

reticulocyte count in HD (possibly result of increased control value). Slight decrease in total leukocyte count in HD, and in lymphocyte count in MD, HD. Moderate increase in neutrophil ct in MD, HD.

Month 3: Minimal, dose-related decrease in erythrocyte ct and PCV in MD and HD, and in Hb conc in LD, MD, HD. Moderate decrease in leukocyte ct in HD, and in lymphocyte ct in LD, MD, HD (dose-related). Moderate to large, non-dose-related increase in neutrophil ct in LD, MD, HD.

Month 6: Minimal, dose-related decrease in erythrocyte ct, Hb conc and PCV in MD and HD. Minimal increase in MCV, MCH in HD. Slight decrease in reticulocyte ct in LD, MD, HD (possibly result of increased control value). Moderate, dose-related decrease in leukocyte ct and lymphocyte ct in LD, MD, HD. Moderate, dose-related decrease in neutrophil ct in LD, MD, HD.

Females:

Month 1: Minimal, dose-related decrease in erythrocyte ct in LD, MD, HD. Minimal decrease in PCV in HD. Minimal increases in MCV in MD and HD, and in MDH in LD, MD and HD. Slight decrease in total leukocyte count and lymphocyte ct in HD. Slight increase in neutrophil ct in HD.

Month 3: Minimal, dose-related decrease in erythrocyte ct and Hb in LD, MD and HD. Minimal, dose-related increase in MCV in LD, MD, HD, and in MCH in MD, HD. Slight decrease in MCHC in HD. Slight non-dose-related increase in reticulocyte ct in LD, MD, HD. Month 6: Minimal, dose-related decrease in erythrocyte ct, Hb conc and PCV in LD, MD and HD. Minimal to slight, dose-related increases in MCV, MCH and reticulocyte ct in LD, MD, HD. Slight, non-dose-related decrease in neutrophil ct in LD, MD, HD.

Coagulation -

Males:

Minimally decreased APTT in MD, HD.

Clinical Chemistry -

Males:

Month 1: Minimal to slight increases in total and ionized Ca in MD, HD. Slight, dose-related increase in ALP in LD, MD, HD. Slight increase in inorganic P in LD, MD, while decrease in HD. Slight, dose-related increase in serum globulin in LD, MD, HD. Slight non-dose-related decreases in AST and triglycerides in LD, MD, HD. Slight increase in K in HD.

Month 3: Minimal increase in total Ca in LD, MD, HD. Minimal increase in ionized Ca in HD. Slight increase in ALP in HD. Slight, dose-related increase in globulin in LD, MD, HD. Minimal decrease in albumin in LD, MD, HD. Minimal increases in BUN in HD, and in creatinine in MD, HD. Moderate, ca. 50% non-dose-related decreases in bilirubin and in triglycerides in all dose groups. Moderate increase in GGT in HD.

Month 6: Minimal increase in total serum Ca in LD, MD, HD, and in inorganic P in MD, HD. Slight increase in ALP in HD. Slight dose-related increase in globulin and decrease in albumin in all dose groups. Slight increase in BUN in HD, and minimal increase in creatinine in MD, HD. Slight decrease in bilirubin in MD, HD.

Females:

Month 1: Minimal increase in total Ca in MD, HD and in ionized Ca in HD. Slight increase in ALP in HD. Slight increase in inorganic P in LD, MD, HD. Slight, dose-related increase in serum globulin in LD, MD, HD. Minimal decrease in albumin in HD. Minimal increase in BUN in MD, HD. Moderate decrease in bilirubin in HD. Slight decrease in ALT in HD, and slight to moderate decreases in AST and triglycerides in MD, HD. Slight decrease in K in HD.

Month 3: Minimal increase in ionized Ca in HD. Slight, non-dose-related increase in P in LD, MD, HD. Slight, dose-related increase in globulin in LD, MD, HD. Minimal decrease in albumin in MD, HD. Minimal increase in BUN in HD. Moderate decrease in bilirubin in MD, HD, and moderate dose-related decrease in triglycerides in all dose groups. Slight non-dose related increase in cholesterol in LD, MD, HD.

Month 6: Slight increase in inorganic P in HD. Minimal to slight dose-related increase in globulin in LD, MD, HD, and decrease in albumin in MD, HD. Minimal decrease in creatinine in HD. Slight decrease in bilirubin in HD. In HD, slight decrease in ALT, and minimal increase in K. Slight non-dose-related increase in cholesterol in LD, MD, HD.

Effects of LY treatment on serum total Ca and ionized Ca

Group		MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	10	30	100	0	10	30	100
Serum Ca (mg/dl)	Day 29	10.1	10.0	10.7*	11.5*	10.4	10.6	11.0*	11.3*
	Day 178-184	10.4	10.6*	10.6*	10.8*	10.5	10.7	10.6	10.6
Serum ionized adjusted Ca (mM)	Day 28	1.47	1.48	1.51*	1.61*	1.49	1.47	1.48	1.57*
	Day 178-184	1.42	1.41	1.44	1.43	1.40	1.37	1.39	1.43

*statistically significant

Urinalysis -*Males:*

Month 1: Increase of 40% (n.s.), 80% in total Ca excretion in MD, HD. Decrease of 30%, 40% in total Cl secretion in MD, HD. Month 3: Increase of 40% in Ca excretion in HD. Slight decreases (10-30%) in total creatinine, K and Cl excretion in MD, HD. Month 6: : Increase of 60%, 50% in total Ca excretion in MD, HD.

Females:

Month 1: Increase of 55% (n.s.), 125% in total Ca excretion in MD, HD. Increase of ca. 30% in P excretion in MD and HD. Moderate, dose-related decrease in total Cl secretion in LD, MD, HD. Month 3: Increase of 150% in Ca excretion in HD. Moderate, ca. 30% increase in P excretion in all dose groups. Slight increase (20-30%) in total Na excretion. Slight increase in urine osmolality in HD. Moderate ca. 50% increase in volume in MD and HD. Month 6: : Increase of 60%, 90% in total Ca excretion, and increase of ca. 25% in P excretion in MD, HD. Increase of 40% in Na excretion, of 25% in K excretion and of 20% in urine osmolality in HD.

**APPEARS THIS WAY
ON ORIGINAL**

Serum PTH (1-34) (Data from Satellite Study Nr. R04396 (Day 0), and Main Study Nr. R01196 (Day 140))

Day	Dose	Sex	C _{0.25h} (ng/ml)	C _{0.5h} (ng/ml)	T _{max}	AUC ₀ (ngxh/ml)	T1/2 (h)
0	0	M	0*				
		F	0.064*				
	10	M	3.78				
		F	3.16				
	30	M	8.39				
		F	6.76				
	100	M	27.1		0.25	22.0	0.42
		F	25.8		0.25	16.1	0.43
140	10	M	3.14				
		F	3.45				
	30	M	10.3				
		F	9.21				
	100	M	57.6	75.0	0.5	53.6	0.44
		F	45.9	52.3	0.5	40.1	0.41

*The predose-level at t=0 on Day 0 was 0 ng/ml in males, and 0.06 ± 0.04 ng/ml (average ± SE, n=12) in females. On Day 140, at t=0, it was 0 ng/ml in males and females (average of n= 3/sex/dose group). All values BLQ were counted as 0 ng/ml.

Immunotoxicity - No LY333334-specific antibodies detected on Day -4 and at necropsy.

Organ Weight (significant changes) -

Males:

Liver: Minimal increase in relative-to-bodyweight (1.05-1.1x) in MD, HD.

Kidney: Minimal increases in absolute and relative weights (1.05-1.1x) in MD, HD.

Females:

Liver: Slight dose-related increase in absolute (1.1-1.2x) and in relative-to-brain weight (1.1-1.2x) in LD, MD, HD. Minimal decrease in relative-to-body weight (1.1x) in HD.

Kidney: Minimal increase in absolute (1.1x) and in relative-to-brain weight (1.1x) in HD.

Spleen: Moderate, dose-related increase in absolute (1.2-1.7x), relative-to-body (1.1-1.5x), and relative-to-brain weight (1.2-1.7x) in LD, MD, HD

Pituitary: Slight decrease in absolute (0.95-0.9x), relative-to-body (0.9-0.8x) and relative-to-brain weight (0.95-0.9x) in LD, MD, HD

Ovary: Slight increases in absolute and relative-to-brain weight (1.15x) in HD.

Femur Length -

Females:

Minimal, but significant increase in LD (1.02x), MD (1.03x) and HD (1.04x). Change not seen in males.

Gross Pathology -

Males:

Spleen: Enlarged (0-0-0-1)
Kidney: Whole tissue alteration (0-1-2-3)
Females:
Liver: Whole tissue alteration (4-6-11-9)
Spleen: Enlarged (0-0-2-4)
Ovary: Cyst (0-1-1-4)
Bone: Thickened (0-0-9-12)

Histopathology - ($n_{\text{examined}} = 15/\text{sex}$ unless indicated otherwise; "?" means 0 animals examined) -

Males:

Whole animal: no substantive tissue alteration (1-0-0-0)
Kidney: Progressive glomerulonephritis (inflammation of glomerular capillary loops), minimal (12-?-0/1-2), slight (3-?-0/1-9) or moderate (0-?-0/1-2). Multifocal mineralization, minimal (0-?-?-4)
Lung: Severe diffuse alveolar hemorrhage (0-?-0/1-1)
Liver: Focal basophilic atypia (0-0-0-1)
Spleen: Extramedullary hematopoiesis, minimal (6-2-0-0), or slight/moderate (0-13-15-15)
Bone:
Femur: Trabecular hypertrophy (=increased thickness of metaphyseal and epiphyseal trabeculae), graded slight (0-15-12-1), moderate (0-0-3-13) or marked (0-0-0-1). Slight and moderate hypertrophy were accompanied by lining with numerous osteoblasts with increased amounts of eosinophilic cytoplasm (osteoblast hypertrophy), while marked trabecular hypertrophy showed less of this effect. Osteoclast numbers were not affected. Cortical bone was thickened in all treated.
Sternum: Trabecular hypertrophy and cortical thickening in all treated

Bone marrow:

Femur: Decreased marrow space, although marrow spaces still present in diaphysis.
Sternum: In HD often complete filling of marrow space near end plates and limited marrow space in middle. In both bones, decreased amounts of adipose tissues in marrow. No apparent change in erythroid and myeloid cell proportions.
Pituitary: Minimal focal mineralization (0-0-0-1/14), Focal hyperplasia (4/14-3-1-0/14), Benign adenoma (1/14-0-1-2/14), Undifferentiated carcinoma (0-1-0-0)

Females:

Whole animal: no substantive tissue alteration (9-0-0-0)
Kidney: Multifocal mineralization, minimal (10-?-?-9) or slight (0-?-?-6). Progressive glomerulonephritis, minimal (7-?-?-11), slight (1-?-?-2)
Liver: Minimal extramedullary hematopoiesis (1-0-1-8)
Spleen: Extramedullary hematopoiesis, minimal (9-0-0-0) slight/moderate (3-12-7-4) or marked (0-3-8-11)
Ovary: Minimal cyst (0-?-?-3)
Vagina: Minimal acute diffuse inflammation (0-?-?-2), Minimal/slight/moderate mucification (3-?-?-7)
Bone:
Femur: Trabecular hypertrophy, slight (0-1-0-0), moderate (0-11-11-2) or marked (0-3-4-13). Slight and moderate hypertrophy were accompanied by lining with numerous hypertrophic osteoblasts, while marked trabecular hypertrophy showed less of this effect. Cortical bone thickening in all treated, especially in HD f. Sternum: Trabecular hypertrophy and cortical thickening in all treated
Bone marrow:
Femur: Decreased marrow space, although spaces still present in diaphysis.
Sternum: In HD often complete filling of marrow space near end plates and limited marrow space in middle. In both bones, decreased amounts of adipose tissues in marrow. No apparent change in erythroid and myeloid cell proportions.
Thyroid: Minimal follicular degeneration (0-?-?-3), Benign C-cell adenoma (0-?-?-1)
Adrenal: Benign unilateral pheochromocytoma (0-0-0-1)
Pituitary: Focal hyperplasia (1/14-1-0-0/14), Benign adenoma (1/14-1-3-0/14)

Endocrinology -

Males:

No difference in testosterone levels among all groups after 6 mo of dosing.

Females:

Methods: For 14 days before necropsy vaginal lavage samples were examined. End of estrus cycle was assigned to the day that the predominant cell type in the lavage was cornified cells, when followed by a day of predominantly leukocytes.

Results: Numbers of animals: in metestrus (2-3-0-1), diestrus I (2-2-2-1), diestrus II (2-2-2-0), proestrus (5-1-0-2), estrus (2-2-2-0), acyclic (1-5-8-7), or at undetermined stage (1-0-1-4). No obvious difference in estradiol levels between animals in/out (different) estrous cycle stages.

SUMMARY

- Vaginal bleeding in a few MD, HD f. Minimal BW decrease in HD m, associated with decreased EFU. Slight BW increase at all doses in f, associated with increased EFU and increased femur length.
- Hematologic changes:
Minimal decreases in erythrocyte count Hb, and PCV in MD, HD m and all treated f. Moderate decreases in leukocyte and lymphocyte ct in LD, MD, HD m. Slight decrease in neutrophil count in LD, MD, HD m and f, more in m than in f. Small increases in MCV, and MCH, and increase in reticulocyte count in LD, MD, HD f.
- Serum Ca, P, ALP:
Effects after 6 mo: Small increase in total Ca in LD, MD, HD m. Ca effect in f at any time less than in m, and no longer seen after 6 mo. Small increase in P in MD, HD m and HD f. Slight increase in ALP in m, at 6 mo only in HD.
- Other serum effects:
Small dose-related increase in serum globulin in m and f and small decrease in albumin. Small decrease in bilirubin in MD, HD m, and in HD f. Slight cholesterol increase in all treated f.
- Urine effects:
50-100% increase in Ca excretion in MD and HD, m and f. Slight P excretion increase in HD f. Also in HD f: increase in Na, K excretion (50%, 25%) and slightly increased urine osmolality.
- Organ weights:
Kidney: minimal increase in MD, HD m, and in HD f. Spleen: dose-related moderate increase in f. Pituitary: slight decrease in MD and HD f. Ovary: slight increase in HD f.
- Pathology:
Ovary cysts in some dosed f. Bone thickened in majority of MD, HD f. Spleen enlarged in 1 HD m, and in some MD, HD f.
- Histopathology:
Kidney: glomerulonephritis with high incidence in HD m, and slight multifocal mineralization in several HD f. Liver: Extramedullary hematopoiesis in LD, MD, HD f. Spleen: Extramedullary hematopoiesis in LD, MD, HD m,f. Bone: Trabecular and cortical hypertrophy in femur and sternum with dose-related incidence and gradation, most prominent in f, and proliferation of osteoblasts in femur. Bone marrow: marked decrease in marrow space in femur and sternum. Pituitary: Focal mineralization in 1 HD m, carcinoma in 1 LD m, adenoma in 1-1-3-0 f. Thyroid and adrenal: follicular degeneration in 3 HD f, C-cell adenoma in 1 HD f, pheochromocytoma in 1 HD f.
- Effects on estrous cycle:
Increased proportion of females become acyclic upon treatment (6-30-50-50%), and in some HD f cycle stage is undeterminable.

- Toxicokinetics:
 HD (100 ug/kg/day)
 Day 0-140:
 C_{max} 25 - 60 ng/ml
 T_{max} 0.25-0.5h
 AUC 20 -45 ng.h/ml
 T_{1/2} 25 min
 C_{max} and AUC slightly higher in m than in f.
 AUC higher on Day 140 than on Day 0 (ca. 2x)

EVALUATION

- BW effects may be endocrine-mediated. Sponsor offers explanation that in females PTH enhances IGF-1 expression, which may lead to increased femur length.
- Decreases in erythrocyte count, Hb and PCV, and decreases in neutrophil and lymphocyte counts supposedly are secondary to bone marrow changes. Effect on neutrophil/lymphocytes was not seen in 3-mo monkey study or 6-wk clinical trial.
- Serum and excretory Ca and P changes are consistent with direct/indirect pharmacological effects of PTH on kidney and intestine (latter through vitD), causing increased Ca/P uptake, increased urinary Ca/P excretion, and net serum Ca/P increase. Since histologic bone effects are more pronounced in f, it is puzzling why the ALP increase - which is probably due to increased bone formation - is observed only in m and not in f, and why serum Ca and P are less increased in f than in m. Hypercalcemia is clinically relevant toxicity.
- Changes in serum bilirubin, albumin and globulin, and cholesterol have unclear cause and significance. Globulin effect was not seen in 3-mo monkey study or 6-week clinical trial.
- Increased urinary Na and K excretion and urine osmolality in f may be related to kidney mineralization seen in f at this dose. Increased kidney weight in m and f is likely to be related to kidney histopathology. Kidney pathology warrants renal function test in clinical setting.
- Decrease in pituitary weight in treated f has unclear cause. Effect was not seen in 3-mo monkey study at doses up to 40 ug/kg.
- Dose-related trabecular and cortical hypertrophy is expected and desired effect of PTH. Females seem more sensitive than males. Reason for this is unclear, and is not a gender difference in exposure. Decreased bone marrow space is secondary to increased bone formation, and is probably the cause of decreased erythrocyte and other blood cell parameters. The effects on spleen weight, and spleen and liver histopathology reflect the compensatory response. The perturbation of blood cell formation may be a clinically relevant toxicity, especially in the course of long term treatment.
- Cause of estrous cycle effects is unclear, but may be endocrine. Clinical significance in target population of postmenopausal women is unclear.
- NOAEL is lower than 10 ukd (LD) based on hematology effects. At 10 ukd in rats, the blood level is 6 to 8 times the human level at 40 ug/day, the high dose in the phase III clinical trial.

CONCLUSION

Main effects observed in this 6-month rat toxicity study:

- Clinical pathology: Hypercalcemia and -phosphatemia
- Target organs: Bone, hematopoietic system, kidney, pituitary
- Other: Endocrine disturbances, possibly epigenetic carcinogenicity

A Chronic Toxicity Study of LY333334 Administered Subcutaneously to Cynomolgus Monkeys for 3 Months (Study #B96-020)
(Toxicology Report 11)

METHODS

Study Number: B96-020
 Duration: 3 months
 Live Phase: 22 April 1996 through 25 July 1996
 Test Article: LY333334
 Chemical Name: Biosynthetic human parathyroid hormone (1-34)
 Bulk lot: 101EM5 was used to prepare formulated lot PPD03757
 Initial Potency: 1.01 mg LY333334/vial
 The potency was assumed to be 1 mg LY333334/vial
 Species: Cynomolgus monkey (*Macaca fascicularis*)
 Initial Body Weights: Males: 2.87 to 3.89 kg
 Females: 2.61 to 3.52 kg
 Initial Age: Approximately 2.5 years
 Route of Administration: Subcutaneous injection
 Frequency of Administration: Once daily
 Number of Animals and Treatment Groups:

<u>Group Number</u>	<u>Test Compound</u>	<u>Dose of LY333334</u> (µg/kg/day)	<u>Animals/Group</u>
1	Vehicle ^a	0 ^b	4 M/4 F
2	LY333334	2	4 M/4F
3	LY333334	10	4 M/4F
4	LY333334	20	3 M/3F
5	LY333334	40	3 M/3F

^a Vehicle: 20 mM sodium phosphate monobasic in saline.

^b Controls: 0.4 mg mannitol/mL of vehicle.

This study was conducted in the Lilly Development Centre, Mont-Saint-Guibert, Belgium.

RESULTS

Mortality – None

Body weight/Food consumption – No treatment effects

Clinical signs – No treatment effects on signs, temperature, or reflexes

Electrocardiography – (Recorded at Pretest; Weeks 3,7,11, at 1-2h (1.5h) and at 24h postdose)

Males –

Increase in R-amplitude (unclear significance).

No effect on heart rate in any dose group, in any Week

No effect on PQ interval in any dose group, at any Week

No effect on QT or QTc interval in any dose group, at any Week

Females –

No effect on heart rate in any dose group, in any Week

No effect on PQ, QT(c) interval, or R-amplitude

		MALES					FEMALES				
Group		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	2	10	20	40	0	2	10	20	40
R (mV)	Pretest	0.7	0.7	1.0	0.9	1.1	0.7	0.5	0.7	0.7	0.3
	Wk3-1.5h	0.5	0.6	1.1	1.3*	1.1*	0.9	0.5	0.8	0.6	0.5
	Wk7-1.5h	0.6	0.7	1.1	1.1*	1.1*	0.7	0.4	0.8	0.6	0.6
	Wk11-1.5h	0.6	0.7	1.2*	1.2*	1.3*	0.7	0.5	0.8	0.7	0.5

*statistically significant effect

Heart rate (auscultation) - (Recorded at Pretest; Weeks 1,2,4,8,13, at 1-2h (1.5h) post dosing; and Weeks 1,13, at 24h post dosing)

Males – No effects

Females – No effects

Ophthalmology – No treatment-related effects

Hematology – (Determined at Pretest = Wk1, and Study Wks 2,6,13, prior to dosing)

RBC, Hgb, Hct: slight decrease in MD,HD males at Wk 13, and in MD, HD females at Wks 6,13

PT: slight increase in MD and HD males, at Wks 2,6,13 (significance unclear)

		MALES					FEMALES				
Group		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	2	10	20	40	0	2	10	20	40
RBC ct ($\times 10^6/\text{L}$)	Wk13	6.73	6.64	6.85	6.37	6.52	6.64	6.47	6.17	5.82*	5.79*
Hgb (g/L)	Wk13	140	139	135	132*	130*	133	136	125	118*	123*
Hct (L/L)	Wk13	0.44	0.44	0.43	0.42	0.42*	0.42	0.44	0.40	0.38*	0.39*
PT (sec)	Wk13	11.4	11.9	11.9	12.1*	12.0*	12.0	11.8	12.0	11.8	12.6

Ionized serum Ca (Determined at Pretest=Wk 2, and at Study Wks 2,4,6,10,13, at 0h,4h,8h post dosing):

Increase in males and females of all dose groups, from Wk 2, at 4h>8h postdose, throughout study. Increase in males and females appeared enlarged over time. Similar changes in pH-adjusted ionized Ca values.

		MALES					FEMALES				
Group		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	2	10	20	40	0	2	10	20	40
Ionized Ca (mmol/L)	Wk6 (4h)	1.45	1.56	1.59	1.62*	1.58	1.40	1.53	1.55*	1.57*	1.58*
	Wk13 (4h)	1.39	1.58*	1.60*	1.60*	1.60*	1.42	1.55	1.65*	1.68*	1.60*

Clinical Chemistry - (Determined at pretest = Wk1, and Wks 2,6,13, prior to dosing)

Total serum Ca – decreases in HMD, HD males and LMD, HMD, HD females from Wk2

Serum P – slight decrease in HD males and all dose females throughout study.

Serum urea – increase in some HD males and HMD females above historical control values in Wks 6,13

Glucose, potassium, chloride – decreases in Wk13 in HD females (18%,8%,3%, respectively as compared to control)

Group		MALES					FEMALES				
		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	2	10	20	40	0	2	10	20	40
Glucose (mmol/L)	Wk13	3.8	3.4	3.8	3.9	3.4	3.5	4.3	3.5	3.1	2.8*
K (mmol/L)	Wk13	4.5	4.3	4.1	4.3	4.3	4.3	4.3	4.0	4.1	3.9*
Total Ca (mmol/L)	Wk6	2.6	2.6	2.5	2.4*	2.3*	2.59	2.54	2.47	2.36*	2.32*
	Wk13	2.5	2.4	2.4	2.3*	2.3*	2.46	2.39	2.32*	2.25*	2.22*
Inorg P (mmol/L)	Wk13	2.0	1.9	1.7	1.9	1.8	1.9	1.8	1.5*	1.7	1.6*
Urea (mmol/L)	Wk13	5.3	5.6	6.1	5.7	6.9	5.8	5.2	6.3	6.1	7.0

Urinalysis (*Determined at pretest = Wk1, and Wks 6,12, from samples collected overnight*) -

No significant treatment-related effects on urine volume, osmolality, pH, glucose, protein, blood, ketone bodies, bilirubin, urobilinogen, urine sediment

Occult blood in feces – No treatment-related effects

Toxicokinetics -

Serum levels of immunoreactive LY were determined On Days 0 or 1, 7, 21, 51, 84 at 1h post dose, and on Days 51, 84 prior to dosing. In animals with LY-specific antibodies, serum LY levels were not reported.

Results:

- Male and female serum levels similar
- Serum concentrations @ 1h post dose: proportional to dose
- Pre-dose levels on Days 51, 84 undetectable in almost all animals
- No apparent accumulation after 3 months at any dose

Group		MALES + FEMALES (average)				
		ctrl	LD	LMD	HMD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	2	10	20	40
Serum concentration (ng/ml) @ 1h post dose	Wk1-13	BLQ	0.55	4.35	11.6	19.2
Human Cmax multiple*		-	3.4x	27x	73x	120x

BLQ=below level of quantitation

*Human Cmax at 20 ug daily dose = 159 pg/mL

The Tmax in monkeys is approximately 0.5h (results from 1-year monkey study). Thus the Cmax multiples in the Table above may be underestimated (Cmax values higher than 1h post-dose values)

Immunotoxicology – (*IgG measured at Pretest, and prior to dosing in Study Wks 2,4,6,8,10,13*)

During pretest 2 animals had measurable IgG levels.

During study, one HMD male and one HD male had quantifiable levels of anti-LY IgG during the treatment period and Day 84, respectively. Levels were similar to the pretest levels.

Thus, LY is not clearly immunogenic within 3 months.

Hepatic enzyme activity – (*measured at study termination*)

No induction of hepatic enzyme activity (EROD, BND, END) or cytochrome P450 content.

Organ weights –

Group		MALES					FEMALES				
		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD

Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	2	10	20	40		0	2	10	20	40
Kidney	Rel wt ($\% \times 10^2$)	44	42	46	51	45		43	47	41	56*	48*
Uterus	Abs wt (g)	-	-	-	-	-		7.7	8.6	6.7	10.2	9.8*
	Rel wt ($\% \times 10^2$)	-	-	-	-	-		24	27	21	32*	34*

Gross pathology –

Group	Dose ($\mu\text{g}/\text{kg}/\text{d}$)	MALES					FEMALES				
		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
		0	2	10	20	40	0	2	10	20	40
N animals		4	4	4	3	3	4	4	4	3	3
Uterus	Red discoloration						0	0	0	0	1
	Red deposit						0	1	0	0	0

Histopathology – (Numbers are incidence in 4 or 3 animals, unless indicated otherwise)

Group	Dose ($\mu\text{g}/\text{kg}/\text{d}$)		MALES					FEMALES				
			ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
			0	2	10	20	40	0	2	10	20	40
N animals			4	4	4	3	3	4	4	4	3	3
Bone (femur)	Increased trabecular bone formation	Minimal	0	2	1	0	0	0	4	0	0	0
		Slight	0	2	3	2	0	0	0	3	1	3
		Mod-Mkd	0	0	0	1	3	0	0	0	2	0
		Total	0	4	4	3	3	0	4	3	3	3
Kidney	Expanded medullary interstitium (bluish)	Minimal	0	0	3	1	1	0	3	2	1	2
		SI-Mod	0	0	0	2	1	0	0	0	2	1
		Total	0	0	3	3	2	0	3	2	3	3
	Cellular basophilia and epithelial regeneration	Minimal	0	0	0	2	0	0	0	2	0	2
		SI-Mod	0	0	0	0	1	0	0	0	2	0
		Total	0	0	0	2	1	0	0	2	2	2
	Tubular dilatation	Minimal	0	0	0	2	1	0	0	0	1	0
		Slight	0	0	0	0	1	0	0	0	0	1
		Total	0	0	0	2	2	0	0	0	1	1
	Medullary mineralization	Minimal	0	0	0	1	1	0	0	0	1	2
		Slight	0	0	0	0	0	0	0	0	1	0
		Total	0	0	0	1	1	0	0	0	2	2
Uterus	Active endometrium	-	-	-	-	-	0	1	1	0	2	
	Enlarged myometrium	-	-	-	-	-	0	0	0	1	0	

Bone change –

Femur: There was an increase in trabecular bone in the femur whose incidence and degree were dose-dependent. The change was characterized by denser and thicker trabecular meshwork with a decrease in intertrabecular space. The effect was especially seen in the metaphysis, but also in epiphysis and diaphysis. Rows of osteoblasts lined segments of trabecular bone.

Sternum: No change observed.

Cortical bone: There was no alteration in cortical bone of femur or sternum.

Renal changes – Incidence and degree appeared dose-related. Expanded medullary interstitium was seen in LD females, and in all other higher dose groups, in males and females. The expanded interstitium was basophilic, suggesting accumulation of ground substance, and was located in the outer stripe of the medulla (in LD and LMD), with extension into the medullary rays as the dose increased (HMD, HD). Epithelial regeneration was observed in collecting ducts and distal tubules of females and males in LMD (f), HMD and HD groups. Tubular dilatation in collecting ducts and tubules, and medullary mineralization were seen in HMD and HD males and females.

CONCLUSIONS

EKG - Slight increase in R amplitude in males at doses ≥ 10 ug/kg; No effect on heart rate, PQ or QT intervals

Hematology – Slight decreases in RBC, Hb, Hct at doses ≥ 20 ug/kg in both sexes at study end.

Increase in PT at doses ≥ 20 ukd in males throughout study

Ionized serum Ca – Increase in all dose groups at 4h and 8h post dose, similar in m and f, throughout study.

Clinical Chemistry – Slight dose-related decrease in total serum Ca and P, in m and f, at all doses throughout study; Increase in urea in some 20 (f) and 40 (m) ukd animals.

TK: No accumulation over 3 months; LY levels @1h post dose varied from 3.4x-120x Cmax levels in humans given a 20 ug daily dose. Tmax is appr. 0.5h, thus multiples probably underestimated.

No clear immunogenicity (LY anti-IgG)

No hepatic enzyme induction

Organ weights: Kidney relative weight increase in females only at 20 and 40 ukd; uterus abs and rel weight increase at 20 and 40 ukd

Histopathology: Femur/cancellous bone: Increased trabecular bone formation in (almost) all animals at all doses, with dose-related severity; trabeculae appeared denser and thicker; osteoblasts lined trabeculae.

Kidney: Dose-related nephropathy consisting of: Bluish expansion of medullary interstitium, with dose-related incidence and degree, in f at all doses ≥ 2 ukd, in m at doses ≥ 10 ukd. Effect was minimal in 3 out of 4 of the 2 ukd females. Localization of lesion extended with dose from outer stripe of medulla (at 2-10 ukd) into medullary rays (at 20-40 ukd). Lesion appeared to represent accumulation of ground substance. At higher doses there was epithelial regeneration of distal tubules/collecting ducts in about half of the animals at doses ≥ 10 ukd, more so in f than in m. Also seen were tubular dilatation and medullary mineralization in 20 and 40 ukd groups, again in about half of the animals. No inflammation was observed.

LOAEL was 2 ukd (3.4 x human Cmax), and was associated with minimal renal medullary interstitial expansion (f), slight increase in blood ionized calcium and decreases in total serum Ca and P (m and f), and increase in trabecular bone formation (m and f).

NOAEL was < 2 ukd

**APPEARS THIS WAY
ON ORIGINAL**

A Chronic Toxicity Study of LY333334 Administered Subcutaneously to Cynomolgus Monkeys for One Year (Study #B96-120)
(Toxicology Report 22)

METHODS

Study: B96-120
 Duration: 1 Year
 Live Phase: 11 February 1997 through 12 February 1998
 Test Article: LY333334
 Chemical Name: Recombinant human parathyroid hormone (1-34)
 Lot Number: Bulk lot: 101EM5 was used to prepare formulated lot PPD03757
 Initial Potency: 0.95 mg LY333334/vial
 The potency was assumed to be 1 mg LY333334/vial for formulation preparation
 Species: Cynomolgus monkey (*Macaca fascicularis*)
 Initial Body Weights: Males: 2.44 to 4.47 kg
 Females: 2.13 to 2.91 kg
 Initial Age: Approximately 2 years
 Route of Administration: Subcutaneous injection
 Frequency of Administration: Once daily
 Number of Animals and Treatment Groups:

Group Number	Test Compound	Daily Dose (µg/kg)	Animals/Sex/Group
1	Control ^a	0 ^b	4
2	LY333334	0.5	4
3	LY333334	2	4
4	LY333334	10	4

^a Controls: 4 mg mannitol/mL of vehicle.

^b Vehicle: 20 mM sodium phosphate monobasic in saline.

This study was conducted at the Lilly Development Centre, Mont-Saint-Guibert, Belgium.

RESULTS

Mortality – None

Body weight/Food consumption – No treatment effects

Signs – No treatment effects on signs, temperature, or reflexes

EKG – (Recorded at Pretest; Week 25 at (1-2h); Week 48 at (1-2h) and at 24h postdose) (1-2h reported as 1.5h)

Males –

Increase in heart rate in all dose groups, significant at Wk 25, MD and HD only

Decrease in PQ interval, in MD and HD, significant at Wk 25, HD

Decrease in QT interval at all doses in Wk 25, but not at Wk48; However, no effect on QTc (corrected QT interval)

Females –

Increase in heart rate in all dose groups, significant at Wk 25 and 4. However, also increase in MD and HD at pretest (Wk 2)

No other effects

Group		MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	0.5	2	10	0	0.5	2	10
Heart rate (bpm)	Pretest	238	242	248	241	225	228	258*	255*
	Wk25-1.5h	206	226	253*	241*	219	231	249*	246*
	Wk48-1.5h	220	233	246	244	208	235*	247*	241*
	Wk48-24h	220	229	242	237	215	239	251	231
PQ (ms)	Pretest	73	73	68	66	No effect			
	Wk25-1.5h	83	88	71	66*	No effect			
	Wk48-1.5h	82	79	76	74	No effect			
	Wk48-24h	80	86	76	74	No effect			
QT (ms)	Pretest	153	147	154	145	No effect			
	Wk25-1.5h	171	145*	143*	148*	No effect			
	Wk48-1.5h	160	161	156	145	No effect			
	Wk48-24h	166	158	153	150	No effect			
QTc (ms)	Any time	No effect				No effect			

*statistically significant effect

Ophthalmology – No treatment-related effects

Clinical pathology -

Sampling Timepoints

Samples for hematology, clinical chemistry, ionized calcium, and urinalysis (overnight and 24-hour) determinations were performed according to the following schedule:

Pretest	Hematology and Clinical Chemistry	Blood (Two and Calcium)	Urine (Overnight)	Urine (24-hour)
	Week 1	Weeks 1, 2	Week 3	Weeks 1, 4
Study	Weeks 4, 10, 24, 39, and 50	Weeks 5, 27, and 52	Weeks 14, 28, 41, and 69	Weeks 16, 24, 39, and 50

During Pretest Week 4, additional samples were collected in order to calculate the creatinine and ocular clearances.

Hematology – (Determined at pretest = Wk1, and Wks 4, 10, 24, 39, 50, prior to dosing)

Males -

RBC, Hgb, Hct – slight decrease in MD, HD at Wks 24, 39, 50, significant in some HD

Females -

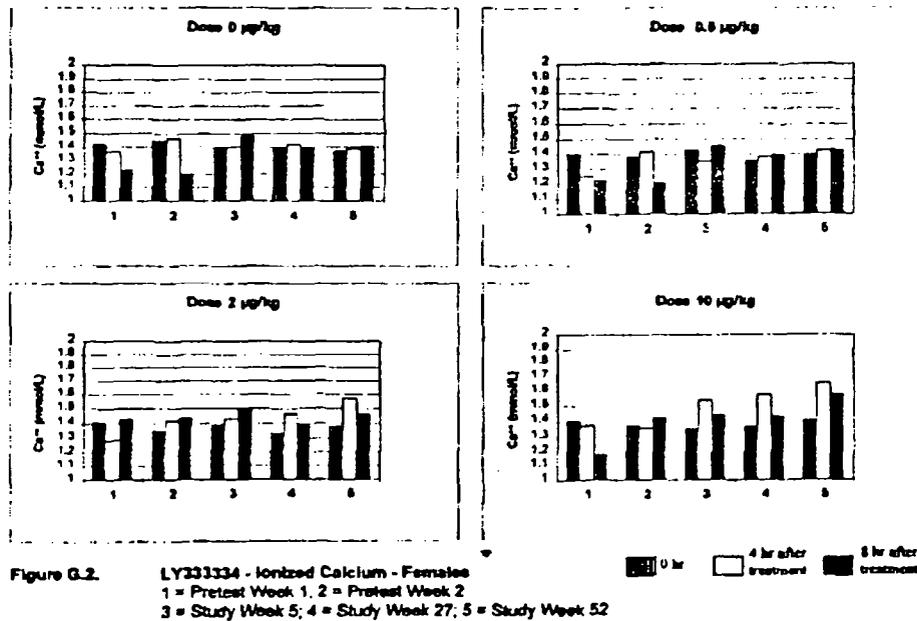
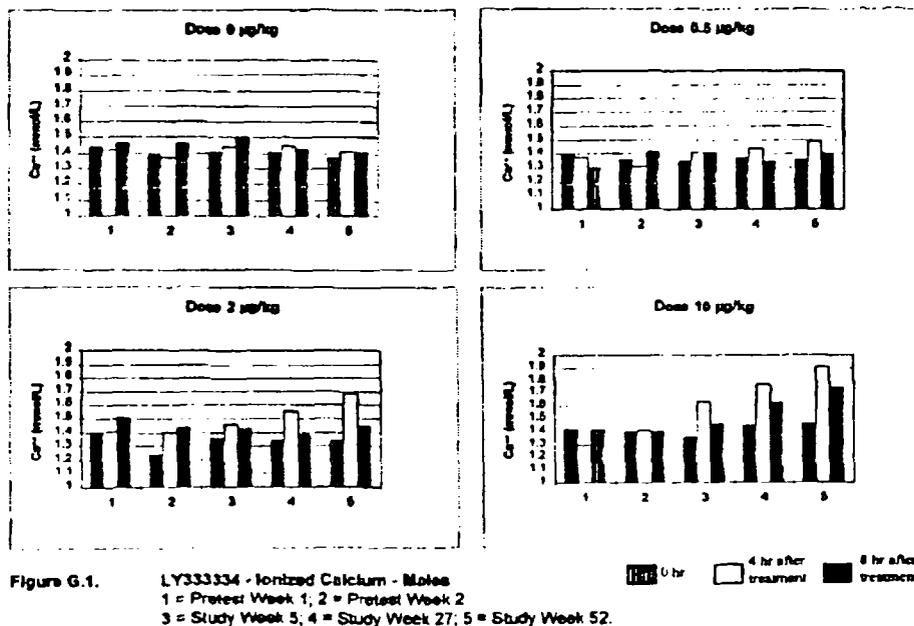
RBC, Hgb, Hct – slight decrease in MD, HD at Wks 10, 24, 39, 50, significant in HD

Group		MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	0.5	2	10	0	0.5	2	10
RBC ct ($\times 10^6/\text{L}$)	Wk24	6.60	6.75	6.36	6.41	6.79	6.55	6.59	5.94
	Wk39	6.63	6.84	6.72	6.48	6.80	6.60	6.60	5.81*
	Wk50	6.63	6.81	6.60	6.48	6.99	6.65	6.74	6.09*
Hgb (g/L)	Wk24	133	135	125	122*	131	128	126	118*
	Wk39	133	135	131	124	130	128	127	116*
	Wk50	138	140	134	128*	138	132	133	124*
Hct (L/L)	Wk10	0.45	0.46	0.46	0.44	0.46	0.49	0.45	0.42*
	Wk24	0.45	0.45	0.43	0.42	0.45	0.45	0.44	0.41*
	Wk39	0.45	0.44	0.45	0.42	0.45	0.45	0.44	0.39*
	Wk50	0.44	0.44	0.44	0.42	0.45	0.45	0.43	0.41*

Ionized serum Ca (Determined at pretest=Wks1, 2, and at study Wks 5, 27, 52, at 0,4,8h post dosing)

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Increase in males and females, all dose groups, from Wk 5, at 4h>8h postdose, throughout study, increasing over time in males (one male #97B-0014 highest value at Wks 27, 52). Similar changes in pH-adjusted ionized Ca values.



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Ionized serum Ca levels

Group		MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	0.5	2	10	0	0.5	2	10
Ionized serum Ca (mmol/L)	Wk52 (4h)	1.41	1.49	1.68	1.91*	1.39	1.42	1.57*	1.65*

Clinical Chemistry - (Determined at pretest = Wk1, and Wks 4, 10, 24, 39, 50, prior to dosing)

Total serum Ca – decreases in males and females in MD, HD until/through Wk39

Serum P – slight decrease (n.s.) in HD males and females from Wk10. Very low value in one HDm (#97B-0014). This animal also had high serum Ca.

Serum urea – increase in HD males at Wk 4, and in some individual MDf or HDm animals at later time points

Bilirubin - decrease in MD, HD females from Wks 10 to 39

Group		MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	0.5	2	10	0	0.5	2	10
Total Ca (mmol/L)	Wk10	2.45	2.46	2.37*	2.34*	2.45	2.46	2.37*	2.34*
	Wk39	2.52	2.56	2.48	2.70	2.56	2.51	2.50	2.40
Urea (mmol/L)	Wk4	5.1	5.5	5.2	7.2*	5.8	5.0	4.9	5.3
	Wk50	5.9	6.7	5.7	7.6	6.9	5.8	7.3	6.5
Bilirubin ($\mu\text{mol}/\text{L}$)	Wk24	2.5	1.8	2.0	1.5	2.8	2.3	1.8*	1.5*
	Wk39	2.3	1.3	1.5	1.3	2.5	1.8	0.5*	1.0*

Urinalysis - (samples collected after 20 ml/kg fluid administration, either overnight or over 24h) -Overnight sampling:

Volume- increased in HD males, with concomitant decreases in urine osmolality, Na, K, creatinine from Wk 10 on. Total electrolyte and creatinine excretion were not affected. At a few time points, similar effects occurred in HD females.

24-hour sampling:

Volume – (males) increased at various time points from Wk10

Electrolytes – (males) decreases in Na, K, throughout study

Ca concentration – No effect, both sexes

Creatinine levels – (males) decreases throughout study

Calcium excretion (24h) – increased in 0h-6h and 6h-24h interval post fluid administration, in HD males and females from Wk10 to end of study. Effect statistically significant, mainly in males, in most 6h-24h periods.

Osmolal clearance and creatinine clearance (24h) – decreases (in normalized values) in HD males at Wk 24 and/or later.

Note: One male(#97B-0014) had diluted urine, decreased electrolyte and creatinine excretion and clearances, indicating renal function impairment

Group		MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	0.5	2	10	0	0.5	2	10
Volume (ml) (overnight)	Wk14	74	103	73	115	82	83	85	94
	Wk41	103	137	98	172*	124	90	121	125
Osmolality (mmol/kg) (o/n)	Wk14	788	621	691	514	555	491	497	431*
	Wk41	667	505	681	421	518	438	467	521
Volume (ml) (6-24h)	Wk50	42	45	49	72*	46	46	50	57
Osmolality (mmol/kg) (0-24h)	Wk50	689	665	634	486*	588	583	563	586

Na (mmol/L) (0-24h)	Wk50	91	78	87	79	83	74	81	85
K (mmol/L) (0-24h)	Wk50	54	58	47	44	39	37	37	37
Ca (mmol/L) (6-24h)	Wk50	15	18	25	22	10	5	12	10
Creatinine (umol/L) (0-24h)	Wk50	8500	8694	7313	4778*	7088	6274	5985	7067
Ca excretion (mmol, 6-24h)	Wk10	0.26	0.42	0.30	0.66*	0.10	0.16	0.14	0.16
	Wk24	0.33	0.26	0.40	0.71*	0.07	0.17	0.39*	0.55*
	Wk50	0.69	0.77	1.20	1.57*	0.46	0.25	0.60	0.55
Osmolal CL (0-24h) (mL/min/kg)	Wk24	0.045	0.048	0.046	0.036*	0.042	0.045	0.043	0.047
	Wk50	0.049	0.042	0.049	0.049	0.049	0.051	0.053	0.048
Creatinine CL (0-24h) (mL/min/kg)	Wk24	3.07	3.02	3.14	2.31	2.94	3.24	3.12	3.42
	Wk50	3.14	2.50	2.84	2.45*	2.82	2.94	2.99	3.19

Pharmacokinetics -

Serum levels of immunoreactive LY were determined during pretest, and in Wks 2, 26, 51, at 0.5, 0.75, 1, 7 hours after dosing. In animals with LY-specific antibodies, serum LY levels were not reported

Results:

- Male and female serum levels similar
- No apparent accumulation after 1 year at any dose
- No apparent change in Cmax or Tmax over 1 year of dosing
- Tmax: 0.53h post dose, independent of dose
- Cmax: linear and proportional to dose

Group		MALES				FEMALES				M+F			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/d)		0	0.5	2	10	0	0.5	2	10	0	0.5	2	10
Cmax (ng/ml)	Wk2	-	0.15	0.87	5.11	-	0.13	0.84	4.00	-	0.14	0.85	4.56
Tmax (h)	Wk2	-	0.5	0.56	0.5	-	0.5	0.5	0.5	-	0.5	0.53	0.5
Cmax	Wk26	-	0.18	0.71	6.72	-	0.20	0.64	3.20	-	0.19	0.67	4.61
Tmax	Wk26	-	0.5	0.63	0.5	-	0.5	0.63	0.58	-	0.5	0.63	0.55
Cmax	Wk51	-	0.11	0.58	4.05	-	0.17	0.68	Nc	-	0.13	0.63	4.05
Tmax	Wk51	-	0.5	0.5	0.5	-	0.5	0.5	Nc	-	0.5	0.5	0.5

Nc=not calculated

Group		MALES + FEMALES (avg)			
		ctrl	LD	MD	HD
Dose (µg/kg/d)		0	0.5	2	10
Cmax (ng/ml)	Wk2-51	-	0.15	0.72	4.41
Human Cmax multiple*		-	0.94x	4.5x	28x

*Human Cmax at 20 ug daily dose = 159 pg/mL

Immunotoxicology – (IgG measured at pretest, and Wks 24, 50)

Dose- and time-dependent increase in incidence

Dose ($\mu\text{g LY333334/kg/day}$)	Response Range (rel. $\mu\text{g IgG/mL}$)	Incidence ^a	
		Week 24	Week 50
0 ^b	BQL ^c	0/8	0/8
0.5	BQL to	2/8	2/8
2	BQL to	0/8	2/8
10	BQL to	3/8	6/8

- ^a Incidence = number of quantifiable responders/total number of animals.
- ^b Vehicle control.
- ^c BQL = Below Quantifiable Limits.

Organ weights -

Group	Dose ($\mu\text{g/kg/d}$)	MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
		0	0.5	2	10	0	0.5	2	10
Kidney	Rel. wt ($\% \times 10^4$)	39	38	39	51*	36	42	41	42
Pituitary	Rel. wt ($\% \times 10^4$)	1.2	1.2	1.6	2.0*	1.8	1.9	2.0	1.7
Thymus	Rel. wt ($\% \times 10^4$)	9.3	15	10	10	6.6	9.8	9.4	14*

Gross pathology -

Group	Dose ($\mu\text{g/kg/d}$)	MALES				FEMALES			
		ctrl	LD	MD	HD	ctrl	LD	MD	HD
		0	0.5	2	10	0	0.5	2	10
Thymus	Red discoloration, or red spot	0	0	0	0	0	1	1	0

Histopathology - (Numbers are incidence in 4 animals, unless indicated otherwise)

Group	Dose ($\mu\text{g/kg/d}$)	N examined		MALES				FEMALES			
				ctrl	LD	MD	HD	ctrl	LD	MD	HD
				0	0.5	2	10	0	0.5	2	10
				4	4	4	4	4	4	4	4
Brain	White matter mineralization		Minimal	0	0	1	1	0	0	0	0
Sternebra	Increased trabecular bone		Minimal	0	0	1	3	0	2	2	2
			Slight	0	0	0	0	0	0	2	2
			Total	0	0	2	4	0	2	4	4
Femur	Increased trabecular bone		Minimal	0	2	1	0	0	3	1	2
			Slight	0	0	1	2	0	0	2	1
			Moderate	0	0	1	2	0	0	1	1
			Total	0	2	3	4	0	3	4	4
	Physis degeneration			0	1	0	1	0	0	0	0
Kidney	Expanded basophilic medullary interstitium (multifocal)		Minimal	0	0	0	0	0	0	1	1
	Expanded basophilic medullary interstitium (diffuse)		Minimal	0	0	0	0	0	1	0	1
			Slight	0	0	0	1	0	0	0	0
			Moderate	0	0	0	2	0	0	0	1
			Total	0	0	0	3	0	1	1	3
	Tubular/interstitial mineralization, multifocal, medulla and cortex		Minimal	0	0	0	3	0	1	1	2
Thymus	Hemorrhage		Minimal to slight	0	0	0	0	0	1	1	0

Bone change -The incidence and degree of the increase in trabecular bone in femur and sternebra was dose-dependent. The change was characterized by denser and thicker trabecular meshwork

with a decrease in intertrabecular space. Numerous osteoblasts lined segments of trabecular bone. There was no alteration in cortical bone.

Kidney lesions – Incidence and degree appeared dose-related. Expanded medullary interstitium was basophilic, suggesting accumulation of ground substance, and was located in the outer stripe of the medulla, with extension into the medullary rays generally increased with dose. All but one HDf with expanded interstitium also had foci of mineralization in the medulla and the cortex, sometimes adjacent to collecting ducts or tubules. The foci were Von Kossa-positive, indicating the presence of calcium. Male #97B-0014 had diffuse expanded interstitium and tubular/interstitial mineralization.

Thymus – The cause of hemorrhage in drug-treated animals was unclear.

Brain – White matter mineralization in 1 MDm and 1 HDm may have been drug-related. The affected HDm also had diffuse expanded interstitium and mineralization in the kidney.

**APPEARS THIS WAY
ON ORIGINAL**

A Special Study to Assess Renal Function in Cynomolgus Monkeys Given LY333334 [rhPTH (1-34)] by Subcutaneous Injection for Approximately 4 Months with a 3-Month Reversibility Period (Study P01597)
(Toxicology Report 27)

METHODS

Study: P01597

Live-phase duration/dates:

Phase	Duration	Dates
Treatment Phase	4 months (119 days)	22 October 1997 to 18 February 1998
Reversibility Phase	3 months (92 days)	18 February to 21 May 1998

Test Article: LY333334

Chemical Name: Recombinant human parathyroid hormone(1-34). This chemical name is abbreviated as rhPTH(1-34)

Lot Number: PPD03521

Assigned Potency: 0.96 mg/vial

Species: Monkey, cynomolgus

Initial body weights: 2.8 to 6.3 kg

Initial age: Young adult

Route of administration: Subcutaneous injection

Frequency of administration: Once daily

Number of animals: Group 01: 4 females

Group 02: 8 females

Treatment group:

Group	LY333334 (µg/kg/day)
01	0 ^a
02	40

^a Placebo = Mannitol Parenteral in a vehicle of 20 mM sodium phosphate buffer in 0.9% Sodium Chloride for Injection, USP.

RESULTS

Survival -

One treated animal (Monkey #83972) was euthanized on Day 114. This animal was removed from treatment on Day 83 (@ ca. 2.5 months) due to severe clinical signs of renal failure. Following discontinuation of LY333334 administration, the animal recovered clinically and was euthanized on Day 114 (@ ca. 4 months) after being evaluated for urinary concentrating capability.

Body Weight -

Not included in this report (used for dose volume calculations only).

Food Consumption -

Monkey 83972: Decrease during third month of treatment (described as 25% to 100% food remaining by visual examination).

Clinical Observations -

Monkey #83972: Severe clinical signs secondary to renal failure including emesis, decreased activity, decreased food consumption, and/or decreased elasticity of skin (dehydration) from

Days 77 through 99. Animal was removed from treatment on Day 83 and became clinically normal by Day 100 following administration of supportive therapy.
Other animals: normal throughout treatment (119 days) and reversibility phase (92 days).

Physical Evaluation -

Monkey #83972: Dehydration, secondary to renal failure, requiring daily supportive treatments until euthanasia on Day 114. Therapeutic intervention included frequent parenteral administration of balanced electrolyte solutions.
Other animals: No remarkable findings at the end of treatment or reversibility phase.

Toxicokinetics -

Serum levels of immunoreactive LY were determined On Days 29 and 89 at 1h post dose

- Levels in control animals undetectable (BLQ)
- Levels on Day 89 much lower than on Day 29 (interference?)

Group	Day	N	Control	LY
Dose ($\mu\text{g}/\text{kg}/\text{d}$)			0	40
Serum concentration (ng/ml) @ 1h post dose (range)	29	8	BLQ	18.55* (10.3-39.1)
	89	7	BLQ	4.68 (0.18-8.94)

BLQ=below level of quantitation

* Level in animal with renal failure and marked nephropathy (#83972)

Level in animal with moderate nephropathy (#83702):

Levels in animals with slight nephropathy (#84852, 83522):

Level in animal with no nephropathy (#85682):

ng/ml

ng/ml)

and

ng/ml)

Pathology Findings in Surviving Animals (Individual data reported only) -

Group	Dose ($\mu\text{g}/\text{kg}$)	Number of Monkeys Evaluated		
		Interim Termination	Treatment Termination	Reversibility Termination
01	0		2	2
02	40	1	4	3

Terminal Necropsy Dates: 13 February 1998 (interim termination,

Monkey 83972 only). 18 February 1998 (treatment termination).

and 21 May 1998 (reversibility termination).

Hematology – (Determined at Pretreatment Day-29 and Day-2; at 2 and 4 months during treatment, and at 1.5 and 3 months during reversibility phase)

RBC, Hgb, Hct: minimal decreases in treated animals during treatment phase.

Serum Ionized Calcium - (Determined at 0h, 4h, 8h, 24h post dosing, at 1, 3, and 4 months during treatment phase)

Slight to marked increase in blood ionized calcium at 4h > 8h post dosing. Increase was 10%-50% compared to 0h baseline value. In one treated animal (#83702), baseline values @ 4 months were increased by appr. 60% compared to values @ 3 months. This monkey had moderate nephropathy.

Note: Values for euthanized Monkey #83972 beyond 1 month (normal increase @4h postdose) were not available since LY treatment was discontinued at 2.5 months.

Clinical Chemistry –

Health Screen (e.g. Glu, Chol, Trig, Alb, BUN, ALP) (Determined at Pretreatment Day-29 and Day-2; at 2 and 4 months during treatment, and at 1.5 and 3 months during reversibility phase)

Monkey #83702: Minimal-to-slight increase in BUN and total serum calcium, and decrease in IP at end of treatment phase (termination of animal). This monkey was diagnosed with moderate nephropathy.

No remarkable effects in other surviving animals.

Renal Function Assessment (Creat, Na, K, Cl, Osm) (Determined at Pretreatment; at 1.5-2 and 3-3.5 months during treatment, and at 1 and 3 months during reversibility phase)

No remarkable findings in surviving animals.

Urinalysis - (Determined at Pretreatment; at 2 and 4 months during treatment, and at 1.5 and 3 months during reversibility phase)

Health Screen -

No treatment-related effects in surviving animals (e.g. Color, Sp Gr, pH, Prot, Glu, Bld)

Renal Function Tests - (Determined at Pretreatment; at 1.5-2 and 3-3.5 months during treatment, and at 1 and 3 months during reversibility phase)

Urinary Acidification Test (pH, Osmolality)- (During 4-6h following nasogastric administration of 0.1g/kg ammonium chloride, and water deprivation) (adequate: pH \leq 5.5)

Slight decrease in average renal acidification capability in treated vs. control surviving animals [pH values @6h: control: 4.92 (n=4); treated 5.63 (n=7)]. Highest values in treated (7.08 and 5.78) occurred in animals with slight nephropathy (#84852 and #83522). However, animal with moderate nephropathy had relatively low value of 5.38. Thus, the relation to treatment is not entirely clear. Reversibility also unclear.

Urinary Concentration Test (Specific Gravity, Osmolality)- (During 2-4h following s.c. administration of 0.4 ug/kg desmopressin, and water deprivation)

No effect of treatment on renal concentration capability in surviving animals.

General Renal Function Test (Vol, Osm, Na, K, Cl, Creat) - (During 20h following nasogastric administration of 0.45% NaCl, and water and food deprivation)

No significant effects on creatinine and osmolality clearance rates or fractional excretion of electrolytes (Na, K, Cl) in surviving animals.

Note:

In the euthanized animal (Monkey#83972) all renal function tests were normal through Month 2. In Monkey #83702 (animal with moderate nephropathy) acidification and concentration ability were normal. However, the general renal function test in this animal showed the lowest values for clearance rates and fractional electrolyte excretion of all animals/all days on Day 93.

Organ Weights -

Minimal increases in relative kidney weight in 5 of 7 treated animals that survived until end of treatment or reversibility phase.

Ranges for relative kidney weight:

Treated: 528 to 607 mg/100g body weight (5 of 7 animals)

Controls: 384 to 508 mg/100 g body weight

Relative kidney weight (mg/100g)

	Control	LY
Dose (ug/kg/day)	0	40
Day 119	452 (N=2)	528 (N=3)*
Day 211	436 (N=2)	519 (N=3)

*one value/organ missing

Note: Kidney weight was not clearly correlated to degree of histopathologic changes in individual animals (listed below)

Gross Pathology -

Group		control	treated
Dose (ug/kg/d)		0	40
Treatment phase (119 days)			
(N animals)		(2)	(5)
Kidney	Whole tissue alteration (pallor/mottling)	2	2

<i>Reversibility phase (211 days)</i>			
(N animals)		(2)	(3)
Kidney	Whole tissue alteration	0	0

Histopathology -**Kidney: Nephropathy****Description of renal lesion(s):**

- medullary interstitial expansion characterized by deposition of pale basophilic material
- multifocal interstitial inflammation
- multifocal mineralization
- multifocal tubular regeneration
- multifocal tubular dilation and inflammation

Grading:

- Minimal: changes were barely detectable and limited to the outer stripe of the medulla
- Slight: changes occurred in the outer stripe of the medulla with minimal extension into the medullary rays
- Moderate: changes occurred in the outer stripe of the medulla with prominent involvement of the medullary rays.
- Marked: extensive involvement of the outer stripe of the medulla, medullary rays, and limited extension into the superficial cortex.

Group		control	Treated
Dose ($\mu\text{g}/\text{kg}/\text{d}$)		0	40
<i>Treatment phase (119 days)</i>			
(N animals)		(2)	(5)
Kidney	No lesions		1
	Slight nephropathy	0	2
	Moderate nephropathy	0	1
	Marked nephropathy	0	1*
<i>Reversibility phase (211 days)</i>			
(N animals)		(2)	(3)
Kidney	Minimal nephropathy	0	3

*Finding in Monkey #83972 that had renal failure and was euthanized on Day 114

- In most animals the medullary interstitial basophilic expansion was accompanied by variable degrees of multifocal mineralization of the medulla, minimal-to-slight interstitial inflammation. Histochemical staining suggested that the basophilic material was a non-sulfated acidic mucopolysaccharide (Alcian blue-positive). Minimal to slight tubular regeneration was seen in two animals with moderate or marked nephropathy, and tubular dilation and inflammation was seen in the one animal with moderate nephropathy.
- At the end of the reversibility phase renal changes included minimal expansion of the medullary interstitium in 3 of 3 monkeys and minimal inflammation and mineralization in 2 of 3 monkeys. In these monkeys the amount of pale basophilic material in the medullary interstitium was marginally increased above that normally observed in the renal medulla. Thus, the nephropathy appeared to be reversible as evidenced by a reduction in the severity and extent of the lesions at the end of the reversibility phase.

**Pathology Findings in Monkey 83972 with Renal Failure -
Monkey #83972:**

- Treatment-related changes: marked azotemia, hypercalcemia (66% increase in serum total calcium on Day 78, compared to mean baseline value), hyposthenuria, polyuria, and dehydration.
- Clinical chemistry: Marked increases in BUN, total calcium and creatinine; increase followed by decrease in inorganic P; and decrease in Na and Cl between Days 78 and Days 89-98. Marked increase in ALP on Days 89 and 93.
- Urinalysis (Days 82 through 113): Slight-to-moderate proteinuria, aciduria, and urine sediment findings of slight-to-moderate numbers of erythrocytes, leukocytes, epithelial cells and/or granular casts, consistent with renal injury.
- Upon treatment discontinuation (Day 83): Clinical recovery, and return of clinical chemistry parameters to normal limits
- Water deprivation test (Day 113): Improvement in urinary concentrating capability.
- Necropsy findings (Day 114): Marked renal histologic changes including medullary interstitial expansion, interstitial inflammation, mineralization, and tubular regeneration. Changes occurred in the outer stripe of the medulla with extension along medullary rays and into the superficial cortex.

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SUMMARY AND EVALUATION OF MONKEY TOXICOLOGY STUDIES

Cardiovascular effects in chronic monkey toxicity studies

Study	Doses (ug/kg/day)	EFFECTS			
		Heart rate	Arterial blood pressure	Other findings	ECG findings
3-month	0, 2, 10, 20, 40	No effect	Not determined	Serum ionized Ca level increased post dosing	R-amplitude increased at doses ≥ 10 ug/kg, 1.5h post dosing (Weeks 3-11, males only)
1-year	0, 0.5, 2, 10	Increased at doses ≥ 2 ug/kg, 1.5h post dosing (Week 25, males only)	Not determined	Serum ionized Ca level increased post dosing	PQ-interval decreased at 10 ug/kg, 1.5h post dosing (Week 25, males only); QT-interval decreased at all doses, 1.5h post dosing (Week 25, males only) No effects on QTc-interval

The ECG effects in monkeys are of unclear significance, since they occurred only in males and the values of the affected parameters generally remained within the range of pretest values.

Nephrotoxicity (clinical pathology findings) in monkey toxicity studies

	3-month toxicity study	1-year toxicity study	4-month reversibility study
Doses	0, 2, 10, 20, 40	0, 0.5, 2, 10	0, 40
N/sex/group	3 or 4	4	4 (control), 8 (treated) (females only)
Ionized serum calcium	Increased at all doses at 4-8h post-dosing	Increased at all doses at 4-8h post-dosing	Increased at 40 ug/kg at 4-8h post-dosing
Serum urea	Increased in some animals at 20 and 40 ug/kg	Increase in males at 10 ug/kg	Increase in animal with moderate nephropathy
Urinalysis	No effects on measured parameters	Increase in volume at 10 ug/kg in males	No effects on measured parameters
Kidney weight (relative to body)	Increased at 20 and 40 ug/kg in females	Increased at 10 ug/kg in males	Increased at end of treatment or reversibility period
Renal histopathology	<ul style="list-style-type: none"> Expanded basophilic medullary interstitium at ≥ 2 ug/kg in females, and at doses ≥ 10 ug/kg in males Cellular basophilia and epithelial regeneration at ≥ 10 ug/kg in females and at ≥ 20 ug/kg in males Tubular dilation and medullary mineralization at ≥ 20 ug/kg in both sexes 	<ul style="list-style-type: none"> Expanded basophilic medullary interstitium at ≥ 0.5 ug/kg in females, and at 10 ug/kg in males Tubular/interstitial mineralization at ≥ 0.5 ug/kg in females and at 10 ug/kg in males 	<ul style="list-style-type: none"> Slight to marked nephropathy in 4/5 animals at end of treatment phase (Marked lesions in animal with renal failure) Minimal nephropathy in 3/3 animals at end of reversibility phase
Renal function		<ul style="list-style-type: none"> Increase in Ca excretion at ≥ 2 ug/kg in both sexes 	<ul style="list-style-type: none"> Renal failure in 1/8 animals treated with 40 ukd (Day 78). Animal was sacrificed prematurely No remarkable effects on renal acidification or concentration ability, or general renal function (creatinine or osmolality clearance) in surviving animals

Dose (ug/kg)	Human C _{max} multiples*	
	3-month study	1-year study
0.5	-	0.94x
2	3.4x	4.5x
10	27x	28x
20	73x	-
40	120x	-

*Human C_{max} at 20 ug daily dose = 159 pg/mL

The incidence and degree of the renal lesions in the 3-month and 1-year study were dose-related. The medullary interstitial expansion was associated with increased deposition of extracellular matrix. This lesion was limited to the outer medulla in the lowest dose groups, but extended into the medullary rays at higher doses. It is unclear if (and which) renal lesions were indirectly due to a stimulation of calcium reabsorption in the distal nephron by LY333334, underlying the observed increase in serum ionized calcium, or if the lesions were due to a direct effect of LY333334 on the renal tissue.

The nephropathy in the 4-month reversibility study consisted of medullary interstitial expansion with deposition of basophilic material, multifocal interstitial inflammation, multifocal mineralization, multifocal tubular regeneration, and multifocal tubular dilation and inflammation. These changes were similar in nature but more extensive than the ones seen in the 3-month toxicity study. Except for one animal with overt renal failure they were not accompanied by renal function deterioration. The histologic lesions in the surviving animals and the impaired renal function in the affected animal were at least partially reversible.

The NOAEL values in the 3-month and 1-year studies were <2 ug/kg, and <0.5 ug/kg, respectively, based on the histologic finding of basophilic expansion of the renal medullary interstitium in the lowest dose groups. The doses of 2 and 0.5 ug/kg represent multiples of the human C_{max} (at the 20 ug daily human dose) of approximately 3.5x and 1x.

The histologic finding of expanded medullary interstitium in the 1-year study occurred at a dose level equivalent to the human 20 ug daily dose and the NOAEL for this finding was not determined. This may indicate a clinical concern for nephrotoxicity. Renal function disturbance was not observed at the lower dose levels used in the 1-year study (1x-5x human C_{max}). However, renal function impairment was suggested by the decreases in creatinine and osmolal clearance rates at the high dose, and by the increased serum urea nitrogen levels in some animals at the mid and high dose (5x-28x human C_{max}) used in this study. Thus, although the results from renal function tests in the 4-month reversibility study at a relatively high dose (>100x human C_{max}) did not indicate renal function impairment, there is a potential clinical concern for renal toxicity after long treatment with LY333334.

In the long term bone quality study in aged (> 9 yrs) ovariectomized monkeys, at doses of 1 and 5 ug/kg, LY333334 (1-8x human AUC at 20 ug/day), no treatment-related renal histopathology changes were observed. This negative result may have been due to the lower Ca content of the diet used in the pharmacology study (0.3% calcium, corresponding to 1734 mg Ca/2000 calories) as compared to the toxicity studies (0.7-0.9% calcium).

CARCINOGENICITY

A carcinogenicity study was carried out by the Sponsor in F344 rats, by administering daily subcutaneous injections of PTH(1-34) for 24 months and evaluating all tissues at the end of the treatment for neoplasm formation.

An Oncogenic Study in Fischer 344 Rats Given LY333334 by Subcutaneous Injection for 2 Years (Studies R00397 and R00497)

(Toxicology Report 36)

GENERAL

Study Title: An Oncogenic Study in Fischer Rats given LY333334 by Subcutaneous Injection for 2 Years
Study Duration: 739 days (males), 743 days (females)
Species and strain: Rat (Fischer 344)
Dosing Route: Subcutaneous injection
Study Number(s): R00397 (males) and R00497 (females)
Study Report: Toxicology Report 36
Volume Numbers: NDA Volumes 1.30-1.38 (Item 5)
Testing Facility: Eli Lilly and Company, Greenfield, Indiana, US
Study Period: April 1997-May 1999
Date of Submission: November 29, 2000 (NDA Submission)
QA Report: Yes
Dose-range-finding study: 6-month toxicity study and 1-year pharmacology study

STUDY PROTOCOL

Species/strain: Rat (F344/NTac)
Number of animals: 60/sex/dose group
Age at start of study: 6 to 7 weeks
Weight at start of study: 100-142g (males), 74-109g (females)
Animal housing: Individually
Animal diet: Certified Rodent Diet 5002 (_____ and tap water, both ad libitum.
Drug Name: LY333334 (teriparatide; rhPTH1-34)
Drug Lot Number(s): PPD03482 (Bulk Lot 067EM5) and PPD04231 (Bulk Lot 026NT8)
Drug Analysis: Periodically during testing phase
Drug Stability: Stable in solution for 24h at room temperature or 5°C.
Dosage form: Solution for s.c. injection
Dosing route: S.C. injection, in the dorsal lumbar area.
Dosing frequency: Once daily
Dose Volume: 1 mL/kg
Dose Solution: Prepared daily at 0, 5, 30, 75 ug/mL (pH 4.40-4.45). Control solution also contained Mannitol Parenteral at 3 mg/mL, the approximate concentration of mannitol present in the dosing solution used for the high dose group.
Vehicle: 20 mM sodium phosphate monobasic (NaH₂PO₄) in 0.9% Sodium Chloride Injection, USP.

Doses:

Group	Designation	N/sex/group	Dose Volume (ml/kg)	Dose (ug/kg/day)
01	Control	60	1	0
02	LD	60	1	5
03	MD	60	1	30
04	HD	60	1	75

Relation to Clinical Use:
CAC Concurrence:

Recommended dose: 20 mg daily dose, by s.c. injection
 Exec CAC recommended doses of 10, 30, 100 ug/kg/day, after Sponsor initially proposed doses of 4, 20, 40 ug/kg/day. Sponsor's dose selection was based on limiting kidney toxicity in a 1-year pharmacology study. Sponsor subsequently selected doses of 5, 30, 75 ug/kg/day, with the high dose based on a 25x animal: human AUC ratio. Concurrence of the Exec CAC with the final dose selection was not obtained.

Interim Sacrifices:
Clinical Observations:

None
 Animals were observed daily for general condition, and weekly (up to 13-17 weeks) or every 2 weeks (after 13-17 weeks) for a more detailed evaluation.

Body Weight:

Rats and food cups were weighed weekly (up to 14-16 weeks) and approximately every 2 weeks thereafter. Body weight, food consumption and efficiency of food utilization (EFU) were calculated as cumulative data.

Food Consumption:
Clinical Pathology:

See "Body Weight"
 Blood samples for evaluation of clinical chemistry and hematology parameters were obtained from moribund animals, and from all surviving animals at study termination after o/n fast. Results on clinical pathology were reported as individual data only.

Morphologic Pathology:

All animals were necropsied, including those that died or were killed moribund. Scheduled necropsies were done on Days 732 through 736 for males, and Days 739 through 743 for females. Tissue specimens were taken from all animals from the tissues listed in the histopathology inventory (appended), and sections were examined from all tissues, from all animals. Final diagnosis status was assigned to lesions that were treatment-related, non-treatment-related but significant to the animals' health, nonneoplastic proliferative lesions, and neoplastic lesions. Gross pathology and histopathology incidence tables were provided for all animals combined (decedents and survivors).

Peer Review:

The initial histopathology evaluation was carried out by two Lilly pathologists. A peer review was performed for all nonosseous tissues by a third Lilly pathologist. A peer review of all bone proliferative lesions was performed by _____

Immunotoxicology:

_____ The results represent the pathologists' consensus. Serum samples collected for TK analysis were also analyzed for LY-333334-specific IgG antibody using an ELISA. Serum samples from all surviving animals at 24 mo were also collected for immunotoxicological evaluation. The ELISA was a sandwich type, and the concentration of LY-specific rat IgG in the samples was reported as "relative" ug/mL because the reference curve was construed using a capture antibody rather than the specific antigen (LY). The QLA (quantitative limit of assay) was _____ relative ug IgG/ml.

Quantitative Bone Analysis:

Analyses were performed in a non-GLP laboratory (Lilly Research

Laboratories, IN). Femoral length, width and weight were measured using calipers and a balance. Femora and L-6 vertebrae from premature decedents and surviving animals were analyzed for BMD by QCT. Histomorphometry of proximal femur was carried out for 6/sex/group. Biomechanical properties of femoral diaphysis, femoral neck and L-6 vertebra were determined for rats surviving to treatment termination. Osteocalcin was determined in sera from surviving animals by RIA.

Toxicokinetics:

Blood was collected from 4/sex/group per sampling time point, at 6, 12, 18 months, and serum concentrations of LY were determined by validated IRMA (immunoradiometric assay).

Statistical Evaluation:

Sponsor's evaluation:

Mortality data: Tarone's one-sided test.

Neoplastic lesions: Survival-adjusted trend test (Peto et al, 1980).

CDER evaluation:

See CDER Biometrics Review (APPENDIX II). There was no statistical evaluation of clinical pathology or non-neoplastic findings.

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RESULTS

Mortality/Survival

SEE FIGURE 1

- Males: Small decrease in survival (increased mortality) in HD, not statistically significant (Sponsor's analysis)
- Females: Statistically significant decrease in survival, i.e., increase in mortality (Sponsor's analysis). This trend remained significant when animals with fatal neoplasm(s) were excluded from analysis.

SURVIVAL – Analysis of all animals

Group	Dose (ug/kg/day)	MALES		FEMALES	
		Number (%)	Number (%)	Number (%)	Number (%)
01	Control	0	17/60 (28%)	28/60 (47%)	
02	LD	5	20/60 (33%)	29/60 (48%)	
03	MD	30	17/60 (28%)	24/60 (40%)	
04	HD	75	14/60 (23%)	21/60 (35%)	
Trend test			P=0.092	P=0.024*	

SURVIVAL – Analysis excluding animals that died of fatal neoplasms

Group	Dose (ug/kg/day)	MALES		FEMALES	
		Number (%)	Number (%)	Number (%)	Number (%)
01	Control	0	17/28 (61%)	28/29 (97%)	
02	LD	5	20/28 (71%)	29/33 (88%)	
03	MD	30	17/30 (57%)	24/27 (89%)	
04	HD	75	14/29 (48%)	21/29 (72%)	
Trend test			P=0.081	P=0.009*	

Figure 1 indicates that in addition to a decreased survival in HD males and MD and HD females, there was also a treatment-related effect on the time of death, with more deaths occurring earlier in the MD and HD groups of both sexes.

Mortality/Cause of death

The most common causes of death were neoplastic and were pituitary adenoma, large granular lymphocytic leukemia and osteosarcoma.

Animals that died of fatal neoplasms (data for all neoplasms, and three most common neoplasms)

Grp	Dose (ug/kg/day)	MALES					FEMALES					
		Deaths due to					Deaths due to					
		Neoplas m(s)	Osteo sarco ma	Pituitary adenoma	LGLL	Other	Neopl asm(s)	Osteo sarco ma	Pituitary adenoma	LGLL	Other	
01	Ctrl	0	32	0	13	16	3	31	0	15	13	3
02	LD	5	32	1	14	13	4	27	3	13	9	2
03	MD	30	30	12	11	5	2	33	6	16	7	4
04	HD	75	31	22	5	2	2	31	8	6	9	8

LGLL= Large granular lymphocytic leukemia

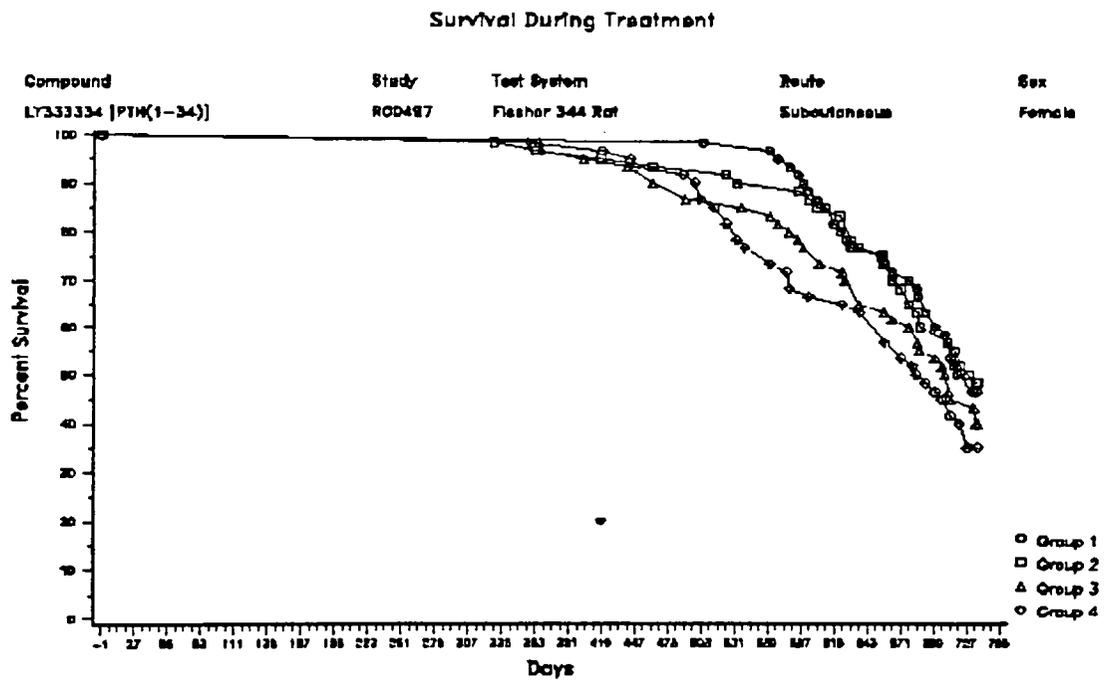
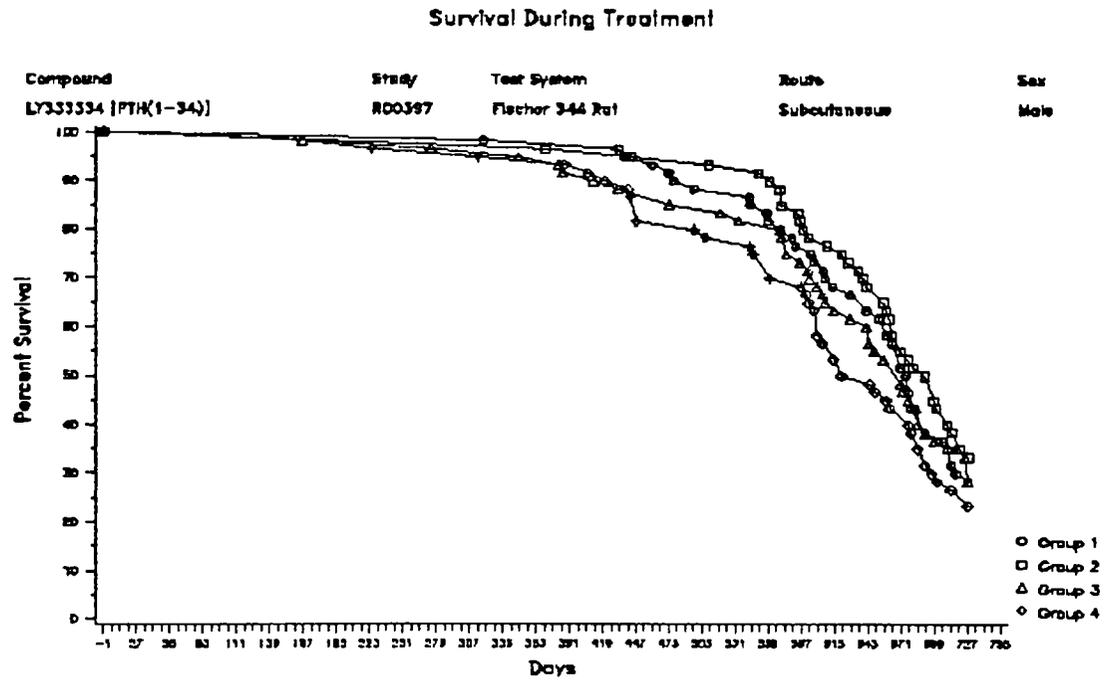


Figure 1 Survival During Treatment

Clinical Observations

LD, MD, HD: Neurological signs, dose-related, generally associated with bone neoplasms
 HD: Rough haircoat, thinness, scabs on tail, generally in second year
 MD, HD males: Leg nodules, mostly in last 6 months of study

Body Weight, Food Consumption and EFUSEE FIGURE 2**MALES: Statistically significant changes (%) as compared to controls after 1 year**

	Control	LD	MD	HD
Body weight (BW) (g)	-			-3.8%
Body weight gain (g)	-			-5.2%
Food consumption (FC) (g/day)	-	+2%	+2.9%	+2.5%
EFU (g/100g)	-		-4.0%	-7.4%

FEMALES: Statistically significant changes (%) as compared to controls after 1 year

	Control	LD	MD	HD
Body weight (BW) (g)	-	+4.5%	+9.0%	+10.4%
Body weight gain (g)	-	+8.5%	+15.4%	+17.3%
Food consumption (FC) (g/day)	-	+2.7%	+5.4%	+6.9%
EFU (g/100g)	-	+5.8%	+9.3%	+10.0%

Note that, although food consumption was slightly increased in both sexes, the efficiency of food utilization (EFU) was affected differently in males (negative) than in females (positive).

Clinical Pathology

(Individual data reported only)

Hematology

No obvious effects on RBC, Hb, Hct, MCV, MCH, MCHC, WBC, leukocyte differential counts, or coagulograms

Clinical chemistry (data from moribund killed animals only)

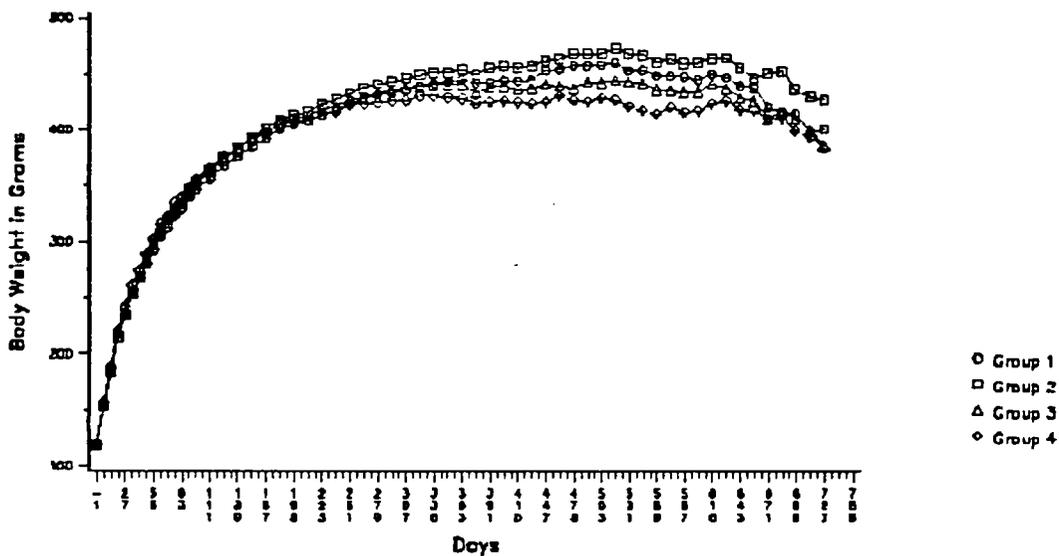
- Overall apparent increase in serum glucose in males
- An increase in serum ALP would be expected due to stimulation of the osteoblast by PTH. However, the data from the moribund animals did not clearly show this. Partly this was because in all dose groups some animals had marked increases in ALP (up to 15-fold). In the control group these animals had a malignant neoplasm such as LGLL or lymphoma, and in the HD (and MD) groups they had osteosarcoma or another tumor such as LGLL. However, several animals with osteosarcoma had no obviously increased ALP values. Thus, there appeared to be no clear correlation between the occurrence of osteosarcoma and serum ALP values. In conclusion, data were insufficient to draw any solid conclusions about serum ALP in non-tumor and tumor-bearing animals.
- No obvious effects on lipids, proteins, BUN, creat, Ca, P, Na, K, Cl, CPK, T Bili, ALT, AST, GGT. Values for some parameters were highly variable (AST, TRIG).

Organ Weights:

No data

Mean Body Weight

Compound	Study	Test System	Route	Sex
LY333334 [PTH(1-34)]	R00397	Flecher 344 Rat	Subcutaneous	Male



Mean Body Weight

Compound	Study	Test System	Route	Sex
LY333334 [PTH(1-34)]	R00497	Flecher 344 Rat	Subcutaneous	Female

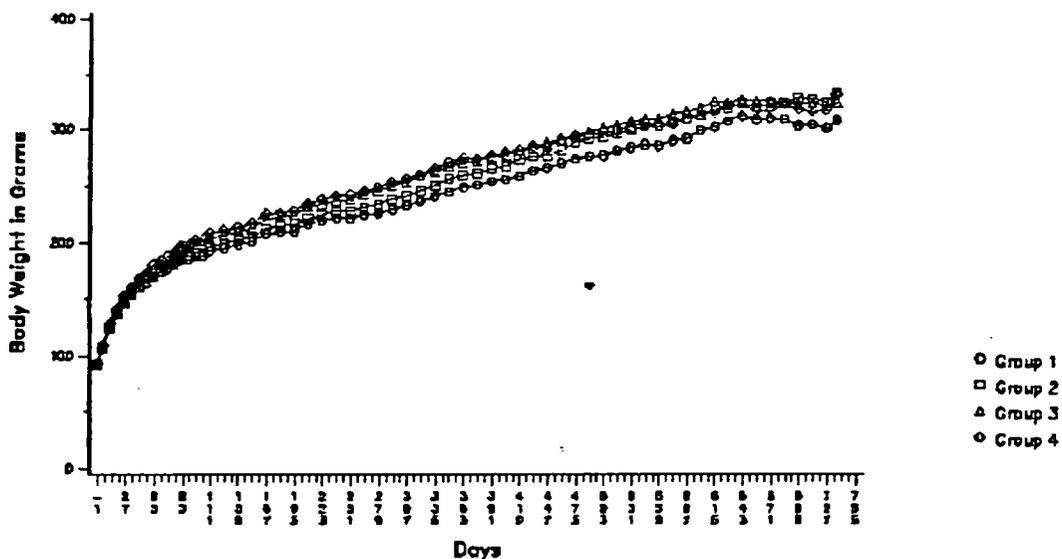


Figure 2 Mean Body Weight During Treatment

Gross pathology:

Findings (Total incidence in all animals, N=60, unless indicated otherwise)

Group		Males				Females			
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Whole animal	Thinness	20	19	30	35	27	21	29	33
	Rough hair coat	28	43	46	55	8	8	19	41
	Scabs on tail	21	30	29	38	8	8	13	15
Kidney	Enlarged	5	7	11	13	0	0	0	0
Urinary bladder	Distention	0	1	3	7	1	4	5	3
Liver	Nodule	1	1	2	4	0	0	1	0
Lung	Nodule	0	2	1	5	0	1	1	1
Spleen	Enlarged	27	18	13	7	24	20	22	21
Testis	Enlarged	24	19	19	10	-	-	-	-
Cervix	Enlarged	-	-	-	-	4	4	12	18
Skin	Nodule	7	6	6	7	1	2	4	4
Bone	Nodule	0	1	17	20	0	3	7	12
	Lesion	1	0	0	3	1	0	1	3
	Whole tissue alteration	8	20	43	44	2	12	46	48

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Histopathology**NON-NEOPLASTIC FINDINGS**

Findings (Total incidence in all animals, N=60, unless indicated otherwise, if N <55)

		Male s					Fema les					Clear drug- related effect*
Group		Ctrl	LD	MD	HD		Ctrl	LD	MD	HD		
N examined		60	60	60	60		60	60	60	60		
Kidney	Chronic progressive nephropathy (DDD in m)	59	51	58	46		59	50	58	48		M
	Pelvic mineralization, minimal to moderate (DDD)	19	42	48	49		8	18	24	39		M,F
	Tubular mineralization, minimal to moderate (DDD in m)	44	53	57	57		20	16	14	21		M
Urinary bladder	Epithelial hyperplasia, minimal to moderate, NO DDD	1	0	3	4		2	0	1	2		M
Liver	Extramedullary hematopoiesis, minimal to slight, (multi)focal, NO DDD	0	1	5	4		10	21	26	29		M,F
Heart	Vascular mineralization, minimal to marked, (multi)focal, NO DDD	0	2	7	7		0	1	2	1		M
	Fibrous cardiomyopathy, or endocardial fibrosis, slight	0	0	0	0		0	0	2	3		
Lung	Vascular mineralization, minimal to slight (f) or moderate (m), (multi)focal NO DDD	12	20	26	26		13	8	14	17		M
	Fibrosis, pleural or septal, slight to moderate	0	0	0	0		0	0	1	2		
Spleen	Increased extramedullary hematopoiesis, minimal to marked (DDD)	23	42	54	55		26	39	46	35		M,F
	Increased hemosiderosis, minimal to moderate (DDD)	0	0	0	0		3	2	3	7		F
Thymus	Epithelial hyperplasia, slight	1	0	0	5		0	0	0	0		
Pancreas	Acinar atrophy, minimal to marked, multifocal (NO DDD!)	22	25	32	36		4	3	8	11		M,F
Ovary	Mineralization, minimal, (multi)focal	0	0	0	0		0	0	1	2		
Mammary gland	Lobular hyperplasia, minimal to moderate, (NO DDD)	0	0	0	0		11/60	21/57	20/59	18/58		F
Bone (stemum)	Cystic cartilaginous degeneration, minimal to severe (DDD in f)	29	24	33	36		23	33	37	47		F
	Osteoblast hyperplasia, slight to marked	0	0	1	1		0	1	1	3		M,F
	Trabecular hypertrophy, minimal to marked	0	50	60	60		14	58	58	60		M,F
Femur	Osteoblast hyperplasia, slight to moderate, focal	0	0	1	0		0	1	0	0		
	Trabecular hypertrophy, minimal to marked	0	60	60	60		17	60	58	58		M,F
	Cartilaginous epiphyseal proliferation, slight	0	0	1	0		0	0	0	0		
	Cartilaginous physeal proliferation, slight	0	0	0	1		0	0	0	0		
Vertebra	Osteoblast hyperplasia, slight	0/48	1/49	0/49	0/42		0	0	0	0		
	Trabecular hypertrophy,	1/48	43/4	47/4	41/4		9/52	52/53	46/48	44/47		M,F

	minimal to marked		9	9	2						
Tibia	Osteoblast hyperplasia, slight to moderate	0	0	0	3		0	0	0	0	
	Trabecular hypertrophy	0	0	0	0		0	0	0	0	
Bone marrow	Hypercellularity, minimal to marked (NO DDD)	12	26	51	52		1	0	0	0	M
Adrenal	Cystic degeneration, minimal to slight, (multi)focal/diffuse	0	2	2	3		0	0	0	0	M
	Medullary hyperplasia, slight	2	3	5	8		3	0	0	4	M
	Cortical hyperplasia, minimal to moderate, (multi)focal, (NO DDD)	3	4	6	5		4	6	5	8	
Thyroid	C-cell hyperplasia, minimal to severe (DDD)	42	43	45	42		47	47	48	44	M,F
Eye	Scleral ossification, minimal to moderate, (multi)focal (DDD)	50	55	57	60		38	44	51	54	M,F

DDD = dose-dependent degree

* = dose-dependent increase in incidence or dose-dependent degree of finding

Incidence of most common fatal non-neoplastic events

Animals that died prematurely due to fatal event

Group		Males				Females			
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Kidney	Chronic progressive nephropathy	4	5	10	4	0	0	0	0
Urinary tract	Inflammatory urinary obstruction	0	0	0	3	0	0	0	0
Non-apparent event		4	4	1	9	1	2	2	3

Non-neoplastic, non-hyperplastic findings with dose-dependent degree in bone

Group		Males				Females			
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Sternum	Trabecular hypertrophy								
	Minimal to slight	0	46	12	5	7	25	2	1
	Moderate	0	12	45	45	6	27	25	15
	Marked to severe	0	0	3	10	1	6	31	44
	TOTAL	0	58	60	60	14	58	58	60
Femur	Trabecular hypertrophy								
	Minimal to slight	0	43	0	0	13	12	0	0
	Moderate	0	16	23	0	2	41	10	2
	Marked to severe	0	1	37	60	2	7	48	56
	TOTAL	0	60	60	60	17	60	58	58
Vertebra	Trabecular hypertrophy								
	Minimal to slight	1	32	10	4	8	17	1	1
	Moderate	0	9	33	19	0	30	18	8
	Marked to severe	0	2	4	18	1	5	27	35
	TOTAL	1/48	43/49	47/49	41/42	9/52	52/53	46/48	44/47
Sternum	Cystic cartilaginous degeneration								
	Minimal	2	2	3	2	12	10	7	6
	Slight	27	22	30	34	6	18	20	26
	Moderate	0	0	0	0	5	5	10	13
	Marked to severe	0	0	0	0	0	0	0	2
	TOTAL	29	24	33	36	23	33	37	47

Non-neoplastic, non-hyperplastic findings with dose-dependent degree in tissues other than bone

Group		Males				Females				Drug-related effect
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD	
N examined		60	60	60	60	60	60	60	60	
Kidney	Chronic progressive nephropathy									
	Minimal to slight	17	7	4	9	42	41	42	45	
	Moderate	28	29	24	35	8	5	8	3	
	Marked to severe	14	22	31	14	1	0	0	0	
	TOTAL	59	51	58	46	59	50	58	48	M
	Pelvic mineralization									
	Minimal	15	28	24	7	7	15	16	24	
	Slight	4	15	23	40	1	3	7	15	
	Moderate	0	0	1	2	0	0	1	0	
	TOTAL	19	42	48	49	8	18	24	39	M,F
Tubular mineralization	Minimal	42	50	47	39	19	16	14	21	
	Slight	2	0	6	17	1	0	0	0	
	Moderate	0	3	4	1	0	0	0	0	
	TOTAL	44	53	57	57	20	16	14	21	M
	Spleen	Increased extramedullary hematopoiesis								
Minimal	12	11	7	2	12	4	3	2		
Slight	10	29	32	36	12	19	14	7		
Moderate	1	2	11	16	1	14	19	22		
Marked to severe	0	0	1	1	1	3	10	14		
TOTAL	23	42	54	55	26	39	46	35	M,F	
Increased hemosiderosis	Minimal	0	0	0	0	1	1	0	1	
	Slight	0	0	0	0	2	1	2	5	
	Moderate	0	0	0	0	0	0	1	1	
	TOTAL	0	0	0	0	3	2	3	7	F
Eye	Scleral ossification									
	Minimal	6	3	1	1	31	34	29	16	
	Slight	44	52	56	59	7	10	22	37	
	Moderate	0	0	0	0	0	0	0	1	
TOTAL	50	55	57	60	38	44	51	54	M,F	

Hyperplastic findings in bone and other tissues

Group		Males				Females			
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Bone (sternum)	Osteoblast hyperplasia								
	Slight	0	0	1	0	0	1	0	1
	Moderate	0	0	0	0	0	0	0	1
Femur	Osteoblast hyperplasia								
	Slight	0	0	0	0	0	1	0	0
	Moderate	0	0	1	0	0	0	0	0
Vertebra	Osteoblast hyperplasia								
	Slight	0	1	0	0	0	0	0	0
Tibia	Osteoblast hyperplasia								
	Slight	0	0	0	2	0	0	0	0
	Moderate	0	0	0	1	0	0	0	0
Whole animal	Osteoblast hyperplasia								
	Slight	0	1	1	2	0	2	0	1
	Moderate	0	0	1	1	0	0	0	1

	Marked	0	0	0	1	0	0	1	1
	TOTAL	0	1	2	4	0	2	1	3
Urinary bladder	Epithelial hyperplasia								
	Minimal	1	0	0	0	1	0	1	0
	Slight	0	0	2	4	0	0	0	0
	Moderate	0	0	1	0	1	0	0	2
	TOTAL	1	0	3	4	2	0	1	2
Adrenal	Medullary hyperplasia								
	Slight	2	3	5	8	3	0	0	4
	TOTAL	2	3	5	8	3	0	0	4
Thyroid	C-cell hyperplasia								
	Minimal to slight	32	36	31	27	41	34	31	30
	Moderate	9	6	13	12	5	10	14	12
	Marked to severe	1	1	1	3	1	3	3	2
	TOTAL	42	43	45	42	47	47	48	44
Thymus	Epithelial hyperplasia								
	Slight	1	0	0	5	0	0	0	0
Mammary gland	Lobular hyperplasia								
	Minimal	0	0	0	0	5	9	9	8
	Slight	0	0	0	0	6	12	10	10
	Moderate	0	0	0	0	0	0	1	0
	TOTAL	0	0	0	0	11/60	21/57	20/59	18/58

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NEOPLASTIC FINDINGS

Findings (Total incidence in all animals, N=60, unless indicated otherwise, if N <55)

Group		Males				Females			
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Whole animal	Osteoblastoma (B)	0	0	2	7	0	1	1	3
	Osteoma (B)	0	0	2	1	0	0	0	1
	Osteosarcoma (M)	0	3	21	31	0	4	12	23
	Large granular lymphocytic leukemia (M)	24	18	6	5	23	22	14	12
Kidney	Osteosarcoma (MS)	0	0	1	2	0	0	0	0
Liver	Osteosarcoma (MS)	0	0	2	7	0	0	0	0
Heart	Osteosarcoma (MS)	0	0	1	1	0	1	0	0
Pericardium	No. examined	0	0	0	1	0	0	1	0
	Osteosarcoma (MS)				1			1	
Aorta	Osteosarcoma (MS)	0	0	2	1	0	0	0	0
Lung	Alveolar/bronchiolar adenoma	0	1	1	1	0	0	0	0
	Alveolar/bronchiolar carcinoma	0	1	0	0	0	0	0	0
	Osteosarcoma (MS)	0	0	10	18	0	1	1	2
Pleura	No. examined	0	0	0	1	0	0	0	0
	Osteosarcoma (MS)	-	-	-	1	-	-	-	-
Mediastinum	No. examined	0	1	1	2	0	0	0	0
	Osteosarcoma (MS)	-	0	1	2	-	-	-	-
Spleen	Osteosarcoma (MS)	0	0	4	3	0	0	0	0
Thymus	Osteosarcoma (MS)	0	0	3	1	0	0	0	0
Pancreas	Islet cell adenoma	4	7	2	5	2	0	2	0
	Islet cell carcinoma	2	1	1	3	1	0	1	0
	Osteosarcoma (MS)	0	0	1	0	0	0	0	0
Stomach	Squamous papilloma (B)	0	0	0	0	0	0	0	1
Jejunum	No. examined	54	54	58	51	58	59	58	56
	Leiomyosarcoma	1	0	0	1	0	0	0	0
Rectum	Osteosarcoma (MS)	0	0	0	0	0	0	0	1
Peritoneum	No. examined	2	1	1	1	0	0	0	0
	Mesothelioma (M)	2	1	1	0	-	-	-	-
Mesentery	No. examined	3	5	7	3	4	0	0	1
	Mesothelioma (M)	1	2	2	0	0	-	-	0
	Osteosarcoma (MS)	0	0	0	1	0	-	-	0
Testis	Interstitial cell tumor (B)	42	37	34	36	-	-	-	-
	Osteosarcoma (MS)	0	0	1	0	-	-	-	-
Ovary	Granulosa cell tumor (M)	-	-	-	-	1	1	1	0
Uterus	Endometrial stromal polyp	-	-	-	-	17	11	8	17
	Endometrial stromal sarcoma (M)	-	-	-	-	3	0	0	0
	Leiomyoma (B)	-	-	-	-	0	0	1	0
	Leiomyosarcoma (M)	-	-	-	-	0	0	2	0
Vagina	Osteosarcoma (MS)	-	-	-	-	0	0	1	1
Skin	Basal cell tumor (B)	3	0	1	2	0	1	0	0
	Basal cell carcinoma (M)	0	0	0	0	0	0	0	1
	Squamous cell papilloma (B)	1	2	1	3	0	2	1	1
	Squamous cell carcinoma (M)	0	0	2	1	0	0	0	0
	Keratoacanthoma (B)	0	1	1	0	0	0	0	0
	Sebaceous adenocarcinoma (M)	0	1	0	1	0	0	0	0
	Fibroma (B)	4	1	1	2	0	0	0	0
	Sarcoma (M)	1	0	0	0	0	0	0	0
	Neurofibrosarcoma (M)	0	0	0	0	0	0	1	1
	Osteosarcoma (MS)	0	0	0	1	0	0	0	0

Subcutis	<i>No. examined</i>	0	1	1	0	1	1	0	2
	Fibroma (B)	-	1	0	-	0	0	-	0
	Lipoma (B)	-	0	1	-	0	0	-	0
	Osteosarcoma (MS)	-	0	0	-	0	0	-	1
Mammary gland	<i>No. examined</i>	48	52	51	48	60	57	59	58
	Adenoma (B)	0	0	0	0	2	1	1	2
	Adenocarcinoma (M)	0	0	0	0	2	2	1	1
	Cystadenoma (B)	0	0	1	0	0	0	0	0
	Fibroadenoma (B)	0	1	1	1	10	19	10	15
	Squamous cell carcinoma	0	0	0	0	1	0	0	0
	Fibroma (B)	0	0	0	0	0	1	0	0
	Lipoma (B)	0	0	0	0	0	1	0	0
Preputial gland	<i>No. examined</i>	0	3	5	1	-	-	-	-
	Adenocarcinoma	0	1	1	0	-	-	-	-
Clitoral gland	<i>No. examined</i>	-	-	-	-	1	1	2	3
	Adenoma	-	-	-	-	0	0	0	1
	Carcinoma	-	-	-	-	0	1	1	1
	Squamous cell carcinoma	-	-	-	-	0	0	0	1
Harderian gland	Fibrosarcoma (M)	0	0	0	1	0	0	0	0
Zymbal's gland	<i>No. examined</i>	0	0	2	0	1	0	0	0
	Adenocarcinoma (M)	0	0	2	0	0	-	-	-
Skeletal muscle	Fibrosarcoma (M)	0	1	0	0	0	0	0	0
	Liposarcoma (M)	1	0	0	0	0	0	0	0
	Osteosarcoma (MS)	0	0	0	2	0	0	0	0
Diaphragm	<i>No. examined</i>	0	1	1	1	0	0	0	0
	Osteosarcoma (MS)	0	0	1	1				
Bone (sternum + other sites)	Osteoblastoma (B)	0	0	0	0	0	1	0	1
	Osteosarcoma (M)	0	0	5	8	0	0	3	2
Femur	Osteoblastoma (B)	0	0	1	4	0	0	0	0
	Osteosarcoma (M)	0	1	3	5	0	0	2	7
Rib	<i>No. examined</i>	0	0	5	4	0	1	2	4
	Osteoma (B)	0	0	1	0	-	0	0	0
	Chondrosarcoma (M)	0	0	1	0	-	0	0	0
	Osteosarcoma (M)	0	0	3	4	-	1	2	4
Vertebra	<i>No. examined</i>	48	49	49	42	52	53	48	47
	Osteoma (B)	0	0	1	1	0	0	0	1
	Liposarcoma (M)	1	0	0	1	0	0	0	0
	Osteosarcoma (M)	0	0	3	6	0	2	5	5
Tibia	<i>No. examined</i>	1	3	14	22	0	0	1	7
	Osteoblastoma (B)	0	0	1	3	-	-	1	2
	Osteosarcoma (M)	0	2	12	14	-	-	0	5
Pelvis	<i>No. examined</i>	0	0	0	1	0	1	2	0
	Osteosarcoma (M)	0	0	0	1	-	1	2	-
Adrenal	Adrenocortical adenoma	2	1	2	4	1	2	1	0
	Adrenocortical adenocarcinoma	0	0	0	0	0	0	1	0
	Pheochromocytoma (B)	8	8	6	6	3	1	1	0
	Pheochromocytoma (M)	0	0	1	0	1	1	1	0
	Osteosarcoma (MS)	0	0	2	1	0	0	0	0
Thyroid	Adenoma (B)	1	0	0	0	0	0	0	0
	C-cell adenoma	0	2	1	3	6	7	8	9
	C-cell carcinoma	1	0	0	1	2	3	1	1
	Follicular cell adenoma (B)	1	1	1	1	0	0	0	0
Parathyroid	Adenoma (B)	0	2	0	1	0	0	0	0
Pituitary	Adenoma	38	42	30	27	40	46	42	29
	Carcinoma	1	0	0	0	2	1	0	0
Ear	<i>No. examined</i>	0	0	1	0	0	0	0	1
	Sebaceous adenoma (B)	0	0	1	0	0	0	0	0
	Osteosarcoma (M)	0	0	0	0	0	0	0	1
Mass 1	<i>No. examined</i>	1	0	0	2	0	0	0	0
	Osteosarcoma (M)	0	0	0	2	-	-	-	-
Mass 2	<i>No. examined</i>	0	0	0	1	0	0	0	0
	Osteosarcoma (M)	0	0	0	1	-	-	-	-
Misc tissue	<i>No. examined</i>	1	0	1	0	0	0	0	1

	Sarcoma (M)	1	0	0	0	-	-	-	0
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(B) = benign; (M) = malignant; (MS) = metastatic

**APPEARS THIS WAY
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Incidence of Frequent Neoplasms in Male Rats (R00397)

Dose ($\mu\text{g}/\text{kg}$)	0	5	30	75	One-Sided Peto's Trend Test ^a on Neoplasm Onset
Number of Animals	60	60	60	60	
Whole Animal					
Osteoma			2	1	$p=.1494^b$
Osteoblastoma			2	7	$p=.0001^{bc}$
Osteosarcoma		3	21	31	$p<.0001^d$
Large granular lymphocytic leukemia	24	18	6	5	$p=.9999^e$
Lung					
Alveolar/bronchiolar adenoma		1	1	1	$p=.2519^b$
Pancreas					
Islet cell adenoma	4	7	2	5	$p=.5000$
Islet cell carcinoma	2	1	1	3	$p=.2978^b$
Testis					
Interstitial cell tumor	42	37	34	36	$p=.5224$
Skin					
Basal cell epithelioma	3		1	2	$p=.6391^b$
Fibroma	4	1	1	2	$p=.8007^b$
Papilloma	1	2	1	3	$p=.1874^b$
Squamous cell carcinoma			2	1	$p=.1124^b$
Mammary Gland					
Fibroadenoma		1	1	1	$p=.2161^b$
Adrenal					
Adrenocortical adenoma	2	1	2	4	$p=.1356^b$
Pheochromocytoma	8	8	6	6	$p=.5718$
Thyroid					
C-cell adenoma		2	1	3	$p=.0553^b$
Follicular cell adenoma	1	1	1	1	$p=.5521^b$
Parathyroid					
Adenoma		2		1	$p=.4517^b$
Pituitary					
Adenoma	38	42	30	27	$p=.9509$
Mesentery and Peritoneum					
Mesothelioma, malignant	2	3	2		$p=.8889^b$

^a With continuity correction.

^b Result based on exact permutation trend test for site/neoplasm combinations with incidence of 10 or less.

^c Statistically significant increasing trend in the 75- $\mu\text{g}/\text{kg}$ group.

^d Statistically significant increasing trends in the 30- and 75- $\mu\text{g}/\text{kg}$ groups.

^e Statistically significant decreasing trends in the 30- and 75- $\mu\text{g}/\text{kg}$ groups ($p \geq 0.995$).

Incidence of Frequent Neoplasms in Female Rats (R00497)

Dose ($\mu\text{g}/\text{kg}$)	0	5	30	75	One-Sided Peto's Trend Test ^a on Neoplasm Onset
Number of Animals	60	60	60	60	
Whole Animal					
Osteoblastoma		1	1	3	$p=.0318^b$
Osteosarcoma		4	12	23	$p<.0001^c$
Large granular lymphocytic Leukemia	23	22	14	12	$p=.9408$
Pancreas					
Islet cell adenoma	2		2		$p=.8130^b$
Uterus					
Endometrial stromal polyp	17	11	8	17	$p=.3928$
Endometrial stromal sarcoma	3				$p=1.000^{b,d}$
Skin					
Papilloma		2	1	1	$p=.3246^b$
Mammary Gland					
Adenoma	2	1	1	2	$p=.4826^b$
Fibroadenoma	10	19	10	15	$p=.1520$
Adenocarcinoma	2	2	1	1	$p=.7504^b$
Adrenal					
Adrenocortical adenoma	1	2	1		$p=.8291^b$
Pheochromocytoma	3	1	1		$p=.9682^b$
Pheochromocytoma, malignant	1	1	1		$p=.7948^b$
Thyroid					
C-cell adenoma	6	7	8	9	$p=.1055$
C-cell carcinoma	2	3	1	1	$p=.7741^b$
Pituitary					
Adenoma	40	46	42	29	$p=.6951$
Carcinoma	2	1			$p=.9795^b$
Ovary					
Granulosa cell tumor, malignant	1	1	1		$p=.7980^b$
Clitoral Gland					
Carcinoma		1	1	1	$p=.2375^b$

^a With continuity correction.

^b Result based on exact permutation trend test for site/neoplasm combinations with incidence of 10 or less.

^c Statistically significant increasing trends in the 30- and 75- $\mu\text{g}/\text{kg}$ groups.

^d Corresponds to two-sided p-value $>.005$.

Incidence of most common/any neoplasms

Data for all neoplasms, and 3 most common neoplasms

		Males				Females			
Group		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Whole animal	Osteosarcoma (M)	0	3	21	31	0	4	12	23
Whole animal	LGLL (M)	24	18	6	5	23	22	14	12
Pituitary	Adenoma (B)	38	42	30	27	40	46	42	29
Testis	Interstitial cell tumor (B)	42	37	34	36	-	-	-	-
All organs	Any neoplasm(s)	???							

LGLL = large granular lymphocytic leukemia

Incidence of most common/any fatal neoplasms

Animals that died of fatal neoplasm (data for all neoplasms, and 3 most common neoplasms)

		Males				Females			
Group		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Whole animal	Osteosarcoma	0	1	12	22	0	3	6	8
Whole animal	LGLL (M)	16	13	5	2	13	9	7	9
Pituitary	Adenoma (B)	13	14	11	5	15	13	16	6
All organs	Any fatal neoplasm(s)	32	32	30	31	31	27	33	31

LGLL = large granular lymphocytic leukemia

Incidence of neoplasm combinations by organ/site and by tissue/type

Findings (Total incidence in all animals, N=60, unless indicated otherwise, if N <55)

		Males				Females			
Group		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
BY ORGAN									
Skin	Squamous cell neoplasm + keratoacanthoma	1	3	4	4	0	2	1	1
	Squamous cell neoplasm + sebaceous neoplasm + keratoacanthoma	1	4	4	5	0	2	1	1
Clitoral gland	No. examined	-	-	-	-	1	1	2	3
	All adenoma + carcinoma	-	-	-	-	0	1	1	3
Thyroid	C-cell adenoma + carcinoma	1	2	1	4	8	10	9	10
BY TYPE									
Whole animal	Any tumor of osteoblast origin (B+M)	0	3	24	36	0	5	13	25
	Chondroma/sarcoma	0	0	1	0	0	0	0	0
	Fibroma/sarcoma	6	3	1	3	0	1	0	0
	Lipoma/sarcoma	2	0	1	1	0	1	0	0
	Chondro + Lipo + Fibro-(sarcoma)	8	3	3	4	0	2	0	0
	Leiomyoma/sarcoma	1	0	0	1	0	0	3	0

Sponsor's Table: Incidences of osteoblast hyperplasia and osteoblastic neoplasms

Group		Males				Females			
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Whole animal	Osteoblast hyperplasia	0	1	2	4	0	2	1	3
	Osteoma	0	0	2	1	0	0	0	1
	Osteoblastoma	0	0	2	7	0	1	1	3
	Osteosarcoma								
	TOTAL	0	3	21	31	0	4	12	23
	Soft tissue metastasis	0	0	10	17	0	1	2	4
	Gross bone nodule/lesion	0	1	17	24	0	3	7	13
	Fatal	0	1	12	22	0	3	6	8
	No. of rats with multiple different bone neoplasms	0	0	1	3	0	0	0	2
	No. of rats with at least one bone neoplasm	0	3	24	36	0	5	13	25

Sponsor's Table: Incidence of osteosarcoma by bone site

Group		Males				Females			
		Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined		60	60	60	60	60	60	60	60
Bone site	Tibia	0	2	12	14	0	0	0	5
	Femur	0	1	3	5	0	0	2	7
	Vertebra	0	0	3	6	0	2	5	5
	Rib	0	0	2	4	0	1	2	4
	Sternum	0	0	3	5	0	0	3	2
	Pelvis	0	0	0	1	0	1	2	0
	Skull	0	0	1	1	0	0	0	1(ear)
	Humerus	0	0	1	1	0	0	0	0
	Single site	0	3	16	22	0	4	10	22
	Multiple sites	0	0	3	7	0	0	2	1
Total incidence		0	3	21	31	0	4	12	23

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Effects on tumor latency**Osteosarcoma**

Analysis of time to death due to fatal osteosarcoma, and of time to diagnosis of fatal/nonfatal osteosarcoma) suggested that in addition to an increase in incidence there was a shortened latency period for this tumor in males, but not clearly in females (see Tables below).

Time to death due to fatal osteosarcoma**Animals that died of fatal osteosarcoma**

Group	Males				Females			
	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined	60	60	60	60	60	60	60	60
Time of death								
Wk 53-78	0	0	0	5	0	0	0	2
Wk 79-91	0	0	5	9	0	1	3	3
Wk 92-103/104 (m/f)	0	1	7	8	0	2	3	3
Wks 53-103/104 (m/f) (TOTAL)	0	1	12	22	0	3	6	8

Time to diagnosis of fatal or nonfatal osteosarcoma

(Also see Figures on next pages).

Animals with osteosarcoma

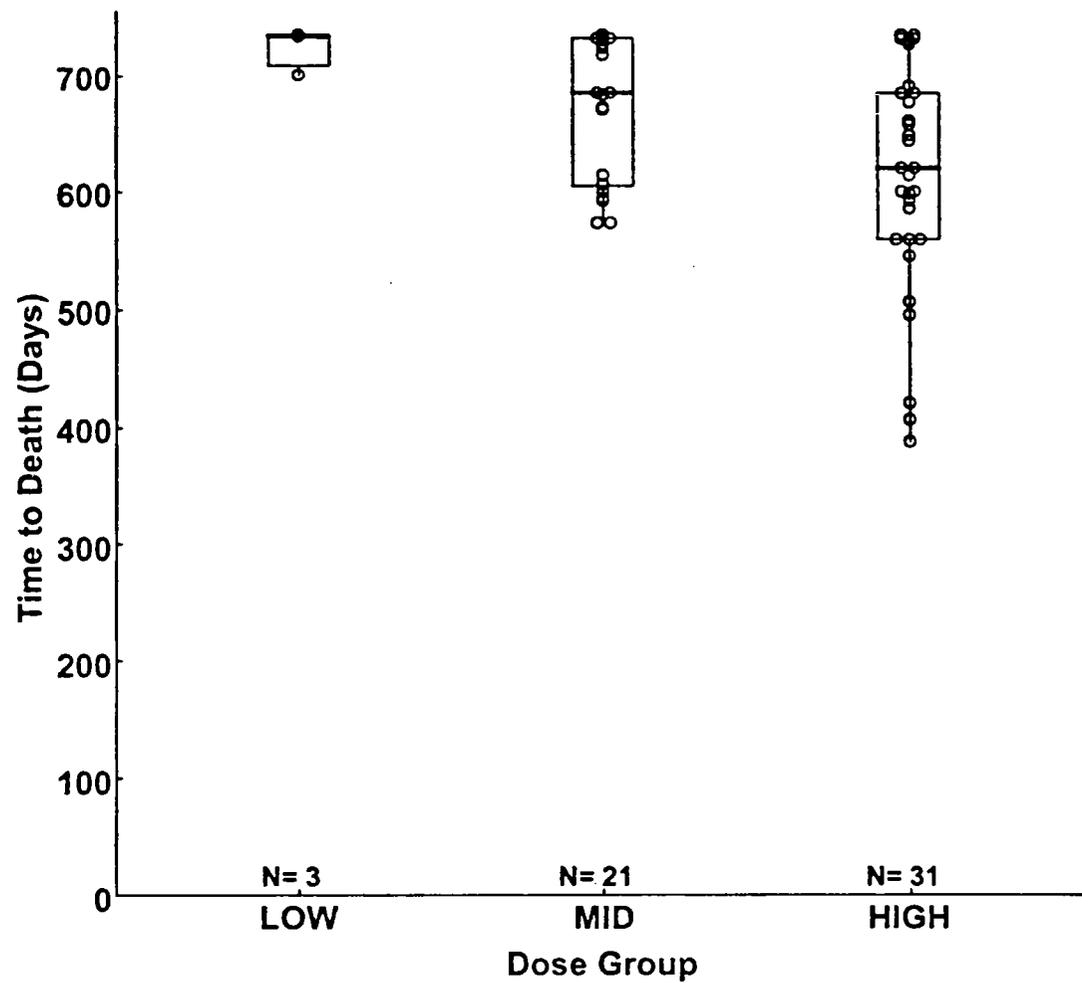
Group	Males				Females			
	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined	60	60	60	60	60	60	60	60
Wk 53-78	0	0	0	6	0	0	0	3
Wk 79-91	0	0	7	11	0	1	4	4
Wk 92-103/104 (m/f)	0	1	8	9	0	2	4	6
Wk 104-105/105-106 (m/f)	0	2	6	5	0	1	4	10
Wks 53-105/106 (m/f) (TOTAL)	0	3	21	31	0	4	12	23

Week 1-52 = Month 1 through 12 = Day 1 through 365
 Week 53-78 = Month 13 through 18 = Day 366 through 547
 Week 79-91 = Month 19 through 21 = Day 548 through 638
 Week 92-103/104 = Month 22 through 24 = Day 639 through 724(m)/731(f)

Week 104-105 (m) = Day 725 through 736 (m), necropsy days (five days) 732-736 (m)
 Week 105-106 (f) = Day 732 through 743 (f), necropsy days (five days) 739-743 (f)

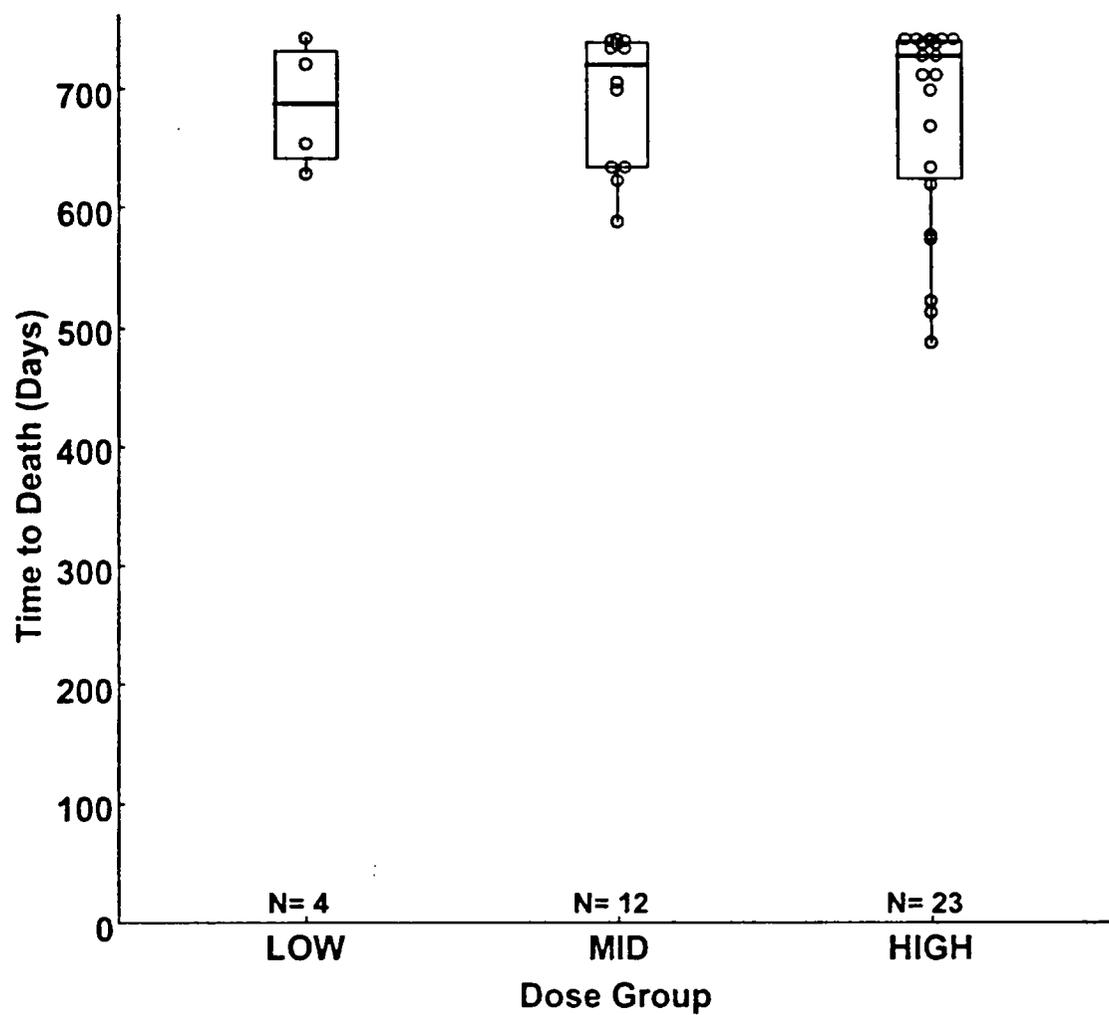
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Figure 1. Time to diagnosis and/or death of osteosarcoma-bearing animals (males)



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Figure 2. Time to diagnosis and/or death of osteosarcoma-bearing animals (females)



Immunotoxicology

In evaluated animals (N=4/sex in LD, MD; N=14-29/sex in HD), blood samples contained <0.082 relative ug IgG/ml (LLQ), at 6, 12, 18, and 24 mo.

Thus, LY33334-specific IgG was not quantifiable in any animal.

Quantitative bone analysis

Bone analyses and serum osteocalcin measurements were done of selected samples/animals. Generally, results were separated for surviving and early-euthanized animals. All data given below are for survivors, except histomorphometry data for proximal femur, which are from early-death animals, and serum osteocalcin data, which are from early-death and postnecropsy animals.

Bone analyses

Whole femur

Dose-dependent increase in length, lateral-medial width and wet weight in all dose groups, up to 6%, 33%, 60% in HD, indicating effect of LY mainly on periosteal expansion. All effects were dose-dependent in that there was a larger effect with increasing dose and a maximal effect at the high dose, except for the effect on femur length in males which was maximal at the low dose. In all dose groups as well as in the controls, male femora were longer, wider and heavier than female ones.

Femoral midshaft

QCT scanning showed reduction of marrow cavity at LD, virtually no marrow and periosteal expansion at MD, further periosteal expansion with altered geometry at HD (Figure 2), and dose-dependent increase in X-sectional area, BMC and BMD.

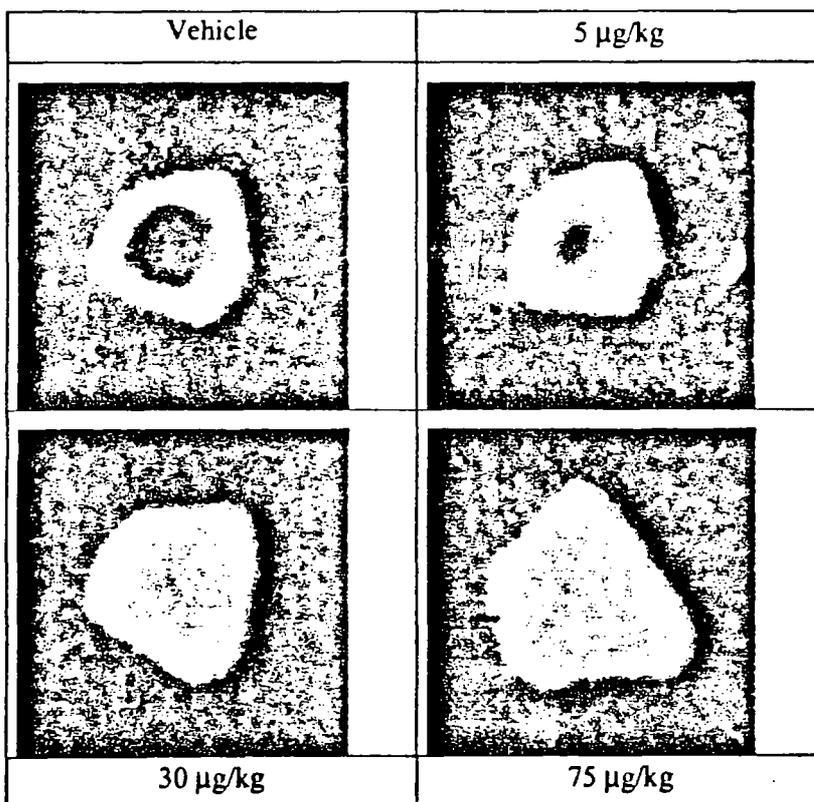


Figure 2

QCT Images of the Femoral Midshaft from Females Treated for Two Years. The midshaft of left femora were analyzed in cross-section by QCT, using voxel dimensions of 150x150x1200 µm. Images show reduction of the marrow cavity at 5 µg/kg, loss of marrow space and expansion of bone at 30 µg/kg, and further expansion of bone area with altered geometry at 75 µg/kg.

BMD (femoral midshaft, survivors, N=?) (approximate values)

	BMD (mg/cc)		% increase in BMD	
	males	females	Males	Females
Control	880	990	-	-
LD	1140*	1260*	30%	27%
MD	1340*	1370*	52%	38%
HD	1370*	1400*	56%	41%

*statistically significant effect

Biomechanical 3-point bending test showed dose-dependent increase in ultimate load, stiffness and intrinsic strength at all dose levels with maximum effect at HD. However, ultimate displacement was significantly reduced to similar extent in all dose groups indicating increased brittleness. Changes in E (Young's modulus) and toughness indicated unclear adverse effects on this part of the bone.

Distal femur

Similar effects as in midshaft on bone macrostructure, i.e., bone marrow space, periosteal expansion and resulting bone thickness. Dose-dependent increase in X-sectional area, BMC and BMD.

BMD (distal femoral metaphyses, survivors, N=?) (approximate values)

	BMD (mg/cc)		% increase in BMD	
	Males	Females	Males	Females
Control	580	670	-	-
LD	780*	990*	34%	48%
MD	1070*	1180*	84%	76%
HD	1220*	1240*	110%	85%

*statistically significant effect

Lumbar vertebrae

QCT analysis showed alteration of structure (increase in size) (Figure 7), and dose-dependent increase in X-sectional area, BMC and BMD.

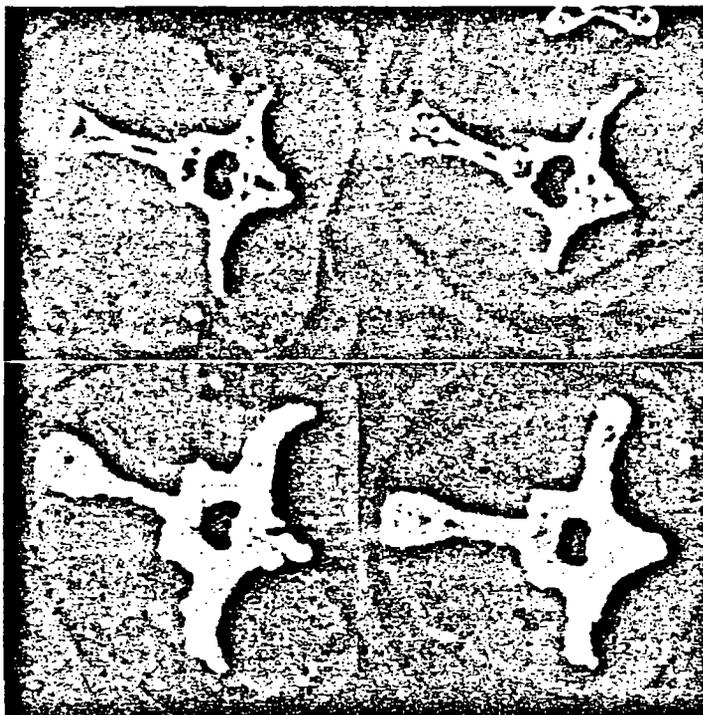


Figure 7

QCT images of L-6 Vertebra from Females. L-6 were scanned in cross-section by QCT, using voxel dimensions of 150x150x150 µm. The top row shows images from two vehicle controls, while the bottom row shows two from the 75 µg/kg group. Considerable alteration of bone architecture was observed for the treated group.

BMD (L-6 vertebrae, survivors, N=?) (approximate values)

	BMD (mg/cc)		% increase in BMD	
	Males	Females	Males	Females
Control	505	640	-	-
LD	600*	790*	19%	23%
MD	660*	825*	31%	29%
HD	720*	860*	43%	34%

*statistically significant effect

Biomechanical compression testing showed marked dose-dependent increases in ultimate load, stiffness, strain (in f), intrinsic strength, Young's modulus and toughness at all dose levels, particularly in females. Near maximal effects were obtained at MD.

Proximal femur

Biomechanical load to failure test of femoral neck indicated dose-dependent increase in strength of the neck in all dose groups, maximal at MD (f) or HD (m).

Transverse and coronal QCT images of proximal femur and femoral neck, respectively, were used to assess histomorphometric parameters. Data showed decrease in marrow area, and increases in X-sectional area, cortical thickness, trabecular area and trabecular connectivity in all treated. Periosteal perimeter was increased modestly (in contrast to femoral midshaft where increase was marked). Data indicated mainly stimulation of endocortical and trabecular bone apposition.

Histomorphometry parameters of proximal femur (males, transverse section)

	X-sectional area (mm ²)	Marrow area (mm ²)	Mean cortical thickness (mm)	Trabecular area (%)	Trabecular number /TV (#/mm ²)	Trabecular node to node/TV (#/mm ²) (connectivity measure)
Control (6)	20	11.4	1.6	22	1.9	1.3
LD (5)	22*	10.7	1.9*	54*	7.2*	8.1*
MD (5)	25*	1.8*	2.7*	46*	4.8*	10.2*
HD (6)	27*	0.7*	2.9*	52*	NE	NE

*statistically significant effect

NE = no estimate

Serum osteocalcin

Levels were dose-dependently elevated, consistent with stimulation of osteoblastic activity. Levels were higher in males than females.

Serum osteocalcin levels (survivors and early-death animals, N=?) (approximate values)

	Serum osteocalcin (ng/ml)	
	Males	Females
Cor.trol	35	21
LD	42	28*
MD	98a	37*
HD	155a	36*

a) levels highly variable and underestimated (some individual levels above quantitation range of assay)

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Increase in BMC and BMD of L-6 vertebrae and femur

L-6 Vertebrae

MALES	L-6 BMC (mg)	Increase	L-6 BMD (mg/cc)	Increase	N tumors
Control	2.7	-	505	-	0
LD	3.9*	1.44x	600	1.19x	0
MD	5.2*	1.93x	660	1.31x	3
HD	6.3*	2.33x	720	1.43x	6

FEMALES	L-6 BMC (mg)	Increase	L-6 BMD	Increase	N tumors
Control	2.3	-	640	-	0
LD	3.4*	1.48x	790*	1.23x	2
MD	4.4*	1.91x	825*	1.29x	5
HD	5.5*	2.39x	860*	1.34x	5

Midshaft femur

MALES	Femoral BMC (mg)	Increase	Femoral BMD (mg/cc)	Increase	N tumors
Control	15	-	880	-	
LD	21.2	1.41x	1140	1.30x	1
MD	30.5	2.03x	1340	1.52x	3
HD	36.5	2.43x	1370	1.56x	5

FEMALES	Femoral BMC (mg)	Increase	Femoral BMD (mg/cc)	Increase	N tumors
Control	12.5	-	990	-	
LD	15.8	1.26x	1260	1.27x	0
MD	20.5	1.64x	1370	1.38x	2
HD	25.0	2.00x	1400	1.41x	7

Distal femur

MALES	Femoral BMC (mg)	Increase	Femoral BMD (mg/cc)	Increase	N tumors
Control	14.5	-	580	-	
LD	23*	1.59x	780*	1.34x	1
MD	37.5*	2.6x	1070*	1.84x	3
HD	48*	3.3x	1220*	2.1x	5

FEMALES	Femoral BMC (mg)	Increase	Femoral BMD (mg/cc)	Increase	N tumors
Control	14	-	670	-	
LD	24.5*	1.75x	990	1.48x	0
MD	33*	2.36x	1180	1.76x	2
HD	37.5*	2.68x	1240	1.85x	7

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Toxicokinetics

For the low dose group, samples were taken at 5, 15, 25, 35, 45, 60, 75, 90, 7h postdose.
For the mid and high dose groups, samples were taken at 5, 15, 30, 45, 60, 90, 120, 150, 7h postdose.

Test compound was detected in serum of 4/sex/group/time point. Data showed that test article was cleared from the system at 7h after dosing. Serum levels were dose-related and differences between males and females were inconsistent. Levels appeared to decrease after 12 months and lowest levels were seen at 18 months. The reason for this decrease is not known.

T_{max} (avg) 0.3h (18 min) (mean, m and f) (most values 15 min)
 $T_{1/2}$ (avg) 0.4h (24 min) (mean, m and f)

C_{max} and AUC values at 3 time points in the study

Group		MALES			FEMALES		
		LD	MD	HD	LD	MD	HD
ug/kg/day		5	30	75	5	30	75
C _{max} (ng/mL)	6 mo	2.3	10	35	1.3	11	20
	12 mo	1.0	5.2	16	2.2	13	28
	18 mo	0.6	3.1	8.3	1.0	6.9	21
AUC (ngxh/mL)	6 mo	1.4	8.7	23	0.8	5.8	14
	12 mo	0.4	4.5	14	1.6	9.2	26
	18 mo	0.6	3.6	14	0.5	4.6	12

Note:

Generally:

C_{max} and AUC: m>f @ 6 months.

C_{max} and AUC: m<f @ 12 months.

C_{max} and AUC: m≤f @ 18 months.

Males: C_{max} and AUC (6 mo)>(12mo)>(18mo)

Females: C_{max} and AUC (6 mo)<(12mo)>(18mo)

Human exposure multiples**Human AUC multiples**

Group	Dose (ug/kg/day)	AUC of PTH(1-34) (ngxh/ml) (Month 6; average m,f)	AUC of PTH(1-34) (ngxh/ml) (Month 12; average m,f)	AUC of PTH(1-34) (ngxh/ml) (Month 18; average m,f)	AUC multiples (rat:human) (Month 6)*	AUC multiples (rat:human) (Month 12)*	AUC multiples (rat:human) (Month 18)*	Average multiple (m+f)
LD	5	1.1	1.05	0.46	3.7x	3.6x	1.6x	3.0x
MD	30	7.3	7.28	4.09	25x	25x	14x	21x
HD	75	18.5	19.94	12.51	63x	68x	42x	58x

*Human AUC=0.295 ngxh/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; study GHAC)

Human C_{max} multiples

Group	Dose (ug/kg/day)	C _{max} of PTH(1-34) (ngxh) (Month 6; average m,f)	C _{max} of PTH(1-34) (ngxh) (Month 12; average m,f)	C _{max} of PTH(1-34) (ngxh/ml) (Month 18; average m,f)	C _{max} multiples (rat:human) (Month 6)*	C _{max} multiples (rat:human) (Month 12)*	C _{max} multiples (rat:human) (Month 18)*	Average multiple (m+f)
LD	5	1.8	1.6	0.8	11x	10x	5x	8.6x
MD	30	10.5	9	5	66x	57x	31x	51x
HD	75	27.5	22	15	173x	138x	94x	135x

*Human C_{max}= 159 pg/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; study GHAC)

STATISTICAL ANALYSIS OF TUMOR FINDINGS

Sponsor's statistical analysis

Survival data:

Sponsor's analysis showed a statistically significant decrease in survival, i.e., increase in mortality, in female rats.

Tumor data:

Sponsor's analysis showed a statistically significant drug-dose-related increase in the incidence of bone neoplasms (osteoblastoma, osteosarcoma) in males and females. A dose-related decrease in incidence was observed for large granular lymphocytic leukemia, in males and females, significant in males only. The decreased incidence of pituitary adenoma observed in both sexes was not statistically significant. An increased incidence in thyroid C-cell adenoma was seen in males and females but was not statistically significant. Note that there was also a dose-related increase in severity of C-cell hyperplasia.

CDER reviewers statistical analysis

Survival data:

Results of the Reviewer's survival data analysis show that there was a positive increase in mortality in female but not in male rats.

Tumor data:

Table 7
Significant Trends in Tumor Incidence for Male and Female Rats

Organ	Tumor	Tumor-Bearing Animals (N)	P-Value
Rat/Male			
Rib (BB)	Osteosarcoma (989)	0,1,3,4	0.004
Femur (BE)	Osteoblastoma (895)	0,0,1,4	0.005
	Osteosarcoma (989)	0,1,3,5	0.004
Bone (BO)	Osteosarcoma (989)	0,0,5,8	<0.001
Tibia (BQ)	Osteoblastoma (895)	0,0,1,3	0.026
Tibia (BQ)	Osteosarcoma (989)	0,2,12,14	<0.001
Vertebra (BV)	Osteosarcoma (989)	0,0,3,6	<0.001
Kidney (KI)	Osteosarcoma (989)	0,0,1,2	0.049
Liver (LI)	Osteosarcoma (989)	0,0,2,7	<0.001
Lung (LU)	Osteosarcoma (989)	0,0,10,18	<0.001
Spleen (SP)	Osteosarcoma (989)	0,0,4,3	0.019
Thyroid (TH)	C-Cell Adenoma	0,2,1,3	0.047
Whole Animal (WA)	Osteoblastoma (895)	0,0,2,7	<0.001
	Osteosarcoma (989)	0,3,21,31	<0.001
Rat/Female			
Rib (BB)	Osteosarcoma (989)	0,1,2,4	0.011
Bone (BO)	Osteosarcoma (989)	0,0,3,2	0.041
Femur (BE)	Osteosarcoma (989)	0,0,2,7	<0.001
Tibia (BQ)	Osteosarcoma (989)	0,0,0,5	<0.001
Vertebra (BV)	Osteosarcoma (989)	0,2,5,5	0.02
Whole Animal (WA)	Osteosarcoma (989)	0,4,12,23	<0.001
	Osteoblastoma (895)	0,1,1,3	0.023

Note: The trends are statistically significant at level 0.05.

The results of the Reviewer's tumor analysis were as follows:

- Statistically positive dose-response relationships in incidence rate of osteoblastoma in femur and whole animal in males.
- Statistically positive dose-response relationships in incidence rate of osteosarcoma in rib, femur, bone, tibia, vertebra, kidney, liver, lung, spleen, and whole animal in males.
- Statistically positive dose-response relationship in incidence rate of osteoblastoma in whole animal in females.
- Statistically positive dose-response relationships in incidence rate of osteosarcoma in rib, bone, femur, tibia, vertebra and whole animal in females.
- Statistically positive dose-response relationship in incidence rate of C-cell adenoma in thyroid in males.

An additional analysis was carried out for a number of combined tumor incidences (thyroid, skin, lung).

The results of the Reviewer's additional tumor analysis were as follows:

- Statistically positive dose-response relationship in incidence rate of combined C-cell adenoma and C-cell carcinoma in the thyroid in males.
- No statistically significant differences in the incidence of any of the tumor combinations in skin and lung between the control and any dose group, based on pairwise comparison.

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SUMMARY AND EVALUATION

Dose selection

The doses used in this study (5, 30, 75 ug/kg/day) were selected by the Sponsor. Initially, Sponsor selected doses of 4, 20 and 40 ug/kg/day based on the results of a 6-month toxicity study. The projected MTD, based on increased bone formation (and resulting secondary effects) and renal disease, was used as a criterion for the high dose selection. Upon review of the dose selection proposal, the Exec CAC felt that the MTD would not be reached at 40 ug/kg/day and recommended doses of 10, 30, and 100 ug/kg/day. Subsequently, Sponsor concluded that the MTD was between 40 and 100 ug/kg/day and decided to use the doses of 5, 30 and 75 ug/kg/day. They also expected that at a high dose of 75 ug/kg/day a rat:human AUC ratio of at least 25x would be attained. The Division concurred with the Sponsor's dose selection, and based on the results of the current study the selection was appropriate.

Mortality

There was a significant dose-related positive trend for increased mortality in females.

Clinical observations

There were thinness, rough hair coat and/or tail scabs in HD groups, leg nodules in MD and HD males in the last 6 months of study, and neurological signs associated with bone neoplasms in all dose groups.

Body weight

Body weight gain was decreased in males, but increased in females. Food consumption was increased in both sexes. EFU was decreased in males but increased in females.

Clinical pathology

In some animals there were marked increases in serum ALP as a result of neoplasm occurrence.

Gross Pathology

There was thinness, rough hair coat and/or tail scabs in all dose groups, kidney enlargement in MD and HD males, urinary bladder distention in all dose groups, liver and lung nodules in all dose groups, cervix enlargement in MD and HD females, and bone nodules and bone tissue alteration in all dose groups.

Histopathology

Non-neoplastic findings

		Dose-related increase in incidence	Dose-related increase in severity
Kidney	Chronic progressive nephropathy	-	M
	Pelvic mineralization	M,F	M
	Tubular mineralization	M	M
Liver	Extramedullary hematopoiesis	M,F	-
Heart	Vascular mineralization	M	-
Lung	Vascular mineralization	M	-
Spleen	Increased extramedullary hematopoiesis	M,F	M,F
	Increased hemosiderosis	F	F
Pancreas	Acinar atrophy	M,F	-
Bone (sternum)	Cystic cartilaginous degeneration	F	F
	Trabecular hypertrophy	M,F	M,F
Femur	Trabecular hypertrophy	M,F	M,F
Vertebra	Trabecular hypertrophy	M,F	M,F
Bone marrow	Hypercellularity	M	-
Adrenal	Cystic degeneration	M	-
Eye	Scleral ossification	M,F	M,F

HYPERPLASIA			
Urinary bladder	Epithelial hyperplasia	M	-
Thymus	Epithelial hyperplasia	M	-
Mammary gland	Lobular hyperplasia	F	-
Bone (sternum)	Osteoblast hyperplasia	M,F	M,F
Tibia	Osteoblast hyperplasia	M	M
Adrenal	Medullary hyperplasia	M	-
Thyroid	C-cell hyperplasia	-	M,F

Neoplastic findings

Single neoplasms

		Dose-related increase in incidence	Dose-related decrease in incidence	Statistically significant (Sponsor)	Statistically significant (CDER)
BONE TUMORS					
Bone (sternum *)	Osteosarcoma (M)	M,F		-	Yes (M,F)
Femur	Osteoblastoma (B)	M		-	Yes (M)
	Osteosarcoma (M)	M,F		-	Yes (M,F)
Rib	Osteoma (B)	M		-	No (M)
	Osteosarcoma (M)	M,F		-	Yes (M,F)
Vertebra	Osteoma (B)	M,F		-	No (M,F)
	Osteosarcoma (M)	M,F		-	Yes (M,F)
Tibia	Osteoblastoma (B)	M,F		-	Yes (M), No (F)
	Osteosarcoma (M)	M,F		-	Yes (M,F)
Pelvis	Osteosarcoma (M)	M,F		-	No (M,F)
OTHER TUMORS					
Whole animal	Large granular lymphocytic leukemia (M)		M,F	Yes (MDm,HDm)	No (M,F)
Pituitary	Adenoma		M,F	No	No (M,F)
Thyroid	C-cell adenoma (B)	M,(F)		No	Yes (M)
Clitoral gland	Carcinoma	F		-	-

Neoplasm combinations

		Dose-related increase in incidence	Dose-related decrease in incidence	Statistically significant (Sponsor)	Statistically significant (CDER)
BY TYPE					
Whole animal	Osteoblastoma (B)	M,F		Yes (HDm)	Yes (M,F)
	Osteoma (B)	M,F		No	No (M,F)
	Osteosarcoma (M)	M,F		Yes (MD,HD)	Yes (M,F)
	Osteosarcoma (MS) in soft tissues	M,F		-	Yes (M)
BY ORGAN					
Skin	Epithelial cell neoplasm	M		-	No (M,F)
Thyroid	C-cell adenoma + C-cell carcinoma	M		-	Yes (M)

The incidence of bone proliferative lesions, including benign osteoblastoma and malignant osteosarcoma was increased in a dose-related manner in all dose groups:

- The increased incidence in (pooled) whole animal osteosarcoma was statistically significant in both males and females based on the Sponsor's statistical analysis (in MD and HD), and based on the statistical analysis by CDER's Biometrics Reviewer.
- The increased incidence in (pooled) whole animal osteoblastoma was statistically significant based on Sponsor's analysis (in HD males), and based on CDER's analysis (both sexes).
- The increased incidences of osteosarcoma at various individual sites were statistically significant in males and females according to CDER's analysis.
- The increased incidences of metastatic osteosarcoma at four soft tissue sites were statistically significant in males according to CDER's analysis.
- The increased incidence of osteoma was not statistically significant.

Other neoplasms:

- The incidence of large granular lymphocytic leukemia was decreased in a dose-related manner in males and females, and the effects was statistically significant based on Sponsor's analysis (in MD and HD males). The decrease may have been due to the decreased survival in MD and HD due to fatal osteosarcoma.
- The incidence of pituitary adenoma was decreased in a dose-related manner in males and females, but the decrease was not statistically significant. This decrease may also have been due to the decreased survival in MD and HD due to fatal osteosarcoma.
- Skin epithelial cell tumor incidence (combination of squamous cell papilloma, adenocarcinoma, and keratoacanthoma, and sebaceous adenocarcinoma) was increased in males, but not in females. The increased incidence was not statistically significant based on CDER's analysis.
- The incidence of thyroid C-cell adenoma was increased in males and females and the incidence of adenoma+carcinoma was increased in males, but according to Sponsor the effect was not statistically significant. However, CDER's analysis showed that the increase in C-cell adenoma and the increase in combined C-cell adenoma and carcinoma in males was statistically significant. It should also be noted that the severity, although not the incidence, of thyroid C-cell cell hyperplasia was increased in both males and females.
- There was an increased incidence of clitoral gland neoplasms. However, since only those few animals with gross lesions were evaluated, it is not clear whether this effect is statistically significant.

In summary, there was a statistically significant positive dose-response relationship in the incidence of:

Bone: Osteosarcoma in males and females
Osteoblastoma in males and females
Thyroid: C-cell adenoma in males
Combined C-cell adenoma and carcinoma in males

Summary of statistically significant neoplastic findings (incidences, N)

Group	MALES				FEMALES			
	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
Dose (ug/kg/day)	0	5	30	75	0	5	30	75
N examined	60	60	60	60	60	60	60	60
Whole animal (bone)								
Osteosarcoma	0	3	21	31	0	4	12	23
Osteoblastoma	0	0	2	7	0	1	1	3
Thyroid								
C-cell adenoma	0	2	1	3				
C-cell adenoma and carcinoma	1	2	1	4				

Tumor latency

Osteosarcoma

There appeared to be a shortened latency period for this tumor in males, but not in females.

Immunotoxicology

Test compound-specific IgG was not quantifiable in any animal at time points between 6 and 24 months. Thus, test article did not appear to induce antibody formation in rats over the course of the study.

Bone analyses

Test compound caused marked skeletal alterations at all doses.

Femoral length, width and weight were increased in both sexes at all dose levels. Femoral bone mass was increased with reduction in bone marrow cavity and endocortical and periosteal expansion in all dose groups. Femoral midshaft strength was increased but brittleness was too. Femoral neck strength was increased. Vertebrae were enlarged, and had markedly increased bone mass and bone

strength at all dose levels. Histomorphometry of proximal femur showed decreased marrow area, increased cortical thickness resulting from endocortical bone apposition, increased trabecular area and increased trabecular connectivity.

Data indicated stimulation of endocortical and/or periosteal, and of trabecular bone apposition.

Human exposure multiples

Human AUC multiples

Group	Dose (ug/kg/day)	AUC of PTH(1-34) (ngxh/ml) (Month 6; average m.f)	AUC of PTH(1-34) (ngxh/ml) (Month 12; average m.f)	AUC of PTH(1-34) (ngxh/ml) (Month 18; average m.f)	AUC multiples (rat:human) (Month 6)*	AUC multiples (rat:human) (Month 12)*	AUC multiples (rat:human) (Month 18)*	Average multiple (m+f)
LD	5	1.1	1.05	0.46	3.7x	3.6x	1.6x	3.0x
MD	30	7.3	7.28	4.09	25x	25x	14x	21x
HD	75	18.5	19.94	12.51	63x	68x	42x	58x

*Human AUC=0.295 ngxh/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; study GHAC)

Human Cmax multiples

Group	Dose (ug/kg/day)	Cmax of PTH(1-34) (ngxh) (Month 6; average m.f)	Cmax of PTH(1-34) (ngxh) (Month 12; average m.f)	Cmax of PTH(1-34) (ngxh/ml) (Month 18; average m.f)	Cmax multiples (rat:human) (Month 6)*	Cmax multiples (rat:human) (Month 12)*	Cmax multiples (rat:human) (Month 18)*	Average multiple (m+f)
LD	5	1.8	1.6	0.8	11x	10x	5x	8.6x
MD	30	10.5	9	5	66x	57x	31x	51x
HD	75	27.5	22	15	173x	138x	94x	135x

*Human Cmax= 159 pg/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; study GHAC)

Data from a previous 6-month rat toxicity study showed that after 140 days levels were approximately 2.5-fold those after a single injection, suggesting some accumulation following long term dosing. In the current study levels appeared to decrease after 6 months with lowest levels seen at 18 months, for unclear reason. T_{max} was ca. 20 minutes, and $T_{1/2}$ was ca. 25 minutes.

The intended human dose is 20 ug, to be administered s.c., once daily. PK studies in humans have shown that the test compound is rapidly absorbed and eliminated, with a T_{max} of ca. 30 minutes, and $T_{1/2}$ of 1 hour. Accumulation did not occur over a 2-week period. Absolute bioavailability is estimated at 95%. Elimination is limited by relatively slower absorption. Exposure was proportional over the 20-80 ug dose range.

Data from the pivotal phase III clinical trial B3H-MC-GHAC showed that after a 20 ug s.c. dose, the median peak serum concentration is 159 pg/mL, and the median systemic exposure (AUC) is 295.5 pgxh/ml. Human exposure multiples (rat AUC:human AUC) were calculated based on these clinical data, and on both 6-month, 12-month and 18-month animal data from the current study.

Historical control data

The following are historical control data for osteosarcoma and thyroid C-cell adenoma in F344 rats from the Sponsor's laboratory (Lilly Research Laboratories) and from the National Toxicology Program (NTP) (published data).

Lilly Historical Control Data, 1993 - 1998

Tumor Type	Overall Incidence ¹ N = 360 per sex		Range of tumor incidence per control group ² N = 60 per sex/study	
	Number of tumors (%)		Number of tumors	
	M	F	M	F
Bone				
Osteosarcoma	1 (.28%)	0	0 - 1	0
Thyroid				
C-cell adenoma	54 (15%)	57 (16%)	3 - 14	5 - 15
C-cell carcinoma	2 (0.56%)	7 (1.9%)	0 - 1	0 - 2

¹ Represents the total number of tumors which have occurred in control rats for all studies combined. Expressed as both absolute number of tumors and percentage.

² The range is the lowest to the highest number of tumors which have been observed in an individual study control group. Expressed as number of tumors.

National Toxicology Program Historical Control Data (*Toxicologic Pathology* 26:428-441; 1998)

	Number of rats evaluated	Number of tumors		Rate (%)		Range (%)	
		M	F	M	F	M	F
Bone							
Osteosarcoma	1354	3	5	0.2	0.4	0-2	0-2
Thyroid							
C-cell adenoma	1347	175	158	13	11.7	2-35	4-22
C-cell carcinoma	1347	24	26	1.8	1.9	0-6	0-4

Calculation of Relative Risk of Osteosarcoma in LY-treated rats

Relative Risk of osteosarcoma in LY-treated rats

		Control	LD	MD	HD
Osteosarcoma Incidence (N/60)	Males	0	3	21	31
	Females	0	4	12	23
Osteosarcoma Incidence (%)	Males	0	5	35	52
	Females	0	6.7	20	38
Relative Risk*	Males	-	25x	175x	260x
	Females	-	34x	100x	190x
	M+F (average)	-	29x	138x	225x

*Based on 0.2% background incidence in control animals (see below)

Background incidence data

Concurrent Control Data	Current 2-year study	Males	0/60	0%
		Females	0/60	0%
Historical Control Data	Lilly Research Laboratories	Males	1/360	0.28%
		Females	0/360	0%
	National Toxicology Program	Males	3/1354	0.22%
		Females	5/1351	0.37%

DISCUSSION

The main findings in this study were increases in bone mass and cellular proliferative lesions of the bone including malignant osteosarcoma in all dose groups, at exposures ranging from ca. 3-40 times expected human exposure. The skeletal effects occurred in a generally dose-related manner. The effect on bone mass was the expected pharmacological effect of daily administration of an active fragment of PTH. The anabolic bone response included increased bone size, bone mass (BMD) and bone strength of femur and vertebrae due to increased trabecular, endocortical and, at some sites, periosteal bone apposition. The structural bone changes were accompanied by a narrowing of bone marrow space. The neoplastic bone lesions were most likely due to chronic hormonal stimulation by PTH(1-34) of its target cell, the osteoblast. Chronic hormonal stimulation is believed to be a potential cause of target cell proliferation (initiation), clonal expansion (promotion), and genetic changes (progression) leading to neoplasm. The neoplastic findings are discussed in more detail below.

The decreased survival in the drug-treated female rats as compared to control animals was not related to a single neoplastic or non-neoplastic cause, and may have been due to the dose-related increase in body weight in females that was not seen in male animals.

Histopathology evaluation showed a number of non-neoplastic alterations in various organs. In the kidney, PTH(1-34) caused increases in incidence and/or severity of renal mineralization and chronic progressive nephropathy. The renal effects were more pronounced in males than in females. The findings were also observed in prior toxicity studies and did not lead to decreased survival. Most likely, the increase in kidney lesions was due to the effect of PTH(1-34) on renal Ca and P reabsorption.

In males, increased incidences of vascular mineralization were observed in heart and lung in all dose groups. Increased scleral ossification of the eye was seen in both sexes. The cause of these changes is unclear, but is probably related to effects of PTH on Ca homeostasis. Measurement of total serum Ca values in part of the animals did not indicate an increase in this parameter. However, data from the 6-month toxicity study indicated marked increases in urinary calcium excretion at doses of 30 and 100 ug/kg/day, suggesting altered calcium handling in PTH-treated animals. In the spleen there was an increased incidence of hemosiderosis in the high dose females but the cause of this lesion is unclear.

All cancellous bones exhibited pronounced trabecular hypertrophy in all dose groups, the microscopic equivalent of increased bone mass (BMD). As a result, and also due to increased endocortical bone formation, there was a decrease in bone marrow and an increase in incidence and/or severity of extramedullary hematopoiesis. In females, there was also an increase in incidence and severity of cystic cartilaginous degeneration in the sternum, that was not seen at other bone sites. The etiology of this cartilage finding is unknown, but may be related to the bone alteration. The increased sternal bone marrow hypercellularity in males may have been due to compression of the medullary hematopoietic cells. However, it is not clear why this did not occur in females.

Hyperplastic findings included a small incidence of osteoblast hyperplasia at various bone sites in both sexes, increased severity of thyroid C-cell hyperplasia in males and females, slightly increased adrenal medullary hyperplasia and increased epithelial hyperplasia of urinary bladder and thymus in males, and slightly increased mammary gland hyperplasia in females.

The bone cell hyperplasia was characterized by single or multiple focal proliferations of well differentiated osteoblast-like cells, often with local osteoid production, but without disruption of bone trabeculae. The osteoblast hyperplasia was probably related to stimulation of the osteoblast by PTH. The thyroid C-cell hyperplasia may have been due to the altered calcium homeostasis caused by PTH and a resulting stimulation of the calcitonin-secreting C-cell. The adrenal medullary hyperplasia in the males was possibly related to the exacerbation of renal disease (mineralization and nephropathy) and has been associated with increased serum calcium levels. The cause of the other hyperplastic lesions is not clear.