) Effect of <=4% methanol detected Principal binding protein in humans was serum albumin
Mouse CD1 Rat SD Rabbit NZ White Dog Beagle Monkey Cynomolgus Man	D4522 KPJ062 In vitro	N/A N/A ZD4522 Lot no C280/1 ADM64957J99 Methanol soln evaporated to dryness	Equilibrium dialysis + D4522 KPV007 D4522 KPV018 D4522 KPV041 Compliant	Plasma from more than 1 male of each species Binding of 78.4, 95.8, 92.5, 85.5, 82.0 and 88.5% in male mouse and rat, female rabbit, and male dog, monkey and human No difference between Western and Japanese male humans

PK/TK summary:

Toxicokinetic studies have been conducted in mouse, rat, rabbit, dog and monkey. Generally, rosuvastatin was rapidly and well absorbed after oral administration. C_{max} and AUC increased with dose proportionally at lower doses and more than proportionally at higher doses. In all species, the pharmacokinetics of rosuvastatin showed considerable inter-animal variability. Similar variation was also observed in humans.

About 90% ZD4522 in plasma binds to protein, mostly serum albumin, in rat, dog, monkey, and human. There were some species differences. At the extremes, free fraction in man was 2.7 times rat and, in the mouse, free fraction was 1.9 times man. Whole body autoradiography (WBA) and tissue distribution studies in the rat show rosuvastatin distribution to be highly selective for the liver. Most tissues, other than the gut and liver, showed radioactivity concentrations at, or below circulating plasma levels. There was a low distribution of rosuvastatin to fetus in rats, but relative higher distribution in fetal tissue was observed in 1/4 fetus in rabbits. However, in the lactating rat, at equivalent timepoints, rosuvastatin was found in milk at concentrations up to 3 times those in plasma.

Rosuvastatin did not induce cytochrome P450s in animals, including rat, dog and mouse, nor inhibit the P450 isoforms in human hepatic microsomes (in vitro). CYP2C9 was the principal P450 involved in rosuvastatin metabolism (2C19, 3A4, and 2D6 were implicated to a lesser extent). These data indicate little potential for interaction of rosuvastatin with co-administered drugs that are metabolized by cytochrome P450s.

Metabolism was a minor route of clearance in rat and dog, where moderate to high absorption has been demonstrated. Similar high ratios of parent drug to metabolite have been demonstrated in all species (including man). The principal metabolites in all species, other than monkey and man, appeared to be derived from metabolism on the pharmacophore (dihydroxyheptenoic acid side chain). Both man and monkey showed the presence of a metabolite, exhibiting demethylation from the nitrogen of the sulfonamide group. Most metabolism was to relatively inactive moieties. The only active (>5% of parent) metabolite demonstrated (N-desmethyl rosuvastatin) in monkey and man had a much lower activity than the parent compound.

The major excretory pathway in all animal species and humans was feces, mostly as unchanged parent compound.

PK/TK conclusions:

All animal species were relevant models for humans, with similar properties in absorption, distribution, and elimination. Monkey was the best animal model in terms of metabolism, because, only monkeys produced same active (>5% of parent) metabolite demonstrated (N-desmethyl rosuvastatin) as man. The human exposure at the highest Sponsor proposed dose of 80 mg was similar to the exposure levels at NOAEL in monkeys, dogs, and mice, but about 3 times the exposure at NOAEL in rats.

IV. GENERAL TOXICOLOGY:

Most studies have been reviewed under IND 56,385 (reviews are attached) and are summarized below. All toxicology studies used the Calcium salt form (marketed) except for a two week male rat study (Y-001-911004) which used a Sodium salt. No difference in toxicity was observed.

Study title: Single oral dose toxicity study of S-4522 in rats
Full review is attached on Page 6 of the initial pharmacology/toxicology review.
Key study findings: rats were treated with S-4522 at a single oral dose of 1000 or 2000 mg/kg. No mortality was noted. Slight increases in GOT, GPT, and CPK were observed. Thickening of transitional epithelium of the renal pelvis and slight inflammatory cell infiltration under the pelvic epithelium in kidney was observed in 1/6 female rat at 2000 mg/kg. No histopathologic findings were reported in liver.

Study title: Single oral dose toxicity study of S-4522 in dogs
Full review is attached on Page 7 of the initial pharmacology/toxicology review.
Key study findings: dogs were treated with S-4522 at a single oral dose of 1000 or 2000 mg/kg. No mortality was noted. No histopathologic findings were reported in kidney and liver.

Study title: Two-week repeated oral toxicity of S-4522 in mice
Full review is attached on Page 39 of the initial pharmacology/toxicology review.
Key study findings: mice were treated with S-4522 at doses of 20, 60, and 200 mg/kg for 17 days. Centrilobular hepatocyte hypertrophy was noted at 200 mg/kg. No histopathologic findings were reported in kidney. NOAEL was determined as 60 mg/kg.

Study title: One-month repeated oral toxicity of S-4522 in rats
Full review is attached on Page 8 of the initial pharmacology/toxicology review.
Key study findings: rats were treated with S-4522 at doses of 15, 50, and 150 mg/kg for 1 months and the high dose group was allowed to recover for 1 month. Single cell necrosis and cytoplasmic eosinophilic change in periportal hepatocyte in liver was noted at ≥ 50 mg/kg. No histopathologic findings were reported in kidney. NOAEL was determined as 15 mg/kg. The findings appeared to be reversible.

Study title: Thirteen-week repeated oral toxicity of S-4522 in mice
Full review is attached on Page 40 of the initial pharmacology/toxicology review.
Key study findings: mice were treated with S-4522 at doses of 20, 60, and 200 mg/kg for 13 weeks. Centrilobular hepatocyte hypertrophy was noted at ≥ 60 mg/kg. No histopathologic findings were reported in kidney. NOAEL was determined as 20 mg/kg. AUC values were 322, 969, and 5610 ng.hr/ml for 20, 60, and 200 mg/kg, respectively (0.7, 2.2, 13X human exposure at 80 mg/day; 1.6, 4.8, 28X human exposure at 40 mg/day; 3.6, 11, 64X human exposure at 20 mg/day; and 8, 24, 140X human exposure at 10 mg/day).

Study title: Three-month repeated oral dose toxicity of S-4522 in rats
Full review is attached on Page 11 of the initial pharmacology/toxicology review.
Key study findings: this study was conducted in Japan and rats were fed with low calcium diet. Rats were treated with S-4522 at doses of 10, 30, and 100 mg/kg for 3 months and the high dose group was allowed to recover for 1 month. Mortality occurred at 100 mg/kg. Hypertrophy of perilobular hepatocytes, diffuse lymphocytes depletion or metamorphosis in the thymus, atrophy of white pulps of the spleen, mucosal hyperkeratosis of the forestomach were noted in premature terminated animals at 100 mg/kg. Hypertrophy of perilobular hepatocytes, perilobular fibrosis, bile duct proliferation, mucosal hyperkeratosis in forestomach were observed at ≥ 30 mg/kg. No apparent histopathologic findings were noted in kidney. NOAEL was determined as 10 mg/kg. No PK data were provided for this study. The toxicity findings in this study was considerable different with the previous 1-month study, indicating diet could significantly influence the toxicity of this compound.

Originally, the Sponsor suggested that toxicity of rosuvastatin in the rat was inversely proportional to the level of Calcium in the diet. Further studies suggested that other variables which were not controlled could contribute to the toxicity. What is clear is that 150 mg/kg/day consistently produced toxicity (clinical observations, body weight, food consumption, plasma liver enzymes, and liver/forestomach histopathology). The data suggested that food type was a major factor.

14 day studies in rats and mice evaluated the effect of CA-1 and MM-6 diets on rosuvastatin toxicity. Rosuvastatin (150 mg/kg) showed toxicity with CA-1 but not with MM-6. Comparison of the diets revealed many differences including total protein, fat, vitamins and essential metal content.

Another study examined the effect of diet on toxicity of rosuvastatin (150 mg/kg/day), simvastatin (200 mg/kg/day) or provastatin (500 mg/kg/day) in rats for 8 or 14 days. A similar effect of diet on toxicity was observed for rosuvastatin and provastatin, but not with simvastatin.

Study title: Thirteen-week repeated oral toxicity of S-4522 in rats
Full review is attached on Page 42 of the initial pharmacology/toxicology review.
Key study findings: rats were treated with S-4522 at doses of 6, 20, and 60 mg/kg for 13 weeks. Bile duct hyperplasia, hepatocyte hypertrophy, centrilobular hepatocyte necrosis were observed in liver at ≥ 20 mg/kg. No apparent histopathologic findings were noted in kidney. NOAEL was determined as 6 mg/kg. AUC values were 240, 1130, and 6720 ng.hr/ml for 6, 20, and 60 mg/kg, respectively (0.6, 2.6, 15X human exposure at 80 mg/day; 1.2, 5.6, 33X human exposure at 40 mg/day; 2.7, 13, 76X human exposure at 20 mg/day; and 6, 28, 168X human exposure at 10 mg/day).

Study title: 91-day dose range finding study in rats

Full review is attached on Page 70 of the review of carcinogenicity study.

Key study findings: this study was conducted in UK using same animal strain and same experimental condition as the 2-year rat carcinogenicity study. Rats were treated with S-4522 at dose of 160 mg/kg for 91 days. Mortality occurred at the first five weeks. Histopathologic findings was observed in liver (diffuse hepatocyte basophilia, single cell necrosis, cytomegaly/karyomegaly), stomach (squamous cell hyperplasia) and kidney (tubular cell degeneration) in decedents. However, animals survived the 91-day treatment appeared to be normal.

Study title: 91-day dose range finding study in rats

Full review is attached on Page 76 of the review of carcinogenicity study.

Key study findings: this study was conducted in UK using same animal strain and same experimental condition as the 2-year rat carcinogenicity study. Rats were treated with S-4522 at doses of 80, 160, 240, and 320 mg/kg for 91 days. Mortality occurred at ≥ 80 mg/kg, indicating 80 mg/kg was above MTD. However, the AUC values at 80 mg/kg (9370 and 4800 ng.hr/ml for males and females, respectively) were about 2 times the value in the 2-year study, indicating these two studies were not fully comparable. Histopathologic findings was observed in liver (diffuse hepatocyte basophilia, single cell necrosis, cytomegaly/karyomegaly), stomach (squamous cell hyperplasia), kidney (tubular cell degeneration), pancreas (acinar cell degeneration), thymus (atrophy), spleen (lymphoid atrophy) bone marrow (marrow atrophy) adrenal (acute zonal cortical congestion, cortical hypertrophy) and lymph nodes (atrophy) in decedents. Only liver and stomach changes were noted in terminal killed animals.

Study title: Six-month repeated oral dose toxicity of S-4522 in rats

Full review is attached on Page 14 of the initial pharmacology/toxicology review.

Key study findings: rats were treated with S-4522 at doses of 2, 6, and 20 mg/kg for 6 months and the high dose group was allowed to recover for 2 month. Histopathologic findings was observed in liver (hypertrophy of perilobular hepatocytes, perilobular fibrosis) at ≥ 6 mg/kg, and kidney (glomerulonephrosis and bladder cystitis) at 2 mg/kg. Generally, the toxicity at high dose was minimal, suggestive of higher dose should have been used in long-term rat study to better identify target organs. The changes appeared to be reversible. NOAEL was determined as 2 mg/kg. AUC values were 149, 424, and 2410 ng.hr/ml for 2, 6, and 20 mg/kg, respectively (0.3, 1, 5.5X human exposure at 80 mg/day; 0.7, 2, 12X human exposure at 40 mg/day; 1.7, 4.8, 27X human exposure at 20 mg/day; and 3.7, 11, 60X human exposure at 10 mg/day).

Study title: Fourteen-day oral dose toxicity in dogs

Key study findings: dogs were treated with S-4522 at doses of 50 or 200 mg/kg for 14 days, with each group consisting of a single male and a single female. Death occurred on day 8 in the male dog dosed with 200 mg/kg/day. The cause of death was thought to be due to hemorrhage of the gastric mucosa, accompanied by histopathological changes in the liver. At this dose level, the dogs showed marked, but transient (even with continued dosing), increases in plasma transaminase activities. In addition, there were decreases in plasma cholesterol and triglycerides, which reflected the response in the species to the pharmacological action of the drug. The NOAEL in this study was 50 mg/kg/day.

Study title: One-month repeated oral dose toxicity in dogs

Full review is attached on Page 17 of the initial pharmacology/toxicology review.
Key study findings: dogs were treated with S-4522 at doses of 10, 30, and 90 mg/kg for 1 months and the high dose group was allowed to recover for 1 month. One female dog was killed moribund (increased GOT, LDH, CPK, creatine, urea nitrogen, total bilirubin; hematoma, thrombus, atrophy of hepatocytes and hyperplasia or the bile duct, hemorrhage of serosa and mucosa of the gallbladder were observed in this animal, suggesting possible muscle and renal toxicity). Histopathologic findings was observed in gallbladder (lamina propia mucosa edema, hemorrhage, and inflammatory cell infiltration), stomach (mucosal erosion and inflammatory cell infiltration), lungs (congestion, hemorrhage and edema), kidneys (necrosis of renal tubular epithelium), adrenals (congestion, hemorrhage, necrosis and hyperplastic responses), Spleen (congestion and atrophy), lymphoid tissues (hemorrhage, inflammation and decreased lymphocytes), bone marrow (hypocellularity), choroid plexus (edema, hemorrhage and partial necrosis in the interstitium of the choroid plexus, without compression of the spinal cord), and eyes (retina dysplasia and loss) at ≥ 30 mg/kg. In addition, an increased number of giant cells in the testicular seminiferous tubules was observed in 1/5 of the 90 mg/kg/day male dogs. NOAEL was determined as 10 mg/kg. AUC values were 1520, 3520, and 19900 ng.hr/ml (note: units for TK were incorrectly labelled as ng.hr/ml on page 19 of original review, it should be µg.hr/ml) for 10, 30, and 90 mg/kg, respectively (3.5, 8, 46X human exposure at 80 mg/day; 8, 18, 99X human exposure at 40 mg/day; 17, 40, 226X human exposure at 20 mg/day; and 38, 88, 498X human exposure at 10 mg/day).

A single incidence of edema, hemorrhage and partial necrosis in the choroid plexus was observed in the moribund killed female dog at 90 mg/kg. Dogs treated with other HMG-CoA reductase inhibitors have demonstrated effects on the vascular system in the central nervous system. Degeneration of the vascular endothelium in the brain with associated focal hemorrhage and perivascular edema, with retrograde axonal degeneration in the optic tract possibly related to focal ischemia produced by the vascular degeneration, has been reported in dogs dosed with lovastatin. Neurological lesions were also seen in a dog dosed with atorvastatin at 280 mg/kg/day and in a 2-year study at 120 mg/kg/day. These lesions were multifocal and considered similar to those described in the brain of dogs given other HMG-CoA reductase inhibitors.

An increased number of giant cells in the testicular seminiferous tubules was observed in one male at 90 mg/kg. In an intravenous study of rosuvastatin, the relative (to body weight) testes and epididymal weights were reduced at the top dose (12 mg/kg/day) and a single dog had a unilateral small area of mild tubular seminiferous degeneration in the testis. The development of similar testicular lesions has been reported in dogs treated with lovastatin and simvastatin, and, thus, this finding is considered likely to be treatment-related.

Study title: Three-month repeated oral dose toxicity in dogs
Full review is attached on Page 21 of the initial pharmacology/toxicology review.
Key study findings: dogs were treated with S-4522 at doses of 7.5, 15, and 30 mg/kg for 3 months and the high dose group was allowed to recover for 1 month. Histopathologic findings was observed in gallbladder (lamina propia mucosa hemorrhage and inflammatory cell infiltration) at all dose levels, and lens (ocular opacities in the anterior portion of the lens) at 30 mg/kg. No apparent renal toxicity was noted. NOAEL cold not be determined based on gallbladder findings. C_{max} values were 350, 410, and 1420 ng/ml for 7.5, 15, and 30 mg/kg, respectively (6, 8, and 27X human exposure at 80 mg/day; 15, 17, and 59X human exposure at 40 mg/day; 35, 40, and 140X human exposure at 20 mg/day; and 85, 100, and 350X human exposure at 10 mg/day).

Study title: Six-month repeated oral dose toxicity in dogs
Full review is attached on Page 23 of the initial pharmacology/toxicology review.
Key study findings: dogs were treated with S-4522 at doses of 1 and 4 mg/kg for 6 months. No apparent treatment related change was noted. NOAEL was determined as 4 mg/kg. AUC values were 122 and 661 ng.hr/ml for 1 and 4 mg/kg, respectively (0.3, 1.5X human exposure at 80 mg/day; 0.6, 3.3X human exposure at 40 mg/day; 1.4, 7.5X human exposure at 20 mg/day; and 3, 17X human exposure at 10 mg/day).

Study title: Twelve-month repeated oral dose toxicity in dogs
Full review is attached on Page 25 of the initial pharmacology/toxicology review.
Key study findings: dogs were treated with S-4522 at doses of 1, 3 and 6 mg/kg for 12 months and the high dose group was allowed to recover for 1 month. Toxicity was observed in gallbladder (lamina propria mucosa hemorrhage) and liver (atrophy of hepatocytes and disarrangement of hepatic cell cords), and eye (retinal dysplasia) at 6 mg/kg. Most changes were reversible. Opacity of the cornea was observed 1/4 male at 1 mg/kg and 2/7 males at 6 mg/kg. NOAEL was determined as 3 mg/kg based on gallbladder findings. AUC values were 237, 703 and 3120 ng.hr/ml for 1, 3 and 6 mg/kg, respectively (0.5, 1.6, 7X human exposure at 80 mg/day; 2.7, 8, 35X human exposure at 20 mg/day; and 6, 17, 78X human exposure at 10 mg/day).

Study title: A preliminary 30-day repeated oral dose toxicity in monkeys
Key study findings: monkeys (1 male and 1 female in each group) were treated with S-4522 at doses of 10 and 30 mg/kg for 30 days. No apparent toxicity was observed. NOAEL was determined as 30 mg/kg. AUC values were 132/72(M/F), and 242/247(M/F) ng.hr/ml for 10 and 30 mg/kg, respectively.

Study title: Six-month repeated oral dose toxicity in monkeys
Full review is attached on Page 27 of the initial pharmacology/toxicology review.
Key study findings: monkeys were treated with S-4522 at doses of 10 and 30 mg/kg for 6 months. Toxicity was observed in testis (decrease in spermatogenic epithelium, vacuolation in seminiferous tubular epithelium), pancreas (vacuolation of acinar cell), adrenal (necrosis of

parenchyma) and thyroid (ectopic thymus) at 30 mg/kg. Kidney findings including basophilic change of renal tubule, degeneration of tubular epithelium in cortex, hyalinous crystalline inclusion body in mucosal and pelvic epithelium were observed in all groups, including controls. NOAEL was determined as 10 mg/kg. AUC values were 246-818, and 733-4180 ng.hr/ml for 10 and 30 mg/kg, respectively (0.6-1.9, 1.7-9.6X human exposure at 80 mg/day; 1.2-4.1, 3.6-21X human exposure at 40 mg/day; 2.8-9.3, 8.3-48X human exposure at 20 mg/day; and 6-20, 18-105X human exposure at 10 mg/day).

Studies on degradation products and impurities in rosuvastatin

Rosuvastatin drug was found to contain an impurity — and a degradation product — Data from the 3 month rat study (Study: F-09-L) which used batch 54 (containing 0.24% rosuvastatin — provide the required toxicology qualification for the product. A batch of rosuvastatin containing — impurity was tested and was also found to be negative in an Ames assay (Study: TMV899) and an *in vitro* cytogenetic study using cultured human hepatocytes (Study: TYX111).

A batch of rosuvastatin containing — degradation product was tested in a 1-month dog study (Study: TAD1148). In this study groups of beagle dogs (3 sex/dose) were orally administered rosuvastatin at doses of 0 or 12 mg/kg/day for 29 days. The only changes noted in treated animals were minor decreases in plasma cholesterol and triglycerides together with minor increases in plasma transaminases. The dose of 12 mg rosuvastatin/kg/day was a NOAEL. In addition, batches of rosuvastatin containing — —)were tested in an Ames assay (Study: TMV900) and a rosuvastatin containing — — in an *in vitro* cytogenetics assay using cultured human hepatocytes (Study: TYX113) and both studies were negative.

These data for the impurity and degradation product provide the necessary toxicology qualification for the clinical trials drug and for the manufactured product. In addition, batches with higher levels of impurity were used in toxicology studies, suggesting less risk concern.

Ranges for specifically controlled organic impurities

	Impurity range (% w/w)				
Impurity	Specification limit	Toxicity studies	Clinical trials	Late development batches	Stability data
ZD4522		—			
ZD4522		—			
ZD4522		—			
ZD4522		—			ND

ND none detected (limit of detection — w/w)

Safety of the metabolite, desmethyl rosuvastatin

In the Cynomolgus monkey, the metabolite, desmethyl rosuvastatin, is present. This metabolite is also found in man. Plasma concentrations of desmethyl rosuvastatin were measured in the monkey following single and multiple doses of rosuvastatin at 10 and 30 mg/kg/day (these are the same doses as used in the 30 day and 6 month toxicology studies in the monkey) in which the NOAEL was 10 mg/kg/day. Concentrations of desmethyl metabolite in the monkey accumulated about 2-fold on multiple dosing, but after a single dose of 10 mg/kg/day the mean C_{max} and $AUC_{(0-24)}$ were 27.5 ng/ml and 160 ng.hr/ml, respectively. Exposure to desmethyl rosuvastatin following a single 80 mg dose in man had a calculated C_{max} of 4.09 ng/ml and $AUC_{(0-t)}$ of 17.2 ng.hr/ml.

DBIT1089 (AstraZeneca Reference Number D4522 KKP064). Mean (± SD) pharmacokinetic parameters of ZD4522 and N-desmethyl metabolite in plasma following single (Day 1) and multiple (Day 10) oral administration of ZD4522 to male monkeys

10 mg/kg/day (Phases 1 and 2)

	Day 1		Day 10	
Parameter	ZD4522	N-desmethyl metabolite	ZD4522	N-desmethyl metabolite
C_{max} (ng/ml)	33.7 ± 12.7	27.5 ± 9.01	62.8 ± 9.51	69.3 ± 29.5
t_{max} (h)	1.88 ± 1.55	1.13 ± 0.629	0.813 ± 0.800	0.875 ± 0.750
AUC(0-t) (ng.h/ml)	223 ± 86.4	160 ± 59.5	415 ± 117	293 ± 89.0
AUC(0-24) (ng.h/ml)	223 ± 86.4	160 ± 59.5	415 ± 117	293 ± 89.0
AUC (ng.h/ml)	220 (n=1)	251 (n=1)	NA	NA
λ_z (/h)	0.141 (n=1)	0.120 (n=1)	0.102 ± 0.00926	0.0817 ± 0.0100
$t_{1/2}$ (h)	4.93 (n=1)	5.78 (n=1)	6.87 ± 0.588	8.57 ± 1.11
R_T	1.04 (n=1)	1.06 (n=1)	NA	NA
R_O	NA	NA	1.93 ± 0.296	1.93 ± 0.555

30 mg/kg/day (Phases 3 and 4)

	Day 1			Day 10	
Parameter	ZD4522[a]	ZD4522[b]	N-desmethyl metabolite	ZD4522	N-desmethyl metabolite
C_{max} (ng/ml)	2990 ± 3770	103 ± 72.0	102 ± 83.9	194 ± 83.0	111 ± 37.2
t_{max} (h)	7.50 ± 11.1	2.25 ± 2.02	1.63 ± 1.60	2.00 ± 0.00	1.38 ± 0.750
AUC(0-t) (ng.h/ml)	4070 ± 3820	459 ± 151	382 ± 178	908 ± 306	549 ± 182
AUC(0-24) (ng.h/ml)	4070 ± 3820	459 ± 151	382 ± 178	908 ± 306	549 ± 182
AUC (ng.h/ml)	NC	NC	NC	NA	NA
λ_z (/h)	NC	NC	NC	0.123 ± 0.0162	0.0979 ± 0.0290
$t_{1/2}$ (h)	NC	NC	NC	5.68 ± 0.757	7.50 ± 2.17
R_T	NC	NC	NC	NA	NA
R_O	NA	NA	NA	2.04 ± 0.480[b]	1.58 ± 0.470

NC = Not calculated; NA = Not applicable

a = Includes all data

b = Excludes anomalous data (1 h data for animals 001M and 003M; 24 h data for animals 003M and 004M)

Toxicology Summary:

Toxicology studies were conducted in rats, mice, dogs, and monkeys. Dose levels used by oral route generally used high initial doses for short durations followed by reduced doses for longer durations, suggesting a potential for progressive toxicity with treatment (dose, duration). The doses evaluated varied in mouse (20 – 200 mg/kg/day), rat (2 – 2000 mg/kg/day), dog (1- 200 mg/kg/day) and monkey (10-30 mg/kg/day). Mortality was not observed in mice or monkey, but drug related mortality was observed in rats at doses ≥ 100 mg/kg/day, and dogs at ≥ 90 mg/kg/day. Consistent changes in clinical chemistry across species included increased liver enzymes, bilirubin and decreased triglycerides. Total cholesterol decreased in dogs and monkeys, but increased in rats as with other statins. Red blood cell parameters increased in rats and dogs, and white blood cell parameter increased in dogs and decreased in rats.

Liver is the major target of rosuvastatin in rats, mice, and dogs. The changes in liver include increases in plasma transaminases, hepatocyte hypertrophy, and cell necrosis. These findings are consistent with the selective distribution of rosuvastatin in liver. The Sponsor suggested that the liver toxicity to the structural/functional components be due to the depletion of cholesterol by prolonged and extensive inhibition of HMG-CoA reductase. Rats were the most sensitive species to the liver toxicity, probably due to its high rate of hepatic synthesis of cholesterol. Generally, liver toxicity was observed at exposure levels about 2, 1, and 7X the human exposure for mice, rats and dogs, respectively, based on the Sponsor proposed high dose of 80 mg/day; about 4, 2, and 16X the human exposure for mice, rats and dogs, respectively, based on the human dose of 40 mg/day; about 11, 5, and 35X the human exposure for mice, rats and dogs, respectively, based on the human dose of 20 mg/day; and about 24, 11, and 78X the human exposure for mice, rats and dogs, respectively, based on the human dose of 10 mg/day. Since liver toxicity was a known class effect for statins, it was closely monitored in clinical studies.

Toxicity on gallbladder and biliary duct, including lamina propia mucosa edema, hemorrhage and inflammatory infiltration, were observed in dogs. That was consistent with the excretion route of rosuvastatin. This toxicity was observed in dogs at 6 mg/kg with exposure levels 7, 16, 35, and 78X the human exposure at human doses of 80, 40, 20, and 10 mg/kg, respectively. Gallbladder toxicity was also observed in mice at 250 mg/kg (>10X human exposure at human dose of 80 mg/day), but less severe than dogs. Gallbladder effects have also been observed with other drugs of this class.

Edema, hemorrhage and partial necrosis in the interstitium of the choroid plexus was observed in one female dog at 90 mg/kg (46X human exposure at human dose of 80 mg/day) that was sacrificed *in extremis* on day 24 of dosing. CNS lesions characterized by perivascular hemorrhage, edema, mononuclear cell infiltration, fibrinoid degeneration of vessel walls in the choroid plexus of the brain stem, and ciliary body of the eye have been observed with several drugs in this class. Toxicity on eyes including opacity of cornea and lens, and retina dysplasia was only observed in dogs at exposure levels 1/2, 1, 3 and 6X the human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively. Lenticular effects have been observed with other drugs of this class.

Forestomach toxicity (mucosal hyperkeratosis) was observed in rats at exposure levels 6, 12, 27, and 60X the human exposure at human doses of 80, 40, 20, and 10 mg/kg, respectively. This anatomical feature is unique to rodents and is therefore not considered clinically relevant.

Toxicity on endocrine organs were noted in testis (decrease in spermatogenic epithelium, giant cells and vacuolation in seminiferous tubular epithelium), pancreas (vacuolation of acinar cell), adrenal (necrosis of parenchyma) and thyroid (ectopic thymus) in monkeys at exposure levels 2, 4, 8, and 18X the human exposure at human doses of 80, 40, 20, and 10 mg/day, respectively. Giant cells and/or mild tubular seminiferous degeneration were also observed in a one-month dog study at dose of 90 mg/kg. The effects on testis in dogs and monkeys have been seen with several drugs in this class.

Renal and muscle toxicity was seen in rats, dogs, rabbits, and monkeys. The toxicity was characterized by blood chemistry changes including increases in creatinine, CPK, urea nitrogen, and histopathologic changes including renal tubular cell degeneration /necrosis, and cardiac or intercostal muscle necrosis. Generally, renal and muscle toxicity was observed in animals dead or moribund killed after high level exposure to rosuvastatin with high multiples of human exposure (about 39 to 46X human exposure for rats and dogs, respectively, based on the human dose of 80 mg/day). The severity and low frequency nature of renal/muscle toxicity of rosuvastatin suggests that some individuals are more susceptible to rosuvastatin, presumably due to the great variations in individual exposure level in both humans and animals. Humans experiencing these type of adverse events had elevated drug plasma levels according to the medical reviewer. In addition, pre-existing condition of renal impairment will significantly enhance the risk of renal toxicity due to increased rosuvastatin plasma levels at such condition. Therefore, the potential risk of renal/muscle toxicity to human can not be excluded. Marked muscle toxicity was reported in humans when Lovastatin was combined with cyclosporin A, an immunosuppressant.

Rosuvastatin has been tested in 5 species and different species showed different toxicity profiles. Generally, dogs and rabbits may be species more sensitive to rosuvastatin induced toxicity, because the exposures in dogs and rabbits are much higher than other species at the same dose level (mg/kg). Therefore, toxicology data from dogs and rabbits may over estimate the human risk. Monkeys are more similar to human in metabolism, because it is the only species that produced the same active metabolite desmethyl rosuvastatin as humans. However, since desmethyl rosuvastatin only accounts for a small proportion of the total plasma concentration, and it is much less active than the parent compound, therefore, the similarity in metabolism and toxicology results do not suggest the monkey as the most predictive animal species for human risk.

In mice, increased liver weights and periportal hypertrophy were seen and in subsequent special toxicity studies, submucosal edema of the gall bladder was observed. In rats, increased liver weights, eosinophilia, scattered and/or periportal necrosis, and intralobular bile duct hypertrophy were seen. Hyperkeratosis of the forestomach mucosa was also seen in the mouse and rat, but since this anatomical feature is unique to rodents, it is not thought to represent a finding relevant to humans. In the dog, effects such as cholecystitis, hepatocellular atrophy and necrosis of gastric mucosa were seen, but the most sensitive organ was the gall bladder, which demonstrated hemorrhage, edema and/or inflammatory cell infiltration in the lamina propria mucosa at doses lower than those associated with the liver effects. Similar gallbladder effects were also seen in the mouse; no similar liver or gallbladder effects were noted in the monkey.

Other histological findings were the appearance of giant cells and/or mild tubular degeneration in the seminiferous tubules of the testis in male dogs and one high-dose male monkey (with a decreased number of germ cells, with vacuolation) and ocular opacities in high-dose male and female dogs. These are observations similar to those seen with other statins.

In the dog the principal target organ was the gall bladder, where edema, hemorrhage and/or inflammatory cell infiltration of the lamina propria mucosae were observed. A NOEL for changes in the gall bladder of 3 mg/kg/day was established in the 12-month study (Study: B-46-L). Minor transient elevations in plasma transaminase activities were seen sporadically. Other findings in the dog, observed at a low incidence, were edema, hemorrhage and partial necrosis in the interstitium of the choroid plexus in one female dog sacrificed *in extremis* (Study: B-20-L), a low incidence of ocular opacities (Study: B-2 I-L), and a low incidence of the appearance of giant cells and/or mild tubular seminiferous degeneration (Study: B-20-L; TAD1018). In addition, increased heart rate, increased T-wave amplitude and other changes secondary to the change in heart rate were observed in high-dose males (12 mg/kg/day) when rosuvastatin was given to dogs intravenously (Study: TAD 1018).

In a 14-day bolus IV study (TAD1018) dogs were dosed 0, 1, 5, and 12 mg/kg/day. There was transient unsteady gait at the top dose (12 mg/kg/day) immediately after injection, with ECG recordings showing evidence of a dose-related, transient, increase in heart rate. At peak effect on day 1, the 3 males at this dose level had increased T-wave amplitude (other changes in PR, QRS or QT intervals were apparent but were secondary to the change in heart rate).
No effect on ECG has been seen in any other study, i.e., the oral studies described above (Study: B-20-L; B-21-L; B-46-L), the special toxicology qualification study (Study: TAD1148 described in Section 3.7.8) and the isolated canine Purkinje fiber study (Study: TSZS) which is described Section 2.5: Safety Pharmacology, where examinations of action potential duration, heart rate and ECG recordings showed no effect on any parameter, including QT interval. The relative (to body weight) testes and epididymal weights were reduced at the top dose and a single dog had a unilateral small area of mild tubular seminiferous degeneration in the testis. The observations described above occurred in the 12 mg/kg/day intravenous dose level. Following this dose the calculated C_0 (22000 ng/ml) and AUC (4050 ng.hr/ml) values were much higher than after oral dosing approximately 475-fold and 12-fold higher, respectively, than those values observed in humans following the maximum proposed clinical dose of 80 mg/day. Similarly at the NOAEL (excluding the increase in heart rate), 5 mg/kg/day the mean C_0 (2130 ng/ml) and mean AUC (234 ng.hr/ml) values were higher than after oral dosing to the dog.

The no observed adverse effect levels (NOAEL) are 20 mg/kg/day in the 13-week mouse study (Study: F-15-L, 0.7X human exposure at human dose of 80 mg/day; 2X human exposure at human dose of 40 mg/day; 4X human exposure at human dose of 20 mg/day; and

8X human exposure at human dose of 10 mg/day), 2 mg/kg/day in the 6 month rat study (Study: F-13-L, 0.3X human exposure at human dose of 80 mg/day; 0.7X human exposure at human dose of 40 mg/day; 2X human exposure at human dose of 20 mg/day; and 4X human exposure at human dose of 10 mg/day), 3 mg/kg/day in the 12-month dog study (Study: B-46-L, 2X human exposure at human dose of 80 mg/day; 3X human exposure at human dose of 40 mg/day; 8X human exposure at human dose of 20 mg/day; and 17X human exposure at human dose of 10 mg/day), and 10 mg/kg/day in the 6-month monkey study (Study: F-14-L, 0.6-2X human exposure at human dose of 80 mg/day; 1-4X human exposure at human dose of 40 mg/day; 3-9X human exposure at human dose of 20 mg/day; and 6-20X human exposure at human dose of 10 mg/day).

V. GENETIC TOXICOLOGY:

Most studies have been reviewed under IND 56,385 (reviews are attached) and are summarized below.

Study title: Reverse mutation test of S-4522 with bacteria
Full review is attached on Page 61 of the initial pharmacology/toxicology review.
Key findings: the study was judged to be valid and the result was negative.

Study title: Micronucleus test of s-4522 with mouse bone marrow cells
Full review is attached on Page 62 of the initial pharmacology/toxicology review.
Key findings: the study was judged to be valid and the result was negative.

Study title: Chromosomal aberration test of s-4522 in cultured Chinese hamster cells
Full review is attached on Page 63 of the initial pharmacology/toxicology review.
Key findings: the study was judged to be valid and the result was negative.

Study title: L5178Y TK +/- mouse lymphoma mutation assay
Key findings: the study was judged to be valid and the result was negative.
Study no: TMV 797
Volume #, and page #: electronic submission, file name tmv797.pdf
Conducting laboratory and location: [redacted]

Date of study initiation: April 1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: ADM60414K99, purity 96.1%
Formulation/vehicle: dried dimethylsulphoxide

Methods:

Strains/species/cell line:L5178Y TK+/- 3.7.2.c cell
Dose selection criteria:
  Basis of dose selection: cytotoxicity
  Range finding studies: the maximum concentration used was 5000 μg/ml in both the presence and absence of S9-mix.
Test agent stability: stable during experiment
Metabolic activation system: S9 from male SD rats treated with phenobarbital and β-naphthoflavone for 3 days.
Controls:
  Vehicle: dried dimethylsulphoxide
  Negative controls: dried dimethylsulphoxide
  Positive controls: ethyl methanesulphonate (EMS): 750 μg/ml without S9
  benzo-ot-pyrene (BP): 2 μg/ml with S9.
Exposure conditions:

Incubation and sampling times: the cultures were treated for 4 or 24 hours at 37°C , then incubated for 48 hours.
Doses used in definitive study: in the 4 hour experiment, concentrations of 313, 625, 1250, 2500, and 5000 μg/ml were used with or without S9. In the 24 hour experiment, 6, 13, 25, 50, 100, and 200 μg/ml were used without S9; 500, 1000, 2000, 2500, 3000, and 4000 μg/ml were used with S9.

Study design: A dose-ranging study was performed to determine the concentrations of ZD4522 to be used in the main mutation assays. Subsequently, two series of exponentially growing suspension cultures of L5178Y cells were treated in duplicate with the solvent control, positive controls or a range of concentrations of ZD4522 for 4 hours in the presence and absence of S9-mix (24 hours in the absence of S9-mix in the second experiment). After removal of the treatment medium, the cells were counted and a sample diluted to determine the survival immediately after treatment. The remaining cells were then cultured to allow any induced mutants to be expressed. During this expression time the growth rate was monitored and, where appropriate, the cells subcultured daily. At the end of the 48-hour expression time, samples were grown in both selective and non-selective medium, and the results obtained used to determine the mutant frequency per viable cell.

Analysis:
No. of replicates: 2
Counting method: under a 10X dissecting microscope

Criteria for positive results: reproducible statistically significant dose-related increases in mutant frequency.

Summary of individual study findings:

Study validity: both positive and negative controls responded appropriately. Testing doses also appeared to be appropriate based on cytotoxicity data.

Study outcome: no reproducible increases in mutant frequency was observed. It was concluded that ZD4522 tested negative in mouse lymphoma assay.

Genetic toxicology summary:

S-4522 was tested in bacterial mutagenicity assay, micronucleus test in the mouse, in vitro chromosome aberration assay using cultured Chinese hamster lung cells, and mouse lymphoma assay in L5178Y TK+/- cells. The results were all negative.

Genetic toxicology conclusions:

ZD4522 tested negative in genotoxic test battery, indicating that it has no mutagenic potential.

Labeling recommendations:

Rosuvastatin tested negative in Ames test, micronucleus test, chromosome aberration assay, and mouse lymphoma assay, suggesting it have no mutagenic or clastogenic potential.

VI. CARCINOGENICITY:

Study title: Mouse oral carcinogenicity study

Full review is attached on Page 12 of the review of carcinogenicity study.

Key study findings: mice were treated with rosuvastatin at doses of 10, 60, and 200 mg/kg for 107 weeks. 400 mg/kg was early terminated due to high mortality. The test species was considered appropriate and the study was concluded to be adequate. Higher incidence of hepatocellular tumors (both adenomas and carcinomas) were observed at the high dose of 200 mg/kg in both sexes.

Study title: Rat oral carcinogenicity study

Full review is attached on Page 40 of the review of carcinogenicity study.

Key study findings: rats were treated with rosuvastatin at doses of 2, 20, 60 and 80 mg/kg for 104 weeks. The test species was considered appropriate and the study was concluded to be adequate. Higher incidence of uterine stromal polyps was observed at 60 and 80 mg/kg in female rats.

Carcinogenicity summary:

In the 2-year oncogenic studies, mice were treated with rosuvastatin at doses of 10, 60, and 200 mg/kg for 107 weeks, and rats were treated with rosuvastatin at doses of 2, 20, 60 and 80 mg/kg for 104 weeks. Non-neoplastic alterations included changes in the forestomach (hyperkeratosis and/or hyperplasia and minor erosion and inflammation of the squamous epithelium) and liver (increased foci of alteration) were observed in both species. Neoplastic alteration were limited to hepatocellular adenomas/carcinomas in the mouse, and in the rat, there was an increased number of uterine stromal polyps at the high dose level.

Carcinogenicity conclusions:

Rosuvastatin tested positive in both rats and mice in the 2-year carcinogenicity studies. Treatment of mice at 200 mg/kg/day was associated with an increased incidence of hepatocellular adenoma/carcinoma in both sexes. Treatment of rats at ≥ 60 mg/kg/day was associated with an increased incidence of uterine stromal polyps, including a single stromal sarcoma in female at 80 mg/kg/day.