

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, epiphysis, 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, epiphysis, 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 of 1 to 2 sternebrae, similar to vertebrae
- Gross lesion: Number and orientation of sections needed to characterize 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						
		Age (Months)						
Study Arm	Study No. Group No.	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

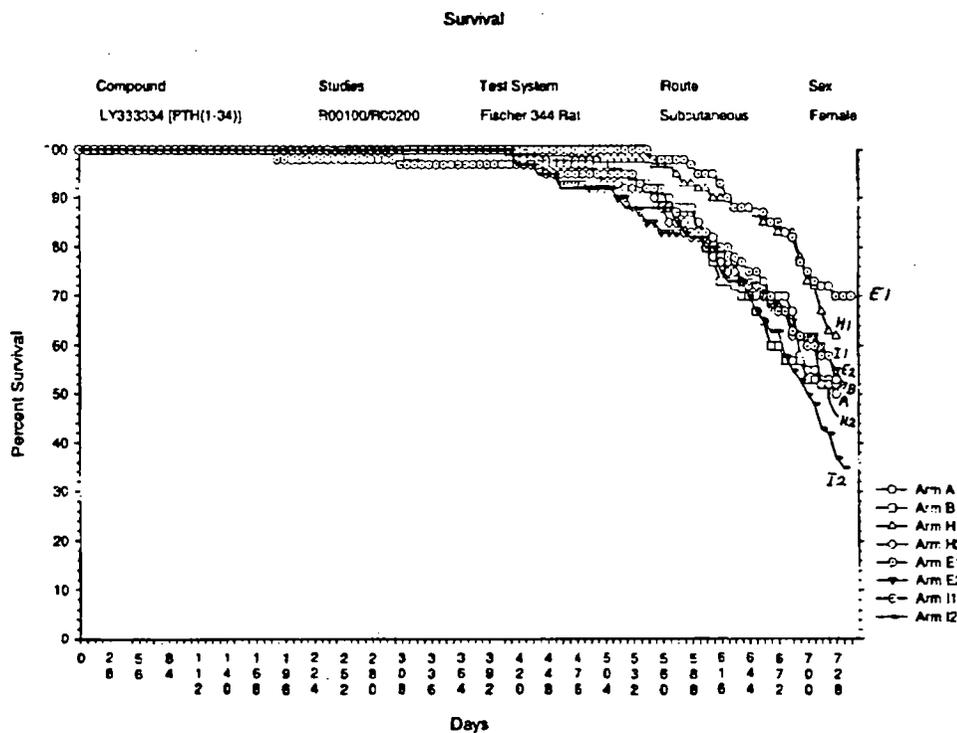
Survival

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 (60 rats/group)							
Study Arm		A	B	E1	E2	H1	H2	I1	I2
Dose ($\mu\text{g/kg}$):		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)

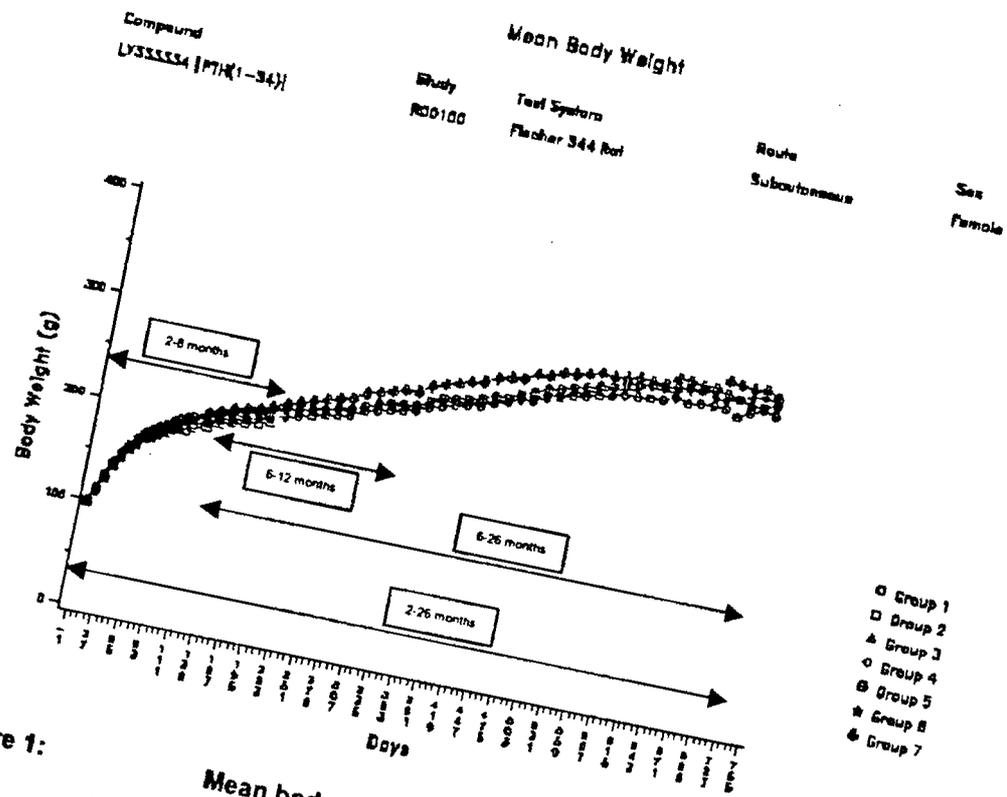


Figure 1:
Mean body weight, Study R00100.

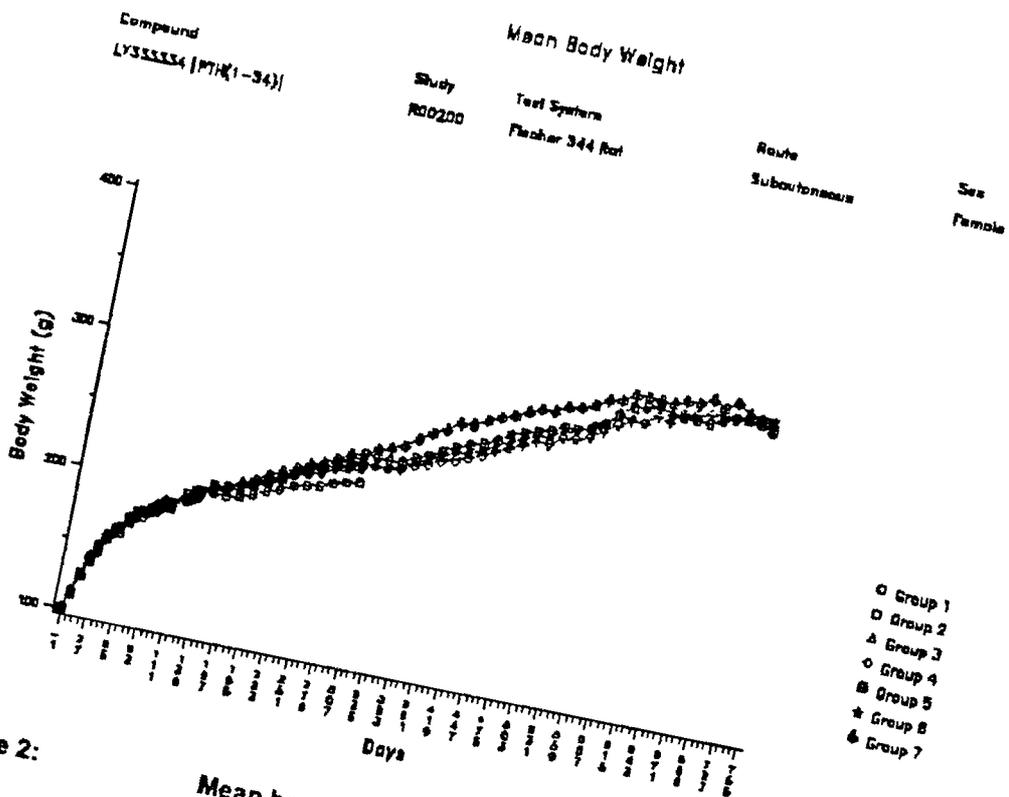


Figure 2:
Mean body weight, Study R00200.

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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

Group	Parameter values			Increase relative to control		
	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	?	?	?	?	?	?
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 (mm ²)	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 (mm ²)	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

Group	Parameter values			Increase relative to control		
	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 (mm ²)	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*	3.06*	-	31%	56%
X-area (mm ²)	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 (mm ²)	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*
Femur midshaft BMC (mg)	10.7	20.7*	10.8	11.6*	11.3	11.6*	15.7*	19.9*
Femur midshaft X-area (mm ²)	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*
L6 vertebra BMC (mg)	2.09	3.91*	1.99	2.14	2.31	2.09	2.87*	3.71*
L6 vertebra X-area (mm ²)	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, H1 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 H1 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, H1 and I2 as compared with vehicle control (Arm A).

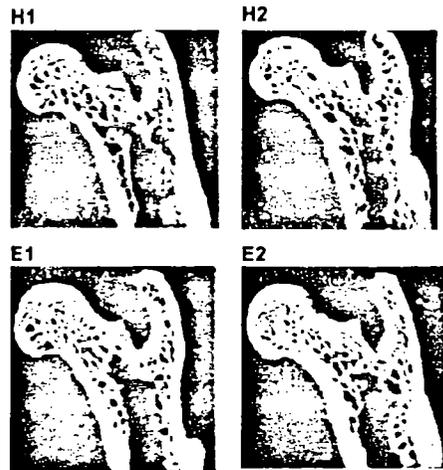


Figure 2: Coronal images of the proximal femur for Arms E and H.

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 (ngxh/ml) (Month 6) (m.f)	AUC (ngxh/ml) (Month 12) (m.f)	AUC (ngxh/ml) (Month 18) (m.f)	AUC (ngxh/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 ngxh/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:

Study R00100	Administered Dose (µg/kg/day)						
	Vehicle	Vehicle	5	30	5	30	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	1.51	9.75	7.39
Day 540, 0.25 hours (ng/mL)	NS	NS	NS	NS	NS	NS	7.64

Study R00200	Administered Dose (µg/kg/day)						
	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 2x0, 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 0-18) (f)	
Study R00100	Grp 1	A	0	5	0		
	Grp 2	F	0	5	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	
	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 Hypertrophys* (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

Abbreviation: 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 ^a								
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

Abbreviation: NA = not applicable.

^a 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 ^a								
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 ^b		2	1	2		5
Total no. of rats with a bone neoplasm	1	12 ^c		2	2	2		6 ^d
Gross bone nodule lesions ^e	1	11			2	2		4

Abbreviations: NA = not applicable, no. = number.

^a Statistically significant with p-value < 0.025 based on 1-sided Cochran-Armitage trend test

^b Number of rats with a specific neoplasm.

^c p=0.0083 (Arm B compared to Arm A).

^d p=0.0010 (Arm B compared to Arm A).

^e p=0.149 (Arms I1 and I2 compared to Arm A).

^f 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						

Abbreviation: 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)
2 nd 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
1 st 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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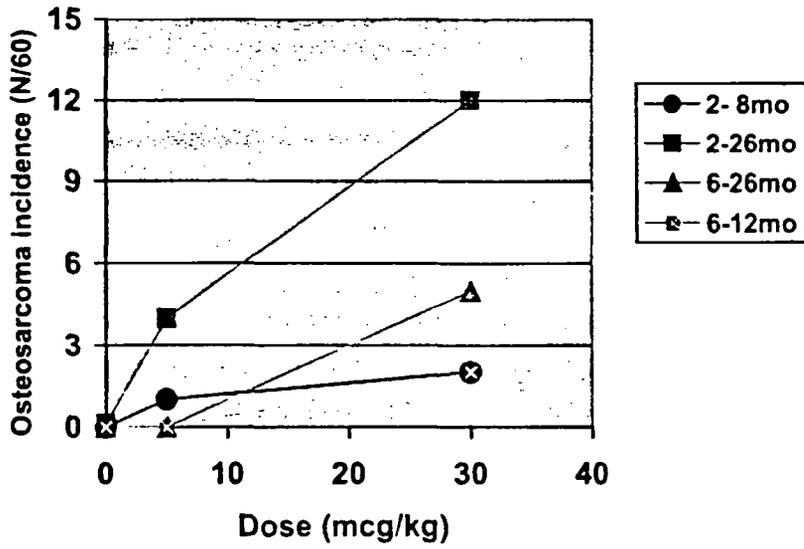
BONE TUMOR DATA

Teriparatide Follow-up Rat Study

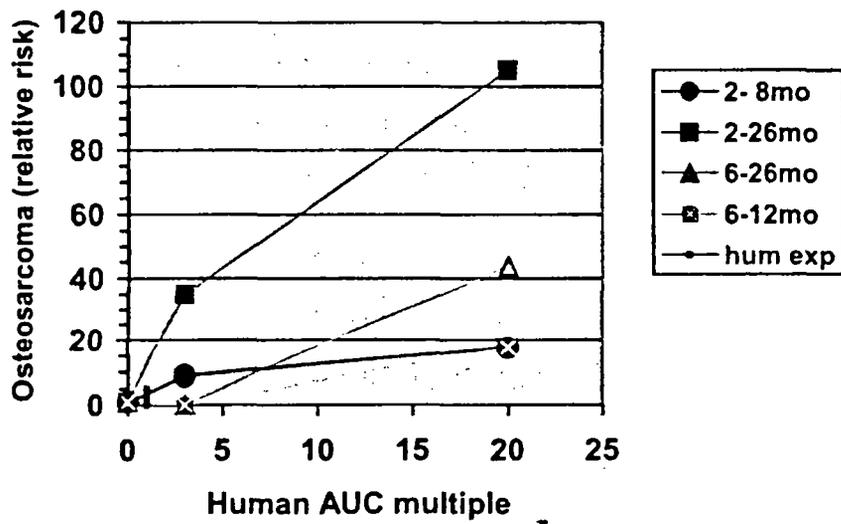
Study Arm	Age (months)					Bone Neoplasms
	2	8	14	20	26	
A	Control n = 60					1 Osteosarcoma
B	Positive Control (30 µg/kg) n = 60					9 Osteosarcoma 2 Osteoma 1 Osteoblastoma
C	Control n = 30		0			
D1	5 µg/kg n = 30		0			
D2	30 µg/kg n = 30		0			
E1	5 µg/kg n = 60					0
E2	30 µg/kg n = 60					2 Osteosarcoma
F	Control n = 30		0			
G1	5 µg/kg n = 30		0			
G2	30 µg/kg n = 30		0			
H1	5 µg/kg n = 60					1 Osteosarcoma 1 Osteoma
H2	30 µg/kg n = 60					2 Osteosarcoma
I1	5 µg/kg n = 60					0
I2	30 µg/kg n = 60					5 Osteosarcoma 1 Osteoma

FIGURE 1. Incidence of bone neoplasms, by study arm

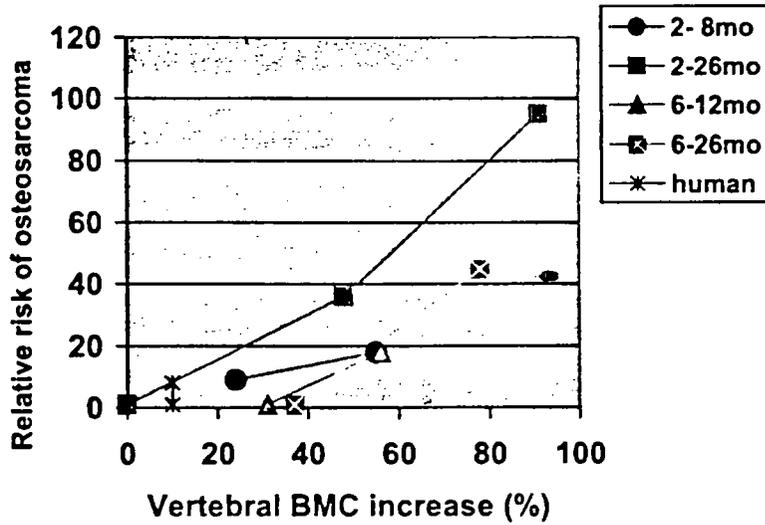
(A) Dose vs. osteosarcoma incidence



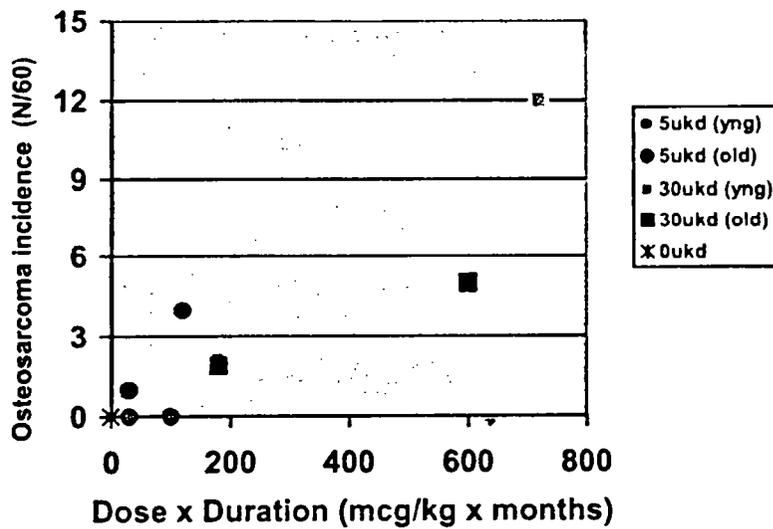
(B) Human AUC multiple vs. risk of osteosarcoma



(C) Vertebral bone mass effect vs. osteosarcoma risk



(D) Dose x Duration vs. osteosarcoma incidence



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	3
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 osteoblast 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 sternbrae, 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 mcg/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 ± sqrtX; 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 than 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 determine this value. A small risk increase in older animals treated with 5 mcg/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 mcg/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 mcg/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 of 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 spontaneous 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
	Multiple 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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/s/

Gemma Kuijpers
11/25/02 12:20:33 PM
PHARMACOLOGIST

Karen Davis-Bruno
11/25/02 12:31:17 PM
PHARMACOLOGIST

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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=1 or 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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ON ORIGINAL**

Teriparatide Follow-up Rat Study

Study Arm	Age (months)					Bone Neoplasms
	2	8	14	20	26	
A	Control n = 60					1 Osteosarcoma
B	Positive Control (30 µg/kg) n = 60					9 Osteosarcoma 2 Osteoma 1 Osteblastoma
C	Control n = 30		0			
D1	5 µg/kg n = 30		0			
D2	30 µg/kg n = 30		0			
E1	5 µg/kg n = 60					0
E2	30 µg/kg n = 60					2 Osteosarcoma
F	Control n = 30		0			
G1	5 µg/kg n = 30		0			
G2	30 µg/kg n = 30		0			
H1	5 µg/kg n = 60					1 Osteosarcoma 1 Osteoma
H2	30 µg/kg n = 60					2 Osteosarcoma
I1	5 µg/kg n = 60					0
I2	30 µg/kg n = 60					5 Osteosarcoma 1 Osteoma

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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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ON ORIGINAL

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:

Reviewer:

Division Name:

HFD #:

Review Completion Date:

Date of submission:

Information to Sponsor:

LY333334 (teriparatide for injection)

Gemma Kuijpers

Division of Metabolic and Endocrine Drug Products

510 (DMEDP)

September 25, 2001

December 19, 2000; August 23, 2001

No

Sponsor:

Drug substance:

Proprietary Name:

Synonyms:

Code Name:

USAN Name:

INN name:

Chemical Name:

Molecular Formula:

Molecular Weight:

Eli Lilly and Company, Indianapolis, IN, USA

Teriparatide (rDNA origin), PTH(1-34)

Forteo™

rhPTH(1-34)

LY333334

Teriparatide

Teriparatide

34-amino acid single chain peptide

C₁₈₁H₂₉₁N₅₅O₅₁S₂

4117.8 Daltons

Drug Product:

Dosage Form:

Dosing Device:

Manufacturer for drug substance:

Manufacturer for dosage form:

rhPTH(1-34), acetic acid, sodium acetate, mannitol, metacresol, sodium acetate, HCl, NaOH, water for injection

Cartridges rhPTH(1-34) Injection (Pen-injector)

3 ml prefilled, delivery device containing a 28-day supply of rhPTH(1-34)

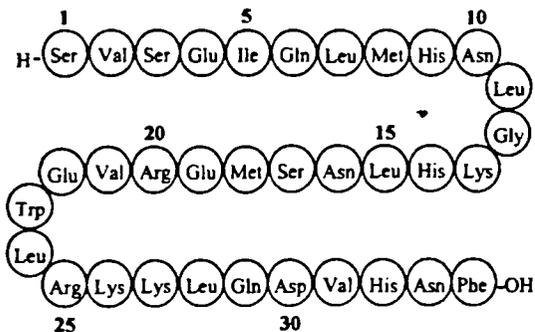
Eli Lilly and Company, Lilly Technology Center, IN, USA

Lilly France S.A., Fegersheim, France

Drug Class:

Structure:

Peptide hormone



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
20 µg daily

Dose:

Route of administration:

Subcutaneous injection (thigh or abdominal wall)

Clinical status:

NDA (Forteo) submitted (November 30, 2000)

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

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

**APPEARS THIS WAY
ON ORIGINAL**

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.

**APPEARS THIS WAY
ON ORIGINAL**

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 evaluation. 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 survived

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 (ug/kg/day)			0	5	30
N animals			30	30	30
BONE					
Trabecular hypertrophy	Femur	Minimal			
		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

Group	Parameter values			Increase relative to control		
	control	LD	HD	control	LD	HD
Dose ($\mu\text{g}/\text{kg}/\text{day}$)	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 (mm ²)	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 (mm ²)	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 evaluation. 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 survived

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 (ug/kg/day)			0	5	30
N animals			30	30	30
BONE					
Trabecular hypertrophy	Femur	Slight	1	22	3
		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

Group	Parameter values			Increase relative to control		
	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)	7.84	8.03	8.66*	-	2.4%	10.5%
L6 vertebra						
BMD(mg/cc)	604	757*	842*	-	25%	39%
BMC (mg)	1.96	2.57*	3.06*	-	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

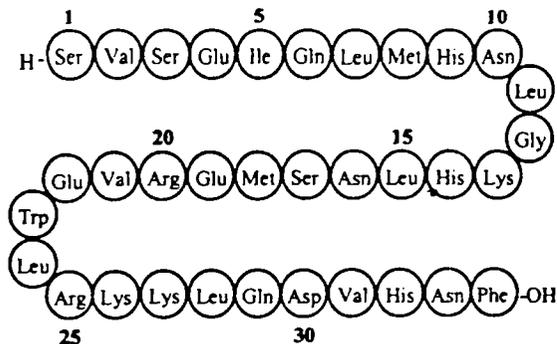
KEY WORDS: Parathyroid hormone, teriparatide, peptide, postmenopausal, osteoporosis, bone, formation, resorption

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: C₁₈₁H₂₉₁N₅₅O₅₁S₂
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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<u>CONTENTS</u>	Page
INTRODUCTION	4
PHARMACOLOGY	
Background.....	5
Mechanism of Action.....	6
General Pharmacology.....	7
Efficacy Pharmacology (Bone Quality)....	8
<i>Monkey Study</i>	10
<i>Rabbit Studies</i>	51
<i>Rat Studies</i>	58
Safety Pharmacology.....	78
GENERAL TOXICOLOGY	
Acute Toxicity Studies.....	81
Chronic Toxicity Studies.....	82
<i>Rat Studies</i>	82
<i>Monkey Studies</i>	89
CARCINOGENICITY	
Rat Carcinogenicity Study.....	108
GENETIC TOXICOLOGY	148
REPRODUCTIVE TOXICOLOGY	150
ADME	168
OVERALL SUMMARY AND EVALUATION	172
RECOMMENDATION	179
LABEL	180
APPENDIX	
Minutes, ECAC Meeting (March 20, 2001)	185
Advisory Committee Briefing Document	187

**APPEARS THIS WAY
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Gemma Kuijpers, Ph.D.
Reviewer

Karen Davis-Bruno, Ph.D.
Team Leader

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 osteoclast 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 cyclase 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 [¹²⁵I](Nle^{8,18}-Tyr³⁴)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 ED₅₀ value of 4.3 ± 1.3 nM (mean s.d.). LY333334 inhibited binding also by maximally 80%, with an ED₅₀ 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) 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 slightly 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 strength 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

Report	Title	In Vivo		In Vitro		Results
		Species, Strain, Gender, & Age	Dose Route	Assay Type	Tissue/Cell Line	
Safety, Efficacy, and Strength of human PTH						
X-95-11	A Study in Ovariectomized Adult Cynomolgus Monkeys (<i>M. fascicularis</i>) 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	Monkey, <i>Macaca fascicularis</i> , 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 2-5%, & spine biomechanical properties by 20-40%. Biomechanical properties of femur neck were increased by 12-23%. 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 rhPTH(1-34) (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 µg/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):	_____
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 (<i>Macaca fascicularis</i>), natural-habitat reared, adult

Initial weight range:	2.77 (mean) \pm 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 μ g/kg/day	PTH1	21	19
Ovariectomized, 12 months LY333334 at 1 μ g/kg/day, then 6 months vehicle once daily	PTH1-W	21	20
Ovariectomized, 18 months LY333334 at 5 μ g/kg/day	PTH5	22	21
Ovariectomized, 12 months LY333334 at 5 μ g/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:

Site	Specifics	Bone Mass		Histomorphometry		Biomechanical Tests
		DEXA	QCT	Trabecular	Cortical	
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%-108%)	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 (cm²), BMC (g), BMD (mg/cm²):

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%

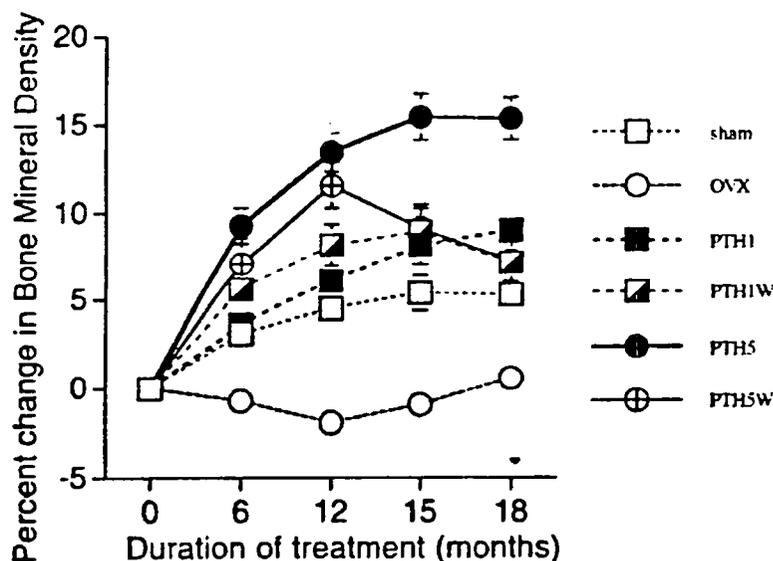


Figure 2.

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 (mm²), BMC (mg/mm), BMD (mg/cm³) (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/cm³Proximal 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: 89.2mm²BMD, 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/cm³Distal 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: 43.4mm²BMD, 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

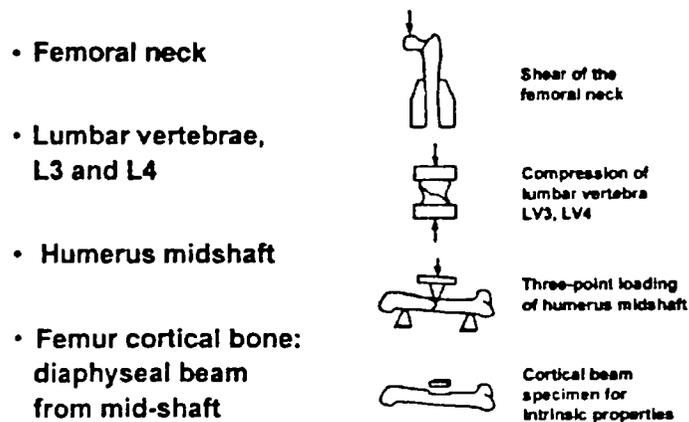


Figure 3. Cartoon to show strategies used in biomechanical testing of the femur neck, lumbar vertebrae (spine), humerus midshaft, and the uniform cortical bone specimen.

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

Spine (Lumbar vertebrae LV3 and LV4)

(Structural properties, i.e., properties dependent on size and shape of specimen)

Yield force (F_y) and stress (σ_y) 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 F_y , with withdrawal associated with lower F_y values. Ultimate, i.e., breaking strength (F_u) 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 \pm SEM of Raw Data)

Measurements	Groups						ANOVA (p-value)	Effects	
	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W		Dose	Withdrawal
Average of L3 and L4 Vertebrae									
A (mm ²)	90.5 \pm 2.1 (21)	86.7 \pm 2.3 (20)	88.3 \pm 2.0 (19)	90.9 \pm 2.3 (20)	87.3 \pm 2.7 (22)	82.8 \pm 2.1 (20)	.15	0.06	ns
F_y (N)	1738 \pm 52 (21)	1499 \pm 94 ^a (20)	1915 \pm 105 ^a (19)	1899 \pm 75 ^a (20)	2113 \pm 77 ^a (22)	1792 \pm 59 ^a (20)	<.0001	ns	<0.05
S (N/mm)	7312 \pm 319 (21)	5805 \pm 476 ^a (20)	7701 \pm 474 ^a (19)	7401 \pm 452 ^a (20)	8012 \pm 367 ^a (22)	7074 \pm 314 ^a (20)	<.005	ns	ns
σ_y (MPa)	19.4 \pm 0.6 (21)	17.3 \pm 1.0 (20)	21.9 \pm 1.3 ^a (19)	21.1 \pm 0.8 ^a (20)	24.6 \pm 1.1 ^a (22)	21.9 \pm 0.9 ^a (20)	<.0001	0.09	0.09
E (MPa)	650 \pm 32 (21)	546 \pm 49 (20)	717 \pm 48 ^a (19)	659 \pm 42 (20)	759 \pm 36 ^a (22)	698 \pm 41 ^a (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.

^s = sham.

^o = ovariectomized.

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

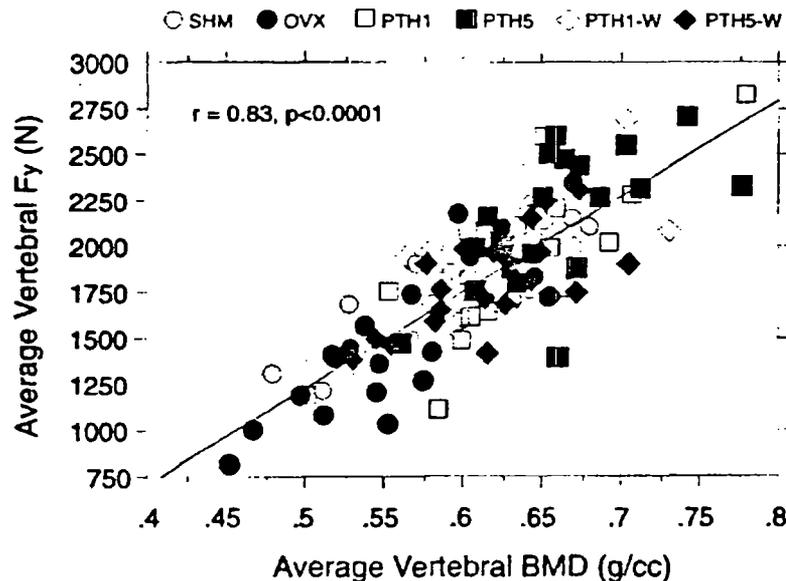


Figure 4.

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

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Humerus midshaft

(Structural properties, i.e., properties dependent on size and shape of specimen)

Ultimate force (F_u), 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 F_u 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 (F_u) 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 \pm SEM of Raw Data)

Measurements	Groups						ANOVA (p-value)	Effects	
	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W		Dose	Withdrawal
Humerus Midshaft									
t (mm)	1.74 \pm 0.04 (21)	1.63 \pm 0.03 ^a (20)	1.68 \pm 0.03 (19)	1.66 \pm 0.04 (20)	1.80 \pm 0.04 ^b (22)	1.72 \pm 0.05 (20)	<.05	<.05	ns
F_u (N)	725 \pm 26 (21)	636 \pm 26 (19)	654 \pm 23 (19)	689 \pm 23 (20)	680 \pm 15 (22)	707 \pm 24 (19)	.08	ns	ns
S (N/mm)	601 \pm 23 (21)	520 \pm 26 (19)	544 \pm 23 (19)	573 \pm 20 (20)	548 \pm 18 (22)	573 \pm 24 (19)	.17	ns	ns
U (mJ)	1797 \pm 85 (21)	1542 \pm 92 (19)	1641 \pm 137 (19)	1751 \pm 84 (20)	1804 \pm 99 (22)	1775 \pm 113 (19)	.41	ns	ns
Proximal Femoral Neck									
F_u (N)	1288 \pm 41 (19)	1105 \pm 53 ^b (18)	1235 \pm 45 ^b (19)	1258 \pm 52 ^b (17)	1362 \pm 30 ^b (21)	1213 \pm 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.

^a = sham.

^b = ovariectomized.

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, $\epsilon_{u\gamma}$ 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 \pm SEM of Raw Data)

Measurements	Groups						ANOVA (p-value)	Effects	
	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W		Dose	Withdrawal
Femoral Diaphyseal Beam Specimen									
σ_u (MPa)	222 \pm 5 (21)	216 \pm 5 (20)	222 \pm 4 (19)	214 \pm 6 (20)	206 \pm 6 (22)	208 \pm 6 (20)	.18	<.05	ns
E (GPa)	17.2 \pm 0.6 (21)	16.4 \pm 0.4 (20)	17.1 \pm 0.4 (19)	16.6 \pm 0.6 (20)	15.4 \pm 0.6 ^a (22)	15.3 \pm 0.6 ^a (20)	<.05	<.01	ns
u (MJ m ⁻³)	5.9 \pm 0.3 (21)	5.8 \pm 0.4 (20)	6.1 \pm 0.4 (19)	5.5 \pm 0.4 (20)	5.4 \pm 0.3 (22)	6.1 \pm 0.4 (20)	.59	ns	ns
E_u	0.035 \pm 0.001 (21)	0.035 \pm 0.002 (20)	0.036 \pm 0.002 (19)	0.034 \pm 0.002 (20)	0.034 \pm 0.002 (22)	0.036 \pm 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.

^a = sham.

^b = ovariectomized.

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Summary Table

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

Data shown as mean ± SEM for 19 to 22 monkeys per group.

Statistical significance, $p < .05$.

^s Versus sham control.

^o 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
Ms.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.wt	µm	Wall width
O.O.P	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 \pm 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
Ca.Ar	24.9 \pm 1.8 (20)	20.9 \pm 1.7 (20)	31.0 \pm 1.8 (18)	31.2 \pm 2.1 (20)	38.0 \pm 2.0 (21)	32.6 \pm 1.8 (19)		.00	ns	<.01	<.001	<.001	<.001
Tb.Tb	126 \pm 8 (20)	116 \pm 6 (20)	135 \pm 6 (18)	148 \pm 9 (20)	162 \pm 11 (21)	140 \pm 8 (19)	log	.00	ns	ns	<.01	<.001	<.01
Tb.N	1.99 \pm 0.09 (20)	1.79 \pm 0.08 (20)	2.33 \pm 0.09 (18)	2.13 \pm 0.10 (20)	2.41 \pm 0.11 (21)	2.35 \pm 0.14 (19)		.00	ns	<.01	<.01	<.001	<.001
Tb.Sc	398 \pm 26 (20)	466 \pm 29 (20)	307 \pm 18 (18)	346 \pm 29 (20)	275 \pm 23 (21)	310 \pm 21 (19)	log	.00	ns	<.001	<.01	<.001	<.001
E.Pm	1.01 \pm 0.30 (20)	2.89 \pm 0.70 (20)	1.83 \pm 0.64 (18)	1.49 \pm 0.29 (20)	1.78 \pm 0.44 (21)	1.82 \pm 0.80 (19)	square root ^a	.16	<.01	ns	ns	ns	ns
Oc.Pm	0.64 \pm 0.24 (20)	1.37 \pm 0.33 (20)	0.90 \pm 0.22 (18)	0.83 \pm 0.18 (20)	1.13 \pm 0.37 (21)	1.49 \pm 0.76 (19)	square root ^a	.48	ns	ns	ns	ns	ns
O.Ar	0.33 \pm 0.06 (20)	0.44 \pm 0.08 (20)	0.74 \pm 0.12 (18)	0.56 \pm 0.09 (20)	0.91 \pm 0.10 (21)	0.84 \pm 0.15 (19)	square root	.00	ns	ns	ns	<.01	<.05
O.Pm	11.6 \pm 1.7 (20)	17.6 \pm 2.7 (20)	23.9 \pm 3.4 (18)	20.7 \pm 2.7 (20)	29.2 \pm 2.6 (21)	26.0 \pm 3.4 (19)		.00	ns	ns	ns	<.05	ns
Ob.Pm	2.5 \pm 0.6 (20)	6.9 \pm 1.7 (20)	6.3 \pm 2.0 (18)	6.1 \pm 1.6 (20)	5.8 \pm 1.4 (21)	7.8 \pm 2.0 (19)	square root ^{a,b}	.35	ns	ns	ns	ns	ns
O.Tb	7.9 \pm 0.4 (20)	8.2 \pm 0.5 (20)	7.9 \pm 0.3 (18)	6.8 \pm 0.3 (20)	7.5 \pm 0.4 (21)	7.7 \pm 0.3 (19)		.16	ns	ns	<.05	ns	ns

Statistical significance was set at $p < .05$.

ns = $p > .05$ and considered not statistically significant.

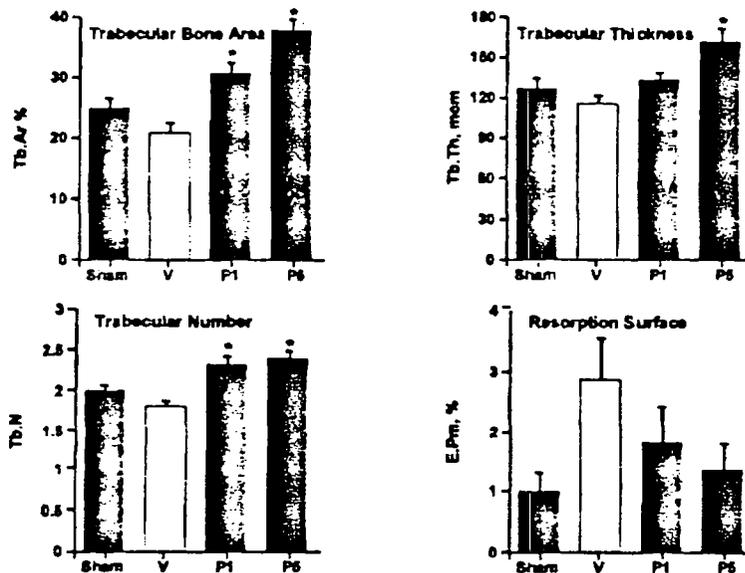


Figure 6.

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 μ g/kg/day (PTH1 shown as P1) or 5 μ g/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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