Spleen congestion	50 (f)	-	
Liver necrosis	100 (f)	50 (m) 100 (f)	(Wk 52)
Liver, hepatocellular vacuolation	-	50 (m), 100 (f)	(Wk 26-52)
Liver, increased eosinophilia	-	50(f), 100 (m)	(Wk 26)
Thymus involution	100 (m)	50	(Wk 26-52)
Vagina, infiltrate	-	5	(Wk 26-52)
Lymph node, pigmented macrophages	-	5 (m), 50 (f)	(Wk 26-52)
Bone marrow, lymphoid germinal center	-	5 (f), 50 (m)	(Wk 26-52)
Testis, juvenile	50	-	

dd dose-dependent
nd not determined

Reversibility of findings at 100 mkd HD after 2 wks (3-mo study), or 4 wks (1-year study):

Reversible were: body weight changes, RBC/Hb/Hct changes (partial in 1-yr study), serum Ca, serum P (partial), ALT, AST, cholesterol, triglyceride, CK changes, urine changes in f (1-year study), thymus involution, liver necrosis

Not reversible were: RBC/Hb/Hct changes in 3-mo study

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EXPOSURE MULTIPLES (PARENT DRUG) IN RAT AND MONKEY TOXICITY STUDIES

RAT (oral toxicity studies)

Study	Study Nr.	Doses (mg/kg/day) M / F	Cmax (ng/mL) M / F	AUC parent (ngxh/mL) M / F	AUC metabolite M7 (ngxh/mL)	**AUC multiple RAT:HUMAN* (parent) M / F**	Comment
Acute	#	1000, 2000					
	#970151	10, 100, 500					
	#100326	1000, 1500					
14-day	# 970018	50		941		**1.5x**	Day 14 values (m only)
		250		6690		**10.3x**	
		500		14700		**23x**	
28-day	#970070	5		81		**0.13x**	Day 28 values, m and f avg
		50		1400		**2.2x**	
		125		6180		**9.5x**	
3-month	#100001	5		49		**0.08x**	Wk 13 values
dietary		15		402		**0.6x**	Dietary DRF study
		50		1520		**2.3x**	
		75		2690		**4.2x**	
6-month	#100082	5		85 / 61		**0.13x / 0.09x**	M / F; Wk 13+26 avg
		25		845 / 1001		**1.3x / 1.5x**	
		100		3450 / 7090 4840 (m+f)		**5.3x / 11x** **7.5x (m+f)**	
24-month	#100209	5 / 5		132 / 144	6360 / 6590	**0.2x / 0.2x**	Carcinogenicity study
		15 / 20		462 / 374	18700 / 33400	**0.7x / 0.6x**	Wk78 values; Dietary study
		50 / 50→35		1620 / 894	37600 / 45000	**2.5x / 1.4x**	
28-day	#101939	0.5		3.2 / 4.7		**0.01x**	D27 values
Juvenile		1.5		24 / 26		**0.04x**	
		5.0		94 / 108		**0.15x**	

MONKEY (oral toxicity studies)

Study	Study Nr.	Doses (mg/kg/d)	Cmax (ng/mL) M / F	AUC parent (ngxh/mL) M / F	AUC metabolite M7 (ngxh/mL)	**AUC multiple MONKEY:HUMAN (parent)***	Comment
DRF	#970142	16		562		**0.9x**	Escalating single doses
		32		869		**1.3x**	
		48		1190		**1.8x**	
1-week	#970147	24		556		**0.9x**	D8 values
DRF		48		1170		**1.8x**	
		96		1330		**2.1x**	
3-month	#100020	5		60 / 74		**0.10x**	D83+D27 avg values (D83 for 150-100gp); M,F avg
		50		900 / 463		**1.1**	
		100		1239/1100		**1.9x**	For multiples: 100 and 150-100gp data pooled
		150-100		1380/1280			
12-month	#100188	5		99	4840	**0.15x**	D180+D358 avg values; M and F avg; M7 (and M5): ca. 50x parent AUC
		50		1055	6100	**1.6x**	
		100		1180	65900 (1.1x)	**1.8x**	AUC multiple (M5+M7) monkey:human: ca. 1.1x

*Human dose 180mg/60kg= ——. AUC=648ngxh/mL; Cmax= ——

DOG (oral toxicity studies)

Study	Study Nr.	Doses (mg/kg/day) m / f	Cmax (ng/mL)	AUC parent (ngxh/mL)	**AUC multiple DOG:HUMAN* (parent)**	Comment
4-day	# 970060	16		97	**0.15x**	Escalating dose study
		32		213	**0.33x**	4-day dosing; 1-wk period
		64		329	**0.51x**	
		96		430	**0.66x**	
		128		552	**0.85x**	
		160		808	**1.2x**	
		200		769	**1.2x**	
28-day	#970078	5		21	**0.03x**	Day 28 values, m and f avg
		50		152	**0.23x**	
		100		503	**0.8x**	
28-day	#101938	0.5		3.4	**0.01x**	D27 values
Juvenile		1.5		9.9	**0.02x**	
		5.0		47.5	**0.07x**	

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HUMAN PK data

PK of parent drug was evaluated in human subjects with chronic renal failure in an ascending, multiple-dose, double-blind, randomized, placebo-controlled study (#20000187). Each subject received a dose for days (once daily), starting with 25 mg or placebo, and if no dose-limiting toxicity occurred after >= 7 doses, the dose was increased by 25 mg. Maximum dose was 300 mg. Doses were given sequentially. Blood samples were taken on study days 4, 6, 7 of each period, and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24h after dosing. N=22 (4f, 18m). Total dosing time was up to 12 weeks. N=17 received AMG073, and N=5 received placebo. N=8 completed all dose levels.

Results: T max was 2-3h. Exposure (Cmax, AUC) was linear with dose up to ——. This suggests doses >200 mg may not provide additional clinical benefit. Steady state concentrations (trough) were achieved by Day 4 of each period.

Pharmacokinetic Results:
Large inter-individual variations were observed in subjects' pharmacokinetic parameters, with some apparent outlying values. For this reason, median pharmacokinetic values were examined and are presented in the following table:

Dose (mg)	N	AUC(0-24) (ng·hr/mL) Median	Range	C_{max} (ng/mL) Median	Range	T_{max} (hr) Median	Range
25	16	76.8		7.22		3.00	
50	16	179		17.2		2.00	
75	16	253		21.6		2.00	
100	16	383		31.1		3.00	
125	15	427		36.5		2.00	
150	15	530		55.5		2.00	
175	13	648		56.6		2.00	
200	11	900		78.3		3.00	
225	12	570		58.6		2.50	
250	11	911		67.0		3.00	
275	9	930		72.1		3.00	
300	7	501		55.7		2.00	

Values are presented as 3 significant figures.
NOTE: Pharmacokinetic parameters were not calculated for Subjects 14, 18, and 110 at the 175-, 150-, and 250-mg doses, respectively, because no day 7 profile was obtained. Parameters for Subject 18 were also not calculated at the 125-mg dose because sample tubes were broken. No parameters at doses > 175 mg were determined for Subject 4 because AMG 073 concentrations were very low or undetectable. The 200-mg dose parameters for Subject 9 were not used in the determination of summary statistics because the day 6 dose was missed.
Source: Appendix 13

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MAIN TOXICITIES: SUMMARY

Tabulation of main toxicity finding and LOAELs in chronic > 1-mo studies

Finding	Rat	Monkey	Indicative of
	LOAEL (mg/kg/day)	LOAEL (mg/kg/day)	
Clinical signs: abnormal breathing, dehydration, salivation	25		Pharmacologic drug effect (hypocalcemia)
Clinical signs: abnormal feces, poor appetite, emesis	-	5-100	
Decrease in serum Ca	5	5	Pharmacologic drug effect
Increase in serum P	5	50	
Decrease in serum PTH	5	5	
Urine Ca increase	5	-	
QTc prolongation	-	5	Hypocalcemia (partly)
BW reduced	25	50	GI/CNS toxicity
FC reduced	25	-	GI/CNS toxicity
RBC, Hb, Hct decrease	25	50	Anemia, bone marrow effects
PT increase	25	50	Coagulopathy/hypocalcemia
APTT incease	100	-	Coagulopathy/hypocalcemia
Triglyceride increase	-	50	?
Serum BUN increase	25	-	Kidney toxicity
Urine Na K decrease	25	(5)	Pharmacologic drug effect
Urine specific gravity decrease	-	50	Diuresis
ALT increase	100	100	Liver toxicity
AST increase	-	100	Liver toxicity
Albumin and/or protein decrease	100	-	Liver toxicity
Creatinine increase	100	-	Renal toxicity
Creatine kinase increase	-	100	Muscle/cardiac toxicity
Testosterone decrease	-	5	
VitD decrease, T3 decrease, T4 increase	-	50	
Hepatic P450 content increase	-	100	Liver enzyme induction
Liver weight increase	100	50	Liver toxicity/enzyme induction
Kidney weight increase	-	50	Kidney toxicity
Testis weight decrease	-	100	
Thymus weight decrease	-	50	
Cecum hyperplasia	5	-	GI toxicity
Heart necrosis	25	-	Cardiotoxicity
Kidney, mineralization	25	-	Renal toxicity
Thymus/spleen lymphoid atrophy	25	50	Other
Cataract formation	100	-	Eye toxicity (hypocalcemia)
Liver necrosis	-	100	Liver toxicity
Testis, atrophy	100	-	Testicular toxicity

AUC multiples in 6-mo rat and 12-mo monkey study (m + f averages)

	Dose (mg/kg/day)	AUC parent (ngxh/mL)	AUC multiple		
			parent	M5 + M7	M2-Glu
Rat	5	99	**0.11x**		
	25	1055	**1.4x**		
	100	1180	**7.5x**	**3.1x**	**(0.2x)**
Monkey	5	73	**0.15x**		
	50	923	**1.6x**		
	100	4840	**1.8x**	**1.1x**	**13x**

SUMMARY AND EVALUATION OF TOXICITY FINDINGS

In animal toxicity studies, single or repeat doses of cinacalcet caused a reduction in serum calcium, and this hypocalcemia is a confounding factor in all toxicity studies. Not only did the hypocalcemia cause pharmacologic/physiologic effects per se, but the presence of hypocalcemia may also have masked CaR-mediated effects of the test compound. It should be noted that patients with secondary HPT on dialysis are monitored for hypocalcemia and this event can be controlled. The drug treatment is intended to reduce increased serum PTH levels but not to levels leading to hypocalcemia. Thus, the hypocalcemia-mediated events in the test animals are not directly relevant. In addition, potential CaR-mediated effects in the treatment population that might resemble effects of increases in serum calcium may have gone undetected in the preclinical studies.

Exposure multiples for parent drug attained in chronic rat and monkey studies were moderate (rat) to low (monkey). Dose administration in animals was limited by the calcium-lowering effect of the drug (rat), or GI toxicity (emesis, monkey). Also, in the monkey, exposure to parent drug did not clearly increase above the high dose of 100 mg/kg/day. The latter may have been due to limited dissolution and absorption, first pass metabolism, and perhaps auto-induction. Exposure multiples for the major human metabolite (M5) were lower than for parent in both rat and monkey. However, multiples for the closely related compound, M7, were very large. Multiples for M2-Glu were adequate in the monkey. The M5, M7 and M2-Glu metabolites are considered inactive at the CaR.

Sponsor attributed several animal toxicity findings to hypocalcemia: clinical signs (salivation, twitching, convulsions) in rats, mice and dogs, GI finding of hyperplasia/inflammation in the rat, decreased hematologic measures (RBC, Hb, Hct) in the monkey, and cataract formation in the rat. Toxicity findings of emesis (dog, monkey), increased liver weight and decreased testosterone (monkey) were ascribed to an action of cinacalcet independent of hypocalcemia. Other findings in toxicity studies were not specifically discussed.

Clinical signs and hypocalcemia
In the single dose studies in rodents, there was reduced activity, abnormal gait and posture, quivering, respiratory signs, tremors. In the 14-day and 28-day rat toxicity studies, numerous clinical signs were observed, especially at the 500 mg/kg/day high dose, and most of them appeared to be signs of CNS- or GI toxicity. Breathing problems, dehydration and salivation persisted at relatively low doses in the 6-month rat study. Hypoactivity and tremors were also seen in single and multiple dose studies in dogs. GI toxicity was seen mainly in dogs and monkeys.

Hypocalcemia leads to nerve excitability (membrane destabilization) and neuromuscular irritability. Thus, respiratory effects, tremors, motor disturbances, convulsions, and GI motility effects in rodents and dogs were probably related to low calcium levels. Excessive salivation in rats, dogs and monkeys may also have been related to hypocalcemia.

In a single dose mouse study, a dose of 200 mg/kg (ca. 6x human dose of 180 mg/day, based on mg/m2) had no proconvulsant effect (defined as potentiation of the effect of electroshock

on the tonic extension of hind limbs). In acute toxicity studies in rats and mice, at oral doses up to 500 mg/kg in mice (6x human mg/m2 dose), and 1500 mg/kg in rat (80x human mg/m2 dose), no convulsions were observed. Serum calcium was transiently decreased, based on data from single dose pharmacology studies in rats. In a 14-day repeat dose rat study (50, 250, 500 mg/kg/day), on Day 6, convulsions were seen in 3/10 animals at the 500 mg/kg/dose (23x human AUC of 648 mgxh/mL @ 180 mg/day). In this study, serum calcium was decreased at all doses from Day 1. In the 6-month rat study with 5, 25, 100 mg/kg/day (up to 7.5 x human AUC), no convulsions were observed. Thus, convulsions were seen in rats at high doses at which there was also serum Ca reduction and may be due to hypocalcemia. The convulsions may also have been the result of an interaction with central neurotransmitter receptors or ion channels (e.g. adrenergic, dopaminergic, GABA-B).

In an acute oral rat toxicity study with 100, 250, 500mg/kg, [redacted] clinical signs of hyper- or hypoactivity, tremors, convulsions, excessive salivation were observed at all doses ≥100 mg/kg (equivalent to ±200 mg/kg parent compound, based on molecular weight comparison). It is unclear whether these effects were due to interaction of this degradant/metabolite with the parathytroid CaR and hypocalcemia-induced CNS effects since serum calcium was not measured, or whether they represent metabolite-specific (CaR-independent) CNS toxicity. The NOAEL for these effects and the multiple of human exposure to the [redacted] metabolite are also unclear.

GI effects

GI toxicity included soft feces and/or GI distension in acute and 14-day mouse and rat studies at high doses, soft feces and poor appetite in monkey (at ≥0.1x human AUC), and vomiting in monkey and dog. In a safety pharmacology study in mice, a dose of 200 mg/kg (6x human mg/m2 dose) increased GI motility. Stomach erosion was seen in rat at 7.5x human AUC. The emesis in dog and monkey occurred at a 0.03x-2x multiple of human exposure (human AUC = 648 ngxh/mL, @180 mg/day) in dog and monkey. Tolerance to emesis developed in the 1-month dog study. The emesis was drug-related and, as suggested by Sponsor, it may have been due to transient hypocalcemia or a CNS effect of cinacalcet. However, a direct role of the CaR in the GI events can not be excluded. The CaR is present along the GI tract and has been implicated in the modulation of gastrointestinal secretion and motility, mineral ion homeostasis, and epithelial cell growth and differentiation (Brown et al, 1998).

In the 6-month rat study, an increased incidence of cecum mucosal hyperplasia/inflammation was observed at al doses including the lowest dose of 5 mg/kg/day (0.1 x human AUC). It was not seen in the 2-year dietary carcinogenicity studies at doses between 5-50 mg/kg/day (0.2-2.5x human AUC). This effect may have been due to interaction with the CaR in the intestine at Cmax/Tmax, or it may have been related to fluctuating drug levels. Gastrointestinal epithelial cells can respond to extracellular Ca and CaR activation with differentiation/proliferation (Brown et al, 1998). The intestinal effect was not seen in dog or monkey and its clinical significance in unclear.

Body weight

Body weight effects in rats (mainly) and monkeys were at least partly related to reduced food consumption. The effects were small in the monkey, and were likely the result of GI toxicity.

Effects were more pronounced in the rat at doses where higher exposures were attained, e.g. body weight gain was minimally affected at 1.5x human AUC (25 mg/kg) in the 6-month rat study, but was decreased by 40% at 7x human AUC (100 mg/kg).

Hematology

In monkeys (3- to 12-month studies) rats (14-day study) and dogs (1-month study), red blood cell parameters (RBC, Hb, Hct) were slightly to moderately decreased at doses ~1-2x human exposure @180 mg/day, an effect that was transient or slowly reversible upon dose discontinuation. The cause of this effect is unclear. Sponsor attributed it to reduced food consumption and body weight gain, or an action on the CaR which has been found in murine bone-marrow derived stem cells. Clinical monitoring has not revealed an effect on Hb or Hct (Study # 20000188). Reduced WBC was also observed in monkeys and rats at higher doses (2-10x human exposure).

Coagulation

Prothrombin time (PT) and APTT were slightly increased in repeat dose rat and monkey studies at the mid or high doses equivalent to ≥1.5x human exposure. The effect was no longer observed after 6 months in monkeys. The effect was slight (10%) but statistically significant. The cause of this finding is unclear, but it may be related to inhibition of calcium-dependent clotting factors or impaired hepatic clotting factor production. There was some indication of liver pathology (increased weight in rat and monkey, and necrosis and vacuolation in monkey).

Ophthalmoscopy

Cataract formation was observed in rats in all repeat dose studies at ≥2x human exposure @180 mg/day. As suggested by Sponsor, cataracts may have been caused by hypocalcemia, and evidence for such an effect of ocular hypocalcemia in animals and humans is present in the published literature (Delamere et al, 1981). Cataracts have been observed in individuals with hypoparathyroidism. Hypocalcemia can cause derangement of lens electrolyte composition. Cataracts were not seen in dog or monkey studies, even though hypocalcemia occurred to a similar degree in monkeys dosed with 100 mg/kg/day (40% reduction). Cataract formation may have been mediated by the CaR which is present in lens epithelial cells (Brown and Macleod, 2001).

Electrocardiography

In a 1-month study in beagle dogs (0, 5, 50, 100 mg/kg/day) there were no effects on EKG parameters, including QT and QTc intervals. QTc intervals were between 0.23 and 0.25 sec @ pre-treatment and @4 wks, in all groups. Serum ionized calcium was decreased maximally 8%, 19%, 20% in LD, MD, HD.

Cmax (Day 28) ranged from [redacted] (up to 1.7x human Cmax), and AUC from 21 to 500 ngxh/mL (up to 0.8x human AUC) in LD-HD.

Effect on serum ionized Ca (pH 7.4 normalized) and serum parent drug (1-month dog study, males)

		Ca predose mg/dL (mmol/L)	Ca post dose (4h) mg/dL (mmol/L)	Cmax (ng/mL) (Tmax=1-2h)
Day 1	Ctrl	5.8 (1.45mM)	5.8 (1.45 mM)	-
	LD	5.9 (1.48 mM)	5.6 (1.40 mM)	

	MD	5.7 (1.43 mM)	4.9 (1.23 mM)	
	HD	5.9 (1.48 mM)	5.0 (1.25 mM)	
Day 28	Ctrl	5.8 (1.45 mM)	5.8 (1.45 mM)	
	LD	5.8 (1.45 mM)	5.5 (1.38 mM)	
	MD	5.1 (1.28 mM)	4.8 (1.20 mM)	
	HD	5.1 (1.28 mM)	4.6 (1.15 mM)	

Cmax human (180 mg/d)

In a 3-month toxicity study in monkeys, QTc was increased dose-dependently at all doses of 5, 50, 100 mg/kg (0.1-2x human AUC @ 180 mg/day). The maximum increase in QTc was from 0.26 sec in controls to 0.32 sec in HD group. Serum ionized calcium was reduced from 1.35 mmol/L in controls, to 0.9 mmol/L (i.e. -35%) in the 100 mg/kg HD group. Sponsor stated that the QTc effects in monkeys were attributed to hypocalcemia, and a linear correlation between serum Ca and QTc was established (rr = -0.7). However, Sponsor also stated that the QTc prolongation was not biologically significant in the monkeys.

QTc data from 3-month monkey study (average, m+f)

	Dose (mg/kg)	Serum ionized Ca (Wk 12) (0-24h avg) (mmol/L)	QTc (Wk 13) (sec)	Cmax (ng/mL)	AUC (ngxh/mL)
Control	0	1.35	0.26		-
LD	5	1.25	0.29		89
MD	50	1.05	0.30		440
HD1 + HD2	100 and 150/100	0.9	0.32		1165

In a 12-month monkey toxicity study, there were also QT and QTc prolongations, most pronounced at 3 and 6 months, but less at 10 and 12 months, at 5, 50, 100 mg/kg/day (0.15-2x human AUC @ 180 mg/day). QRS interval appeared unchanged. QT prolongation was due to a lengthening of the ST segment. The maximal QT(c) effect was 60 msec (0.26sec in controls to 0.32 sec @100 mg/kg/day) when serum ionized calcium was reduced from 1.35 to 0.8 mM. It is unclear why the QT effect in monkeys is attenuated after 6 months despite continuing hypocalcemia. Sponsor ascribed the QT effect in the monkey to hypocalcemia, and the diminution of the effect upon long term (>6 month) treatment to loss of responsiveness of the heart to low calcium.

QTc data from 12-month monkey study (males)

	Dose (mg/kg)	Wk 26		Wk 52		Cmax (ng/mL) Wk26+52	AUC (ngxh/mL) Wk26+52
		Serum Ca (0-24h avg) (mmol/L)	QTc (sec)	Serum Ca (0-24h avg) (mmol/L)	QTc (sec)		
Control	0	1.35	0.26	1.35	0.26		-
LD	5	1.20	0.29	1.15	0.27		99
MD	50	0.90	0.31	0.87	0.28		1055
HD	100	0.85	0.32	0.80	0.28		1180

The cause of the discrepancy between dog and monkey QT data is unclear.

An association of the QT increase with hypocalcemia is likely. The relationship between calcium and QT(c) in monkeys was similar as has been described for patients with hypoparathyroidism and hypocalcemia (Bronsky et al, 1961) and for volunteers given citrate to lower serum calcium (Davis et al, 1995). In these cases, a decrease in serum ionized calcium of 0.5 mmol/L was associated with an increase in QT(c) of 60 msec, and a decrease of 0.2 mM with an increase in Qta(c) of 34 msec, respectively. This compares to an increase of 60 msec in the monkey. However, it can not be excluded that the QT increase due to cinacalcet may partially be due to an other drug-related event. In an *in vitro* safety pharmacology study, KATP channel and two voltage dependent K channels (Kv4.3, Kv1.5) which may play a role in cardiac conduction were inhibited by 96%, 45%, 20%, respectively, by 500 ng/mL. Blockage of these channels might lead to calcium-independent effects on cardiac conduction. HERG channels were minimally inhibited by 12% at 500 ng/mL (1.27uM) prompting moderate clinical concern. Other *in vitro* studies have not been conducted.

Serum chemistry findings (calcium, phosphorus)
The main finding was a dose-dependent reduction in serum calcium in all animal species at all dose levels tested in toxicity studies (down to 0.03x human exposure). Increases in serum P were observed at the same doses or a level higher. The Ca and P changes were at least partly related to the intended pharmacologic effect of the drug, i.e., suppression of parathyroid PTH release. PTH stimulates renal reabsorption of calcium across the distal convoluted tubule and inhibits reabsorption of phosphate in the proximal tubule, hence reduction in PTH lowers serum Ca and increase serum P. Decreases in serum PTH were observed at the same dose levels as the decrease in calcium, but a clear dose-dependence was not established in either rat, dog or monkey. In the rat effects on calcium may also have been mediated by increase in serum calcitonin and suppression of bone resorption. The effects on Ca and P may also have been mediated by other CaR than those in the parathyroid gland e.g. in the kidney. It has been suggested that calcium can inhibit its own reabsorption in the distal tubule (Blankenship et al, 2001), and a calcimimetic may exert this same action.

In the repeat dose rat studies the changes in calcium and phosphate lead to increases in CaxP product, while in the monkey this was not observed. The increased CaxP product was also observed in rat and mouse 24-month carcinogenicity studies, where vascular or cellular mineralization occurred in several soft tissues. Kidney mineralization (pelvis diverticulum) was seen in the 6-month rat study at 25-100 mg/kg/day doses (≥1.5x human AUC), possibly resulting from increased serum CaxP product, or from distal tubule precipitate formation due to increases in urine calcium concentrations.

Liver
A number of observations suggest a potential for liver effects and/or toxicity. There were slight increases in serum ALT and AST at the high dose (100 mg/kg/day) in 3-month and 12-month monkey studies (2x human AUC, respectively). ALT was also increased in repeat-dose rat studies at doses as low as 50 mg/kg/day (2.2x human AUC). Serum protein and/or albumin were decreased in 1-month and 6-month rat studies rats (100 mg/kg/day, 7.5x human AUC) and albumin was decreased in the 150/100 mg/kg group in the 3-month monkey study (2x human AUC). Liver weight increase was seen in >1-month studies in rats and monkeys at 25

and 50 mg/kg/day (1.5-2x human AUC). Liver necrosis and liver cell changes that were possibly drug-related occurred in the 12-month high dose monkeys (2x human AUC).

In the 1-year monkey study, hepatic cytochrome P450 content was increased in the high dose group (100 mg/kg/day) after 6 and 12 months (1.8-1.4x control value). Also, CL/F (l/h/kg) was increased at 6 and 12 months as compared to Day 1, in the 100 mg/kg group as compared to control. Sponsor concluded that this suggests modest induction of hepatic P450 enzymes. However, in the 3-month monkey study no dose-related increases in CL/F was observed after 1 or 3 months as compared to Day 1. In the rat, in the 6-month study, no evidence of significant induction based on parent drug clearance or liver weight was observed.

The P450 increase, liver weight changes and increased clearance in the monkey suggest a potential for induction or auto-induction of cinacalcet-metabolizing enzymes. In humans, there was no indication of induction or auto-induction of hepatic P450 enzymes based on the linear dose response of plasma exposure (Cmax, AUC) up to doses of 180 mg/day. However, the observed saturation of exposure to parent drug at higher doses may be partially explained by auto-induction of metabolism at higher dose levels. Induction and/or auto-induction could affect the clearance of drugs metabolized by the various CYP450 enzyme isoforms including those involved in the metabolism of cinacalcet (3A4, 1A2, 2C9).

Kidney
Renal effects were observed in the rat, dog and monkey. In the rat, serum BUN was increased (1.4x human AUC) and creatinine was increased (7.5x human AUC) accompanied by kidney mineralization (1.4x human AUC). In the monkey, kidney weight was increased in the monkey (2x human AUC), and there were slight tubualr changes. The cause of the weight increase was unclear. Urine volume was increased in dogs and monkeys and urinary calcium excretion was increased in rats and dogs. The volume and ion excretion effects were probably due to pharmacologic effects on the kidney CaR, which is thought to play a role in calcium reabsorption and urine concentrating ability.
Renal toxicity is particularly relevant for patients with primary hyperparathyroidism.

Other findings
In rats, in 2-week, 1-month and 6-month toxicity studies, histopathologic findings of myocardial necrosis/degeneration were observed at doses of 1.4x human AUC and higher. The cause of this effect is unclear. It may be secondary to myocardial effects of hypocalcemia, or to a block of protective KATP potassium currents resulting in Ca overload and myocardial damage. Creatine kinase increase and muscle degeneration occurred at 100 mg/kg (2x human AUC) in 3- to 12-month monkey toxicity studies. The data suggest a potential for myocardial, cardiovascular or muscle toxicity.

Endocrine changes were observed in the 12-month monkey study. Serum testosterone levels were markedly decreased at all doses of 5-100 mg/kg/day (0.1-2x human AUC @ 180 mg/day) in the 1-year monkey study. This was accompanied by testicular weight decrease at 100 mg/kg/day. Testicular tubular atrophy or degeneration was also observed in the 1-and 6-month rat studies at 50-100 mg/kg/day (3-7.5x human AUC @ 180 mg/day), and the 1-month dog study at 100 mg/kg/day (0.8x human AUC @ 180 mg/day). In monkeys, Vitamin D was

decreased and thyroid T3 was decreased and T4 increased at 50 mg/kg/day (1.5x human AUC). The cause of these endocrine organ effects is unclear. Sponsor reported no testicular changes in clinicla trials (Study20000188).

In the 6-month rat toxicity study, there were effects on bone size (reduced length, increased diameter), but BMD of trabecular or cortical bone was not clearly affected. The increase in bone diameter may have been due to periosteal expansion possibly as a result of fluctuating PTH levels.

In juvenile 3-week old rats, cortical bone area was increased at the expense of trabecular bone, possibly due to decreased cortical bone resorption in the fast growing skeleton.
In juvenile 10-week old dogs, after 1 month of recovery from 1 month of treatment, there was slightly increased incidence of left ventricular arterial hypertrophy, and myocardial fibrosis, at a dose leading to 1/10 of human exposure. The etiology of this finding is unclear, but might be related to irreversible KATP channel blockage.

Findings of unclear origin were thymus weight and histology changes, bone marrow changes, low incidences of histological changes in monkeys of unclear significance.

PK and AUC multiples
PK studies showed qualitatively similar metabolite profiles in all species, with parent drug comprising a minor part (<1%) of total drug related material. However, there were large quantitative differences in metabolite levels between species, and the multiples for the main metabolite observed in humans (M5) were about half those for parent in rats and monkeys. A larger multiple (13x) for the minor metabolite (M2-Glu) in humans was achieved in the chronic monkey studies. Metabolite multiples for the dog are unknown.
The calculated multiples of human AUC are based on median human exposure and are subject to large variation.

The M5 and M7 metabolites were >300-fold less active on the CaR in an in vitro system. The M2 metabolite did not interact with the CaR. However, both parent and metabolites may contribute to other (toxic) effects unrelated to CaR activation. The toxicity of M6 (circulating in humans) is likely to be similar to that of M5 and M7. The differences in toxicities between rats and monkeys may have been partly due to species differences in exposure to parent and metabolites and species differences in biological response to these compounds.

In all species, upon dealkylation of the parent, naphthalene-group containing metabolites are formed at unknown levels. Upon acute oral and subchronic inhalation, naphthalene (active ingredient in moth balls) can cause toxicity in humans including neurotoxicity (convulsions), GI effects, hepatic effects, renal effects and ocular effects including cataracts. In rats, at oral doses of 400 mg/kg for 3 months, clinical signs, kidney and thymus lesions, and anemia have been observed. Interestingly, there appears to be some similarity between these effects and the preclinical toxicities observed with cinacalcet. The acute rat toxicity study with . ——— suggested potential CNS toxicity of this metabolite/cleavage product with unknown effect on serum calcium.

CONCLUSIONS

Rat, dog and monkey studies with cinacalcet were carried out by the oral route using daily dose administration. In studies of ≥3 month duration in rats and monkeys, exposure to parent drug was up to 7.5 and 1.8 times, respectively, the exposure attained in humans treated for secondary hyperparathyroidism with the maximal dose of 180 mg/day. Exposures in humans at the highest dose proposed for primary hyperparathyroidism (360 mg/day) was in a similar range as the exposure at 180 mg/day. Thus, multiples for this dose/indication are similar as those for the secondary HPT indication.

Preclinical studies were suboptimal due to the dose-limiting effects of hypocalcemia and GI toxicity (MTD). GI toxicity was either hypocalcemia-related or the result of drug-related GI or CNS toxicity. The main target organs of cinacalcet identified in studies with drug-induced hypocalcemia are CNS, heart (EKG), GI tract, eye, liver, kidney, intestine, endocrine/ reproductive organs, bone marrow, thymus, spleen, lymph nodes.

Apart from hypocalcemia, the data from animal toxicity studies suggest the following potential clinical safety concerns: GI toxicity, hematologic effects (anemia), hepatic toxicity, renal toxicity, liver enzyme induction, myocardial and skeletal muscle toxicity, endocrine effects (testosterone, thyroid hormones, Vitamin D). Moreover, it can not be excluded that the EKG abnormalities, CNS toxicity (seizures), and cataracts observed in animals may have a calcium-independent component.

Other toxicities not predicted by animal data due to CaR activation and resembling hypercalcemia may also occur in humans. A theoretical concern is the potential effects on mGluR and $GABA_BR$ in brain or pancreas, which might lead to interference with central nervous system electrical activity or insulin secretion.

Because metabolism is an essential feature in cinacalcet PK, the potential for toxicity is enhanced in cases where systemic exposure to parent and/or metabolite is increased, e.g. in hepatic or renal impairment.

3.4.3. Genetic toxicology

An Ames assay was performed with Salmonella Typhimurium (TA98, TA100, TA1535, TA 1537), at concentrations up to 250 µg/plate in the absence of S-9, and at concentrations up to 750 µg/plate in the presence of S-9. Concentrations up to 750 ug/plate were tested in E. Coli (WP2 uvrA), with and without S-9. Concentrations were selected based on results of toxicity assays performed at doses up to 5,000 µg/plate. Cinacalcet tested negative in all strains with and without metabolic activation.

A HGPRT forward gene mutation test was performed using CHO cells. Cinacalcet was not mutagenic in this assay with and without metabolic activation.

A chromosomal aberration assay was carried out in CHO cells, with and with out S-9. Cells were harvested at 4 and 20 hours. Cinacalcet was negative for induction or structural and numerical chromosome aberrations.

A mouse micronucleus study was conducted at doses up to 200 mg/kg orally. Cinacalcet did not induce micronucleus formation in this assay.

Summary

Cinacalcet tested negative *in vitro* in the Ames test in S.typhimurium and E.Coli, the HGPRT mammalian cell gene mutation assay, and the CHO cell chromosome aberration assay, with and without metabolic activation, and *in vivo* in the mouse micronucleus assay.

3.4.4. Carcinogenicity

NOTE: For brief summary of carcinogenicity studies, see Executive CAC meeting minutes (APPENDIX)

Adequacy of the carcinogenicity studies and appropriateness of the test model:
Carcinogenicity studies in rats and mice were performed using the dietary route, because of the difficulty dosing animals for 2 years via gavage due to behavioral changes (agitation, sensitivity) resulting from the pharmacologic effect of hypocalcemia. Dietary dosing provides for fewer fluctuations in serum drug and calcium concentrations. Doses were adequate and were selected based on 3-month dietary studies (EXEC CAC Meeting Minutes September 22, 1998). The high dose in the female rat study was adjusted after 1 year of study due to excessive body weight effects, in consultation with the EXEC CAC (Meeting Minutes January 11, 2000). The presence of cinacalcet in initial plasma samples from control animals in rat and mouse studies was most likely the result of post sample collection contamination. This issue was reviewed by the Division and it was concluded that the integrity of the studies was maintained (EOP-2 Meeting Minutes November 9, 2001). Both studies were adequate and acceptable.

3.4.4.1. Mouse Carcinogenicity Study

Study title: 104 Week Carcinogenicity Study of AMG 099073-01 in Mice with Administration by Diet.

Key study findings:

- CD-1 mice were dosed with 0, 15, 50, 100 mg/kg/day (males), or 0, 30, 70, 200 mg/kg/day (females) in the diet for 104 weeks.
- Doses resulted in parent AUC levels of 109, 366, 941 (m) ngxh/mL and 198, 401, 1050 (f) ngxh/mL, equivalent to 0.2x, 0.6x, 1.5x (males) and 0.3x, 0.6x, 1.6x (females) the human AUC (648 ngxh/mL) at a daily dose of 180 mg/day.
- There were no significant effects of cinacalcet on survival. Convulsions and agitation related to hypocalcemia were seen in MD (m) and HD (m,f). An increased incidence of cataracts and lens opacities was seen in MD (m) and HD (m,f).
- Cinacalcet induced a dose-dependent reduction in body weight gain (gr) in MD and HD (m,f). Food consumption (gr/day) was minimally decreased in MDm and HD m,f. Efficiency of food utilization was decreased in all treated. The HD was the maximum tolerated dose (MTD) based on the body weight effects.
- There was a sustained, dose-dependent decrease in serum Ca, and increase in serum P, accompanied by increases in CaxP product. Serum PTH and ionized Ca were decreased in all treated groups.
- There were decreases in serum bilirubin in MD and HD (f).
- Kidney weight was decreased in HDm.
- Mineralization was observed in the kidney (in males only) at all dose levels, and in several other organs (at low incidences) in male and female MD and HD groups. Vascular mineralization was increased in testis in HD group. Mineralization events were probably partly related to the increased CaxP product.

- There were no statistically significant increases in tumor incidences in males or female, according to trend analysis (CDER). There were small, non-statistically significantly increased incidences of erythroid leukemia and pituitary adenoma (intermediate lobe) in HD females. Both tumors were increased above historical control range. There was also an increase in incidence of hyperplasia in the pituitary gland (intermediate) in MD and HD females above historical control range.
- Leukemia (erythroid) and pituitary adenoma (intermediate lobe) in HD females appeared to be biologically significant findings.

Study no.: 100250 (—— Study Nr. 454446)
Conducting laboratory: ——————(main study including TK of parent); Amgen USA (TK metabolite and TK data analysis)
Date of study initiation: November 18, 1998 (Start of dosing: January 6, 1999)
GLP compliance: Yes (—————— Amgen TK metabolite study: FDA USA)
QA report: Yes
Drug, lot #, % purity: AMG 099073-01, Lot Nr. 709001 (used January 6, 1999 until May 26, 1999), and Lot 9D0682 BAN AM9AD-02 (used June 1, 1999 until study end)
CAC concurrence: Yes

Methods

Doses:

Main Study

Dose Group	Males			Females		
	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers
1	Control I*	0	1-60	Control I*	0	301-360
2	Control II*	0	61-120	Control II*	0	361-420
3	Low	15	121-180	Low	30	421-480
4	Intermediate	50	181-240	Intermediate	70	481-540
5	High	125	241-300	High	200	541-600

*= Control groups were given rodent diet alone

Toxicokinetic Study

Dose Group	Males			Females		
	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers
6	Control*	0	601-666	Control*	0	865-930
7	Low	15	667-732	Low	30	931-996
8	Intermediate	50	733-798	Intermediate	70	997-1062
9	High	125	799-864	High	200	1063-1128

*= Control groups were given rodent diet alone

Basis of dose selection (MTD): MTD (HD) and AUC
Species/strain: CD-1 (Crl:CD-1™ ——)BR) mice
Number/sex/group: 60 (main study): 60 (TK study)
Age: 6 weeks

Route: oral (diet)
Formulation: Formulated diet prepared once weekly by adding formulated premix to blank diet. Formulated diet level (ppm) was based on body weight and food consumption data to achieve constant dose levels (mg/kg), and adjusted once weekly for first 13 weeks, once monthly thereafter. Diet analysis performed at 14 time points.
Frequency of dosing: Daily (diet)
Diet: Rat and Mouse [redacted]
Animal housing: 1 per cage in suspended cages, 12h light/dark cycle
Drug stability/homogeneity: Certificate provided for bulk test article (App 2)
Dual controls employed: Yes (main study)
Interim sacrifices: None
Deviations from original study protocol:

Observation times
Mortality: Twice daily
Clinical signs: Once weekly (including masses)
Body weights: Once weekly (first 13 weeks), and once every 4 weeks (after 13 wks)
Food consumption: Once weekly (first 13 weeks), and once every 4 weeks over 1-wk period (after 13 wks)
Ophthalmoscopy: Pretreatment, and Wks 26, 53, 78, 102
Laboratory Investigations: Week 105-106 (hematology and clinical chemistry)
Ionised calcium/pH: Wks 5, 26, 53 (samples from TK animals)
PTH: Wks 27, 54 (from TK animals)
TK studies: Wks 1, 20, 53, 71. Plasma was analysed for AMG 073 by LC-MS/MS at [redacted], and for metabolite AMG 102664-00 (trifluoromethyl-cinnamic acid) by LC-MS/MS at Amgen (Report App. 31). Surviving TK animals sacrified at Wk 71.
Terminal evaluation: Main study animals killed after 104 weeks of treatment, and gross necropsy performed. Tissues weighted (part) and examined histologically (Tissue Inventory Appended)
Histopathology: Peer review: Yes
Statistical analysis: Pairwise comparison against combined control, for all parameters. "Peto analysis was not performed on the histopathology data because there were no observed differences in the pattern of mortality between the groups and no increase in neoplastic lesions found in any tissue in any of the incidence tables generated."
Statistical trend analysis was performed by CDER Biometrics Reviewer (J. Buenconsejo) based on the method described by Peto et al (1980) and exact permutation test developed by Division of Biometrics II (CDER).

Results
Mortality:

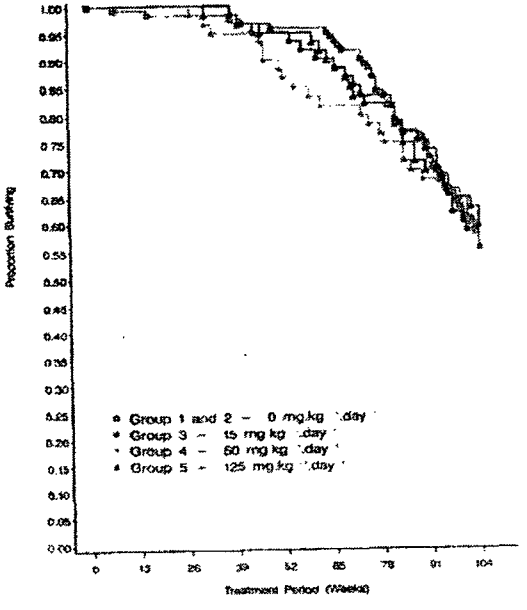
Group	Dose level (mg/kg/day)	N survivors		N premature deaths	
	M/F	M	F	M	F
1	0/0	32	23	28	37
2	0/0	35	21	25	39

3	15/30	35	24	25	36
4	50/70	35	21	25	39
5	125/200	36	23	24	37

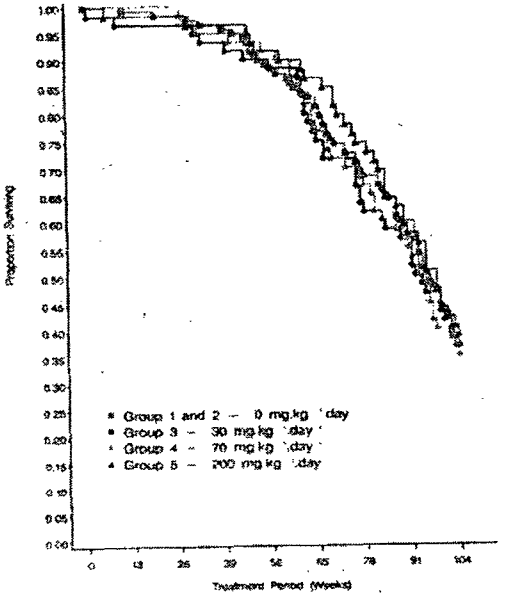
No significant differences control vs. treated

8 Figures

AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Mice with Administration by Diet
Figure 1 Kaplan Meier Survival Curve: Males



AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Mice with Administration by Diet
Figure 2 Kaplan Meier Survival Curve: Females



Clinical signs:

Males, incidence of finding at any time in study

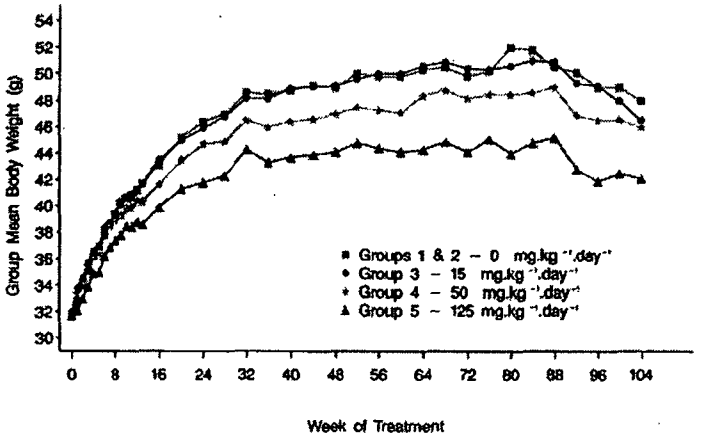
Group	1	2	3	4	5
N	60	60	60	60	60
Dose (mg/kg/day)	0	0	15	50	125
Agitated	7	13	15	18	19
Convulsions	2	6	8	13	10
Eyes opaque	4	4	5	6	12

Females, incidence of finding at any time in study

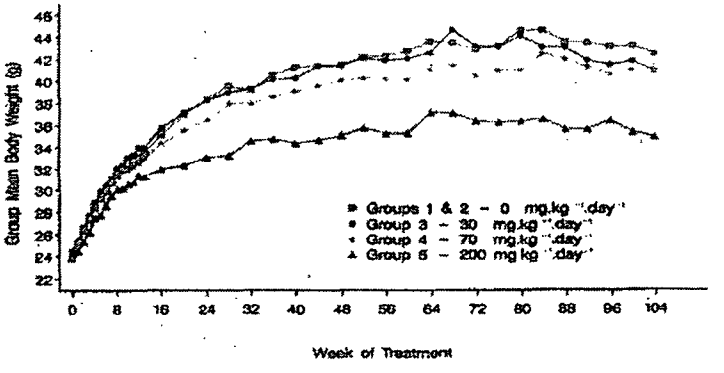
Group	1	2	3	4	5
N	60	60	60	60	60
Dose (mg/kg/day)	0	0	30	70	200
Convulsions	3	4	6	3	13
Eyes opaque	10	12	6	7	13

Body weights:

AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Mice with Administration by Diet
Figure 3 *Group Mean Body Weight (g): Males*



AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Mice with Administration by Diet
Figure 4 *Group Mean Body Weight (g): Females*



Body weight, Males (gr)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	15	50	125
Body weight				
Week 0 (pretrial)	31.6	32.0	31.7	31.7

Week 52	50.0	49.6	47.5*	44.8***
Week 104	48.0	46.5	46.0	42.1***
Body weight gain				
Wk 0-104	16.7	14.8	14.5*	10.6***
	100%	*89%*	*87%*	*63.5%*

Body weight, Females (gr)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	30	70	200
Body weight				
Week 0 (pretrial)	23.8	24.5	24.4	24.3
Week 52	42.2	42.1	40.3	35.8***
Week 104	42.5	40.9	41.1	35.0***
Body weight gain				
Wk 0-104	19.0	16.3	17.0	11.4***
	100%	*86%*	*89%*	*60%*

Food consumption:

FC minimally reduced in MD and HD, in several weeks throughout study. In some weeks, effect was statistically significant in HD.

Food consumption, Males (gr/animal/day, over 1-week period)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	15	50	125
Week 10	6.4	6.1	6.2	6.3
Week 20	5.8	5.6	5.5*	5.2***
Week 40	5.8	5.9	5.7	5.5**
Week 60	6.0	5.9	5.8	5.5***
Week 80	5.7	5.7	5.8	5.7
Week 104	5.5	5.5	5.4	5.7
Time Average (these weeks only)	*5.9 (100%)*	*5.8 (98%)*	*5.7 (97%)*	*5.65 (96%)*

Food consumption, Females (gr/animal/day, over 1-week period)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	30	70	200
Week 11	6.1	6.1	5.9	5.5***
Week 24	5.7	5.8	5.6	5.3**
Week 44	5.5	5.7	5.4	4.9***
Week 64	5.4	6.0**	5.6	5.0*
Week 80	5.5	5.7	5.5	5.4
Week 104	5.3	5.3	5.7	5.5
Time Average (these weeks only)	*5.6 (100%)*	*5.7 (102%)*	*5.6 (100%)*	*5.3 (94%)*

Relative food consumption (gr/kg/day) was increased dose-dependently in all dose groups (m and f), suggesting a drug-related decrease in efficiency of food utilization (EFU) in all groups.

Ophthalmoscopy
Wk 53: Incidence of lens opacities increased in HDm and HDf
Wk 78: Incidence of lens opacities, including cataracts, increased in MDm, HDm
Wk 102: Incidence of lens opacities, including cataracts, increased in LDm, HDm, HDf.

Achieved dosages
These values were calculated using nominal diet concentrations (see APP 6,Tables 7-8). Since diet concentrations were actually lower than nominal, actual achieved dosages were very close to nominal.

Group means achieved dosages (mg/kg/day)

MALES, Group	3	4	5
Target dose (mg/kg/day)	15	50	125
Achieved dose (mg/kg/day)	16	54	136
(% of target dose)	(109)	(108)	(109)
FEMALES, Group	3	4	5
Target dose (mg/kg/day)	30	70	200
Achieved dose (mg/kg/day)	33	77	220
(% of target dose)	(110)	(110)	(105)

Analysis of formulated diets:
Concentrations of test compound in formulated diets (ppm) were in range of +/-14% of the nominal concentrations, in Wks 13, 20, 28, 29, 30, 47, 53, 65, 78, 90, 102. At most times the diet concentration were approximately 5-10% lower than nominal.

Test article purity:
Lot Nr. 709001: 99.7%,
Lot Nr. 9D0682: 99.9%

Hematology:
No significant effects on RBC, WBC, platelet parameters

Clinical Chemistry:
Males, clinical chemistry, Wk 105

Group	1+2	3	4	5
Dose (mg/kg/day)	0	15	50	125
Ca	2.74	2.58**	2.32***	1.98***
Phos	2.06	2.25	2.70***	3.33***
Alk P	382	301	176	238
T.Bi	3.3	2.9	3.1	2.9

Females, clinical chemistry, Wk 105

Group	1+2	3	4	5

Dose (mg/kg/day)	0	30	70	200
Ca	2.73	2.47***	2.28***	1.91***
Phos	2.14	2.48*	3.06***	3.44***
Alk P	205	123	156	121
T.Bi	3.3	3.2	2.5*	2.2**

Calcium x Phosphorus product

104-week Mouse Carcinogenicity Study (100250)				
Dose (mg/kg)	0	15	50	125
Ca x P – Week 105 Males	93.86	96.53	104.16	109.64
Ca x P – Week 105 Females	97.15	101.86	116.01	109.26

Ionized Calcium:

Samples over 24-h period at 1600 pm, 2200 pm, 0400 am, 1200 pm, in Wks 5, 26, 53

- ☐ Ionized calcium decreased at all time points, in all dose groups
- ☐ Lowest values seen at 2200 h to 0400 h
- ☐ Values generally lowest in Wk 53

Males, ionized calcium (mmol/L, 2200h)

Group				
Dose (mg/kg/day)	0	15	50	125
Wk 5	1.20	1.09	0.96	0.84
Wk 26	1.15	1.02	0.94	0.81
Wk 53	1.19	1.10	0.96	0.81

Females, ionized calcium (mmol/L, 2200h)

Group				
Dose (mg/kg/day)	0	30	70	200
Wk 5	1.23	1.12	1.01	0.78
Wk 26	1.17	1.00	0.91	0.83
Wk 53	1.25	1.05	0.87	0.77

Figure 7

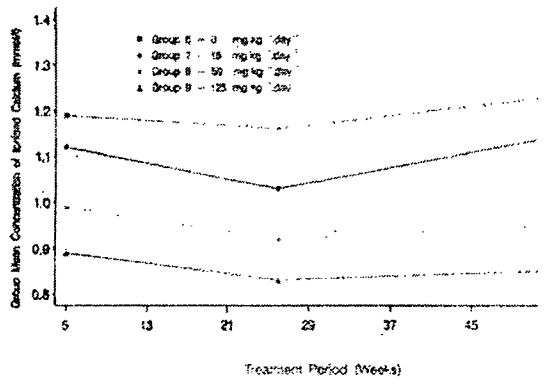
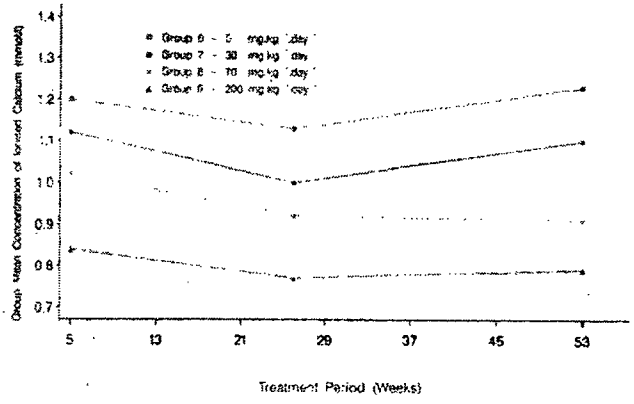


Figure 8



PTH:

Dose-related decrease in all dose groups

Males, serum PTH (pg/mL)

Group				
Dose (mg/kg/day)	0	15	50	125
Wk 26	44	21	3.3	1.0
Wk 53	37	8.2	3.5	2.0

Females, serum PTH (pg/mL)

Group				
Dose (mg/kg/day)	0	30	70	200
Wk 26	24	18	3.9	2.0
Wk 53	27	14	3.3	9.2

Toxicokinetics:

Control samples contained AMG 073 parent, but not metabolite (AMG 102664-00, i.e., M5), particularly in Wk 1. This was most likely due to post-collection contamination, although the source of the contamination is unclear.

There was no obvious increase in AMG073 or metabolite levels over time

Exposure to metabolite M5 was 10-20x exposure to parent drug

Systemic exposure

AUC Males (ngxh/mL)

Group				
Dose (mg/kg/day)	0	15	50	125
PARENT AMG 099073-00				
Wk 1	76	188	594	954
Wk 20	10	138	285	901
Wk 53	23	292	443	1130
Wk 71	2.1	109	366	941
METABOLITE AMG 102664-00				
Wk 1	0	798	3450	6690
Wk 20	0	916	5280	7530
Wk 53	0	989	3470	8450
Wk 71	0	1100	3510	12400

AUC Females (ngxh/mL)

Group				
Dose (mg/kg/dy)	0	30	70	200
PARENT AMG 099073				
Wk 1	70	185	467	992

Wk 20	0	239	398	929
Wk 53	23	292	443	1130
Wk 71	0	198	401	1050
METABOLITE AMG 102664-00				
Wk 1	0	1690	5720	13200
Wk 20	0	3080	10300	10800
Wk 53	0	2220	7890	20000
Wk 71	0	4170	11500	13900

Organ weights:

Males

Group				
Dose (mg/kg/day)	0	15	50	125
Body weight (gr)	46	45	45	41***
Absolute weight (gr)				
Kidney	0.84	0.87	0.83	0.72***
Relative weight (BW covariant)				
Kidney	0.83	0.86	0.83	0.75**

Females

Group				
Dose (mg/kg/day)	0	30	70	200
Body weight (gr)	41	40	40	34***
Absolute weight (gr)				
Lung	0.29	0.27	0.29	0.24**
Salivary glands	0.17	0.17	0.15**	0.15**
Relative weight (BW covariant)				
Salivary glands	0.17	0.17	0.15**	0.15**

Gross pathology:

MALES					
Group		1+2	3	4	5
Dose (mg/kg/day)		0	5	15	35
N examined		120	60	60	60
Mass(es)		22	9	6	7
Eye	Opaque (one/both)	12	7	8	11
Harderian gland	Dark	1	3	4	7

FEMALES					
Group		1+2	3	4	5
Dose (mg/kg/day)		0	5	20	50/35
N examined		120	60	60	60

Mass(es)		19	9	6	7
Harderian gland	Speckled	1	2	2	5
	Dark	3	3	5	8

Histopathology:

Non-neoplastic:

MALES

Group		1+2	3	4	5	
Dose (mg/kg/day)		0	5	15	35	
N examined*		120	60	60	60	
Lung	Lymphoid hyperplasia	0	0	1	3*	↑
Lymph node (mesenteric)	Lymphoid hyperplasia	8	5	12*	9	↑
Heart	Progressive cardiomyopathy	50	19	20	9***	↓
	Mineralization, myocardial	0	0	1	0	
	Perivasculitis	1	1	1	3	
Pituitary gland	Cyst	12	0*	1	2	↓
Pancreas	Hyperplasia, ductular, scattered	0	0	3*	0	
Testis	Tubular mineralization	41	9*	12	13	↓
	Tubular atrophy	51	15*	20	18	
	Vascular mineralization	0	1	1	10*	
Epididymis	Mineralization	5	1	3	6	
Kidney	Mineralization (minim-mod)	23	19	26**	31***	↑
Stomach	Inflammation	2	3	2	3	
	Squamous cyst	0	0	2	2	↑
Eye	Corneal mineralization	1	2	1	2	
Harderian gland	Porphyrin secretion	0	2	2	4*	↑
Sciatic nerve	Mineralization	0	0	1	2	

FEMALES

Group		1+2	3	4	5	
Dose (mg/kg/day)		0	5	15	35	
N examined*		120	60	60	60	
Lung	Lymphoid hyperplasia	4	2	2	6	↑
Lymph node (mesenteric)	Lymphoid hyperplasia	9	7	3	8	↑
Lymph node (mediastinal)	Lymphoid hyperplasia	0	2	0	2*	↑
Heart	Mineralization, myocardial	0	0	2	1	
	Progressive cardiomyopathy	11	11	10	1	↓
Pituitary gland	Hyperplasia, diffuse, intermediate lobe	2	1	3	6*	↑
	Hyperplasia, diffuse, intermediate lobe	0	0	0	0	
	Hyperplasia, focal, anterior	0	1	0	3*	↑

	lobe					
	Hyperplasia, diffuse, anterior lobe	0	1	1	1	↑
	Cyst	3	0	0	0	↓
Uterus	Hyperplasia, stromal	0	3*	2	1	↑
	Atypical hyperplasia, focal	0	0	0	1	
Kidney	Mineralisation	4	1	3	1	
	Eosinophilic globules, tubular cell	2	0	1	3	
Stomach	Inflammation	3	3	1	3	
	Inflammatory cell infiltration	0	0	3*	0	
Salivary gland	Inflammatory cell infiltration	5	5	11*	6	
Pancreas	Hyperplasia, ductular, scattered	0	1	0	1	
Mammary gland	Cystic hyperplasia	1	1	2	3	↑
Eye	Corneal mineralization	0	0	0	2	
Harderian gland	Porphyrin secretion	4	4	3	8*	↑
Sternum	Atrophy, marrow	0	0	4*	1	
Sciatic nerve	Mineralization	0	0	0	2	

*In several cases, N examined was slightly less than the specified 120-60-60-60. However, relative to each other, the numbers remained fairly constant. Thus, only absolute incidences are given in the Tables.

Small incidences of mineralization or vascular mineralization were observed in MD and/or HD group (males and/or females) in several organs (lung, thymus, heart, aorta, adrenal, epididymis, ovary, pancreas, eye, brain, sciatic nerve).

Neoplastic:

MALES

Group		1+2	3	4	5	p-value* (trend test, exact)	Historical control incidence range (%)
Dose (mg/kg/day)		0	15	50	125		
N examined		120	60	60	60		
BW gain (%)		100%	89%	87%	64%		
Hematopoietic system	Lymphoma, follicular centre cell (M)	3	1	1	3	>0.05	
	Leukemia, erythroid (M)*	0	1	0	0	> 0.05	
Spleen	Haemangiosarcoma	0	0	2	1	> 0.05	
Kidney	Tubular cell adenoma (B)	0	0	0	2	0.042	0-5.1%
Femur	Osteoma	1	0	0	0		
Tail	Osteosarcoma	1	0	1	0		

FEMALES

Group		1+2	3	4	5	p-value*	Historical

						(trend test, exact)	control incidence range (%)
Dose (mg/kg/day)		0	30	70	200		
N examined		120	60	60	60		
BW gain (%)		100%	86%	89%	60%		
Hematopoietic system	Leukemia, erythroid (M)*	0	0	0	2 (3.3%)	0.048	0-0%
	Lymphoma, follicular centre cell (M)	1	0	1	1	>0.05	
Pituitary	Adenoma, intermediate lobe (B)	0	0	0	2 (3.3%)	0.066	0-0.9%
	Adenoma, anterior lobe (B)	3	1	0	3	>0.05	0-8.2%
Combined	Adenoma, intermediate lobe and anterior lobe (B)	3	1	0	5	0.040	
Uterus	Stromal polyp (B)	0	0	0	2	0.041	0-22%
	Stromal sarcoma (M)	3	1	4	1	>0.05	0-2.5%
Combined	Stromal polyp and sarcoma	3	1	4	3	>0.05	
Bone (other)	Osteoma	0	0	1	0		
	Osteosarcoma	2	2	0	1		
Tail	Osteosarcoma	0	1	0	0		

*finding not significant if p >0.025 (rare tumor), or p>0.005 (common tumor)
Historical control incidences from 12 experiments (1994-2001)

Note: Leukemias should not be combined in the mouse (McConnell et al, 1986). Also, there were no other leukemias with increased incidence in the mouse (m or f).

NOTES:

- There were no effects on # or % of animals with tumors, or on # of tumors
- There were no increases in incidence of tumors of the same histomorphogenic type (e.g. haemangiosarcoma), when combined for different anatomic sites.

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104 Week Carcinogenicity Study of AMG 099073-01 in Mice with Administration by Diet
Table 27 Summary of Tumour Findings: All Animals Combined

	TUMOUR TABLE							
	Males				Females			
GROUP / DOSE	Cont 0 mg/kg /day	Grp 3 15 mg/kg /day	Grp 4 50 mg/kg /day	Grp 5 125 mg/kg /day	Cont 0 mg/kg /day	Grp 3 30 mg/kg /day	Grp 4 70 mg/kg /day	Grp 5 200 mg/kg /day
NUMBER OF ANIMALS	120	60	60	60	120	60	60	60
NUMBER OF ANIMALS WITH TUMOURS	96	45	45	38	80	34	40	36
NUMBER OF ANIMALS WITH SINGLE TUMOURS	54	20	23	17	52	20	25	27
NUMBER OF ANIMALS WITH MULTIPLE TUMOURS	42	25	22	21	28	14	15	9
NUMBER OF ANIMALS WITH BENIGN TUMOURS	56	32	28	27	35	18	18	15
NUMBER OF ANIMALS WITH MALIGNANT TUMOURS	65	27	29	23	61	26	29	25
NUMBER OF ANIMALS WITH METASTASISING TUMOURS	5	2	1	2	2	1	1	1
TOTAL NUMBER OF TUMOURS	189	90	86	81	121	54	58	46
TOTAL NUMBER BENIGN TUMOURS	114	58	52	53	48	24	24	19
TOTAL NUMBER OF MALIGNANT TUMOURS	75	32	34	28	73	30	34	27
TOTAL NUMBER OF METASTASISING TUMOURS	5	2	1	2	2	1	1	1
% ANIMALS WITH TUMOURS	80	75	75	63	67	57	67	60
% ANIMALS WITH SINGLE TUMOURS	45	33	38	28	43	33	42	45
% ANIMALS WITH MULTIPLE TUMOURS	35	42	37	35	23	23	25	15
% ANIMALS WITH BENIGN TUMOURS	47	53	47	45	29	30	30	25
% ANIMALS WITH MALIGNANT TUMOURS	54	45	48	38	51	43	48	42
% ANIMALS WITH METASTASISING TUMOURS	4	3	2	3	2	2	2	2

Control groups (1 and 2) are combined

Summary of findings:

Group		1+2	3	4	5
Dose (mg/kg/day)	M	0	15	50	125
	F	0	30	70	200
Agitation and/or convulsions	M				
	F				
Body weight gain decrease	M,F				
Food consumption decrease	M,F				
Lens opacities	M,F				
Decrease in serum PTH	M,F				
Decrease in serum Ca/ionized Ca	M,F				
Increase in serum P	M,F				
Decrease in bilirubin	F				
Kidney rel weight decrease	M				
Salivary gland rel. weight decrease	F				
Harderian gland, dark/speckled	M,F				

Histopathology

Non-neoplastic findings:

- Lymphoid hyperplasia in lung and lymph node, MD and/or HD, males and females
- Heart, significant decrease in incidence of progressive cardiomyopathy in HDm and HDf
- Testis, increase in vascular mineralization in HD, significant
- Testis, decrease in tubular atrophy and tubular mineralization, in all dose groups without dose-dependence
- Kidney, significant increase in incidence of mineralization (all grades) in males only, in all dose groups
- Harderian gland, significant increase in porphyrin secretion in HDm and HDf

Neoplastic findings:

- Sponsor submitted historical control data from control groups of CD-1 mice from 12 two-year studies (1994-2001). The current study was included in the historical database.
- Incidences given below are for (combined controls, LD, MD, HD)
- Reviewer evaluated tumor findings in light of the notion that body weight was decreased in the HD groups with potential decrease in tumor rates.

- For rare tumors (control incidence <1%) the p-value cut off is 0.025, for common tumors (>1%) the p-value cut off is 0.005.

Tumor findings:

- Leukemia, erythroid, in females (0-0-0-2, i.e., 0-0-0-3.3%) was increased in the HD group. The finding was not statistically significant according to CDER trend test (p=0.0423, > 0.025 for rare tumor) or pairwise comparison ——— However, erythroid leukemia has not been observed in the historical control database. The historical control range for all types of leukemias combined was 0-1% (average 0.1% (1/979), 1/100 non-erythroid leukemia in 1 control experiment). Based on incidence rate of 3.3% in HD group, and absence of tumor from historical controls, Reviewer feels the finding was biologically significant.
- Pituitary intermediate, adenoma, in females (0-0-0-2, i.e., 0-0-0-3.3%) was increased in the HD group above the historical control range (range 0-0.9%, average 0.1% (1/979), 1/117 occurrence in 1 control group). The finding was not statistically significant according to CDER trend test (p=0.0658, > 0.025 for rare tumor) or pairwise comparison ———. The incidence of diffuse hyperplasia in the pituitary (intermediate lobe) (1.7-1.7-5-10%) was above historical control range for (focal) hyperplasia (range 0-2%, avg 0.4%), in MD and HD. Note that historical control data for diffuse hyperplasia were not provided. Based on the size of the tumor effect in the HD group (33x average historical control incidence, 3.5x highest control incidence of 0.9%) and the parallel presence of hyperplasia in this part of the gland, Reviewer feels the finding was biologically significant.
- Ptuitary anterior, adenoma, in females (3-1-0-3, i.e., 5-1.7-0-5%) was within the historical control range (range 0-8.2%, average 2.5%). The finding was not statistically or biologically significant.
- Uterine stromal sarcoma incidence (3-1-4-1, i.e., 1.5-1.7-6.7-1.7%) was increased in the MD group outside the historical control range (0-2.5%, avg 1.8%). The tumor finding was not statistically significant according to CDER trend test (p>0.05). Although a reduction in body weight may have artificially suppressed tumor incidence in the HD group, there was a lower incidence in the HD group. Based on this, and the size of the effect in the MD group (2-3x highest incidence in historical control experiment), Reviewer concludes that the increased incidence in the MD group was not biologically significant.
- Uterine stromal polyp incidence, female (0-0-0-2, i.e., 0-0-0-3.3%) was within historical control range (0-22%, avg 6.2%), and was not statistically or biologically significant.
- Combined incidence of uterine polyp and stromal sarcoma (3-1-4-3, i.e., 2.5-1.7-6.7-5%) was inside historical control range (0-24%) and not statistically or biologically significant.
- Lymphoma, follicular centre cell, males (3-1-1-3, i.e., 2.5-1.7-1.7-5%) was not statistically significant. The historical control range for combined lymphoma was 2-22.5% for 12 experiments. The control incidence of this particular type of lymphoma in 2 experiments was 4% and 2.5%. The size of the effect in the HD group indicates that the finding was not biologically significant.
- Kidney tubular cell adenoma, males (0-0-0-2, i.e., 0-0-0-3.3%), was inside historical control range (0-5.1%) and was not statistically or biologically significant.
- Spleen hemangiosarcoma, males (0-0-2-1, i.e., 0-0-3.3-1.7%) was inside historical control range (approximately 0-5%) and was not statistically or biologically significant.

Conclusion

Potentially biologically significant tumor findings:

- Leukemia, erythroid (f)
- Pituitary, intermediate, adenoma (f)

NOTE: Upon discussion of the study findings with the Exec CAC (Dec 16, 2003), the Committee concluded that the leukemia and pituitary adenoma in the female mouse may have been biologically significant and treatment-related, but need not be included in the label based on low incidences and lack of statistical significance.

Histopathology inventory

	Organ weight	Histology
Adrenals		X
Aorta		X
Bone Marrow smear		X
Bone (femur, rib)		X
Brain	X	X
Cecum		X
Cervix		
Colon		X
Duodenum		X
Epididymis		X
Esophagus		X
Eye		X
Fallopian tube		
Gall bladder		X
Gross lesions		X
Harderian gland		X
Heart	X	X
Ileum		X
Injection site		
Jejunum		X
Kidneys	X	X
Lachrymal gland		
Larynx		
Liver	X	X
Lungs	X	X
Lymph nodes, cervical		
Lymph nodes, mandibular		
Lymph nodes, mesenteric		X
Mammary Gland		X
Nasal cavity		
Optic nerves		X
Ovaries	X	X

Pancreas		X
Parathyroid		X
Peripheral nerve		
Pharynx		
Pituitary		X
Prostate	X	X
Rectum		X
Salivary gland, submaxillary (mandibular)	X	X
Sciatic nerve		X
Seminal vesicles		X
Skeletal muscle		X
Skin		X
Spinal cord		X
Spleen	X	X
Sternum		X
Stomach		X
Testes	X	X
Thymus		X
Thyroid		X
Tongue		X
Trachea		X
Urinary bladder		X
Uterus		X
Vagina		X
Zymbal gland		X

3.4.4.2. Rat Carcinogenicity Study

Study title: 104 Week Carcinogenicity Study of AMG 099073-01 in Rats with Administration by Diet.

Key study findings:

- SD rats were dosed with 0, 0, 5, 15, 35 mg/kg/day (males), or 0, 0, 5, 20, 50/35 mg/kg/day (females), in the diet, for 104 weeks. The female high dose was reduced from 50 mg/kg/day to 35 mg/kg/day in Week 63 due to excessive reduction in body weight gain.
- Doses resulted in parent AUC levels of 132, 462, 1620 (m) ngxh/mL and 144, 374, 894 (f) ngxh/mL, equivalent to 0.2x, 0.7x, 2.5x (males) and 0.2x, 0.6x, 1.4x (females) the human AUC (648 ngxh/mL) at a daily dose of 180 mg/day.
- Cinacalcet had no significant effects on survival. Agitation related to hypocalcemia was seen in all treatment groups (m,f). An increased incidence of cataracts and lens opacities was seen in MDf and HDm,f.
- Cinacalcet induced a dose-dependent reduction in body weight gain (gr) in MD and HD (m,f), and parallel but smaller decreases in absolute food consumption (gr/day). Efficiency of food utilization was decreased in MD and HD. The HD was the maximum tolerated dose (MTD) based on the body weight effects.
- There was a sustained dose-dependent decrease in serum Ca, and increase in serum P, accompanied by increases in CaxP product. Ionized Ca was decreased in all treated groups, serum PTH was decreased in MD and HD.
- There were increases in neutrophil count in MD and HD (m), decreases in serum triglycerides in MD and HD (m,f), and increases in serum bilirubin in MD and HD (f).
- Kidney weight was decreased in HD (m), and in MD and HD (f).
- There was an increased incidence and degree of vascular mineralization in lung and heart in all dose groups (m and f), and in kidney in MD and HD (m,f). In the heart, ossification was seen in MD and HD (m,f). In several tissues, mineralization occurred at low incidences in HD groups. Mineralization events were probably related to the increased CaxP product.
- There was a statistically significant positive trend in combined lymphoma incidence in female rats. However, based on historical control data, the effect was not biologically significant. There were no statistically significant increases in other tumor incidences in males or females, according to trend analysis (CDER). There were small non-statistically significant increases in incidence of lung bronchio-alveolar carcinoma (males) and combined malignant lymphoma (males). However, based on historical control data, these effects were not biologically significant. There was a dose-related decrease in thyroid C-cell adenoma and in parathyroid adenoma in males and females. This was likely related to the suppression of the release of calcitonin (the antagonistic hormone of PTH) from thyroid C-cells, and the suppression of PTH release from parathyroid cells.

Study no.: 100209 ([redacted]: Study No. 454430)
Conducting laboratory: [redacted] (main study including TK of parent); Amgen, USA (TK metabolite and TK data analysis)
Date of study initiation: October 30, 1998 (Start of dosing: November 19, 1998)

GLP compliance: Yes [redacted] Amgen TK metabolite study: FDA USA)

QA report: Yes

Drug, lot #, % purity: AMG 099073-01, Lot Nr. 709001 (used November 19, 19989 until May 19, 1999), and Lot 9D0682 BAN AM9AD-02 (used May 26, 1999 until study end)

CAC concurrence: Yes

Methods

Doses: 0, 5, 15, 35 mg/kg/day (males)
0, 5, 20, 50/35 mg/kg/day (females)

Main Study

Dose Group	Males			Females		
	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers
1	Control I*	0	1-60	Control I*	0	301-360
2	Control II*	0	61-120	Control II*	0	361-420
3	Low	5	121-180	Low	5	421-480
4	Intermediate	15	181-240	Intermediate	20	481-540
5	High	35	241-300	High	50/35•	541-600

*= Control groups were given rodent diet alone
•= This change in dose level took effect from the start of Week 63 of treatment

Toxicokinetic Study

Dose Group	Males			Females		
	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers	Treatment ($mg.kg^{-1}.day^{-1}$)		Animal Numbers
6	Control *	0	601-630	Control *	0	721-750
7	Low	5	631-660	Low	5	751-780
8	Intermediate	15	661-690	Intermediate	20	781-810
9	High	35	691-720	High	50/35•	811-840

*= Control group was given rodent diet alone.
•= This change in dose level took effect from the start of Week 63 of treatment

Basis of dose selection (MTD): MTD (HD) and AUC
Species/strain: Sprague-Dawley (Crl:CD^{TM}BR) rats
Number/sex/group: 60 (main study): 60 (TK study)
Age: 6 weeks
Route: oral (diet)
Formulation: Formulated diet prepared once weekly by adding formulated premix to blank diet. Formulated diet level (ppm) was based on body weight and food consumption data to achieve constant dose levels (mg/kg), and adjusted once weekly for first 13 weeks, once monthly thereafter. Diet analysis performed at 14 time points.
Frequency of dosing: Daily (diet)
Diet: Rat and Mouse [redacted]
Animal housing: up to 5 per cage in suspended cages, 12h light/dark cycle
Drug stability/homogeneity: Certificate provided for bulk test article (App 2)
Dual controls employed: Yes (main study)
Interim sacrifices: None

Deviations from original study protocol: Dose in female high dose group was changed from 50 mg/kg/day to 35 mg/kg/day on Wk 63 of treatment, due to excessive reduction in body weight gain in this group. The change was made in consultation with the EXEC CAC.

Observation times

Mortality: Twice daily
Clinical signs: Once weekly (including masses)
Body weights: Once weekly (first 13 weeks), and once every 4 weeks (after 13 wks)
Food consumption: Once weekly (first 13 weeks), and once every 4 weeks over 1-wk period (after 13 wks)
Ophthalmoscopy: Pretreatment, and Wks 26, 52, 78, 104
Laboratory Investigations: Week 52 and 104 (hematology and clinical chemistry)
Ionised calcium/pH: Wks 1, 26, 35, 52, 65 (samples from TK animals)
PTH: Wks 1, 26, 52 (from TK animals, Grps 6,7,8,9)
TK studies: Wks 1, 21, 52, 78, 35. Plasma was analysed for AMG 073 by LC-MS/MS at ——— (project Nr. 366837, Amgen Study No. 100327), and for metabolite AMG 102664-00 (M7, trifluoromethyl-cinnamic acid) (Wk 52, 78 samples only) by LC-MS/MS at Amgen (Report App. 40). Surviving TK animals sacrified at Wk 78.
Terminal evaluation: Main study animals killed after 104 weeks of treatment, and gross necropsy performed. Tissues weighted (part) and examined histologically (Histopathology Inventory Appended)
Processing of fixed tissues: Slide production was carried out by ———
Histopathology: Peer review: Yes, by Sponsor's pathologist
Statistical analysis: Pairwise comparison against combined control, using various two-sided tests, for all parameters. "Peto analysis was not performed on the histopathology data because there were no observed differences in the pattern of mortality between the groups and no increase in neoplastic lesions found in any tissue in any of the incidence tables generated."
P-values: *<0.05, ** < 0.01, *** <0.001.
Statistical trend analysis was performed by CDER Biometrics Reviewer (J. Buenconsejo) based on the method described by Peto et al (1980) and exact permutation test developed by Division of Biometrics II (CDER).

Results

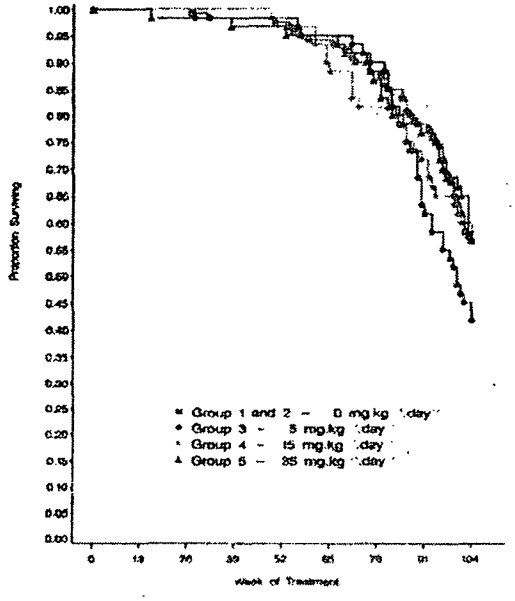
Mortality:

Group	Dose level (mg/kg/day)	N survivors		N premature deaths	
	M/F	M	F	M	F
1	0/0	28	26	32	34
2	0/0	39	27	21	33
3	5/5	25	27	35	33
4	15/20	35	26	25	34
5	35/50→35	34	33	26	27

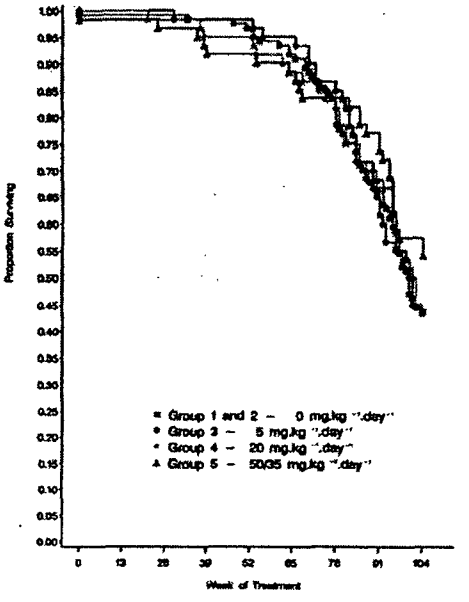
No significant differences in mortality of control vs. treated

8 Figures

AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Rats wi
Administration by Diet
Figure 1 Kaplan-Meier Survival Curve: Males



AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Rats with
Administration by Diet
Figure 2 Kaplan-Meier Survival Curves: Females



Dose level for Group 5 was reduced from 50 mg/kg/day to 35 mg/kg/day in Week 53 of treatment.

Clinical signs:

Males, incidence of finding at any time in study

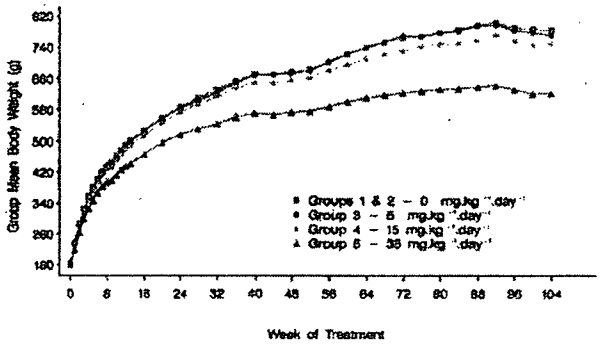
Group	1	2	3	4	5
N	60	60	60	60	60
Dose (mg/kg/day)	0	0	15	50	125
Agitated	9	10	16	22	20
Skin discoloured	8	4	11	15	15

Females, incidence of finding at any time in study

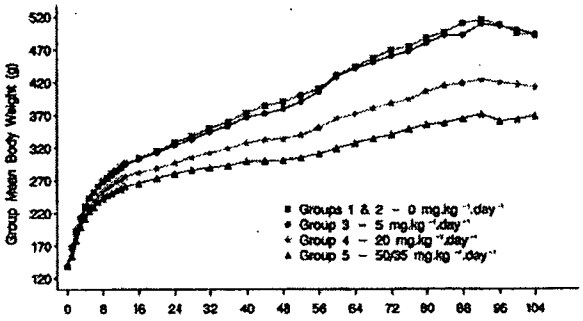
Group	1	2	3	4	5
N	60	60	60	60	60
Dose (mg/kg/day)	0	0	30	70	200
Agitated	4	6	11	12	16

Body weights:

AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Rats with Administration by Diet
Figure 3 Group Mean Body Weight (g): Males



AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Rats with Administration by Diet
Figure 4 Group Mean Body Weight (g): Females



Dose level for Group 5 was reduced from 50 mg/kg⁻¹.day⁻¹ to 35 mg/kg⁻¹.day⁻¹ in Week 49 of treatment

Body weight, Males (gr)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	5	15	35
Body weight				
Week 0 (pretrial)	184	184	188	183
Week 52	685	681	663	577***
Week 104	785	771	749	624
Body weight gain				
Wk 0-104	600	588	561	443***
	100%	*98%*	*94%*	*74%*

Body weight, Females (gr)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	5	15	50/35
Body weight				

Week 0 (pretrial)	138	139	138	142*
Week 52	400	390	340***	305***
Week 104	491	493	412***	369***
Body weight gain				
Wk 0-104	354	353	273***	228***
	100%	*99.7%*	*77%*	*64%*

Food consumption:

FC (gr/animal/day) slightly reduced in MD and HD groups, in several weeks throughout study. In some weeks, effect was statistically significant. Effect similar in m and f

Food consumption, Males (gr/animal/day, over 1-week period)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	5	15	35
Week 10	27.2	26.5	26.2	24.1***
Week 20	26.5	26.2	26.3	24.1***
Week 40	26.8	26.9	25.4*	23.5***
Week 60	28.6	27.1	27.8	25.4***
Week 80	28.5	29.0	29.3	26.4*
Week 104	29.3	31.3	27.8	27.0*
Time Average (these weeks only)	*27.8 (100%)*	*27.8 (100%)*	*27.1 (97%)*	*25.1 (90%)*

Food consumption, Females (gr/animal/day, over 1-week period)

Group	1+2	3	4	5
N	120	60	60	60
Dose (mg/kg/day)	0	5	20	50/35
Week 10	21.3	20.2*	19.1***	18.1***
Week 20	19.5	19.2	18.1***	17.3***
Week 40	20.4	20.3	18.8**	17.8***
Week 60	22.8	23.1	21.6	24.0
Week 80	23.8	24.6	22.5	22.1
Week 104	24.2	24.3	26.0	23.7
Time Average (these weeks only)	*22.0 (100%)*	*21.9 (100%)*	*21.0 (95%)*	*20.5 (93%)*

Relative food consumption (gr/kg/day) was increased dose-dependently in MD and HDm,f, suggesting a drug-related decrease in efficiency of food utilization (EFU) in these groups. Thus, the decrease in food consumption only partially explained the reduced body weight gain.

Ophthalmoscopy

Wk 52, 78, 104: Incidence of lens abnormalities, including opacities and cataracts increased in HDm and MDf, HDf

Achieved dosages

These dosages were calculated using nominal diet concentrations (see APP 6, and Tables 7-8). Since diet concentrations were actually lower than nominal, actual achieved dosages were very close to nominal.

Group means achieved dosages (mg/kg/day)

MALES, Group	3	4	5	
Target dose (mg/kg/day)	5	15	35	
Achieved dose (mg/kg/day)	5.6	17	39	
(% of target dose)	(102)	(103)	(109)	
FEMALES, Group	3	4	5	
Target dose (mg/kg/day)	5	20	50 / 35	
Achieved dose (mg/kg/day)				
(% of target dose)	102	100	100 / 114	

Analysis of formulated diets:
Concentrations of test compound in formulated diets (ppm) were in range of +/- 10% of the nominal concentrations, in Wks 1, 19, 21, 35, 36, 37, 52, 65, 78, 90, 102. On average, concentrations were ca. 5% lower than nominal.

Test article purity
Batch Nr. 709001: 99.7% (Wk 13), 100% (Wk 52)
Batch No. 9D0682: 100% (Wk 52), 99.8%

Hematology:

Minimal changes in MCH (↓), MCV (↓), MCHC (↑) in males in Wks 52, 104, statistically significant in HD and/or HD. Similar effects in females in Wk 52 only.
Slight dose-related on WBC and neutrophils in males
Minimal decrease in Hct in MD and HD males in Wk 104

Males, hematology

Group	1+2	3	4	5
Dose (mg/kg/day)	0	5	15	35
WEEK 52				
WBC	9.89	11.1	10.6	11.7**
Neutrophils	1.8	2.2	2.3*	3.3***
WEEK 104				
WBC	10.4	13.4	12.8*	13.2**
Neutrophils	3.4	5.4	4.9**	5.6***

Clinical Chemistry:
Decrease in Ca, increase in P
Increase in bilirubin in females
Minimal decreases in serum Cl and K in males and females, mostly in Wk 52
Decrease in triglycerides, males and females (possibly due to BW reduction)
Small decrease in glucose, in HD males and females, Wk 52 (possibly due to BW reduction)

Males, clinical chemistry

Group	1+2	3	4	5
Dose (mg/kg/day)	0	5	15	35
WEEK 52				
Ca	2.94	2.68***	2.50***	2.17***
Phos	1.68	2.1***	2.6***	3.1***
Trig	1.5	1.3	1.4	1.0**
WEEK 104				
Ca	2.91	2.68***	2.45***	2.05***
Phos	1.47	1.75***	2.14***	2.60***

Females, clinical chemistry

Group	1+2	3	4	5
Dose (mg/kg/day)	0	5	15	35
WEEK 52				
Ca	3.19	3.02***	2.69***	2.28***
Phos	1.7	1.9***	2.4***	3.0***
Trig	2.5	2.4	1.7***	1.1***
T.Bi	2.1	2.0	2.5**	2.8***
WEEK 104				
Ca	2.99	2.81	2.67****	2.35***
Phos	1.6	1.7	1.9**	2.4***
Trig	2.8	2.1	1.4*	1.1***
T.Bi	2.1	2.4	2.9	2.5

Calcium x Phosphorus product

104-week Rat Carci Study (100209)				
Dose (mg/kg)	0	5	15	35[a]
Ca x P - Wk 52 Males	82.13	93.58	106.00	110.06
Ca x P - Wk 52 Females	90.18	96.92	106.91	113.36
Ca x P - Wk 104 Males	71.13	77.99	87.18	88.63
Ca x P - Wk 104 Females	77.56	81.30	83.47	93.39

[a] female dose reduced from 50 mkd to 35 mkd at Wk63

Ionized Calcium:

Samples over 24-h period at 1600 pm, 2200 pm, 0400 am, 1200 pm, in Wks 1, 26, 35, 52, 65

- ☐ Ionized calcium decreased in all Weeks, at all time points, in all dose groups
- ☐ Lowest values seen at 2200 to 0400 in Week 1; Values similar over 24-h period in later Weeks
- ☐ Decreases smaller in Week 1 as compared to later Weeks; Values/decreases generally similar in all Weeks from Week 26
- ☐ Wk 65 values increased in various groups, including HD females who received lowered dose level from Wk 63 (50/35 mkd)

☐ Wk 35 values only from Grp 6 (controls)

Mean concentrations over 24-h periods, Wks 1, 26, 52, 65

Group No. (mg.kg^{-1}.day^{-1})	Mean Concentration (mmol.l^{-1}) (% of Control)							
	Sampling Timepoints (Weeks)							
	Week 1		Week 26		Week 52		Week 65	
	♂	♀	♂	♀	♂	♀	♂	♀
6 (0)	1.43	1.45	1.33	1.35	1.31	1.37	1.36	1.40
7 (5)	1.34 (94)	1.32 (91)	1.23 (92)	1.21 (90)	1.16 (89)	1.23 (90)	1.27 (93)	1.28 (91)
8 (15♂, 20♀)	1.18 (83)	1.19 (82)	1.00 (75)	1.02 (76)	1.01 (77)	1.05 (77)	1.06 (78)	1.10 (79)
9 (35♂, 50/35♀)	1.07 (75)	1.07 (74)	0.90 (68)	0.87 (64)	0.90 (69)	0.89 (65)	0.95 (70)	1.01 (72)

The mean concentration was calculated by taking the mean of means from the 4 timepoints (1600, 2200, 0400 and 1200) sampled at each of weeks 1, 26, 52 and 78

Figure 7 AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Rats with Administration by Diet
Group Mean Concentration of Ionised Calcium (mmol.l^{-1}): Males

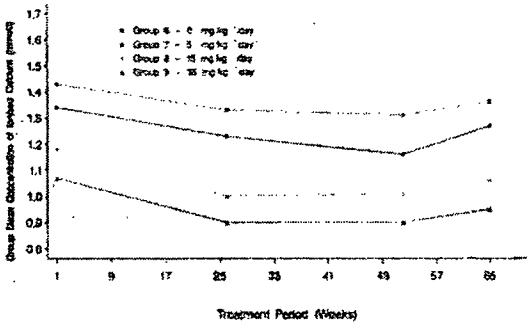
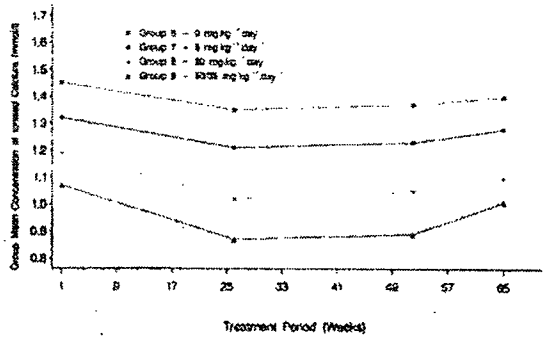


Figure 8 AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Rats with Administration by Diet
Group Mean Concentration of Ionised Calcium (mmol.l^{-1}): Females



PTH:

No significant in LD (5mkd) groups
Some reduction in MD (15 and 20 mkd) groups
Decrease in HD groups
Standard deviations were large (10-50%)

Males, serum PTH (pg/mL)

Group				
Dose (mg/kg/day)	0	5	15	35
Wk 1	89	74	85	59
Wk 26	75	66	55	48
Wk 52	67	83	46	47

Females, serum PTH (pg/mL)

Group				
Dose (mg/kg/day)	0	5	20	50/35
Wk 1	98	112	119	88
Wk 26	65	73	54	48
Wk 52	61	63	69	45

Toxicokinetics:

- Control samples contained AMG 073 parent, but not metabolite AMG102664 (M7, trifluoromethyl-cinnamic acid), particularly in Wk 1. This was most likely due to post-collection contamination (since systemic exposure would have resulted in considerable metabolite levels), although the source of the contamination is unclear.
- In males, at the HD of 35 mkd, exposure to parent appeared to increase over time, and exposure to metabolite was decreased. There was no obvious increase in AMG073 or metabolite levels over time at other dose levels, or in females
- Upon decrease in female HD from 50 to 35 mkd at Wk 63 exposure to metabolite was decreased
- Average exposure to metabolite (ngxh/mL) was **50-fold** higher than to parent

Systemic exposure

AUC Males (ngxh/mL)

Group				
Dose (mg/kg/day)	0	5	15	35
PARENT AMG 099073-00				
Wk 1	54	172	388	491
Wk 21	0	124	370	693
Wk 52	0	111	396	1050
Wk 78	0	132	462	1620
METABOLITE AMG 102664-00				
Wk 1	3.4	6220	18700	78000
Wk 21	ND	5010	18750	49500
Wk 52	0	7080	22000	40200
Wk 78	0	6360	18700	37600

AUC Females (ngxh/mL)

Group				
Dose (mg/kg/dy)	0	5	20	50/35
PARENT AMG 099073				
Wk 1	75	211	394	862
Wk 21	3	91	280	657
Wk 52	0	95	373	737
Wk 78	0	144	374	894
METABOLITE AMG 102664-00				
Wk 1	14	6260	23700	35500

Wk 21	0	7910	33800	101000
Wk 52	0	9820	39300	93200
Wk 78	0	6590	33400	45000

Organ weights:

Males

Group				
Dose (mg/kg/day)	0	5	15	35
Body weight (gr)	780	766	743	620
Absolute weight (gr)				
Adrenal	0.10	0.08	0.08	0.06***
Kidney	4.6	4.9	4.5	3.9***
Liver	22	23	21	19***
Lung	2.4	2.5	2.3	2.2*
Thymus	0.16	0.17	0.14	0.12***
Relative weight (BW covariant)				
Kidney	4.5	4.8	4.5	4.1**

Females

Group				
Dose (mg/kg/day)	0	5	20	50/35
Body weight (gr)	479	485	405***	363***
Absolute weight (gr)				
Heart	1.5	1.6	1.5	1.4**
Kidney	3.1	3.0	2.7***	2.3***
Liver	15	15	13**	12***
Thymus	0.14	0.17	0.12	0.11**
Relative weight (BW covariant)				
Brain	2.1	2.0	2.1	2.2***
Kidney	3.0	3.0	2.8*	2.5***

Gross pathology:

MALES

Group		1+2	3	4	5
Dose (mg/kg/day)		0	5	15	35
N examined		120	60	60	60
Mass(es)		45	15	17	17
Epididymis	Mass(es)	0	0	1	5
Testes	Flaccid	10	8	8	14
Liver	Prominent lobulation	8	3	1	0

FEMALES

Group		1+2	3	4	5