CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-318

PHARMACOLOGY REVIEW(S)

A SPECIAL CHRONIC STUDY IN FEMALE FISCHER 344 RATS GIVEN LY333334 (TERIPARATIDE) BY SUBCUTANEOUS INJECTION FOR UP TO 2 YEARS

NDA:	**21,318 (Teriparatide)**
Sponsor:	Eli Lilly and Company
Test compound:	LY333334 (rhPTH1-34, teriparatide)
Testing facility:	Eli Lilly and Company, IN
Study numbers:	R00100 and R00200
Study period:	March 2000-March 2002
Study duration:	2 years (R00100-R00200: 736 days-743 days)
Report Date:	June 2002
Submission Date:	July 30, 2002
Due date:	March 20, 2003
Toxicology Report:	43
QA/GLP compliance:	Statements included
Review Date:	November 20, 2002

BACKGROUND

This two-part study was a follow up to a previous 2-year rat carcinogenicity study with the same compound, LY333334 (teriparatide). In that study a marked dose-related incidence of osteosarcoma was observed in male and female animals. Tumors were observed at all subcutaneously injected doses (5, 30, 75 mcg/kg/day) and were accompanied by large increases in bone mass. There were also drug-related increases in osteoblastoma and osteoma incidence. Human exposure multiples were 3x, 21x, 58x (based on AUC comparison with the human dose of 20 mcg). The study was reviewed for NDA 21,318 (Gemma Kuijpers, September 6, 2001), and presented to the Exec CAC on March 20, 2001.

The Sponsor initiated a second carcinogenicity study in female rats in March 2000, to investigate the tumorigenic effect of daily sc injections of PTH1-34 when a relatively short term treatment of 6-months was employed starting at two different ages. Treatment was initiated in either 6-7 week old growing animals (as had been done in the first study) or in 6-month old full-grown animals. Two doses (5, 30 mcg/kg/day) were used. A positive control arm (30 mcg/kg/day, 24 month treatment) to replicate the findings of the first study was included. Animals undergoing a 6-month treatment period were sacrificed immediately after treatment or after a drug-free follow-up period of 14 or 18 months. An arm in which animals were treated with 30 mcg/kg/day, from 6 months of age, for 20 months was also included. The study protocol was submitted on October 5, 1999, and reviewed by the Exec CAC on October 29, 1999, and December 29, 1999. The Sponsor believed at the time that "treatment of rats for a shorter-than-lifespan duration, i.e. 6 months, will produce the expected pharmacologic effect on bone but will not result in osteosarcoma" (Submission November 24, 1999)

The NDA for teriparatide was submitted to DMEDP for the indication of treatment of osteoporosis in postmenopausal women and men (November 29, 2000). The proposed human dose is 20 mcg/day (sc injection). The NDA was given an approvable status (AE) with requests for amended labeling, a risk management plan and resolution of manufacturing facility deficiencies on October 2, 2001. The P/T Reviewer recommended the NDA was approvable pending the results of the second rat carcinogenicity study. The Sponsor submitted a complete response including labeling changes to the action on November 15, 2001. On April 18, 2002, the label including a black box warning based on the animal finding of osteosarcoma was negotiated with the Division. On May 16, 2002, the Sponsor was informed that the NDA was approvable pending a favorable inspection of the manufacturing facilities. The report of the second rat carcinogenicity study was submitted on July 30, 2002. On September 19, 2002, a second complete response was submitted and the Sponsor proposed —— It was also noted that the manufacturing deficiencies were resolved.
Following is the review of the second rat study. The proposed label is appended to this review.

METHODS

Species/strain:	Rats (female F344/NTac)
Number of animals:	N=30 or 60/group
Age at treatment start:	6-7 weeks (appr. 2 months)
Housing:	Individual
Diet:	Certified Rodent Diet 5002 ——
Drug lot #:	PPD04231 and CTM00840
Dosing route:	Subcutaneous injection, daily (alternating right and left dorsal lumbar areas)
Dosage form:	Solution, 1 ml/kg
Vehicle:	20 mM sodium phosphate buffer in 0.9% NaCl Injection, USP (Mannitol 1.2 mg/ml in controls and HD)
Dose groups:	Control, LD, HD (0, 5, 30 mcg/kg/day)
Duration of treatment:	6, 20, or 24 months (see STUDY DESIGN) Study R100: all groups starting treatment at appr. 2 months of age (Day 0), and terminated on Day 736 Study R200: all groups starting treatment at appr. 6 months of age (Day 121), and terminated on Day 743
CAC concurrence:	Study protocol was discussed with the EXEC CAC on October 29 and December 20, 1999
Study objectives:	Determine if limited duration of treatment (6 months) followed by 14- or 18-month withdrawal results in an increase in bone neoplasms Determine if treatment during the rapid skeletal growth phase (2-6 months) causes this species to be particularly susceptible to the development of bone neoplasms
Primary endpoints:	Bone mass Gross and microscopic pathology of bone (femur, tibia, vertebra, sternum)
Toxicokinetics:	Serum concentrations of immunoreactive teriparatide were determined predose and 0.25h after dosing (appr. Tmax), by —— on Days 0, 154, 280, and 540 or 551 (Months 0, 5, 9, 18). Number of animals evaluated was N=5-7 for predose and N=5-7 (other animals) for 0.25h samples. Same animals were evaluated in different sampling months.
Observations:	Survival, condition, behavior: daily. Clinical signs and palpable lesions: weekly (for 14 weeks) or biweekly (after that). Body weight and food consumption: weekly (for 14 weeks) or biweekly (after that).
Bone analysis:	Femur, tibia, lumbar vertebra in 70% ethanol, for QCT (quantitative computed tomography) parameters BMD, X-area, BMC
Necropsy:	Animals were necropsied at study termination as shown in STUDY DESIGN. Most tissues and all gross lesions were collected and preserved in formalin. Bone was preserved in 70% ethanol for QCT.
Histopathology:	Tissues processed were 4 bone sites (see below), and masses/nodules. Some bone neoplasms from animals in arm B were processed for immunohistochemistry of PTH receptors. Sections of all processed tissues from all animals were examined.
Data analysis:	Statistical analysis was carried out on data of the following combinations of groups: Groups A and B; Groups A, H1, H2; Groups A, E1, E2; Groups A, I1, I2. Tests used were Cochran-Armitage trend test for dose-related increases for animals that died before study end, and test for comparison of mortality rates. Description of tests was not clear.

Bone histopathology
Just as in the original study, histological examination of bone was performed as follows:
- fixation in 10% formalin (femur, tibia, vertebra, sternum, gross bone lesions)
- trimming using bone saw
- decalcification in formic acid

- paraffin embedding of tissue blocks
- sectioning at 5-um thickness and staining with H&E
- microscopy of one longitudinal section of each bone site

Size and composition of each section:
- Femur: Longitudinal section evaluated of distal femur including cartilage, epipysis, physis, metaphysis, part of diaphysis, so that appr. 50% of the length of the bone was evaluated
- Tibia: Longitudinal section evaluated of proximal tibia including cartilage, epipysis, physis, metaphysis, part of diaphysis, so that appr. 30-40% of the length of the bone was evaluated
- Vertebra: Longitudinal section evaluated of a single lumbar vertebra (L2 or L3) including endplate and body, so that entire length of a vertebra was evaluated.
- Sternebra: Longitudinal section evaluated of1 to 2 sternebrae, similar to vertebrae
- Gross lesion: Number and orientation of sections needed to charatcterize lesion determined by pathologist

Comment on histopathology methods:
The sampling method applied is normal for this type of study, but also means that only a small fraction of the skeleton is examined microscopically. Thus, it is possible that small tumors are missed (in both treated and controls) using this method.

STUDY DESIGN:

Study Design Summary

Study Arm	Study No Group No	Age (Months) 2	8	14	20	26	Age During Treatment (Months)
A	R00100 01	Vehicle Control n = 60					
B	R00100 07	Positive Control (30 µg/kg) n = 60					2 - 26
C	R00200 01	Vehicle Control n = 30					
D1	R00200 02		5 µg/kg n = 30				6 - 12
D2	R00200 03		30 µg/kg n = 30				6 - 12
E1	R00200 04		5 µg/kg n = 60				6 - 12
E2	R00200 05		30 µg/kg n = 60				6 - 12
F	R00100 02	Vehicle Control n = 30					
G1	R00100 03	5 µg/kg n = 30					2 - 8
G2	R00100 04	30 µg/kg n = 30					2 - 8
H1	R00100 05	5 µg/kg n = 60					2 - 8
H2	R00100 06	30 µg/kg n = 60					2 - 8
I1	R00200 06		5 µg/kg n = 60				6 - 26
I2	R00200 07		30 µg/kg n = 60				6 - 26

Abbreviation: No = number.

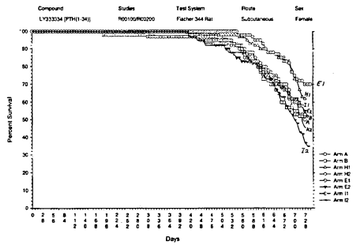
RESULTS

Survival
Interim sacrifices: All animals in the interim sacrifice groups survived until scheduled necropsy
Terminal sacrifices: Although survival appeared higher in arms E1 and H1 (6-month 5 mcg/kg treatment arms) there was no statistically significant treatment effect. Survival in arm I2 appeared lower than in the other arms.

Survival (N/ no. group)

Study Arm	A	B	E1	E2	H1	H2	I1	I2
Dose (µg/kg)	0	30	5	30	5	30	5	30
Treatment Duration (Months)	NA	24	6	6	6	6	20	20
Age During Treatment (Months)	NA	2 - 26	6 - 12	6 - 12	2 - 8	2 - 8	6 - 26	6 - 26
Rate (%):	50	52	70	52	62	52	53	35
Number:	30/60	31/60	42/60	31/60	37/60	31/60	32/60	21/60

Abbreviation: NA = not applicable.



Clinical observations
Palpable masses (hindleg or ventral thorax)

Arm	N
A	1
B	6
I2	1
TOTAL	8

Body weight (Figures 1 and 2)
Interim sacrifices: Small increases in BW and BWG in treated animals (up to 7% in BW in groups D2 and G2)
Terminal sacrifices: Small increases in BW and BWG in rats treated from 2 months of age (up to 8% and 14% in group B, @ 12mo)

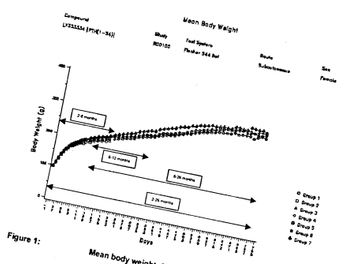


Figure 1: Mean body weight, Study R00100.

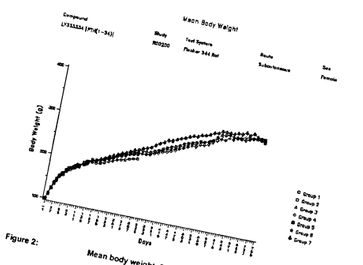


Figure 2: Mean body weight, Study R00200.

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Quantitative bone analysis
Interim sacrifices: Dose-dependent increases in BMD, BMC, X-area and length in L6 vertebra and femur midshaft, similar for animals treated from 2-8 and 6-12 months.
Terminal sacrifices: Large increases in bone parameters in long term treatment arms (2-26 or 6-26 months). No significant bone changes remaining at 26 months when treatment was for 6 months with 5 mcg/kg/day, and small changes remaining when treatment was with 30 mcg/kg/day

Data for interim sacrifices:

Young animals: Effects at end of 2-8 month treatment period:
Increases in femoral length, and increases in bone mass in femoral midshaft and lumbar vertebra, in LD and HD

	Parameter values				Increase relative to control	
Group	Control	LD	HD		control	LD	HD
Arm	F	G1	G2		F	G1	G2
Treatment (months)	NA	2-8	2-8		NA	2-8	2-8
Dose (µg/kg/day)	0	5	30		0	5	30
N animals	7	7	7		7	7	7
Tumor(s) found at study end						2 (vertebra)	2 (vertebra, rib)
Femur							
Length	32.8	33.3*	33.9*		-	1.5%	3.4%
Femur midshaft							
BMD(mg/cc)	939	991*	1095*		-	5.5%	16.6%
BMC (mg)	8.68	9.34*	11.59*		-	7.6%	33.5%
X-area (mm2)	7.70	7.86	8.82*		-	2.1%	14.6%
L6 vertebra							
BMD(mg/cc)	572	671*	767*		-	17%	34%
BMC (mg)	1.80	2.23*	2.79*		-	24%	55%
X-area (mm2)	20.9	22.1*	24.2*		-	10.6%	16%

Older animals: Effects at end of 6-12 month treatment period:
Increases in femoral length, and in bone mass in femoral midshaft, lumbar vertebra and proximal tibia, in LD and HD

	Parameter values				Increase relative to control	
Group	control	LD	HD		control	LD	HD
Dose (µg/kg/day)	0	5	30		0	5	30
Arm	C	D1	D2		C	D1	D2
Treatment (months)	NA	6-12	6-12		NA	6-12	6-12
N animals	8	8	8		8	8	8
Tumor(s) found at study end						No	2 (femur, vertebra)
Femur							
Length	34.2	34.7*	35.2*			1.5%	2.9%
Femur midshaft							
BMD(mg/cc)	962	1043*	1216*		-	8.4%	26%
BMC (mg)	9.04	10.04*	12.61*		-	11.1%	39%
X-area (mm2)	7.84	8.03	8.66*		-	2.4%	10.5%
L6 vertebra							
BMD(mg/cc)	604	757*	842*		-	25%	39%
BMC (mg)	1.96	2.57*	3.08*		-	31%	56%
X-area (mm2)	21.7	22.6	24.2*		-	4.2%	11.5%
Proximal Tibia							
BMD(mg/cc)	891	1133*	1250*		-	27%	40%
BMC (mg)	1.46	2.21*	2.58*		-	51%	77%
X-area (mm2)	11.0	13.0*	13.7*		-	18%	24.6%

Data for terminal sacrifice:

All animals: Effects at end of study:
Increases in femoral length, and bone mass in femoral midshaft and lumbar vertebra

Group	Control	HD	LD	HD	LD	HD	LD	HD
Dose (µg/kg/day)	0	30	5	30	5	30	5	30
Arm	A	B	E1	E2	H1	H2	I1	I2
Treatment (months)	NA	2-26	6-12	6-12	2-8	2-8	6-26	6-26
N animals	8	8	8	8	8	8	8	8
Tumors found	1 (tibia)	12 (multiple sites)	0	2 (femur, vertebra)**	2 (vertebra)	2 (vertebra, rib)	0	6 (tibia, vertebra, sternum, scapula)
Femur								
Length	35	35	35	35	35	35	35	35
Femur midshaft								
BMD(mg/cc)	973	1417*	970	1014*	991	988	1323*	1448*
BMC (mg)	10.7	20.7*	10.8	11.6*	11.3	11.6*	15.7*	19.9*
X-area (mm2)	9.2	12.2*	9.3	9.5	9.5	9.7*	9.9*	11.5*
L6 vertebra								
BMD(mg/cc)	579	851*	574	591	600	573	758*	828*
BMC (mg)	2.09	3.91*	1.99	2.14	2.31	2.09	2.87*	3.71*
X-area (mm2)	24	30.6*	23.1	24.1	25.4	24.3	25.2	29.7*

*significantly different from concurrent control (p<0.05)

Association of tumors with bone mass effect

Group	Ctrl	HD	LD	HD	LD	HD	LD	HD		LD	MD	HD
Dose (µg/kg/day)	0	30	5	30	5	30	5	30		5	30	75
Arm	A	B	E1	E2	H1	H2	I1	I2		-	-	-
Treatment (months)	NA	2-26	6-12	6-12	2-8	2-8	6-26	6-26		2-26	2-26	2-26
N animals	8	8	8	8	8	8	8	8		<10	<10	<10
Vertebra BMC increase* at end of treatment (%)	-	87%	31%	56%	24%	55%	37%	78%		48%	91%	139%
Vertebra BMC increase* at end of study (%)	-	87%	-5%	2%	11%	0%	37%	78%		48%	91%	139%
# Bone tumors (osteosarcoma + other)	1	9+3	0	2	1+1	2	0	5+1		4+1	12+1	23+2

*increase as compared to concurrent control

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Proximal femur structure at 26 months

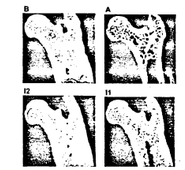

Figure 1: **Representative coronal images of the proximal femur for Arms A, B, I1 and I2.**

The vehicle control (Arm A) is in the upper right panel. The positive control Arm B is in the upper left panel. The 5-µg/kg group of Arm I1 is in the bottom right panel. The 30-µg/kg group of Arm I2 is in the bottom left panel. Marked alterations in bone architecture were observed with long-term teriparatide treatment including substantial reduction in marrow spaces for Arms B, I1 and I2 as compared with vehicle control (Arm A).

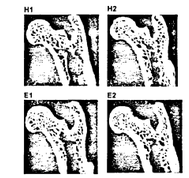

Figure 2: **Coronal images of the proximal femur for Arms E and H.**

A representative image of the 5-µg/kg group of Arm H1 is shown in the upper left panel. An image of the 30-µg/kg group of Arm H2 is shown in the upper right panel. An image of the 5-µg/kg group of Arm E1 is shown in the bottom left panel. An image of the 30-µg/kg group of Arm E2 is shown in the bottom right panel. Images and histomorphometry parameters for the withdrawal groups (Arms E and H) were generally similar to vehicle controls (Arm A), which is shown in Figure 1.

Biomechanical analysis of bone in groups F, G1, G2 showed that bone strength of vertebra and femur (proximal and midshaft) was positively correlated to dose and BMD.

Histomorphometry of femoral neck of 26-month old animals showed increases in various static parameters.

Toxicokinetics

First Study
Human AUC multiples (first study)

Group	Dose (ug/kg/day)	AUC (ngxhr/ml) (Month 6) (m,f)	AUC (ngxhr/ml) (Month 12) (m,f)	AUC (ngxhr/ml) (Month 18) (m,f)	AUC (ngxhr/mL) (average)	AUC multiples (Months 6-18) (m+f)
LD	5	1.1	1.05	0.46	0.9	3.0x
MD	30	7.3	7.28	4.09	6.2	21x
HD	75	18.5	19.94	12.51	17	58x

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

Human Cmax multiples (first study)

Group	Dose (ug/kg/day)	Cmax (ng/ml) (Month 6) (m,f)	Cmax (ng/ml) (Month 12) (m,f)	Cmax (ng/ml) (Month 18) (m,f)	Cmax (ng/mL) (average)	Cmax multiples (Months 6-18) (m+f)
LD	5	1.8	1.6	0.8	1.4	8.6x
MD	30	10.5	9	5	8.2	51x
HD	75	27.5	22	15	21.5	135x

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

For the 5 and 30 mcg/kg/day doses, average Cmax values of 1.4 and 8.2ng/mL were associated with AUC multiples of 3x and 21x.

Second Study
Plasma levels at 15 min post dosing:

	Administered Dose (µg/kg/day)					
Study R00100	Vehicle	Vehicle	5	30	5	30
	Group 01	Group 02	Group 03	Group 04	Group 05	Group 06	Group 07
Day 0, 0.25 hours (ng/mL)	NS	NS	0.66	6.84	NS	NS	NS
Day 154, 0.25 hours (ng/mL)	NC	NC	1.36	11.49	1.31	9.75	7.39
Day 540, 0.25 hours (ng/mL)	NS	NS	NS	NS	NS	NS	7.64

	Administered Dose (µg/kg/day)						
Study R00200	Vehicle	5	30	5	30	5	30
	Group 01	Group 02	Group 03	Group 04	Group 05	Group 06	Group 07
Day 154, 0.25 hours (ng/mL)	NC	1.26	8.63	1.12	10.21	1.15	8.79
Day 248, 0.25 hours (ng/mL)	NC	1.67	12.99	NS	NS	NS	NS
Day 551, 0.25 hours (ng/mL)	NS	NS	NS	NS	NS	1.25	8.85

Abbreviations. NC = no calculation due to insufficient measurable concentrations to determine mean value; NS = no sample collected on this date.

Cmax values (15 min post dosing)

Study	Arm	Dose	Months	Cmax (ng/mL) (average)	Average values Dose (mcg/kg)	Cmax (ng/mL) (Months 6-18) (f)
Study R00100						
Grp 1	A	0	5	0		
Grp 2	F	0	5	0	0	0
Grp 3	G1	5	0.5	1.0	5	1.2
Grp 4	G2	30	0.5	9.2	30	8.8
Grp 5	H1	5	5	1.3		
Grp 6	H2	30	5	9.8		
Grp 7	B	30	5.18	7.5		
Study R00200						
Grp 1	C	0	5.9	0	0	0
Grp 2	D1	5	5.9	1.5	5	1.3
Grp 3	D2	30	5.9	10.8	30	9.9
Grp 4	E1	5	5	1.1		
Grp 5	E2	30	5	10.2		
Grp 6	I1	5	5.18	1.2		
Grp 7	I2	30	5.18	8.8		

Human Cmax multiples (second study)

Group	Dose (ug/kg/day)	Cmax average	Cmax multiples (f)
LD	5	1.3	8.2x
MD	30	9.4	59x

Since the Cmax multiples in the second study (8.2x, 59x)were very similar to those in the first study (8.6x, 51x), the AUC multiples at 5 and 30 mcg/kg in the second study were probably also very similar to the AUC multiples in the first study (3x and 21x).

PTH receptor staining
No PTH receptors were observed upon immunostaining of osteosarcoma tissues of 8 animals, while they were present in a positive control tissue (prostate carcinoma). PTH receptor staining was observed in osteoblasts adjacent to tumor tissue.

Morphologic Pathology
Main findings
- Trabecular hypertrophy
- Bone neoplastic lesions and bone proliferative lesions

Both findings were dependent on dose and treatment duration, i.e., cumulative exposure. However, relation to age of animals at treatment-initiation was unclear.

Trabecular hypertrophy
Trabecular hypertrophy was characterized by diffusely thickened trabeculae in metaphysis and epiphysis (correlated with the necropsy observation of "hard" bone) and increased prominence of osteoblasts. Grading was based on subjective evaluation of trabecular number and thickness and associated reduction in marrow space.

Interim sacrifices (6-mo treatment arms without follow-up):
Assessment at 4 bone sites (femur, tibia, vertebra, sternum)
Trabecular hypertrophy was similar when treatment was started @2mo or @6mo, for femur, tibia, vertebrae. In sternum effect was more pronounced when started @6mo.

Incidence of Trabecular Hypertrophy* (Continued)

Study Arm:	C	D1	D2	F	G1	G2
Dose (µg/kg):	0	5	30	0	5	30
Treatment Duration (Months):	NA	6	6	6	6	6
Age During Treatment (Months):	NA	6 - 12	6 - 12	2 - 8	2 - 8	2 - 8
Age at Necropsy (Months):	12	12	12	8	8	8
Vertebra						
Minimal	1	1	0	0	7	0
Slight	1	22	5	0	20	15
Moderate	0	7	22	0	3	15
Marked	0	0	3	0	0	0
Sternum						
Minimal	1	9	0	0	23	1
Slight	1	16	4	0	7	25
Moderate	0	5	23	0	0	4
Marked	0	0	2	0	0	0

Abbreviations: NA = not applicable.
* Number of rats with finding.

Terminal sacrifices (treatment arms terminated at 26 months):
Assessment subjectively compiled for 4 sites (femur, tibia, sternum, vertebra).

Incidence of Trabecular Hypertrophy*

Study Arm:	A	B	E1	E2	H1	H2	I1	I2
Dose (µg/kg):	0	30	5	30	5	30	5	30
Treatment Duration (Months):	NA	24	6	6	6	6	20	20
Age During Treatment (Months):	NA	2 - 26	6 - 12	6 - 12	2 - 8	2 - 8	6 - 26	6 - 26
Trabecular hypertrophy								
Minimal	11	0	18	29	11	27	1	0
Slight	6	0	9	13	8	4	15	1
Moderate	4	48	1	3	1	1	40	47
Marked	0	11	0	0	0	0	2	12

Abbreviations: NA = not applicable.
* Number of rats with finding.

Trabecular hypertrophy with dose-dependent severity was observed in almost all animals at all doses in the previous study.

Bone neoplastic lesions
Treatment-related lesions observed: osteosarcoma (mainly), osteoma, osteoblastoma.
Incidence was dependent on dose and treatment duration, but not clearly on age at treatment initiation.

Incidence of Primary Bone Neoplasms*

Study Arm:	A	B	E1	E2	H1	H2	I1	I2
Dose (µg/kg):	0	30	5	30	5	30	5	30
Treatment Duration (Months):	NA	24	6	6	6	6	20	20
Age During Treatment (Months):	NA	2 - 26	6 - 12	6 - 12	2 - 8	2 - 8	6 - 26	6 - 26
Osteoma		2			1			1
Osteoblastoma		1						
Osteosarcoma	1	9[a]		2	1	2		5
Total no. of rats with a bone neoplasm	1	12[b]		2	2	2		6[c]
Gross bone nodule lesions[d]	1	11			2	2		4

Abbreviations: NA = not applicable, no. = number.
*Statistically significant with p-value < .025 based on 1-sided Cochran-Armitage trend test
a Number of rats with a specific neoplasm.
b p< .0003 (Arm B compared to Arm A)
c p< 0.0010 (Arm B compared to Arm A).
d p< 0149 (Arms H and I2 compared to Arm A).
e Number of rats with a grossly observed bone nodule, bone lesion, or metastatic lesion, which was diagnosed as a primary bone neoplasm.

Incidence of Primary Bone Neoplasms by Bone Site

Study Arm:	A	B	E1	E2	H1	H2	I1	I2
Dose (µg/kg):	0	30	5	30	5	30	5	30
Treatment Duration (Months):	NA	24	6	6	6	6	20	20
Age During Treatment (Months):	NA	2 - 26	6 - 12	6 - 12	2 - 8	2 - 8	6 - 26	6 - 26
Site of bone neoplasm								
Vertebra		5		1	2	1		2
Tibia	1	3						2
Femur		2		1				
Rib		3				1		
Sternum		1						1
Mandible		1						
Humerus scapula		1						1
Single site[a]	1	10		2	2	2		6
Multiple sites[b]		2						

Abbreviations: NA = not applicable.
a Number of rats with bone neoplasms associated with a single bone site.
b Number of rats with bone neoplasms (osteosarcomas) associated with multiple bone sites.

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Animals with bone tumors

Arm	Study	Grp	Animal Nr.	Day of necropsy	Mode of sacrifice	Diagnosis	Gross nodule	Tumor characteristics
A (control)	0100	1	1062	555	Killed	Tibia osteosarcoma nodule	Yes	Tibia
B (30ukd, 24 mo)	0100	7	7051	645	killed	Rib osteosarcoma	Yes (1x1cm)	Thorax
		7	7056	596	Killed	Scapula-humerus OS	Yes (1-3cm)	Shoulder
		7	7072	590	Killed	Femur, rib, vertebra, tibia OS (Not mentioned which vertebra)	Yes in femur and rib No in vertebra No in tibia	Vertebral tumor arising from medulla extending into spinal canal, however, no spinal cord depression noted Tibial tumor not specified
		7	7081	628	Killed	Vertebra and tibia OS	Yes in proximal tibia- No in vertebra	Tumor of vertebra growing throughout bone in a unspecified dorsal vertebra; no spinal cord depression
		7	7084	674	Killed	Rib OS	Yes (1-3cm)	Near sternum
		7	7091	546	Killed	Femur OS	Yes	Femoral midshaft
		7	7094	708	Killed	Vertebra OS	Yes (1-3cm)	Thoracic part of spine at last rib; only this vert examined?
		7	7098	631	Killed	Mandible OS	Yes (1-3cm)	Left mandible
		7	7153	660	Killed?	Sternum	Yes (0.6-1cm)	Sternum (unspecified)
		7	7090	734	Terminal	Tibia osteoblastoma	No	No remarks on tumor
		7	7157	660	Killed	Vertebral osteoma	Yes (1-3cm)	Lumbosacral junction
		7	7160	736	Terminal	Vertebral osteoma	Yes (1-3cm)	T11-T12 tumor projecting ventrally from vertebral body
H1 (5ukd, 6mo)	0100	5	5052	695	Killed	Vertebral osteosarcoma (posterior thorax)	Yes	Posterior thorax
			5067	733	Terminal	Vertebral osteoma	Yes (0.6-1cm)	T5-T6
H2 (30 ukd, 6mo)	0100	6	6065	678	Killed	Vertebra mixed osteosarcoma osteoma	Yes (0.6-1cm)	mid thorax
		6	6088	491	Killed	Rib osteosarcoma	Yes (0.6-1cm)	Right posterior rib in thorax
E2 (30 ukd, 6mo)	0200	5	5084	743	Terminal	Femur OS	No	Femur (exact location not specified)
		5	5160	719	Killed	Vertebral OS	No	T7-8 (spinal cord depression observed). Tumor projecting into spinal canal.
I2 (30 ukd, 20mo)		7	7057	638	Killed	Tibia OS	Yes (103)	Proximal tibia
		7	7094	742	Terminal	Tibia OS	Yes (0.6-1cm)	Proximal tibia
		7	7096	742	Terminal	Scapula OS	Yes (1-3cm)	Scapula
		7	7151	735	Killed	Sternum OS	No	Tumor in medulla of an unspecified sternebra
		7	7073	740	Terminal	Vertebral osteoma	Yes (0.5 cm)	At T6, ventral and dorsal
		7	7161	716	Killed	Vertebra OS	No	Posterior thorax; anterior lumbar region (spinal cord depression observed, 0.5cm)

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Time of tumor detection

When treatment was started at 6 months there was a 20 month-period follow-up after start of treatment, as compared to 24 months when treatment was started at 2 months.
In group B (30 mcg/kg/day, 2-26 mo) the average time-to-grossly-observed-tumor was 642 days, i.e., 21 months. Thus 21 months on average was needed for a tumor to manifest itself as gross nodule in this group. The time-to(all)-tumor was 650 days.
In group I2 (30 mcg/kg/day, 6-26mo) the average time-to-grossly-observed-tumor was 716 days, i.e, 596 days or 19.5 months after start of treatment, and the time to-(all)-tumor was 719 days. In this group 2/6 tumors were microscopically detected.
In group E2 (30 mcg/kg/day, 6-12mo) the time-to-tumor was 731 days, i.e., 611 days or 20 months after start of treatment (end of study). Both tumors in E2 were microscopically detected.

There were relatively more animals with only microscopic (as compared to grossly observed) tumors in the groups starting treatment at 6 months (2/2 in E2, 2/6 in I2) than in the groups starting at 2 months (1/12 in B, 0/2 in H1, 0/2 in H2). However in the original study with treatment from 2-26 months there was a fair proportion of animals with only microscopic tumors in all dose groups (1/5 in LD, 6/13 in MD, 13/25 in HD).

The average time-to-tumor (observed due to death of animal) appeared longer in those groups starting treatment at 6 months (E2, I2) rather than 2 months (B, H1, H2, 1st study arms) (725 days vs. 669 days). This suggests that there may have been more chance of missing a treatment-related tumor in the later treatment arms (E1, E2, I1, I2), whether as a gross nodule or a microscopic lesion. This would seem plausible since tumors grow exponentially.

Grp	Dose (ukd)	Treatment period (mo)	Bone tumors (N)	Time to tumor detection (days)	Time to tumor detection (avg., days)	Time to end of study (days)	Average time between tumor detection and end of study (days)
2nd study							
A	0	NA	1	555	555	-	-
B	30	2-26	12	546-736	650	736	86
H1	5	2-8	2	695-733	714	736	22
H2	30	2-8	2	491-678	585	736	151
E1	5	6-12	0	no tumors	-	743	-
E2	30	6-12	2	719-743	731	743	13
I1	5	6-25	0	no tumors	-	743	-
I2	30	6-26	6	638-742	719	743	25
1st study							
LD	5	2-26	5	629-743	697	743	46
MD	30	2-26	13	589-742	698	743	45
HD	75	2-26	25	488-743	675	743	68

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BONE TUMOR DATA

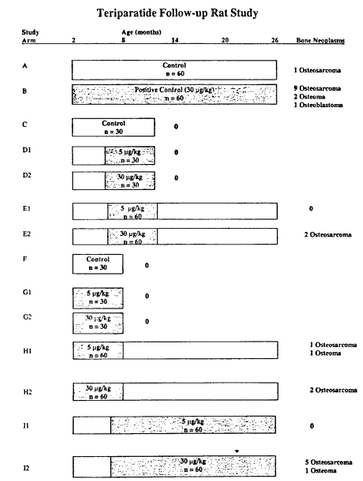
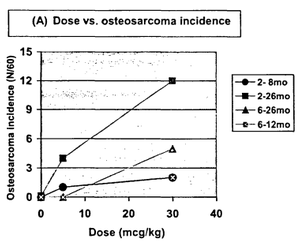
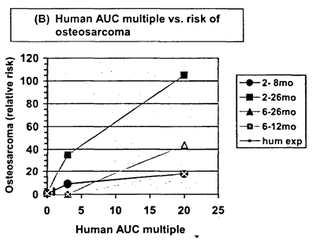
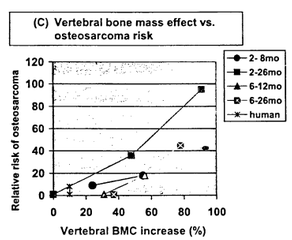
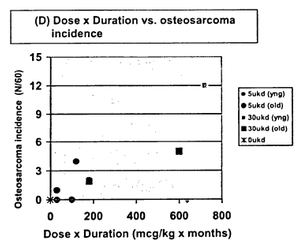


FIGURE 1. Incidence of bone neoplasms, by study arm









Bone proliferative lesions

Three different lesions appeared to be treatment-related: osteoblast hyperplasia, stromal proliferation, and stromal vascular proliferation.

Focal osteoblast hyperplasia consisted of focal increases in well differentiated, osteoblast-like cells often with evidence of local osteoid production, but without significant disruption of preexisting bone trabeculae. The focal increase in osteoblast numbers was in excess of the increased prominence of osteoblasts associated with the trabecular hypertrophy.

Stromal proliferation consisted of a proliferation of spindle-shaped stromal cells that filled marrow spaces in a focal area with loose fibrous connective tissue and varying proportions of vascular components. The vascular components were thin walled (capillaries and/or vascular sinuses). The reaction often appeared to be accompanied by localized and limited resorption of trabecular bone and sometimes contained small foci of osteoid deposition. The tissue reaction was proliferative, but had no dysplastic features. The stromal proliferation was presumed to include committed osteoprogenitor cells of the osteoblastic lineage, based upon the scattered focal areas of osteoid deposition. The observation was reported to be seen usually at one bone site in any given animal, and was seen in either femur, tibia, vertebra, or sternum (with approximately equal frequencies).

Stromal vascular proliferation was observed occasionally, and consisted of stromal proliferation that had a more prominent vascular component .

Incidence of Nonneoplastic Proliferative Lesions in Bone*

Study Arm	A	B	E1	E2	H1	H2	I1	I2
Dose (µg/kg)	0	30	5	30	5	30	5	30
Treatment Duration (Months)	NA	24	6	6	6	6	20	20
Age During Treatment (Months)	NA	2 - 26	6 - 12	6 - 12	2 - 8	2 - 8	6 - 26	6 - 26
Focal osteoblast hyperplasia		1						3
Focal stromal proliferation								
Minimal							2	3
Slight		5		1			4	5
Moderate		2		1			2	7
Focal stromal vascular proliferation								
Moderate marked						1		2

Abbreviation. NA = not applicable.
* Number of rats with lesion.

Preneoplastic lesions and neoplasms

Group	A	B	E1	E2	H1	H2	I1	I2
Osteoblast proliferative lesions	0	8	0	2	0	1	8	15
Osteoblast tumors	1	12	0	2	2	2	0	6

Small incidences of treatment-related focal ostoblast hyperplasia were observed in the previous study, but stromal proliferation was not reported.

Other treatment-related bone lesions in the current study included cartilaginous dystrophy (cartilage degeneration) in tibia and femur, apparently in association with trabecular hypertrophy.

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SUMMARY AND EVALUATION

The results of the second rat carcinogenicity study with teriparatide confirm the results obtained in the first study. That study showed that teriparatide causes osteosarcomas and other bone tumors upon 24-month treatment at exposures ranging from 3 to 60 times the human exposure at the proposed 20-mcg clinical dose. The findings of the previous study carried out with 5, 30, 75 mcg/kg/day are shown in the APPENDIX.

Effects on bone tumors

The results of the second study show that bone tumors can be induced upon 6-month treatment of younger rats with 5 or 30 mcg/kg, and upon 6- or 20-month treatment of older rats with 30 mcg/kg. Treatment of older animals for 6 or 20 months with 5 mcg/kg did not induce a significant increase in bone tumors. The 5 and 30 mcg/kg/day doses lead to 3 and 21 times human exposure, respectively.

Effects on bone quality

There was a significant effect of teriparatide on bone mass and the compound induced trabecular hypertrophy at several bone sites. These effects were reversed upon treatment withdrawal so that animals treated for 6 months early in life no longer had significant increases in bone at the time of study termination. This indicates that osteoblast stimulation rather than the presence of an excessive amount of bone mineral in the vicinity of the osteoblast is the cause of the bone tumors

Spontaneous bone neoplasm incidence

The vehicle control incidence of osteosarcoma in the current study was 1/60. In 7 previous control groups from other studies by the Sponsor and in the first study with teriparatide there were no osteosarcomas in female vehicle-treated control groups (N=60/grp). Thus, the combined concurrent and historical control (HC) rate is 1/540, i.e. 0.0019, or 0.19%. Historical control data from Eli Lilly and the NTP database for female rats are given in the APPENDIX.

Effect of survival

The survival curves showed that mortality was similar in the long term groups A, B, H2, E2, and I1, but was somewhat less in groups H1 and E1, and somewhat higher in I2. This might explain why a relatively strong tumor response was seen in group H1 as compared to H2. It also suggests that the absence of tumors in arms E1 and I1 was not likely to be due to decreased survival. The lower survival in group I2 could mean that the tumor incidence in that group (N=6) was slightly underestimated. Mortality was not significantly associated with bone tumors.

Effect of observation period

It is possible that tumors were missed in the groups starting treatment at 6 months (E, I) due to insufficient observation time (14 months as compared to 18 months in H, and 20 months as compared to 24 months in B). Also, the microscopic evaluation included only a small part of the skeleton, i.e., part of a femur and tibia, and 1-2 vertebrae and sternebrae, and microscopic tumors could be missed for this reason. However, it should be noted that potential underdetection due to limited sampling applies to all groups including the controls. Also, the proportion of microscopically detected tumors was not increased in the later treatment groups when compared to all early treatment groups from both studies. Nevertheless, caution is warranted in the interpretation of the low tumor incidences (0-2) in the arms starting treatment at later age since there may have been underdetection of bone tumors in those groups due to shorter follow up. A period of 4 months may be significant for the growth of a tumor, and the potential failure to detect 1 tumor in any of those low incidence groups has a significant impact on the interpretation of the results.

Preneoplastic lesions

Osteoblast hyperplasia and focal stromal proliferation were seen in several treatment arms at 30 mcg/kg and in group I1 at 5 ucg/kg. Although Sponsor states that it is not clear whether this is a preneoplastic lesion, Reviewer feels that this finding suggests that the 5 mcg/kg dose would eventually lead to bone tumor formation.

Interpretation of tumor data

The Sponsor concluded from this second study that the induction of bone tumors was dependent on dose and treatment duration, but not clearly on age of animal at treatment initiation. Sponsor also concluded that the 5 mcg/kg dose is a NOEL (i.e., NOAEL) for bone tumor formation in mature (6-month old) rats, based on the finding that there were no tumors in the older animals treated with the 5 mcg/kg dose. Sponsor integrated this contention in the label.

Reviewer agrees with Sponsor that tumor incidence is dose-related and treatment duration related. However because of the rare incidence of bone tumors, the new data do not give us accurate quantitative information on the tumor response at low doses and short durations. Reviewer feels that the conclusion that 5mcg/kg is the NOAEL in older animals is not justified. The study was not powered to detect modest increases in the incidence of these rare tumor(s), and with a group size of N=60 false negatives are possible.
At the time of study design it was communicated to the Sponsor that it needed adequate power to provide confidence that a negative result implies the absence of a drug-related risk (Memo; Ron Steigerwalt 9/14/99)

The following table estimates how many tumors are to be expected in groups of N=60 at given levels of risk increase. Calculation of the number of animals needed to detect a risk increase is based on the "rule of three".

Osteosarcoma risk increase	Expected incidence in a group of N=540	Expected incidence in a group of N=60 (X)	Expected range in a group of N=60 (X ± sqrX; rounded)	Number of animals to be studied to detect this increase
1x	1/540	0.11	0-1	1620
3x	3/540	0.33	0-1	540
5x	5/540	0.55	0-2	324
8x	8/540	0.9	0-2	203
10x	10/540	1.1	0-2	162
15x	15/540	1.7	0-3	108
20x	20/540	2.2	1-4	81
30x	30/540	3.2	1-5	54

In order to reliably detect a 25-fold risk/incidence increase in a tumor with a 0.2% background rate (from 0.2% to 5% or 1/20) one would have to study 3x20=60 animals in which one would then expect 3 tumors ("rule of 3"). To detect a 10-fold increase (from 0.2% to 2% or 1/50) one would have to study 3x50=150 animals. A 5-fold risk increase would need 300 animals. Conversely, in a group of 60 animals, if 1 tumor is seen (1/60=1.7%) that represents a 1/60:1/480= 8-fold risk increase, and 2 tumors represent a 16-fold risk increase. This suggests that a group of N=60 is not big enough to detect a relatively small 5- or 10-fold risk increase. The absence of tumors in the two 5 mcg/kg/day groups starting at later age (N=120) suggests that in those groups risk was, on average, <10-fold increased.

Assuming the dose-response curve between 5 and 30 mcg/kg is linear, the incidence at the 0 ug/kg dose is 0 (reasonable assumption), and the time of treatment onset is not a critical factor, the number of tumors expected and observed in 60 animals at 5 ucg/kg, is as follows (calculation based on 30 mcg/kg/day data):

Tumors at 5 mcg/kg

Age at treatment onset (months)	Incidence of osteosarcoma	Treatment duration		
		6mo	20mo	24mo
2	Expected if dose-linear response between 5-30 ukd	0.3	-	2
2	Observed	1	-	4
6	Expected if dose-linear response between 5-30 ukd	0.3	0.8	-

6	Observed	0	0	-

This suggests that, although younger animals may be more responsive to the low dose than the older ones, the observed results are also consistent with the hypothesis of dose-linearity regardless of age at treatment onset.

Alternatively, one can consider the hypothesis that treatment starting at 2 months has no threshold level with regard to bone tumor incidence, whereas treatment starting at 6 months does. The data could be in agreement with this hypothesis since the young animals treated with 5 mcg/kg had tumors whereas the older ones did not. However, the apparent absence of a dose response in animals treated from 2-8 months (2 tumors in both 5- and 30 mcg/kg dose groups) and the fact that the 30 mcg/kg dose causes 2 osteosarcomas in both young and older animals seems to contradict this hypothesis (see table below). Apparently, the uncertainty in the low incidence data is large and the data do not provide convincing support for the hypothesis. Although 2/60 tumors is a strong indication of a treatment effect, outcomes of 0/60 or 1/60 tumors are difficult to interpret. The data also suggest that effect of age at treatment onset is unclear.

Bone tumor incidence with 6 month treatment duration

	Onset	Young (2 mo)	Old (6 mo)
Dose (mcg/kg)			
5		2	0
30		2	2

Reviewer feels that the most appropriate conclusion from this study is that the risk in the LD animals was increased by less then 10-fold. In retrospect the study was not adequately designed to establish a tumor NOAEL, and large groups of e.g. 500 animals would have been needed to determined this value. A small risk increase in older animals treated with 5 ucg/kg/day is supported by the occurrence of stromal proliferation in group I2. The conclusion is also based on the possibility that tumor incidence was underestimated in the groups with shorter observation time (E and I). The role of age at treatment initiation remains unclear.
It should also be noted that even if a NOAEL was firmly established this value could not be directly extrapolated to humans, and safety factors would have to be introduced to predict a "safe" dose in humans.

Statistical analysis

A statistical analysis of the second study data using a logistic regression model was carried out by the Statistics Reviewer (Joy Mele, M.S.) to estimate the probability of finding a bone tumor as a function of dose, duration, age at treatment onset and observation period (i.e., time between treatment onset and study end). With a vehicle control value of 1/60 (or a substituted value of 0/60), dose and treatment duration were found to be the only significant factors related to the probability of developing an osteosarcoma. The analysis showed that the odds ratio of finding a tumor at 5 ucg/kg/day was 1.79 (CI 1.21, 2.65), regardless of treatment duration. This indicated that, based on the data, there was a low risk of osteosarcoma in all 5 ug/kg/day groups that was significantly higher than in the controls.

Risk assessment

It is unclear how we can extrapolate the findings in the two rat studies to humans, and a quantitative risk assessment based on the animal data remains difficult. There is some degree of uncertainty with regard to the choice of the relevant risk parameter (although relative risk seems more appropriate than absolute risk), and it is likely that there are differences in susceptibility of rats vs humans and interindividual differences. Because of these uncertainties and differences, safety factors are often introduced in risk assessment in order to calculate a "safe" dose. These types of calculations can be carried out for the hormonal bone carcinogenic effect of teriparatide. However, they are speculative and quantitatively unreliable.

With regard to duration of treatment Sponsor has argued that, since humans will be treated for only 3% of their lifespan while the rats in the studies were treated for 20%-80% of theirs, and the

NOAEL was concluded to be 5 mcg/kg for mature rats, the proposed clinical treatment regimen is safe. However, it may be more appropriate to compare treatment duration in terms if bone turnover cycles or bone mass effect. A treatment time of 6 months in the rat (5 bone turnover cycles) is equivalent to 500-1000 days in humans, i.e., 1.4-2.8 years. Graph C shows how tumor incidence is related to the pharmacodynamic effect of teriparatide on bone (BMD or BMC). Since the proposed 20 mcg dose causes about a 10% increase in BMD and BMC in humans in the lumbar spine, this relationship suggests that human risk could be increased anywhere between 1- and 5-fold.

CONCLUSION

Teriparatide can cause bone tumors in female rats when treatment is started in immature or mature animals. The effect is dependent on dose and treatment duration. Due to the low spontanous rate of osteosarcoma and other bone tumors in the rat, a threshold or NOAEL dose for bone tumor formation due to teriparatide treatment could not be established. The main conclusion from this study is that a limited duration of treatment of mature rats can cause osteosarcoma. It can not be excluded that the proposed clinical treatment regimen is associated with a finite risk for osteosarcoma in the projected treatment population.

RECOMMENDATION

AE (Approvable)

The results of the second rat carcinogenicity study suggest that osteosarcomas and other bone tumors can be induced by teriparatide in older rats. Moreover, there is insufficient evidence for a threshold level regarding tumor risk in rats. The Reviewer concludes that clinical use of the compound for a duration of 2 years at the proposed dose of 20 mcg/day (a dose equivalent to 1/3 times the lowest tested rat dose of 5 mcg/kg/day) is potentially associated with an increased risk of osteosarcoma or other osteoblastic bone tumor formation in humans. Based on this potential risk the Reviewer recommends that the NDA can be approved if the Clinical Reviewer feels that the risk is acceptable and is communicated in a proper manner in the product label (including a black box warning and restrictions as specified in the draft label of November 20, 2002).

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APPENDIX

FIRST STUDY RESULTS

Incidences of osteoblast neoplasms and hyperplasia

		Males				Females			
Group		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	**0**	**3**	**21**	**31**	**0**	**4**	**12**	**23**
	Gross bone nodule/lesion	0	1	17	24	0	3	7	13
	Total no. of rats with a bone neoplasm	0	3	24	36	0	5	13	25
	No. of rats with multiple different bone neoplasms	0	0	1	3	0	0	0	2

Incidence of osteosarcoma by bone site

		Males				Females			
Group		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
	Mutiple sites	0	0	3	7	0	0	2	1
Total incidence		0	3	21	31	0	4	12	23

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OSTEOSARCOMA HISTORICAL CONTROL DATA

Data Source	Database	Sex	Incidence (N/N)	Incidence (%)
First teriparatide study	Eli Lilly	Females	0/60	0%
Second teriparatide study	Eli Lilly	Females	1/60	1.67%
Historical controls	Eli Lilly	Females	0/420	0%
	NTP 1998	Females	5/1354	0.4%
	NTP 2002	Females	1/909	0.1%
Current* plus historical control values (*teriparatide studies)	Eli Lilly	Females	1/540	0.19%

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PHARMACOLOGY/TOXICOLOGY REVIEW OF NDA SUBMISSION

NDA 20815
Eli Lilly
FORTEO™ (teriparatide) (rhPTH1-34) (LY333334)

Submission date: May 10, 2002
Review date: May 14, 2002

STATUS REPORT FOR A SPECIAL 2-YEAR STUDY WITH LY333334 (TERIPARATIDE) IN FEMALE FISCHER 344 RATS
(Studies R001011 and R00200)

On May 10, 2002, Lilly submitted a status report for the recently completed 2-year study with LY333334 (teriparatide) in female Fischer 344 rats. The study was initiated in March 2000 as a follow-up for a standard two-year rat carcinogenicity study with teriparatide in which animals were treated from 6-8 weeks of age with three doses of teriparatide for 24 months.

Methods
The new study design included a negative and a positive control arm, and various arms with treatment starting at either 6 weeks of age or 6 months of age, and lasting either 6 months, or 20-24 months (doses 5 or 30 ug/kg/day). The animals in the 6-month treatment arms were immediately sacrificed or continued off treatment for the remainder of the study (Figure 1).
The bone sampling and histology methods included collection of femur, tibia, sternum, vertebra, and of all bone lesions (nodules, masses, other). Examined were:

- Femur : longitudinal section (distal part) comprising approximately 50% of length of bone
- Tibia: longitudinal section (proximal part) comprising approximately 30-40% of length of bone
- Vertebra: longitudinal section including entire length of single vertebra
- Sternum: longitudinal section including length of 1-2 sternebrae
- Bone lesions: specimens to be determined by pathologist

This method implies that bone tumor incidence is probably underestimated, in both control and treated groups, as is the case for all tumor lesions in this type of study.
Previous PK data have shown that the exposure to teriparatide in rats treated with 5 and 30 ukd is expected to be approximately 3 and 20 times the exposure of humans given a 20 ug daily dose.

Results
The results show that upon histologic examination of 4 bone sites (femur, sternum, rib, vertebra) and all other grossly apparent bone lesions, osteosarcomas were observed in the following arms: the negative control arm (n=1), positive controls (n=9), 6-month treatment arm with 30 ukd starting at 6 months of age (n=2), 6-month treatment arms with 5 or 30 ukd starting at 6 weeks of age (n=1or n=2), and the continuous treatment arm with 30 ukd starting at 6 months of age (n=5) (Figure 1).

The data show that teriparatide can elicit bone tumors in rats treated from a young or older age, and can cause tumors to appear upon a relative short term treatment of 6 months when that treatment is started at either young or older age. However, the incidence of tumors was lower in the 6-month treatment arms vs. the 20-24 treatment arms and generally lower in the 5 ukd vs. 30 ukd groups.

No tumors were observed at 5 ukd when treatment was for 6 months starting a 6 months of age. Although the latter finding is somewhat reassuring because it most closely resembles the clinical treatment (2 years in postmenopausal women or men with osteoporosis) the appearance of tumors in the other short term treatment arms (E2, H1 and H2) including the one in which treatment is starting at the older age (E2) is very disconcerting.

The overall data suggest that the tumor response is positively correlated to (1) dose and (2) treatment-duration, but not clearly to (3) age-at-treatment-initiation.

Conclusions
The conclusion from these data is that teriparatide causes osteosarcomas in rats in a dose-dependent and treatment duration-dependent manner. The Reviewer also interprets these data to signify that treatment of postmenopausal women or men with teriparatide for 2 years at a 20 mcg daily dose puts patients at increased risk for osteoblast neoplasms. However, the magnitude of the absolute or relative risk is not clear. A quantitative estimation of this risk based on extrapolation from the current animal data would be fraught with considerable uncertainty.

Further review and evaluation of these data upon submission of the final report (July 2002) is warranted before definite conclusions are drawn. Particularly the ability to detect bone tumors with the employed sampling and histology methods needs to be carefully evaluated, and further sampling may be needed.

Questions to Sponsor
The Reviewer sent the following questions to Lilly on May 13 and May 14, 2002:

1. How many of the bone tumors were detected in prematurely deceased/sacrificed animals vs. terminally sacrificed animals?
2. How many of the bone tumors were fatal vs. non-fatal?
3. How many of the bone tumors were detected by gross necropsy with subsequent microscopy vs. microscopy only (both for prematurely deceased/sacrificed and terminally sacrificed animals)?
4. At which bone site (femur, tibia, vertebra, sternum, other) were the reported tumors observed?
5. What was the incidence of osteoblast hyperplasia and of trabecular hypertrophy in the 14 study groups?

Gemma Kuijpers, Ph.D.
HFD-510

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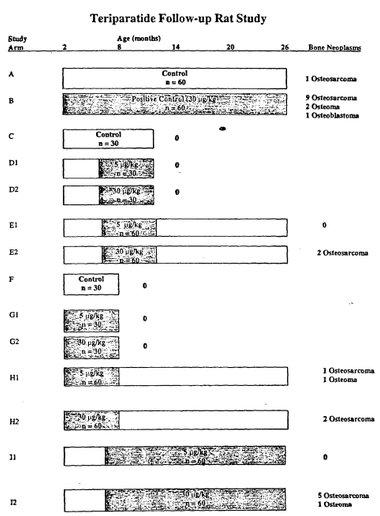

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/s/

Gemma Kuijpers
5/23/02 02:21:11 PM
PHARMACOLOGIST

Karen, please sign if you think this review is ready

Karen Davis-Bruno
5/24/02 11:57:47 AM
PHARMACOLOGIST

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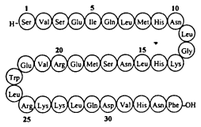
REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

Interim Reports on a Special Chronic Study in Female Fischer 344 Rats Given LY333334 For up to 2 Years

KEY WORDS: Parathyroid hormone, teriparatide, peptide, postmenopausal, osteoporosis, bone, formation, resorption

IND NUMBER:	
DRUG:	**LY333334 (teriparatide for injection)**
Reviewer:	Gemma Kuijpers
Division Name:	Division of Metabolic and Endocrine Drug Products
HFD #:	510 (DMEDP)
Review Completion Date:	September 25, 2001
Date of submission:	December 19, 2000; August 23, 2001
Information to Sponsor:	**No**
Sponsor:	Eli Lilly and Company, Indianapolis, IN, USA
Drug substance:	Teriparatide (rDNA origin), PTH(1-34)
Proprietary Name:	Forteo™
Synonyms:	rhPTH(1-34)
Code Name:	LY333334
USAN Name:	Teriparatide
INN name:	Teriparatide
Chemical Name:	34-amino acid single chain peptide
Molecular Formula:	$C_{181}H_{291}N_{55}O_{51}S_2$
Molecular Weight:	4117.8 Daltons
Drug Product:	rhPTH(1-34), acetic acid, sodium acetate, mannitol, metacresol, sodium acetate, HCl, NaOH, water for injection
Dosage Form:	Cartridges rhPTH(1-34) Injection (Pen-injector)
Dosing Device:	3 ml prefilled, delivery device containing a 28-day supply of rhPTH(1-34)
Manufacturer for drug substance:	Eli Lilly and Company, Lilly Technology Center, IN, USA
Manufacturer for dosage form:	Lilly France S.A., Fegersheim, France
Drug Class:	Peptide hormone
Structure:	



Indication:

Clinical formulation: Sterile solution containing per ml: 250 µg/ml rhPTH(1-34), 0.41 mg acetic acid, 0.1 mg sodium acetate, 45.4 mg mannitol, 3 mg metacresol, and water for injection

Dose: 20 µg daily
Route of administration: Subcutaneous injection (thigh or abdominal wall)
Clinical status: NDA (Forteo) submitted (November 30, 2000)

Gemma Kuijpers, Ph.D.
Pharmacology Reviewer, HFD-510

Cc:
IND Arch
HFD-510
HFD-510/Kuijpers/Davis-Bruno/Hedin

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In a two year carcinogenicity study in the F344 rat LY333334 caused a marked incidence of osteosarcoma and other bone proliferative lesions. In that study animals were treated subcutaneously for 24 months, with 0, 5, 30, 75 ug/kg/day, from an age of 6-7 weeks, and bone tumors were first found by palpation after 17 months in the high dose groups and after 20 months in the low and mid dose groups.

A second 24-month follow-up carcinogenicity study in female F344 rats was initiated in March 2000, and the protocol of this study was reviewed by the Exec CAC (date). The study is intended to investigate the role of treatment duration and age of animals at treatment onset. The protocol includes the following treatment arms:

STUDY R00100				
Group	Dose	N	Treatment period	Follow-up period
1	Control	60	2-26 months (vehicle)	-
7	Positive control	60	2-26 months	-
2	Control	30	2-8 months (vehicle)	-
3	5 ug/kg	30	2-8 months	-
4	30 ug/kg	30	2-8 months	-
5	5 ug/kg	60	2-8 months	8-26 months
6	30 ug/kg	60	2-8 months	8-26 months
STUDY R00200				
1	Control	30	6-12 months (vehicle)	-
2	5 ug/kg	30	6-12 months	-
3	30 ug/kg	30	6-12 months	-
4	5 ug/kg	60	6-12 months	12-26 months
5	30 ug/kg	60	6-12 months	12-26 months
6	5 ug/kg	60	6-26 months	-
7	30 ug/kg	60	6-26 months	-

Sponsor submitted two interim reports with the data from the 6-months treatment arms in which animals were sacrificed immediately after treatment (Study R00100, Groups 2,3,4; Study R00200, Groups 1,2,3). This review evaluates the results submitted in the two interim reports.

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Interim Report on a Special Chronic Study in Female Fischer 344 Rats Given LY333334 For up to 2 Years (Study R00100)
(Toxicology Report 38)
Submission date: December 19, 2000

METHODS
Female F344 rats (6-7 weeks of age) (N=30/group) were dosed daily with s.c. injections of 0, 5, 30 ug/kg/day, for 6 months. Examinations included (1) survival, general physical condition, behavior (daily) and (2) muscle tone, pelage condition, eyes, respiration, posture, excreta, locomotion, visible/palpable lesions or growths (weekly for 2 weeks, then biweekly), and (3) body weight and food consumption. Necropsy included examination of body surfaces, orifices, cavities, viscera. Soft tissues and 4 bones (femur, tibia, sternum, lumbar vertebra) were preserved in formalin. Bones were decalcified and processed for histologic evaluaiton. Femur, tibia and L-6 lumbar vertebra were also preserved in 70% ethanol for pQCT evaluation (cross-section) of X-area, BMC, BMD.

RESULTS
Survival
All animals suvived

Clinical Observations
No treatment-related signs

Body weight and food consumption
Slight increase in body weight in HD group from Week 10-25 (5%-7% relative to controls)

Morphologic Pathology
Trabecular hypertrophy(diffusely thickened trabeculae in metaphysis and epiphysis), correlated with necropsy observation of hard bones, most prominent in femur and tibia. Hypertrophy associated with marked reduction of bone marrow spaces. Normal marrow elements were generally present. No cellular proliferative lesions of bone observed.

Group			control	LD	HD
Dose (µg/kg/day)			0	5	30
N animals			30	30	30
BONE					
Trabecular	Femur	Minimal			
hypertrophy		Slight		28	
		Moderate		2	30
	Tibia	Minimal			
		Slight		26	
		Moderate		2	30
	Vertebra	Minimal		7	
		Slight		20	15
		Moderate		3	15
	Sternum	Minimal		23	1
		Slight		7	25
		Moderate			4

Quantitative Bone Analysis
Femur, Vertebra:
Increases in: (a) femoral length, and femoral midshaft BMD, BMC, X-area, and (b) lumbar vertebra BMD, BMC, X-area, in both LD and HD

	Parameter values			Increase relative to control		
Group	control	LD	HD	control	LD	HD
Dose (µg/kg/day)	0	5	30	0	5	30
N animals	30	30	30	30	30	30
Femur						
Length	32.8	33.3*	33.9*	-	1.5%	3.4%
Femur midshaft						
BMD(mg/cc)	939	991*	1095*	-	5.5%	16.6%
BMC (mg)	8.68	9.34*	11.59*	-	7.6%	33.5%
X-area (mm2)	7.70	7.86	8.82*	-	2.1%	14.6%
L6 vertebra						
BMD(mg/cc)	572	671*	767*	-	17%	34%
BMC (mg)	1.80	2.23*	2.79*	-	24%	55%
X-area (mm2)	20.9	22.1*	24.2*	-	10.6%	16%

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Interim Report on a Special Chronic Study in Female Fischer 344 Rats Given LY333334 For up to 2 Years (Study R00100)
(Toxicology Report 39)
Submission date: April 5 and August 23, 2001

METHODS
Female F344 rats (6-7 weeks initial age) (N=30/group) were given daily with s.c. injections of vehicle for approximately 4 months, and then (at approximately 6 months of age) dosed with 0, 5, 30 ug/kg/day, for 6 months. Examinations included (1) survival, general physical condition, behavior (daily) and (2) muscle tone, pelage condition, eyes, respiration, posture, excreta, locomotion, visible/palpable lesions or growths (weekly for 2 weeks, then biweekly), and (3) body weight and food consumption. Necropsy included examination of body surfaces, orifices, cavities, viscera. Soft tissues and 4 bones (femur, tibia, sternum, lumbar vertebra) were preserved in formalin. Bones were decalcified and processed for histologic evaluaiton. Femur, tibia and L-6 lumbar vertebra were also preserved in 70% ethanol for pQCT evaluation (cross-section) of X-area, BMC, BMD.

RESULTS
Survival
All animals suvived

Clinical Observations
No treatment-related signs

Body weight and food consumption
Slight increase in body weight in LD and HD from Week 8 of dose administration (5%-7% relative to controls)

Morphologic Pathology
Trabecular hypertrophy (diffusely thickened trabeculae in metaphysis and epiphysis), and marked reduction of bone marrow spaces. Normal marrow elements were present. No cellular proliferative lesions of bone observed.

Group			control	LD	HD
Dose (µg/kg/day)			0	5	30
N animals			30	30	30
BONE					
Trabecular	Femur	Slight	1	22	3
hypertrophy		Moderate		7	25
		Marked			2
	Tibia	Slight	1	22	3
		Moderate		8	25
		Marked			2
	Vertebra	Slight	1	22	5
		Moderate		7	22
		Marked			3
	Sternum	Minimal	1	9	
		Slight	1	16	4
		Moderate		5	23
		Marked			2

Quantitative Bone Analysis
Femur, Vertebra, Tibia:
Increases in: (a) femoral length, and femoral midshaft BMD, BMC, X-area, (b) lumbar vertebra BMD, BMC, X-area, and (c) proximal tibial BMD, BMC, X-area

	Parameter values			Increase relative to control		
Group	control	LD	HD	control	LD	HD
Dose (µg/kg/day)	0	5	30	0	5	30
N animals	8	8	8	8	8	8
Femur						
Length	34.2	34.7*	35.2*		1.5%	2.9%
Femur midshaft						
BMD(mg/cc)	962	1043*	1216*	-	8.4%	26%
BMC (mg)	9.04	10.04*	12.61*	-	11.1%	39%
X-area (mm2)	7.64	8.03	8.66*	-	2.4%	10.5%
L6 vertebra						
BMD(mg/cc)	604	757*	842*	-	25%	39%
BMC (mg)	1.96	2.57*	3.06*	-	31%	56%
X-area (mm2)	21.7	22.6	24.2*	-	4.2%	11.5%
Proximal Tibia						
BMD(mg/cc)	891	1133*	1250*	-	27%	40%
BMC (mg)	1.46	2.21*	2.58*	-	51%	77%
X-area (mm2)	11.0	13.0*	13.7*	-	18%	24.6%

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EVALUATION

The 6-months treatment arms for which these interim reports were submitted were carried out to confirm the pharmacologic effect of treatment on bone and the lack of proliferative lesions after a 6-month treatment period. The treatment arm in which animals were dosed from 6-12 months of age (second interim report) is basically a duplicate of a 6-month toxicity study carried out earlier in the development program (doses 0, 10, 30, 100 ug/kg) in which trabecular hypertrophy and lining of trabeculae with numerous hypertrophic osteoblasts but no proliferative lesions were observed.

The current two interim reports describe that no proliferative bone lesions were observed in femur, tibia, sternum, and lumbar vertebrae of female rats treated with 5 or 30 ug/kg for 6 months, either from 1.5 months or 6 months of age. However, a marked dose-dependent effect on bone mass of the femur (midshaft) and spine (L6-vertebra) paralleled by the histologic finding of trabecular hypertrophy was observed after treatment of both the younger and older animals. Quantitatively this effect was slightly larger in the older animals.

It should be noted that although no microscopic lesions were after 6 months of treatment the sampling methods are not sufficiently comprehensive to fully ensure that no hyperplasia or small neoplastic lesions are present anywhere in the skeleton. Thus, they are no assurance that 6 months of treatment will not lead to delayed neoplasia. The results of the follow-up groups treated for 6 months and continued for another 14-18 months are therefore essential to evaluate and interpret the aggregate results of this study.

As of September 15, 2001, this study is at the 18-month time point. Initiation was in March 2000. So far, no clinically palpable bone lesions have been observed in the long term treatment groups. If the results of the initial study are reproduced in this follow-up study it can be expected that in the current two dose groups (5 and 30 ug/kg/day) bone tumors will be observed in October-November 2001.

CONCLUSION

The results of this study so far are as expected, and support the pharm/tox recommendation (AE) for NDA ' — ,, submitted on November 30, 2000 (Review dated September 6, 2001).

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/s/

Gemma Kuijpers
10/22/01 03:10:21 PM
PHARMACOLOGIST

Karen Davis-Bruno
10/22/01 03:37:51 PM
PHARMACOLOGIST

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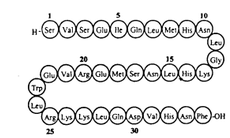
REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS: Parathyroid hormone, teriparatide, peptide, postmenopausal, osteoporosis, bone, formation, resorption

NDA NUMBER:	21,318
DRUG:	TERIPARATIDE INJECTION (FORTEO™)
Reviewer:	Gemma Kuijpers
Division Name:	Division of Metabolic and Endocrine Drug Products
HFD #:	510 (DMEDP)
Review Completion Date:	September 6, 2001
Date of submission:	November 29, 2000
Date received (CDR):	November 30, 2000
Information to Sponsor:	Yes (X) (Labeling Comments)
Sponsor:	Eli Lilly and Company, Indianapolis, IN, USA
Drug substance:	Teriparatide (rDNA origin), PTH(1-34)
Proprietary Name:	Forteo™
Synonyms:	rhPTH(1-34)
Code Name:	LY333334
USAN Name:	Teriparatide
INN name:	Teriparatide
Chemical Name:	34-amino acid single chain peptide
Molecular Formula:	$C_{181}H_{291}N_{55}O_{51}S_2$
Molecular Weight:	4117.8 Daltons
Drug Product:	rhPTH(1-34), acetic acid, sodium acetate, mannitol, metacresol, sodium acetate, HCl, NaOH, water for injection
Dosage Form:	Cartridges rhPTH(1-34) Injection (Pen-injector)
Dosing Device:	3 ml prefilled, delivery device containing a 28-day supply of rhPTH(1-34)
Manufacturer for drug substance:	Eli Lilly and Company, Lilly Technology Center, IN, USA
Manufacturer for dosage form:	Lilly France S.A., Fegersheim, France
Drug Class:	Peptide hormone
Structure:	



Indication: ——

Clinical formulation:	Sterile solution containing per ml: 250 µg/ml rhPTH(1-34), 0.41 mg acetic acid, 0.1 mg sodium acetate, 45.4 mg mannitol, 3 mg metacresol, and water for injection
Dose:	20 µg daily
Route of administration:	Subcutaneous injection (thigh or abdominal wall)
Disclaimer - use of sponsor's material:	Tables and Figures from the electronic NDA submission have been copied for use in this review
Relevant INDs/NDAs/DMFs:	—— (teriparatide) DMF —

RECOMMENDATION CODE: **AE**

Cc:
NDA Arch
HFD-510
HFD-510/Kuijpers/Davis-Bruno/Hedin

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Gemma Kuijpers, Ph.D.
Reviewer

Karen Davis-Bruno, Ph.D.
Team Leader

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INTRODUCTION

Parathyroid hormone is a peptide hormone that has an essential physiological role in calcium homeostasis. PTH tends to protect the system against hypocalcemia by stimulating osteoclastic bone resorption, calcium uptake from the intestine and Ca and P retention in the kidney, while calcitonin protects against hypercalcemia by antagonistic mechanisms. The endogenous hormone is PTH(1-84) and is secreted from the parathyroid gland in response to a decrease in plasma calcium levels.

Depending on the mode of administration, parathyroid hormone can increase or decrease bone mass, and numerous studies with amino-terminal PTH fragments in both animals and humans have shown that when given in intermittent fashion these peptides can be anabolic to the skeleton. For example, daily injection of synthetic PTH(1-34) in the rat at normocalcemic doses stimulates bone formation, and increases bone mass and bone strength. This "paradoxal" anabolic effect on bone is conditional upon intermittent, usually once daily, administration and is not seen with continuous infusion. The mechanism of this anabolic action of PTH is unknown, but is probably based on an increase in the activity and number of bone forming osteoblasts.

The current NDA (#21,318) is for teriparatide injection, or Forteo™, for the indication of treatment of osteoporosis in postmenopausal women and in men. Teriparatide, or LY333334, is a biosynthetic recombinant product and is identical in sequence to the 34 N-terminal amino acids of natural human PTH (rhPTH(1-34)). The proposed treatment regimen is once daily subcutaneous injection with a dose of 20 ug.

Clinical trials with the test compound were discontinued by the Sponsor in December 1998, after 17-23 months of the 2-year pivotal Phase III trial GHAC had been completed, because of the preclinical finding of osteosarcomas in rats treated with teriparatide in a 24-month carcinogenicity study. Nevertheless, the Sponsor arrived at the conclusion that the rat findings were unlikely to be clinically relevant, and an NDA with truncated clinical data was submitted on November 29, 2000.

Part of the study reviews in this NDA review are from previous IND reviews: —— Review Dates: September 25, 1995, and February 5, 1997) and are inserted as indicated in the main text of the review.

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PHARMACOLOGY

BACKGROUND

Parathyroid hormone

Parathyroid hormone (PTH) is secreted from four parathyroid glands adjacent to the thyroid gland in the neck. The hormone is stored in both chief cells and oxyphil cells. PTH, vitamin D and calcitonin have critical roles in calcium (Ca) homeostasis. The secretion of PTH is regulated by the serum Ca concentration via a negative feedback mechanism, i.e., increases in serum Ca inhibit secretion and decreases in serum Ca stimulate it. Mediating this effect is the extracellular calcium receptor (CAR). The effect of increased serum PTH is an increase in serum Ca level.
The secreted form of PTH is a 84-amino-acid peptide with a Mw of 9300. Intact PTH(1-84) is predominantly cleared in the kidney and the liver, where it is cleaved at the 33-34 and 36-37 position. N-terminal fragments have not been demonstrated in blood, while C-terminal ones have. The classic activities of PTH are encoded in the N-terminal 1-34 region. However, recent evidence also suggests specific roles for the C-terminal fragments.

Action of PTH

The anti-hypocalcemic action of PTH manifests itself in three target organs, i.e., kidney, bone and gut. The effect on the intestine is indirect and mediated by a stimulation of renal vitamin D production resulting in increased intestinal calcium absorption. In the kidney, PTH increases the reabsorption of calcium in the distal nephron, and decreases the reabsorption of phosphate in the proximal tubule. In bone, PTH acts both on bone resorbing osteoclasts and bone forming osteoblasts. Generally, a decrease in serum Ca is thought to induce a PTH-mediated increase in Ca resorption. The actions of PTH are mediated by the PTH-1 receptor in kidney and bone. The primary PTH-1 receptor binding domain on the hormone is PTH(18-34). A separate PTH(35-84) receptor for C-terminal regions may exist mediating a totally different set of actions in different tissues. Because of the pharmacological action of PTH, hypercalcemia and hypercalciuria are expected adverse events with administration of high doses of PTH.

Action of PTH in bone

The overall effect of PTH in bone depends on whether it is administered continuously or intermittently. Sustained exposure to PTH activates the osteoblast PTH-receptor which leads to an indirect paracrine activation of the osteoclast. This results in an increase in bone turnover and a net effect of accelerated bone resorption and Ca release. Since the first experiments done in the 1930's by Hans Selye in the rat, however, it has been confirmed by numerous experiments in animal species and humans that intermittent, daily injection of bioactive PTH fragments predominantly stimulates the osteoblast resulting in an anabolic effect on bone, i.e., bone growth. The PTH injections have to be a certain time apart in order for it to have an anabolic effect on bone, supposedly because of a suppression or obliteration of osteclast activation. When PTH is administered at closer than daily intervals or infused continuously, the balance shifts and the net response is bone resorption rather than formation. The precise mechanisms involved in the catabolic and anabolic effects of PTH are unknown. The different actions may be explained by a differential expression of osteoblastic genes such as those encoding for OPG, FGF-2, IGF-1, IGF-binding proteins, IL-6, or IL-11.

PTH receptor mediated events

The receptor that is activated by PTH(1-84) or PTH(1-34) is the osteoblast PTH/PTHrP receptor which stimulates at least 2 intracellular enzymes: adenylyl cyclase (AC) which promotes cAMP production thus causing protein kinase A activation, and phospholipase-C (PLC) which breaks down PIP2 into diacylglycerols and IP3. The latter two compounds stimulate protein kinase C (PKC) and Ca release from intercellular stores, respectively. The signal molecules (AC/PKA and PKC) trigger a plethora of events likely to include the expression of osteogenesis-driving genes. There is some evidence that the AC/cAMP/PKA stimulation is pivotal for the anabolic bone effect of PTH.

MECHANISM OF ACTION

A variety of studies was carried out to obtain insight in the mechanism of action of PTH and to identify the intracellular and/or extracellular mediators of the anabolic action of PTH. Binding of PTH to the G-protein coupled PTH/PTHrP receptor is known to activate two intracellular signal transduction pathways (adenylate cylcase and phospholipase C), leading to activation of PKA and PKC. The two protein kinases can phosphorylate specific proteins which are believed to lead to further downstream effects. However, it is not known how these receptor-mediated early responses lead to the eventual process of bone formation and bone mass increase.

There is evidence that in vivo the increase in bone mass following intermittent PTH administration is associated with an increase in the number of osteoblasts which occurs to be partly due to a conversion of bone lining cells to osteoblasts, and possibly an inhibition of osteoblast apoptosis. An increase in stromal cell differentiation into osteoblasts upon PTH treatment has also been demonstrated. However, the molecular mechanisms that mediate the PTH-induced bone formation remain unknown.

A number of studies showed that single injections of PTH can cause transient effects (stimulation or inhibition) on early response genes in bone (c-fos, c-jun, c-myc, IL-6, histone H4), and that daily PTH treatment can upregulate marker genes for bone formation (collagen type-1, osteocalcin, MMP-9). One study indicated that continuous and intermittent exposure have differential effects on gene expression in rat femur, which may be related to the different biological responses to PTH in bone depending on its mode of administration, e.g. the increase in osteoclastic resorption with continuous but not intermittent exposure.
However, the relation between the genetic responses and the cellular and tissue responses in the bone remains unclear.

In conclusion, the cellular and molecular mechanisms mediating the anabolic action of PTH or related peptides on bone have not been elucidated.

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GENERAL PHARMACOLOGY

Comparison of LY333334 [PTH(1-34)] with Synthetic Human PTH(1-34): Binding to the PTH Receptor and Stimulation of cAMP Synthesis
(Nonclinical Pharmacology Report 01)
(From IND Review September 25, 1995)

RESULTS
The binding of 1 nM [^{125}I](Nle^{8,18}Tyr^{34})humanPTH(1-34) to adenovirus-transformed human kidney 293 cells, which had been transfected with the human PTH receptor, was determined. The synthetic hPTH(1-34) peptide inhibited binding by maximally 80%, with an ED_{50} value of 4.3 1.3 nM (mean s.d.). LY333334 inhibited binding also by maximally 80%, with an ED_{50} of 3.0 0.1 nM. Synthetic hPTH(1-34) and LY333334 both stimulated the production of cAMP in IBMX-treated human osteosarcoma cells (SaOS-2) with equal potency.

CONCLUSION
In two biological PTH-responsive in vitro systems, LY333334 produced an equivalent response as synthetic human PTH(1-34).

Comparison of The Anabolic Effects of LY333334 and Synthetic Human Pth(1-34) on The Long Bones of Young Male Rats
(Nonclinical Pharmacology Report CG3-04)
(From IND Review September 25, 1995)

METHODS
Male viral antibody-free Sprague-Dawley rats, age ca. 4 weeks, weighing 70 g, were untreated (n=6, baseline controls), or treated (n=10/dose group) for 18 days with either synthetic PTH(1-34) ([redacted] or LY 333334 at **0, 16, 80** ug/kg/day by s.c. injection. Rats were killed 2 h after last injection, and long bones were resected. Proximal tibia bone parameters were determined by quantitative CT.

RESULTS
Body weight gain was increased significantly by 20% in HD PTH(1-34), and non-significantly by 10% in HD LY 333334, while femur length was the same in all groups. Tibia bone parameters, BMD, BMC, voxels and cross-sectional area, were significantly and equivalently increased by both PTH(1-34) and LY 333334 (10-30% in LD, 20-50% in HD). In LD, tibia BMC and BMD were increased slightly, but significantly more in LY 333334-treated than in PTH(1-34)-treated animals. Femur trabecular bone mass, i.e. dry weight, was increased similarly with PTH(1-34) and LY 333334. In LD-HD, the increase was 50-90% for trabecular and 15-30% for cortical bone. Femur neck resistance to fracture was also increased similarly by both agents, in LD (ca. 25%) and HD (ca. 50%). Three-point loading/bending of the femur shaft was slighly increased in LD by PTH(1-34) but not by LY333334, and in HD by both agents. Stiffness was not affected by any treatment.

CONCLUSION
In the rat, LY 333334 causes increases in bone mass, density, mineral content and strenght of femur and tibia, generally to the same extent as equivalent doses of synthetic PTH(1-34).

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EFFICACY PHARMACOLOGY

Numerous studies published in the literature over the past 20 years have shown that in the rat intermittent (usually once daily) administration of a synthetic 1-34 N-terminal amino acid fragment of human PTH (hPTH1-34) has an anabolic effect on bone, as determined by bone calcium content, ash weight, or BMD. The effect has been seen in males and females, in intact, ovariectomized or orchidectomized animals. In most studies treatment duration varied from 12 days to 6 months. In virtually all studies the effect on cancellous bone was more pronounced than on cortical bone. In monkeys, treatment with PTH1-34 (3x/week) for 3-6 months also increased trabecular BMD to a larger extent than cortical BMD.

The anabolic, bone-building effect of PTH(1-34) occurs when the hormone is administered in an "intermittent" fashion, i.e., as opposed to a continuous way of administration, and a once daily subcutaneous injection has been the most frequently employed dosing regimen with an anabolic effect. The anabolic effect is thought to be due to preferential stimulation of the bone forming cell, the osteoblast, and is characterized by increased bone formation, or bone apposition, on trabecular and cortical bone surfaces, and increased bone mass and strength.

For this NDA, the Sponsor submitted a variety of reports of bone studies carried out in various animal species (rats, rabbits, monkeys, mice). Most studies were carried out with rhPTH(1-34), or LY333334, a recombinant form of the N-terminal 1-34 amino acid fragment of human PTH, while some were done with synthetic PTH1-34, PTH- or PTHrP analogues, or with combinations of LY333334 with other bone active agents. In this review LY333334 refers to rhPTH(1-34).
Various measurements were made to provide information on efficacy and safety of the drug treatment (bone mass, size, strength and histomorphometry).

Two long term studies in ovariectomized animals were carried out in rats and in monkeys, as recommended by the FDA Guidelines for the Evaluation of Preclinical and Clinical Agents used in the Prevention or Treatment of Postmenopausal Osteoporosis. Shorter studies were carried out in aged female rats, male rats, rabbits and mice. The areas of concern addressed were cortical bone effects, long term effects, high dose effects, and effects of drug withdrawal. The long term bone quality studies carried out in the monkey (18-month study) and the rat (12-month study), the rabbit studies (2 studies, 70 and 140 days) and part of the other rat studies (treatment duration up to 12 months) were considered most relevant for evaluation of bone efficacy and safety pertinent to the proposed clinical indication. Therefore, these studies are discussed in detail in this NDA review.

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PIVOTAL BONE QUALITY STUDIES

Species	Report/Study#	NDA Volume	Page	Study description	Prevention/ Treatment regimen
MONKEY	X95-11	13,14	44,4	1-yr ovx monkey study	Prevention
RABBIT	CG3-06	15	3	140-day study in mature intact rabbits	N/a
	CG3-13	15	49	35-, 70-, or 140-day study in 9-mo old intact rabbits	N/a
RAT	R00796/R04296	15	83	1-year study in 6-mo old ovx'ed or male F344 rats	Prev/Treatmt
	BN5-01	15	231	6-mo study in aged 9-mo old SD rats	Prevention
	BN5-02	15	282	3-mo study in osteopenic (1-mo ovx'ed) 7-mo old SD rats	Treatment
	BN5-09	16	39	3- to 9-mo study in intact young (1.5-mo old) or mature (6-mo old) F344 rats	N/a
	R43-01	16	91	Study in young SD male rats on effect of anabolic or catabolic injection protocols on PK profile	N/a
	CG3-02	16	126	Study in young SD male rats on relation between frequency of daily injections and anabolic bone effect	N/a

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LONG TERM MONKEY BONE QUALITY STUDY

		In Vivo		In Vitro		
Report	Title	Species, Sexes, Gender, & Age	Dose/Route	Assay Type	Tissue/ Cell Line	Results
Safety, Efficacy, and Strength of human PTH						
X-95-11	A Study in Ovariectomized Adult Cynomolgus Monkeys (*M. fascicularis*) Given LY333334 Once Daily by Subcutaneous Injections for 18 Months or for 12 Months Followed by 6 Months of Observation to Assess Effects on Bone	Monkeys: *Macaca fascicularis*, female, ca 9-11 years, OVX	1 & 5 µg/kg day SC for 18 months or for 12 months followed by 6 months withdrawal			LY333334 dose dependently increased total body bone mineral content by 5-15%, spine bone mass by 10-20%, spine cross-sectional area by 2-3%, & spine biomechanical properties by 20-40%. Biomechanical properties of femur neck were increased by 12-27%. Histomorphometry of distal & mid-radius, mid-humerus, second lumbar vertebra, & femur neck showed increased bone turnover results in remodeling transients manifest as increased porosity & intra-trabecular remodeling. There were no effects on material biomechanical properties of bone or on cortical bone strength. There was no cortical bone thinning or weakening, no woven bone, & no marrow fibrosis. Benefits on bone mass & strength were sustained after withdrawal of treatment. Monkeys remained normocalcemic. There were no changes in kidney histology. Serum measures of calcium homeostasis were returned to sham control values in treated OVX monkeys. Pharmacokinetics showed no accumulation of LY333334 over time & consistent clearance

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A Study in Ovariectomized Adult Cynomolgus Monkeys (*M. fascicularis*) Given LY333334 Once Daily by Subcutaneous Injections for 18 Months or for 12 Months Followed by 6 Months of Observation to Assess Effects on Bone (Study Nr. X-95-11)
(Non-Clinical Pharmacology Report X-95-11)

INTRODUCTION
This study in OVX adult cynomolgus monkeys was carried out to obtain information on the long term safety and efficacy of rhPTH1-34 (LY) on bone, in particular cortical bone. Concerns about the safety of PTH on cortical bone stem from the cortical thinning reported in small observational studies of hyperparathyroid patients, in which increases in trabecular bone were often accompanied by reduction in cortical bone ("cortical steal phenomenon"). Histomorphometric studies of larger animals, such as dogs and monkeys, have also shown that PTH increases activation frequency and porosity in osteonal cortical bone. The consequences of these changes on the biomechanical properties of bone have not been tested.
The present study was designed to assess cancellous and cortical bone responses to LY333334 (1 and 5 ug/kg/day), including bone mass, biomechanical properties and bone histomorphometry in the ovariectomized monkey. The study was also designed to examine the effect of withdrawal after LY333334 treatment on bone mass, strength, and architecture.

METHODS

Study:	X-95-11
Live-phase duration:	18 months
Live-phase dates:	12 February 1996 through 3 October 1997
Study site (in vivo phase):	[redacted]
Test article:	Recombinant human parathyroid hormone (1-34), LY333334
Chemical name:	rhPTH (1-34)
Lot number and potency: PPD03521:	102%
Species and strain:	Monkey cynomolgus (*Macaca fascicularis*), natural-habitat reared, adult

Initial weight range:	2.77 (mean) ±0.03 (SEM) kg females
Initial age:	Skeletally mature adults with no open growth plates or skeletal abnormalities that would interfere with bone mass measurements (9-11 years old, based on dentition)
Number of animals:	128 at baseline, 121 that completed the study (4 died/ euthanized/removed during live phase, 3 excluded at necropsy)
Animal diet:	Diet made at test facility, containing 0.3% calcium, 0.3% phosphorus, and 250 IU VitD3/100g diet (ca. 1750 mg Ca/2000 calories)
Surgery:	Sham or bilateral ovariectomy
Test compound:	LY333334 (0.1 ml/kg)
Start of treatment:	Day after surgery
Dosing route:	Subcutaneous
Frequency and duration of dosing:	Once daily; 18 months
Vehicle:	20 mM sodium phosphate
Sampling:	Blood and urine samples at 0, 3, 6, 9, 12, 15, 18 months. Blood samples collected 22-24h after dosing, urine collected over 1-day after dosing
Bone densitometry:	At 0, 6, 12, 15, 18 months; DEXA (vertebrae and whole body) or pQCT (radius, tibia, vertebra)
Bone strength:	At 18 months; Compression (vertebrae), bending (mid humerus, femoral beam specimens), shear force (femoral neck)
Bone histomorphometry:	Fluorochrome labels given at 6, 15, and 18 months
PK sampling:	At 1, 7, 11, 17 months (10, 20, 40, 60, 90, 150, 180, 240 minutes after dosing)
Samples/organs taken at necropsy:	Serum, kidneys, lumbar and thoracic vertebrae, humerus, femur, radius, tibia, calvaria

Treatment groups

Group	Abbreviation	Monkeys at Outset (n=128)	Monkeys in Final Analyses (n=121)
Sham-ovariectomized control, 18 months vehicle	Sham	21	21
Ovariectomized control, 18 months vehicle	OVX	22	20
Ovariectomized, 18 months LY333334 at 1 µg/kg/day	PTH1	21	19
Ovariectomized, 12 months LY333334 at 1 µg/kg/day, then 6 months vehicle once daily	PTH1-W	21	20
Ovariectomized, 18 months LY333334 at 5 µg/kg/day	PTH5	22	21
Ovariectomized, 12 months LY333334 at 5 µg/kg/day, then 6 months vehicle once daily	PTH5-W	21	20

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Bone sites evaluated

Sites used to assess safety and efficacy of LY333334 on the skeleton:

		Bone Mass		Histomorphometry		Biomechanical
Site	Specifies	DEXA	QCT	Trabecular	Cortical	Tests
Whole body	BMC	-				
Iliac biopsies	6 months			+		
	15 months			+		
Lumbar vertebrae (spine)	LV2	-		+	-	
	LV3	-				+
	LV4	-		+		+
	LV5		+			
Tibia	Proximal		+			
Femur	Neck			+	+	+
	Midshaft				+	
	Midshaft cortical "beam"					+
Radius	Distal		+	+	+	
	Midshaft		+		-	
Humerus	Midshaft				-	+

DEXA analyses, most serum, and urine chemistries were measured at 3-month intervals. QCT was conducted at 0, 12, and 18 months, except LV5 which was scanned only at 18 months. Biomechanical tests and histomorphometry (except for iliac biopsies at 6 and 15 months) were conducted at 18 months.

Destination of bones/organs at necropsy:

Biomechanics	LV3, LV4; Right femur; Right humerus
Histomorphometry	Left humerus; LV2; Left femur; Left radius; Iliac crest biopsy
High resolution pQCT	LV5 (subset of animals)
Histopathology (Lilly):	Kidneys
Storage (Lilly):	LV1 and LV5, TV12-13, right radius, tibiae

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RESULTS
Data were reported for 128 (baseline) and 121 (completed study) monkeys.

Body weight
No effect of treatment. All monkeys gained weight (4-9%).

Ovariectomy result
Ovx reduced serum estradiol (sham levels ca. 50 pg/ml) to levels near or below assay detection limit (5-10 pg/ml) except for 2 monkeys whose ovariectomy was incomplete and who were excluded from analysis.

Biochemistry
Calcium homeostasis – Effect at 18 months

	Effect of OVX (relative to sham)	Effect of LY (relative to OVX)	Effect of LY withdrawal (relative to LY)	Comments
Total serum Ca	Increase* (3%)	No effect	No effect	-
Serum Ca (ionized)	No effect	No effect	No effect	-
Serum P	Increase* (14%)	Decrease* (ca. 18%)	Increase#	LY effect not dose-dependent
Serum intact PTH	No effect	Decrease* (ca. 50%)	Increase#	LY effect not dose-dependent
Serum calcitriol	Decrease* (29%)	Increase* (ca. 33%)	Decrease#	LY effect not dose-dependent
Urine cAMP/creat	No effect	Increase* (35%-105%)	Decrease#	LY effect dose-dependent
Urine Ca/creat	No effect	No effect	No effect	-
Serum creat	No effect	No effect	No effect	-
Serum BUN	No effect	No effect	No effect	-

n.s. = not significant
*statistically significant effect
#Effect of LY withdrawal not tested for significance

Bone turnover markers – Effect at 18 months

	Effect of OVX (relative to sham)	Effect of LY (relative to OVX)	Effect of LY withdrawal (relative to LY)	Comments
Serum ALP	Increase* (56%)	Small increase (n.s.)	No effect	LY effect appeared dose-dependent
Serum osteocalcin	Increase* (75%)	Small increase (n.s.)	No effect	LY effect not dose-dependent
Urinary CrossLaps (C-terminal collagen fragments)	Increase* (50%)	Small increase (n.s.)	No effect	LY effect not dose-dependent

n.s. = not significant
*statistically significant effect
#Effect of LY withdrawal not tested for significance

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Bone mass

DEXA measurements: Projected area (cm^2), BMC (g), BMD (mg/cm^2):

Spine (Lumbar Vertebrae LV2-LV4)
Projected area (cm2)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
0 months	7.07	6.95	7.07	7.08	7.07	7.00
18 months	7.36	7.17	7.37	7.33	7.42	7.24

*significantly different from OVX

Change in projected area (%)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
0-18mo	4.1%	3.2%	4.2%	3.7%	5.2%*	3.4%

*significantly different from OVX

BMD (mg/cm2)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
0 months	572	558	578	578	571	576
18 months	601	561	631	618	657	616

*significantly different from OVX

Change in BMD (%)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
0-18mo	5.3%*	0.6%	9.1%*	7.2%*	15.4%*	7.3%*

*significantly different from OVX

Change in BMC (%)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
0-18mo	9.7%	3.9%	13.7%	11.4%	20.3%	11.4%

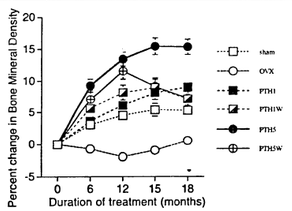

Figure 2. Changes over time in bone mineral density of the spine (lumbar vertebrae, LV2-LV4) in sham controls and ovariectomized adult monkeys, *M. fascicularis*, given vehicle or LY333334 at 1 µg/kg/day (PTH1) or 5 µg/kg/day (PTH5) for 18 months or at 1 µg/kg/day (PTH1-W) or 5 µg/kg/day (PTH5-W) for 12 months, after which treatment was withdrawn for a further 6 months. Note the relative osteopenia of OVX controls compared to sham; the magnitude of increase induced by LY333334 after 18 months, compared to either sham or OVX controls; and the maintenance of the increase following treatment withdrawal between 12 and 18 months (PTH1-W and PTH5-W). BMD of PTH-treated monkeys was significantly different from OVX controls, p<.05.

Whole body
Projected area, change from 0-18 months (%)

Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
3.5%	2.8%	1.6%	1.5%	4.7%	4.5%

Sham baseline value: 323cm²

BMD, change from 0-18 months (%)

Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
8.8%*	3.5%	6.2%	9.1%*	11.4%*	6.8%*

*significantly different from OVX
Sham baseline value: 313 mg/cm2

pQCT measurements: X-area (mm^2), BMC (mg/mm), BMD (mg/cm^3) (different bone zones):

Midshaft Radius
X-area, change from 0-18 months (%)

Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
-1.7%	+3.1%	+5.9%	+5.6%	+7.4%*	+5.6%

*significantly different from OVX
Sham baseline value: 23.5mm2

BMD, change from 0-18 months (%)

Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
+3.2%	+0.3%	-4.2%	+1.0%	-2.1%	-1.6%

Sham baseline value: 949 mg/cm3

Proximal Tibia
X-area, change from 0-18 months (%)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
Total	+3.0%	+2.8%	+4.0%	+1.2%	+0.8%	-2.9%

Sham baseline value: 89.2mm2

BMD, change from 0-18 months (mg/cm3 values)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
Zone I	+10	+1	+11	+9	+7	-8
Zone II	+48	+3	+33	+63	+77*	+39
Zone III	+15	-21	+26	+39*	+130*	+88*
Zone IV	+5	-10	+25*	+14	+78*	+58*

*significantly different from OVX
Sham baseline values: Zone I (outermost/cortical): 798 mg/cm3, Zone II: 832 mg/cm3, Zone III: 367 mg/cm3, Zone IV (innermost/cancellous): 133 mg/cm3

Distal Radius
X-area, change from 0-18 months (%)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
Total	-4.8%	-1.7%	-2.1%	+2.7%	-1.4%	-3.2%

Sham baseline value: 43.4mm2

BMD, change from 0-18 months (mg/cm3 values)

	Sham	OVX	PTH1	PTH1W	PTH5	PTH5W
Zone I	+36*	-12	-16	-6	-21	+8
Zone II	+107	+70	+28	+62	+55	+47
Zone III	+72	+55	+24	+44	+67	+39
Zone IV	-22	-21	-10	0	+26*	-20

*significantly different from OVX
Sham baseline values: Zone I (outermost/cortical): 814 mg/cm3, Zone II: 865 mg/cm3, Zone III: 456 mg/cm3, Zone IV (innermost/cancellous): 230 mg/cm3

Bone strength

Testing methods

- **Femoral neck**
- **Lumbar vertebrae, L3 and L4**
- **Humerus midshaft**
- **Femur cortical bone: diaphyseal beam from mid-shaft**

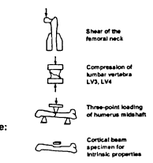

Figure 3. Cartoon to show strategies used in biomechanical testing of the femur neck, lumbar vertebrae (spine), humerus midshaft, and the uniform cortical bone specimen.

Table B1 **Variables Reported for the Third and Fourth Lumbar Vertebrae (L3 and L4), Humerus Midshaft, Proximal Femoral Neck, and Femoral Beam Specimens**

Variable	Units	Description
Lumbar Vertebrae, L3 and L4		
A	mm^2	Cross-sectional area
F_y	N	Yield force is the force at a 0.2% offset
S	N/mm	Slope of the linear portion of the force-displacement curve (stiffness)
σ_y	MPa	Yield stress
E	MPa	Young's modulus
Humerus Midshaft		
t	mm	Average cortical thickness
F_u	N	Ultimate force is the maximum force the specimen can withstand
S	N/mm	Slope of the linear portion of the force-displacement curve (stiffness)
U	mJ	Area under the load-displacement curve (work)
Proximal Femoral Neck		
F_u	N	Ultimate force is the maximum force the specimen can withstand
Femur Diaphyseal Beam Specimens		
σ_u	MPa	Ultimate stress
E	GPa	Young's modulus
u	MJ/m^3	Toughness
ε_u		Ultimate strain

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Spine (Lumbar vertebrae LV3 and LV4)
(Structural properties, i.e., properties dependent on size and shape of specimen)
Yield force (Fy) and stress (σy) of lumbar vertebrae and related biomechanical parameters were decreased by OVX, and dose-dependently increased by LY treatment (Table B3). The effects were significant in all LY groups. Yield force was significantly larger in the PTH5 group than in sham. There was also a significant effect of LY withdrawal on Fy, with withdrawal associated with lower Fy values.
Ultimate, i.e., breaking strength (Fu) of vertebrae was not given because some vertebrae did not break.
Young's modulus (E) was decreased by OVX, and significantly and dose-dependently increased by LY. Withdrawal of Ly reversed this effect.
There was a nearly significant dose effect on cross-sectional area (A) with the higher dose of PTH associated with a reduced area. This was mainly due to a decrease in the PTH5W group (Table B3). Note however that the X-area is not an accurate measure but an estimate.

Table B3 Average of Third and Fourth Lumbar Vertebrae Data (Mean ± SEM of Raw Data)

Measurements	Groups						ANOVA	Effects	
	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W	(p-value)	Dose	Withdrawal
Average of L3 and L4 Vertebrae									
A (mm²)	90.5 ± 2.1 (21)	86.7 ± 2.3 (20)	88.3 ± 2.0 (19)	90.9 ± 2.3 (20)	87.3 ± 2.7 (22)	82.8 ± 2.1 (20)	.15	0.06	ns
F_y (N)	1738 ± 52 (21)	1499 ± 94* (20)	1915 ± 105* (19)	1899 ± 75* (20)	2113 ± 75*# (22)	1792 ± 59* (20)	<.0001	ns	<0.05
S (N/mm)	7312 ± 319 (21)	5605 ± 476* (20)	7701 ± 474# (19)	7401 ± 452# (20)	8012 ± 367# (22)	7074 ± 314# (20)	<.005	ns	ns
σ_y (MPa)	19.4 ± 0.6 (21)	17.2 ± 1.0 (20)	21.9 ± 1.2# (19)	21.1 ± 0.6# (20)	24.0 ± 1.1## (22)	21.9 ± 0.9# (20)	<.0001	0.09	0.09
E (MPa)	650 ± 32 (21)	546 ± 49 (20)	717 ± 48# (19)	659 ± 42 (20)	759 ± 36# (22)	698 ± 41# (20)	<.01	ns	ns

Number of specimens per group are shown in parentheses.
Statistical significance was set at p<.05.
ns = p>.05 and considered not statistically significant.
* = sham
# = ovariectomized.

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There was a good correlation between spinal BMD of LV2-LV4 and average yield force (Fy) of L3 and L4 (Figure 4). The correlation appeared to be similar for all groups.

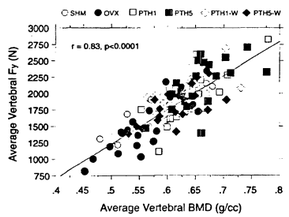

Figure 4. Correlation between vertebral bone mineral density at the spine and biomechanical properties, shown as yield force, r = 0.83, p<.0001.

Humerus midshaft
(Structural properties, i.e., properties dependent on size and shape of specimen)
Ultimate force (Fu), stiffness (S) or work to failure (U) were not statistically significantly affected by OVX or LY treatment (Table B4). However, values for all three parameters were lower in OVX than in sham, and higher in LY-treated than in OVX. The absence of a (net) effect on Fu and work to failure indicated that PTH did not weaken cortical bone. However, it is likely that this was at least partially due to the increased thickness (geometry change) of the humeral bone.
Humeral thickness was decreased in OVX vs. sham. There was a significant dose effect on thickness (t), with the higher dose of PTH associated with an increased thickness as compared to OVX (Table B4).

Femoral Neck
(Structural properties, i.e., properties dependent on size and shape of specimen)
Ultimate force (Fu) was decreased in OVX, and increased vs. OVX in LY-treated. The effect was significant for all LY groups except the PTH5W group (Table B4).

Table B4 Humerus Midshaft and Proximal Femoral Neck Data (Mean ± SEM of Raw Data)

Measurements	Groups						ANOVA	Effects	
	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W	(p-value)	Dose	Withdrawal
Humerus Midshaft									
t (mm)	1.74 ± 0.04 (21)	1.63 ± 0.03* (20)	1.68 ± 0.03 (19)	1.66 ± 0.04 (20)	1.80 ± 0.04# (22)	1.72 ± 0.05 (20)	<.05	<0.05	ns
F_u (N)	725 ± 26 (21)	636 ± 26 (19)	654 ± 23 (19)	689 ± 23 (20)	680 ± 15 (22)	707 ± 24 (19)	.08	ns	ns
S (N/mm)	601 ± 23 (21)	520 ± 26 (19)	544 ± 23 (19)	573 ± 20 (20)	548 ± 18 (22)	573 ± 24 (19)	.17	ns	ns
U (mJ)	1797 ± 85 (21)	1542 ± 92 (19)	1641 ± 157 (19)	1751 ± 84 (20)	1804 ± 99 (22)	1775 ± 113 (19)	.41	ns	ns
Proximal Femoral Neck									
F_u (N)	1288 ± 41 (19)	1105 ± 53* (18)	1255 ± 45# (18)	1258 ± 52# (17)	1362 ± 30# (21)	1213 ± 42 (20)	<.005	ns	ns

Number of specimens per group are shown in parentheses.
Statistical significance was set at p<.05.
ns = p>.05 and considered not statistically significant.
* = sham.
# = ovariectomized.

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Femoral midshaft beam specimens
(Material properties, i.e., properties intrinsic to bone tissue and independent on size and shape of specimen)
There was no effect on ultimate stress (σ_u) in OVX (vs. sham). However, there was a significant dose effect on σ_u, with the higher dose of PTH associated with a decreased value (Table B5).
Young's modulus (E), i.e., the intrinsic stiffness of bone, was decreased in the high dose LY groups as compared to sham and OVX. The difference between PTH5 and sham was statistically significant. There was a significant dose effect on E with the higher dose of PTH associated with a decreased value. E was also slightly lower in OVX vs. sham (Table B5). The parameter, E, reflects the elastic properties of the bone material, and represents the slope of the load-deformation curve. A decrease in this slope indicates that it takes less force to bend the bone to a certain degree.
The parameters u (toughness) and ultimate strain, ε_u, were not significantly affected by OVX or PTH. The results indicate a tendency of PTH5 to weaken cortical bone material, possibly due to an increase in cortical porosity.

Table B5 Femoral Diaphyseal Beam Specimen Data (Mean ± SEM of Raw Data)

Measurements	Groups						ANOVA	Effects	
	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W	(p-value)	Dose	Withdrawal
Femoral Diaphyseal Beam Specimen									
σ_u (MPa)	222 ± 5 (21)	216 ± 5 (20)	222 ± 4 (19)	214 ± 6 (20)	206 ± 6 (22)	208 ± 6 (20)	.18	<0.05	ns
E (GPa)	17.2 ± 0.6 (21)	16.4 ± 0.4 (20)	17.1 ± 0.4 (19)	16.9 ± 0.6 (20)	15.4 ± 0.6# (22)	15.3 ± 0.6# (20)	<.05	<0.01	ns
u (MJ/m³)	5.9 ± 0.3 (21)	5.3 ± 0.4 (20)	6.1 ± 0.4 (19)	5.5 ± 0.4 (20)	5.4 ± 0.3 (22)	6.1 ± 0.4 (20)	.59	ns	ns
ε_u	0.035 ± 0.001 (21)	0.035 ± 0.002 (20)	0.036 ± 0.002 (19)	0.034 ± 0.002 (20)	0.034 ± 0.002 (22)	0.036 ± 0.002 (20)	.70	ns	ns

Number of specimens per group are shown in parentheses.
Statistical significance was set at p<.05.
ns = p>.05 and considered not statistically significant.
* = sham.
# = ovariectomized.

Summary Table

	Biomechanical Results					
	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W
Femoral neck ultimate force (N)	1288 ± 41°	1105 ± 53ª	1235 ± 45°	1258 ± 52°	1362 ± 30°	1213 ± 42
Lumbar vertebrae yield force (N)	1738 ± 52°	1499 ± 94ª	1915 ± 105°	1899 ± 73°	2113 ± 77°ª	1792 ± 59°
Humerus ultimate force (N)	725 ± 26	636 ± 26	654 ± 23	689 ± 23	680 ± 15	707 ± 24
Humerus cortical porosity (%)	1.3 ± 0.1°	2.6 ± 0.3ª	4.7 ± 1.1ª	2.3 ± 0.4	6.7 ± 0.9°ª	6.5 ± 1.0°ª
Humerus cortical thickness (mm)	1.74 ± 0.04	1.63 ± 0.03ª	1.68 ± 0.03	1.66 ± 0.04	1.80 ± 0.04°	1.72 ± 0.05

Data shown as mean ± SEM for 19 to 22 monkeys per group.
Statistical significance, p<.05.
ª Versus sham control.
° Versus OVX control.

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Bone histomorphometry

Iliac crest (trabecular bone analysis):
Values for various parameters (10 parameters at 6 months, 23 parameters at 15 months) were reported.

Table D1 Definition of Histomorphometric Variables for Cancellous Bone Measurements From Iliac Crest Biopsies

Variable	Units	Description
Cn.Ar	%	Cancellous bone area
Tb.Th	µm	Trabecular thickness
Tb.N	# /mm	Trabecular number
Tb.Se	µm	Trabecular separation
E.Pm	%	Erosion perimeter normalized to total cancellous perimeter
Oc.Pm	%	Osteoclast perimeter normalized to total cancellous perimeter
O.Ar	%	Osteoid area normalized to cancellous area
O.Pm	%	Osteoid perimeter normalized to total cancellous perimeter
Ob.Pm	%	Osteoblast perimeter normalized to total cancellous perimeter
L.O.Pm	%	Proportion of osteoid perimeter labeled with a fluorochrome
Ma.Pm	%	Mineralizing perimeter normalized to total cancellous perimeter
MAR	µm/day	Mineral apposition rate
Aj.AR	µm/day	Adjusted apposition rate
Omt	days	Osteoid maturation time
BFR/BV	%/yr	Bone formation rate normalized to cancellous bone volume
BFR/BS	µm/day	Bone formation rate normalized to total cancellous perimeter
BFR/Tt.BV	%/yr	Bone formation rate normalized to tissue (bone + marrow) volume
FP	days	Formation period
Rs.P	days	Resorption period
Rm.P	days	Remodeling period
Ac.f	cycles/yr	Activation frequency
W.wi	µm	Wall width
Ot.Th	µm	Osteoid thickness

* Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595-610.

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6 month data (Table D2, Figure 6):

Effect of OVX (relative to sham):
- Non-statistically significant (n.s.) decrease in Cn.Ar., and n.s. increase in Tb.Se.
- N.s. increase in normalized E.Pm (p<0.1) (3-fold) and Oc.Pm
- N.s. increases in normalized O.Ar, O.Pm, Ob.Pm.

What this means: Ovariectomy causes increased cancellous bone resorption and formation activity (turnover), resulting in a statistically non-significant decrease in cancellous bone

Effect of LY treatment (relative to OVX):
- Significant increase in Cn.Ar., Tb.Th., Tb.N., Tb.Se
- Significant increase on O.Ar and O.Pm.
- N.s. decrease in normalized E.Pm, Oc.Pm
- No effect on normalized Ob.Pm

What this means: PTH causes decreased bone resorption and increased bone formation, resulting in a significant increase in cancellous bone

Effect of LY withdrawal:
- No significant effects

What this means: PTH withdrawal did not reverse the changes in the static histomorphometry parameters effected by PTH

Table D2 Iliac Crest Biopsy Data (Mean ± SEM of Raw Data) at 6 Months (n = 118)

	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W	Transformation	ANOVA p-value	Bonferroni-Adjusted p-value Sham	PTH1	PTH1-W	PTH5	PTH5-W
Cn.Ar	24.9 ± 1.8 (20)	20.9 ± 1.7 (20)	31.0 ± 1.8 (18)	31.2 ± 2.1 (20)	34.6 ± 2.0 (21)	32.6 ± 1.8 (19)		.00	ns	<.01	<.001	<.001	<.001
Tb.Th	126 ± 8 (20)	116 ± 6 (20)	133 ± 6 (18)	144 ± 9 (20)	162 ± 11 (21)	140 ± 8 (19)	log	.00	ns	ns	<.01	<.001	<.1
Tb.N	1.99 ± 0.09 (20)	1.79 ± 0.08 (20)	2.33 ± 0.09 (18)	2.13 ± 0.10 (20)	2.41 ± 0.11 (21)	2.35 ± 0.14 (19)		.00	ns	<.01	<.1	<.001	<.001
Tb.Se	398 ± 26 (20)	466 ± 29 (20)	307 ± 18 (18)	346 ± 29 (20)	275 ± 23 (21)	316 ± 21 (19)	log	.00	ns	<.001	<.01	<.001	<.001
E.Pm	1.61 ± 0.36 (20)	2.69 ± 0.76 (20)	1.83 ± 0.64 (18)	1.49 ± 0.29 (20)	1.29 ± 0.44 (21)	1.62 ± 0.60 (19)	square root*	.16	<.1	ns	ns	ns	ns
Oc.Pm	0.64 ± 0.14 (20)	1.37 ± 0.33 (20)	0.90 ± 0.22 (19)	0.83 ± 0.18 (20)	1.13 ± 0.27 (21)	1.49 ± 0.76 (19)	square root*	.46	ns	ns	ns	ns	ns
O.Ar	0.37 ± 0.06 (20)	0.94 ± 0.06 (20)	0.74 ± 0.12 (18)	0.56 ± 0.09 (20)	0.91 ± 0.10 (21)	0.84 ± 0.15 (19)	square root	.00	ns	ns	ns	<.01	<.05
O.Pm	11.6 ± 1.7 (20)	17.6 ± 2.7 (20)	21.9 ± 2.4 (18)	20.7 ± 2.7 (20)	29.2 ± 2.6 (21)	26.8 ± 3.4 (19)		.00	ns	ns	ns	<.05	ns
Ob.Pm	2.5 ± 0.6 (20)	6.9 ± 1.7 (20)	6.3 ± 2.0 (18)	6.1 ± 1.6 (20)	9.8 ± 1.4 (21)	7.8 ± 2.0 (19)	square root*	.35	ns	ns	ns	ns	ns
O.Th	7.4 ± 0.4 (20)	8.2 ± 0.5 (20)	7.9 ± 0.3 (18)	6.8 ± 0.3 (20)	7.3 ± 0.4 (21)	7.7 ± 0.3 (19)		.16	ns	ns	<.05	ns	ns

Statistical significance was set at p<.05.
ns = p>.05 and considered not statistically significant.

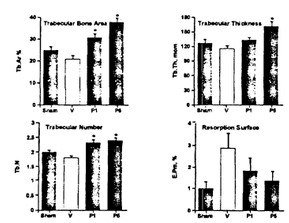

Figure 6. Histomorphometric data in iliac biopsies taken 6 months after starting treatment in control sham and OVX (V) groups and in ovariectomized monkeys treated with LY333334 at 1 µg/kg/day (PTH1 shown as P1) or 5 µg/kg/day (PTH5 shown as P5). Note the increase in trabecular bone area (Tb.Ar, %), trabecular thickness (Tb.Th, mcm) and trabecular number (Tb.N), together with the absence of significant differences between groups in resorption surface (E.Pm, %). Statistical significance, * p<.05.

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15-month data (Table D3, Figure 7):

Effect of OVX:
- Significant increase in E.Pm (2-fold)
- Significant increases in O.Pm, Ms.Pm, BFR/BV, all appr. 2-fold.
- N.s decrease in Cn.Ar., and n.s. increase in Tb.Se.
- N.s. increases in Oc.Pm
- N.s. increases in O.Ar, Ob.Pm
- N.s. decreases in Rs.P >FP, and in Rm.P
- N.s. slight increase in W.Wi (10%).

What this means: Ovariectomy causes increased cancellous bone resorption and formation, resulting in non-significantly decreased cancellous bone. It also appears to cause a decrease in remodeling (resorption and formation) periods. There is a paradoxical increase in wall width.

- Effect of LY treatment:
- Significant increases in Cn.Ar., Tb.Th., Tb.N., and decrease in Tb.Se
- Significant increases in O.Ar, O.Pm, Ob.Pm, Ms.Pm, BFR/BS, BFR/Tt.BV (see figure below)
- Slight increase in E.Pm and Oc.Pm
- N.s. increase in BFR/BV, Ac.F
- N.s. further decrease in Rs.P and Rm.P
- N.s. slight increase in W.Wi in all PTH groups (ca. 10%).

What this means: PTH causes increased osteoclastic resorption activity and significantly increases osteoblastic formation activity, resulting in a significant increase in cancellous bone. PTH also causes a further decrease in remodeling (resorption) period. The increase in wall width indicates a positive bone balance and reflects the net remodeling changes occurring at the level of the BMU.

Effect of LY withdrawal:
- Decreases in Cn.Ar., Tb.N., and increase in Tb.Se
- Decreases in O.Ar, O.Pm, Ms.Pm, BFR/BS, BFR/Tt.BV (see figure below)
- Decreases in E.Pm and Oc.Pm
- No effect on BFR/BV or Ac.F
- Reversal of Rs.P to OVX/sham levels
- No change in W.Wi

What this means: PTH withdrawal partially reversed the increases in osteoclastic resorption and osteoblastic formation activity, resulting in a partial reversal of the increase in cancellous bone. PTH withdrawal did not reverse the increased wall width brought about by PTH.

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Table D3 Iliac Crest Biopsy Data (Mean ± SEM of Raw Data) at 15 Months (n = 121)

	Sham	OVX	PTH1	PTH1-W*	PTH5	PTH5-W*
Ac.Ar	26.8 ± 1.7 (21)	22.3 ± 1.9 (20)	30.4 ± 2.5 (19)	31.2 ± 1.9 (20)	46.5 ± 2.5 (21)	33.0 ± 2.5 (19)
Tb.Th	147 ± 7 (21)	137 ± 7 (20)	159 ± 7 (19)	157 ± 8 (20)	190 ± 13 (21)	152 ± 11 (19)
Tb.N	1.81 ± 0.07 (21)	1.59 ± 0.09 (20)	2.41 ± 0.12 (19)	2.00 ± 0.09 (20)	2.64 ± 0.13 (21)	2.18 ± 0.09 (19)
Tb.Sc	422 ± 24 (21)	540 ± 51 (20)	279 ± 29 (19)	367 ± 29 (20)	207 ± 16 (21)	319 ± 20 (19)
E.Pm	1.92 ± 0.44 (21)	4.12 ± 0.78 (20)	4.40 ± 0.48 (19)	4.32 ± 0.73 (20)	4.64 ± 0.64 (21)	3.65 ± 0.46 (19)
Oc.Pm	0.96 ± 0.17 (21)	1.31 ± 0.25 (20)	1.66 ± 0.22 (19)	1.50 ± 0.28 (20)	1.46 ± 0.29 (21)	1.15 ± 0.23 (19)
O.Ar	0.65 ± 0.14 (21)	0.87 ± 0.11 (20)	1.96 ± 0.23 (19)	1.08 ± 0.15 (20)	1.81 ± 0.15 (21)	1.09 ± 0.12 (19)
U.Pm	23.9 ± 3.0 (21)	35.4 ± 3.0 (20)	46.1 ± 2.5 (19)	34.9 ± 3.7 (20)	46.1 ± 2.4 (21)	36.4 ± 3.2 (19)
Ob.Pm	2.09 ± 0.46 (21)	5.31 ± 1.02 (20)	9.82 ± 0.97 (19)	10.82 ± 1.66 (20)	9.56 ± 1.85 (21)	9.92 ± 1.44 (19)
L.O. Pm	56.2 ± 4.3 (21)	64.6 ± 5.0 (20)	65.9 ± 4.7 (19)	63.6 ± 4.4 (20)	74.8 ± 3.2 (21)	64.7 ± 5.0 (19)
Mc.Pm	12.7 ± 1.8 (21)	22.5 ± 2.4 (20)	29.9 ± 2.3 (19)	22.3 ± 2.4 (20)	35.9 ± 2.2 (21)	24.3 ± 2.9 (19)
MAR	0.72 ± 0.03 (21)	0.77 ± 0.03 (20)	0.75 ± 0.03 (19)	0.96 ± 0.05 (19)	0.81 ± 0.03 (21)	0.88 ± 0.05 (19)
Aj.AR	0.41 ± 0.05 (21)	0.50 ± 0.05 (20)	0.51 ± 0.04 (19)	0.62 ± 0.07 (19)	0.61 ± 0.04 (21)	0.58 ± 0.06 (19)
Omt	12 ± 1 (21)	12 ± 1 (20)	15 ± 2 (19)	10 ± 1 (19)	11 ± 1 (21)	10 ± 1 (19)
BFR/BV	42.9 ± 6.6 (21)	78.0 ± 9.1 (20)	92.8 ± 9.3 (19)	93.5 ± 14.4 (19)	104.1 ± 11.3 (21)	93.4 ± 13.8 (19)
BFR/BS	3517 ± 544 (21)	6449 ± 812 (20)	8422 ± 740 (18)	7937 ± 1004 (19)	10768 ± 661 (21)	7993 ± 1139 (19)
BFR/Tb.BV	11.1 ± 1.9 (21)	17.1 ± 2.3 (20)	33.0 ± 3.2 (18)	26.9 ± 3.9 (19)	48.0 ± 4.9 (21)	29.2 ± 3.8 (19)
FP	200 ± 36 (21)	139 ± 10 (20)	165 ± 13 (19)	136 ± 15 (19)	121 ± 9 (21)	142 ± 19 (19)
Rs.P	19.5 ± 7.5 (21)	15.3 ± 2.4 (20)	11.3 ± 1.7 (19)	21.2 ± 5.6 (19)	11.4 ± 1.7 (21)	16.4 ± 3.8 (19)
Rm.P	227 ± 41 (20)	155 ± 11 (20)	150 ± 14 (18)	157 ± 19 (19)	132 ± 9 (21)	159 ± 21 (19)
Ac.F	2.49 ± 0.32 (21)	2.56 ± 0.18 (20)	2.62 ± 0.22 (18)	2.86 ± 0.33 (19)	3.08 ± 0.26 (21)	3.01 ± 0.43 (19)
W.Wi	57.8 ± 2.6 (21)	62.2 ± 3.5 (20)	67.1 ± 2.9 (19)	71.0 ± 2.5 (20)	66.4 ± 3.0 (21)	65.5 ± 3.3 (19)
O.Th	8.62 ± 0.58 (21)	9.11 ± 0.51 (20)	10.59 ± 1.19 (19)	8.91 ± 0.47 (20)	8.36 ± 0.42 (21)	8.14 ± 0.38 (19)

Number of specimens per group are shown in parentheses.
* Withdrawal data represent changes measured in monkeys treated for 12 months with LY333334 and then withdrawn from treatment for 3 months.

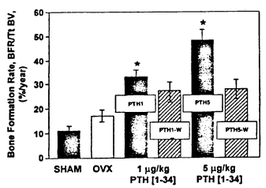

Figure 7. Bone formation rate measured in iliac crest biopsies taken at 15 months from sham controls and ovariectomized adult monkeys, *M fascicularis*, given vehicle or LY333334 at 1 µg/kg/day (PTH1) or 5 µg/kg/day (PTH5) for 18 months or at 1 µg/kg/day (PTH1-W) or 5 µg/kg/day (PTH5-W) for 12 months, after which treatment was withdrawn for a further 3 months. Note the increase in bone formation rates during active treatment in PTH1 and PTH5 and their reversion towards control values in PTH1-W and PTH5-W. Statistical significance, * p<.05.

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Additional data on tunneling, cortical thickness in iliac crest:

Table 1 Incidence of Tunneling in Iliac Crest Biopsies

Treatment Duration	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W
6 months	9.5 (21)	10.0 (20)	41.2 (17)	25.0 (20)	50.0 (20)	38.1 (21)
15 months	4.8 (21)	5.0 (20)	47.4 (19)	10.0 (20)	60.0 (20)	5.0 (20)

Number of specimens per group are shown in parentheses. Incidence in percent.

Conclusion: PTH caused a marked increase in iliac crest tunneling

Table 4 Porosity and Cortical Thickness (Mean ± SEM) of the Cortical Bone of the Iliac Crest Biopsies

	Sham	OVX	PTH1	PTH1-W*	PTH5	PTH5-W*
Porosity (%)						
6 months	2.3 ± 0.3 (21)	2.8 ± 0.5 (18)	3.6 ± 0.7 (16)	3.3 ± 0.4 (19)	2.5 ± 0.5 (21)	2.7 ± 0.4 (17)
15 months	1.2 ± 0.2[a] (19)	1.1 ± 0.1[b] (18)	1.0 ± 0.1[c] (16)	1.1 ± 0.2[c] (17)	1.1 ± 0.2[c] (17)	1.3 ± 0.3[c] (14)
Cortical Thickness (µm)						
6 months	433.9 ± 11.7	403.2 ± 15.4	482.2 ± 22.6	452.0 ± 24.6	500.0 ± 28.1	399.3 ± 26.7
15 months	430.6 ± 17.7	446.6 ± 21.3	388.5 ± 20.3[c]	399.7 ± 24.4	418.2 ± 33.1	440.6 ± 25.8

Number of specimens per group shown in parentheses.
* Withdrawal data represent changes measured in monkeys treated for 12 months with LY333334 and then withdrawn from treatment for 3 months.
a p<.05 vs. 6 months
c p<.01 vs. 6 months

Conclusion: PTH did not increase porosity in the iliac crest, unlike what was seen in the mid-humerus (see below, Table E4). At 6 months, cortical thickness was increased by PTH1 and PTH5, although the effect was not statistically significant. However, after 15 months of treatment the previously increased cortical thickness was decreased in both PTH1 and PTH5 groups to below- sham levels.

PTH did not affect the incidence of woven bone in specimens of the iliac crest at 15 months relative to 6 months.

These results indicate that it is unclear what the result of long term PTH treatment will be on a mixed bone site, like the iliac crest or the femoral neck, in terms of bone strength. However, histomorphometry (below, Table F16) and strength (above, Table B4) data for the femoral neck suggest that at this particular bone site adverse effects on bone density or strength do not occur.

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Humerus midshaft
Measurements were performed on periosteal, endocortical and intracortical surfaces of cortical bone from humerus mid-diaphysis

Table E1 Definition of Histomorphometric Variables for Cortical Bone Measurements of the Humerus*

Variable	Units	Description
Ac.F	cycles/year	Activation frequency
BFR/BS.Ec	µm/day	Bone formation rate, endocortical surface referent
BFR/BS.Ps	µm/day	Bone formation rate, periosteal surface referent
BFR/BV	%-year	Bone formation rate, bone volume referent
FP	days	Formation period
L.On.N/Ct.A	#/mm2	Number of fluorochrome labeled osteons per unit cortical area
MAR	µm/day	Mineral apposition rate, intracortical
MAR.Ec	µm/day	Mineral apposition rate, endocortical surface
MAR.Ps	µm/day	Mineral apposition rate, periosteal surface
MS/BS.Ec	%	Mineralizing endocortical surface normalized to total endocortical surface
MS/BS.Ps	%	Mineralizing periosteal surface normalized to total periosteal surface
O.Wi	µm	Osteoid width
Rs.N/Ct.A	#/mm2	Number of resorption spaces per unit cortical area
W.Wi	µm	Osteonal wall width
omt	days	Osteoid maturation time
Po	%	Porosity, the percentage of bone area occupied by spaces
B.Ar	mm2	Bone area, the total area within the periosteal surface
Ct.Ar	mm2	Cortical area, the area of bone within the periosteal surface (includes porosities)
Me.Ar	mm2	Medullary cavity area

* Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. *J Bone Miner Res* 2:595-610

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18 month data (Table E4):
Effect of OVX (relative to sham):

- Significant increases in Ac.F, BFR/BV, L.On.N/Ct.A, MS/BS.Ec, MS/BS.Ps, Rs.N/Ct.A, Po
- N.s. increase in BFR/BS.Ec, BFR/BS.Ps, O.Wi, Me.Ar
- No effect on Ct.Ar

Effect of LY treatment (relative to OVX):

- Significant increases in L.On.N/Ct.A, MS/BS.Ec (but not MS/BS.Ps), Rs.N/Ct.A, Po, Ct.Ar
- N.s. increases in Ac.F, BFR/BS.Ec* (but not BFR/BS.Ps), BFR/BV* (*=only in PTH5 grp), B.Ar
- N.s. decrease in O.Wi, Me.Ar
- No effect on BFR/BS.Ps

Effect of LY treatment withdrawal:

- Significant decrease in Ac.F, BFR/BV, L.OnN/.Ct.A, MS/BS.Ec, MS/BS.Ps (all but last of these parameters were increased by both OVX and LY, and were reversed below OVX levels, back to sham level, by LY withdrawal)
- Some other parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e. BFR/BS.Ec, BFR/BS.Ps, Rs.N/Ct.A)
- Decrease in porosity after withdrawal of PTH1 but not PTH5
- No effect on Ct.Ar or B.Ar

What this means:
Ovariectomy caused an increase in periosteal and endocortical bone formation and intracortical resorption, i.e. increased bone turnover. Ovariectomy caused increased porosity but had no effect on cortical bone area.
PTH5 but not PTH1 caused a further increase in endocortical but not periosteal bone formation. PTH caused an increase in cortical area apparently due to decreased medullary and increased bone area. PTH1 and PTH5 both increased cortical porosity. This appeared to be the result of an increase in intracortical resorption.

The effect of PTH withdrawal was to decrease bone formation to below-OVX levels and occurred in both periosteal and endocortical zones. PTH1 withdrawal reversed the increased porosity. However, within the time frame studied, PTH5 withdrawal had no consequences for the treatment-induced effects on cortical area or porosity.

Table E4 Intracortical Measurements (Mean ± SEM of Raw Data) of the Humerus at 18 Months (n = 121)[a]

Variable	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W
Ac.F	1.85 ± 0.43 (19)	6.06 ± 0.76 (19)	7.69 ± 1.14 (19)	3.17 ± 0.49 (19)	9.09 ± 0.79 (21)	2.14 ± 0.32 (20)
BFR/BS.Ec	7.1 ± 2.2 (3)	20.9 ± 5.0 (15)	18.1 ± 3.3 (18)	14.9 ± 3.3 (10)	34.0 ± 4.3 (20)	13.9 ± 6.0 (8)
BFR/BS.Ps	3.8 ± 1.4 (5)	9.1 ± 2.0 (14)	8.5 ± 2.6 (17)	3.6 ± 1.1 (12)	9.0 ± 1.4 (17)	5.8 ± 2.0 (5)
BFR/BV	2.1 ± 0.5 (19)	9.2 ± 1.2 (19)	9.2 ± 1.4 (19)	4.5 ± 0.8 (19)	13.5 ± 1.2 (21)	2.3 ± 0.4 (20)
FP	82.7 ± 9.4 (19)	65.9 ± 4.5 (19)	63.4 ± 2.3 (19)	65.5 ± 2.8 (19)	61.7 ± 2.5 (21)	82.5 ± 10.3 (20)
L.On.N/Ct.A	0.28 ± 0.06 (21)	1.03 ± 0.12 (19)	1.26 ± 0.16 (19)	0.52 ± 0.08 (20)	1.47 ± 0.10 (21)	0.40 ± 0.06 (20)
MAR	0.91 ± 0.08 (19)	1.07 ± 0.04 (19)	0.98 ± 0.03 (19)	1.04 ± 0.06 (19)	1.07 ± 0.02 (21)	0.88 ± 0.06 (20)
MAR.Ec	0.48 ± 0.11 (5)	0.75 ± 0.06 (15)	0.66 ± 0.04 (18)	0.66 ± 0.05 (10)	0.75 ± 0.03 (20)	0.61 ± 0.06 (9)
MAR.Ps	0.62 ± 0.11 (5)	0.69 ± 0.06 (14)	0.99 ± 0.23 (17)	0.54 ± 0.04 (12)	0.66 ± 0.04 (17)	0.83 ± 0.07 (5)
MS/BS.Ec	3.1 ± 1.4 (21)	21.0 ± 4.1 (19)	25.2 ± 3.9 (19)	12.0 ± 3.3 (20)	42.3 ± 5.2 (21)	9.2 ± 3.5 (20)
MS/BS.Ps	1.8 ± 0.8 (21)	10.0 ± 2.4 (19)	8.6 ± 1.3 (19)	4.0 ± 1.1 (20)	11.5 ± 2.1 (21)	2.0 ± 0.8 (20)
O.Wi	3.77 ± 0.20 (21)	4.04 ± 0.21 (19)	3.66 ± 0.15 (19)	4.03 ± 0.18 (20)	3.72 ± 0.11 (21)	3.75 ± 0.19 (20)
Rs.N/Ct.A	0.12 ± 0.04 (21)	0.21 ± 0.03 (19)	0.28 ± 0.04 (19)	0.12 ± 0.02 (20)	0.45 ± 0.05 (21)	0.17 ± 0.04 (20)
W.Wi	63.2 ± 3.0 (21)	68.6 ± 3.5 (19)	61.4 ± 1.8 (19)	66.0 ± 2.3 (19)	65.4 ± 2.1 (21)	63.0 ± 1.7 (20)
omt	4.58 ± 0.29 (19)	3.87 ± 0.24 (19)	3.76 ± 0.16 (19)	3.96 ± 0.14 (19)	3.5 ± 0.13 (21)	4.82 ± 0.55 (20)
Po	1.32 ± 0.13 (21)	2.61 ± 0.32 (19)	4.65 ± 1.10 (19)	2.31 ± 0.36 (20)	6.65 ± 0.94 (21)	6.46 ± 0.97 (20)
B.Ar	53.1 ± 1.2 (21)	52.8 ± 1.6 (19)	54.2 ± 1.4 (19)	55.3 ± 1.4 (20)	55.4 ± 1.3 (21)	57.9 ± 2.0 (20)
Ct.Ar	37.4 ± 0.8 (21)	35.4 ± 1.2 (19)	37.6 ± 0.9 (19)	38.1 ± 1.1 (20)	40.6 ± 0.9 (21)	40.8 ± 1.3 (20)
Me.Ar	15.7 ± 0.9 (21)	17.5 ± 1.0 (19)	16.6 ± 0.9 (19)	17.3 ± 0.7 (20)	14.9 ± 1.1 (21)	17.1 ± 1.4 (20)

Number of specimens per group are shown in parentheses.
Although the total sample size was n = 121 monkeys, it was not always possible to measure each parameter due to label escape. Missing measurements also prevent calculation of derived variables, reducing the sample size for some variables.
a The total n for some variables is less than 121 due to missing values.

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Evaluation of three different cortical zones in the mid-humerus revealed that porosity was largest and/or most increased by LY treatment near the endocortical surface, and least near the periosteal surface (Fig. 10, below). Porosity in this endocortical zone was also largest to begin with (see Sham data).

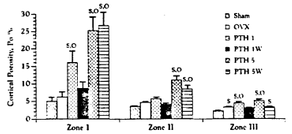

Figure 10. Localization of porosities to predominantly the endocortical region of the midshaft humerus in sham controls and ovariectomized adult monkeys, *M fascicularis*, given vehicle or LY333334 at 1 µg/kg/day (PTH1) or 5 µg/kg/day (PTH5) for 18 months or at 1 µg/kg/day (PTH1-W) or 5 µg/kg/day (PTH5-W) for 12 months, after which treatment was withdrawn for a further 6 months. (A) In the PTH-treated groups, porosity increased most near the endocortical surface (Zone I). Porosity was significantly greater in PTH1 and PTH5 than in sham or OVX near the periosteal surface (Zone III) as well, but the increases were much smaller. Zone I is the inner (endocortical) one-third of the cortical diameter; Zone II is the intermediate one-third, and Zone III is the outer (periosteal) one-third of the diameter. S= p<.05 vs sham; O = p<.05 vs OVX. Data are expressed as Mean ± SEM. (B) If porosity were uniformly distributed throughout the cross-section, the reduction in strength of the humerus would have been greater than when the porosity is primarily distributed close to the endocortical surface, as it was in PTH-treated monkeys. For PTH1 and PTH5, a uniform distribution of porosity would have caused a significant decrease in strength of the humerus (* p<.05). Because there was a gradient of porosity that decreased toward the periosteal surface, no significant reductions in strength were predicted. Data were expressed as Mean ± SEM.

In a recently published paper by Burr et al. (J. Bone Miner. Res. 16(1), 2001) the issue of cortical porosity, its distribution and its effect on strength was addressed further. Data from the current monkey study on architecture and strength of the humerus midshaft were used to support the hypothesis that the dose-dependent increase in cortical porosity upon intermittent administration of PTH(1-34) does not reduce the strength or stiffness of cortical bone. According to the data, PTH (1 and 5 ug/kg/day) dose-dependently increased cortical bone turnover and intracortical porosity. This would be expected to result in a decrease in cortical BMD. The increase in porosity occurred mainly in the endocortical zone. This increase in porosity is theoretically expected to result in a decrease in bone strength (based on an estimation of Young's modulus in each zone). However, the decrease would not be expected to be as large as when porosity would have been uniformly distributed throughout the cortical zones.
The data also showed that PTH caused addition of new bone and increased cortical area and thickness and increased bone formation at the endocortical surface, which was statistically significant

at the high dose (see Table E4). This would offset the adverse effect of increased porosity on strength. Actual strength measurements confirmed the predictions based on bone architecture (see Table B4, above). The effect of PTH withdrawal for 6 months was that at the low dose the increased porosity was reversed to near OVX levels, while at the high dose it was partially maintained (Table E4). The absence of reversal in the endocortical zone could indicate a temporary uncoupling of resorption and formation. However, strength measurements did not suggest adverse effects after PTH withdrawal at either dose.

It should be noted that in the published study only at the high dose of PTH a significantly increased thickness of cortical bone was observed. Treatment with the low dose, which is paralleled by a plasma AUC of PTH(1-34) similar to the expected AUC in humans treated with 20 ug/day, did not significantly affect cortical thickness. This raises concern about the generalization of the humerus findings. It is conceivable that at the intended human dose an increase in endocortical porosity and an associated decrease in cortical bone BMD leads to decreased cortical bone strength (when the increased porosity is not balanced by a sufficient increase in bone thickness). Nevertheless, in the current monkey study the humeral strength was not significantly affected/decreased at the low dose (Table B4), possibly because there was a slight non-significant increase in thickness that balanced the increased porosity. However, since the humerus was the only cortical bone site assessed for strength, the theory that PTH will not pose an increased cortical fracture risk may not be applicable to all cortical bone sites.

In fact, histomorphometry data for the midshaft radius from this study showed that PTH causes a slight decrease in BV/TV while bone area (ie thickness) was not significantly affected (Table F7). Bone densitometry also showed a decrease in BMD). However, according to this technique X-area was dose-dependently increased. A decrease in BV/TV also occurred in the cortical part of the distal radius (Table G3) while BMD of this site was slightly decreased by PTH1 in the middle zones. Therefore, it is unclear whether this bone site may be subject to deleterious effects of PTH on bone strength. Histomorphometry data on the femoral midshaft (Table F10) suggested that BV/TV and B.Ar (thickness) were not affecetd by PTH.

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Mid-Radius, Mid-Femur, Vertebra, and Femoral Neck (18-month data)

Table F1 Definition of Histomorphometric Variables for Cortical Bone Measurements of the Midshaft Femur and Radius*

Variable	Units	Description
T.Ar	mm^2	Tissue area, the total area within the periosteal surface
Ma.Ar	mm^2	Marrow area, the area of the medullary cavity
Md.Ar	mm^2	Mineralized area, the area of mineralized bone, excluding porosities
Vd.Ar	mm^2	Void area, the area of porosities in the cortex
Ps.Pm	mm	Periosteal bone perimeter
On.Pm	mm	Osteonal bone perimeter
Ec.Pm	mm	Endocortical bone perimeter
Po	%	Porosity, the percentage of mineralized area occupied by porosities
Md.V/TV	%	Bone volume, mineralized area as a percentage of tissue area
Ps.MS/Md.S	%	Periosteal mineralizing surface
Ps.MAR	µm/day	Periosteal mineral apposition rate
Ps.BFR/Md.S	mm^3/mm^2/day	Periosteal bone formation rate, surface referent
Ps.BFR/Md.V	%/year	Periosteal bone formation rate, bone volume referent
On.MS/Md.S	%	Osteonal mineralizing surface
On.MAR	µm/day	Osteonal mineral apposition rate
On.BFR/Md.S	mm^3/mm^2/day	Osteonal bone formation rate, surface referent
On.BFR/Md.V	%/year	Osteonal bone formation rate, bone volume referent
Ec.MS/Md.S	%	Endocortical mineralizing surface
Ec.MAR	µm/day	Endocortical mineral apposition rate
Ec.BFR/Md.S	mm^3/mm^2/day	Endocortical bone formation rate, surface referent
Ec.BFR/Md.V	%/year	Endocortical bone formation rate, bone volume referent
Tt.BFR/Md.V	%/year	Sum of periosteal, osteonal, and endocortical BFR/BV

* Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595-610.

Table F2 Definition of Histomorphometric Variables for Structural and Label Measurements of Vertebra and Femur Neck*

Variable	Units	Description
Md.V/TV	%	Bone volume, the mineralized area as a percentage of tissue area
Md.S/Md.V	mm/mm^2	Mineralized tissue surface-volume ratio
Tb.Th	µm	Trabecular thickness
Tb.N	mm^{-1}	Trabecular number
Tb.Sp	µm	Trabecular separation
MS/MS.S	%	Mineralizing surface
MAR	µm/day	Mineral apposition rate
BFR/MS.S	mm^3/mm^2/day	Bone formation rate, surface referent
BFR/MS.V	%/year	Bone formation rate, bone volume referent
BFR/TV	%/year	Bone formation rate, tissue volume referent
Ps.MS/Md.S	%	Periosteal mineralizing surface
Ps.MAR	µm/day	Periosteal mineral apposition rate
Ps.BFR/Md.S	mm^3/mm^2/day	Periosteal bone formation rate, surface referent

* Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595-610.

Table F3 Definition of Histomorphometric Variables for Histological Measurements of the Vertebra*

Variable	Units	Description
BV/TV	%	Bone volume as a percentage of tissue volume
BS/BV	mm/mm^2	Bone surface/volume ratio
Tb.Th	µm	Trabecular thickness
Tb.N	mm^{-1}	Trabecular number
Tb.Sp	µm	Trabecular separation
It.Th	µm	Interstitial thickness
OV/BV	%	Osteoid volume as a percentage of bone volume
OV/TV	%	Osteoid volume as a percentage of tissue volume
O.Th	µm	Osteoid thickness
W.Th	µm	Wall thickness, the thickness of completed bone packets
OS/BS	%	Osteoid surface as a percentage of bone surface
ES/BS	%	Eroded surface as a percentage of bone surface
Oc.S/BS	%	Osteoclastic surface as a percentage of bone surface
Ac.f	cycles/year	Activation frequency
Aj.AR	µm/day	Adjusted apposition rate
FP	days	Formation period
Rs.P	days	Resorption (osteoclastic) period
Rv.P	days	Reversal period
Mt	days	Mineralization lag time
Omt	days	Osteoid maturation time

* Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595-610.

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Mid-Radius (Table F7):
Measurements of static and dynamic parameters in cortical bone from the midshaft radius:

Effect of OVX (relative to sham):
- Significant increases in Po, On.MS/Md.S, On.BFR/Md.S, On.BFR/Md.V
- N.s. increase in Vd.Ar, On.Pm, Ps.MS/Md.S, Ps.BFR/Md.S, Ps.BFR/Md.V, and Ec.BFR-parameters
- No effect on T.Ar, Ma.Ar, Md.Ar (bone area), Md.V/TV

Effect of LY treatment (relative to OVX):
- Significant increases in T.Ar, Ma.Ar, Vd.Ar, On.Pm, Ec.Pm, Po, On.BFR/Md.V, Ec.MS/Md.S, Ec.BFR/Md.S and Ec.BFR/Md.V
- Significant decrease in Md.V/TV (=BV/TV) !
- N.s. slight increases in Ps.BFR/Md.S and Ps.BFR/Md.V
- No effect on Md.Ar

Effect of LY treatment withdrawal (relative to OVX):
- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e., Vd.Ar, On.Pm, Po, Ps.Ms/Md.S, Ps.BFR/Md.S, Ps.BFR/Md.V, On.BFR/Md.S, On.BFR/Md.V, and Ec.BFR parameters).
- No further effect on or reversal of decreased Md.V/TV (BV/TV). No effect on Md.Ar

Conclusion: Ovariectomy caused increased cortical bone turnover and porosity but had no effect on bone area. PTH caused increased endocortical and osteonal bone formation and increased cortical porosity. PTH decreased fractional bone volume (Md.V/TV), but did not affect total bone area. PTH withdrawal decreased periosteal bone formation to below OVX levels! A similar finding was obtained in the humerus midshaft. However, withdrawal did not reverse the decrease in fractional bone volume (BV/TV) or bone area.

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Note: 3 out of 21 mid-radius specimens from the PTH5 group had intramedullary bone formation (ring of mineralized bone under endocortical surface present in the medullary area)

Table F7 Static and Dynamic Histomorphometry of the Midshaft Radius (n = 121, Mean ± SEM of Raw Data) and Bonferronni-Adjusted Group Comparisons (128-Monkey Population)

	Sham n = 14*	OVX n = 19*	PTH1 n = 19*	PTH5-W n = 17*	PTH5 n = 21*	PTH-W n = 21*	Trans-formation	Over-all p	Bonferroni-Adjusted p, OVX vs. Sham	PTH1	PTH5-W	PTH5	PTH-W
T.Ar	38.4 ± 0.4	39.4 ± 0.5	39.2 ± 0.6	38.8 ± 0.5	38.8 ± 0.4	39.8 ± 0.6		.06	ns	ns	ns	ns	ns
Ma.Ar	6.74 ± 0.30	7.77 ± 0.29	6.94 ± 0.29	5.66 ± 0.31	4.91 ± 0.32	5.35 ± 0.23		.02	ns	< .05	< .01	ns	< .01
Md.Ar	15.2 ± 0.5	14.2 ± 0.3	15.3 ± 0.4	15.2 ± 0.3	15.3 ± 0.3	14.5 ± 0.3		.03	ns	ns	ns	ns	ns
Vd.Ar	0.57 ± 0.09	0.97 ± 0.09	0.97 ± 0.11	0.68 ± 0.09	1.21 ± 0.13	0.91 ± 0.16	*	.00	ns	ns	ns	< .01	ns
Ps.Pm	17.2 ± 0.2	16.5 ± 0.3	17.4 ± 0.3	17.2 ± 0.3	17.2 ± 0.3	17.4 ± 0.2		.09	ns	ns	ns	ns	ns
On.Pm	26.6 ± 1.6	35.1 ± 2.1	42.4 ± 3.4	35.1 ± 2.2	32.5 ± 2.1	38.7 ± 2.1		.00	ns	< .1	ns	< .001	ns
Ec.Pm	7.75 ± 0.29	8.79 ± 0.32	8.15 ± 0.33	8.03 ± 0.27	7.83 ± 0.26	8.66 ± 0.40		.06	ns	< .05	< .05	ns	< .001
Po	2.35 ± 0.43	3.67 ± 0.35	3.00 ± 0.42	3.05 ± 0.33	7.21 ± 0.71	5.61 ± 1.13	log	.00	< .05	ns	ns	< .01	ns
Md.V/TV	75.2 ± 1.6	77.4 ± 1.1	72.3 ± 1.4	73.7 ± 1.2	73.1 ± 1.3	71.5 ± 1.5		.02	ns	< .05	ns	< .1	< .01
Ps.MS/Md.S	4.5 ± 1.3	12.8 ± 3.4	15.4 ± 3.1	7.4 ± 1.2	13.5 ± 2.8	9.3 ± 1.1	square root	.01	< .1	ns	ns	ns	ns
Ps.MAR	0.71 ± 0.12	0.91 ± 0.09	0.97 ± 0.06	0.78 ± 0.12	0.61 ± 0.09	0.65 ± 0.07	*	.02	ns	ns	ns	ns	ns
Ps.BFR/Md.S	12.8 ± 3.4	20.0 ± 9.6	26.1 ± 8.9	21.0 ± 4.2	32.2 ± 7.2	15.4 ± 2.9	square root	.01	ns	ns	ns	ns	ns
On.MS/Md.S	1.76 ± 0.42	3.21 ± 1.04	3.51 ± 0.94	2.00 ± 0.49	3.85 ± 0.97	3.95 ± 0.61	square root	.07	ns	ns	ns	ns	ns
On.MAR	0.3 ± 1.1	26.3 ± 2.6	26.1 ± 1.5	24.4 ± 3.0	24.2 ± 1.9	20.3 ± 2.4	*	.00	< .05	ns	ns	ns	ns
On.BFR/Md.S	0.97 ± 0.16	8.14 ± 0.64	8.99 ± 0.83	5.84 ± 0.94	7.81 ± 0.92	6.84 ± 0.65	*	.01	< .1	ns	ns	ns	ns
Ec.MS/Md.S	24 ± 5	104 ± 10	107 ± 5	97 ± 12	114 ± 7	79 ± 11	*	.00	< .05	ns	ns	ns	ns
Ec.BFR/Md.S	4.7 ± 1.0	24.9 ± 3.3	29.7 ± 2.5	22.5 ± 3.7	41.0 ± 5.4	19.9 ± 2.7	square root	.00	< .001	ns	ns	ns	< .01
Ec.MS/Md.S	2.3 ± 1.7	12.1 ± 4.2	19.7 ± 6.7	13.2 ± 4.7	34.7 ± 5.7	17.8 ± 4.3	*	.00	ns	ns	ns	< .05	ns
Ec.MAR	0.47 ± 0.17	0.79 ± 0.09	0.65 ± 0.09	0.75 ± 0.08	0.79 ± 0.07	0.66 ± 0.06	*	.34	ns	ns	ns	ns	ns
Ec.BFR/Md.S	4.7 ± 2.4	17.2 ± 4.5	16.5 ± 2.4	24.4 ± 3.4	29.2 ± 3.5	11.9 ± 2.4	square root	.00	ns	ns	< .1	ns	ns
Ec.MS/Md.V	9.24 ± 0.74	1.01 ± 0.67	3.25 ± 1.15	2.85 ± 0.99	6.23 ± 1.36	3.21 ± 1.12	*	.00	ns	ns	ns	< .05	ns
Ec.BFR/Md.V	0.6 ± 1.4	19.5 ± 4.1	26.8 ± 3.1	20.8 ± 4.0	51.1 ± 4.2	26.7 ± 3.2	square root	.00	< .001	ns	ns	< .001	ns

n = p < .1

* Leverene: Ps.MAR (n = 16), On.MAR (n = 18) and Ec.MAR (n = 4).

* Leverene: Ps.MAR (n = 16) and Ec.MAR (n = 11).

* Leverene: Ec.MAR (n = 15).

* Leverene: Ec.MAR (n = 16).

* Leverene: Ps.MAR (n = 21) and Ec.MAR (n = 21).

* Leverene: Ps.MAR (n = 19) and Ec.MAR (n = 14).

* Data resistant to transformation, analyzed using Kruskal-Wallis test

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Midshaft Femur (Table F10):
Measurements of static and dynamic parameters in midshaft femur

Effect of OVX (relative to sham):
- Significant increases in On.Pm, Po, Ps.MS/BS, Ps.BFR, On.MS/BS, On.MAR, On.BFR/BS, On.BFR/BV, and Ec.BFR parameters
- N.s. increase in Vd.Ar, Ps.MAR
- No effect on T.Ar, Ma.Ar, B.Ar, BV/TV

Effect of LY treatment (relative to OVX):
- Significant increases in Vd.Ar, On.Pm, Po, On.MS/BS, On.BFR/BS, On.BFR/BV, and Ec.BFR parameters
- N.s. increase in B.Ar
- N.s. increases in Ps.MS/BS, Ps.BFR/BS and Ps.BFR/BV at high dose only
- No effect on T.Ar, Ma.Ar, BV/TV !

Effect of LY treatment withdrawal (relative to OVX):
- Some parameters were reversed significantly below OVX levels (Ps.MS/BS, Ps.BFR/BS, Ps.BFR/BV, On.MAR, On.BFR/BS)
- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e., Vd.Ar, On.Pm, Po, On.BFR/BV, Ec. BFR parameters)
- No effect on T.Ar, Ma.Ar, B.Ar, BV/TV

Conclusion: Ovariectomy caused increased cortical bone turnover and porosity but did not affect bone area. PTH caused increased endocortical, periosteal and osteonal bone formation and increased cortical porosity. However, PTH did not affect fractional bone volume (BV/TV) and slightly increased total bone area. PTH withdrawal decreased bone formation parameters to below OVX (near-sham) levels. PTH withdrawal did not affect specific bone volume (BV/TV) or total bone area. Similar findings were obtained in the humerus and midshaft radius.

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Table F10 Static and Dynamic Histomorphometry of the Midshaft Femur (n = 121, Mean ± SEM of Raw Data) and Bonferronni-Adjusted Group Comparisons (121-Monkey Population)

	Sham n = 21*	OVX n = 24*	PTH1 n = 13	PTH5-W n = 17	PTH5 n = 21	PTH-W n = 24*	Trans-formation	Over-all p	Bonferroni-Adjusted p, OVX vs. Sham	PTH1	PTH5-W	PTH5	PTH-W
T.Ar	38.5 ± 1.0	39.2 ± 1.6	39.7 ± 0.9	38.4 ± 1.4	38.2 ± 1.5	42.3 ± 1.4							
Ma.Ar	17.3 ± 0.8	16.5 ± 1.0	17.5 ± 0.7	16.7 ± 1.0	17.1 ± 1.0	17.9 ± 1.2	log	.34	ns	ns	ns	ns	ns
B.Ar	22.3 ± 0.7	21.5 ± 1.0	22.3 ± 0.8	21.8 ± 0.7	21.7 ± 0.6	23.8 ± 0.6		.29	ns	ns	ns	ns	ns
Vd.Ar	0.36 ± 0.03	0.76 ± 0.10	1.04 ± 0.17	0.73 ± 0.14	1.48 ± 0.43	1.51 ± 0.41	*	.00	< .1	ns	ns	< .01	ns
Ps.Pm	25.2 ± 0.3	25.3 ± 0.4	25.7 ± 0.2	26.3 ± 0.4	26.1 ± 0.3	25.9 ± 0.4		.30	ns	ns	ns	ns	ns
On.Pm	36.1 ± 5.7	51.9 ± 3.7	79.2 ± 7.8	50.8 ± 5.7	93.5 ± 8.4	67.9 ± 5.6	log	.00	< .05	< .1	ns	< .001	ns
Ec.Pm	14.7 ± 0.7	15.7 ± 0.4	14.8 ± 0.4	16.2 ± 0.6	14.8 ± 0.7	14.6 ± 0.5	*	.29	ns	ns	ns	ns	ns
Po	1.09 ± 0.10	2.42 ± 0.31	4.79 ± 0.96	2.07 ± 0.36	6.55 ± 1.04	4.77 ± 1.06	*	.00	< .05	ns	ns	< .01	ns
BV/TV	85.2 ± 1.1	81.8 ± 1.3	82.7 ± 1.2	82.1 ± 1.1	83.6 ± 1.3	80.8 ± 1.5		.23	ns	ns	ns	ns	ns
Ps.MS/BS	2.2 ± 0.8	11.5 ± 2.1	13.1 ± 2.8	4.8 ± 1.2	22.8 ± 4.8	1.8 ± 0.7	*	.00	< .05	ns	ns	ns	< .05
Ps.MAR	0.54 ± 0.07	0.76 ± 0.07	0.62 ± 0.07	0.58 ± 0.09	0.50 ± 0.09	0.05 ± 0.07	*	.01	ns	ns	ns	ns	ns
Ps.BFR/BS	9.6 ± 2.3	41.7 ± 12.9	30.7 ± 4.9	11.7 ± 3.1	79.6 ± 17.0	3.3 ± 1.1	*	.00	< .05	ns	ns	ns	< .05
Ps.BFR/BV	0.47 ± 0.20	3.57 ± 1.12	2.24 ± 0.47	0.96 ± 0.43	5.97 ± 1.54	0.25 ± 0.08	*	.00	< .05	ns	ns	ns	< .05
On.MS/BS	5.3 ± 1.7	17.0 ± 2.4	19.0 ± 2.1	9.4 ± 1.7	27.4 ± 2.1	11.5 ± 1.6	square root	.00	< .001	ns	< .1	< .01	ns
On.MAR	0.91 ± 0.12	1.28 ± 0.04	1.08 ± 0.03	1.22 ± 0.06	1.14 ± 0.03	1.04 ± 0.03	*	.00	< .05	< .05	ns	ns	< .05
On.BFR/BS	21.6 ± 6.4	77.5 ± 10.7	75.5 ± 8.5	39.8 ± 9.3	114.8 ± 9.4	45.8 ± 5.9	*	.00	< .001	ns	< .05	< .01	< .05
Ec.BFR/BS	2.8 ± 1.0	14.6 ± 2.6	18.2 ± 3.1	5.8 ± 1.3	33.7 ± 4.7	9.2 ± 2.6	square root	.00	< .001	ns	ns	< .001	ns
Ec.MS/BS	5.5 ± 2.7	12.5 ± 3.7	29.7 ± 5.0	6.8 ± 1.3	30.8 ± 5.6	5.0 ± 2.6	*	.00	ns	< .1	ns	< .05	ns
Ec.MAR	0.64 ± 0.06	0.78 ± 0.04	0.84 ± 0.06	0.79 ± 0.03	0.78 ± 0.06	0.71 ± 0.09	*	.03	ns	ns	ns	ns	ns
Ec.BFR/BS	13.2 ± 7.6	30.0 ± 14.7	85.0 ± 19.4	16.2 ± 4.1	134.2 ± 27.9	14.0 ± 5.9	*	.00	ns	< .1	ns	< .05	ns
Ec.BFR/BV	0.29 ± 0.17	2.00 ± 0.77	3.71 ± 0.77	0.79 ± 0.34	5.31 ± 1.04	0.66 ± 0.27	*	.00	ns	ns	ns	< .01	ns
Ec.MS/BV	3.9 ± 2.2	20.2 ± 3.4	24.5 ± 3.3	8.7 ± 1.5	44.9 ± 5.5	10.2 ± 1.7	*	.00	< .05	ns	ns	< .1	ns

n = p < .1

* Leverene: Ps.MAR (n = 14), On.MAR (n = 17) and Ec.MAR (n = 14).

* Leverene: Ps.MAR (n = 17), On.MAR (n = 19) and Ec.MAR (n = 17).

* Leverene: Ps.MAR (n = 19) and Ec.MAR (n = 17).

* Leverene: Ps.MAR (n = 17), On.MAR (n = 19) and Ec.MAR (n = 17).

* Data resistant to transformation, analyzed using Kruskal-Wallis test

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LV2
(A) Measurements of static and dynamic parameters in both cortical and cancellous vertebral bone
(Table F12):
Cortical bone:
Effect of OVX (relative to sham):
- Significant increases in BFR/Md.S, BFR/Md.V, BFR/TV
- N.s. increase in Md.S/Md.V, MS/Md.S, MAR, Ps.MS/Md.S, Ps.BFR/Md.S
- No effect on Md.V/TV (=BV/TV)

Effect of LY treatment (relative to OVX):
- Significant increases in Md.V/TV, BFR/TV (increase in BV/TV also observed with DEXA)
- N.s. increases in Md.S/Md.V, MS/Md.S, BFR/Md.S, BFR/Md.V, Ps.MS/Md.s, Ps.BFR/Md.S
- No effect on MAR

Effect of LY treatment withdrawal (relative to OVX):
- Parameters were reversed towards OVX levels (static) or below-OVX levels (dynamic), but were not significantly different from OVX (i.e., Md.V/TV, Md.S/Md.V, MS/Md.S, BFR's, Ps.MS/Md.S, Ps.BFR/Md.S)

Conclusion: OVX increased cortical and periosteal bone formation, but not cortical bone volume density. PTH increased both cortical and periosteal bone formation and increased cortical bone volume density. PTH withdrawal caused reversal of cortical and periosteal bone formation to below-OVX levels, and reversal of bone volume density to above-OVX levels.

Cancellous bone:
Effect of OVX (relative to sham):
- No significant effects

Effect of LY treatment (relative to OVX):
- Significant increases in Md.V/TV, Tb.N., MS/Md.S, BFR's
- Significant decrease in Tb.Sp.
- N.s. increases in Tb.Th

Effect of LY treatment withdrawal (relative to OVX):
- Static parameters were reversed towards OVX levels (i.e., Md.V/TV, Tb.Th, Tb.N., Tb.Sp,). Dynamic parameters (MS/Md.S, BFR's) were reversed but did not reach levels below OVX as was seen in cortical bone

Conclusion: Ovariectomy had no effect on cancellous bone turnover, volume density or trabecular parameters. PTH caused increased trabecular density, cancellous bone density and cancellous bone formation parameters. PTH withdrawal caused partial reversal of these parameters. There were no specific data on the effect of PTH on bone resorption.

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(B) Measurements of histology/label-derived data in LV2 cancellous bone (Table F14):
Effect of OVX (relative to sham):
- N.s. increase in O.Th, Oc.S/BS, FP, Rs.P, Mlt
- No effects on BV/TV or trabecular parameters

Effect of LY treatment (relative to OVX):
- Significant increases in BV/TV, Tb.N., Ac.F
- Significant decreases in Tb.Sp, O.Th
- N.s. increase in OV/TV, OS/BS
- N.s. decrease in Oc.S/BS, but no effect on ES/BS
- N.s. decreases in FP, Rs.P

Effect of LY treatment withdrawal (relative to OVX):
- Some parameters were reversed towards OVX levels, but were not significantly different from OVX (i.e., BV/TV, Tb.N., Tb.Sp, OV/TV, O.Th)

Conclusion: Ovariectomy caused an increase in relative osteoclast surface, but did not affect cancellous bone turnover, bone volume or trabecular density. Ovariectomy lengthened the remodeling period. PTH increased cancellous bone formation, bone volume and trabecular density. PTH decreased osteoid thickness. The effect on bone resorption was unclear. PTH withdrawal caused partial reversal of the increased bone volume and trabecular density, and the decreased osteoid thickness. The nature of the effect of PTH on osteoid thickness is unclear.

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Table F12 Static and Dynamic Histomorphometry of LV2 (n = 118, Mean ± SEM of Raw Data) and Bonferronni-Adjusted Group Comparisons (121-Monkey Population)

	Tissue	Sham n = 9[a]	OVX n = 8[a]	PTH1 n = 9[a]	PTH1-W n = 8[a]	PTH5 n = 9[a]	PTH5-W n = 9[a]	Trans-formation	Over-all p	Bonferroni-Adjusted p: OVX vs. Sham	OVX vs. PTH1	OVX vs. PTH1-W	OVX vs. PTH5	OVX vs. PTH5-W
MAR/Ts	Cortical	66.2 ± 3.9	62.8 ± 2.4	69.2 ± 2.0	65.5 ± 1.7	72.1 ± 1.5	67.6 ± 1.6		.21	ns	< .01	ns	< .01	ns
	Cancellous	26 ± 2	33 ± 3	35 ± 3	34 ± 3	39 ± 3	37 ± 3	log	.09	ns	< .002	< .002	< .002	< .01
MS/MAS	Cortical	6.2 ± 0.3	7.2 ± 0.5	6.6 ± 0.7	7.2 ± 0.4	8.2 ± 0.4	7.2 ± 0.5		.61	ns	ns	ns	ns	ns
	Cancellous	23 ± 2	32 ± 3	31 ± 3	29 ± 3	31 ± 2	32 ± 3		.31	ns	ns	ns	ns	ns
Ob.Th	Cancellous	97 ± 9	85 ± 5	97 ± 5	105 ± 6	106 ± 4	94 ± 5	reciprocal	.34	ns	ns	ns	ns	ns
Ob.N	Cancellous	2.7 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	log	.99	ns	ns	ns	ns	ns
Ob.Sp	Cancellous	293 ± 11	313 ± 23	292 ± 9	296 ± 14	294 ± 13	299 ± 17		.99	ns	ns	ns	ns	ns
MS/BS	Cortical	16.2 ± 1.9	22.7 ± 2.8	26.4 ± 2.3	19.2 ± 1.6	18.8 ± 2.3	18.4 ± 2.7		.07	ns	ns	ns	< .1	ns
	Cancellous	19 ± 2	19 ± 2	22 ± 2	16 ± 1	20 ± 2	19 ± 2		.50	ns	ns	ns	< .05	ns
OV/AR	Cortical	0.64 ± 0.03	0.76 ± 0.04	0.83 ± 0.06	0.75 ± 0.04	0.74 ± 0.04	0.77 ± 0.06		.01	ns	ns	ns	ns	ns
	Cancellous	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	log	.57	ns	ns	ns	ns	ns
MR/MAS	Cortical	39.1 ± 5.0	44.8 ± 7.0	75.4 ± 10.9	54.9 ± 4.9	70.3 ± 7.4	49.4 ± 7.8		.06	< .05	ns	ns	ns	ns
	Cancellous	43 ± 3	39 ± 3	52 ± 7	45 ± 4	44 ± 4	42 ± 4	log	.97	ns	ns	ns	ns	ns
OV/BV/MAS	Cortical	39 ± 4	36 ± 4	70 ± 17	48 ± 5	45 ± 3	35 ± 4	square root	.05	< .05	ns	ns	ns	ns
	Cancellous	49 ± 19	84 ± 10	112 ± 16	86 ± 12	110 ± 14	88 ± 10	square root	.09	ns	ns	ns	< .05	ns
BV/TV	Cortical	16.8 ± 2.2	29.6 ± 7.6	46.8 ± 9.1	25.8 ± 3.1	47.8 ± 5.5	32.8 ± 7.6	square root	.05	< .05	< .1	ns	< .05	ns
	Cancellous	23 ± 4	29 ± 2	36 ± 3	26 ± 4	47 ± 6	34 ± 2	square root	.00	ns	< .05	ns	< .001	ns

[illegible]

[a] n = 8 for:
[illegible]

Table F14 LV2 Cancellous Histological and Combined Histological/Label Derived Data (n = 118, Mean ± SEM of Raw Data) and Bonferronni-Adjusted Group Comparisons (121-Monkey Population)

	Sham n = 9	OVX n = 8	PTH1 n = 9	PTH1-W n = 8	PTH5 n = 9	PTH5-W n = 9	Trans-formation	Over-all p	Bonferroni-Adjusted p: OVX vs. Sham	OVX vs. PTH1	OVX vs. PTH1-W	OVX vs. PTH5	OVX vs. PTH5-W
OV/TV	25.4 ± 1.4	26.1 ± 1.9	36.8 ± 1.4	30.2 ± 3.1	36.7 ± 1.7	29.6 ± 1.5		.06	ns	< .001	ns	< .001	ns
OV/BV	19.7 ± 0.7	18.6 ± 0.8	18.8 ± 0.6	18.7 ± 0.6	18.3 ± 0.6	19.5 ± 0.7		.24	ns	ns	ns	ns	ns
O.Th	103 ± 3	110 ± 3	114 ± 4	112 ± 4	111 ± 3	105 ± 4		.24	ns	ns	ns	ns	ns
O.N	2.47 ± 0.10	2.41 ± 0.09	3.29 ± 0.11	2.72 ± 0.16	3.34 ± 0.15	2.87 ± 0.12		.00	ns	< .001	ns	< .001	< .05
O.Sp	319 ± 28	320 ± 31	305 ± 9	244 ± 12	264 ± 14	297 ± 13	log	.06	ns	< .001	< .1	< .001	< .05
ES	51.4 ± 2.7	55.8 ± 3.2	59.6 ± 4.1	50.1 ± 3.9	56.8 ± 3.5	54.2 ± 3.1	log	.34	ns	ns	ns	ns	ns
ES/BS	2.87 ± 0.45	2.24 ± 0.29	1.51 ± 0.31	3.38 ± 0.17	2.38 ± 0.34	1.84 ± 0.29	log	.22	ns	ns	< .05	ns	ns
OS/TV	0.56 ± 0.16	0.79 ± 0.07	0.74 ± 0.12	0.43 ± 0.06	0.85 ± 0.14	0.54 ± 0.09	square root	.09	ns	ns	ns	ns	ns
OS/BS	6.44 ± 0.56	7.18 ± 0.27	6.37 ± 0.75	6.54 ± 0.27	5.37 ± 0.17	6.37 ± 0.27	"	.01	ns	ns	ns	< .05	ns
Ac.Th	35.5 ± 0.5	27.3 ± 0.5	27.8 ± 0.8	26.5 ± 0.7	27.3 ± 0.5	35.4 ± 0.6		.39	ns	ns	ns	ns	ns
Ac.BS	15.4 ± 2.2	14.7 ± 1.8	15.5 ± 2.5	11.4 ± 1.6	15.9 ± 3.1	15.4 ± 2.2	square root	.07	ns	ns	ns	ns	ns
Ac.BV	53.2 ± 3.7	43.1 ± 2.8	44.2 ± 3.1	47.9 ± 2.5	47.9 ± 2.7	49.9 ± 2.2	"	.11	ns	ns	ns	ns	ns
Ac.S/BS	1.34 ± 0.19	1.34 ± 0.23	1.31 ± 0.15	1.55 ± 0.23	1.43 ± 0.24	1.78 ± 0.27	square root	.61	ns	ns	ns	ns	ns
Ac.F	1.96 ± 0.10	1.79 ± 0.23	2.43 ± 0.29	2.17 ± 0.34	3.06 ± 0.7	2.09 ± 0.23	log	.04	ns	ns	ns	< .05	ns
Ac.AR	0.82 ± 0.12	0.73 ± 0.06	1.17 ± 0.23	1.21 ± 0.17	1.06 ± 0.11	0.96 ± 0.13	log	.06	ns	ns	< .05	ns	ns
FP	33.6 ± 4.2	45.3 ± 11.6	27.8 ± 4.6	23.5 ± 3.1	26.3 ± 2.7	27.3 ± 7.6	log	.06	ns	ns	< .05	ns	ns
Rs.P	3.45 ± 0.73	8.83 ± 1.92	2.77 ± 0.41	5.75 ± 0.92	2.91 ± 0.76	4.28 ± 1.07	"	.09	ns	ns	ns	< .1	ns
Mlt.P	197 ± 30	114 ± 17	96 ± 22	237 ± 142	72 ± 10	123 ± 23	log	.11	ns	ns	ns	ns	ns
Mlt	11.2 ± 2.4	14.6 ± 3.2	8.2 ± 1.3	7.5 ± 1.1	7.8 ± 0.8	8.1 ± 0.9	log	.01	ns	< .05	< .05	< .05	ns
Ac.ES	18.5 ± 1.1	16.7 ± 0.4	15.9 ± 0.7	15.5 ± 0.6	15.7 ± 0.5	15.6 ± 0.4	"	.45	ns	ns	ns	ns	ns

[a] More than 25% of the groups numerically distributed and transformation was ineffective, analyzed using Kruskal-Wallis test

[b] Results of Levene's test on the variable indicate a lack of homogeneity of variances (p < .05) that was not corrected by transformation, analyzed using Kruskal-Wallis test

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Qualitative evaluation of vertebrae
Bright field examination:
- In 4 of 121 animals (2 PTH1, 2 PTH5) there was focal or diffuse osteoidosis (thickened osteoid) (osteoid = organic unmineralized bone matrix) or osteomalacia (=impaired mineralization with excess osteoid accumulation) in the vertebral section.
- In 6 of 121 animals (scattered through groups) there were small ventral osteophytes (osseous outgrowths).
- Trabecular tunneling was seen in all groups (Table F6). The incidence of score (0) and score (+) tunneling was decreased, and of score (++) and score (+++) increased in PTH1 and PTH5 relative to OVX. Score (+++) tunneling occurred only in PTH treated. Withdrawal of PTH appeared to decrease the extent of tunneling.

Polarized light examination:
- No abnormalities of lamellar structure observed in sections with or without histological abnormalities (osteoidosis, osteophyte).

Table F6 Trabecular Tunneling Summary

Trabecular Tunneling Score	Total Cases	Number of Cases in Group: Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W
-	31	11	8	1	7	1	3
+	35	3	7	3	10	0	12
++	36	5	5	10	4	7	5
+++	19	0	0	5	0	13	1

Conclusion: In a few animals, PTH caused osteoidosis/osteomalacia. PTH caused a marked but reversible increase in the incidence and severity of trabecular tunneling. There were no abnormalities in lamellar structure.

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Femoral neck
Measurements of static and dynamic parameters in femoral neck (Table F16):
Note: No periosteal double label observed in any section

Cortical bone:
Effect of OVX (relative to sham):
- Significant increase in Ps.MS/Md.S
- N.s. increase in MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV
- No effect on Md.V/TV, Md.S/Md.V

Effect of LY treatment (relative to OVX):
- Significant increase in Md.S/Md.V (BS/BV)
- N.s. increase in BFR/Md.V, BFR/TV, Ps.MS/Md.S
- No effect on Md.V/TV

Effect of LY treatment withdrawal (relative to OVX):
- Some parameters were reversed significantly below OVX levels (MS/Md.S, BFR/Md.S)
- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e., BFR/Md.V, BFR/TV, Ps.MS/Md.S)

Conclusion: Ovariectomy and PTH both caused increases in cortical and periosteal bone formation but no changes in cortical bone volume density. Bone formation parameters were decreased by PTH withdrawal to OVX or below-OVX (i.e. sham) levels.

Cancellous bone:
Effect of OVX (relative to sham):
- Nearly significant increase in Tb.Sp
- N.s. decrease in Tb.Th, Tb.N.
- N.s. increase in MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV
- No effect on Md.V/TV, Md.S/Md.V

Effect of LY treatment (relative to OVX):
- Significant increase in Md.V/TV, Tb.N., MS/Md.S, BFR/TV
- Significant decrease in Tb.Sp
- N.s. increase in Tb.Th, BFR/Md.S, BFR/Md.V
- No effect on Md.S/Md.V

Effect of LY treatment withdrawal (relative to OVX):
- Parameters were reversed to OVX or below-OVX levels, but were not significantly different from OVX (i.e., Md.V/TV, Tb.Sp, MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV)

Conclusion: Ovariectomy increased cancellous bone formation and decreased trabecular density but had no net effect on cancellous bone volume density. PTH increased both cancellous bone formation and trabecular and bone volume density. PTH withdrawal reversed the increases in cancellous bone formation and bone volume density. There were no specific data on resorption.

Table F16 Static and Dynamic Histomorphometry of the Femur Neck (n = 120, Mean ± SEM of Raw Data) and Bonferronni-Adjusted Group Comparisons (121-Monkey Population)

	Tissue	Sham n = 9	OVX n = 8	PTH1 n = 9	PTH1-W n = 8	PTH5 n = 9	PTH5-W n = 9	Trans-formation	Over-all p	Bonferroni-Adjusted p: OVX vs. Sham	OVX vs. PTH1	OVX vs. PTH1-W	OVX vs. PTH5	OVX vs. PTH5-W
MS/TV	Cortical	93.5 ± 1.4	93.5 ± 1.1	90.7 ± 0.5	92.8 ± 0.3	94.3 ± 0.3	93.2 ± 0.3		.06	ns	ns	ns	ns	ns
	Cancellous	43.8 ± 1.6	50.6 ± 1.7	42.4 ± 1.1	47.8 ± 2.2	46.7 ± 1.9	46.1 ± 2.6		.02	ns	< .05	< .05	ns	< .05
MS/MAS	Cortical	3.7 ± 0.2	3.9 ± 0.2	3.3 ± 0.7	4.1 ± 0.2	4.5 ± 0.2	4.7 ± 0.3		.20	ns	< .01	ns	ns	< .1
	Cancellous	33.5 ± 0.9	33.5 ± 0.4	33.5 ± 0.4	33.4 ± 0.7	33.2 ± 0.7	33.5 ± 0.7		.67	ns	ns	ns	ns	ns
Ob.Th	Cancellous	154 ± 7	153 ± 5	154 ± 7	155 ± 7	152 ± 5	154 ± 6	log	.51	ns	ns	ns	ns	ns
Ob.N	Cancellous	2.9 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.1 ± 0.1		.00	ns	< .001	< .01	< .01	< .01
Ob.Sp	Cancellous	281 ± 11	249 ± 14	254 ± 7	275 ± 12	252 ± 7	252 ± 7	log	.02	ns	< .05	< .05	< .05	< .05
MS/MAS	Cortical	17.3 ± 2.2	17.3 ± 1.4	21.8 ± 2.2	12.8 ± 2.0	26.2 ± 2.5	14.2 ± 2.9	square root	.00	ns	ns	< .01	ns	< .1
	Cancellous	14.5 ± 2.1	17.3 ± 1.9	22.7 ± 2.6	19.3 ± 2.4	31.8 ± 2.5	15.1 ± 2.6		.00	ns	ns	ns	< .01	ns
OV/AR	Cortical	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1		.37	ns	ns	ns	ns	ns
	Cancellous	0.7 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1		.24	ns	ns	ns	ns	ns
MR/MAS	Cortical	50.3 ± 8.9	74.1 ± 14.6	66.8 ± 7.3	41.9 ± 7.6	84.3 ± 9.9	39.2 ± 6.5	square root	.20	ns	ns	< .05	ns	< .05
	Cancellous	37.2 ± 6.7	74.8 ± 8.7	58.6 ± 8.7	45.3 ± 5.7	82.8 ± 8.3	41.1 ± 5.7		.00	ns	ns	ns	< .05	ns
OV/BV/MAS	Cortical	18.6 ± 2.4	35.2 ± 5.4	33.5 ± 4.2	18.2 ± 3.6	38.7 ± 5.1	18.1 ± 2.3	square root	.00	ns	ns	< .1	ns	ns
	Cancellous	49.4 ± 7.6	69.9 ± 8.5	77.3 ± 11.9	59.3 ± 9.5	107.8 ± 14.6	52.9 ± 9.4	square root	.01	ns	ns	ns	ns	ns
BV/TV	Cortical	16.9 ± 2.2	28.2 ± 4.6	30.6 ± 3.4	16.9 ± 3.2	36.3 ± 4.5	16.5 ± 2.8	square root	.00	ns	ns	< .1	ns	ns
	Cancellous	20.5 ± 3.2	35.1 ± 5.6	42.1 ± 5.3	26.8 ± 4.1	50.6 ± 7.7	26.1 ± 4.1	square root	.00	ns	ns	ns	< .01	ns
Ac.MAS	Cortical	0.9 ± 1.1	20.2 ± 3.6	22.7 ± 2.7	17.1 ± 2.7	24.2 ± 2.7	17.5 ± 2.6		.00	< .01	ns	ns	ns	ns

[illegible]

[a] Over-all p < .05

[b] Results of Levene's test on the variable indicate a lack of homogeneity of variances (p < .05) that was not corrected by transformation, analyzed using Kruskal-Wallis test

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Distal Radius

Table G1 Definition of Histomorphometric Variables for Structural and Label Measurements of Distal Radius*

Variable	Units	Description
MS/TV	%	Bone volume, the mineralized area as a percentage of tissue area
MS/MV	mm/mm^3	Mineralized tissue surface volume ratio
Tb.Th	μm	Trabecular thickness
Tb.N	mm^{-1}	Trabecular number
Tb.Sp	μm	Trabecular separation
MS/MAS	%	Mineralizing surface
MAR	μm/day	Mineral apposition rate
BFR/MAS	$mm^3/mm^2/day$	Bone formation rate, surface referent
BFR/MV	%/year	Bone formation rate, bone volume referent
BFR/TV	%/year	Bone formation rate, tissue volume referent
Ps.MS/MAS	%	Periosteal mineralizing surface
Ps.MAR	μm/day	Periosteal mineral apposition rate
Ps.BFR/MAS	$mm^3/mm^2/day$	Periosteal bone formation rate, surface referent

* Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595-610.

Measurements of static and dynamic parameters in distal radius (Table G3):

Cortical bone:
Effect of OVX (relative to sham):
- Significant increase in MS/Md.S, MAR, BFR/Md.S, BFR/Md.V, BFR/TV, Ps.MS/Md.S, Ps.BFR/Md.S
- No effect on Md.V/TV, Md.S/Md.V

What this means:
OVX caused an increase in intracortical and periosteal bone formation, without consequence for bone surface or volume density (i.e. increased turnover but resorption balances formation)

Effect of LY treatment (relative to OVX):
- Significant increase in Md.S/Md.V (BS/BV),
- N.s. increase in BFR/Md.V, BFR/TV, Ps.MS/Md.S, Ps.BFR/Md.S
- Small but n.s. decrease in Md.V/TV

What this means:
PTH caused increased intracortical and periosteal bone formation, with consequently increased intracortical bone surface and a small effect on bone volume density.

Effect of LY treatment withdrawal (relative to OVX):
- Some parameters were reversed to OVX levels, but were not significantly different from OVX (i.e., Md.S/Md.V, MS/Md.S, BFR/Md.V, BFR/TV, Ps.MS/Md.S, Ps.BFR/Md.S)
- No effect on Md/V/TV

What this means:
PTH withdrawal decreased cortical and periosteal bone formation to OVX or below-OVX levels. Withdrawal reversed the increase in bone surface degsity. Bone volume density remained unaffected.

Conclusion: Intracortical and periosteal bone formation were increased by ovariectomy, and further increased by PTH treatment, and this appeared to cause a small decrease on cortical bone volume density. Bone formation parameters were reversed by PTH withdrawal.

Cancellous bone:
Effect of OVX (relative to sham):
- Significant increase in MS/Md.S, MAR, BFR/Md.S, BFR/Md.V, BFR/TV
- N.s. increase in Tb.Sp
- N.s. decrease in Tb.N.
- No effect on Md.V/TV, Md.S/Md.V, Tb.Th

What this means:
OVX caused an increase in bone formation (turnover) without a significant effect on trabecular bone parameters or cancellous bone volume or surface density.

Effect of LY treatment (relative to OVX):
- Significant increase in Md.V/TV, Tb.N
- Significant decrease in Tb.Sp
- N.s. increase in MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV
- No effect on Md.S/Md.V, Tb.Th

What this means:
PTH caused an increase in cancellous bone formation with consequently increased bone volume density and improved trabecular parameters.

Effect of LY treatment withdrawal (relative to OVX):
- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e., Md.V/TV, Tb.N, Tb.Sp, MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV)

What this means:
PTH withdrawal caused a decrease in bone formation to OVX or below-OVX levels. Withdrawal partially reversed bone volume and trabecular parameters.

Conclusion: Cancellous bone formation but not bone volume was increased by OVX, whereas both bone formation and bone volume were increased by PTH treatment. Bone formation, volume density and trabecular parameters were reversed by PTH withdrawal.

Table G3 **Distal Radius Data n=119 (Mean + SEM of Raw Data) and Bonferronni-Adjusted Group Comparisons (121 Monkey Population)**

	Tests	Sham n = 27	OVX n = 20	PTH1 n = 18	PTH1-W n = 20[a]	PTH5 n = 21	PTH5-W n = 19	Trans-formation	Overall p	Sham	OVX vs. PTH1	OVX vs. PTH1-W	PTH5	PTH5-W
MS/V.Ts	Cortical	56.8 ± 2.7	46.7 ± 2.5	65.2 ± 2.3	56.6 ± 2.6	47.2 ± 1.8	56.5 ± 2.5		.13	ns	<.05	ns	ns	ns
	Cancellous	17.5 ± 1.6	16.1 ± 2.0	19.7 ± 1.2	19.5 ± 1.1	22.1 ± 1.2	19.5 ± 1.1		.06	ns	ns	ns	<.05	ns
MS/Md.S	Cortical	8.7 ± 0.7	8.1 ± 0.9	12.4 ± 0.9	8.9 ± 0.7	12.0 ± 0.9	9.8 ± 0.9	square root	.01	ns	<.05	ns	<.05	ns
	Cancellous	27.3 ± 1.1	24.5 ± 1.3	26.9 ± 1.2	25.2 ± 0.9	24.1 ± 1.2	25.3 ± 1.2	log	.29	ns	ns	ns	ns	ns
Tb.Th	Cancellous	79.8 ± 2.7	84.8 ± 4.1	79.6 ± 3.5	81.1 ± 3.1	86.1 ± 3.3	83.6 ± 4.1		.22	ns	ns	ns	ns	ns
Tb.N	Cancellous	2.5 ± 0.1	2.9 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	2.6 ± 0.1		.01	ns	<.05	<.05	<.05	ns
Tb.Sp	Cancellous	391 ± 25.7	471 ± 36	352 ± 29.5	365 ± 26	318 ± 19.4	380 ± 25		.01	ns	<.05	<.05	<.05	ns
MS/V.Ts	Cortical	8.7 ± 2.2	32.8 ± 2.9	30.2 ± 2.3	29.4 ± 3.8	32.5 ± 2.6	37.6 ± 3.3		.00	<.01	ns	ns	ns	ns
	Cancellous	11.1 ± 2.4	29.1 ± 2.3	31.1 ± 2.4	30.5 ± 4	30.5 ± 2.5	24.3 ± 3.3		.00	<.01	ns	ns	ns	ns
V/ar	Cortical	0.5 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0		.00	<.01	ns	ns	ns	ns
	Cancellous	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.6 ± 0	0.6 ± 0.0	0.6 ± 0.0		.00	<.01	ns	ns	ns	ns
BFR/BS	Cortical	16.7 ± 4.8	90.7 ± 9.7	72.2 ± 6.7	78.6 ± 10.7	80.7 ± 8.1	76.0 ± 10.3		.00	<.01	ns	ns	ns	ns
	Cancellous	22.8 ± 5.2	67.1 ± 6.3	63.9 ± 6.2	59.5 ± 8	61.2 ± 5.6	52.6 ± 7.6		.00	<.01	ns	ns	ns	ns
BFR/BV	Cortical	16.5 ± 4.2	68.8 ± 7.1	87.1 ± 9.2	69.9 ± 9.7	89.8 ± 11.1	68.2 ± 8.5		.00	<.01	ns	ns	ns	ns
	Cancellous	66 ± 12.2	198 ± 18.7	176 ± 19.6	169 ± 22	151 ± 16	123 ± 19.6		.00	<.01	ns	ns	ns	ns
BFR/TV	Cortical	8.8 ± 2.3	43.9 ± 4.7	49.4 ± 4.9	41.6 ± 5.8	49.7 ± 5.7	39.4 ± 5.1		.00	<.01	ns	ns	ns	ns
	Cancellous	9.8 ± 2.6	29.5 ± 3.2	29.3 ± 3.5	27.8 ± 4	43.9 ± 3.5	23.1 ± 3.4	square root	.00	<.0006	ns	ns	<.05	ns
MS/BS/MS	Cortical	7.9 ± 2.8	24.5 ± 2.3	29.1 ± 3.9	16.8 ± 2.5	29.6 ± 2.7	20.5 ± 3.0		.01	<.05	ns	ns	ns	ns
Ob.S/BS	Cortical	0.5 ± 0.1	6.6 ± 0.9	9.0 ± 0.6	8.5 ± 0.9	8.7 ± 0.7	9.0 ± 0.1		.00	ns	ns	ns	ns	ns
Ob.S/BS/MS	Cortical	16.3 ± 5.7	32.0 ± 4.7	30.6 ± 5.2	50.2 ± 4.9	30.8 ± 4.6	43.9 ± 6.6		.01	<.05	ns	ns	ns	ns

ns = p > .1

[a] Excepted: Ps MAR (n=15)

[b] Excepted: Ps MAR (n=15)

[c] Data normal w/ equal variances, analyzed using Kruskal-Wallis test

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Three-Dimensional Modeling of the Effects of Recombinant PTH(1-34) on the Bone Distribution of Lumbar Vertebra from Cynomolgus Monkeys
(Non-Clinical Pharmacology Report BN5-08)

A sophisticated 3D histomorphometry study was carried out of lumbar vertebrae, using high resolution QCT and computerized modeling techniques to estimate voxel (bone element) BMD distribution and effective vertebral strain.

Methods
Test animals: 6 groups (sham control, OVX, PTH5, PTH5W), N=7/group.
QCT: BMC, X-area, BV/TV, TbTh, TbN, TbSp, connectivity
Scanning: Voxels, modeled into tetrahedral finite elements (490x490x500 um)
Calculation: BMD and Young's modulus for each bone element, determining density distribution
Analysis: Linear elastic stress analysis (compression), determining vertebral stress and strain distribution

Results
QCT: Bone mass

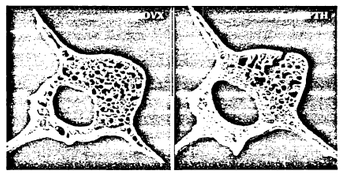

Figure 1 **Representative scans of monkey L-5 vertebra. Excised L-5 were scanned serially by quantitative computed tomography (QCT), using 70 x 70 x 500 µm voxels. Middle slices of OVX and PTH5 are shown as examples.**

- L5 cross section (500 um middle slice, Fig.1): Small n.s. decrease in BMD by OVX, 21% increase relative to OVX by PTH5
- L5 midsagittal section (centrum): Small n.s. decrease in BMD by OVX, 30% increase in BMD by PTH5 relative to OVX, 7% decrease in BMD by PTH5W relative to PTH5
- Bone area and volume not affected (not shown)

Histomorphometry (by QCT) (middle slice in cross-section)
- Significant decrease of BV/TV and TbN, increase in TbSp, and decrease in connectivity by OVX
- Significant increase in structural parameters BV/TV, Tb.Th, Tb/N, decrease in Tb.Sp, and increase in connectivity parameters (node density) by PTH5
- No effect on any parameter of PTH5W relative to PTH5

Neural canal/histology

- Area of neural canal cross sectional area not affected by OVX or PTH.
- Bone response of PTH mainly located along endocortical and trabecular but not periosteal surface

Density distribution of bone elements (voxels) (Fig.4)

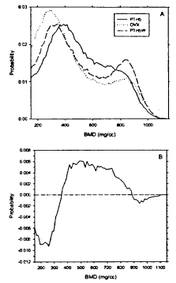

Figure 4 **Histogram analysis of BMD for whole L-5 vertebra. BMD distributions were pooled and are shown for PTH5, OVX, and PTH5W vertebra (A). A bimodal distribution of voxels (490 x 490 x 500 µm) was observed. A difference histogram was generated to highlight differences between PTH5 and OVX (B). These histograms show that PTH5 reduced the amount of low density bone by increasing the amount of medium density bone, compared to OVX. Withdrawal of PTH for 6 months (PTH5W) induced a redistribution of medium density bone into lower and higher density bone, with the result that PTH5W had less low density and more high density bone than OVX.**

- No effect of OVX
- Decrease in proportion of low density voxels and increase in medium density voxels by PTH5 (shift of low density bone to medium density bone). Thus, PTH appeared to convert low density bone into medium-density bone.
- Decrease in medium density voxels and increase in both low- and high density voxels by PTH5W (relative to PTH5). Result was that there were more high density elements in PTH5W than in PTH5 and OVX groups.
- No hypermineralization (BMD range of PTH5 similar to sham or OVX)

Strain distribution (centrum,mid-sagittal) (Fig. 7)

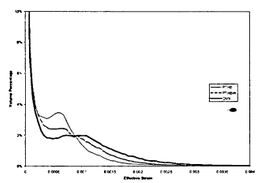

Figure 7 **Analysis of the effective strain distribution for PTH5, PTH5W, and OVX Vertebra (Centrum) loaded in compression. The strain histograms show that fewer bone elements in PTH5 vertebra experience strain greater than 0.001 compared to OVX. The average strain for PTH5 was 36% less than OVX. The strain histogram for PTH5W was intermediate between PTH5 and OVX with average strain 23% below OVX.**

- Reduction of average strain by PTH5. Reduction bone elements under high strain (>0.0008) by PTH5 relative to OVX, and increase of bone elements under moderate strain (0.0002-0.0008) by PTH5 relative to OVX. This indicates a greater resistance to failure for vertebrae loaded in compression.
- Distribution in PTH5W group intermediate between OVX and PTH5

Note:
The previous compression analysis results of LV3-LV4 (Study X-95-11) are in accordance with the strain distribution results obtained in this study. Shift to high (and low) density bone elements in PTH5W group maybe due to relatively large unstability of mid-density elements (e.g. some of these elements maybe porous cortical bone since PTH increases porosity, and these may fill up after PTH withdrawal).

Conclusions
PTH treatment caused micro-structural bone changes in vertebra which were accompanied by increased strength (decreased likelihood to fail) in both PTH5 and in PTH5W groups relative to OVX.

Serum Pharmacokinetics of LY333334 in Adult Ovariectomized Female Cynomolgus Monkeys Following a Daily Subcutaneous Dose of 1 or 5 ug/kg LY333334 for 17 Months
(ADME Report 15)

Methods
Serum pharmacokinetics of immunoreactive LY333334 in adult ovariectomized (OVX) female cynomolgus monkeys following daily subcutaneous dosing of 1 or 5 µg/kg for 1,7,11, or 17 months was reported. Serum concentrations of immunoreactive LY333334 were determined by a validated immunoradiometric assay (IRMA) in samples collected at 0, 0.17, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, and 4 hours after dosing, in the PTH1 and PTH5 groups.

Results
The serum concentrations of immunoreactive LY333334 in nearly all monkeys prior to dosing were determined to be BQL. Most of the serum levels were also BQL 3 hours postdose for 1 µg/kg or 4 hours postdose for 5 µg/kg.

For the 1 ug/kg/d group, with regard to proportion of BQL values a satisfactory time interval was established to be from 0 to 1 hour for the AUC0-t calculation. For the 5 ug/kg/d group, the time interval from 0 to 2 hours was established for the AUC0-t calculation.

Table 11 **Estimated Mean AUC_{0t} for Each Dose Group and Month**

Dose (µg/kg)		1 month AUC_{0t}[1] (ng·mL*hr)	7 month AUC_{0t} (ng·mL*hr)	11 month AUC_{0t} (ng·mL*hr)	17 month AUC_{0t} (ng·mL*hr)
1	Mean	0.25	0.46	0.29	0.24
	SE[2]	0.06	0.22	0.03	0.02
	N[3]	15	14	13	13
5	Mean	2.03	2.53	2.45	1.65
	SE	0.31	0.28	0.15	0.18
	N	13	16	14	16

[1] AUC_{0t}= AUC^{t} from 0 to t where t=1 hour for the 1 µg/kg group and t=2 hours for the 5 µg/kg group.
[2] SE = standard error.
[3] N = total number of animals used in the sparse sampling scheme.

Note: AUC was increased more than proportionally with dose in the range of 1 ug/kg/d to 5 ug/kg/d. This was also seen in a 2-year rat carcinogenicity study, in the dose range of 5-30 ug/kg/day, which is associated with an AUC range (0.5-7.5 g/mL*hr) similar to the one observed in this monkey study.

The data indicated:
- There was no accumulation of LY333334 after 17 months of once daily subcutaneous dosing.
- There was no time dependent trends in mean AUC for either the 1 or 5 µg/kg dose group, suggesting unchanged exposure upon repeated dosing for 17 months.

Human exposure multipes

Human exposure multiples in 18-month monkey study with doses of 1 and 5 ug/kg/day

Dose (ug/kg/day)	Monkey AUC of PTH(1-34) (ngxhr/ml) (Month 11)	Monkey AUC of PTH(1-34) (ngxhr/ml) (Month 17)	AUC multiples (monkey:human) (Month 11)*	AUC multiples (monkey:human) (Month 17)*
1	0.29	0.24	0.98x	0.81x
5	2.45	1.85	8.3x	6.3x

*Human AUC=0.295 ngxhr/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; Clinical trial GHAC)

Conclusion
Exposure in the PTH1 group was similar to expected human exposure, while exposure in the PTH5 group was approximately 7 times the expected human exposure.

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Histopathologic Evaluation of Kidneys From Ovariectomized Female Cynomolgus Monkeys Given LY333334 Daily by Subcutaneous Injection for up to 18 Months (Study P03196)
(Toxicology Report 10)

Ovariectomized cynomolgus monkeys were given LY333334 doses of 1 or 5 µg/kg for 18 months or for 12 months with a 6-month withdrawal period. At necropsy, kidneys were collected and submitted to Lilly Research Laboratories for histologic processing and evaluation.
There were no gross findings in control or treated animals. A variety of renal histopathologic lesions were present in control and treated groups. The most frequent finding was subacute multifocal inflammation which occurred in almost all animals.

Conclusion
LY333334 had no gross or histopathologic effects in the kidneys of OVX monkeys treated for 12 or 18 months. The no-observed-effect level (NOEL) for renal histologic changes was ≥5 µg/kg/day.

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SUMMARY AND EVALUATION OF MONKEY BONE QUALITY STUDY

In this long term bone quality study, mature ovariectomized cynomolgus monkeys were treated with LY333334 (1 or 5 ug/kg/day) for 18 months, or for 12 months with a subsequent 6-month period in which treatment was withdrawn. Results were compared with those from sham-operated untreated animals.
The PK of LY333334 in the OVX animals did not appear to change over time. There was no drug accumulation over the course of the treatment period, and the AUC values for the 1 and 5 ug/kg/day groups were approximately 0.3 and 2.2 ngxh/ml, respectively. This represents human exposure multiples of approximately 1x and 7x the expected exposure at the intended clinical dose of 20 ug/day.
After 18 months, effects of LY333334 on serum biochemistry included decreases in serum phosphorus and endogenous PTH, increases in serum calcitriol and urinary cAMP/creatinine ratio. There were no effects on serum Ca (total or ionized), serum creatinine or serum BUN. Assessment of bone turnover markers showed small, although not statistically significant increases in serum alkaline phosphatase, osteocalcin, and urinary C-terminal collagen fragments (Crosslaps).
PTH at 1 or 5 ug/kg/day had no significant histopathology effects in the ovariectomized monkey.

Bone mass
Ovariectomy resulted in a relative osteopenia, as the intact animals gained bone mass, while the ovariectomized ones remained stable or gained less bone mass than the sham animals.

In the spine, LY333334 caused a gradual dose-dependent increase in projected BMD (mg/cm^2). At 18 months, the BMD in both PTH treatment groups (PTH1 and PTH5) was larger than the BMD of the sham animals. PTH also increased the projected area (cm^2) of the spine in the high dose group.
In the whole body, PTH caused a dose-dependent increase in projected BMD with no significant effects on projected area.

In the midshaft of the radius there was a decrease in volumetric BMD (mg/cm^3)in both PTH groups, and a dose-dependent increase in cross-sectional area (X-area, mm^2).

In the proximal tibia, there were marked dose-dependent increases in BMD (mg/cm3) of the innermost cancellous zone and the two intermediate zones. In the outher, cortical zone BMD was not significantly affected by PTH. There was no effect on X-area.

In the distal radius, a small decrease in BMD was observed in the outer cortical zone and a dose-dependent increase was seen in the innermost cancellous zone. There were no clear effects on the BMD of the intermediate zones. There was no effect on X-area.

Generally, the effect of LY333334 withdrawal on bone mass consisted of either no or a small reversal after withdrawal of the low dose (PTH1W), and a significant reversal after withdrawal of the high dose (PTH5W).

Bone strength
In the lumbar vertebrae (L3 and L4 average data) LY333334 caused a dose-dependent increase in yield strength, yield stress, and Young's modulus, so that the values for these parameters in the PTH5 group were larger than in the sham group. Spinal BMD (g/cm3) was significantly related to vertebral yield strength (r=0.83), and the correlation appeared to be similar for all treatment groups including sham and OVX.

Data from a high resolution QCT study showed that PTH treatment caused micro-structural bone changes in vertebra which were accompanied by increased strength (decreased likelihood to fail) in both PTH5 and in PTH5W groups relative to OVX.

In the humerus midshaft, ultimate force, stiffness and work to failure were slightly but not significantly decreased by OVX and increased dose-dependently although not significantly by LY333334. The

humeral midshaft cortical thickness was increased by LY333334, significantly so in the PTH5 group. The effect on ultimate force (F_u) is likely to be related to the effect on thickness, which is presumed to offset an increase in cortical porosity by PTH (see histomorphometry results below).

In the femoral neck ultimate force (Fu) was significantly decreased in the OVX group, and significantly and dose-dependently increased in both PTH groups.

In femoral midshaft beam specimens whose properties are not dependent on bone geometry, ultimate stress appeared to be decreased in the PTH5 group. Young's modulus was slightly decreased in the OVX group, and further decreased by LY333334, so that this parameter was significantly lower in PTH5 groups than in the sham group. The data indicated a drug-related decrease in intrinsic stiffness and a concomitant decrease in ultimate stress of the cortical bone material. As in the humerus, this may have been due to an increase in cortical porosity (see histomorphometry data below).

Generally, the effects of LY333334 withdrawal on bone strength were inconsistent. In some instances, there was a reversal of the effects of PTH, and in other cases there was no difference between treatment and withdrawal groups. This appeared to depend on the bone site evaluated and the dose of PTH used.

Histomorphometry
In the iliac crest, LY333334 increased bone formation and either decreased or increased bone resorption depending on the duration of treatment. Regardless of the effect on resorption, treatment with PTH resulted in a significant increase in cancellous trabecular bone. LY333334 also appeared to induce a small increase in wall width (bone balance). LY333334 caused an increase in tunneling and a transient increase in cortical thickness with levels actually decreased below OVX after 15 months. The increase in tunneling was also observed in vertebral trabeculae. Cortical porosity in the iliac crest was not affected.

In the humerus midshaft, LY333334 increased endocortical but not periosteal bone formation, which resulted in an increase in cortical area. LY333334 at both doses increased cortical porosity, apparently due to increased intracortical resorption.
The histomorphometry and biomechanical strength data for the humerus midshaft suggest that increased cortical haversian remodeling and increased cortical porosity induced by PTH do not necessarily have deleterious effects on bone strength. This is due in part to the porosity being mainly located in the endocortical zone where it exerts relatively small effects on bone strength, and the fact that the increased porosity can be accompanied by a PTH-induced increase in cortical thicknesss due to a stimulation of cortical bone apposition.
However, an increase in porosity (decrease in BMD) may not be offset by an increase in thickness at all cortical bone sites. Thus, the long term cortical bone effects and safety remain a concern for PTH treatment.

Histomorphometry was also carried out of the mid-radius, the mid-femur, the vertebrae, the femoral neck and the distal radius. Although at all bone sites PTH increased bone formation, the effect of this increase on the structure of the bone varied with the different bone sites and type of bone (cortical or cancellous). Generally, the increased bone turnover/formation induced by PTH at cortical bone sites caused increased porosity and either a decrease (mid-radius, distal radius cortex), increase (vertebral cortex), or no effect (femoral shaft, femoral neck cortex) on fractional bone volume (BV/TV).
The anatomical site of the increased cortical bone formation (periosteal, endocortical, intracortical) and the effect of PTH on cortical bone area or thickness depended on the bone site under evaluation. Bone area or thickness was increased in humerus and possibly midradius and vertebrae, while it was unchanged in midfemur and distal radius. The latter data are from either densitometric or histomorphometric assessments.

By contrast, the increased bone formation induced by PTH in cancellous bone always resulted in an increase in cancellous bone volume and improved trabecular architecture (more, thicker, and less separated trabeculae).

The effect of PTH withdrawal always consisted of a decrease in bone formation rate and related parameters, sometimes to levels below those in the OVX group (particularly after PTH5 withdrawal). Moreover, if effects on fractional bone volume (BV/TV) had been observed with PTH treatment (e.g. in vertebrae, cancellous bone of femoral neck and distal radius), withdrawal would usually reverse these effects, although this did not happen in the midradius (and perhaps the cortical bone of the distal radius) where PTH caused a decrease in this parameter and withdrawal did not reverse that effect.

The predictive value of the preclinical monkey study data was generally supported by the BMD and fracture data obtained in the pivotal clinical trial B3D-MC-GHAC (17-23 month study in postmenopausal women). In trial GHAC, after ca. 21 months of treatment with 20 ug/day, BMD was markedly increased at the lumbar spine (9% as compared to placebo), and the risk of one or more new vertebral fractures was significantly decreased (by 65%). The BMD was also increased albeit less pronounced at sites with a relative larger contribution of cortical bone (hip, femoral neck, intertrochanter area and Ward's triangle). However, in the distal radius BMD was decreased as compared to placebo (average -1% at 20 ug, -2% at 40 ug). The decrease was larger in the radial midshaft (mainly cortical bone), and was statistically significant at this site at the 40 ug/day dose. Further pQCT evaluation of the radius (proximal area) indicated that periosteal and endosteal circumferences and total bone area were increased while BMC was unchanged, which would reduce BMD but increase computed measures of bone strength. The latter appears to be analogous to the effects of LY333334 in the monkey humerus and radius.

The potential implication of the preclinical data that cortical bone strength might be impaired so far has not been borne out by clinical fracture data. The incidence of fragility fractures at nonvertebral sites (hip, wrist, ankle, humerus, rib, foot, pelvis) was either unaffected (humerus, foot) or decreased (other sites) in trial GHAC. However, the number of fractures was too small to demonstrate a statistically significant fracture risk reduction at individual nonvertebral sites. Transiently increased cortical porosity in the human iliac crest confirms that this phenomenon also takes place in humans.

The decrease in clinical non-vertebral fractures may be due to the positive effect of LY333334 on cancellous bone which is present at most non-vertebral sites (e.g. femoral neck/hip), combined with a generally positive effect on cortical bone thickness or area and a relatively small adverse effect of an increase in porosity. Thus, although the small numbers of non-vertebral fractures preclude a solid conclusion, the clinical data suggest that there is no major safety problem in cortical bone.

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RABBIT BONE QUALITY STUDIES
Two studies were conducted in intact rabbits, to evaluate the effects of LY333334 on cortical bone properties. Unlike the rat, the rabbit is a species in which there is osteonal (Haversian, cortical) bone remodeling, as in monkey and human. The remodeling period in the rabbit is ca. 70 days, compared to ca. 90 days in primates.

Report	Title	In Vivo: Species, Strain, Gender, & Age	In Vivo: Dose Route	In Vitro: Assay Type	In Vitro: Tissue/ Cell Line	Results
CG3-06	Histomorphometric and Biomechanical Analyses of the Anabolic Effects of LY333334 on the Cortical Bone of Ovary-Intact Adult Rabbits	Rabbit, New Zealand, ovary-intact female, mature	10 & 40 µg/kg day SC once daily for 140 days			LY333334 increased biomechanical measures of strength in rabbit cortical bone by restructuring bone architecture via appositional bone formation, favorable geometric changes, & reformation of pre-existing matrix with new matrix due to increased advention frequency in long bones. The high dose was associated with a transient increase in serum calcium which remained within physiologic range.
CG3-13	Effects of Human PTH (1-34), LY333334, on Bone Mass, Remodeling, and Mechanical Properties of Cortical Bone During the First Remodeling Cycle in Rabbits	Rabbit, New Zealand, ovary-intact female, mature	10 µg/kg day SC once daily for 35 (P35), 70 (P70), or 140 (P140) days			Cortical bone histomorphometry of rib/rad mid-shaft showed intracortical remodeling was significantly increased by PTH without significant increase of cortical porosity in first remodeling cycle. This was associated with significantly greater cortical area & bone strength in P35 & P70 groups due to stimulation of periosteal bone formation rate. By 140 days, surface remodeling effects declined so there was no difference between control & treated groups. PTH increased cancellous bone volume & biomechanical properties in 2nd lumbar vertebra. Although endocortical remodeling increases within first remodeling period of 70 days, the greater cortical area due to periosteal periosteal expansion increases cortical bone strength.

Histomorphometric and Biomechanical Analyses of the Anabolic Effects of LY333334 on the Cortical Bone of Ovary-Intact Adult Rabbits
(Nonclinical Pharmacology Report CG3-06)
Note: The results of this study were published by Hirano et al. (J.Bone Min.Res., Vol.14,#4, p536) (1999)

METHODS
Female, ovary-intact New Zealand white rabbits ( ) (n=6/group), approximately 9 months old, weighing 3.25 to 3.75 kg, were treated with LY333334 (10 or 40 ug/kg/day, PTH10 or PTH40) or vehicle by subcutaneous injection, once daily, on 5 days per week, for 140 days (approximately 2 remodeling cycles). Bone mass data (pQCT or pDEXA) were collected on proximal femur, femur mid-shaft, and lumbar vertebra, LV4. Biomechanical assays of strength were done on femur mid-shaft and lumbar vertebra, LV5. Intrinsic material properties were assessed by acoustic microscopy on the humerus mid-shaft. Histomorphometry was done on tibia mid-shaft and lumbar vertebra, LV3.

RESULTS
Body weight gain was decreased in treated groups, and there was a slight loss of body weight at PTH40. At 4h after the last injection, a small increase of 1.4 mg/dL in serum calcium was observed in the PTH40 group. Serum alkaline phosphatase increased by 2-fold in the PTH40 group. Serum phosphate did not vary significantly between groups.

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In the cortical mid-shaft of the femur, bone mineral density (BMD), bone mineral content (BMC), and cross-sectional bone area (X-area) were increased by PTH. In the proximal femur, BMD and BMC, but not X-area, were increased dose-dependently. In LV4 there were no effects of PTH on BMD, BMC or X-area (Table 3).

Table 3 **Bone Mass and Geometry of Lumbar Vertebra, LV4, Proximal Femur and Femur Mid-Shaft in Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10 µg/kg (PTH10) or 40 µg/kg (PTH40) Once Daily, for 5 Months[a]**

	Control	PTH10	PTH40
Vertebra LV4			
Bone mineral density vBMD, mg/cm^3	482 ± 34	465 ± 20	463 ± 26
Bone mineral content BMC, mg	53 ± 2	54 ± 3	54 ± 3
Cross-sectional area XSA, mm^2	98 ± 10	97 ± 3	97 ± 2
Voxels	1130 ± 116	1114 ± 32	1118 ± 26
Proximal Femur			
Bone mineral density aBMD, g/cm^2	0.251 ± 0.002	0.285 ± 0.009[b]	0.305 ± 0.010[b]
Bone mineral content BMC, g	0.99 ± 0.02	1.12 ± 0.04[b]	1.21 ± 0.04[b]
Cross-sectional area XSA, mm^2	3.94 ± 0.09	3.92 ± 0.05	3.99 ± 0.12
Mid femur			
Bone mineral density vBMD, mg/cm^3	929 ± 21	989 ± 20	1051 ± 19[b]
Bone mineral content BMC, mg	53 ± 2	60 ± 2[b,c]	70 ± 2[b]
Cross-sectional area XSA, mm^2	48 ± 2	51 ± 2	55 ± 1[b]
Voxels	2210 ± 100	2353 ± 87	2532 ± 55[b]

a Data are expressed as mean ± SEM for 6 rabbits per group, except for total bone mineral content for LV5 where n=5
b p≤.05 compared with control.
c p≤.05 compared with PTH 1-34) 40 µg/kg/day.

In the femoral midshaft, bone strength measured as ultimate force, stiffness, work to failure and apparent cross-sectional moment of inertia (CSMI) was increased by PTH and all parameters were significantly increased in the PTH40 group. Young's modulus was significantly lower in the PTH40 than in the PTH10 group but was not different from controls (Fig. 1).

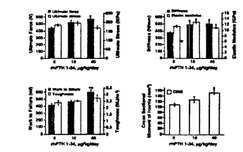

Figure 1 **Biomechanical data obtained from in vitro strength testing by 3-point bending of the femur diaphyseal mid-shaft of ovary-intact rabbits, given vehicle (0) or PTH once daily for 5 months. Statistical significance, p<.05, * vs control (0); # PTH at 10 µg/kg vs 40 µg/kg.**

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In the lumbar vertebrae, there were no significant effects of PTH on biomechanical properties (Table 4).

Table 4 **Biomechanical Measures of Strength in Lumbar Vertebra, LV5, of Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10 µg/kg (PTH10) or 40 µg/kg (PTH40) Once Daily for 5 Months***

	Control	PTH10	PTH40
Vertebra LV5			
Ultimate force (Fu, N)	1539 ± 112	1653 ± 72	1552 ± 92
Stiffness (S, slope, N/mm)	5684 ± 409	5705 ± 433	5670 ± 384
Cortical thickness (t, µ)	10.0 ± 0.5	9.8 ± 0.3	10.4 ± 0.4
Cross-sectional area (ASA, mm²)	67 ± 3	65 ± 3	68 ± 3
Ultimate stress (σ_u, Mpa)	23 ± 1	26 ± 2	23 ± 1
Young's modulus (E)	861 ± 90	856 ± 55	872 ± 65
Toughness (u, MJ/m³)	0.46 ± 0.05	0.58 ± 0.07	0.45 ± 0.03

* Data are expressed as mean ± SEM for 6 rabbits per group except for total bone mineral content for LV5 where n=5. No statistically significant differnces were detected between groups

PTH did not alter intrinsic material properties of cortical bone (humerus intrinsic stiffness).

Histomorphometry was carried out in tibial midhaft and lumbar vertebrae. In the cortical bone of the tibial mid-shaft LY caused a marked dose-dependent increase in intracortical activation frequency (AcF) (Table 6). LY also caused a dose-dependent increase in cortical porosity (Fig. 2). The increased porosity in both PTH10 and PTH40 groups was predominantly in the endocortical (Zone 1) region (Fig. 3). The increased porosity probably reflects the "birth" of multiple new sites of bone turnover.

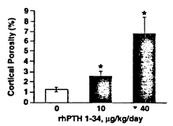

Figure 2 Cortical porosity in histologic cross-sections of the tibial mid-shaft of ovary-intact female rabbits given vehicle (0) or PTH once daily for 5 months. Statistical significance, p<.05, * vs control.

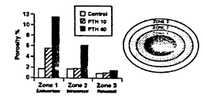

Figure 3 Spatial localization of cortical porosities into one of three concentric zones, arbitrarily superimposed on histologic cross-sections of the tibial mid-shaft of rabbits given vehicle or PTH once daily for 5 months. Note that porosities preferentially cluster within the endocortical area (Zone 1) of cortical bone. Because of this localization, minimal effect may be expected on cross-sectional moment of inertia (CSMI). As shown in Figure 1, CSMI is measure of strength, increased due to increased surface bone formation on endosteal and periosteal surfaces.

In the tibial midshaft, LY increased bone formation on periosteal (PsMS/BS, PsBFR/BS) and endocortical (EcMS/BS, EcBFR/BS) surfaces, in either low and high dose or only high dose groups (Table 5). Intracortically, the number of resorption sites (RsN/Ct.Ar) was increased in the PTH40 group, and bone formation rate (BFR/BV) was increased at both doses (Table 6). Bone area (B.Ar=total area within periosteal surface including marrow area) was increased in the PTH40 group, and marrow area (Ma.Ar) was slightly but not significantly decreased by PTH. Cortical area (Ct.Ar=area of bone within periosteal surface including porosities) and %cortical area (%Ct.Ar=Ct.Ar/B.Ar) were significantly increased in both groups.

Table 5 **Periosteal and Endocortical Bone Remodeling of Tibial Mid-Shaft in Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10 µg/kg (PTH10) or 40 µg/kg (PTH40) Once Daily for 5 Months***

Parameters	Abbreviations	Control	PTH10**	PTH40**
Endocortical mineral surface	EcMS/BS (%)	8.8 ± 1.0	13.7 ± 1.5	20.2 ± 5.8
Endocortical mineral thickness	EcOTh (µm)	7.4 ± 2.4	3.7 ± 2.3	8.1 ± 5.9
Periosteal mineral apposition rate	PsMAR (µm/day)	0.33 ± 0.17	0.38 ± 0.08	0.46 ± 0.14
Endocortical mineral apposition rate	EcMAR (µm/day)	1.33 ± 0.22	0.79 ± 0.16*	1.32 ± 0.15
Periosteal mineralizing surface	PsMS/BS (%)	3.8 ± 1.9	8.2 ± 2.1	22.3 ± 2.7**
Endocortical mineralizing surface	EcMS/BS (%)	29.4 ± 6.6	32.6 ± 8.2	55.7 ± 10.4*
Periosteal bone formation rate	PsBFR/BS (µm³/µm²/day)	0.02 ± 0.02	0.03 ± 0.01	0.16 ± 0.04**
Endocortical bone formation rate	EcBFR/BS (µm³/µm²/day)	0.04 ± 0.10	0.31 ± 0.10	0.72 ± 0.12**

* Data are expressed as mean ± SEM for 6 rabbits per group.
* p<.05 compared with control.
** p<.01 compared with PTH (1-34) 10 µg/kg/day

Table 6 **Intracortical Bone Remodeling of Tibial Mid-Shaft in Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10 µg/kg (PTH10) or 40 µg/kg (PTH40) Once Daily for 5 Months***

Parameters	Abbreviations	Control	PTH10	PTH40
Resorption cavity number	RsN/Ct.Ar (#/mm²)	0.014 ± 0.013	0.015 ± 0.004	0.097 ± 0.036**
Labeled osteon number	LOsN/Ct.Ar (#/mm²)	0.011 ± 0.006	0.027 ± 0.006	0.215 ± 0.094**
Osteoid thickness	OTh (µm)	4.92 ± 0.5**	5.42 ± 0.3**	5.16 ± 0.2**
Mineral apposition rate	MAR (µm/day)	1.19 ± 0.20	1.36 ± 0.13*	1.60 ± 0.12*
Bone formation rate	BFR/BV (%/yr)	0.5 ± 0.3	8.3 ± 2.7**	21.4 ± 3.0**
Activation frequency	AcF (#/mm²/yr)	1.8 ± 1.0	15.1 ± 5.0*	43.8 ± 10.5**
Bone area	B.Ar (mm²)	29.3 ± 1.3	33.3 ± 1.9*	37.8 ± 2.7*
Marrow area	Ma.Ar (mm²)	12.7 ± 0.7	11.9 ± 1.0	10.7 ± 1.0
Cortical area	Ct.Ar (mm²)	16.4 ± 0.9	21.3 ± 1.2*	27.1 ± 2.0**
% Cortical area	%Ct.Ar (%)	56.4 ± 1.5	64.2 ± 1.0*	71.6 ± 1.5**

* Data are expressed as mean ± SEM for 6 rabbits per group.
* p<.05 compared with control.
** p<.01 compared with PTH (1-34) 10 µg/kg/day.

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In the centrum of LV3, most formation parameters [osteoid surface (OS/BS), osteoblast surface (ObS/BS), osteoid volume (OV/TV), mineralizing surface (MS/BS), and bone formation rate (BFR/BS)] increased with PTH treatment with significant effects in the PTH40 group. Moreover, eroded surface (ES/BS) and osteoclast surface)OcS/BS) were increased in both groups. There was no effect on osteoid thickness (OTh). Fractional bone volume (BV/TV) and trabecular thickness (Tb.Th) were not changed upon 5 months of PTH treatment. Tunneling resorption and peritrabecular fibrosis were not observed in any of the groups.

SUMMARY

In the femoral midshaft, LY333334 (10 or 40 ukd) caused an increase in X-area and BMD, and an increase in strength with an increase in cross-sectional moment of inertia (CSMI).
In the tibial midshaft, LY increased intracortical bone turnover and cortical porosity, particularly in the endocortical zone, and increased cortical bone area. In the tibial midshaft LY increased periosteal and endocortical bone formation.
In the vertebrae, LY increased cancellous bone turnover, but LY had no effect on whole tissue BMD, X-area, biomechanical properties including compressive strength, or vertebral cortical bone thickness. LY caused a slight increase in serum calcium and an increase in serum alkaline phosphatase at the high dose.

Comments on cortical porosity, moment of inertia, and bone strength
An increase in CSMI can occur despite increased intracortical porosity if the increased porosity is compensated for by a sufficient increase in bone thickness (the latter of which was observed in the femur). Also, if the increased porosity is located relatively close to the bone's bending axis (as suggested by tibial data), it will have relatively little effect on CSMI. In fact, in a separate study the actual CSMI in the femur was re-calculated on the basis of the porosity distribution measured in the tibia, and it was shown that this actual CSMI was only slightly affected by the increase in porosity (Fig.5, from:*"Changes in Geometry and Cortical Porosity in Adult, Ovary-Intact Rabbits after 5 Months Treatment with LY333334 (hPTH 1-34), by Hirano et al. (2000), Calcif. Tissue Int. 66, pp456-460).*

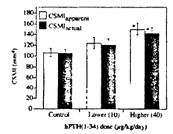

Fig. 5. Data are expressed as mean ± SEM. *P < 0.05 versus control.. Both $CSMI_{proximal}$ and $CSMI_{total}$ in the higher dose PTH group were significantly greater than in controls. $CSMI_{total}$ was smaller than $CSMI_{proximal}$ in the same group by 1.3% in the control group, 2.3% in the lower dose PTH group, and 4.8% in the higher dose PTH group due to porosity.

However, the main reason for the increase in midfemur strength upon treatment with PTH was the increased X-area. Since the femur was the only site tested for strength, it is not clear whether increased strength occurred only in the femur or would also be seen in the tibia or other cortical bone sites. In other words, it cannot be excluded that increased porosity with a resulting decrease in cortical BMD could lead to a decrease in (CSMI and) bone strength at some sites upon treatment with PTH. This potential adverse effect would occur if sufficient compensation by enhanced periosteal bone apposition and increased bone thickness is not achieved.

CONCLUSIONS
Treatment of intact rabbits with 10 or 40 ukd LY333334 for 140 days caused an increase in femoral midshaft cortical bone strength and thickness, and an increase in tibial midshaft cortical bone turnover, surface apposition, thickness and (mainly endocortical) porosity. The results of the study suggest that the effect of PTH on cortical bone strength at any site will be determined by the balance between the effect on cortical surface bone apposition and thickness and the effect on bone turnover and porosity.

Effects of Human PTH (1-34), LY333334, on Bone Mass, Remodeling, and Mechanical Properties of Cortical Bone During the First Remodeling Cycle in Rabbits
(Nonclinical Pharmacology Report CG3-13)

Based on the results of the first rabbit study, a second study was carried out in rabbits to examine the effects of PTH on the remodeling dynamics and mechanical properties of cortical bone during the first remodeling cycle (70 days) after the initiation of treatment.
The first study had shown that LY increases cortical bone mass and mechanical strength after 140 days of treatment. However, cortical porosity also increased. If cortical porosity increases prior to the change in geometry, there may be a transient decrease in cortical bone strength that could make the bone more susceptible to fracture in the early phase of treatment.
Note: The manuscript (Mashiba et al.) describing this study was submitted to J Clin Endocrinology Metab (2000)

METHODS
Intact, 9-month-old female New Zealand white rabbits were randomized into groups of 10 animals each. A baseline control group was sacrificed at the start of the experiment. The PTH-treated groups were given rhPTH(1-34) at 10 µg/kg daily subcutaneously for 35 (P35), or 70 (P70) days. Age-matched control groups (V35, V70) were injected with vehicle.

RESULTS
There was no effect of LY (10 ug/kg/day) on serum Ca, P, or alkaline phosphataser.
There were no sustained effects on X-area or BMD in whole femur, femoral midshaft or whole tibia. On D35 tibial BMD, and on D70 femoral midshaft BMD were slightly increased. Vertebral BMD was not measured.
In tibial midshaft there was increased cortical remodeling, as indicated by increased resorption cavity number, osteoid area, labeled osteon number, Ac.F, and BFR as compared to age-matched vehicle controls at both 35 and 70 days. Tibial midshaft strength (Fu, stiffness) was increased at 35 and 70 days. Work to failure (AUC) was (non-significantly) increased on D35 but not affected on D70. Cortical area was increased at D35 and D70. Porosity was slightly but not significantly increased at both days. Medullary area was unaffected. Tibial periosteal bone formation was increased at D35 and D70, endocortical bone formation at D35 only. Results from the previous study (CG3-06) had shown that by 140 days the surface effects were no longer significant at the 10 ug/kg/day dose.
These results indicate increased endocortical, periosteal and intracortical bone formation without a significant porosity increase in the first remodeling cycle, with a net result of increased bone thickness and bone strength.
In the lumbar vertebra, LY did not have a sustained effect on cancellous bone volume or bone turnover. However, it did increase Fu, stiffness, and work to failure (AUC) on both D35 and D70.
In the femur midshaft, LY increased Fu, stiffness and AUC (extrinsic properties) on D35 and D70, and increased ultimate stress, modulus and toughness (intrinsic properties) on D70.

CONCLUSIONS
Upon start of treatment of intact rabbits with 10 µg/kg LY333334, cortical bone turnover and bone apposition in the tibia were increased within the first remodeling period (70 days). However, this did not lead to significantly increased porosity or an adverse effect on cortical bone strength. In fact, treatment caused an increased cortical bone thickness and increased cortical bone strength in the

tibial midshaft during the whole remodeling period. Bone strength was also increased in the femoral midshaft throughout the 70-day study period.

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RAT BONE QUALITY STUDIES

Report	Title	In Vivo: Species, Strain, Gender, & Age	In Vivo: Dose Route	In Vitro: Assay Type	In Vitro: Tissue/ Cell Line	Results
R00/96 & R04/96	A Study in Intact Male and Ovariectomized Female Fischer 344 Rats Given Recombinant Human Parathyroid Hormone, rhPTH 1-34 (LY333334), for 1 Year to Assess Effects on Bone	Rat, F344, intact male & OVX female, ca 8 months	b & 40 µg/kg/day SC once daily for 12 months			LY333334 was anabolic in skeletons of intact males & OVX female rats, increasing bone mass & biomechanical strength properties of bone at clinically relevant sites such as spine & femur neck as well as in proximal tibia & femur mid-shaft. Increases in bone mass & strength exceeded those of age-matched sham & OVX controls. In femoral midshaft in rats given the higher dose, ultimate strain decreased despite increase in stiffness & ultimate load strength. No abnormal histopathology was found in tibia diaphyseal-metaphyseal junctional area. Systemic growth was slightly decreased in males but not affected in OVX females. There were no differences in femur length, but LY333334 did not alter length of tibia or femur in either sex. In treated rats, serum calcium increased by 5-10% at 6 months & then remained stable; serum alkaline phosphatase increased by 35-50%.
BN5-01	Recombinant Human Parathyroid Hormone (1-34) Effects on Bone Mass, Architecture, and Quality in Aged Ovariectomized Rats	Rat, Sprague-Dawley, female, ca 9 months, OVX	b or 40 µg/kg/day for 6 months SC			PTH stimulated bone formation in trabecular & cortical bone sites with improved biomechanical properties in vertebra, femoral neck, & diaphysis

Report	Title	In Vivo: Species, Strain, Gender, & Age	In Vivo: Dose Route	In Vitro: Assay Type	In Vitro: Tissue/ Cell Line	Results
BN5-02	Skeletal Effects of LY333334 in Ovariectomized Rats with Established Osteopenia	Rat, Sprague-Dawley, female, 3-6 months + 1 month post-OVX	0 to 80 µg/kg/day for 0.5 to 6 months SC			Osteopenic rats were permitted to lose bone due to ovariectomy before initiation of dosing. PTH restored bone in the proximal tibia & distal femur to above sham & baseline levels with a minimally efficacious dose of 0.3 to 1 µg/kg/day.
BN5-09	Skeletal Effects of LY333334 in Young and Mature Ovary-Intact Rats	Rat, F344 & Sprague Dawley, female, 1.5 to 6 months	0-75 µg/kg/day for 3-9 months SC			Bone mass was dose dependently increased in response to LY333334 with little change in geometry. Intact rats showed increasing responsiveness to LY333334 with maturation.
R43-01	Pharmacokinetic Profile of Human Parathyroid Hormone (1-34) (LY333334) and Serum Chemistry in Rats After Anabolic or Catabolic Injection Protocols	Rat, Sprague Dawley, male, ca 5 weeks	80 µg/kg once/day or 13.3 µg/kg 6 x/day at 10 min or 1 hr intervals; SC			Anabolic or catabolic response of bone to LY333334 may be primarily determined by the length of time serum concentrations remain above baseline & only secondarily by C_{max} obtained.
CG3-02	The Frequency of Daily Injections Determines Induction of an Anabolic Response in Bone by Human Synthetic and Biosynthetic (LY333334) Parathyroid Hormone Fragment, hPTH 1-34, in Young Rats	Rat, Sprague Dawley, male, 4-6 weeks	Synthetic hPTH(1-34) or LY333334 at 80 µg/kg/day given as 1, 3, or 6 injections within 1 hr or within a 6-8 hr period each day, or 3 injections at 80 µg/kg over 6-hr period (daily dose 240 µg/kg/day) SC			PTH increased bone mass & strength when given at 80 µg/kg/day in 1, 3, or 6 doses within a 1-hr period. If given as 80 µg/kg/day (total daily dose 240 µg/kg), or as 6-day over 6-8 hr (total daily dose of 80 µg/kg/day), bone mass decreased consistent with a catabolic response. Together with companion report (R43-01) these data suggest that response in bone to PTH (1-34) may be primarily determined by length of time serum concentrations of PTH remain above baseline & only secondarily by C_{max} obtained.

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LONG TERM RAT BONE QUALITY STUDY

A Study in Intact Male and Ovariectomized Female fischer Rats Given Recombinant Human Parathyroid Hormone, rhPTH 1-34 (LY333334) for 1 Year to Assess Effects on Bone (Study Nr. R00796/R04296)
(Non-Clinical Pharmacology Report RN00796/R04296)

METHODS

Studies:	R00796 (1-year treatment data) R04296 (baseline data)
Live-phase duration:	1 year
Live-phase dates:	R00496: 27 February 1996 through 27 February 1996
R00796:	12 February 1996 through 11 February 1997
Test article:	Recombinant human parathyroid hormone (1-34), LY333334 (Lilly)
Chemical name:	rhPTH (1-34)
Lot number and potency:	PPD03482; 1.02 mg/vial
Species and strain:	Fischer 344 rats
Initial body weight range:	Intact males: 323 to 383g; sham females: 167 to 197 g; OVX females: 196 to 236 g
Initial age:	12-14 weeks at time of surgery (sham or OVX)
Age at study start:	18-20 weeks (Study R00796); 20-22 weeks (Study R04296) (4-5 months)
Time of surgery to study start:	6 weeks
Route:	Subcutaneous
Frequency of administration:	Once daily
Number of animals:	80 males; 40 sham females; 83 OVX females (3 additional intact males and 3 OVX females were added for blood level evaluations)

Treatment groups:

Treatment groups:

Group Identification	Treatment	Number Animals Males	Females
	Study R04296		
IntactB	Intact, vehicle control (baseline)	10	
OVXB	Ovariectomized (OVX), vehicle control (baseline)		10
ShamB	Sham-operated, vehicle control (baseline)		10
	Study R00796		
IntactV	Intact, vehicle control	30	
Intact8	Intact, 8 µg LY333334/kg	20	
Intact40	Intact, 40 µg LY333334/kg	20	
OVXV	Ovariectomized, vehicle control		30
ShamV	Sham-operated, vehicle control		30
OVX8	Ovariectomized, 8 µg LY333334/kg		20
OVX40	Ovariectomized, 40 µg LY333334/kg		20

RESULTS

Body weight

LY reduced body weight gain in intact male rats. BW was increased in OVX females as compared to sham controls at study start, and remained increased throughout the study. LY had no effect on BW gain in OVX females.

Serum calcium and alkaline phosphatase

LY333334 increased serum calcium by 5% to 10% compared to vehicle controls (male and female) after 6 months of treatment and then remained stable until the end of the study. Ovariectomy increased serum alkaline phosphatase by 30% to 50% compared to sham controls. LY333334 further increased alkaline phosphatase by 30% to 50% at 40 µg/kg in OVX rats. A similar increase was observed for Intact40 male rats. These effects are consistent with the known actions of PTH.

Femur length

The femurs and tibia were longer in males than females at all times in the study. LY333334 did not alter linear bone growth in males, but slightly increased the length of the long bones in OVX8 and OVX40 as compared to OVXV or ShamV.

Bone Mass

Bone mass of proximal tibiae was analyzed by dual energy x-ray absorptiometry (DXA), while mass of femurs was analyzed by quantitative computed tomography (QCT). No data were collected for the vertebrae.

In the cancellous bone-enriched proximal tibia, LY333334 dose dependently increased bone mineral content (BMC) and bone mineral density (BMD) by up to 2- or 3-fold (at 40 ukd) in both intact males and OVX females compared to their respective controls. LY also increased projected area by up to 5-10% at 40 ukd (Tables G1 and G3).
In the cortical bone of the femur mid-shaft, LY333334 dose dependently increased BMC by approximately 200% and 70%; BMD by approximately 67% and 55%; and cross-sectional area by approximately 22% and 10% in intact males and OVX females, respectively, compared to their controls (Figure 1, and Tables G2 and G4).

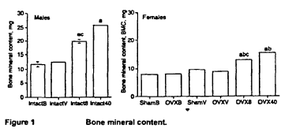

Figure 1 Bone mineral content.

Bone mineral content of the femur mid-shaft of intact male and ovariectomized female rats after treatment for 1 year with once daily LY333334 at 8 or 40 µg/kg/day. Data are shown as mean ± sem for 20 to 30 rats/group. Statistical significance, p<.05, a: vs controls (IntactV for males, OVXV for females); b: vs ShamV for females; c: OVX8 vs OVX40.

Table G1 **Dual Energy X-Ray Absorptiometry Analysis of Proximal Tibiae From Male Fischer 344 Rats Treated With LY333334**

Group[a]	Length (cm)	Bone Mineral Density (g/cm²)	Bone Mineral Content (g)	Projected Area (cm²)
IntactB	1.420 ± 0.020[c,d,e]	0.119 ± 0.002[d,e]	0.105 ± 0.003[c,d,e]	0.879 ± 0.015[c,d,e]
IntactV	1.480 ± 0.010[b]	0.130 ± 0.001[b,e]	0.126 ± 0.003[b,d,e]	0.964 ± 0.011[b]
Intact8	1.495 ± 0.014[b]	0.236 ± 0.002[b,c,e]	0.231 ± 0.003[b,c,e]	0.981 ± 0.009[b]
Intact40	1.472 ± 0.016[b]	0.326 ± 0.003[b,c,d]	0.320 ± 0.006[b,c,d]	0.978 ± 0.011[b]

a Mean ± SEM.
b Significantly different from IntactB, p<.05.
c Significantly different from IntactV, p<.05.
d Significantly different from Intact8, p<.05.
e Significantly different from Intact40, p<.05.

Table G2 **Quantitative Computed Tomography Analysis of Femoral Mid-Shaft From Male Fischer 344 Rats Treated With LY333334**

Group[a]	Bone Mineral Density (mg/cc)	Bone Mineral Content (g)	X-Area (mm²)	Voxel Number
IntactB	890 ± 31[c,d,e]	11.8 ± 0.90[d,e]	10.9 ± 0.37[c,d,e]	501 ± 17[c,d,e]
IntactV	796 ± 9[b,d,e]	12.6 ± 0.18[d,e]	13.3 ± 0.19[b,d,e]	609 ± 9[b,d,e]
Intact8	1161 ± 6[b,c,e]	19.8 ± 0.71[b,c,e]	14.2 ± 0.50[b,c,e]	678 ± 8[b,c,e]
Intact40	1332 ± 7[b,c,d]	25.8 ± 0.31[b,c,d]	16.2 ± 0.18[b,c,d]	742 ± 9[b,c,d]

a Mean ± SEM.
b Significantly different from IntactB, p<.05.
c Significantly different from IntactV, p<.05.
d Significantly different from Intact8, p<.05.
e Significantly different from Intact40, p<.05.

Table G3 **Dual Energy X-Ray Absorptiometry Analysis of Proximal Tibiae From Female Fischer 344 Rats Treated With LY333334**

Group[a]	Length (cm)	Bone Mineral Density (g/cm²)	Bone Mineral Content (g)	Projected Area (cm²)
OVXE	1.300 ± 0.045	0.099 ± 0.001[d,e]	0.073 ± 0.002[b,c,d,e]	0.736 ± 0.026
ShamE	1.250 ± 0.027[b,d,e]	0.112 ± 0.002[c,d,e]	0.078 ± 0.001[c,d,e]	0.698 ± 0.018[b,c,d,e]
OVXV	1.300 ± 0.011	0.110 ± 0.001[c,d,e]	0.082 ± 0.001[c,d,e]	0.749 ± 0.007
ShamV	1.300 ± 0.005	0.140 ± 0.007[b,d,e]	0.103 ± 0.002[b,d,e]	0.770 ± 0.006
OVX8	1.333 ± 0.011[b,e]	0.176 ± 0.002[b,c,e]	0.137 ± 0.002[b,c,e]	0.779 ± 0.007[b]
OVX40	1.330 ± 0.013[b,e]	0.224 ± 0.003[b,c,d]	0.176 ± 0.002[b,c,d]	0.787 ± 0.006[b]

a Mean ± SEM.
b Significantly different from OVXV, p<.05.
c Significantly different from ShamV, p<.05.
d Significantly different from OVX8, p<.05.
e Significantly different from OVX40, p<.05.

Table G4 **Quantitative Computed Tomography Analysis of Femoral Mid-Shaft From Female Fischer 344 Rats Treated With LY333334**

Group[a]	Bone Mineral Density (mg/cc)	Bone Mineral Content (g)	X-Area (mm²)	Voxel Number
OVXE	836 ± 9[c,d,e]	7.98 ± 0.14[c,d,e]	7.96 ± 0.13[b,e]	366 ± 5[b,e]
ShamE	860 ± 9[a,c,d,e]	7.89 ± 0.17[c,d,e]	7.65 ± 0.15[b,e]	351 ± 6.7[b,e]
OVXV	812 ± 7[c,d,e]	8.92 ± 0.11[c,d,e]	9.15 ± 0.08	420 ± 8.6
ShamV	904 ± 13[b,d,e]	9.64 ± 0.30[b,d,e]	8.88 ± 0.19	408 ± 8.6
OVX8	1105 ± 10[b,c,e]	12.9 ± 0.18[b,c,e]	9.74 ± 0.09[b,e]	447 ± 4.2[b,e]
OVX40	1258 ± 20[b,c,d]	15.3 ± 0.35[b,c,d]	10.1 ± 0.13[b,e]	464 ± 6.0[b,e]

a Mean ± SEM.
b Significantly different from OVXV, p<.05.
c Significantly different from ShamV, p<.05.
d Significantly different from OVX8, p<.05.
e Significantly different from OVX40, p<.05.

In summary, LY333334 increased bone mass (BMC and BMD) and bone geometry in a dose-dependent manner in both cancellous and cortical bone regions of intact male and ovariectomized female Fischer 344 rats.

Bone Strength

Bone strength was determined in lumbar vertebrae (compression), femur midshaft (3-point bending), and femur neck (load to failure). X-area, BMD and BMC of femoral midshaft were determined by pQCT.

Biomechanical parameters (Figure H1)

F_u	ultimate load
d_u	ultimate displacement (1/brittleness)
S	stiffness
U	work to failure
S_u	ultimate stress (inversely proportional to I)
I	moment of inertia (increases with t)
E	Young's modulus
u	modulus of toughness
ε_u	ultimate strain (in vertebra: d_u/l)
t	cortical thickness or, in vertebra: specimen thickness

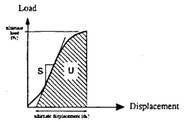

Figure H1 Derivation of biomechanical parameters from the load/displacement curves.

Note: Ultimate load (F_u), which reflects the extrinsic bone strength, is the maximum height of the curve. Ultimate displacement (d_u), which is the reciprocal of bone brittleness, is the maximum width of the curve. Stiffness (S) is the maximum slope and work to failure (U) is the area under the curve.

Data for vertebrae and femur

Biomechanical strength

1. In lumbar vertebra L-6 LY333334 increased strength by approximately <200% in males (Figure 2) and females (Figure 3).
2. In the femur neck, LY333334 increased strength by 150% to 175% in males (Figure 2) and by 140% to 175% in females compared to OVXV or by 120% to 150% compared to ShamV (Figure 3).
3. In the femur mid-shaft, LY333334 increased strength by ≥250% in both males and females (Figure 2,3).

Other biomechanical parameters

Ultimate strain decreased slightly at the higher dose (Intact40 and OVX40), while stiffness, Young's modulus, ultimate load, and ultimate stress (strength) increased for both males and females (Tables H1-H2).

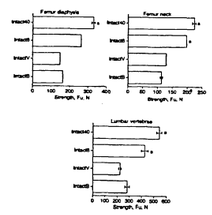

Figure 2 Biomechanical strength - males.

Biomechanical strength, measured as ultimate load (Fu) of the femur diaphyseal mid-shaft (cortical bone site), femur neck (cortical and trabecular bone), and lumbar vertebrae (trabecular bone-enriched site) in male rats, after treatment for 1 year with once daily LY333334 at 8 or 40 µg/kg/day. Data are shown as mean ± sem for 20 to 30 rats/group. Statistical significance, p<.05, a: vs IntactV controls.

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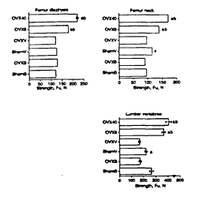


Figure 3 Biomechanical strength - females.

Biomechanical strength, measured as ultimate load (Fu) of the femur diaphyseal mid-shaft (cortical bone site), femur neck (cortical and trabecular bone), and lumbar vertebrae (trabecular bone-enriched site) in ovariectomized female rats after treatment for 1 year with once daily LY333334 at 8 or 40 µg/kg/day. Data are shown as mean ± sem for 20 to 30 rats/group. Statistical significance, p<.05, a: vs OVXV; b: vs ShamV; c: OVX8 vs OVX40.

Table H1 **Biomechanical Parameters for Male Rats Treated With LY333334**

Variable	IntactB	IntactV	Intact8	Intact40
	Femur Diaphyseal Midshaft*			
F_u (N)	162 ± 3	147 ± 2	262 ± 4[b,c]	329 ± 9[b,c]
S (N/mm)	413 ± 12	363 ± 9[b]	653 ± 10[b,c]	806 ± 20[b,c]
U (mJ)	72 ± 2	63 ± 2	84 ± 4[b,c]	78 ± 4[c]
d_u (mm)	0.69 ± 0.02	0.68 ± 0.02	0.53 ± 0.02[b,c]	0.44 ± 0.01[b,c]
S_u (Mpa)	239 ± 6	220 ± 2[b]	272 ± 6[b,c]	277 ± 9[b,c]
E (GPa)	7.8 ± 0.2	10.9 ± 0.3[b]	13.1 ± 0.6[b,c]	11.9 ± 0.4[b]
u (MJ/m³)	8.3 ± 0.3	4.7 ± 0.1[b]	4.6 ± 0.2[c]	3.8 ± 0.2[b,c]
ε_u	0.053 ± 0.002	0.034 ± 0.001[b]	0.028 ± 0.001[b,c]	0.025 ± 0.001[b,c]
	Femoral Neck*			
F_u (N)	113 ± 4	129 ± 2[b]	197 ± 3[b,c]	223 ± 6[b,c]
S (N/mm)	115 ± 8	406 ± 34[b]	449 ± 30[b]	499 ± 18[b,c]
U (mJ)	63 ± 5	42 ± 3[b]	62 ± 6[c]	58 ± 4[c]
d_u (mm)	1.08 ± 0.07	0.55 ± 0.03[b]	0.58 ± 0.05[b]	0.49 ± 0.03[b]
	Lumbar Vertebrae*			
F_u (N)	282 ± 19	222 ± 10	424 ± 27[b,c]	541 ± 21[b,c]
S (N/mm)	2412 ± 258	1623 ± 93[b]	2605 ± 23[b,c]	2972 ± 21[b]
U (mJ)	25.6 ± 1.1	24.1 ± 1.5	63.5 ± 6.6[b,c]	81.9 ± 6.7[b,c]
d_u (mm)	0.16 ± 0.01	0.19 ± 0.01	0.26 ± 0.03[b,c]	0.25 ± 0.02[b,c]
S_u (Mpa)	32.5 ± 1.0	22.0 ± 0.8[b]	40.9 ± 2.1[b,c]	43.9 ± 1.5[b,c]
E (MPa)	726 ± 67	582 ± 37	941 ± 90[c]	882 ± 63[c]
u (MJ/m³)	1.17 ± 0.06	0.67 ± 0.03[b]	1.63 ± 0.14[b,c]	1.77 ± 0.08[b,c]
ε_u	0.059 ± 0.004	0.033 ± 0.003	0.064 ± 0.007[c]	0.070 ± 0.004[c]

a Mean ± sem.
b Significantly different than IntactB.
c Significantly different than IntactV.

Table H2 **Biomechanical Parameters For Female Rats Treated With LY333334**

Variable	ShamB	OVXB	ShamV	OVXV	OVX8	OVX40
			Femur Diaphyseal Midshaft*			
F_u (N)	121 ± 3	129 ± 2	127 ± 2	121 ± 2	180 ± 3[b,c,d]	222 ± 5[b,c,d]
S (N/mm)	342 ± 7	351 ± 9	435 ± 10[b]	402 ± 6[b,c,d]	618 ± 13[b,c,d]	783 ± 12[b,c,d]
U (mJ)	45 ± 3	60 ± 4[b]	32 ± 1[b]	35 ± 2[b]	44 ± 3[c,d]	39 ± 2[c,d]
d_u (mm)	0.58 ± 0.02	0.69 ± 0.03[b]	0.41 ± 0.01[b]	0.46 ± 0.02[b,c,d]	0.41 ± 0.02[b,c,d]	0.32 ±0.01[b,c,d,e]
S_u (Mpa)	240 ± 4	258 ± 6	236 ± 3[b,c]	252 ± 3[b,c,d]	225 ± 4[b,c]	272 ± 6[c]
E (GPa)	10.3 ± 0.1	9.6 ± 0.3	10.7 ± 0.2[c]	9.2 ± 0.2[b,d]	11.0 ± 0.2[c,e]	11.6 ± 0.2[b,c,d,e]
u (MJ/m³)	7.0 ± 0.3	8.8 ± 0.4[b]	4.4±0.1[b,c]	4.7± 0.2[b,c]	5.0 ± 0.2[b,c]	3.9 ± 0.2[b,c,e]
ε_u	0.041 ± 0.002	0.050 ± 0.002[b]	0.031± 0.001[b,c]	0.035 ± 0.001[b,c,d]	0.032 ± 0.001[b,c]	0.026 ± 0.001[b,c,d,e]
			Femoral Neck*			
F_u (N)	96 ± 3	91 ± 3	116 ± 2[b,c]	97 ± 3[d]	139 ± 3[b,c,d,e]	171 ± 3[b,c,d,e]
S (N/mm)	130 ± 10	129 ± 9	296 ± 16[b,c]	256 ± 16[b,c]	364 ± 42[b,c,d,e]	391 ± 20[b,c,d,e]
U (mJ)	41 ± 3	39 ± 3	32 ± 2[b]	23 ± 1[b,d]	46 ± 3[c,e]	46 ± 3[c,e]
d_u (mm)	0.83 ± 0.05	0.82 ± 0.06	0.50 ± 0.03[b,c]	0.45 ± 0.02[b,c]	0.53 ± 0.04[b,c]	0.51 ± 0.03[b,c]
			Lumbar Vertebrae*			
F_u (N)	265 ± 16	172 ± 9[b]	222 ± 11[c]	167 ± 10[d]	366 ± 14[b,c,d,e]	408 ± 25[b,c,d,e]
S (N/mm)	2233 ± 285	1233 ± 179[b]	1780 ± 118[b]	1485 ± 80[b]	2735 ± 163[c,d,e]	2443 ± 195[c,d,e]
U (mJ)	23.0 ± 2.1	23.5 ± 3.9	23.7 ± 2.1	17.6 ± 1.5[b]	40.4 ± 3.5[b,c,d,e]	55.1 ± 4.3[b,c,d,e]
d_u (mm)	0.17 ± 0.02	0.22 ± 0.04	0.18 ± 0.01	0.17 ± 0.01[c]	0.18 ± 0.01[c]	0.23 ± 0.01[b,d,e]
S_u (Mpa)	40.2 ± 1.7	27.9 ± 1.3[b]	30.7 ± 1.2[b]	21.7 ± 0.8[b,c,d]	45.0 ± 1.1[b,c,d,e]	46.7 ± 1.5[b,c,d,e]
E (MPa)	737 ± 72	519 ± 81[b]	687 ± 46	546 ± 32[b,d]	1004 ± 68[b,c,d,e]	810 ± 57[c,e]
u (MJ/m³)	1.86 ± 0.13	1.59 ± 0.26	1.14 ± 0.09[b,c]	0.82 ± 0.07[b,c,d]	1.67 ± 0.11[c,e]	2.19 ± 0.15[b,c,e]
ε_u	0.076 ± 0.006	0.097 ± 0.019	0.063 ± 0.004[c]	0.060 ± 0.004[c]	0.063 ± 0.003[c]	0.081 ± 0.004[c,e]

a Mean ± sem.
b Significantly different than ShamB.
c Significantly different than OVXB.
d Significantly different than ShamV.
e Significantly different than OVXV.

Data on correlation between BMC and Fu (femur midshaft)
Morphometry and BMC/BMD data (QCT) for the femur midshaft are shown in Tables H3-H4.
The moment of inertia of the femoral midshaft increased by 47% in OVX40 females and by 104% in Intact40 males. This increase was related to the increase in femoral thickness (t). These findings indicate that the agent substantially increased periostea bone formation.
Figure H2 shows the relation between femoral midshaft ultimate load and BMC. This suggests that bone strength was significantly correlated with total bone mineral, which is proportional to BMD and bone area (or size).

Table H3 **Bone Morphometry and Mineral Content for Male Rats Treated with LY333334**

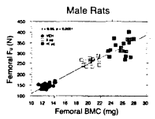
Variable	IntactB	IntactV	IntactB	Intact40
		Femur		
t (mm)	0.60 ± 0.01	0.54 ± 0.01	1.04 ± 0.01[b,c]	1.46 ± 0.06[b,c]
I (mm4)	3.74 ± 0.08	3.59 ± 0.11[b]	8.53 ± 0.33[b,c]	11.45 ± 0.42[b,c]
Cortical area (mm2)	6.78 ± 0.11	8.63 ± 0.00[b]	10.36 ± 0.15[b,c]	12.10 ± 0.24[b,c]
Total area (mm2)	8.13 ± 0.11	10.39 ± 0.09[b]	11.46 ± 0.17[b,c]	12.79 ± 0.18[b,c]
BMC (mg)	10.9 ± 0.2	12.8 ± 0.3[b]	20.6 ± 0.3[b,c]	23.8 ± 0.3[b,c]
BMD (mg/cc)	860 ± 11	791 ± 3[b]	1161 ± 6[b,c]	1232 ± 7[b,c]

a Mean ± sem.
b Significantly different than IntactB.
c Significantly different than IntactV.

Table H4 **Bone Morphometry and Mineral Content for Female Rats Treated with LY333334**

Variable	ShamB	OVXB	ShamV	OVXV	OVX8	OVX40
			Femur			
t (mm)	0.52 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.52 ± 0.01[b]	0.75 ± 0.01[b,c,d,e]	1.10 ± 0.02[b,c,d,e]
I (mm4)	2.35 ± 0.07	2.74 ± 0.12	2.87 ± 0.06[b,c]	3.10 ± 0.10[b,c,d]	3.97 ± 0.16[b,c,d,e]	4.55 ± 0.08[b,c,d,e]
Cortical area (mm2)	3.73 ± 0.08	3.94 ± 0.10	4.14 ± 0.09[b]	4.11 ± 0.07[b]	5.56 ± 0.06[b,c,d,e]	7.06 ± 0.11[b,c,d,e]
Total area (mm2)	6.12 ± 0.13	6.45 ± 0.13	6.97 ± 0.11[b,c]	7.21 ± 0.07[b,c]	7.59 ± 0.10[b,c,d,e]	7.81 ± 0.14[b,c,d,e]
BMC (mg)	7.49 ± 0.17	7.98 ± 0.14	9.30 ± 0.23[b,c]	8.92 ± 0.13[b,c,d]	12.92 ± 0.16[b,c,d,e]	15.06 ± 0.23[b,c,d,e]
BMD (mg/cc)	860 ± 9	836 ± 9	867 ± 7[b,c]	812 ± 7[b,c]	1105 ± 10[b,c,d,e]	1241 ± 8[b,c,d,e]

a Mean ± sem.
b Significantly different than ShamB.
c Significantly different than OVXB.
d Significantly different than ShamV.
e Significantly different than OVXV.



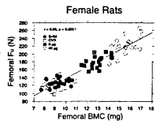


Figure H2 **Correlation between ultimate load of the femur (F_u) and the diaphyseal bone mineral content (BMC).**

Note: Data from baseline rats (early controls) were not included in the above figures, thus the comparisons were limited to same age and size rats.

Biomechanical characteristics of different bone sites
Similar responses to LY333334 on bone strength were seen in females and males, however, the effect of LY333334 was site specific. Figures H3,4,5 graphically show the different biomechanical characteristics of rat vertebrae (H3), femoral neck (H4), and femoral midshaft (H5).
In lumbar vertebrae, LY333334 increased both ultimate load and ultimate displacement causing a large increase in work to failure and a reduction in brittleness (Figure H3). In the femoral neck, LY333334 increased ultimate load without changing ultimate displacement, thus work to failure was increased but there was no change in brittleness (Figure H4). In the femur, LY333334 increased ultimate load while decreasing ultimate displacement. The bones became more brittle with treatment and work to failure was only marginally improved (Figure H5). The latter result was unexpected.

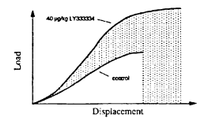


Figure H3 **Effects of LY333334 on rat vertebrae.**

Note: Shaded area indicates the gain in work to failure.

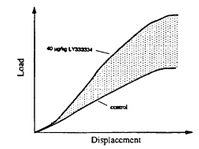


Figure H4 **Effects of LY333334 on rat femoral necks.**

Note: Shaded area indicates the gain in work to failure

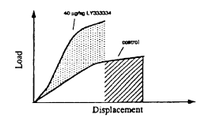


Figure H5 **Effects of LY333334 on rat femoral bending test.**

Note: Shaded area indicates the gain in work to failure and the striped area represents the less in work to failure.

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In summary, LY333334 significantly and dose-dependently increased bone strength of the femur, the femoral neck and lumbar vertebra. LY333334 also changed the biomechanical characteristics of the three different bone sites in a different manner.

Histomorphometry
Data shown are for the proximal tibia of baseline rats only, which were killed approximately 8 weeks after surgery (Study R04296).

- Trabecular bone volume (BV/TV, Tb.N) in OVX females and intact males was statistically significantly lower than in sham females.This confirmed that OVX rats were osteopenic at the initiation of dosing.
- Bone turnover (BFR), i.e, bone formation and bone resorption measures, were significantly higher in OVX females and male rats than in sham females.
- Osteoid volume in male rats was approximately 10% (0.1x) that measured in females. The reason for this was unclear as there were no equivalent differences between sexes in bone formation measures.
- Because of an error in specimen trimming and processing, data were not collected from specimens at the end of the study.

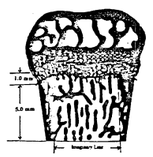

Figure H1 **Schematic diagram of longitudinal section cut through upper end of tibia of a young rat. The picture illustrates measured areas between 1 and 5 mm from growth plate towards the midshaft and between the imaginary lines.**

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Table H1 **Histomorphometry of Proximal Tibia of Untreated Sham and Ovariectomized (OVX) Females, 8 Weeks after Surgery, and Intact Male Rats at Study Start**

Measurement	Units	Baseline Sham[a]	Baseline OVX[a]	Baseline Male[a]
Bone volume (BV/TV)	%	14.6 ± 0.8	5.2 ± 0.5[b]	8.8 ± 1.1[c]
Trabecular thickness (TbTh)	µ	47.5 ± 1.0	45.5 ± 1.4	50.3 ± 1.8
Trabecular number (TbN)		3.1 ± 0.2	1.1 ± 0.1[b]	1.7 ± 0.2[c]
Trabecular space (TbSp)	µ	285 ± 17	911 ± 17[b]	602 ± 80[c]
Osteoid volume (OV/BV)	%	3.1 ± 0.2	4.3 ± 0.2	0.3 ± 0.0
Osteoid surface (OS)	%	25.9 ± 1.5	33.3 ± 1.5	29.8 ± 1.2
Osteoid thickness (OTh)	µ	3.6 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
Osteoblast + surfaces (Ob.S/BS)	%	6.8 ± 0.8	16.2 ± 2.0[b]	11.6 ± 1.7[c]
Mineralizing surfaces (MS/BS)	%	25.9 ± 1.5	33.3 ± 1.5[b]	29.8 ± 1.2
Mineralization rate (MAR)	µ/day	1.11 ± 0.04	1.45 ± 0.03[b]	1.38 ± 0.04[c]
Bone formation rate (BFR/BS)	µ/day	83 ± 6	137 ± 6[b]	118 ± 6[c]
Bone formation rate (BFR/BV)	%/year	351 ± 26	575 ± 38[b]	473 ± 27[c]
Bone formation rate (BFR/TV)	%/year	50.51 ± 4.0	30.7 ± 2.4[b]	39.8 ± 4.2[c]
Formation period (FP)	days	11.2 ± 0.6	13.5 ± 0.5[b]	11.2 ± 0.6[c]
Mineralization time (OMT)	days	3.3 ± 0.1	2.6 ± 0.1	2.8 ± 0.1
Resorption surface (Rs/BS)	%	8.5 ± 1.3	13.2 ± 1.6	12.5 ± 1.2
Eroded surface (ES/BS)	%	8.9 ± 1.3	14.4 ± 1.7[b]	13.0 ± 1.3
Osteoclast + surface (Oc.S/BS)	%	0.32 ± 0.05	1.24 ± 0.23[b]	0.51 ± 0.07[c]
Quiescent surface (QS/BS)	%	65.3 ± 2.5	52.3 ± 2.3[b]	57.2 ± 1.6[c]

a 10 group.
b Statistical significance, p<.05, sham female versus OVX female.
c Statistical significance, p<.05, intact male versus sham female.

Toxicokinetics
Blood samples were collected predose and 15 min postdose (near T_{max}), after 1, 151, 334 days (Day 0, 150, 333) of dosing, from 3 animals/sex/treatment group per time point. LY was assayed [redacted]. Quantitation limit was [redacted].

Serum levels of LY333334 (ng/ml) in F344 rats treated with 8 or 40 ug/kg

	8 ug/kg				40 ug/kg			
Day	Predose	15 min	Human C_{max} multiple*	*Human AUC multiple***	predose	15 min	Human C_{max} multiple*	*Human AUC multiple***
0	NR	NR	-	-	0 (BQL)	10.93	69x	*31x*
150	0 (BQL)	3.16	20x	9x	0 (BQL)	13.47	85x	*38x*
333	0 (BQL)	3.29	21x	9.5x	0 (BQL)	14.71	93x	*42x*

NR= not reported, insufficient sample volume
BQL = below quantitation limit
* Human C_{max} (15 min) = 159 pg/ml, at 20 ug daily dose
** Extrapolated from 2-year rat carcinogenicity study AUC and Cmax data (Human AUC = 295 pgxh/ml, at 20 ug daily dose)

The predose serum concentrations of LY333334 after 6 and 12 months of treatment were below the quantitation limit. This suggested there was no accumulation of LY333334 upon repeated dosing during the study.
The serum concentrations of immunoreactive LY333334 at 15 min (0.25h) postdose, were dose dependent. No differences were observed between serum levels between males and females or between Days 0, 150, 333.

Morphologic Pathology
Kidneys from terminally necropsied animals treated with 0 or 40 ug/kg (males: IntactV or Intact40, females: OVXV or OVX40, n=19 or 20/group) were weighted and examined histologically.
All intact males and several females had progressive glomerulonephrosis (PGN). In the OVX40 group, the incidence of PGN was increased as compared to OVXV, from 10/20 to 19/20. No other effects of LY333334 on kidney histology were noted.

CONCLUSIONS
In conclusion, LY333334 significantly increased bone mass and bone strength in intact male and OVX female rats after 1 year of once daily treatment.

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OTHER RAT BONE STUDIES

Recombinant Human Parathyroid Hormone (1-34) Effects on Bone Mass, Architecture, and Quality in Aged Ovariectomized Rats
(Nonclinical Pharmacology Report BN5-01)
Note: Preliminary results of this study were published by Sato et al. (Endocrinology, Vol. 138, #10, p4330, 1997)

This study was carried out to evaluate the effect of LY in older ovariectomized rats, and was powered adequately for biomechanical analysis.

METHODS
Study was carried out in "aged" 9-month old SD rats (N=26 baseline group,N=35/treatment group). Groups included baseline animals, sham controls, OVX controls, OVX given 8 ug/kg LY, or OVX given 40 ug/kg LY, once daily by s.c. injection. Treatment duration was 6 months. Bone mass measurements were carried out by QCT (during in-life phase of study and at study termination), and histomorphometry and biomechanical analyses were carried out of bones removed at the end of the study.

RESULTS
Bone mass
In life QCT analysis of proximal tibial metaphysis showed a small (ca. 5%) age-dependent reduction in bone BMD. Ovariectomy caused a larger reduction in tibial BMD (15%). LY dose-dependently increased tibial BMD up to 2x the OVX control value, at 40 ug/kg, after 5 months.
In the femoral metaphysis (distal femur) and diaphysis, OVX significantly decreased BMD, and LY increased BMD above base-line levels at both doses (2-3x OVX control values) (Figure 3).

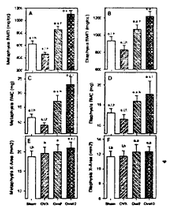

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Figure 3 **Analysis of femora by QCT. The metaphysis and mid-diaphysis of femora were examined at termination by QCT, using voxel dimensions of 0.147 x 0.147 x 1.2 mm. Data are mean ± standard deviation. Significant differences from Sham, OVX, OVX8 and OVX40 are designated "a", "o", "l", and "h", respectively (Fisher PLSD, p<0.011). Analysis of the**

Histomorphometry
Cortical bone
In both tibial and femoral diaphyses cortical thickness or area were slightly reduced by OVX. LY markedly increased cortical thickness, up to 1.8x at 40 ug/kg (femur), and decreased medullary area, concomitant with an increase in moment of inertia. As shown by dynamic histomorphometry, bone formation rate at the periosteal and endocortical surface of the tibial midshaft was increased by OVX, and further increased markedly by LY in a dose-dependent manner (2-3x).
Cancellous bone
Histomorphometric analysis showed that in the metaphyses of the tibia OVX caused a marked reduction in BV/TV and Tb.N but not Tb.Th. LY largely improved all trabecular bone parameters (BV/TV, Tb.N, Tb.Sp, Tb.Th) so that LY (40 ug/kg)-treated tibial trabeculae were much thicker and closer together than control (sham) trabeculae. LY also dose-dependently increased trabecular connectivity. Another effect of LY was an increase in bone formation (MS/BS, BFR/TV) parameters indicating a stimulation of osteoblastic activity. Histomorphometric analysis of cancellous vertebral bone showed similar effects on trabecular bone architecture as in tibial metaphysis.

Bone strength
Femoral sites
Biomechanical analysis of femoral diaphysis and femoral neck showed decreased bone strength in OVX animals, including a decrease in Young's modulus (E) (slope of the deformation-load curve). LY dose-dependently improved structural ("extrinsic") properties (Fu, S) and material ("intrinsic") properties (σu, E, U) above OVX and sham values. Only εu was unaffected or minimally decreased by LY (Table 7). This was in accordance with data from the 1-year study in which this parameter (ultimate strain) in the femoral diaphysis was decreased after 1 year treatment of initially 4- to 5-mo old rats. Femoral neck strength was unaffected by OVX, but was increased by LY.

Table 7 **Biomechanical Analyses of the Femoral Diaphysis and Femoral Neck**

Group[a]	F_u	S	σ_u	E	U	ε_u	Neck F_u
Baseline	177o,l,h	521o,l,h	318l,o	9.00o	5.60o	0.038	116l,h
	5	11	9	0.23	0.28	0.001	3
Sham	175o,l,h	574o,h,l,h	309o	9.62o	5.09o	0.036	116l,h
	5	13	9	0.22	0.28	0.001	3
OVX	142o,h,l,h	466o,h,l,h	249o,h,l,h	7.80o,h,l,h	3.90o,h,l,h	0.035	115l,h
	3	11	6	0.22	0.23	0.001	3
OVX8	213o,a,h,h	696l,o,a,h,h	309o,h	9.43o,h	5.19o	0.036	152o,a,h,h
	5	18	5	0.14	0.26	0.001	5
OVX40	247o,a,h,l	813o,a,h,l	332o,a,h,l	10.13o,h,l	5.27o	0.034	164o,a,h,l
	4	14	6	0.12	0.22	0.001	4
ANOVA	< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.015	<0.0001

a Femora diaphyses were examined by 3-point bending to measure the ultimate force (F_u), stiffness (S), ultimate stress (σ_u), Young's modulus (E), work to failure (U), and ultimate strain (ε_u). Femora necks were loaded to failure to measure the ultimate force (F_u). Data are mean followed by SEM (n=23 to 33). Significant differences from baseline, Sham, OVX, OVX8, or OVX40 are depicted as "b", "s", "o", "l", or "h", respectively.

Vertebrae
Vertebral strength, i.e., both structural and material biomechanical properties, were decreased by OVX and increased in a dose-dependent manner above sham and baseline levels by LY.

LY caused a small increase in body weight and uterine weight of OVX animals.

Skeletal Effects of LY333334 in Ovariectomized Rats with Established Osteopenia
(Nonclinical Pharmacology Report BN5-02)

METHODS
This study was carried out in a 'delayed-dosing intervention model", i.e., 6-month old SD rats that were ovariectomized and subsequently permitted to lose bone for 1 month. In most experiments treatment was carried out by daily s.c. administration of LY (rhPTH1-34) or synthetic PTH1-34 , at doses between 0 and 80 ug/kg/day, for 3 months. Bone mass measurements were carried out by QCT (during in-life phase of study and at study termination), and histomorphometry and biomechanical analyses were carried out of bones removed at the end of the study.

RESULTS
Bone mass
QCT analysis of he proximal tibial metaphysis showed a decrease in BMC and BMD upon ovariectomy. Treatment with 0.1,0.3, 1, 3, 10, 30 ug/kg/d of LY for 3 months restored BMD in a dose-dependent manner, to sham or above-sham levels at doses >= 3 ug/kg (Figure 2). The efficacy of LY continued to increase to the highest dose used of 80 ug/kg. X-area of the metaphysis was not affected by OVX or LY.

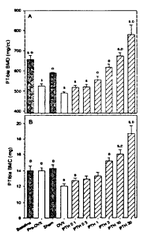

Figure 2 **Dose-dependent effects of LY333334 in osteopenic ovariectomized rats. The proximal tibial metaphysis was analyzed by QCT, using voxel dimensions of 148 x 148 x 1200 µm. Treatment was initiated after 1 month post-ovariectomy (7 months of age) and continued for the following 3 months. Groups included baseline, pretreatment OVX (Pre-OVX), Sham, OVX, and LY333334 at 0.1 to 30 µg/kg/day subcutaneously, as indicated. Plotted data are mean ± standard deviations with group sizes of n=7 to 8. Significant differences from Sham and OVX are depicted as "a" and "o", respectively (p<0.05, Fisher's PLSD). LY333334 increased BMD and BMC dose dependently to significantly beyond OVX, Sham, and Baseline levels. (9721)**

QCT of the femora showed qualitatively similar results, although the effects of OVX and LY, particularly in the midshaft area, were less pronounced.

Histomorphometry
Histomorphometry of the proximal tibial metaphysis showed some interesting results (Table 3).

Table 3 **Histomorphometry of LY333334 Effects on the Proximal Tibial Metaphysis**

Group[a]	Bn/Pm	Ob.S/BS	Es/BS	Oc.S/BS	N.Oc/BS
Sham	36.6±1.9•	0.73±0.18•	4.30±0.23•	1.03±0.11	0.235±0.024•
OVX	11.1±1.5ª	8.68±2.14ª	7.63±1.21ª	2.61±0.57	0.618±0.147ª
PTH 0.03	6.8±0.9ª	7.89±1.68ª	9.30±1.50ª	3.73±0.83ª	0.835±0.173ª
PTH 0.3	9.5±1.4ª	7.93±2.22ª	9.30±1.65ª	3.57±0.90ª	0.758±0.181ª
PTH 3	25.9±3.3•°	7.33±2.41ª	8.10±0.51ª	2.28±0.34	0.497±0.071

a The proximal tibial metaphysis was analyzed by histomorphometry following treatment. Groups included Sham, OVX, and LY333334 (PTH) at 0.03, 0.3, and 3 µg/kg/day subcutaneously. Parameters analyzed included cancellous bone perimeter (Bn/Pm), osteoblast surface (Ob.S/BS), eroded surface (Es/BS), osteoclast surface (Oc.S/BS), and osteoclast number (N.Oc/BS), as previously described (Parfitt et al. 1987). Data are mean ± standard errors with group sizes of n=7 to 9. Significant differences from Sham or OVX are depicted as "•", "ª", respectively (p<0.05, Fisher's PLSD). (9607)

Bone perimeter was decreased by OVX, and further decreased by 0.03-0.3 ug/kg LY, but increased by 3 ug/kg LY. Bone turnover, as indicated by the one formation and the three resorption parameters in Table 3, was increasd by OVX, as expected. LY at doses of 0.03-0.3 ug/kg appeared to stimulate resorption, while at 3 ug/kg it decreased resorption. Formation (Ob.S/BS) was not significantly affected but it should be noted that any effect on osteoblast surface per se will be masked by an effect on BS, particularly at the higher doses at which bone perimeter and BMD are increased.

Bone strength
Biomechanical analysis of the femur showed a slight decrease in femoral midshaft strength due to OVX, and a dose-dependent increase in femoral midshaft thickness and strengt, and in femoral neck strength, after 3 monts of treatment with LY at doses of 0.3-30 ug/kg (Table 4)

Table 4 **LY333334 Effects on the Biomechanical Properties of the Femora**

Group[a]	t	F_y	S	Neck F_y
Sham	.664±.010	163±5•	457±11	108±8
OVX	.621±.018	145±4ª	438±19	108±9
PTH 0.3	.643±.011	160±6	464±37	113±5
PTH 3	.683±.009•°	169±9•	476±12	133±3•
PTH 30	.906±.027•°	196±10•°	601±21•°	149±9•°

a LY333334 effects on the biomechanical properties of femora were evaluated by 3-point bending of the femoral midshaft and load to fracture of the femoral neck. Femora were isolated from osteopenic, ovariectomized rats that were dosed for 3 months, starting 1 month post-surgery. Groups included Sham, OVX, and human PTH (1-34) (LY333334) at 0.3, 3, and 30 µg/kg/day subcutaneously. Parameters analyzed for the femora diaphysis included cortical thickness (t), ultimate force (F_y), and stiffness (S). In addition, femoral neck strength was analyzed in shear (F_y). Data are mean followed by SEM (n=7 to 8). Significant differences from Sham or OVX are depicted as "•", "ª", respectively (p<0.05, Fisher's PLSD). (9565/9568)

Skeletal [illegible]
(Nonclinical Pharmacology Report BN5-09)

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The skeletal effects of human recombinant parathyroid hormone PTH (1-34) (LY333334) were evaluated in ovary-intact rats, as a follow-up to the 2-year oncogenicity study of LY333334 in male and female Fischer 344 rats.

Study 9904 (*F344 rats, age at treatment start 1.5 month, treatment duration 9 months, doses 0, 0.03, 0.3, 5, 30, 75 ukd*):
In 1.5 month old F344 rats, the BMD of the proximal tibial metaphysis increased 2-fold in the absence of drug treatment from 1.5 to 10.5 months of age (age effect). LY333334 (0.03-75 ug/kg/day) treatment for 9 months, from 1.5 months of age, induced a dose-dependent increase in proximal tibial BMD, from 3 months after treatment initiation, of up to ca. 40% at 75 ukd. This treatment also caused an increase in wet weight of the femora of up to ca. 20% at 75 ukd. QCT images of the femur showed that LY caused some thickening of the cortex associated with somewhat reduced marrow space. This is in contrast to the findings in the 2-year carcinogenicity study, where marrow space was completely lost and femoral bone geometry was altered after 2 years of 30 or 75 ukd of LY treatment. This suggests that these changes occurred after at least 9 month of treatment.
Over 9 months, femoral BMD was increased by ca. 35% in vehicle controls. LY (5, 30, 75 ukd) caused an additional femoral BMD increase of up to 10% in 75 ukd treated animals (Table 1).
At these doses LY caused increased femoral midshaft strength (Fu) and stiffness (S) (as compared to vehicle controls) but had no effect on ultimate displacement (i.e., brittleness). In the femoral neck Fu was increased by 30 and 75 ukd LY. Vertebral BMD and strength were also significantly increased by LY up to 30% at doses of 5, 30 and 75 ukd (Table 1). Some reduction of both femoral neck and vertebral BMD and or strength was seen at low doses of PTH (0.03 and 0.3 ukd), possible related to a decrease in ultimate strain. Mature, 10.5 month old F344 rats had substantially lower osteocalcin levels and PTH immunoreactivity compared to young animals. The former is consistent with an age-dependent reduction in osteoblastic activity. Dose-dependent elevation of osteocalcin levels by LY333334 of up to 120% at 75 ukd indicated stimulation of osteoblastic activity.

BMD of femoral midshaft and lumbar vertebrae, after 9 months of treatment of 1.5 month old intact rats with LY333334

	Femur midshaft				Lumbar vertebrae			
Group/Dose	BMD (mg/cc)	% change	BMC (mg)	% change	BMD (mg/cc)	% change	BMC (mg)	% change
Baseline	760	-	0.61	-	460	-	0.98	-
Vehicle	1080	-	1.15	-	557	-	1.77	-
0.03	1075	-1%	1.17	+1.7%	552	-1%	1.72	-3%
0.3	1075	-1%	1.16	+1%	556	+0%	1.77	0%
5	1105	+2%	1.17	+1.7%	615	+10%	1.94	+10%
30	1130	+5%	1.26	+10%	690	+24%	2.30	+30%
75	1180	+9%	1.35	+17%	714	+28%	2.51	+42%

Study 9937 (*SD rats, age at treatment start 1.5 months, treatment duration 3 months, doses 0, 0.3, 5, 30, 75 ukd*):
LY increased proximal tibial BMD and femoral midshaft BMD at 30 and 75 ug/kg/day. The effect was larger at the cancellous tibial site. Femoral diaphyseal and femoral neck strength were also increased by LY doses of 30 and 75 ug/kg/day. At 75 ukd, femoral midshaft ultimate displacement and strain were slightly decreased.

Study 9907 (*SD rats, age at treatment start 6 months, treatment duration 3 months, doses 0, 0.03, 0.1, 0.3, 1, 3, 30 ukd*):
LY increased proximal tibial BMD at 3 and 30 ukd, as in the younger animals (Study 9937). However, the mature animals appeared to be slightly more responsive (effect at lower dose, and larger effect) than the young animals. LY also increased femoral midshaft BMD at 3 and 30 ukd. The effect was larger at the cancellous tibial site. Femoral diaphyseal strength was not affected at doses up to 30 ukd, but femoral neck strength was increased by 30 ukd. At 3 and 30 ukd, femoral midshaft ultimate displacement and strain were slightly decreased. Vertebral BMD was increased at 30 ukd.

Other results
Peak bone mass
Longitudinal analyses suggested that intact SD and F344 female rats attain peak bone mass (BMD) in the proximal tibia at about 7 months of age, and in the femoral midshaft between 9 and 13 months of age.

Intact vs. OVX rats
With respect to the effect on proximal tibial BMD, mature intact SD rats (aged 6 months) were less responsive to LY than mature ovariectomized animals (aged 7 months). However, BMD in intact rats was larger than in OVX rats at every dose of LY tested.

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STUDIES ON EFFECT OF DOSING FREQUENCY

Pharmacokinetic Profile of Human Parathyroid Hormone (1-34) (LY333334) and Serum Chemistry in Rats After Anabolic or Catabolic Injection Protocols

Nonclinical Pharmacology Report R43-01

Summary

Intermittent administration of human parathyroid hormone (PTH) (1-34) has been shown to increase bone mass in animals and humans. However, if given continuously, or if administered at high doses, a net catabolic response is observed. Different delivery protocols for PTH(1-34) were evaluated in this report in order to correlate serum biochemical and PTH(1-34) pharmacokinetic profiles with the anabolic or catabolic response in bone. Subcutaneous injection protocols for biosynthetic human PTH(1-34) (LY333334) which resulted in either an anabolic or catabolic response in bone, were administered to 100 g male rats, and serum was collected at different times after dosing. After a single, subcutaneous dose of LY333334 (80 µg/kg), which gave an anabolic bone response after 18 days of daily treatment, serum Ca rapidly decreased to a nadir (10 minutes), followed by return to baseline by 40 minutes. Serum phosphorous also decreased but the decline was slower (nadir at 60 to 120 minutes). Serum PTH(1-34) (determined using an immunoradiometric assay) rose rapidly to a peak (10 minutes) (18.6 ± 4.2 ng/mL) and then quickly declined to background levels (240 minutes). LY333334 (13.3 µg/kg/ injection) given as 6 injections (10 minutes intervals), which produced an anabolic bone response after 18 days of daily treatment, gave a longer duration serum Ca and phosphorous depression with a lower nadir than the single dose regimen. Serum PTH(1-34) rose to 2.5 ± 0.6 ng/mL 5 minutes after the first dose and gradually increased to 5.6 ± 0.7 ng/mL 5 minutes after the last dose. Levels returned to baseline by 240 minutes after the first injection. When LY333334 (13.3 µg/kg/injection) was administered for 6 injections (1 hour intervals), a catabolic response was observed in bone after 18 days of treatment. Serum Ca and phosphorous decreased after each injection followed by an increase just prior to the next dose. Serum PTH(1-34) rose to a C_{max} 5 minutes after each dose (2.2-3.8 ng/mL) and declined to 2-10% of the peak values prior to the next dose but remained above baseline until 420 minutes after the first dose. The response in bone to PTH(1-34) may be primarily determined by the length of time serum concentrations remain above baseline and only secondarily by the C_{max} obtained.

The Frequency of Daily Injections Determines Induction of an Anabolic Response in Bone by Human Synthetic and Biosynthetic (LY333334) Parathyroid Hormone Fragment, hPTH 1-34, in Young Rats.

Nonclinical Pharmacology Report CG3-02

Summary

Parathyroid hormone (PTH), given once daily, stimulates bone formation to increase bone mass in a variety of rodent models and osteoporotic humans. When used to treat patients with osteoporosis, PTH must be given by once daily injection. In assessing the feasibility of alternate delivery systems, such as an electrophoretic patch, we found it was not technically possible to deliver an efficacious dose once daily. The purpose of this study was to assess the bone mass of young rats given either synthetic human parathyroid fragment, hPTH 1-34, or biosynthetic human parathyroid fragment, hPTH 1-34, LY333334, as multiple doses within either 1h or 6-8h periods, by daily subcutaneous (sc) injections for 12 or 18 days.

Results showed that when PTH, as a total daily dose of 80µg/kg/d, was given as 6 injections within 1h daily, for 12 or 18 days, PTH increased bone mass of proximal tibias equivalently to the significant increase measured after once daily injections in young rats. When PTH was given as 3 injections over an 8h day, at the same total daily dose as once daily injections (i.e. 80µg/kg/d), a complex response was observed. Bone mass, expressed as BMD, did not differ significantly from controls. However, the increase in bone mineral content and cross-sectional area resulted in an equivalent increase in resistance to fracture of the femur neck as the positive controls. When injections of PTH were increased to 6/day at the same daily dose of 80µg/kg/d over a 6-8h period for 18 days, PTH was catabolic and decreased bone mass. We concluded that, if an efficacious dose of PTH was to be given in multiple injections, these had to be delivered rapidly within a short time period, to induce an increase in bone mass. More prolonged exposure to the hormone, by multiple injections over a 6-8h period, resulted in significant loss of bone mass that more closely resembled the response observed when PTH is given by continuous sc infusion.

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OTHER EFFICACY PHARMACOLOGY STUDIES
Several other studies with LY and combinations of LY with other products were carried out in rats, and some studies with LY were done in mice. Selected studies are briefly discussed in this review.

Rats
(Report CG3-8)
The anabolic effects of human PTH(1-34) at 8- ug/kg, given by s.c. injection for 12 days, were independent of the gender, breed and strain in normal and mutant disease-prone rats. The bones of all mutants had similar responses to hPTH1-34.

(Report BN5-03)
A raloxifene analogue had an additive effect on cancellous bone mass when given in combination with PTH(1-34). After discontinuation of PTH, the analogue prevented rapid bone loss that was observed in the controls.

(Report CG3-15)
After treatment with PTH(1-34) in combination with a raloxifene analogue (RA) of ovx rats, for 3 months, maintenance therapy with RA alone, reduced PTH, or the two together all resulted in reductions in the treatment-enhanced BMD. Thus, discontinuation of PTH treatment reverses the previously obtained increase in bone mass.

(Report CG1-16)
In ovx rats, discontinuation of PTH(1-34) therapy, rapidly results in a decrease in bone mass. Estrogen and raloxifene maintained bone mass after PTH withdrawal. These results apparently contradict the results from study CG3-15 (above).

Mice
(Report BN5-07)
Ovx mice from 9 different strains were treated with LY333334 (10 ug/kg) fro 2 months. There were variable positive and negative effects of aging, ovariectomy, and LY333334 on total body or femoral BMC, and other parameters (body weight, lean fat mass), depending on mouse strain.

(Report W53-26)
In adult male mice PTH(1-34) two out of the five tested strains responded positively to PTH treatment with respect to femoral diaphyseal midshaft BMC and X-area. The study indicates mouse strain sensitivity of the bone formation effect by PTH.

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SAFETY PHARMACOLOGY

Safety pharmacology studies with LY33334 were carried out in adult male rats (CV effects), female beagle dogs (CV effects), and adult male mice (CNS and behavioral effects). Summary reviews of these studies are included in this review.

		In Vivo		In Vitro		
Report	Title	Species, Strain, Gender, & Age	Dose Range	Assay Type	Tissue/ Cell Line	Results
Safety Pharmacology Study Reports						
General Pharmacology Report 1	The Acute Cardiovascular Effects of LY333334 [PTH (1-34)] Administered Subcutaneously to Conscious Male Sprague Dawley Rats	Rats Sprague Dawley, male, adult	LY333334 3, 10, 30, 100, 300, & 1000 µg/kg single dose SC			The no-observed-effect level for cardiovascular changes (increased heart rate and decreased blood pressure) was 4.3 µg/kg of LY333334
General Pharmacology Report 2	An Acute Cardiovascular Toxicity Study With LY333334 Administered Subcutaneously in Conscious Instrumented Female Beagle Dogs	Dogs, Beagle, female, 12 to 21 months	LY333334 6 µg/kg single dose SC			The female beagle dog demonstrated a consistent and reproducible decrease in arterial pressure and increases in left ventricular inotropic state and heart rate after treatment with 6 µg/kg LY333334 that is consistent with compound-induced vasodilation and compensatory physiological homeostatic mechanisms
General Pharmacology Report 3	The Acute Behavioral Profile of LY333334 Following Subcutaneous Administration in Male CD-1 Mice	Mouse, CD-1, male, adult	LY333334 10, 30, & 100 µg/kg single dose SC			LY333334 at doses ≤100 µg/kg would not be expected to produce secondary pharmacology related to central nervous system (CNS) and behavioral functions such as changes in body temperature, ambulatory and nonambulatory activity levels, CNS depressants, and convulsive thresholds

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The Acute Cardiovascular Effects of LY333334 [PTH(1-34) Administered Subcutaneously to Conscious Male Sprague Dawley Rats (Studies RV0595 and RV0795)
(General Pharmacology Report 1)

METHODS
LY333334 was administered subcutaneously to conscious male Sprague Dawley rats (300-600g) at doses of 100, 300 or 1000 µg/kg (N=4/group) (Study RV0595), or nominal dose levels of 3, 10 and 30 µg/kg (actual levels 0.5, 4, 23 ug/kg) (Study RV0795). Cardiovascular parameters measured were heart rate, systolic, diastolic, and mean arterial pressure, and arterial pulse pressure. Data were acquired from 5h before to 4 h after dosing.

RESULTS
Transient redness of extremities at all doses. Increase in heart rate and decrease in blood pressure (systolic, diastolic, and mean arterial pressure) at dose levels of 30 (real dose level 22.8), 100, 300, and 1000 µg/kg. Cardiovascular effects were observed during the first 2 hours after dosing. The no-observed-effect level was 4.3 µg/kg (nominal level 10 ug/kg). This dose is equivalent to approximately 1.5x the human exposure at a 20 ug daily dose.

CONCLUSION
LY333334 has a vasorelaxing and hypotensive action and causes an increased heart rate in rats at doses ≥ 23 ug/kg.

An Acute Cardiovascular Toxicity Study With LY333334 Administered Subcutaneously in Conscious Instrumented Female Beagle Dogs (Study DV0196)
(General Pharmacology Report 2)

METHODS
LY333334 (6 ug/kg) was administered subcutaneously to conscious female beagle dogs. Cardiovascular parameters measured were heart rate, left ventricular inotropic state, systolic, diastolic, and mean arterial pressure, and arterial pulse pressure. The peak value of the first derivative of left ventricular pressure (dP/dtmax) was used as an index of left ventricular inotropic state. Electrocardiograms were also recorded. Serum ionized calcium levels were determined approximately 6 hours after dosing

RESULTS
Decrease in arterial pressure (systolic, diastolic, mean arterial pressure, arterial pulse pressure), increase in left ventricular inotropic state, and increase in heart rate. All effects were maximal during the first 2 hours after dosing. Heart rate was statistically elevated during the first 6 hours after dosing. Serum ionized calcium levels were significantly elevated 6 hours after dosing.
There were no significant treatment-related electrocardiographic effects. The increase in left ventricular inotropic state and heart rate could be due a reflex increase in sympathetic outflow in response to a decrease in total peripheral resistance due to vasodilation.

CONCLUSION
LY333334 causes vasodilation, hypotension and a compensatory increase in heart rate in female beagle dogs at 6 ug/kg.

Acute cardiovascular effects of LY333334 in rats and dogs

Species	Study type	Doses (ug/kg/day)	EFFECTS			
			Heart rate	Arterial blood pressure	Other findings	ECG findings
RAT (Sprague-Dawley), male	Single dose	0, 0.5, 4, 23, 100, 300, 1000	Increased at doses ≥ 23 ug/kg	Decreased at doses ≥ 23 ug/kg	Transient redness of extermities	-
DOG (Beagle), female	Single dose	0, 6	Increased	Decreased	Left ventricular inotropic state increased; Serum ionized Ca level elevated postdosing	No abnormalities

Thus, in rats and dogs, effects on heart rate and blood pressure were consistent with drug-induced vasodilation and a compensatory cardiac response.

The Acute Behavioral Profile of LY333334 Following Subcutaneous Administration in Male CD-1 Mice (Studies PN9830 and PN9836)
(General Pharmacology Report 3)

METHODS
LY333334 was administered subcutaneously to CD-1 mice at doses of 0 (vehicle), 10, 30, or 100 µg/kg (10 males/treatment group).

RESULTS
No overt clinical signs or changes in body temperature at any dose examined. No changes in spontaneous ambulatory and nonambulatory activity levels. Hexobarbital-induced sleep times were not affected by administration of LY333334, indicating that LY333334 does not produce central nervous system (CNS) depression or inhibit hepatic enzymes involved in hexobarbital metabolism.

No changes in convulsive thresholds were observed as evaluated by the administration of electroshock or pentylenetetrazol.

CONCLUSION
LY333334 has no acute adverse effects on the central nervous system in the mouse at doses ≤100 ug/kg.

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GENERAL TOXICOLOGY

ACUTE TOXICITY STUDIES

Acute Toxicity Of LY333334 Administered Subcutaneously to F344 Rats (Study Nr. R07695)
(Toxicology Report 04)
(From IND Review September 25, 1995)
METHODS
Fischer 344 (F344/NHsd) rats (5/sex/dose group), age 9-10 weeks, weight 130-170 g, were fasted for 18 h, and then given a s.c. injection of **0, 100, 300, 1000 ug/kg**, and observed for 14 days.
RESULTS
No deaths occurred. Redness of extremities was seen in all treated within 15 minutes after dosing, and was reversed within 2 h. Body weight gain was the same for controls and treated. No gross abnormalities were detected at necropsy. Median LD was >1000 ug/kg.

Acute Toxicity of LY333334 Administered Intravenously to F344 Rats (Study Nr. R07595)
(Toxicology Report 05)
(From IND Review September 25, 1995)
METHODS
Fischer 344 rats (5/sex/dose group), age 9-10 weeks, weight 130-170 g, were fasted for 17h, and then given an intravenous dose of **0, 300 ug/kg**, and observed for 14 days.
RESULTS
No deaths occurred. Redness of extremities was seen in all treated immediately after dosing, and was reversed within 2 h. Body weight gain was the same for controls and treated. No gross abnormalities were detected at necropsy. Median LD was >300 ug/kg.

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CHRONIC TOXICITY STUDIES

A Chronic Toxicity and Blood Level Study in Rats given LY333334 by S.C. Injection for 6 Months (Study Nrs. R01196 and R04396)
(Toxicology Report 12).
(From IND Review February 7, 1997)

METHODS
Fischer 344 rats (n=15/sex/dose group), weight 200-450 gr, age 27-28 weeks, were administered, by subcutaneous injection, **0, 10, 30, 100 ug/kg/day** (control, LD, MD, HD) LY333334. In the satellite toxicokinetic Study Nr. R04396, blood samples were taken on Day 0 from n= 3/sex (control), 6/sex (LD, MD), or 15/sex (HD), at 0h (pre-dose) in control group, at 0h and 0.25 h (T_{max}) in LD and MD, and at 0-3h (10 samples) in HD. In the main toxicity Study Nr. R01196 samples were taken on Day 140 from n=15/sex (LD, MD, HD), at 0h and 0.25h in LD and MD, and at 0-3h (10 samples) in HD. Samples were assayed for immunoreactive PTH(1-34) by immunoradiometry. Testosterone and estradiol were also assayed by RIA.

RESULTS
Clinical signs -
In 2 MD f, and 1 HD f, blood in vaginal lavage sample during last 2 wks of treatment period. Vaginal bleeding in 1 HD f.

Mortality –
1 MD m killed on Day 170 due to hindleg weakness: upon necropsy, animal had spinal cord injury. 1 HD m died on Day 56 for unknown cause: animal had nasal/pulmonary hemorrhage. No drug-related mortality.

Body Weight -
Males:
After 6 months, significant decrease in body weight (BW) as compared to control in HD (0.96x control value); non-significant (n.s.) decrease in BW gain in HD (0.24x). After 3 months significant decrease in BW in HD (0.95x) and significant decrease in BW gain in HD (0.7x). In first 5 wks, slight decrease in BW in all groups including control.
Females:
After 6 months, treatment-related, significant increase in BW in LD, MD, HD (1.07x, 1.1x, 1.1x); increase in BW gain in LD (n.s.), MD, HD (1.4x, 1.7x, 1.8x). After 3 months significant increase in BW in MD, HD (1.05x, 1.05x) and increase in BW gain in MD (n.s.) and HD (1.5x, 1.65x). In first 2 wks, minimal decrease in BW in all groups including control.

Food Consumption -
Males:
Relative food consumption (RFC) (g/kg/day) significantly increased in HD in wks 12-26 (1.03-1.04x). EFU significantly decreased in HD in wks 5-26 (0.7x after 26 wks).
Females:
FC significantly increased in HD in wks 4-10 and wks 14-26 (1.06x after 26 wks). RFC significantly decreased in LD, MD, HD in wks 21-26 (0.96x, 0.95x, 0.97x after 26 wks). EFU significantly increased in LD, MD, HD in wks 22-26, 10-26, 3-26 respectively (1.4x, 1.6x, 1.7x after 26 wks)

Vital Signs - No data.

Ophthalmoscopy - No compound-related effects.

Hematology - (*significant changes*) (samples taken at Month 1,3,6 from N= 10,10,15 or 14)
Males:
Month 1: Minimal decreases in Hb conc, PCV, MCV and MCH in MD, HD. Slight decrease in

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

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/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.

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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 - ($n_{examined}$ =15/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 basophylic 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), estrud (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 -phospatemia
- 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:	

Group Number	Test Compound	Dose of LY333334 (µg/kg/day)	Animals/Group
1	Vehicle*	0[a]	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

* Vehicle: 20 mM sodium phosphate monobasic in saline.
[a] 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 (µg/kg/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 (µg/kg/d)		0	2	10	20	40	0	2	10	20	40
RBC ct (x10E12/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 (µg/kg/d)		0	2	10	20	40	0	2	10	20	40
Ionized Ca	Wk6 (4h)	1.45	1.56	1.59	1.62*	1.58	1.40	1.53	1.55*	1.57*	1.58*
(mmol/L)	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)

		MALES					FEMALES				
Group		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
Dose (µg/kg/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

		MALES + FEMALES (average)				
Group		ctrl	LD	LMD	HMD	HD
Dose (µg/kg/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 –

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

Dose (µg/kg/d)		0	2	10	20	40	0	2	10	20	40
Kidney	Rel wt (%x10[3])	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 (%x10[6])	-	-	-	-	-	24	27	21	32*	34*

Gross pathology –

		MALES					FEMALES				
Group		ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
Dose (µg/kg/d)		0	2	10	20	40	0	2	10	20	40
N animals		4	4	4	3	3	4	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)

			MALES					FEMALES				
Group			ctrl	LD	LMD	HMD	HD	ctrl	LD	LMD	HMD	HD
Dose (µg/kg/d)			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	Minimal	0	2	1	0	0	0	4	0	0	0
	bone formation	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	Minimal	0	0	3	1	1	0	3	2	1	2
	interstitium (bluish)											
		Sl-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	Minimal	0	0	0	2	0	0	0	2	0	2
	epithelial regeneration	Sl-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 ≥ 2ukd, 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

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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*	0[a]	4
2	LY333334	0.5	4
3	LY333334	2	4
4	LY333334	10	4

* Controls: 4 mg mannitol/mL of vehicle.
[a] 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

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/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	245	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:

	Screening visit / Clinical Chemistry, Day and Calcium	Period 1	Period 2	Period 3
Period	Week 1	Weeks 4 & 5	Week 3	Weeks 1 & 4
Blood	Weeks 4, 10, 24, 39 and 50	Weeks 1, 25, and 52	Weeks 14, 26, 41, and 49	Weeks 10, 24, 39, and 50
Urine (24-hour)			Week 3	Weeks 1 & 4

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

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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

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/d)		0	0.5	2	10	0	0.5	2	10
RBC ct (x10E12/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*)

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.

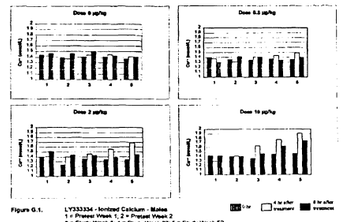

Figure G.1. LY333334 - Ionized Calcium - Males
1 = Pretest Week 1, 2 = Pretest Week 2
3 = Study Week 5, 4 = Study Week 27, 5 = Study Week 52

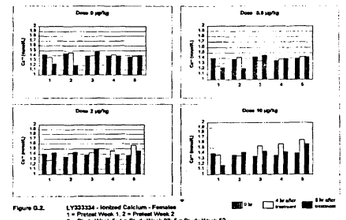

Figure G.2. LY333334 - Ionized Calcium - Females
1 = Pretest Week 1, 2 = Pretest Week 2
3 = Study Week 5, 4 = Study Week 27, 5 = Study Week 52

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Ionized serum Ca levels

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/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

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/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 (umol/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

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/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

		MALES				FEMALES				M+F			
Group		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

		MALES + FEMALES (avg)			
Group		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 (µg LY333334/kg/day)	Response Range (rel. µg IgG/mL)	Incidence [a] Week 24	Incidence [a] 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 -

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/d)		0	0.5	2	10	0	0.5	2	10
Kidney	Rel. wt (%x10^2)	39	38	39	51*	36	42	41	42
Pituitary	Rel. wt (%x10^2)	1.2	1.2	1.6	2.0*	1.8	1.9	2.0	1.7
Thymus	Rel. wt (%x10^2)	9.3	15	10	10	6.6	9.8	9.4	14*

Gross pathology –

		MALES				FEMALES			
Group		ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/d)		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)

			MALES				FEMALES			
Group			ctrl	LD	MD	HD	ctrl	LD	MD	HD
Dose (µg/kg/d)			0	0.5	2	10	0	0.5	2	10
N examined			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, mutifocal, 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.

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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*
02	40

* 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 (µg/kg/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): ng/ml
Levels in animals with slight nephropathy (#84852, 83522): ng/ml and
Level in animal with no nephropathy (#85662): ng/ml

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

Group	Dose (µg/kg)	Number of Monkeys Evaluated: Interim Termination	Treatment Termination	Reversibility Termination
01	0	1	2	2
02	40	1	4	3

Terminal Necropsy Dates: 13 February 1998 (interim termination, Monkey #3972 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 4-5h following nasogastric administration of 0.1g/kg ammonium chloride, and water deprivation) (adequate: pH≤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 nephopathy (#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)	518 (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 (µg/kg/d)		0	40
Treatment phase (119 days)			
(N animals)		(2)	(5)
Kidney	Whole tissue alteration (pallor/mottling)	2	2

Reversibility phase (211 days)			
(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 (µg/kg/d)		0	40
Treatment phase (119 days)			
(N animals)		(2)	(5)
Kidney	No lesions	1	
	Slight nephropathy	0	2
	Moderate nephropathy	0	1
	Marked nephropathy	0	1*
Reversibility phase (211 days)			
(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	• 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	• 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	• 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		• Increase in Ca excretion at ≥2 ug/kg in both sexes	• 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 Cmax 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 interstitum 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 [redacted] 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 phospate monobasic (NaH_2PO_4) 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: Recommended dose: 20 mg daily dose, by s.c. injection
CAC Concurrence: 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: None
Clinical Observations: 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: See "Body Weight"
Clinical Pathology: 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 [redacted] The results represent the pathologists' consensus.
Immunotoxicology: 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 [redacted] 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 Number (%)	FEMALES 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 Number (%)	FEMALES 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*

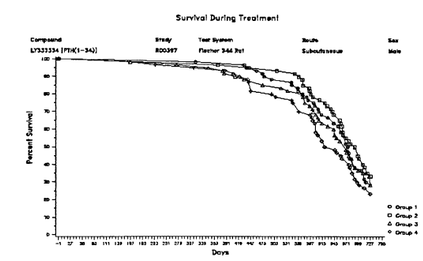
Figure 1 indicates that in addition to a decreased survival in HD males and MD and HD females, there was also a treatment-related effect on the time of death, with more deaths occurring earlier in the MD and HD groups of both sexes.

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)

			MALES					FEMALES				
			Deaths due to					Deaths due to				
Grp		Dose (ug/kg/day)	Neoplasm(s)	Osteosarcoma	Pituitary adenoma	LGLL	Other	Neoplasm(s)	Osteosarcoma	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



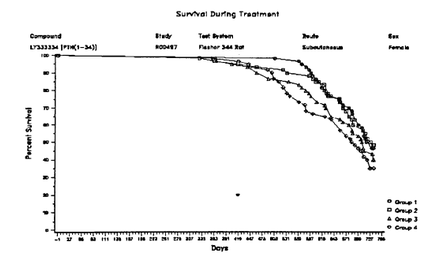


Figure 1 Survival During Treatment

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 EFU
SEE 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%

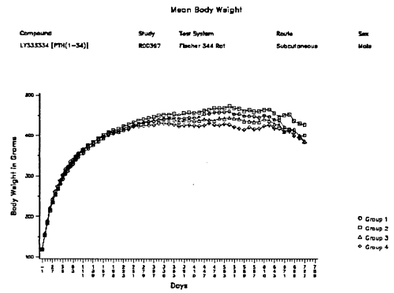
Note that, although food consumption was slightly increased in both sexes, the efficiency of food utilization (EFU) was affected differently in males (negative) than in females (positive).

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



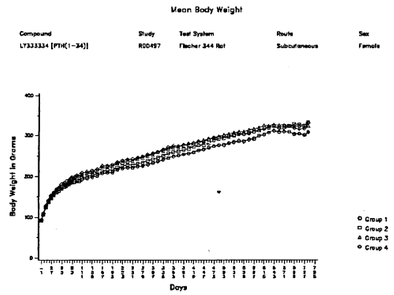


Figure 2 Mean Body Weight During Treatment

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

		Males				Females			
Group		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)

		Males				Females				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 (sternum)	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

		Males				Females			
Group		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

		Males				Females			
Group		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

		Males				Females				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									
	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

		Males				Females			
Group		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
	Marked	0	0	▼0	1	0	0	1	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)

		Males					Females			
Group		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	*No. examined*	*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	*No. examined*	*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	*No. examined*	*0*	*3*	*5*	*1*	-	-	-	-
	Adenocarcinoma	0	1	1	0	-	-	-	-
Clitoral gland	*No. examined*	-	-	-	-	*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	*No. examined*	*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	*No. examined*	*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	*No. examined*	*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	*No. examined*	*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	*No. examined*	*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	*No. examined*	*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	*No. examined*	*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	*No. examined*	*1*	*0*	*0*	*2*	*0*	*0*	*0*	*0*
	Osteosarcoma (M)	0	0	0	2	-	-	-	-
Mass 2	*No. examined*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*
	Osteosarcoma (M)	0	0	0	1	-	-	-	-
Misc tissue	*No. examined*	*1*	*0*	*1*	*0*	*0*	*0*	*0*	*1*

	Sarcoma (M)	1	0	0	0	-	-	-	0

(B) = benign; (M) = malignant; (MS) = metastatic

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Incidence of Frequent Neoplasms in Male Rats (R00397)

Dose (µg /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[b,c]
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-µg/kg group.
[d] Statistically significant increasing trends in the 30- and 75-µg/kg groups.
[e] Statistically significant decreasing trends in the 30- and 75-µg/kg groups (p ≥0.995).

Incidence of Frequent Neoplasms in Female Rats (R00497)

Dose (µg /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-µg/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-(sarcoxma	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

		Males				Females			
Group		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

		Males				Females			
Group		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

	Males				Females			
Group	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

	Males				Females			
Group	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)

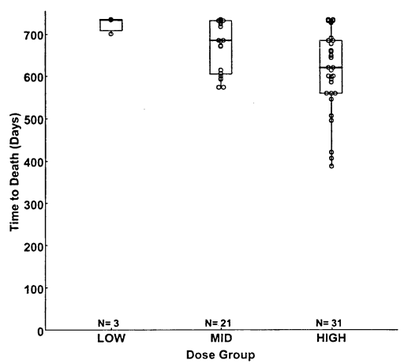

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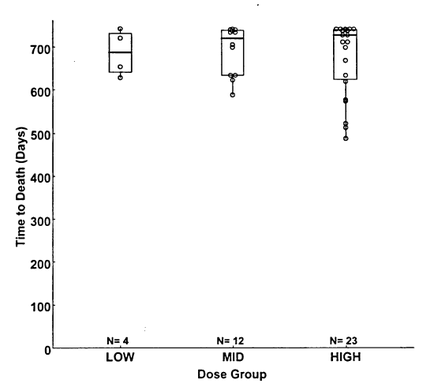
Figure 2. Time to diagnosis and/or death of osteosarcoma-bearing animals (females)



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.

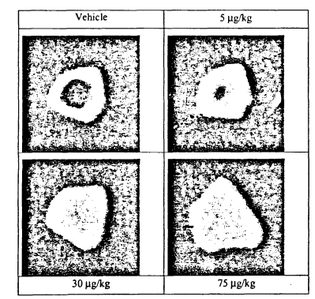


Figure 2 QCT Images of the Femoral Midshaft from Females Treated for Two Years. The midshaft of left femora were analyzed in cross-section by QCT, using voxel dimensions of 150x150x1200 µm. Images show reduction of the marrow cavity at 5 µg/kg, loss of marrow space and expansion of bone at 30 µg/kg, 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	% 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	% 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.

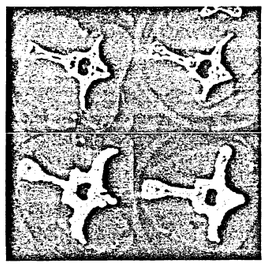


Figure 7 **QCT images of L-6 Vertebra from Females. L-6 were scanned in cross-section by QCT, using voxel dimensions of 150x150x150 µm. The top row shows images from two vehicle controls, while the bottom row shows two from the 75 µg/kg group. Considerable alteration of bone architecture was observed for the treated group.**

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

	BMD (mg/cc)		% increase in BMD	% 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 (mm2)	Marrow area (mm2)	Mean cortical thickness (mm)	Trabecular area (%)	Tabecular number /TV (#/mm2)	Trabecular node to node/TV (#/mm2) (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
Control	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)

Cmax and AUC values at 3 time points in the study

		MALES				FEMALES		
Group		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:
Cmax and AUC: m>f @ 6 months.
Cmax and AUC: m<f @ 12 months.
Cmax and AUC: m≤f @ 18 months.
Males: Cmax and AUC (6 mo)>(12mo)>(18mo)
Females: Cmax and AUC (6 mo)<(12mo)>(18mo)

Human exposure multiples

Human AUC multiples

Group	Dose (ug/kg/day)	AUC of PTH(1-34) (ngxhr/ml) (Month 6; average m,f)	AUC of PTH(1-34) (ngxhr/ml) (Month 12; average m,f)	AUC of PTH(1-34) (ngxhr/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 ngxhr/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) (ngxhr) (Month 6; average m,f)	Cmax of PTH(1-34) (ngxhr) (Month 12; average m,f)	Cmax of PTH(1-34) (ngxhr/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)

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 signifiant 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.

APPEARS THIS WAY
ON ORIGINAL

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 al 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 (stemum)	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 (stemum)	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)

	MALES				FEMALES			
Group	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) (ngxhr/ml) (Month 6; average m,f)	AUC of PTH(1-34) (ngxhr/ml) (Month 12; average m,f)	AUC of PTH(1-34) (ngxhr/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 ngxhr/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) (ngxhr) (Month 6; average m,f)	Cmax of PTH(1-34) (ngxhr) (Month 12; average m,f)	Cmax of PTH(1-34) (ngxhr/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 Number of tumors (%)		Range of tumor incidence per control group[2] N = 60 per sex/study 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 (mineralizarion and nephropathy) and has been associated with increased serum calcium levels. The cause of the other hyperplastic lesions is not clear.

Neoplastic findings included osteosarcoma, osteoblastoma, osteoma at various bone sites. These tumor findings are discussed further below. Besides bone tumors, there was a dose-related increase in the incidence of combined skin epithelial cell neoplasms in males, and of thyroid C-cell adenoma and combined C-cell adenoma and adenocarcinoma in males.
The thyroid C-cell tumor findings were statistically significant. However, it should be noted that the statistical significance of the combined tumor data was due to the increased incidence of adenoma only. Although the significance of the thyroid finding can be questioned in light of historical control data, it may be a biologically plausible outcome that is probably the direct or indirect result of the hormonal stimulation of calcium metabolism by PTH. The increased severity of C-cell hyperplasia in both males and females confirms the significance of the finding.
The increased tumor incidence in the skin was not statistically significant.
The apparent increase in combined clitoral gland carcinoma in females could not be tested since the finding was made only in animals with gross lesions and animals without gross lesions were not examined.

PTH(1-34) caused statistically significant increases in osteosarcoma and osteoblastoma and a statistically non-significant increase in osteoma. Osteosarcomas were seen in both the appendicular and the axial skeleton. In males the tumor was most frequently seen in the tibia, while in females it was most commonly found in the vertebrae. Multiple osteosarcomas at different bone sites were seen in 14% of the animals diagnosed with osteosarcoma. In the majority of animals with osteosarcoma gross bone nodules, bone lesions or metastatic lesions were also observed. Some animals had metastatic osteosarcoma without the identification of a primary bone site.

When considering historical control data, the osteosarcoma finding was highly significant. In 720 control animals only 1 osteosarcoma was detected in a male rat (1/360) according to the Sponsor's historical control database. The historical control data from the Sponsor and the NTP combined indicate that the control (background) incidence of osteosarcoma in rats is approximately 0.25% in males, and 0.17% in females. This would yield an average background incidence for both sexes of 0.2%. Based on this 0.2% background incidence the risk of osteosarcoma in rats was increased by PTH(1-34) from ca. 30x in the low dose to 225x in the high dose groups.

Osteosarcomas included a broad spectrum of malignant osteoblastic lesions. Many formed large bone nodules, while others were only microscopically observed intramedullary lesions. The osteosarcomas were characterized by invasive, polygonal to fusiform neoplastic mesenchymal cells which produced osteoid matrix and uncalcified bone. Intramedullary tumors often replaced hematopoietic cells and disrupted bone trabeculae.
Osteblastoma has not been described previously in the rat, and is an uncommon benign bone neoplasm in humans. The diagnosis was made on the basis of histological similarity to the human lesion. These tumors were sharply demarcated, contained large active osteoblasts, often arranged along bony trabeculae with minimal atypia. The neoplasm replaced a region of pre-existing bone, but was minimally invasive.
Osteoma in the rat is a benign tumor characterized by a sharply demarcated expansive neoplasm composed of dense variably mineralized lamellar bone with well-differentiated osteoblasts on the outer bone trabeculae.

Osteosarcomas were first detected by clinical or gross observations of bone nodules after approximately 17 months in the high dose groups, and 20 months in the low and mid dose groups. However, the earliest occurrence of an osteosarcoma was in a male rat in the high dose groups that died at 13 months from a fatal osteosarcoma in the vertebra. Apart from the dose-related increase in incidence, the time to death of the osteosarcoma-bearing animals was reduced in a dose-related manner, at least in the males.
The time of tumor initiation is unclear and depends on the duration of the various stages of the multi-stage tumorigenesis process. However, based on the data from this study it is likely that in the rat tumor initiation and promotion can take place at any time between 6-12 months after start of the daily PTH treatment.

The mechanism of the bone tumor formation in the animals as a result of intermittent PTH exposure is not clear. Likewise, the precise mechanism of the anabolic effect of PTH on bone has not been elucidated. Signal transduction following PTH/PTHrP-receptor activation is mediated by adenylyl cyclase/cAMP/PKA and phospholipase-C/PKC activation. The signal molecules (AC/PKA and PKC) trigger a plethora of events, which, in the case of an intermittent dosing regimen, are likely to include the expression of osteogenesis-driving genes. These events are thought to involve many intermediate factors (FGF-2, IGF-1, IGF-binding proteins, IL-6, IL-11, OPG). Most likely, as a result of long term repetitive PTH stimulation and in conjunction with the stimulation of bone formation some processes are activated that increase the likelihood of neoplastic transformation. Notably, PKC has been associated with cell proliferation and differentiation, and a potential side effect of prolonged stimulation of PKC activity, such as by the phorbol ester TPA, is the promotion of tumor formation.

It has been suggested that upon intermittent dosing, the differential effect on osteoblastic gene expression involves stimulation of growth factor production (FGF-2), which stimulates preosteoblast proliferation, and stimulation of IGF-1 production, which prevents apoptosis of postmitotic osteoblasts (Whitfield et al, 2000). These events and/or the activation of PKC may be linked to an increased susceptibility of the osteoblast to neoplastic transformation.

It is unlikely that the osteosarcoma induction by PTH(1-34) is a species-specific effect. In studies with an analogue of PTHrP that were terminated prematurely osteosarcomas were seen in both rats and mice after 30-50 weeks of treatment.
Other peptide hormones have also been known to induce neoplastic events in the rat when administered chronically (gonadotrophins and ovarian neoplasia, gastrin and ECL-omas). Stimulation of the target cells for these peptide hormones has also been associated with human neoplasm formation.

A main issue is the relevance of the osteosarcoma findings in rats for humans, particularly because an increased tumor incidence was observed in the low dose groups of both sexes at a human exposure multiple of only 3x. A discussion of the relevance needs to take several factors into consideration: species-specificity, duration of treatment, onset of treatment, etc. The Sponsor contends that the effects of PTH in the rat are different in extent and nature than those in humans. Their explanation of the bone proliferative lesions is that PTH is tumorigenic on rat bone because of the sustained hormonal stimulation of the osteoblast starting at a young age during a time of skeletal growth and continuing throughout most of the rat's life span. A precise mechanism of action is not elaborated upon.

According to Sponsor, "the findings from the 2-year rat study are unlikely to be predictive of an increased risk of osteosarcoma in postmenopausal women receiving a limited duration of PTH(1-34) therapy". This conclusion is based on the fact that the rats were treated from a young age (6-7 weeks), starting during their rapid growth phase, for a total of up to two years, which is 80-90% of their life span. In comparison, humans are to be treated for less than 24 months, which is 2-3% of their lifespan. The conclusion is also based on the finding that 2-year treatment of the rat bone at all doses including the low dose induces "exaggerated pharmacologic" effects on bone mass, with "skeletal effects at all dose levels in excess of the desired therapeutic bone effect in humans".

Although this is consistent with the data and the bone effect in rats is marked, this argument cannot be used to dismiss the findings and justify the conclusion that the osteosarcoma findings are not relevant for humans. While the bone effect is quantitatively larger in rats than in humans, as is to be expected in a chronic toxicity study, there are no known qualitative differences between rat and human osteoblasts with respect to their response of intermittent PTH stimulation. Therefore, the results of the rat study may simply have uncovered a potential adverse effect that could possibly occur also when treatment is for a shorter time period, at doses that cause a less pronounced bone effect, possibly with a lag time after treatment discontinuation.

In a previous 6-month study (doses 10, 30, 100 ukd) and a 1-year pharmacology study (doses 8 and 40 ukd) in rats osteosarcomas were not observed. Also, in an 18-month study in cynomolgus

monkeys (1 and 5 ukd) bone proliferative lesions have not been observed. However, the animals in these studies were not followed up for y extebnt of time and/or were not evaluated by rigorous histopathological examination as the animals in the current oncogenicity study, and the possibility of preneoplastic changes being elicited after a shorter-than-lifetime treatment cannot be excluded. For that reason the Sponsor is currently carrying out a follow-up carcinogenicity study in rats to elucidate the effect of animal age at onset of treatment and the effect of treatment duration. In this study part of the animals will be treated for a shorter term (6 months) and followed up for an extended period of time after treatment discontinuation. The final results of this 2-year follow-up study are expected in 2002.

Human dose administration is similar as carried out in the animals, namely once daily by sc injection. Human Phase III trials with the test compound were discontinued in December 1998 after 17-23 months treatment (2-year protocol), when the first osteosarcomas in rats were diagnosed after detection by clinical or gross observation. Patients were followed up for 2 years after treatment discontinuation. No osteosarcomas during the study or during the follow up phase have been reported. Also, in other small clinical studies with intermittent PTH(1-34) dosing of up to 1 or 2 years, carried out over the last 25 years, osteosarcomas have not been reported.

In humans, hyperparathyroidism (HPT) is a pathologic state in which the parathyroid gland on a continuous base secretes excessive amounts of PTH. In this case, bone mass is generally decreased rather than increased, although in mild cases stimulation of bone formation and cancellous bone preservation have also been reported. No association has been found between osteosarcoma and hyperparathyroidism in humans. This suggests that in humans chronically elevated PTH levels do not induce osteosarcoma. However, the currently proposed treatment is a daily injection with transiently elevated PTH levels, and it can not be excluded that the intermittent/pulsatile nature of dosing is at the root of the animal tumor findings, paralleling the anabolic effect of this administration regimen. Unfortunately, results from long term animal studies with continuous dosing regimens are not available.

In conclusion, the relevance of the animal osteosarcoma finding for humans is not clear. Considering the occurrence of the tumor at exposure levels near expected human exposure, the osteosarcoma finding upon long term treatment of rats with PTH(1-34) constitutes a major clinical concern.

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NDA 21,318
Forteo
Rat Carcinogenicity Study

Histopathology Inventory

Species: Rat	Pathology	
	Tissues retained and examined in all animals (per protocol)	Tissues examined in some animals
Abnormalities	X	
Adipose tissue		X
Adrenals	X	
Aorta	X	
Blood smear*	X	
Bone (sternum)	X	
Bone (femur)1	X	
Bone (vertebra)2	X	
Bone (tibia)3	X	
Bone (pelvis)3	X	
Bone (rib)3	X	
Bone marrow (sternum)	X	
Brain stem	X	
Cerebrum	X	
Cerebellum	X	
Cervix	X	
Cecum	X	
Clitoral gland		X
Colon	X	
Duodenum	X	
Epididymides	X	
Ear		X
Esophagus	X	
Eyes	X	
Eyelid		X
Gall bladder		
Harderian glands	X	
Heart	X	
Ileum	X	
Injection site	X	
Jejunum	X	
Kidneys	X	
Lacrimal glands		
Larynx		
Liver	X	
Lips		X
Lung	X	
Lymph nodes, submaxillary	X	
Lymph nodes, mesenteric		X
Lymph nodes, sup cv		X
Mammary gland	X	
Mediastinum		X
Meninges		X
Mesentery		X
Optic nerves		
Ovaries	X	
Pancreas	X	
Pericardium		X
Pituitary	X	

Parathyroids	X	
Pharynx		
Pleura		X
Preputial gland		X
Prostate	X	
Rectum*	X	
Salivary gland	X	
Sciatic nerve	X	
Seminal vesicles	X	
Skeletal muscle	X	
Skin	X	
Spinal cord	X	
Spleen	X	
Stomach	X	
Suboutis		X
Teeth		
Testes	X	
Thymus	X	
Thyroid	X	
Tongue	X	
Tonsils		
Trachea	X	
Urethra		X
Urinary bladder	X	
Uterus	X	
Vagina	X	
Vessel		X
Zymbal glands		X

* Blood films examined only as necessary

1 Femur collected and examined in all animals

2 Lumbar vertebrae collected from all animals that died after Day 607, and from animals that died before Day 607, if available

3 Tissues (tibia, pelvis, rib) collected/examined when abnormal

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GENETIC TOXICOLOGY

The Effect of LY333334 on The Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells (Study 950411MLA3939)
(Toxicology Report 01)
(From IND Review September 25, 1995)
METHODS
LY333334 was tested at concentrations of **29, 78, 156, 313, 625, 1250, 5000 ug/ml** with and without metabolic activation by the S9 fraction from [redacted] treated rats, to determine its ability to induce mutations at the TK locus in mouse lymphoma cells. No cytotoxic response was observed at concentrations up to 5000 ug/ml. The positive controls used were ethylmethanesulfonate (EMS) in the non-activated, and 3-methylcholanthrene (3-MC) in the activated assay. Test article was incubated [redacted] Assay was done in [redacted] No statistical test was performed, and a positive result was defined as an increase in mutation index of at least 2-fold control value at two successive dose levels.
RESULTS
Incubation with LY333334 had no effect on the mutation frequency of cell cultures at all doses tested. Positive controls were clearly positive in both non-activated and activated assay with mutation indices of 12 and 9, respectively, similar as historical controls.
CONCLUSION
LY333334 is not mutagenic in the L5178Y TK$^{+/-}$ mouse lymphoma cell assay.

The Effect of LY333334 on the Induction of Reverse Mutations in Salmonella typhimurium and Escherichia coli Using the Ames Test (Study 950412AMS3939)
(Toxicology Report 02)
(From IND Review September 25, 1995)
METHODS
LY333334 was tested at concentrations of **313, 625, 1250, 2500, 5000 ug/ml** with and without metabolic activation by S9 fraction from [redacted]-induced rats, to determine its ability to induce reverse mutations in histidine auxotrophs of Salmonella typhimurium,LT-2 (TA 1535, TA 1537, TA 98, TA 100), and a tryptophan auxotroph of Escherichia coli (WP2uvrA). No cytotoxic response was observed at concentrations up to 5000 ug/ml. The positive controls used were [redacted] 7) for the non-activated, and [redacted] for the activated assay. Test was conducted [redacted] with an incubation time of [redacted] Each concentration of test article and controls was tested in [redacted] No statistical test was performed, and a positive result was defined as an increase in the number of revertants to at least twice the vehicle control value at two successive concentrations.
RESULTS
Incidence of revertant colonies after treatment with LY333334 was approximately equal to the values for vehicle controls, for each tester strain of S. typhimurium and E. coli. Positive controls all produced increases in revertants consistent with expected results.
CONCLUSION
LY333334 is not mutagenic in the Ames bacterial mutagenesis test.

The Effect Of LY333334 Given Subcutaneously for 2 Consecutive Days on the Induction of Micronuclei in Bone Marrow of ICR Mice (Study 950405MNT3939)
(Toxicology Report 03)
(From IND Review September 25, 1995)
METHODS
Hsd (ICR) mice (n=5/sex/group), age 8-9 weeks, weight 26-36g, were given doses of 0, 1000, 3000, 10000 ug/kg for 2 consecutive days by subcutaneous injection. Vehicle was 20 mM monobasic [redacted] Positive control was [redacted] mg/kg). Ca. 24 hours after the second dose, femoral bone marrow was collected on a slide

(1slide/femur), and the number of normochromatic erythrocytes (NME), anucleate polychromatic erythrocytes (PCE) and micronucleated polychromatic erythrocytes (MPCE) was determined.
RESULTS
Both in males and females, the PCE/NCE ratio was similar to vehicle controls (1.0 and 1.3, respectively). The MPCE/1000 PCE ratio was not affected by LY333334 in males or females (Trend test p-values 0.37 and 0.94, respectively). This indicates that LY333334 is not toxic to bone marrow.
CONCLUSION
LY333334 is not clastogenic and does not interact with the mitotic spindle in the bone marrow micronucleus test.

The Effect of LY333334 on the In Vitro Induction of Chromosome Aberrations in CHO Cells
(Study 950412CAB3939)
(Toxicology Report 06)
(From IND Review September 25, 1995)
METHODS
CHO cells were cultured in [redacted] and incubated for [redacted] **5000 ug/ml** in the absence or presence of an [redacted] Positive controls used were 0.5 g/ml mitomycin C for the non-activated assay, and 15 g/ml cyclophosphamide for the activated assay. Cells from 2-3 cultures/dose group were harvested after 1 cell cycles (18-24 h), treated with [redacted] and prepared for microscopy. 100 cells were scored for vehicle controls and treated, 25 cells for positive controls.
RESULTS
LY333334 was not toxic to CHO cells at tested concentrations. In the non-activated assay, the incidence of cells with aberrations including gaps ranged from 2-3% at all doses tested. Positive control mitomycin C yielded 64% aberrant cells. In the activated assay, incidence of cells with aberrations including gaps ranged from 0-1% at all doses tested. Treatment with cyclophosphamide, the positive control, gave 44% aberrant cells. Highest concentration of LY333334 (5000 ug/ml) induced an increase in the % cells with diplochromosomes from 0.5% (control) to 3.5%. However, this is not known to be correlated to chromosomal damage. Thus, LY333334 had no significant effect on the percentage of aberrant CHO cells in the presence or absence of metabolic activation.
CONCLUSION
LY333334 is not clastogenic in the CHO chromosome aberration assay.

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REPRODUCTIVE TOXICOLOGY

The reproductive toxicity of LY333334 was assessed in a Segment I study in rats, Segment II studies in rats, mice and rabbits, and a Segment III study in rats. Studies are listed in Table 5.

Table 5 Developmental and Reproductive Toxicity Studies with LY333334

Report Number	Species, Strain Study Type	Number Group, Age	Dose (µg/kg/day)	Route of Administration	Duration of Treatment	Parameters Evaluated	Observations
29	Rat, CD Segment I	20 Males; 13 weeks 20 Females; 9 weeks	Males 0, 30, 100, 300 Females; not treated	Subcutaneous	5 weeks: 4 weeks prior to and during cohabitation until termination	Survival Clinical observations Body weight Food consumption Testicular weight and morphology Sperm concentration, motility Mating Fertility	↓ Prostate weight in males at 300 µg/kg. Redness of the extremities in all treated males. No adverse effects on body weight, food consumption, mating, fertility, testicular weight, testicular morphology, sperm concentration, or sperm motility. NOAEL = 300 µg/kg
15	Rat, CD Segment I	20 Males 20 Females; 9 weeks	Males not treated Females; 0, 30, 100, 300	Subcutaneous	2 weeks prior to and during cohabitation until postmating Day 6	Survival Clinical observations Body weight Food consumption Mating Fertility Gestation survival Fetal parameters	↑ Body weight and food consumption in females at 300 and 300 µg/kg. Redness of the extremities in all treated females. No effects on maternal reproductive or fetal parameters. NOAEL = 300 µg/kg

Report Number	Species, Strain Study Type	Number Group, Age	Dose (µg/kg/day)	Route of Administration	Duration of Treatment	Parameters Evaluated	Observations
14	Rat, CD Segment II	25 Pregnant females; 12 weeks	0, 30, 225, 1000	Subcutaneous	Gestation Days 6 through 17	Survival Clinical observations Body weight Food consumption Reproductive and uterine parameters Fetal viability, weight, and morphology	Redness of the extremities in all treated females. No treatment-related effects on maternal body weight, food consumption, reproductive and uterine parameters, or on fetal viability, weight, or morphology. NOAEL = 1000 µg/kg
17	Mouse, CD-1 Segment II	30 Pregnant females; 12 weeks	0, 30, 225, 1000	Subcutaneous	Gestation Days 6 through 15	Survival Clinical observations Body weight Food consumption Reproductive and uterine parameters Fetal viability, weight, and morphology	↑ Food consumption at 225 and 1000 µg/kg. Redness of the extremities in all treated females. No treatment-related effects on maternal body weight, reproductive and uterine parameters, or on fetal viability, weight, or morphology. NOAEL = 1000 µg/kg

Report Number	Species, Strain Study Type	Number Group, Age	Dose (µg/kg/day)	Route of Administration	Duration of Treatment	Parameters Evaluated	Observations
37	Rabbit, New Zealand White Segment II (pilot)	5 Pregnant females; 7 months	0, 3, 10, 30, 100	Subcutaneous	Gestation Days 7 through 19	Survival Clinical observations Body weight Food consumption Reproductive and uterine parameters Fetal viability, weight and external morphology	↓ Survival at 100 µg/kg. ↑ Blood ionized calcium in all treated groups. Totally resorbed fetuses in all surviving females at 10 µg/kg and 1 female at 3 µg/kg. ↓ Live fetuses per litter at 3 µg/kg. NOAEL = 1.3 µg/kg
35	Rat, CD Segment III/III	25 Pregnant females, adult	0, 30, 225, 1000	Subcutaneous	Gestation Day 6 through Postpartum Day 20	Survival Clinical observations Body weight Food consumption Gestation length Litter size Progeny survival, growth, development, behavior, reproductive performance, external and gross anatomical examination	Redness of the extremities in all treated females; ↓ Pup body weight on PND 14 in F_1 females at 1000 µg/kg. ↓ Motor activity on PND 23 and 60 for F_1 at 1000 µg/kg. Growth retardation in F_1 males at 1000 µg/kg and F_1 females at 225 µg/kg. Maternal/Reproductive NOAEL = 1000 µg/kg Developmental NOAEL = 30 µg/kg

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A Segment I Reproduction Study of LY333334 Administered Subcutaneously to Male CD Rats
(Study R19797)
(Toxicology Report 20)

The purpose of this Segment I male reproduction study was to evaluate the reproductive toxicity of LY333334 following administration to adult male rats.

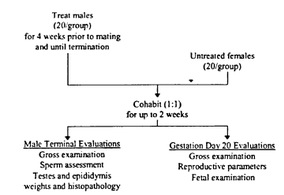
Study:	R19797
Live-phase duration:	2 months
Live-phase dates:	20 October through 21 December 1997
Test article:	LY333334
Chemical name:	Biosynthetic human parathyroid hormone (1-34)
Lot number:	PPD03521
Assigned potency:	0.96 mg/vial
Species and strain:	Rat, CD
Body weight range at	Males: 354 to 397 g
live-phase initiation:	Females: 192 to 230 g
Age at distribution:	Males: approximately 13 weeks Females: approximately 9 weeks
Route:	Males: subcutaneous injection Females: untreated
Number of animals:	20/sex/group
Treatment period:	4 weeks prior to mating continuing for a total of at least 7 weeks

Treatment groups:

Group	Dose of LY333334 (µg/kg/day)
00	0*
01	30
02	100
03	300

*Sodium phosphate monobasic in saline

Study Design:



Parameters evaluated: Clinical signs of toxicity, mortality, behavior, body weight, food consumption, mating performance, fertility, sperm concentration, sperm motion characteristics, maternal reproductive parameters, fetal parameters (external examination for anomalies only), male organ weights, male organ pathology

RESULTS

MALES
Clinical signs: Red extremities in all LY-treated males appr. 30 min after dosing.
Mortality: None
Body weight: No significant effects throughout live phase
Food consumption: No significant effects during premating
EFU: No significant effects during premating

Mating performance and fertility:
No significant differences in evidence of mating (#) (all 20 males in each group mated), fertile matings (#) (20-20-19-18), and time to mating (days).

Sperm concentration:
No treatment-related differences in cauda epididymal weights, cauda epididymal sperm numbers, or sperm concentration expressed as number of sperm per gram of tissue.

Sperm motion:
The following sperm motion parameters were collected for each animal:
- Percent motile sperm
- Percent progressively motile sperm
- Percent linearity
- Percent straightness
- Curvilinear velocity
- Average path velocity
- Straight-line velocity
- Beat/cross frequency
- Amplitude of lateral head displacement

Slight (<6%) but statistically significant decrease in amplitude of lateral head displacement in males of the 100- and 300-µg/kg groups, similar in both groups, as compared to the control group.

No treatment-related differences in percent motility, percent progressively motile, percent linearity, percent straightness, curvilinear velocity, average path velocity, straight-line velocity, or beat/cross frequency.

Organ weights:
The following organs were weighed from all animals that were necropsied at treatment termination:
- Testes
- Epididymis (left)
- Prostate

Statistically significant, treatment-related reductions in absolute and relative (to body weight) weights of the prostate in the 300-µg/kg group of 13% and 14%, respectively. The effect was not clearly associated with significant morphologic prostate changes.

No treatment-related differences in testicular or epididymal weights.

Group		00	01	02	03
Dose (ug/kg/day)		0	30	100	300
Body	Terminal weight (g, average of n=20)	525	537	529	528
Prostate	Absolute wt (g, average of n=20)	1174	1085	1095	1020* (-13%)
	Relative wt (mg/100g, average of n=20)	225	203	208	193* (-14%)

*p≤0.05

Pathology:
All animals were necropsied, and the following tissues from control and high-dose (300 µg/kg) animals were examined microscopically:
- Testis
- Epididymis
- Prostate

Gross pathology
No significant treatment-related gross changes were observed.

Histopathology
Histopathology changes (animal incidence, n/20)

Group		00	03
Dose (ug/kg/day)		0	300
N		20	20
Prostate	Interstitial inflammation, slight, (multifocal	7	1
	Tubular inflammation, slight, focal	0	2

The histopathology findings in the prostate were slight and not clearly related to the prostate weight effect.

FEMALES
Clinical signs: No remarkable findings
Mortality: None
Body weight: No treatment effects on gestational body weight or body weight gain in the untreated females
Food consumption: No data for females

Maternal reproductive parameters:
Cesarean sections were performed on Gestation Day 20.
No effect on number of corpora lutea, implantations and preimplantation loss.
No effect on fetal viability (per litter incidence and litter incidence of: #live fetuses, #resorptions, #non-live implants)

OFFSPRING
Fetal parameters:
No adverse effects on fetal weight, fetal gender, or occurrence of fetal runts.

Fetal anomalies:
Incidence of malformations and deviations:

Anomalies (external examination only; no visceral/skeletal examination)

	Conceptuses (litters) affected			
Group	00	01	02	03
Dose (ug/kg/day)	0	30	100	300
#Examined	275 (20)	294 (20)	291 (19)	265 (18)
MALFORMATIONS				
Abdomen: visceral organs protruding	0 (0)	0 (0)	0 (0)	1 (1)*
Chest: visceral organs protruding	0 (0)	0 (0)	0 (0)	1 (1)*
DEVIATIONS				
Head/Neck: hematoma	2 (2)	2 (2)	4 (2)	6 (3)
Perineum: tail curved	0 (0)	1 (1)	1 (1)	0 (0)
Perineum: tail kinked	0 (0)	0 (0)	0 (0)	1 (1)

* malformations observed in the same (1) fetus

The visceral organ protrusion is rare and was not mentioned in the Sponsor's historical control database. However, the finding has been observed occasionally in the CD rat according to the MARTA historical control database (1992-1994). Since this was a single finding it is no convincing evidence of a treatment-related effect.
The head/neck hematoma incidence was within Sponsor's historical control range (total of 79/14494 conceptuses affected; range 0-8 conceptuses/litter, 0-7 litters; Eli Lilly database, 1989-1997).
The tail findings were not mentioned in the Sponsor's historical control database. However, the finding has been observed in the CD rat according to the MARTA historical control database (1992-1994). Since these were single findings with no clear dose-relationship they constitute no convincing evidence of treatment-related effects.

There were no fetuses with external variations.

CONCLUSIONS
Postdosing redness of extremities (vasodilation) at all doses.
Small, but statistically significant decrease in prostatic weight in males of the 300-µg/kg grou.
The no-observed-adverse-effect level (NOAEL) for paternal and reproductive toxicity was 300 µg/kg, the highest dose tested in this study.

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A Segment I Reproduction Study in Female CD Rats Administered LY333334 Subcutaneously (Study R16697)
(Toxicology Report 15)

The purpose of this Segment I female study was to evaluate reproductive performance and developmental outcome following administration of LY333334 to female CD rats

METHODS
Female Crl:CD(SD) rats (20/group) received subcutaneous injections of 0, 30, 100, or 300 ug/kg/day from 2 weeks prior to mating through a maximum 2-week cohabitation period and continuing through Gestation/Postmating Day 6. Rats in the control group received vehicle . Clinical signs of toxicity, body weight, and food consumption parameters were measured. Females were killed on Gestation Day 20 for evaluation of maternal reproductive parameters and fetal viability, weight, gender, and external morphology.

RESULTS
All dams survived until scheduled termination.
A treatment-related clinical sign was redness of the extremities (vasodilation) in all treated animals following injection of LY333334.
During the 2-week premating period there were increases in body weight and/or food consumption parameters in the 100- and 300-ug/kg groups.
There were no treatment-related effects on mating performance (% succesful mating), fertility indices (% fertile mating) or time-to-mating.
There were no treatment effects on (# or %) corpora lutea, implantations, preimplantation loss, or on (#) live or dead fetuses/litter, resorptions/litter, or live implants/litter (ie fetal viability).
There were no treatment effects on fetal weight, gender, or incidence of fetal runts.
There were no fetuses with external malformations, deviations or variations.

CONCLUSIONS
Postdosing redness of the extremities (vasodilation) in all treated animals.
Increases in maternal body weight and/or food consumption were observed at doses ≥100 ug/kg.
There were no adverse effects on maternal reproductive parameters or fetal parameters.
The no-observed-adverse-effect level (NOAEL) for maternal, reproductive, and developmental toxicity was 300 ug/kg.

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A Segment II Developmental Toxicity Study of LY333334 Administered Subcutaneously to Pregnant CD Rats (Study R13297)
(Toxicology Report 14)

The purpose of this Segment II developmental toxicity study was to evaluate maternal reproductive parameters and fetal outcome following subcutaneous administration of LY333334 to pregnant CD rats during organogenesis.

METHODS
Female Crl:CD(SD) rats (25/group) received injections of 0, 30, 225, or 1000 ug/kg/day on Gestation Days 6 through 17. Rats in the control group received vehicle . Clinical signs, body weight and food consumption were measured. Females were killed on Gestation Day 20 for evaluation of maternal reproductive parameters, and fetal viability, weight, gender, and external morphology.

RESULTS
All dams survived until scheduled termination.
Redness of the extremities was noted 30 minutes after dosing in all or almost all animals of the LY333334 treatment groups. There were no other treatment-related clinical signs.
Maternal body weight and food consumption parameters were not significantly affected.
There were no treatment effects on (# or %) corpora lutea, implantations, preimplantation loss, or on (#) live or dead fetuses/litter, resorptions/litter, or live implants/litter (ie fetal viability).
There were no treatment effects on fetal weight, gender, or incidence of fetal runts.
There were no significant treatment-related effects on the fetal or litter incidences of combined malformations, deviations or variations.

However, there were increases above historical control values in two skeletal deviations in the 30 and/or 225 ug/kg dose groups:

Skull-calvaria occipital bone- incomplete ossification:
Conceptus incidence (Ctrl-LD-MD-HD): 6-19-8-8 (hist ctrl range 0-10/litter)
Skull-hyoid bone-unossified:
Conceptus incidence (Ctrl-LD-MD-HD): 1-7-5-2 (hist ctrl range (0-3/litter)

However, the incidence of these skeletal deviations was not dose-related, and they are therefore unlikely to be treatment effects.

CONCLUSIONS
Postdosing redness of the extremities was noted in all LY333334 treatment groups.
There were no adverse effects of LY treatment on maternal reproductive parameters, or on fetal parameters.
The no-observed-adverse-effect level (NOAEL) for both maternal reproductive and developmental toxicity was 1000 ug/kg/day, the highest dose used in the study.

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A Segment II Developmental Toxicity Study in Pregnant CD-1 Mice Given LY333334 Subcutaneously (Study M09497)
(Toxicology Report 17)

This Segment II developmental toxicity study evaluated maternal reproductive parameters and fetal outcome following administration of LY333334 to pregnant CD-1 mice during organogenesis.

METHODS
Female Crl:CD-1[ICR] mice (30/group) received subcutaneous injections of 0, 30, 225, or 1000 ug/kg/day on Gestation Days 6 through 15. Mice in the control group received subcutaneous injections of the vehicle . Clinical signs of toxicity, body weight, and food consumption parameters were measured. Females were killed on Gestation Day 18 for evaluation of maternal reproductive parameters, and fetal viability, weight, gender, and morphology were examined.

RESULTS
All dams survived until scheduled termination. Redness of the extremities was noted postdosing in all animals from the 225- and 1000-ug/kg groups and one animal from the 30-ug/kg group. Body weight parameters were not affected by LY333334 treatment during organogenesis (Gestation Days 6-16), but was slightly decreased during Gestation Days 16-18.
Relative food consumption (g/day/10g body weight) was slightly increased during Gestation Days 6-16 in the 225- and 1000-ug/kg groups
There were no significant treatment effects on implantations, or on (#) live or dead fetuses/litter, resorptions/litter, or nonlive implants/litter (ie fetal viability).
There were no treatment effects on fetal weight, gender, or incidence of fetal runts.
There were no significant treatment-related effects on the fetal or litter incidences of combined malformations, deviations or variations.

However there were apparently small treatment-related increases in numbers of conceptuses affected with the following individual anomalities:

Malformation (external):
Eyelid open: incidence 0/354 - 0/330 - 1/355 - 2/359 (hist ctrl: 1/1879, range 0-1/litter; 8 litters examined)
Deviations (skeletal):
Axial skeleton-rib cage-rib-interrupted: incidence 1-1-1-3 (hist ctrl: 1/741, range 0-1/litter; 8 litters)
Axial skeleton-vertebral column-presacral vertebra-extra: incidence 0-0-0-2 (hist ctrl: 1/741, range 0-1/litter; 8 litters)
Skull-calvaria-occipital bone incomplete ossification: incidence 4/166 - 12/159 - 13/171 - 12/169 (hist ctrl: 13/741, range 0-5/litter; 6 litters)
Variation (skeletal):
Axial skeleton-rib cage-rib-extra: incidence 35/166 - 46/159 - 62/171 - 58/169 (hist ctrl: 147/741; range 0-35/litter; 6 litters)

The malformation (eyelid open) appeared treatment-related. Since only the incidence in the HD group (2) was above the historical control value (0-1), the NOAEL for this effect would be 225 ug/kg.
The skeletal deviation (rib interrupted) appeared treatment-related. The incidence in the HD group (3) was above the concurrent (1) and historical control value (0-1). The NOAEL for this effect would thus be 225 ug/kg.
The skeletal deviation (extra vertebra) appeared treatment-related. The incidence in the HD group (2) was above the concurrent (0) and historical control value (0-1). The NOAEL for this effect would thus be 225 ug/kg.
The skeletal deviation (incomplete ossification of skull bone) occurred with increased but not dose-related incidence in all treatment groups. It is therefore unclear if this effect was treatment-related.
The skeletal variation (extra rib) appeared treatment-related. The incidence was appr 2x the historical control incidence in the MD and HD groups. The NOAEL for this effect would thus be 30 µg/kg.

CONCLUSIONS
Postdosing redness of the extremities was noted in the 225- and 1000-ug/kg/day treatment groups.
The no-observed-adverse-effect level (NOAEL) for maternal reproductive was 1000 ug/kg/day, the highest dose used in the study.
The no-observed-adverse-effect level (NOAEL) for fetal developmental toxicity was 30 ug/kg/day.
Fetal anomalies included one external malformation (eyelid open) with increased incidence in the 1000 µg/kg group, and a number of skeletal deviations and variations (interrupted rib, extra vertebra, extra rib) with increased incidences in the groups dosed with ≥225 ug/kg.

APPEARS THIS WAY
ON ORIGINAL

A Pilot Developmental Toxicity Study of LY333334 Administered by Subcutaneous Injection to Pregnant New Zealand White Rabbits (Study B00497)
(Toxicology Report 37)

The purpose of this pilot developmental toxicity study was to evaluate maternal toxicity, maternal reproductive parameters, and fetal outcome following administration of LY333334 to pregnant rabbits during organogenesis.

METHODS
LY333334 was injected subcutaneously, once daily, to pregnant New Zealand white rabbits (n=5/group) at doses of 0, 3, 10, 30, or 100 ug/kg/day on Gestation Days (GD) 7 through 19. Control rabbits received vehicle. Blood samples were collected at 0, 2, 4, 6, and 8 hours postdose on GD 7 and 19 from 3/group for measurement of blood ionized calcium. The rabbits were euthanized on GD 28 for evaluation of maternal reproductive parameters and the fetuses were assessed for viability, weight, and external and skeletal morphology.

RESULTS
Mortality
In the 100 ug/kg/day group, 2 rabbits died on GD 16 and 18, and the 3 remaining rabbits were euthanized on GD 18 for humane reasons. These animals had depressed food consumption, body weight loss, reactivity to touch, and blood in waste trays. Exact cause of death was not determined.

Clinical signs
Peripheral vasodilation in all treatment groups
Red material in the waste tray, usually indicative of embryotoxicity, was commonly observed. Vaginal blood loss was seen in all animals of the 100 ukd group.

	Number of Animals Affected				
Observation Dose (µg/kg/day):	0[a]	3	10	30	100
Death	0	0	0	0	2
Euthanized	0	0	0	0	3
Clinical observations					
Blood (red material) in waste tray	0	1	5	5	5
Feces decreased	2	1	1	4	5
Increased reactivity to touch	0	0	0	0	5
Urine absent	0	0	0	2	2
Urogenital/vaginal orifice staining	0	0	0	0	5
Vasodilation of the ears	0	5	5	5	5

[a] Vehicle.

Body weight
Dose-related decreases in body weight gain throughout the treatment period in all treatment groups

	Mean Body Weight Gain (g)				
Gestation Days Dose (µg/kg/day):	0[a]	3	10	30	100
GD 7-10	-16	14	-25	-83	-93
GD 7-20	82	-36	-126	-342	--[b]
GD 20-28	126	97	169	220	--[b]

[a] Vehicle.
[b] Body weight data were not available due to euthanasia or death on or before GD 18.

Food consumption

Reduction in the 30- and 100-ug/kg/day groups.

	Mean Food Consumption (% change relative to control)			
Time Period Dose (µg/kg/day):	3	10	30	100
GD 4-7	24	61	20	35
GD 7-10	-3	33	-21	-51
GD 10-14	18	38	-43	-56
GD 14-17	-2	19	-53	-49
GD 24-28	29	52	36	--[a]

[a] Food consumption data were not available due to euthanasia or death on or before GD 18.

Blood ionized calcium
Increase in all treatment groups on both GD 7 and 19. Table summarizes % increase over baseline. Blood ionized calcium levels were increased in all treated, with the nominally highest increase (peak level) in the 3ukd group at 2h postdose, but the longest duration of increase in the 30 and 100 ukd groups lasting up to 8h postdose. Thus, the higher dose groups appeared to respond in a delayed and prolonged manner. The level attained in the 100 ukd group was particularly low even at 8h postdose.

	pH Adjusted Calcium[a]				
Administered Dose (µg/kg/day):	0[b]	3	10	30	100
Day 7 Time Point (hr)					
0	1.89	1.91	2.17	2.16	2.16
2	1.93 (2.1)	2.30 (20.4)	2.47 (13.8)	2.37 (9.7)	2.16 -
4	1.90 (0.5)	2.01 (5.2)	2.50 (15.2)	2.51 (16.2)	2.18 (0.9)
6	1.81 -	1.96 (2.6)	2.35 (8.3)	2.46 (13.9)	2.24 (3.7)
8	1.90 (0.5)	1.96 (2.6)	2.17 -	2.31 (6.9)	2.28 (5.6)
Day 19 Time Point (hr)					
0	2.04	2.11	1.97	1.89	--[c]
2	1.89 -	2.61 (23.7)	2.31 (17.3)	2.04 (7.9)	--[c]
4	1.90 -	2.49 (18.0)	2.26 (14.7)	2.18 (15.3)	--[c]
6	1.91 -	2.00 -	2.10 (6.6)	2.11 (11.6)	--[c]
8	1.87 -	1.95 -	2.06 (4.6)	2.03 (7.4)	--[c]

[a] mmol/L (% increase from 0-hour baseline).
[b] Vehicle.
[c] Blood samples were not available due to euthanasia or death on or before GD 18.

Maternal reproductive parameters

Resorptions:
Incidence of resorptions markedly increased in the 3-ug/kg/day group. All conceptuses in the groups given 10 or 30 ug/kg/day were resorptions.
No other significant effects.

Parameter[a] Dose (µg/kg/day):	Observation				
	0[b]	3	10	30	100
Pregnant females	5	5	5	5	5
Corpora lutea	9.2	9.8	7.8	9.6	--[c]
Implantations	9.0	9.0	6.4[d]	9.8	--[c]
Preimplantation loss/dam	0.2	0.8	1.4	0	--[c]
Resorptions (early and late)	0.6	6.4	6.4	9.8	--[c]
Resorptions/litter (%)	6.7	69*	100*	100*	--[c]
Litters with resorption sites only	0	1	5*	5*	
Live fetuses/litter	8.4	2.6*	0*	0*	--[c]

* = p<.05.
[a] All data expressed as mean values for the group, except for the number of pregnant females and dead fetuses.
[b] Vehicle.
[c] Maternal data were not available due to euthanasia or death on or before GD 18.
[d] Significantly different from the 30-µg/kg group, p<.05.

Fetal parameters

Fetal weight and external and skeletal morphology:
No effects in the 3 ug/kg/day group. This was the only LY333334 treatment group that yielded viable fetuses.

Affected implants (total of resorptions, dead fetuses, and malformed live fetuses) (%):
Increased in all groups.

Malformations:
Conceptus and litter incidence increased in 3 ug/kg/day group. Malformations included absent incisor, and/or absent left vertebral arch (in two viable concepituses), and umbilical torsion (in a late resorption). Since the sample size in the 3-ug/kg group was low (n=13 fetuses) compared to the controls (n=42 fetuses) and there were no evaluated fetuses in the other treatment groups it is unclear whether the increase in malformation incidence in the 3 ug/kg/day group was significant. It is also unclear whether these malformations were due to treatment-related maternal toxicity or hypercalcemia-induced embryotoxicity or a selective developmental effect.

Parameter[a]	Administered Dose (µg/kg/day)				
	0[b]	3	10	30	100
Fetal weight (g)	35.97	37.61	--[c]	--[c]	--[d]
Number of conceptuses with malformations	0	3	--[c]	--[c]	--[d]
Number of fetuses with multiple malformations	0	1	--[c]	--[c]	--[d]
Affected implants/litter (%)	6.7	73*	100*	100*	--[d]
Malformations-conceptus (litter) incidences					
Umbilical torsion	0 (0)	1 (1)			
Cervical vertebra – absent left arch	0 (0)	1 (1)			
Maxilla – absent incisor	0 (0)	2 (2)			

* = p<.05.
[a] All data are expressed as mean values for the group, except for malformation frequency.
[b] Vehicle.
[c] Fetal data were not available because all conceptuses were resorptions.
[d] Fetal data were not available due to euthanasia or death on or before GD 18.

CONCLUSIONS
Maternal exposure to LY333334, as evidenced by increased blood ionized calcium levels and peripheral vasodilation, was demonstrated in all treatment groups.

The treatment-related mortality and morbidity in the 100 ug/kg/day parental animals may have resulted from perturbations in calcium homeostasis, in conjunction with the marked reductions in food consumption and body weight observed in this group.

Circulating calcium ions readily cross the placenta into fetal circulation and maternal hypercalcemia results in fetal hypercalcemia, which induces suppression of fetal parathyroid function. However, the placenta blocks passage of both PTH and calcitonin from the maternal to he fetal circulation.

Hypercalcemia in rats and humans is associated with fetal growth retardation, abortion or stillbirth, and neonatal and maternal morbidity. Thus, the embryotoxicity that occurred in all treatment groups may have resulted from the elevations in maternal blood ionized calcium observed following LY333334 administration, with the duration of the elevation being the primary determinant for the toxicity. The reductions in food consumption and body weight may have also been contributing factors to the embryotoxicity.

The maternal deaths and embryotoxicity in the 100 ukd group was somewhat paradoxical considering that blood Ca levels did not attain very high levels within the 8h post dose measurement period.

It is unclear whether the malformations observed in 3 fetuses from the 3 ug/kg/day group were due to treatment-related maternal toxicity or embryotoxicity or whether they were a selective developmental effect related to suppression of fetal PTH secretion.

A no-observed-adverse-effect level (NOAEL) was not determined in this study.

In rats and mice, adverse effects on reproductive or developmental effects were not observed with doses of LY333334 up to 1000 ug/kg/day. This difference may be due to the fact that the New Zealand white rabbit has higher baseline serum calcium levels than other mammalian species. This may highten the rabbit's sensitivity to treatment with PTH peptides, since they will cause further increases in serum Ca. These high levels of serum Ca may be particularly toxic to the rabbit fetus during pregnancy.

Assuming that the rabbit findings were due to hypercalcemia they are unlikely to represent a clinical safety concern, because hypercalcemia has not been found to be an important side effect of LY333334 therapy in humans administered 20 ug/day for up to 23 months.

Because the hypercalcemia cannot be separated from any direct effects of LY333334 in the rabbit, the Sponsor felt that this species is not a good model for testing developmental toxicity to LY333334.

APPEARS THIS WAY
ON ORIGINAL

Study of the Effects of LY333334 on Embryo/Fetal and Postnatal Development, Including Maternal Function, in the Rat (Study WIL-353001)
(Toxicology Report 35)

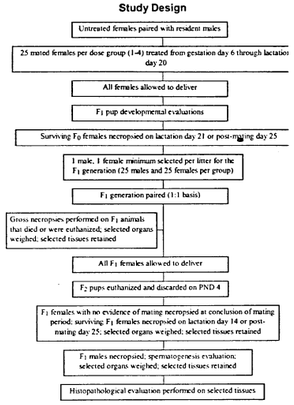
The purpose of this study was to determine the potential of LY333334 to induce developmental toxicity in rats after maternal exposure during the period of major organogenesis; and to determine the potential adverse effects of maternal LY333334 exposure from implantation to weaning on pregnancy, parturition and lactation of the maternal animals, and on the growth, viability, and development of the F1 and F2 neonates. Reproductive performance of the F1 generation was also evaluated.

METHODS
LY333334 was administered by subcutaneous injection to pregnant Crl:CD(SD)IGS BR female rats (n=25/group), once daily, from gestation day 6 through lactation day 20, at doses of 0, 30, 225 and 1000 ug/kg/day. Dose volume was 1 ml/kg. Control animals (n=25) received vehicle [redacted]

Surviving F0 [redacted] females were allowed to deliver and rear their offspring to lactation day 21. F1 pups (one male and one female per litter, n=25/sex/group), 9-14 days old, were randomly selected for the F1 generation, and evaluated for sensory function, behavior, physical and functional development, and estrous cycling. Other F1 pups were euthanized and necropsied on PND 21. Selected F1 animals were mated when 85-90 days old, and surviving F1 females were allowed to deliver. F2 pups were euthanized and discarded on postnatal day (PND) 4. F1 females were necropsied on lactation day 14. F1 males were euthanized following the last necropsy of the F1 females.

APPEARS THIS WAY
ON ORIGINAL

Study Design



RESULTS
F0 maternal generation
Clinical signs/Survival
Reddened and warm extremities at the time of dosing with dose-related incidence in the treated groups. No other treatment-related clinical signs.
No adverse effect on F0 gestation or the process of parturition (dystocia). All F0 females survived to the scheduled necropsy.

Body weight/Food consumption
No effects on mean body weights, body weight changes and food consumption in the treated group F0 females.

F0 necropsy data
No internal findings related to test article administration at any dose level.

F1 litter data

No effect on F1 numbers of pups born per litter, percentages of males per litter, live litter sizes on PND 0, postnatal pup survival (percent per litter) and general physical condition of the pups. Necropsy findings for pups that died or were euthanized on PND 21 apparently not treatment-related.

F1 pup body weight
Mean F1 female pup body weights reduced in the 1000 ug/kg/day group on PND 7, 14* and 21 (ca. 5% reduction at each time interval).
Mean F1 male pup body weights reduced in the 1000 ug/kg/day group on PND 14 and 21 (6% and 3%, respectively).
Mean F1 pup body weights unaffected on PND 1 and 4 (females) and 1,4 and 7 (males).
Mean F1 pup body weights (male and female average) reduced in the 1000 ug/kg/day group on PND 14* and 21 (7% and 8% reduction).
*statistically significant effect

F1 development and behaviour
No effects on pre-weaning indicators of physical and functional development (pinnal detachment, surface righting response and eye opening) of the F1 pups in the treated groups.
No treatment-related effects on achievement of vaginal patency.
Slight but statistically significant delay in achievement of balanopreputial separation in males in the 1000 ug/kg/day group (Mean day of acquisition: 43-43.5-43.8-44.5* days)
No significant adverse effects in auditory startle test response, and learning and memory (Biel maze swimming trial).
Motor activity was reduced in the 1000 ug/kg/day group males and females on PND 23 and 60. The reduction was similar for total activity and ambulatory activity separatedly.

Motor activity: Total activity (counts)

	PND 23	PND 60
Males	-26%	-20%*
Females	-23%	-18%

*statistically significant effect

F1 generation
Clinical signs/Survival
One F1 male and two F1 females in the 225 ug/kg/day group died or were euthanized in extremis. The morbies were not attributed to the test article since no deaths occurred in the 1000 ug/kg/day group. No remarkable clinical signs reported in any of the F1 animals.

Body weight
WEEKLY
Males
Body weight gain significantly reduced in the 1000 ukd group during weeks 7-8 and 8-9, but not after that through week 23. Body weight significantly reduced during weeks 8,9,10,11 (7-11%). No effect on body weight or body weight gain at 30 or 225 ukd.
Females
Body weight gain significantly reduced in the 1000 ukd group during weeks 7-8, 8-9, and 9-10, but not after that through week 15 (end of premating period). Body weight significantly reduced throughout premating period, i.e., weeks 7-15 (9-12%).
Body weight gain significantly reduced in the 225 ukd group during weeks 8-9 and 11-12, but not at other times. Body weight significantly reduced from week 9-15 (4-8%).
No effect on body weight or body weight gain at 30 ukd.

GESTATION
Females
Mean body weights reduced in1000 ukd F1 females throughout gestation, but body weight gains unaffected. Mean body weights reduced in225 ukd F1 females on GD 0,6,9, but body weight gains

again unaffected. The decreased body weights were attributed to the above described premating effect and not to an effect on gestational body weight gain.
No effects on body weight or body weight gain in 30 ukd F1 females

LACTATION
Females
Mean body weights reduced in1000 ukd F1 females throughout lactation, but body weight gains unaffected. The decreased body weights were attributed to the above described premating effect and not to an effect on gestational or lactational body weight gain.
No effects on body weight or body weight gain in 30 and 225 ukd F1 females.

F1 reproductive parameters and organ weights
Estrous cycles, mating and (male or female) fertility, the duration of gestation, parturition, spermatogenic endpoints and mean reproductive organ weights in the F1 rats of the treated groups not significantly affected by maternal treatment.

F1 necropsy data
No treatment-related F1 necropsy findings.

F2 litter data
No effects on F2 numbers of pups born per litter, percentages of males per litter, live litter sizes on PND 0, pup body weights, and the general physical condition of the pups. Postnatal pup survival (percent per litter) over the birth-PND 4 time interval was slightly but not significantly reduced in the 1000 ukd group as compared to controls (97.5%-98.6%-96.8%-94.2%) because 1 female had total litter loss on PND 1. Necropsy findings for pups that died appeared not teatment-related.

CONCLUSIONS
NOAEL for F0 maternal and reproductive toxicity was 1000 ug/kg/day.
NOAEL for F1 developmental toxicity was 30 ug/kg/day (F0 maternal dose)

Developmental toxicity consisted of growth retardation (body weight gain and/or body weight reduction) and reduced motor activity.

Growth retardation occurred in the F1 males of the 1000 ug/kg/day group and in the F1 females in the 225 and 1000 ug/kg/day groups during the initial part of the premating growth period.
As a consequence, body weights for males in the 1000 ug/kg/day group were significantly reduced for approximately one month compared to the control group. Body weights for females in the 225 ug/kg/day group were significantly reduced during much of the growth period. Body weights for females in the 1000 ug/kg/day group were significantly reduced throughout the growth, gestation and lactation periods.

Motor activity was reduced in F1 male and female rats at the 1000 ug/kg/day dose level through 2 months of age.

APPEARS THIS WAY
ON ORIGINAL

SUMMARY AND EVALUATION OF REPROTOXICITY STUDIES

In Segment I reproduction studies in male and female CD rats, no significant effects were observed after administration of subcutaneous doses of 30 to 300 µg/kg/day.

In a Segment II developmental toxicity study in pregnant CD rats, there were no significant findings at daily subcutaneous doses of 30 to 1000 µg/kg.
In a Segment II developmental toxicity study in pregnant CD-1 mice, there was an increase in the incidence of one malformation (eyelid open) at a daily subcutaneous dose of 1000 ug/kg. There were also increases in the incidences of a number of skeletal deviations and variations (interrupted rib, extra vertebra, extra rib) at subcutaneous doses ≥ 225 µg/kg.

In a pilot Segment II developmental study in pregnant New Zealand white rabbits, vaginal bleeding and total resorption of all fetuses was observed in all animals given subcutaneous doses of 10 to 100 µg/kg. At 100 µg/kg, 2 pregnant animals died and the remaining 3 animals were euthanized. These animals had excessive reductions in food consumption and body weight. At 3 ug/kg there was an increase in the incidence of resorptions, with 1 of 5 animals having all conceptuses resorbed, and a resulting reduction in litter size. The embryotoxicity observed in pregnant rabbits was considered to have resulted from the elevations in blood ionized calcium following LY333334 administration. Since sustained hypercalcemia is not expected to be a side effect of LY333334 therapy in humans, these findings in rabbits are not considered to represent a relevant clinical safety concern for non-pregnant women or for men.

In a combined Segment II/Segment III perinatal and postnatal study in CD rats, there was no maternal and reproductive toxicity at doses of 30 to 1000 µg/kg/day. However, there was developmental toxicity in the F1 progeny which consisted of mild growth retardation in F1 male rats at 1000 ug/kg and in F1 female rats at 225 and 1000 µg/kg/day, and reduced motor activity in F1 male and female rats at 1000 µg/kg/day.

APPEARS THIS WAY
ON ORIGINAL

ADME

Absorption, Distribution, Metabolism, and Excretion (ADME)
The pharmacokinetics of LY333334 after single and repeated subcutaneous doses was evaluated in male and female rats and in cynomolgus monkeys. Administration of LY333334 over 3 months did not appear to have a significant effect on induction of liver metabolizing enzymes. Based on information in the published literature, liver, kidney, and bone are primarily responsible for the metabolism and clearance of PTH.

Pharmacokinetics and Toxicokinetics
Pharmacokinetic and toxicokinetic studies with LY333334 administered by the subcutaneous route indicate that LY333334 is rapidly absorbed and eliminated from the circulation in all species studied. Generally, Tmax was approximately 15 to 30 minutes after dose administration and T½ was approximately 15 to 30 minutes. When multiple dose levels were administered, AUC and Cmax was found to increase with dose. No evidence for accumulation was observed after daily repeated dosing in rats (up to 18 months) or monkeys (up to 17 months). There were no apparent gender differences in the single-dose serum pharmacokinetics of LY333334 in the rat or the monkey at doses of 10 µg/kg or less.

Bioavailability
Absolute bioavailability studies were conducted in male and female rats and monkeys. Bioavailability was estimated at 61% and 55% for male and female rats, respectively, administered 10 µg/kg LY333334 by the subcutaneous and intravenous routes. In an earlier study, estimated bioavailability following a subcutaneous dose of 300 µg/kg was 8.5% for female rats and 20% for male rats. In cynomolgus monkeys, bioavailability following a 10 µg/kg subcutaneous dose was estimated at 39% for males and 34% for females. These studies in rats and monkeys demonstrated that LY333334 was well absorbed from the subcutaneous space, and that bioavailability was comparable between males and females at the 10 µg/kg dose level.

Metabolism, Distribution, and Excretion
Distribution, metabolism, and excretion studies were not performed with LY333334. The ultimate fate of this peptide is expected to be metabolism into individual constituent amino acids, which are then reincorporated into the normal protein pool. However, there is a large amount of information in the published literature describing the metabolism and clearance of PTH(1-84), PTH(1-34), and related peptides, indicating that liver, kidney, and bone are the major sites of metabolism and clearance for PTH(1-84) and its amino- and carboxy-terminal fragments. In rat, dog, cow, and man, sites of cleavage have been identified at amino acids 33 and 37. These data are consistent with generation of peptide metabolites similar in structure to the bioactive amino-terminal domain, PTH(1-34).
Despite extensive efforts, the PTH(1-34) fragment has not been unequivocally identified as a natural metabolite that circulates in animals or humans. Although peptides with molecular weights less than that of PTH(1-84) with PTH-like bioactivity have been isolated, the identity of these peptides has not been definitively determined.

APPEARS THIS WAY
ON ORIGINAL

The following tables summarize the results obtained for the pharmacokinetic parameters C_{max}, T_{max}, $T_{1/2}$ and $AUC_{(0-\tau)}$, in repeat-dose studies in rats (6 weeks, 6 months, 24 months) and monkeys (3 months, 4 months, 1 year, 18 months). The results on C_{max} and AUC were utilized to calculate multiples of human C_{max} and AUC values attained in repeat dose animal studies.

Table 4 Summary of Pharmacokinetic Parameters for Male and Female Rats After Daily Subcutaneous Administration of LY333334 for up to 18 Months

Report/ Duration	Time of Sampling	Gender	Dose (µg/kg)	Pharmacokinetic Parameters C_{max} (ng/mL)	T_{max} (minutes)	$T_{1/2}$ (minutes)	$AUC_{(0-\infty)}$ (ng•hour/mL)
ADME 4 6 weeks	Day 8	M	10	2.3	15	ND[b]	ND
		F		1.6	15	ND	ND
	Day 43	M		4.6	15	ND	ND
		F		2.8	15	ND	ND
	Day 8	M	30	7.1	15	ND	ND
		F		6.9	15	ND	ND
	Day 43	M		12.0	15	ND	ND
		F		9.6	15	ND	ND
	Day 8	M	100	30.0	15	13	22.9
		F		24.0	15	13	15.7
	Day 43	M		64.0	15	14	45.0
		F		35.0	15	11	26.3
	Day 8	M	300	112.0	30	ND	ND
		F		86.0	15	ND	ND
	Day 43	M		195.0	15	ND	ND
		F		120.0	5	ND	ND
ADME 7 6 months	Day 0	M	10	3.8[a]	ND	ND	ND
		F		3.2[a]	ND	ND	ND
	Day 140	M		3.1[a]	ND	ND	ND
		F		3.5[a]	ND	ND	ND
	Day 0	M	30	8.4[a]	ND	ND	ND
		F		6.8[a]	ND	ND	ND
	Day 140	M		10.3[a]	ND	ND	ND
		F		9.2[a]	ND	ND	ND
	Day 0	M	100	27.1	15	25	22.0
		F		25.8	15	26	16.1
	Day 140	M		75.0	30	26	53.6
		F		52.3	30	25	40.1

continued

Table 4 (continued) Summary of Pharmacokinetic Parameters for Male and Female Rats After Daily Subcutaneous Administration of LY333334 for up to 18 Months

Report/ Duration	Time of Sampling	Gender	Dose (µg/kg)	Pharmacokinetic Parameters C_{max} (ng/mL)	T_{max} (minutes)	$T_{1/2}$ (minutes)	$AUC_{0-\infty}$ (ng•hour/mL)
ADME 23 18 months	6 months	M	5	2.3	15	19	1.4[c]
		F		1.3	15	15	0.8[c]
	12 months	M		1.0	15	11	0.4[c]
		F		2.2	15	26	1.6[c]
	18 months	M		0.6	15	34	0.6[c]
		F		1.0	15	10	0.5[c]
	6 months	M	30	10.3	15	37	8.7[c]
		F		10.9	15	16	5.8[c]
	12 months	M		5.2	30	28	4.5[c]
		F		12.5	15	20	9.2[c]
	18 months	M		3.1	15	32	3.6[c]
		F		6.9	15	27	4.6[c]
	6 months	M	75	35.4	15	20	23.5[c]
		F		20.1	15	24	13.9[c]
	12 months	M		16.4	30	28	13.7[c]
		F		28.4	30	31	25.6[c]
	18 months	M		8.3	30	62	14.0[c]
		F		21.1	15	27	11.9[c]

Abbreviations: M = males; F = females; iv = intravenous; sc = subcutaneous; kg = kilogram; mL = milliliter; C_{max} = maximum concentration; T_{max} = time of maximum concentration, T ½ = half-life, AUC 0-∞ = area under the curve extrapolated to infinity; pg = picogram; ng = nanogram.
[a]Samples collected at 0.25 hour post dose (T_{max} was assumed to be 0.25 hour).
[b] ND = not determined.
[c]AUC 0 to τ, where τ is the next dose (24 h).

Comment on TK observations written by Reviewer's pet rat:
"Tee',.k =-p77gggggggrfof000uttv "

Table 5 Summary of Pharmacokinetic Parameters for Male and Female Cynomolgus Monkeys After Daily Subcutaneous Administration of LY333334 up to 17 months

ADME Report /Duration	Time of sampling	Gender	Dose (µg/kg)	Pharmacokinetic Parameters C_{max} (ng/mL)	T_{max} (minutes)	AUC_{0-t} (ng•hour/mL)
ADME 9 3 months	(Days 0/1,7,21,51,84)	M	2	0.5-0.7[a]	ND[b]	ND
		F		0.5-0.7[a]	ND	ND
		M	10	4.0-6.0[a]	ND	ND
		F		3.5-5.0[a]	ND	ND
		M	20	14.9-18.6[a]	ND	ND
		F		6.8-9.8[a]	ND	ND
		M	40	18.5-31.5[a]	ND	ND
		F		16.2-23.9[a]	ND	ND
ADME 14 1 year	Week 2	M	0.5	0.2	30	ND
		F		0.1	30	ND
	Week 26	M		0.2	30	ND
		F		0.2	30	ND
	Week 51	M		0.1	30	ND
		F		0.2	30	ND
	Week 2	M	2	0.9	34	ND
		F		0.8	30	ND
	Week 26	M		0.7	38	ND
		F		0.6	38	ND
	Week 51	M		0.6	30	ND
		F		0.7	30	ND
	Week 2	M	10	5.1	30	ND
		F		4.0	30	ND
	Week 26	M		6.7	30	ND
		F		3.2	35	ND
	Week 51	M		4.1	30	ND
		F		ND	ND	ND
ADME 15[e] 17 months	1 month	F[d]	1	ND	ND	0.25
	7 months	F[d]		ND	ND	0.46
	11 months	F[d]		ND	ND	0.29
	17 months	F[d]		ND	ND	0.24
	1 month	F[d]	5	ND	ND	2.03
	7 months	F[d]		ND	ND	2.53
	11 months	F[d]		ND	ND	2.45
	17 months	F[d]		ND	ND	1.85
ADME 19 3 months	1 month	F	40	18.6[c]	ND	ND
	3 months			4.7[c]	ND	ND

Abbreviations: M = males; F = females; kg = kilogram; mL = milliliter; C_{max} = maximum concentration; T_{max} = time of maximum concentration, T ½ = half-life, AUC 0-t = area under the curve to time "t"; pg = picogram; ng = nanogram; [a] Immunoreactivity measured at a single time-point 1 hour postdose. Values are the ranges observed at the indicated dose level over the duration of the study; [b] ND = not determined; [c] Immunoreactivity measured at a single time-point 1 hour post-dose; [d] Ovariectomized; [e] Sampling conducted using a sparse sampling scheme with a single sample per animal

OVERALL SUMMARY AND EVALUATION

INTRODUCTION

Parathyroid hormone (PTH) can differentially affect bone mass depending on its mode of administration. Given intermittently (once daily) PTH has an anabolic action on bone, stimulating bone formation, and increasing bone mass and bone strength. Although the exact mechanisms involved are unknown, the anabolic action is thought to be based on a selective increase in the activity and number of osteoblasts leading to new bone apposition on previously quiescent surfaces. By contrast, continuous infusion or closely spaced injections of PTH predominantly activate the osteoclast and are associated with a shift in the response from bone formation to bone resorption and a decrease in bone mass. The different biological responses to PTH in bone are ultimately believed to be mediated by the (pre)osteoblast and a differential effect on its cellular activity and gene expression.
Teriparatide (Forteo™) is a biosynthetic recombinant product identical in sequence to the first 34 amino acids of endogenous human PTH. The compound appears to bind to the same receptor and have similar biological actions as endogenous PTH(1-84) and synthetic PTH(1-34). The current NDA is submitted for [redacted] and [redacted]. A variety of pharmacology and toxicology studies were performed generally with daily subcutaneous administration to evaluate the nonclinical efficacy and safety of the compound[1].

EFFICACY PHARMACOLOGY

The most clinically relevant efficacy pharmacology study was an 18-month bone quality study in cynomolgus monkeys. The study was designed to address the efficacy and safety of teriparatide on vertebral and non-vertebral (cancellous, cortical) bone in a species with cortical bone remodeling. In particular this study was carried out to ensure that the increase in trabecular bone is not achieved at the expense of cortical bone.

Teriparatide at doses of 1 and 5 µg/kg (equivalent to approximately 1x and 7x the human exposure at the 20 µg daily clinical dose) dose-dependently increased bone mass in the vertebrae and in the cancellous bone of the distal radius and femoral neck. This increase was associated with increased bone formation and improved trabecular architecture and lead to increased bone strength of the lumbar spine and the femoral neck. Data suggested that there was new bone apposition on existing trabecular surfaces and bone resorption inside the trabeculae (intratrabecular tunneling) with an overall increase in trabecular connectivity and bone mass.
The data indicate that teriparatide improves cancellous bone quality, and support the efficacy and safety of once daily teriparatide treatment with regard to its effects on cancellous bone.

Vertebral properties in monkeys treated with teriparatide

	Sham	OVX				
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
BMD (Change from baseline, %)	5.3%*	0.6%	9.1%*	15.4%*	7.2%*	7.3%*
Yield force F_y (N)	1738	1499	1915*	2113*	1899*	1792*
BV/TV (%)	26	23	33*	35*	30*	27*
Tb N (mm^{-1})	2.7	2.5	3.4*	3.6*	2.9*	2.9*
BFR/BV	23	20	36*	47*	26	24

*significantly different from OVX
PTH1 = treatment with 1 ug/kg/day, and PTH5 = treatment with 5 ug/kg/day, for 18 months
PTH1W = treatment with 1 ug/kg/day, and PTH5W = treatment with 5 ug/kg/day, for 12 months, with 6 month withdrawal period

[1] For a more extensive summary of bone quality, cardiovascular toxicity, nephrotoxicity and carcinogenicity data please refer to the "Advisory Committee Briefing Document" (July 27, 2001) (APPENDIX)

Femoral neck properties in monkeys treated with teriparatide

	Sham	OVX				
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	1738	1499	1915*	2113*	1899*	1792*
BV/TV (%)	44	39	54*	57*	48*	50*
Tb.N (mm^{-1})	2.9	2.6	3.5*	3.4*	3.1*	3.3*
BFR/TV (%/year)	21	25	42	59*	27	26

*significantly different from OVX
PTH1 = treatment with 1 ug/kg/day, and PTH5 = treatment with 5 ug/kg/day, for 18 months
PTH1W = treatment with 1 ug/kg/day, and PTH5W = treatment with 5 ug/kg/day, for 12 months, with 6 month withdrawal period

In cortical bone, teriparatide dose-dependently stimulated intracortical (osteonal) bone remodeling and increased intracortical porosity, while simultaneously increasing endocortical and/or periosteal bone formation with unclear effects on resorption. In the humerus midshaft this resulted in a slight increase in bone thickness and morphometric cortical bone area and a slight decrease in medullary area. At this cortical bone site, biomechanical strength was increased at the high dose of 5 µg/kg but the effect was not statistically significantly. Biomechanical testing of femoral diaphyseal specimens showed a slight impairment in the material properties of the cortical bone at the 5 µg/kg dose, possibly due to an increase in cortical porosity.

Humerus midshaft properties in monkeys treated with teriparatide

	Sham	OVX				
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	725	636	654	680	689	707
Cortical thickness (mm)	1.74	1.63	1.68	1.80*	1.66	1.72
Porosity (%)	1.3*	2.6	4.7	6.7*	2.3	6.5*
Cortical Area (mm^2)	37	35	38	41*	38	41*
Medullary Area (mm^2)	16	18	17	15	17	17
MS/BS (Endocortical) (%)	3.1	21	25	42*	12	9.2
MS/BS (Periosteal) (%)	1.8	10	8.6	11.5	4.0	2.0

*significantly different from OVX

Data suggested that treatment with teriparatide can result in two opposing effects in cortical bone; increased endocortical porosity due to increased intracortical remodeling, and altered bone thickness due to periosteal and/or endosteal surface effects. In the mid-humerus this lead to an increase in bone strength because the dominating effect was an increase in cortical thickness resulting in an increase in cross-sectional moment of inertia (CSMI). The increase in porosity was observed at all cortical bone sites evaluated but effects on bone thickness and area were not evident at all sites. Since bone strength is determined by the balance between thickness and porosity it is therefore unclear whether the results obtained in the humerus (the only cortical bone for which strength was determined) can be generalized to all cortical sites. It is conceivable that strength is maintained or improved at sites where bone thickness is sufficiently increased (humerus, mid radius) but impaired at sites were increased porosity is unopposed by increased thickness (mid femur). Uncertainly about the effect of teriparatide on cortical thickness is corroborated by the morphometric data on a delayed decrease in cortical thickness of the iliac crest.

Withdrawal of teriparatide treatment after 12 months in the monkey had different effects in cancellous and cortical bone. At predominantly cancellous sites (vertebra and femoral neck), bone mass and bone strength were usually decreased after withdrawal, and histomorphometric parameters of bone formation were reduced. At cortical bone sites, strength and thickness were not affected after treatment withdrawal, while porosity and parameters of bone formation were largely reversed. Apparently, teriparatide withdrawal lead to a termination of the increase in intracortical remodeling and bone surface mineral apposition reflected by the reduction in cortical bone formation. However, this was not associated with a reversal of the established changes in endocortical and/or periosteal bone perimeter within the time of the withdrawal period (6 months). The different effects of withdrawal may be related to the shorter duration of the bone turnover cycle in cancellous than in cortical bone.

Thus, although the data from the monkey study do not suggest a significant safety issue for cortical bone, some concern about the effects of long term treatment with teriparatide on the quality of cortical bone remains.

In the rabbit tibial and femoral midshaft similar results were obtained as in the monkey humerus. Although at these cortical bone sites porosity was increased bone strength was increased as a result of a compensatory increase in bone thickness. Vertebral BMD and strength in this species were not affected by teriparatide. Although the lack of a vertebral effect suggests that the rabbit is not a good animal model for testing of teriparatide the cortical bone data confirm the conclusion from the monkey study that the compound improves the biomechanical strength of cortical bone.

The data from the various rat studies demonstrated that teriparatide increased cancellous and cortical bone mass and strength. However, unlike rabbit, monkey and human, the rat is a species that lacks Haversian (osteonal) remodeling. Therefore the data on cortical bone in the rat have limited clinical relevance.

The predictive value of the monkey study data was generally supported by the clinical bone mass and fracture data obtained in the large phase III osteoporosis trials (GHAC, GHAJ). These studies demonstrated marked efficacy of teriparatide to increase vertebral BMD and reduce vertebral fractures. In addition, the incidence of nonvertebral fragility fractures was reduced at most appendicular skeletal sites although the effect was less than in the axial skeleton and appeared to vary by site.

SAFETY PHARMACOLOGY

Acute cardiovascular effects of teriparatide were evaluated in rats and dogs. In these species, the vasodilatory, hypotensive action of teriparatide was maximal at 2h post dosing. A compensatory increase in heart rate was observed at ≥23 µg/kg and at 6 µg/kg in the rat and dog, respectively. In a 3-month toxicity study in cynomolgus monkeys increases in R-amplitude in electrocardiograms were observed at 10 µg/kg in males, and in a 12-month monkey toxicity study PQ interval decreases were seen at 10 µg/kg in males. Slight increases in heart rate were observed in the 12-month study at 2 µg/kg and 10 µg/kg in males only. These preclinical data indicate that teriparatide can cause vasodilation resulting in hypotension and a compensatory heart rate increase. Based on their transient nature the ECG changes observed in the monkey are of unclear significance. Clinical trial data have shown increases in pulse rate within 6 hours after dosing but no clinically significant effects on the rates of syncope, dizziness or vertigo.

Renal pathology with dose-related incidence and severity was observed in cynomologus monkeys in 3- month and 12-month toxicity studies. The doses used in these studies were 2, 10, 20, 40 µg/kg and 0.5, 2, 10 µg/kg, respectively. Interstitial medullary expansion was associated with increased deposition of extracellular matrix in the outer medulla of the lowest dose groups (0.5 µg/kg and 2 µg/kg) but extended into the medullary rays at higher doses. It is unclear if the renal lesions were a direct effect of teriparatide or an indirect consequence of increased calcium reabsorption in the distal nephron or of the observed increase in serum ionized calcium.
In a 4-month reversibility toxicity study in monkeys at 40 ug/kg nephropathy was evidenced by medullary interstitial expansion with deposition of basophilic material, multifocal interstitial inflammation, multifocal mineralization, multifocal tubular regeneration and multifocal tubular dilatation and inflammation. One animal had acute renal failure, accompanied by declining renal function. Renal function parameters were not affected in the other monkeys. The kidney findings including renal failure appeared partially reversible.

The expanded medullary interstitium following chronic dosing in monkeys was observed at the low doses in both the 3-month and the 12-month toxicity study (0.5 µg/kg and 2 µg/kg). Since these

doses represent exposures of only 1x-3.5x the human exposure at the 20-µg dose (based on C_{max}) this may constitute a clinical concern for renal toxicity with long term dosing. Renal findings were, however, not observed in a long-term 18-month monkey bone quality study at doses of 1 and 5 µg/kg (equivalent to 1x-7x the human AUC at 20 µg/day). The dietary calcium content in the bone pharmacology study was lower (0.3%) than that used in the toxicity studies (0.7-0.9%), and this difference has been proposed to explain the discrepancy in the study findings. Thus, the histologic renal lesions observed in the toxicity studies were probably related to post-dose hypercalcemia.
In the largest phase III clinical trial (GHAC) there were transient and small but statistically significant increases in post-dose serum calcium and in the frequency of hypercalcemia. However, there has been no indication of renal toxicity (serum creatinine, creatinine clearance, serum urea nitrogen).

GENERAL TOXICOLOGY

Chronic toxicity was assessed in 6-month rat and 12-month monkey toxicity studies. The toxicities observed were related to the pharmacologic activity of teriparatide. Target organs were the kidney and bone/marrow.
In the 6-month rat toxicity study (10, 30, 100 µg/kg) dose-related trabecular and cortical hypertrophy was observed at all doses and was the desired effect of teriparatide. Females were more sensitive than males despite similar exposures. Decreased bone marrow space was secondary to increased bone formation and was the likely cause of the decreased hematology parameters observed in all dose groups, which may have clinical significance when long term treatment is intended. The increase in spleen weight and the splenic and hepatic extramedullary hematopoiesis in all dose groups probably represented compensatory responses. Likewise the hypercalcemia and hyperphosphatemia at 30 and 100 µg/kg were expected consequences of PTH agonist activity. An increased incidence of glomerular nephritis in males and kidney mineralization in females was observed at 100 µg/kg/day. The NOAEL in this rat study was <10 µg/kg based on hematologic effects. This dose is equivalent to 5-10x the human exposure at the 20 µg dose, based on AUC.

In the 12-month monkey toxicity study (0.5, 2, 10 µg/kg) diffuse expanded basophilic medullary interstitium (minimal-moderate) was observed in all female dose groups (0.5, 2, 10 µg/kg) and in high dose males (10 µg/kg). A similar incidence pattern as for medullary expansion was observed for cortical or medullary tubular/interstitial mineralization. Relative kidney weights were increased significantly in high dose males but not females. Erythrocyte parameters were decreased in males and females at 10 µg/kg. As in the rat, this was probably related to the dose-related increase in trabecular bone in the femur and sternebra, which was characterized by a denser and thicker trabecular meshwork with a decrease in intertrabecular space. Transient increases in serum ionized calcium in all dose groups, and decreases in total serum calcium and phosphorus at 2 and 10 µg/kg, were related to the pharmacologic effect of the test compound. A dose- and time-dependent increase in the proportion of animals with measurable anti-teriparatide immunoglobulin (IgG) levels was also observed. The NOAEL in this monkey study was 0.5 µg/kg, based on renal histopathologic effects. This dose is equivalent to 1x the human exposure at the 20 µg dose, based on C_{max}.

The toxicities observed at the lowest adverse effect dose levels were due to the expected pharmacological effect of the test compound on calcium metabolism. In clinical trials, related adverse effects have been absent or minor and have not elicited any significant clinical concern.

CARCINOGENICITY

The main result obtained in the two-year rat carcinogenicity study was that teriparatide causes osteosarcomas. Two-year subcutaneous dosing of the rat with 5, 30, or 75 µg/kg/day revealed cellular proliferative lesions of the bone including malignant osteosarcoma in all dose groups at exposure levels equivalent to 3x-60x the human exposure at a clinical dose of 20 µg/day. At the

highest dose the incidence of osteosarcoma reached 52% in males and 38% in females (average 45%).

Incidence of osteoblastic neoplasms and osteoblast hyperplasia in rat carcinogenicity study

	MALES				FEMALES			
Group	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined	60	60	60	60	60	60	60	60
Dose (ug/kg/day)	0	5	30	75	0	5	30	75
Human exposure multiple (AUC based)*	-	3x	21x	58x	-	3x	21x	58x
No. of animals with								
Osteosarcoma	0	3	21	31	0	4	12	23
Osteoma	0	0	2	1	0	0	0	1
Osteoblastoma	0	0	2	7	0	1	1	3
Osteoblast hyperplasia	0	1	2	4	0	2	1	3
Historical control data on the incidence of osteosarcoma in the rat (Lilly database)	1/360	-	-	-	0/360	-	-	-

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

The time to osteosarcoma appearance appeared to be dose-related and the earliest time of tumor diagnosis was 21, 19 and 13 months for the 5, 30, 75 µg/kg groups, respectively. Notably, some tumors may have gone undetected since not all tumors resulted in gross lesions, not all bones were examined histopathologically and examined bones were not completely evaluated. In addition, the exact time of tumor induction is unknown since tumors were detected prior to study termination only by detection of gross lesions or premature death.

Examination of historical control databases revealed that the background incidence of osteosarcoma in rats is extremely low. According to the Sponsor's database (6 studies, 5-year period) there was 1 osteosarcoma in 360 male rats (0.28%) and none in 360 females (0%). The National Toxicology Program historical control database indicates an incidence of 0.22% in males and 0.37% in females. Using an average background incidence of 0.2% it can be calculated that the relative risk of osteosarcoma in the current study varied from 30x in the 5 µg/kg group to 225x in the 75 µg/kg groups. Since this is a substantial relative risk increase at near human exposure the finding constitutes a major clinical concern.

The Sponsor has suggested that osteosarcoma formation in the rat is associated with the exaggerated pharmacological response, i.e., the increase in bone mass, of the rat skeleton to teriparatide. Compared to monkeys and humans the increase in bone mass in rats after 24 months of treatment was indeed marked at all doses, particularly at non-vertebral sites. At the end of the study the BMC of the mid-femur in female rats in the 5- and 30-µg/kg groups (~3x, 20x the human exposure at the 20 µg dose) was increased by 25% and 65%, respectively. By contrast, postmenopausal women treated for ~21 months with 40 µg/day had an increased midshaft radius BMC of only 2%. On the other hand, the increase in vertebral BMC in the 5 µg/kg female dose group (3x the human exposure at 20 µg/day) was 50%, which represented a much lower multiple of the increase in humans treated with 20 µg/day (10%) than at the cortical site. However, vertebral tumors but not femoral tumors were observed in the 5 µg/kg group. It should also be noted that the tumors in the rat must have been induced at a time when the increase in BMC was far less than the increase measured at the end of study. Moreover, since the NOAEL or threshold for the tumor induction has not been determined osteosarcomas may arise at doses associated with smaller BMC increases.

Although the increase in bone mass in the rats that developed bone tumors was larger than the increase expected in humans, the difference is quantitative rather than qualitative. Therefore, in the opinion of this Reviewer it is not justified to conclude that the tumors have no clinical relevance. In fact, the critical issue is a qualitative one, namely whether the rat osteoblast responds in the same manner to intermittent activation with teriparatide as the human osteoblast. As there is increased osteogenesis in both the rat and the human, there is reason to believe that the osteoblast response to

teriparatide is similar in the two species, and it is an entirely plausible hypothesis that the induction of bone tumors is the result of chronic, intermittent hormonal stimulation of the osteoblast. This chronic stimulation may cause target cell proliferation and confer a selective growth advantage to precancerous or initiated cells, i.e., cause tumor promotion. Thus, the osteosarcoma finding in the rat may very well be relevant for all species that respond to intermittent treatment with an increase in bone formation and apposition, including humans. Support for this hypothesis is provided by data from studies employing intermittent injection of a PTHrP analogue with a similar pharmacodynamic effect as teriparatide, showing that both rats (Sprague-Dawley) and mice (CD-1) develop osteosarcomas.

The molecular and genetic events underlying the bone tumor induction by teriparatide in rats are unknown, and so are the mechanisms underlying the anabolic action of PTH on bone. Based upon nonclinical pharmacology data, osteoblastic gene expression differs upon continuous and intermittent activation of the PTH receptor, possibly due to homologous desensitization of the receptor with continuous activation. Concomitantly with increased bone formation this differential gene expression may be associated with increased osteoblast or osteoprogenitor cell proliferation and osteoblastic tumorigenesis in the case of an intermittent administration regimen. Based on evidence that increased bone formation upon intermittent dosing is due to inhibition of osteoblast apoptosis, it is also possible that an anti-apoptotic action may contribute to an increased chance of neoplastic cell transformation.

No bone tumors have been observed in other animal studies, such as a 6-month chronic rat toxicity study (n=15/sex/group) with doses up to 100 µg/kg/day, and an 18-month female monkey pharmacology study (n=40/group) with doses of 1 and 5 µg/kg. However, negative tumor results from these studies have limited relevance since these studies were not designed to detect carcinogenicity. They were of relatively short duration (rat study), encompassed limited bone evaluations (monkey study) or utilized an insufficiently large sample size (monkey study). In particular, the absence of radiographically detectable tumors in the monkey study was not entirely reassuring, since the bone samples examined by microscopic evaluation were merely sections taken from small specimens intended for histomorphometric evaluation and small neoplastic lesions may have easily escaped detection. Moreover, even if the bone evaluations had been all-inclusive, the number of animals used in the monkey study was too small to detect with reasonable probability even a large increase (10x-100x) in bone tumor risk, assuming a low background incidence in this species of e.g. 1 in 10,000. Therefore, these negative results do not provide sufficient evidence of a lack of tumor risk in humans treated for an extended period of time. Similarly, although in humans treated with teriparatide in the clinical trials no osteosarcomas have been reported, the number of patients treated for >1 year (~1150) was too small to detect even a large increase in the risk of this tumor due to the very low background incidence in the human population (1 in 250,000).

Although Sponsor has argued otherwise, the absence of a clinical association between hyperparathyroidism and osteosarcoma is not entirely reassuring either. The intended therapeutic use of teriparatide is intermittent dosing causing pulsatile increases in serum PTH(1-34) levels while in hyperparathyroidism endogenous PTH levels are chronically elevated. This difference in pharmacokinetics is related to differences in pharmacodynamics at the tissue and cellular level and is potentially related to the bone tumor finding.

In conclusion, the rat osteosarcomas can not be dismissed as clinically irrelevant without knowing the mechanism of tumor formation and without any information about the presence or absence of this mechanism in humans.

In order to address the effects of (1) age/skeletal maturity at treatment onset and (2) treatment duration the Sponsor is currently conducting a follow-up carcinogenicity study in female rats using doses of 5 and 30 µg/kg. In this study, groups of animals are treated starting at two different ages (1.5 or 6 months), and for two different treatment periods (6 months or 20-24 months). Part of the animals treated for 6 months will be followed up for the remainder of the study. The results of this follow-up rat study will be available by mid-2002. Sponsor is also conducting an additional monkey

study in which ovariectomized animals are treated with 5 µg/kg teriparatide for 18 months, and followed up for 3 years. At the end of this study bones will be evaluated for the presence of proliferative lesions. The study is done in order to investigate whether there is a lag time in tumor development.

GENETIC TOXICOLOGY

Teriparatide did not exhibit any genotoxic potential in a standard test battery (bacterial mutagenicity assay, mouse lymphoma assay, chromosomal aberration test in CHO cells, mouse micronucleus test) of *in vitro* (with or without metabolic activation) and *in vivo* genotoxicity assays. These data indicate that teriparatide is not genotoxic.

REPRODUCTIVE TOXICOLOGY

In Segment I reproduction studies in male and female CD rats, no significant effects were observed after administration of subcutaneous doses of 30 to 300 µg/kg/day.

In a Segment II developmental toxicity study in pregnant CD rats, there were no significant findings at daily subcutaneous doses of 30 to 1000 µg/kg.
In a Segment II developmental toxicity study in pregnant CD-1 mice, there was an increase in the incidence of one malformation (eyelid open) at a daily subcutaneous dose of 1000 µg/kg. There were also increases in the incidences of a number of skeletal deviations and variations (interrupted rib, extra vertebra, extra rib) at subcutaneous doses ≥ 225 µg/kg.

In a pilot Segment II developmental study in pregnant New Zealand white rabbits, vaginal bleeding and total resorption of all fetuses was observed in all animals given subcutaneous doses of 10 to 100 µg/kg. At 100 µg/kg, 2 pregnant animals died and the remaining 3 animals were euthanized. These animals had excessive reductions in food consumption and body weight. At 3 µg/kg there was an increase in the incidence of resorptions, with 1 of 5 animals having all conceptuses resorbed, and a resulting reduction in litter size. The embryotoxicity observed in pregnant rabbits was considered to have resulted from the elevations in blood ionized calcium following teriparatide administration. Since sustained hypercalcemia is not expected to be a side effect of teriparatide therapy in humans, these findings in rabbits are not considered to represent a relevant clinical safety concern for non-pregnant women or for men.

In a combined Segment II/Segment III perinatal and postnatal study in CD rats, there was no maternal and reproductive toxicity at doses of 30 to 1000 µg/kg/day. However, there was developmental toxicity in the F1 progeny which consisted of mild growth retardation in F1 male rats at 1000 µg/kg and in F1 female rats at 225 and 1000 µg/kg/day, and reduced motor activity in F1 male and female rats at 1000 µg/kg/day.

RECOMMENDATION

The results submitted to NDA 21,318 on the pharmacologic effects of teriparatide (rhPTH(1-34), FORTEO™) support the efficacy and safety of the compound for the treatment of osteoporosis.

The results submitted on the toxicologic effects of teriparatide, excluding those obtained in the rat carcinogenicity study, also support the safety of the compound for the treatment of osteoporosis.

However, the finding of osteosarcoma obtained in the rat carcinogenicity study does not support the safety of teriparatide for long term use in postmenopausal women and men with osteoporosis.

The currently ongoing follow-up rat carcinogenicity study addresses two issues, (1) effect of animal age at treatment onset and (2) effect of treatment duration, that are critically important for further evaluation of the bone tumor finding and its clinical relevance.

This Reviewer feels that the pending results from the follow-up rat carcinogenicity study need to be available to the Agency before a decision to approve the product for marketing in the treatment population can be made.

For that reason, this Reviewer recommends the NDA to be approvable (AE) pending the results from the follow-up rat carcinogenicity study.

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APPENDIX

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Executive CAC
March 20, 2001

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Frank Sistare, Ph.D., HFD-910, Alternate Member
Jim Farrelly, Ph.D., HFD-530, Alternate Member
Karen Davis-Bruno, Ph.D., Team Leader
Gemma Kuijpers, Ph.D., Presenting Reviewer

Author of Draft: Gemma Kuijpers

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21,318
Drug Name: Forteo
Sponsor: Eli Lilly

LY333334 (Forteo®) is the recombinant (1-34)-amino-acid fragment of human parathyroid hormone (rhPTH(1-34). When administered by daily s.c. injections, this compound causes an increase in osteoblastic bone formation resulting in increased bone mass and bone strength. The compound is under review for marketing for the indication of treatment of osteoporosis in postmenopausal women and men. One 2-year carcinogenicity study in the rat was carried out using doses of **0, 5, 30, 75 ug/kg/day**.

RAT CARCINOGENICITY STUDY:
The main result of the study was a dose-related statistically significant ($p<0.05$) increase in incidence of osteoblastoma and osteosarcoma at various bone sites in males and females in all dose groups. No bone tumors were seen in the control groups. The human exposure multiple in the dose groups varied from 1.6x to 42x. There was also a statistically significant positive dose-response relationship in the incidence of thyroid C-cell adenoma in males.

Executive CAC Recommendations and Conclusions:
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•
•

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\
/Division File, HFD-510
/KDavisBruno, HFD-510
/GKuijpers, HFD-510
/RHedin, HFD-510
/ASelfried, HFD-024

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ADVISORY COMMITTEE BRIEFING DOCUMENT
Pharmacology/Toxicology

NDA 21,318
FORTEO™
LY333334

July 27, 2001

INTRODUCTION

The intermittent administration of PTH causes an anabolic bone response in animals and in humans. It is generally thought that the anabolic effect of intermittent PTH is due to stimulation of osteoblastic bone formation preceding and/or dominating osteoclastic bone resorption. However, the precise molecular mechanism of the anabolic action of PTH is unknown.

The osteoblast is the major target cell for PTH in bone. The cell possesses high-affinity surface membrane PTH/PTHrP receptors which are coupled to adenylyl cyclase and phospholipase C. Activation of the two associated signal transduction pathways leads to alterations in osteoblastic function and gene expression. Most likely the anabolic action of PTH on bone is mediated by a combination of intracellular responses triggered specifically by intermittent receptor occupation.

The Sponsor has developed recombinant human PTH(1-34) (rhPTH1-34), or LY333334, as a therapeutic agent for the treatment of osteoporosis in postmenopausal women and men.
In order to evaluate preclinical efficacy and safety LY333334 was tested extensively in a variety of pharmacology and toxicology studies. The studies were generally done by daily subcutaneous administration.

In the pharmacological studies carried out in monkeys, rats and rabbits, all species responded to intermittent LY333334 dosing with an increase in bone formation at various skeletal sites. The long term monkey bone quality study was the most clinically relevant study. In this study, a thorough assessment of the efficacy and safety of daily dosing with LY333334 on both cancellous and cortical bone was carried out.

In the toxicity studies in rats, monkeys, rabbits and mice, the effects of LY333334 were frequently related to the pharmacological effect of PTH on bone and mineral homeostasis. The main findings prompting clinical concern were cardiovascular toxicity, nephrotoxicity, and carcinogenicity. LY333334 was not genotoxic.

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BONE EFFICACY/SAFETY

Preclinical and clinical studies have shown that intermittent treatment with PTH injections increases vertebral bone mass and strength. However, the effect of this treatment on non-vertebral, predominantly cortical bone sites is not as clear. Sponsor carried out pharmacology studies with LY333334 in rats, monkeys and rabbits to evaluate bone quality. The monkey and rabbit studies were particularly designed to address the issue of non-vertebral bone efficacy and safety in remodeling species.

Adult skeletally mature ovariectomized (OVX) monkeys were treated with LY333334 at daily dose of 1 or 5 ug/kg (PTH1, PTH5) for 18 months, or for 12 months followed by a withdrawal period of 6 months (PTH1W, PTH5W). Ovary-intact New Zealand rabbits were treated with daily doses of 10 or 40 ug/kg, for up to 5 months. Rat studies were carried out in ovariectomized and intact animals, starting treatment at different ages, for time periods up to 2 years. Bone quality was assessed by X-ray densitometry, histomorphometry and biomechanical testing. Monkey study data described are from end of study.

Monkey study

In the vertebrae, LY333334 dose-dependently increased vertebral cancellous bone mass (BMC and BMD) and bone strength (yield force), mainly due to an increase in trabecular number and connectivity (TABLE 1). Bone formation rate (BFR/BV) was increased without a clear effect on bone resorption parameters. BMD was significantly correlated with yield force in the spine (r=0.83).

TABLE 1. Vertebral bone properties

	Sham Vehicle	OVX Vehicle	PTH1	PTH5	PTH1W	PTH5W
BMD (Change from baseline, %) (0-18mo)	5.3%*	0.6%	9.1%*	15.4%*	7.2%*	7.3%*
Yield force F_y (N)	1738	1499	1915*	2113*	1899*	1792*
BV/TV (%)	26	23	33*	35*	30*	27*
Tb N (mm^{-1})	2.7	2.5	3.4*	3.6*	2.9*	2.9*
BFR/BV	23	20	36*	47*	26	24

*significantly different from OVX

In proximal tibia and distal radius, pQCT (peripheral quantitative computed tomography) measurements showed that BMD was increased in the middle and/or innermost zones (cancellous bone), whereas significant differences in the outermost zone (cortical bone) could not be resolved. Cross-sectional bone area at these bone sites was not significantly affected.

Histomorphometry of the distal radius indicated that in cancellous bone fractional bone volume (BV/TV) was increased associated with an increase in trabecular number and bone formation (TABLE 2). In cortical bone BV/TV was decreased while bone surface to volume ratio (BS/BV) and bone formation rate (BFR/TV) were increased.

TABLE 2. Distal radius histomorphometry data

	Sham Vehicle	OVX Vehicle	PTH1	PTH5	PTH1W	PTH5W
CANCELLOUS						
BV/TV (%)	17	16	20	22*	19	19
Tb N (mm^{-1})	2.3	1.9	2.6*	2.6*	2.4*	2.3
BFR/BV (%/year)	9.0*	26	33	42*	28	32
CORTICAL						
BV/TV (%)	56	62	53*	58	58	57
BS/BV (mm/mm^3)	8.7	8.1	12.4*	11.0*	9.5	9.9
BFR/TV (%/year)	8.8*	42	45	50	42	36

*significantly different from OVX

In the femoral neck, LY333334 significantly increased ultimate bone strength, and increased cancellous BV/TV associated with an increase in bone formation and trabecular number (TABLE 3). In

cortical bone there were no significant dose-dependent effects of LY333334 treatment on BV/TV, BS/BV or BFR/TV. BMD data for this site were not available.

TABLE 3. Femoral neck properties

	Sham Vehicle	OVX Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	1738	1499	1915*	2113*	1899*	1792*
CANCELLOUS BONE						
BV/TV (%)	44	39	54*	57*	48*	50*
Tb N (mm^{-1})	2.9	2.6	3.5*	3.4*	3.1*	3.3*
BFR/TV (%/year)	21	25	42	59*	27	26

*significantly different from OVX

In the midshaft humerus bone strength (ultimate force, F_u) was not significantly affected by either dose of LY333334 (TABLE 4). Macroscopic cortical thickness was increased by LY333334, significantly at 5 ug/kg. Histomorphometrically, LY333334 treatment increased cortical porosity (Po), number of mineralizing osteons and activation frequency. Total bone area (B.Ar) and cortical bone area including porosities (Ct.Ar) were increased, and medullary cavity area (Me.Ar) was slightly decreased. Endocortical, but not periosteal, mineralizing surface (MS/BS) and BFR were increased. All effects were more pronounced at the high dose. There were no significant effects on osteoid or wall width. There were no data on bone perimeters. BMC and BMD of the humerus were not determined.

TABLE 4. Humerus midshaft properties

	Sham Vehicle	OVX Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	725	636	654	680	689	707
Cortical thickness (mm)	1.74	1.63	1.68	1.80*	1.66	1.72
Po (%)	1.3*	2.6	4.7	6.7*	2.3	6.5*
B.Ar (mm^2)	53	53	54	55	55	58
Ct.Ar (mm^2)	37	35	38	41*	38	41*
Me.Ar (mm^2)	16	18	17	15	17	17
MS/BS.Ec (%)	3.1	21	25	42*	12	9.2
BFR/BS.Ec (um/day)	7.1	21	18	34	15	14

*significantly different from OVX

Although cortical porosity was increased, the mechanical strength of the mid humerus was not significantly affected or slightly increased since the LY333334-induced increase in porosity was concentrated near the inner endocortical surface zone and since LY333334 increased cortical bone thickness. The latter two phenomena lead to a (relative) increase in the moment of inertia, which caused an increase in bone strength.
In the midshaft radius, pQCT measurement showed a significant increase in cross-sectional area, concomitant with a slight non-statistically significant decrease in BMD in LY333334-treated groups. As in the humerus, cortical porosity was significantly increased. Moreover, bone area and medullary area were increased, but mineralized area (excluding porosities) was not significantly affected. Endocortical perimeter was slightly increased, and endocortical bone formation was increased. Periosteal perimeter was slightly but not significantly increased.
In the midshaft femur porosity was increased, while bone area, medullary area, mineralized area (excluding porosities), and periosteal and endocortical perimeters were not significantly affected.

Biomechanical testing of femoral diaphyseal beam specimens containing both endocortical and periosteal bone showed that the material properties of this bone site (ultimate stress, Young's modulus) were slightly decreased at the high dose of LY333334 (TABLE 5). Dose effects for both parameters were significant according to a two-way ANOVA model.The effects might be due to an increased endocortical porosity.

TABLE 5. Femoral diaphyseal beam specimen properties

	Sham Vehicle	OVX Vehicle	PTH1	PTH5	PTH1W	PTH5W
σ_u (Mpa)	222	216	222	206	214	208
E (Gpa)	17.2	16.4	17.1	15.4*	16.6	15.3*

* significantly different from sham

In the cancellous bone of the iliac crest, LY333334 caused a dose-dependent increase in bone formation at 6 months followed by an increase in bone formation and resorption at 15 months, and increases in trabecular parameters (density, thickness) at both time points. In the cortical bone both doses of LY333334 caused increased cortical thickness at 6 months. However, this effect was reversed after 15 months at which time thickness was lower than in the sham or OVX groups. Porosity was not significantly affected by LY333334 at either time point.

The incidence and degree of tunneling (intratrabecular remodeling) was dose-dependently and reversibly increased in vertebrae and iliac crest by LY333334. The incidence of woven in iliac crest biopsies bone was also increased at 15 months.

Rabbit study

In the tibial midshaft, treatment with LY333334 (10 or 40 ug/kg/day) for 20 weeks (2 remodeling cycles) increased intracortical bone remodeling and cortical porosity, particularly in the endocortical zone. At this site, LY333334 also increased cortical bone area, and increased both endocortical and periosteal bone formation. In the femoral midshaft, LY333334 caused a dose-dependent increase in X-area and apparent BMD, and an increase in strength in parallel with an increase in cross-sectional moment of inertia (CSMI). In the vertebrae, resorption and formation parameters were increased, but BMD, strength and cortical thickness were not affected.
In the tibial midshaft, treatment with LY333334 (10 ug/kg) for 5 or 10 weeks (1/2-1 remodeling cycle) increased endocortical, periosteal and intracortical bone formation without a significant increase in porosity. The net result was increased bone thickness and strength. Femoral midshaft strength was also increased by LY333334.

Rat studies

In rats, LY333334 generally increased BMC, BMD and projected or cross-sectional area of proximal tibia, femur (midshaft, neck, distal end) and vertebrae. Bone strength of lumbar vertebrae, femoral neck and midshaft were increased with a significant correlation between femoral midshaft BMC and ultimate load to failure (Fu). However, the biomechanical characteristics of these three sites were affected differently by the treatment with LY333334. Notably, brittleness was decreased in the vertebrae, unaffected in the femoral neck, but increased in the femoral diaphysis.
Histomorphometry data indicated that in cancellous bone LY333334 increased bone formation and trabecular morphometric parameters (density, thickness,connectivity). In cortical bone LY333334 increased periosteal and endocortical bone formation, and increased bone thickness and moment of inertia.

Conclusions

The data from the monkey study suggest that LY333334 at doses of 1 and 5 ug/kg, equivalent to approximately 1x and 8x the human exposure at 20 ug per day, increases bone mass in the vertebrae and in the cancellous bone of the distal radius and femoral neck. This increase is associated with increased bone formation and improved trabecular architecture and leads to increased bone strength of the lumbar spine and the femoral neck.

The effects of LY333334 on cortical bone are not as clear. The data suggest that LY333334 dose-dependently stimulates intracortical (osteonal) bone remodeling and porosity, while simultaneously increasing endocortical bone formation with unclear effects on resorption. At the humerus midshaft these effects were accompanied by a slight increase in bone thickness and morphometric cortical area and a slight decrease in medullary area. The location of the increased porosity to the endocortical bone zone in combination with the increased bone thickness presumably caused a

preservation of the cross sectional moment of inertia (CSMI) which could explain why bone strength at this site was not significantly affected.

At other cortical bone sites, there were inconsistent effects on BMD and fractional bone mass (BV/TV), and on histomorphometrically determined parameters of total bone tissue area, cortical mineralized area, medullary area, periosteal and endocortical perimeter (midshaft radius, radial cortex, mid femur, femoral neck cortex). However, at all cortical bone sites evaluated, porosity was increased. Bone strength was not measured at sites other than the humerus.
At the high dose of LY333334 there was a slight deterioration in the intrinsic or material properties of mid femoral bone, σ_u (ultimate stress) and E (Young's modulus), which may be due to increased porosity in the endocortical bone zone.

Taken together, these data suggest that treatment with LY333334 can cause two different effects in cortical bone: (1) increased endocortical porosity due to increased intracortical remodeling and (2) altered bone thickness. In the mid humerus, this lead to a maintenance of bone strength due to the dominating effect of an increase in cortical thickness and area. However, it is not clear whether this result can be generalized for all cortical bone sites. The increased porosity was observed at all evaluated cortical bone sites but the effects on bone thickness and bone area were not evident at all sites. As bone strength is determined by the balance between the two opposing effects it is conceivable that strength is maintained or increased at sites where bone thickness and area are sufficiently increased (e.g. humerus, mid radius) but impaired at sites where increased porosity is not counterbalanced by increased cortical bone thickness and/or area (e.g. midfemur). Uncertainty about the effects of LY333334 on cortical thickness is corroborated by the data on cortical thickness of the iliac crest.

Similar data as in the monkey humerus were obtained in the rabbit tibial and femoral midshaft, at which sites porosity was increased but bone strength was increased due to a compensating increase in bone thickness apparently preceding the increase in porosity. However, the clinical relevance of the data obtained in the intact rabbit model is not clear since vertebral BMD and strength in this species were not affected by LY333334.

The data from the rat studies showed that in this species LY333334 causes increases in cancellous and cortical bone mass and strength. However, unlike rabbits, monkey and humans, the rat lacks Haversian (osteonal) remodeling. Therefore, the data on cortical bone in the rat have limited clinical relevance.

The monkey study results and the BMD and fracture data obtained in clinical trial GHAC (postmenopausal women) generally support the predictive value of the preclinical monkey study data. In trial GHAC, after ca. 21 months of treatment with 20 ug/day, BMD was markedly increased at the lumbar spine (9% as compared to placebo), and the risk of one or more new vertebral fractures was significantly decreased (by 65%). The BMD was also increased albeit less pronounced at sites with a relative larger contribution of cortical bone (hip, femoral neck, intertrochanter area and Ward's triangle). However, in the distal radius BMD was decreased as compared to placebo (average -1% at 20 ug, -2% at 40 ug). Further pQCT evaluation of the radius (proximal area) indicated that periosteal and endosteal circumferences and total bone area were increased while BMC was unchanged, which would reduce BMD but increase computed measures of bone strength. The latter appears to bear some analogy to the effects of LY333334 in the monkey humerus and radius. The incidence of clinical fragility fractures at nonvertebral sites (hip, wrist, ankle, humerus, rib, foot, pelvis) was either unaffected (humerus, foot) or decreased (other sites). However, the number of fractures was too small to demonstrate a statistically significant fracture risk reduction at individual nonvertebral sites. Transiently increased cortical porosity in the human iliac crest confirms that this phenomenon also takes place in humans.

The possible implication of the preclinical data of a decrease in cortical bone strength so far has not been borne out by clinical data. This may be due to the positive effect of LY333334 on cancellous bone which is present at most skeletal bone sites (e.g. femoral neck/hip), combined with a generally

positive effect on cortical bone thickness or area and a relatively small adverse effect of an increase in porosity. Thus, although the small numbers of non-vertebral fractures preclude a solid conclusion, the clinical data suggest that there is no major safety problem in cortical bone.

CARDIOVASCULAR TOXICITY

The acute cardiovascular effects of LY333334 were evaluated in rats and dogs. Electrocardiography was carried out in the acute toxicity study in dogs, and in 3-month and 1-year repeated-dose studies in monkeys. All doses were administered subcutaneously. Results are shown in TABLE 1.

TABLE 1. Cardiovascular effects of LY333334 in rats, dogs, and monkeys

Species	Study type	Doses (ug/kg/day)	EFFECTS			
			Heart rate	Arterial blood pressure	Other findings	ECG findings
RAT (Sprague-Dawley), male	Single dose	0, 0.5, 4, 23, 100, 300, 1000	Increased at doses ≥ 23 ug/kg	Decreased at doses ≥ 23 ug/kg	Transient redness of extermities	-
DOG (Beagle), female	Single dose	0, 6	Increased	Decreased	Left ventricular inotropic state increased; Serum ionized Ca level elevated postdosing	No abnormalities
MONKEY (Cynomolgus); male and female	3-month, repeated dose	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, repeated dose	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

In rats and dogs, effects on heart rate and blood pressure were maximal during first 2 hours after dosing. The effects in these species are consistent with drug-induced vasodilation and a compensatory cardiac response.

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.

In clinical trials orthostatic hypotension and a decrease in QTc interval upon dosing with LY333334 have been observed.

NEPHROTOXICITY

Renal pathology was observed in male and female cynomolgus monkeys (age 2-2.5 years) treated daily with subcutaneous doses of LY333334 for 3 months or 1 year. In a special 4-month toxicity study in female monkeys with a 3-month reversibility period, renal function was further evaluated. The data from these studies are summarized in TABLE 1.

TABLE 1. Clinical pathology and histopathology findings in monkey toxicity studies

	3-month toxicity study	1-year toxicity study	4-month reversibility study
Doses (ug/kg/day)	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 nitrogen	Increased in some animals at 20 and 40 ug/kg	Increased in two males at 10 ug/kg, and in one female at 2 ug/kg	Increased in one 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	• 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	• 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	• 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	• No specific tests don	• Increase in Ca excretion at ≥2 ug/kg in both sexes • Slight decreases in creatinine and osmolal clearance at 10 ug/kg in males, after 3-6 months	• Renal failure in 1/8 animals treated with 40 ukd (Day 78). The animal was sacrificed prematurely • No remarkable effects on urine acidification or concentration ability in surviving animals • No significant effects on general renal function (creatinine or osmolality clearance, or fractional electrolyte excretion) in surviving animals

Dose (ug/kg)	Human C_{max} multiples*	
	3-mo study	12-mo study
0.5	-	0.94x
2	3.4x	4.5x
10	27x	28x
20	73x	-
40	120x	-

*Human Cmax 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 interstitum 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 two-year subcutaneous carcinogenicity study in Fisher 344 rats was carried out with LY333334 doses of 5, 30, and 75 ug/kg/day (low dose, mid dose and high dose). The number of animals treated was 60/sex/group. The animals were treated from an age of 6-7 weeks for the duration of 24 months. Histologic evaluation of tissues was carried out of all animals that were euthanized at the end of the study and all animals that died or were killed prematurely.

Bone tumor findings

LY333334 caused an increase in the incidence of osteosarcoma in all dose groups in a dose-dependent manner. LY333334 also caused an increase in the incidence of other osteoblast neoplasms (osteoblastoma and osteoma), and an increase in the incidence of osteoblast hyperplasia. In some animals multiple neoplasms occurred (TABLE 1). The increased incidences of osteosarcoma and osteoblastoma were statistically significant (trend test).

TABLE 1. Incidence of osteoblastic neoplasms and osteoblast hyperplasia at all bone sites

	Males					Females			
Group	Contr	LD	MD	HD		Contr	LD	MD	HD
N examined	60	60	60	60		60	60	60	60
No. of animals with									
Osteosarcoma	0	3	21	31		0	4	12	23
Osteoma	0	0	2	1		0	0	0	1
Osteoblastoma	0	0	2	7		0	1	1	3
Osteoblast hyperplasia	0	1	2	4		0	2	1	3
No. of animals with bone neoplasm(s)	0	3	24	36		0	5	13	25
Osteosarcoma: Historical control incidence	1/360					0/360			

In several, but not in all cases, the osteosarcomas presented as clinically palpable bone nodules. These lesions were first detected in the high dose groups after 17 months in the study. Metastatic tumors were detected predominantly in males at soft tissue sites such as lung, liver, kidney and spleen. In a subset of animals the osteosarcoma was fatal and lead to early death of the animal (TABLE 2).

TABLE 2. Incidence of gross lesions, metastasis, and fatal osteosarcoma

	Males					Females			
Group	Ctrl	LD	MD	HD		Ctrl	LD	MD	HD
N examined	60	60	60	60		60	60	60	60
Osteosarcoma characteristics									
Total incidence	0	3	21	31		0	4	12	23
Gross bone nodule/lesion	0	1	17	24		0	3	7	13
Soft tissue metastasis	0	0	10	17		0	1	2	4
Fatal	0	1	12	22		0	3	6	8

Osteosarcomas were detected at various bone sites. Identification occurred either upon gross observation of a bone nodule, or upon microscopic evaluation of four routinely examined bone sites: femur (distal end), tibia (proximal end), sternum (one or two sternebrae), and vertebrae (one lumbar vertebra).
The most common site for osteosarcoma in males was the tibia and in females the vertebra (TABLE 3). The larger incidence in the mid dose and high dose males as compared to females was mainly due to the incidence of tibial tumors in the male dose groups. The increased incidences of osteosarcomas were statistically significant in tibia, femur, vertebra, rib and sternum in both males and females (trend test).

TABLE 3. Incidence of osteosarcoma by bone site

	Males					Females			
Group	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
Other (pelvis, skull, humerus)	0	0	2	3		0	1	2	1
Single site	0	3	16	22		0	4	10	22
Multiple sites	0	0	3	7		0	0	2	1

Time-to-death of tumor-positive animals

In addition to the osteosarcoma incidence, the time-to-death of animals with osteosarcoma appeared to be dose-dependent (Figure 1A,B). The data points represent all animals that were diagnosed with osteosarcoma, i.e. animals that died or were euthanized prematurely due to any event, and animals that were sacrificed at study termination. Most animals that died early did so because the osteosarcoma was fatal, although some died early for another cause and the osteosarcoma was detected as a coincidence. The earliest bone tumor diagnosed was a vertebral osteosarcoma in a high dose male which died after 13 months of treatment. The tumor was fatal but was only detected upon microscopic examination. The data suggest that osteosarcomas in the high dose groups either originated earlier or grew faster than the tumors in the low dose groups. This dose effect was more pronounced in males than in females.

Figure 1A. Time-to-death of animals diagnosed with osteosarcoma (males)

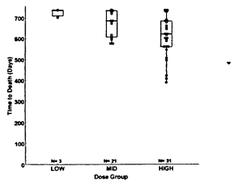
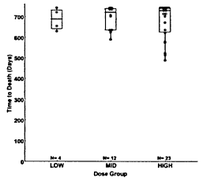


Figure 1B. Time-to-death of animals diagnosed with osteosarcoma (females)



Bone tumor location

Osteosarcomas that were associated with gross bone nodules were often several mm in diameter and invasive in character, and the anatomical site of origin of these tumors could not be established. However, for those tumors for which a site of origin could be determined the most common anatomical location was intramedullary. The most common bone site was the metaphysis. Rarely, tumors were located in the diaphysis and one tumor was limited to the epiphysis. In contrast to intramedullary neoplasms, periosteal or parosteal surface osteosarcomas were only seen in two cases in the tibia.

Pharmacodynamic effect of LY333334

It is of interest to consider the association between bone tumor incidence and the pharmacodynamic effect of LY333334. As expected LY333334 induced a change in bone architecture through increased bone formation associated with an increase in BMC and BMD in all dose groups.
In the femoral midshaft, QCT scanning showed that LY333334 caused a dose-dependent increase in endocortical bone apposition, resulting in a reduction of the marrow cavity, and periosteal bone expansion (Figure 2).

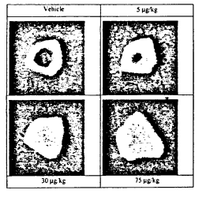


Figure 2 QCT images of the Femoral Midshaft from Females Treated for Two Years. The midshaft of left femurs were analyzed in cross section by QCT, using voxel dimensions of 150x150x1200 µm. Images show reduction of the marrow cavity at 5 µg/kg, loss of marrow space and expansion of bone at 30 µg/kg, and further expansion of bone area with altered geometry at 75 µg/kg.

In the proximal femur and vertebrae the morphologic effect of LY333334 were also dose-dependent and consisted of an increase in trabecular thickness and number, an increase in cortical and bone thickness, and a decrease in marrow area (Figure 11).

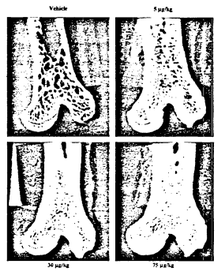


Figure 11 High Resolution QCT images of the Proximal Femur. Representative coronal images at 24x24x24 µm resolution are shown of the vehicle, 5, 30, and 75 ug/kg groups of females. Images show significant loss of marrow space due to increased cortical thickness, trabecular number, trabecular thickness, and trabecular connectivity.

In femur (midshaft and distal part) and vertebrae, there was a dose-dependent increase in BMC, X-sectional area and BMD (TABLE 4). Bone strength was increased accordingly.

TABLE 4. BMC and BMD of femur and vertebrae at end of study (approximate values for females)

	Femur midshaft		Distal femur		L-6 Vertebra	
	BMC % increase	BMD % increase	BMC % increase	BMD % increase	BMC % increase	BMD % increase
Control	-	-	-	-	-	-
LD	28%	27%	75%	48%	48%	23%
MD	68%	38%	140%	76%	91%	29%
HD	100%	41%	170%	85%	140%	34%

LY333334 Toxicokinetics

The serum levels of LY333334 in the rats were used to calculate the ratio of C_{max} and AUC between the rats and humans treated with 20 ug LY333334, the intended clinical dose of PTH1-34. LY333334 serum levels were clearly dose-related but there were inconsistent differences between males and females and inconsistent changes over time. Levels appeared to decrease, in males after 6 months and in females after 12 months, and lowest levels were seen in both sexes at 18 months. The reason for these inconsistencies is not known. Therefore, the C_{max} and AUC multiples should be considered mere approximations.

TABLE 5. Human C_{max} and AUC multiples

Rat:human C_{max} and AUC multiples based on average values for males and females, at 12 and 18 months

Group	Dose (ug/kg/day)	Cmax multiples (rat:human)		AUC multiples (rat:human)	
		Month 12	Month 18	Month 12	Month 18
LD	5	7x	5x	3.6x	1.6x
MD	30	57x	31x	25x	14x
HD	75	138x	94x	68x	42x

*Human Cmax= 159 pg/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; study GHAC)
*Human AUC=0.295 ngxhr/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; study GHAC)

Conclusions

Long term treatment of the rat with LY333334 caused bone tumors at all doses tested. The majority of tumors observed were osteosarcomas. The tumor incidence was dependent on the dose, and tumors appeared to occur earlier in the higher dose groups. An NOAEL for the tumorigenesis was not established since tumors occurred in all dose groups. It is possible that some tumors remained undetected since (1) not all tumors resulted in gross bone lesions and not all bones were microscopically examined, and (2) microscopically examined bones were not evaluated completely.

The fact that the animals were treated from a young age and for a large part of their life time might be taken to suggest that relatively short term treatment of an adult human population is unlikely to carry an increased risk for bone tumor formation. However, long term rodent carcinogenicity studies are carried out with relatively large numbers of animals over an extended period of time specifically for the purpose of identifying a potential risk. Therefore, the positive finding in the current carcinogenicity study coupled with the fact that the tumors were seen at very small human exposure multiples and the fact that an NOAEL was not established raises considerable concern.

It should also be noted that, although near-lifetime treatment was employed in the rodent study, tumor initiation was not systematically evaluated and tumors were only detected when the animal died prematurely or had evidence of a gross abnormality or was sacrificed at study termination. However, from the data on time-to-death due to fatal bone tumors it can be concluded that the earliest time of tumor initiation at the cellular level in the low, mid and high dose groups was less than 21, 19 and 13 months.

In contrast to the 2-year rat carcinogenicity study, bone tumors were not detected in femur or sternum of rats treated with LY333334 for 6 months at doses up to 100 ug/kg/day, or in the proximal tibia of male or ovariectomized female animals treated for 1 year with 8 or 40 ug/kg. Moreover, in skeletally mature ovariectomized monkeys treated for 18 months with 1-5 ug/kg/day (1-8x AUC at 20 ug human dose) no bone neoplasms were observed by X-ray radiography or histology evaluation of various bone sites. However, these findings were obtained in studies with relatively small number of animals (15-20/dose group) using evaluation of a limited number of bone sites (rat) or relatively small histologic bone samples (monkey), uncompensated for by a prolonged treatment duration. Thus, the negative results from shorter term rodent or intermediate term primate studies, although somewhat reassuring, are from studies that were not designed to detect potential carcinogenicity. Therefore they do not constitute sufficient evidence that there is no increased risk of tumor formation in humans treated for e.g. 2 years.

The Sponsor has argued that the tumor formation in the rat is associated with the exaggerated response of the rat skeleton to LY333334 in terms of its pharmacodynamic effect, i.e., bone mass increase. This is consistent with the data submitted. However, it is unclear where the threshold for tumor development is regarding dose and treatment duration.

The increase in bone mass in the rat study was marked at all doses tested. Particularly for the nonvertebral sites the effect appeared to be very large in comparison to what has been observed in monkeys and humans. For example, after 24 months of dosing the BMC of the mid femur in the

females at the low and mid dose (approximately 2x and 20x the 20 ug/day human exposure) was increased by 30% and 70%. Femoral tumor incidence in these dose groups was 0/60 and 2/60. By contrast, in monkeys treated for 18 months with doses up to 5 ug/kg (8x the 20 ug/day human exposure), the midshaft radius BMC was not increased, while in humans treated for an average of 21 months with a dose of 40 ug/day the mid radius BMC was increased by only 2%.

However, at the vertebral site in the female rat the increase in BMC at the low dose of 5 ug/kg (2x the 20 ug/day human exposure) was ca. 50% which was only 5-fold the increase in humans treated with 20 ug/day (10%). In this dose group, vertebral tumors were observed in 2/60 animals whereas none occurred in 60 concurrent controls or in 360 historical control animals. However, vertebrae were not routinely evaluated in the historical control animals.

It should be noted that the increases in BMC for the animals are increases at the end of the study. In the female low dose group mentioned above one of the vertebral tumors was fatal at ca. 21 months in the study, and was evident as a 0.5 cm nodule in the spinal canal. Initiation of the tumor must have occurred before 21 months at which time the increase in BMC was probably less than 48%, since it has been shown that treatment of the intact female rat for 9 months with 5 ug/kg leads to an increase in vertebral BMC of 10%.

Although the rat osteosarcomas occurred at sites where the increase in bone mass was larger than observed in humans or monkeys, the NOAEL or threshold for the tumor induction has not been determined. Thus it can not be excluded that tumors would arise at doses leading to a smaller BMC increase. Moreover, it is also possible that the bone mass effect is not related to the tumor induction since the two events may be mediated by intracellular events that are at least partially uncoupled. In that case, the large extent of the bone mass effect in the rat would not be relevant.

The absence of a clinical association between hyperparathyrodism and osteosarcoma in humans is no reassurance that the intended treatment, i.e., intermittent dosing with LY333334, is not associated with an increased human tumor risk. The pharmacokinetics and the pharmacodynamic effects of PTH in hyperparathyroidism, a condition with chronically elevated PTH levels, are different from those upon intermittent dosing with exogenous PTH(1-34). Preclinical data are available which indicate that intermittent PTH receptor activation can induce a differential effect on osteoblastic gene expression that is distinct from the effect of continuous activation, possibly due to the lack of homologous receptor desensitization in the case of intermittent receptor activation. This differential genetic response may be linked to the tumor formation, and the similarly increased osteoblastic bone formation activity that occurs in both cases may therefore be irrelevant.

The mechanism responsible for the osteosarcoma formation is not known. Likewise, the mechanism of the anabolic effect of intermittent PTH on bone has not been elucidated. Possibly, in conjunction with the increased osteogenesis, the repeated stimulation of the osteoblast by PTH increases cell proliferation and the increased cell division drives the accumulation of genetic errors leading to neoplastic transformation. Signal transduction following PTH receptor activation is mediated by adenylyl cyclase/cAMP/PKA and phospholipase-c/PKC activation, and PKC has been associated with cell proliferation and the promotion of tumor formation.

Hormonal agents have elicited positive responses in rodent carcinogenicity studies without parallel increases in human tumor incidences. For example, chronically elevated TSH levels cause thyroid follicular hyperplasia and neoplasia in rats but not in humans. By contrast, however, elevated peptide hormone levels can induce tumor formation in the rat and increase tumor risk in humans. Specifically, there is evidence that chronically increased gastrin levels, which cause tumors of ECL (enterochromaffin-like) cells in the gastric corpus in rats, increase the incidence of ECL-cell carcinoid tumors in hypergastrinemic patients with Zollinger-Ellison syndrome, or in humans with sustained hypergastrinemia following antisecretory therapy with proton pump inhibitors.

With the currently available data it is not justified to conclude that it is unlikely that the bone tumors in rats predict an increased tumor risk in humans. Ultimately, the animal findings can not be dismissed

without knowledge of the mechanism of the tumor formation and without information as to whether or not this mechanism also occurs in humans. Therefore, the animal osteosarcoma finding causes a concern for an increased risk of bone neoplasms in humans treated with LY333334. The data, however, can not predict the extent of the human risk.

To address the issues of animal age at treatment onset and length of treatment the Sponsor is currently conducting an extensive follow-up carcinogencity study in female rats using doses of 5 and 30 ug/kg. The study includes dose groups that are treated either from 2 months or 6 months of age, either for 6 months or for 24 or 20 months. In order to uncover a possible lag time in the neoplastic transformation of the osteoblast animals treated for 6 months are followed up by a withdrawal period of 18 or 14 months. The results of this study will be available in 2002.

In conclusion, although the relevance of the rodent osteosarcoma finding for humans remains unclear, the data suggest a carcinogenic potential of intermittent LY333334 injection.

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