

Neoplastic findings included osteosarcoma, osteoblastoma, osteoma at various bone sites. These tumor findings are discussed further below. Besides bone tumors, there was a dose-related increase in the incidence of combined skin epithelial cell neoplasms in males, and of thyroid C-cell adenoma and combined C-cell adenoma and adenocarcinoma in males.

The thyroid C-cell tumor findings were statistically significant. However, it should be noted that the statistical significance of the combined tumor data was due to the increased incidence of adenoma only. Although the significance of the thyroid finding can be questioned in light of historical control data, it may be a biologically plausible outcome that is probably the direct or indirect result of the hormonal stimulation of calcium metabolism by PTH. The increased severity of C-cell hyperplasia in both males and females confirms the significance of the finding.

The increased tumor incidence in the skin was not statistically significant.

The apparent increase in combined clitoral gland carcinoma in females could not be tested since the finding was made only in animals with gross lesions and animals without gross lesions were not examined.

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

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

Osteosarcomas included a broad spectrum of malignant osteoblastic lesions. Many formed large bone nodules, while others were only microscopically observed intramedullary lesions. The osteosarcomas were characterized by invasive, polygonal to fusiform neoplastic mesenchymal cells which produced osteoid matrix and uncalcified bone. Intramedullary tumors often replaced hematopoietic cells and disrupted bone trabeculae.

Osteoblastoma has not been described previously in the rat, and is an uncommon benign bone neoplasm in humans. The diagnosis was made on the basis of histological similarity to the human lesion. These tumors were sharply demarcated, contained large active osteoblasts, often arranged along bony trabeculae with minimal atypia. The neoplasm replaced a region of pre-existing bone, but was minimally invasive.

Osteoma in the rat is a benign tumor characterized by a sharply demarcated expansive neoplasm composed of dense variably mineralized lamellar bone with well-differentiated osteoblasts on the outer bone trabeculae.

Osteosarcomas were first detected by clinical or gross observations of bone nodules after approximately 17 months in the high dose groups, and 20 months in the low and mid dose groups. However, the earliest occurrence of an osteosarcoma was in a male rat in the high dose groups that died at 13 months from a fatal osteosarcoma in the vertebra. Apart from the dose-related increase in incidence, the time to death of the osteosarcoma-bearing animals was reduced in a dose-related manner, at least in the males.

The time of tumor initiation is unclear and depends on the duration of the various stages of the multi-stage tumorigenesis process. However, based on the data from this study it is likely that in the rat tumor initiation and promotion can take place at any time between 6-12 months after start of the daily PTH treatment.

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

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

It is unlikely that the osteosarcoma induction by PTH(1-34) is a species-specific effect. In studies with an analogue of PTHrP that were terminated prematurely osteosarcomas were seen in both rats and mice after 30-50 weeks of treatment.

Other peptide hormones have also been known to induce neoplastic events in the rat when administered chronically (gonadotrophins and ovarian neoplasia, gastrin and ECL-omas). Stimulation of the target cells for these peptide hormones has also been associated with human neoplasm formation.

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

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

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

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

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

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

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

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

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Rat Carcinogenicity Study

Histopathology Inventory

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

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

* Blood films examined only as necessary

- 1 Femur collected and examined in all animals
- 2 Lumbar vertebrae collected from all animals that died after Day 607, and from animals that died before Day 607, if available
- 3 Tissues (tibia, pelvis, rib) collected/examined when abnormal

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GENETIC TOXICOLOGY

The Effect of LY333334 on The Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells (Study 950411MLA3939)

(Toxicology Report 01)

(From IND Review September 25, 1995)

METHODS

LY333334 was tested at concentrations of 29, 78, 156, 313, 625, 1250, 5000 ug/ml with and without metabolic activation by the S9 fraction from -treated rats, to determine its ability to induce mutations at the TK locus in mouse lymphoma cells. No cytotoxic response was observed at concentrations up to 5000 ug/ml. The positive controls used were ethylmethanesulfonate (EMS) in the non-activated, and 3-methylcholanthrene (3-MC) in the activated assay. Test article was incubated . Assay was done in . No statistical test was performed, and a positive result was defined as an increase in mutation index of at least 2-fold control value at two successive dose levels.

RESULTS

Incubation with LY333334 had no effect on the mutation frequency of cell cultures at all doses tested. Positive controls were clearly positive in both non-activated and activated assay with mutation indices of 12 and 9, respectively, similar as historical controls.

CONCLUSION

LY333334 is not mutagenic in the L5178Y TK⁺ mouse lymphoma cell assay.

The Effect of LY333334 on the Induction of Reverse Mutations in Salmonella typhimurium and Escherichia coli Using the Ames Test (Study 950412AMS3939)

(Toxicology Report 02)

(From IND Review September 25, 1995)

METHODS

LY333334 was tested at concentrations of 313, 625, 1250, 2500, 5000 ug/ml with and without metabolic activation by S9 fraction from -induced rats, to determine its ability to induce reverse mutations in histidine auxotrophs of Salmonella typhimurium, LT-2 (TA 1535, TA 1537, TA 98, TA 100), and a tryptophan auxotroph of Escherichia coli (WP2uvrA). No cytotoxic response was observed at concentrations up to 5000 /ml. The positive controls used were

 7) for the non-activated, and for the activated assay. Test was conducted with an incubation time of . Each concentration of test article and controls was tested in . No statistical test was performed, and a positive result was defined as an increase in the number of revertants to at least twice the vehicle control value at two successive concentrations.

RESULTS

Incidence of revertant colonies after treatment with LY333334 was approximately equal to the values for vehicle controls, for each tester strain of S. typhimurium and E. coli. Positive controls all produced increases in revertants consistent with expected results.

CONCLUSION

LY333334 is not mutagenic in the Ames bacterial mutagenesis test.

The Effect Of LY333334 Given Subcutaneously for 2 Consecutive Days on the Induction of Micronuclei in Bone Marrow of ICR Mice (Study 950405MNT3939)

(Toxicology Report 03)

(From IND Review September 25, 1995)

METHODS

Hsd:(ICR) mice (n=5/sex/group), age 8-9 weeks, weight 26-36g, were given doses of 0, 1000, 3000, 10000 ug/kg for 2 consecutive days by subcutaneous injection. Vehicle was 20 mM monobasic

Positive control was mg/kg. Ca. 24 hours after the second dose, femoral bone marrow was collected on a slide

(1slide/femur), and the number of normochromatic erythrocytes (NME), anucleate polychromatic erythrocytes (PCE) and micronucleated polychromatic erythrocytes (MPCE) was determined.

RESULTS

Both in males and females, the PCE/NCE ratio was similar to vehicle controls (1.0 and 1.3, respectively). The MPCE/1000 PCE ratio was not affected by LY333334 in males or females (Trend test p-values 0.37 and 0.94, respectively). This indicates that LY333334 is not toxic to bone marrow.

CONCLUSION

LY333334 is not clastogenic and does not interact with the mitotic spindle in the bone marrow micronucleus test.

The Effect of LY333334 on the In Vitro Induction of Chromosome Aberrations in CHO Cells

(Study 950412CAB3939)

(Toxicology Report 06)

(From IND Review September 25, 1995)

METHODS

CHO cells were cultured in _____ and incubated for _____. Positive controls used were 0.5 g/ml mitomycin C for the non-activated assay, and 15 g/ml cyclophosphamide for the activated assay. Cells from 2-3 cultures/dose group were harvested after 1 cell cycles (18-24 h), treated with _____ and prepared for microscopy. 100 cells were scored for vehicle controls and treated, 25 cells for positive controls.

RESULTS

LY333334 was not toxic to CHO cells at tested concentrations. In the non-activated assay, the incidence of cells with aberrations including gaps ranged from 2-3% at all doses tested. Positive control mitomycin C yielded 64% aberrant cells. In the activated assay, incidence of cells with aberrations including gaps ranged from 0-1% at all doses tested. Treatment with cyclophosphamide, the positive control, gave 44% aberrant cells. Highest concentration of LY333334 (5000 ug/ml) induced an increase in the % cells with diplochromosomes from 0.5% (control) to 3.5%. However, this is not known to be correlated to chromosomal damage. Thus, LY333334 had no significant effect on the percentage of aberrant CHO cells in the presence or absence of metabolic activation.

CONCLUSION

LY333334 is not clastogenic in the CHO chromosome aberration assay.

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REPRODUCTIVE TOXICOLOGY

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

Table 5 Developmental and Reproductive Toxicity Studies with LY333334

Report Number	Species, Strain, Study Type	Number/Group, Age	Dose ($\mu\text{g}/\text{kg}/\text{day}$)	Route of Administration	Duration of Treatment	Parameters Evaluated	Observations
20	Rat, CD Segment I	20 Males: 13 weeks 20 Females: 9 weeks	Males: 0, 30, 100, 300 Females: not treated	Subcutaneous	7 weeks: 4 weeks prior to and during cohabitation until termination	Survival Clinical observations Body weight Food consumption Testicular weight and morphology Sperm concentration, motility Mating Fertility	↓ Prostate weight in males at 300 $\mu\text{g}/\text{kg}$ Redness of the extremities in all treated males. No adverse effects on body weight, food consumption, mating, fertility, testicular weight, testicular morphology, sperm concentration, or sperm motility NOAEL = 300 $\mu\text{g}/\text{kg}$
15	Rat, CD Segment I	20 Males 20 Females: 9 weeks	Males: not treated Females: 0, 30, 100, 300	Subcutaneous	2 weeks prior to and during cohabitation until postmating Day 6	Survival Clinical observations Body weight Food consumption Mating Fertility Gestation survival Fetal parameters	↑ Body weight and food consumption in females at 100 and 300 $\mu\text{g}/\text{kg}$ Redness of the extremities in all treated females. No effects on maternal reproductive or fetal parameters NOAEL = 300 $\mu\text{g}/\text{kg}$
14	Rat, CD Segment II	25 Pregnant females: 12 weeks	0, 30, 225, 1000	Subcutaneous	Gestation Days 6 through 17	Survival Clinical observations Body weight Food consumption Reproductive and uterine parameters Fetal viability, weight, and morphology	Redness of the extremities in all treated females. No treatment-related effects on maternal body weight, food consumption, reproductive and uterine parameters, or on fetal viability, weight, or morphology NOAEL = 1000 $\mu\text{g}/\text{kg}$
17	Mouse, CD-1 Segment II	30 Pregnant females: 12 weeks	0, 30, 225, 1000	Subcutaneous	Gestation Days 6 through 15	Survival Clinical observations Body weight Food consumption Reproductive and uterine parameters Fetal viability, weight, and morphology	↑ Food consumption at 225 and 1000 $\mu\text{g}/\text{kg}$ Redness of the extremities in all treated females. No treatment-related effects on maternal body weight, reproductive and uterine parameters, or on fetal viability, weight, or morphology NOAEL = 1000 $\mu\text{g}/\text{kg}$
37	Rabbit, New Zealand White Segment II (pilot)	5 Pregnant females: 7 months	0, 3, 10, 30, 100	Subcutaneous	Gestation Days 7 through 19	Survival Clinical observations Body weight Food consumption Reproductive and uterine parameters Fetal viability, weight and external morphology	↓ Survival at 100 $\mu\text{g}/\text{kg}$ ↑ Blood ionized calcium in all treated groups. Totally resorbed fetuses in all surviving females at ≥10 $\mu\text{g}/\text{kg}$ and 1 female at 3 $\mu\text{g}/\text{kg}$ ↓ Live fetuses per litter at 3 $\mu\text{g}/\text{kg}$ NOAEL = < 3 $\mu\text{g}/\text{kg}$
35	Rat, CD Segment II/III	25 Pregnant females: adult	0, 30, 225, 1000	Subcutaneous	Gestation Day 6 through Postpartum Day 20	Survival Clinical observations Body weight Food consumption Gestation length Litter size Progeny survival, growth, development, behavior, reproductive performance, external and gross internal examination	Redness of the extremities in all treated females. ↓ Pup body weight on PND 14 in F_1 females at 1000 $\mu\text{g}/\text{kg}$ ↓ Motor activity on PND 23 and 60 for F_1 at 1000 $\mu\text{g}/\text{kg}$ Growth retardation in F_1 males at 1000 $\mu\text{g}/\text{kg}$ and F_1 females at ≥225 $\mu\text{g}/\text{kg}$ Maternal/Reproductive NOAEL = 1000 $\mu\text{g}/\text{kg}$ Developmental NOAEL = 30 $\mu\text{g}/\text{kg}$

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

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

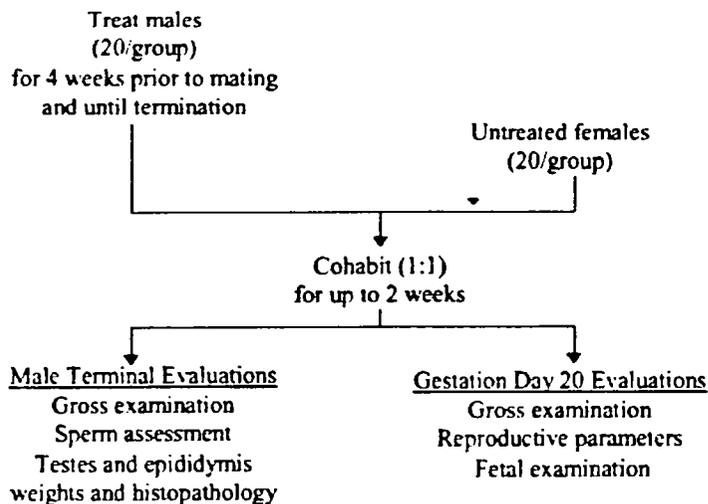
Study: R19797
 Live-phase duration: 2 months
 Live-phase dates: 20 October through 21 December 1997
 Test article: LY333334
 Chemical name: Biosynthetic human parathyroid hormone (1-34)
 Lot number: PPD03521
 Assigned potency: 0.96 mg/vial
 Species and strain: Rat, CD
 Body weight range at live-phase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

Treatment groups:

Group	Dose of LY333334 ($\mu\text{g}/\text{kg}/\text{day}$)
00	0*
01	30
02	100
03	300

*Sodium phosphate monobasic in saline

Study Design:



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

RESULTS

MALES

Clinical signs: Red extremities in all LY-treated males appr. 30 min after dosing.

Mortality: None

Body weight: No significant effects throughout live phase

Food consumption: No significant effects during premating

EFU: No significant effects during premating

Mating performance and fertility:

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

Sperm concentration:

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

Sperm motion:

The following sperm motion parameters were collected for each animal:

- Percent motile sperm
- Percent progressively motile sperm
- Percent linearity
- Percent straightness
- Curvilinear velocity
- Average path velocity
- Straight-line velocity
- Beat/cross frequency
- Amplitude of lateral head displacement

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

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

Organ weights:

The following organs were weighed from all animals that were necropsied at treatment termination:

- Testes
- Epididymis (left)
- Prostate

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

No treatment-related differences in testicular or epididymal weights.

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

*p≤0.05

Pathology:

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

- Testis
- Epididymis
- Prostate

Gross pathology

No significant treatment-related gross changes were observed.

Histopathology

Histopathology changes (animal incidence, n/20)

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

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

FEMALES

Clinical signs:

No remarkable findings

Mortality:

None

Body weight:

No treatment effects on gestational body weight or body weight gain in the untreated females

Food consumption:

No data for females

Maternal reproductive parameters:

Cesarean sections were performed on Gestation Day 20.

No effect on number of corpora lutea, implantations and preimplantation loss.

No effect on fetal viability (per litter incidence and litter incidence of: #live fetuses, #resorptions, #non-live implants)

OFFSPRING

Fetal parameters:

No adverse effects on fetal weight, fetal gender, or occurrence of fetal runts.

Fetal anomalies:**Incidence of malformations and deviations:****Anomalies (external examination only; no visceral/skeletal examination)**

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

* malformations observed in the same (1) fetus

The visceral organ protrusion is rare and was not mentioned in the Sponsor's historical control database. However, the finding has been observed occasionally in the CD rat according to the MARTA historical control database (1992-1994). Since this was a single finding it is no convincing evidence of a treatment-related effect.

The head/neck hematoma incidence was within Sponsor's historical control range (total of 79/14494 conceptuses affected; range 0-8 conceptuses/litter, 0-7 litters; Eli Lilly database, 1989-1997).

The tail findings were not mentioned in the Sponsor's historical control database. However, the finding has been observed in the CD rat according to the MARTA historical control database (1992-1994). Since these were single findings with no clear dose-relationship they constitute no convincing evidence of treatment-related effects.

There were no fetuses with external variations.

CONCLUSIONS

Postdosing redness of extremities (vasodilation) at all doses.

Small, but statistically significant decrease in prostatic weight in males of the 300- μ g/kg group.

The no-observed-adverse-effect level (NOAEL) for paternal and reproductive toxicity was 300 μ g/kg, the highest dose tested in this study.

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

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

METHODS

Female CrI:CD(SD) rats (20/group) received subcutaneous injections of 0, 30, 100, or 300 ug/kg/day from 2 weeks prior to mating through a maximum 2-week cohabitation period and continuing through Gestation/Postmating Day 6. Rats in the control group received vehicle

Clinical signs of toxicity, body weight, and food consumption parameters were measured. Females were killed on Gestation Day 20 for evaluation of maternal reproductive parameters and fetal viability, weight, gender, and external morphology.

RESULTS

All dams survived until scheduled termination.

A treatment-related clinical sign was redness of the extremities (vasodilation) in all treated animals following injection of LY333334.

During the 2-week pre-mating period there were increases in body weight and/or food consumption parameters in the 100- and 300-ug/kg groups.

There were no treatment-related effects on mating performance (% successful mating), fertility indices (% fertile mating) or time-to-mating.

There were no treatment effects on (# or %) corpora lutea, implantations, preimplantation loss, or on (#) live or dead fetuses/litter, resorptions/litter, or live implants/litter (ie fetal viability).

There were no treatment effects on fetal weight, gender, or incidence of fetal runts.

There were no fetuses with external malformations, deviations or variations.

CONCLUSIONS

Postdosing redness of the extremities (vasodilation) in all treated animals.

Increases in maternal body weight and/or food consumption were observed at doses ≥ 100 ug/kg.

There were no adverse effects on maternal reproductive parameters or fetal parameters.

The no-observed-adverse-effect level (NOAEL) for maternal, reproductive, and developmental toxicity was 300 ug/kg.

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

(Toxicology Report 14)

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

METHODS

Female Crl:CD(SD) rats (25/group) received injections of 0, 30, 225, or 1000 ug/kg/day on Gestation Days 6 through 17. Rats in the control group received vehicle

. Clinical signs, body weight and food consumption were measured. Females were killed on Gestation Day 20 for evaluation of maternal reproductive parameters, and fetal viability, weight, gender, and external morphology.

RESULTS

All dams survived until scheduled termination.

Redness of the extremities was noted 30 minutes after dosing in all or almost all animals of the LY333334 treatment groups. There were no other treatment-related clinical signs.

Maternal body weight and food consumption parameters were not significantly affected.

There were no treatment effects on (# or %) corpora lutea, implantations, preimplantation loss, or on (#) live or dead fetuses/litter, resorptions/litter, or live implants/litter (ie fetal viability).

There were no treatment effects on fetal weight, gender, or incidence of fetal runts.

There were no significant treatment-related effects on the fetal or litter incidences of combined malformations, deviations or variations.

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

Skull-calvaria occipital bone- incomplete ossification:

Conceptus incidence (Ctrl-LD-MD-HD): 6-19-8-8 (hist ctrl range 0-10/litter)

Skull-hyoid bone-unossified:

Conceptus incidence (Ctrl-LD-MD-HD): 1-7-5-2 (hist ctrl range (0-3/litter)

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

CONCLUSIONS

Postdosing redness of the extremities was noted in all LY333334 treatment groups.

There were no adverse effects of LY treatment on maternal reproductive parameters, or on fetal parameters.

The no-observed-adverse-effect level (NOAEL) for both maternal reproductive and developmental toxicity was 1000 ug/kg/day, the highest dose used in the study.

**APPEARS THIS WAY
ON ORIGINAL**

CONCLUSIONS

Postdosing redness of the extremities was noted in the 225- and 1000-ug/kg/day treatment groups. The no-observed-adverse-effect level (NOAEL) for maternal reproductive was 1000 ug/kg/day, the highest dose used in the study.

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

**APPEARS THIS WAY
ON ORIGINAL**

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

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

METHODS

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

RESULTS

Mortality

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

Clinical signs

Peripheral vasodilation in all treatment groups

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

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

^a Vehicle.

Body weight

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

Gestation Days	Dose ($\mu\text{g}/\text{kg}/\text{day}$):	Mean Body Weight Gain (g)				
		0 ^a	3	10	30	100
GD 7-10		-16	14	-25	-83	-93
GD 7-20		82	-36	-126	-342	- ^b
GD 20-28		126	97	169	220	- ^b

^a Vehicle.

^b Body weight data were not available due to euthanasia or death on or before GD 18.

Food consumption

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

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

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

Blood ionized calcium

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

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

^a mmol/L (% increase from 0-hour baseline).

^b Vehicle.

^c Blood samples were not available due to euthanasia or death on or before GD 18.

Maternal reproductive parameters

Resorptions:

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

Parameters ^a	Dose ($\mu\text{g}/\text{kg}/\text{day}$):	Observation				
		0 ^b	3	10	30	100
Pregnant females		5	5	5	5	5
Corpora lutea		9.2	9.8	7.8	9.6	— ^c
Implantations		9.0	9.0	6.4 ^d	9.8	— ^c
Preimplantation loss/dam		0.2	0.8	1.4	0	— ^c
Resorptions (early and late)		0.6	6.4	6.4	9.8	— ^c
Resorptions/litter (%)		6.7	69*	100*	100*	— ^c
Litters with resorption sites only		0	1	5*	5*	
Live fetuses/litter		8.4	2.6*	0*	0*	— ^c

* = $p < .05$.

^a All data expressed as mean values for the group, except for the number of pregnant females and dead fetuses.

^b Vehicle.

^c Maternal data were not available due to euthanasia or death on or before GD 18.

^d Significantly different from the 30- $\mu\text{g}/\text{kg}$ group, $p < .05$.

Fetal parametersFetal weight and external and skeletal morphology:

No effects in the 3 ug/kg/day group. This was the only LY333334 treatment group that yielded viable fetuses.

Affected implants (total of resorptions, dead fetuses, and malformed live fetuses) (%):

Increased in all groups.

Malformations:

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

Parameters	Administered Dose ($\mu\text{g}/\text{kg}/\text{day}$)				
	0 ^b	3	10	30	100
Fetal weight (g)	35.97	37.61	— ^c	— ^c	— ^d
Number of conceptuses with malformations	0	3	— ^c	— ^c	— ^d
Number of fetuses with multiple malformations	0	1	— ^c	— ^c	— ^d
Affected implants/litter (%)	6.7	73*	100*	100*	— ^d
Malformations-conceptus (litter) incidences					
Umbilical torsion	0 (0)	1 (1)			
Cervical vertebra – absent left arch	0 (0)	1 (1)			
Maxilla – absent incisor	0 (0)	2 (2)			

* = $p < .05$.

^a All data are expressed as mean values for the group, except for malformation frequency.

^b Vehicle.

^c Fetal data were not available because all conceptuses were resorptions.

^d Fetal data were not available due to euthanasia or death on or before GD 18.

CONCLUSIONS

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

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

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

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

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

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

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

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

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

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

**APPEARS THIS WAY
ON ORIGINAL**

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

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

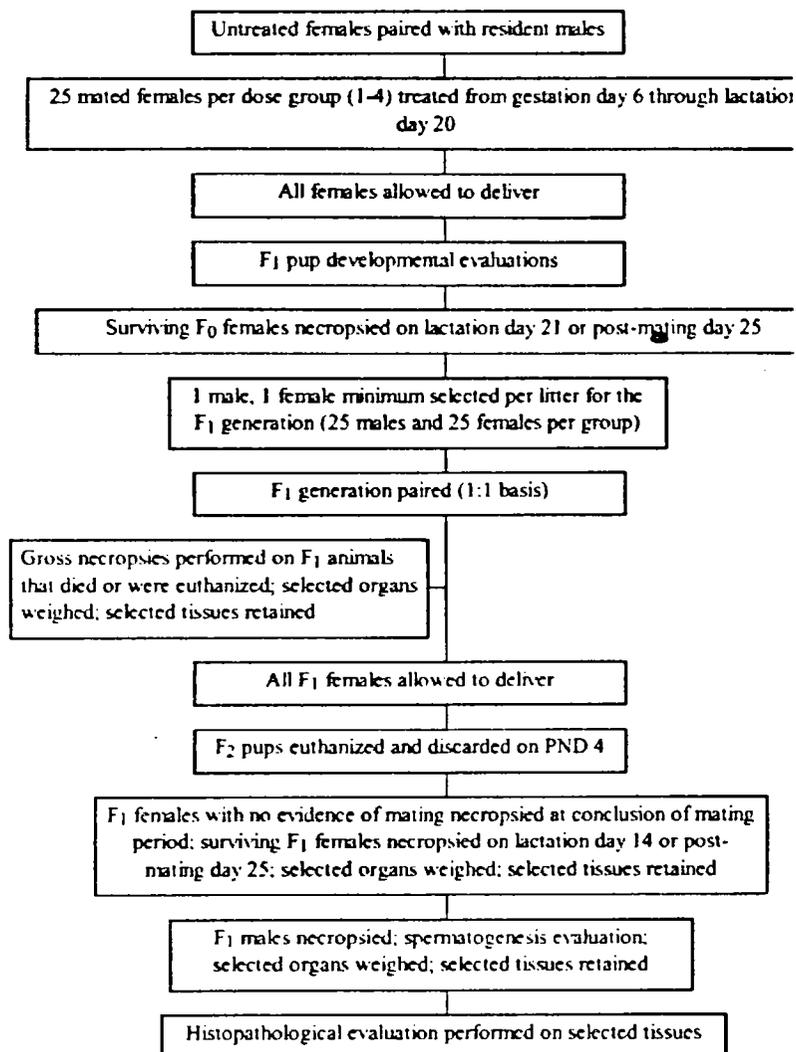
METHODS

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

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

**APPEARS THIS WAY
ON ORIGINAL**

Study Design



RESULTS

F0 maternal generation

Clinical signs/Survival

Reddened and warm extremities at the time of dosing with dose-related incidence in the treated groups. No other treatment-related clinical signs.

No adverse effect on F0 gestation or the process of parturition (dystocia). All F0 females survived to the scheduled necropsy.

Body weight/Food consumption

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

F0 necropsy data

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

F1 litter data

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

F1 pup body weight

Mean F1 female pup body weights reduced in the 1000 ug/kg/day group on PND 7, 14* and 21 (ca. 5% reduction at each time interval).

Mean F1 male pup body weights reduced in the 1000 ug/kg/day group on PND 14 and 21 (6% and 3%, respectively).

Mean F1 pup body weights unaffected on PND 1 and 4 (females) and 1,4 and 7 (males).

Mean F1 pup body weights (male and female average) reduced in the 1000 ug/kg/day group on PND 14* and 21 (7% and 8% reduction).

*statistically significant effect

F1 development and behaviour

No effects on pre-weaning indicators of physical and functional development (pinnal detachment, surface righting response and eye opening) of the F1 pups in the treated groups.

No treatment-related effects on achievement of vaginal patency.

Slight but statistically significant delay in achievement of balanopreputial separation in males in the 1000 ug/kg/day group (Mean day of acquisition: 43-43.5-43.8-44.5* days)

No significant adverse effects in auditory startle test response, and learning and memory (Biel maze swimming trial).

Motor activity was reduced in the 1000 ug/kg/day group males and females on PND 23 and 60. The reduction was similar for total activity and ambulatory activity separately.

Motor activity: Total activity (counts)

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

*statistically significant effect

F1 generation

Clinical signs/Survival

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

Body weight

WEEKLY

Males

Body weight gain significantly reduced in the 1000 ukd group during weeks 7-8 and 8-9, but not after that through week 23. Body weight significantly reduced during weeks 8,9,10,11 (7-11%). No effect on body weight or body weight gain at 30 or 225 ukd.

Females

Body weight gain significantly reduced in the 1000 ukd group during weeks 7-8, 8-9, and 9-10, but not after that through week 15 (end of pre-mating period). Body weight significantly reduced throughout pre-mating period, i.e., weeks 7-15 (9-12%).

Body weight gain significantly reduced in the 225 ukd group during weeks 8-9 and 11-12, but not at other times. Body weight significantly reduced from week 9-15 (4-8%).

No effect on body weight or body weight gain at 30 ukd.

GESTATION

Females

Mean body weights reduced in 1000 ukd F1 females throughout gestation, but body weight gains unaffected. Mean body weights reduced in 225 ukd F1 females on GD 0,6,9, but body weight gains

again unaffected. The decreased body weights were attributed to the above described pre-mating effect and not to an effect on gestational body weight gain.

No effects on body weight or body weight gain in 30 ukd F1 females

LACTATION

Females

Mean body weights reduced in 1000 ukd F1 females throughout lactation, but body weight gains unaffected. The decreased body weights were attributed to the above described pre-mating effect and not to an effect on gestational or lactational body weight gain.

No effects on body weight or body weight gain in 30 and 225 ukd F1 females.

F1 reproductive parameters and organ weights

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

F1 necropsy data

No treatment-related F1 necropsy findings.

F2 litter data

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

CONCLUSIONS

NOAEL for F0 maternal and reproductive toxicity was 1000 ug/kg/day.

NOAEL for F1 developmental toxicity was 30 ug/kg/day (F0 maternal dose)

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

Growth retardation occurred in the F1 males of the 1000 ug/kg/day group and in the F1 females in the 225 and 1000 ug/kg/day groups during the initial part of the pre-mating growth period.

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

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

**APPEARS THIS WAY
ON ORIGINAL**

SUMMARY AND EVALUATION OF REPROTOXICITY STUDIES

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

In a Segment II developmental toxicity study in pregnant CD rats, there were no significant findings at daily subcutaneous doses of 30 to 1000 µg/kg.

In a Segment II developmental toxicity study in pregnant CD-1 mice, there was an increase in the incidence of one malformation (eyelid open) at a daily subcutaneous dose of 1000 µg/kg. There were also increases in the incidences of a number of skeletal deviations and variations (interrupted rib, extra vertebra, extra rib) at subcutaneous doses ≥ 225 µg/kg.

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

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

**APPEARS THIS WAY
ON ORIGINAL**

ADME

Absorption, Distribution, Metabolism, and Excretion (ADME)

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

Pharmacokinetics and Toxicokinetics

Pharmacokinetic and toxicokinetic studies with LY333334 administered by the subcutaneous route indicate that LY333334 is rapidly absorbed and eliminated from the circulation in all species studied. Generally, T_{max} was approximately 15 to 30 minutes after dose administration and T_{1/2} was approximately 15 to 30 minutes. When multiple dose levels were administered, AUC and C_{max} was found to increase with dose. No evidence for accumulation was observed after daily repeated dosing in rats (up to 18 months) or monkeys (up to 17 months). There were no apparent gender differences in the single-dose serum pharmacokinetics of LY333334 in the rat or the monkey at doses of 10 µg/kg or less.

Bioavailability

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

Metabolism, Distribution, and Excretion

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

**APPEARS THIS WAY
ON ORIGINAL**

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

Table 4 Summary of Pharmacokinetic Parameters for Male and Female Rats After Daily Subcutaneous Administration of LY333334 for up to 18 Months

Report/ Duration	Time of Sampling	Gender	Dose ($\mu\text{g}/\text{kg}$)	Pharmacokinetic Parameters			
				C_{max} (ng/mL)	T_{max} (minutes)	$T_{1/2}$ (minutes)	$AUC_{0-\infty}$ ($\text{ng}\cdot\text{hour}/\text{mL}$)
ADME 4 6 weeks	Day 8	M	10	2.3	15	ND ^b	ND
		F		1.6	15	ND	ND
	Day 43	M		4.6	15	ND	ND
		F		2.8	15	ND	ND
	Day 8	M	30	7.1	15	ND	ND
		F		6.9	15	ND	ND
	Day 43	M		12.0	15	ND	ND
		F		9.6	15	ND	ND
	Day 8	M	100	30.0	15	13	22.9
		F		24.0	15	13	15.7
	Day 43	M		64.0	15	14	45.0
		F		35.0	15	11	26.3
	Day 8	M	300	112.0	30	ND	ND
		F		86.0	15	ND	ND
Day 43	M		195.0	15	ND	ND	
	F		120.0	5	ND	ND	
ADME 7 6 months	Day 0	M	10	3.8 ^a	ND	ND	ND
		F		3.2 ^a	ND	ND	ND
	Day 140	M		3.1 ^a	ND	ND	ND
		F		3.5 ^a	ND	ND	ND
	Day 0	M	30	8.4 ^a	ND	ND	ND
		F		6.8 ^a	ND	ND	ND
	Day 140	M		10.3 ^a	ND	ND	ND
		F		9.2 ^a	ND	ND	ND
	Day 0	M	100	27.1	15	25	22.0
		F		25.8	15	26	16.1
	Day 140	M		75.0	30	26	53.6
		F		52.3	30	25	40.1

continued

Table 4 (continued) Summary of Pharmacokinetic Parameters for Male and Female Rats After Daily Subcutaneous Administration of LY333334 for up to 18 Months

Report/ Duration	Time of Sampling	Gender	Dose ($\mu\text{g}/\text{kg}$)	Pharmacokinetic Parameters			
				C_{max} (ng/mL)	T_{max} (minutes)	$T_{1/2}$ (minutes)	$\text{AUC}_{0-\infty}$ (ng \cdot hour/mL)
ADME 23	6 months	M	5	2.3	15	19	1.4 ^c
		F		1.3	15	15	0.8 ^c
18 months	12 months	M		1.0	15	11	0.4 ^c
		F		2.2	15	26	1.6 ^c
	18 months	M		0.6	15	34	0.6 ^c
		F		1.0	15	10	0.5 ^c
	6 months	M	30	10.3	15	37	8.7 ^c
		F		10.9	15	16	5.8 ^c
	12 months	M		5.2	30	28	4.5 ^c
		F		12.5	15	20	9.2 ^c
	18 months	M		3.1	15	32	3.6 ^c
		F		6.9	15	27	4.6 ^c
	6 months	M	75	35.4	15	20	23.5 ^c
		F		20.1	15	24	13.9 ^c
	12 months	M		16.4	30	28	13.7 ^c
		F		28.4	30	31	25.6 ^c
	18 months	M		8.3	30	62	14.0 ^c
		F		21.1	15	27	11.9 ^c

Abbreviations: M = males; F = females; iv = intravenous; sc = subcutaneous; kg = kilogram; mL = milliliter; C_{max} = maximum concentration; T_{max} = time of maximum concentration, $T_{1/2}$ = half-life.

$\text{AUC}_{0-\infty}$ = area under the curve extrapolated to infinity; pg = picogram; ng = nanogram.

^aSamples collected at 0.25 hour post dose (T_{max} was assumed to be 0.25 hour).

^bND = not determined.

^c $\text{AUC}_{0-\tau}$, where τ is the next dose (24 h).

Comment on TK observations written by Reviewer's pet rat:

"Tee',k =-p77gggggggrfof000utv"

Table 5 Summary of Pharmacokinetic Parameters for Male and Female Cynomolgus Monkeys After Daily Subcutaneous Administration of LY333334 up to 17 months

ADME Report /Duration	Time of sampling	Gender	Dose (µg/kg)	Pharmacokinetic Parameters		
				C _{max} (ng/mL)	T _{max} (minutes)	AUC _{0-t} (ng•hour/mL)
ADME 9 3 months	(Days 0/1,7,21,51,84)	M	2	0.5-0.7 ^a	ND ^b	ND
		F		0.5-0.7 ^a	ND	ND
		M	10	4.0-6.0 ^a	ND	ND
		F		3.5-5.0 ^a	ND	ND
		M	20	14.9-18.6 ^a	ND	ND
		F		6.8-9.8 ^a	ND	ND
		M	40	18.5-31.5 ^a	ND	ND
		F		16.2-23.9 ^a	ND	ND
ADME 14 1 year	Week 2	M	0.5	0.2	30	ND
		F		0.1	30	ND
	Week 26	M	0.5	0.2	30	ND
		F		0.2	30	ND
	Week 51	M	0.5	0.1	30	ND
		F		0.2	30	ND
	Week 2	M	2	0.9	34	ND
		F		0.8	30	ND
	Week 26	M	2	0.7	38	ND
		F		0.6	38	ND
	Week 51	M	2	0.6	30	ND
		F		0.7	30	ND
	Week 2	M	10	5.1	30	ND
		F		4.0	30	ND
	Week 26	M	10	6.7	30	ND
		F		3.2	35	ND
	Week 51	M	10	4.1	30	ND
		F		ND	ND	ND
ADME 15 ^c 17 months	1 month	F ^d	1	ND	ND	0.25
	7 months	F ^d		ND	ND	0.46
	11 months	F ^d		ND	ND	0.29
	17 months	F ^d		ND	ND	0.24
	1 month	F ^d	5	ND	ND	2.03
	7 months	F ^d		ND	ND	2.53
	11 months	F ^d		ND	ND	2.45
	17 months	F ^d		ND	ND	1.85
ADME 19 3 months	1 month	F	40	18.6 ^c	ND	ND
	3 months	F		4.7 ^c	ND	ND

Abbreviations: M = males; F = females; kg = kilogram; mL = milliliter; C_{max} = maximum concentration; T_{max} = time of maximum concentration, T_{1/2} = half-life, AUC 0-t = area under the curve to time "t"; pg = picogram; ng = nanogram; ^a Immunoreactivity measured at a single time-point 1 hour postdose. Values are the ranges observed at the indicated dose level over the duration of the study; ^b ND = not determined; ^c Immunoreactivity measured at a single time-point 1 hour post-dose; ^d Ovariectomized; ^e Sampling conducted using a sparse sampling scheme with a single sample per animal

OVERALL SUMMARY AND EVALUATION

INTRODUCTION

Parathyroid hormone (PTH) can differentially affect bone mass depending on its mode of administration. Given intermittently (once daily) PTH has an anabolic action on bone, stimulating bone formation, and increasing bone mass and bone strength. Although the exact mechanisms involved are unknown, the anabolic action is thought to be based on a selective increase in the activity and number of osteoblasts leading to new bone apposition on previously quiescent surfaces. By contrast, continuous infusion or closely spaced injections of PTH predominantly activate the osteoclast and are associated with a shift in the response from bone formation to bone resorption and a decrease in bone mass. The different biological responses to PTH in bone are ultimately believed to be mediated by the (pre)osteoblast and a differential effect on its cellular activity and gene expression.

Teriparatide (Forteo™) is a biosynthetic recombinant product identical in sequence to the first 34 amino acids of endogenous human PTH. The compound appears to bind to the same receptor and have similar biological actions as endogenous PTH(1-84) and synthetic PTH(1-34). The current NDA is submitted for _____ and _____.

A variety of pharmacology and toxicology studies were performed generally with daily subcutaneous administration to evaluate the nonclinical efficacy and safety of the compound¹.

EFFICACY PHARMACOLOGY

The most clinically relevant efficacy pharmacology study was an 18-month bone quality study in cynomolgus monkeys. The study was designed to address the efficacy and safety of teriparatide on vertebral and non-vertebral (cancellous, cortical) bone in a species with cortical bone remodeling. In particular this study was carried out to ensure that the increase in trabecular bone is not achieved at the expense of cortical bone.

Teriparatide at doses of 1 and 5 µg/kg (equivalent to approximately 1x and 7x the human exposure at the 20 µg daily clinical dose) dose-dependently increased bone mass in the vertebrae and in the cancellous bone of the distal radius and femoral neck. This increase was associated with increased bone formation and improved trabecular architecture and lead to increased bone strength of the lumbar spine and the femoral neck. Data suggested that there was new bone apposition on existing trabecular surfaces and bone resorption inside the trabeculae (intratrabecular tunneling) with an overall increase in trabecular connectivity and bone mass.

The data indicate that teriparatide improves cancellous bone quality, and support the efficacy and safety of once daily teriparatide treatment with regard to its effects on cancellous bone.

Vertebral properties in monkeys treated with teriparatide

	Sham		OVX			
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
BMD (Change from baseline, %)	5.3%*	0.6%	9.1%*	15.4%*	7.2%*	7.3%*
Yield force F _y (N)	1738	1499	1915*	2113*	1899*	1792*
BV/TV (%)	26	23	33*	35*	30*	27*
Tb.N (mm ⁻¹)	2.7	2.5	3.4*	3.6*	2.9*	2.9*
BFR/BV	23	20	36*	47*	26	24

*significantly different from OVX

PTH1 = treatment with 1 µg/kg/day, and PTH5 = treatment with 5 µg/kg/day, for 18 months

PTH1W = treatment with 1 µg/kg/day, and PTH5W = treatment with 5 µg/kg/day, for 12 months, with 6 month withdrawal period

¹ For a more extensive summary of bone quality, cardiovascular toxicity, nephrotoxicity and carcinogenicity data please refer to the "Advisory Committee Briefing Document" (July 27, 2001) (APPENDIX)

Femoral neck properties in monkeys treated with teriparatide

	Sham		OVX			
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	1738	1499	1915*	2113*	1899*	1792*
BV/TV (%)	44	39	54*	57*	48*	50*
Tb.N (mm ⁻¹)	2.9	2.6	3.5*	3.4*	3.1*	3.3*
BFR/TV (%/year)	21	25	42	59*	27	26

*significantly different from OVX

PTH1 = treatment with 1 ug/kg/day, and PTH5 = treatment with 5 ug/kg/day, for 18 months

PTH1W = treatment with 1 ug/kg/day, and PTH5W = treatment with 5 ug/kg/day, for 12 months, with 6 month withdrawal period

In cortical bone, teriparatide dose-dependently stimulated intracortical (osteonal) bone remodeling and increased intracortical porosity, while simultaneously increasing endocortical and/or periosteal bone formation with unclear effects on resorption. In the humerus midshaft this resulted in a slight increase in bone thickness and morphometric cortical bone area and a slight decrease in medullary area. At this cortical bone site, biomechanical strength was increased at the high dose of 5 μ g/kg but the effect was not statistically significantly. Biomechanical testing of femoral diaphyseal specimens showed a slight impairment in the material properties of the cortical bone at the 5 μ g/kg dose, possibly due to an increase in cortical porosity.

Humerus midshaft properties in monkeys treated with teriparatide

	Sham		OVX			
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	725	636	654	680	689	707
Cortical thickness (mm)	1.74	1.63	1.68	1.80*	1.66	1.72
Porosity (%)	1.3*	2.6	4.7	6.7*	2.3	6.5*
Cortical Area (mm ²)	37	35	38	41*	38	41*
Medullary Area (mm ²)	16	18	17	15	17	17
MS/BS (Endocortical) (%)	3.1	21	25	42*	12	9.2
MS/BS (Periosteal) (%)	1.8	10	8.6	11.5	4.0	2.0

*significantly different from OVX

Data suggested that treatment with teriparatide can result in two opposing effects in cortical bone; increased endocortical porosity due to increased intracortical remodeling, and altered bone thickness due to periosteal and/or endosteal surface effects. In the mid-humerus this led to an increase in bone strength because the dominating effect was an increase in cortical thickness resulting in an increase in cross-sectional moment of inertia (CSMI). The increase in porosity was observed at all cortical bone sites evaluated but effects on bone thickness and area were not evident at all sites. Since bone strength is determined by the balance between thickness and porosity it is therefore unclear whether the results obtained in the humerus (the only cortical bone for which strength was determined) can be generalized to all cortical sites. It is conceivable that strength is maintained or improved at sites where bone thickness is sufficiently increased (humerus, mid radius) but impaired at sites where increased porosity is unopposed by increased thickness (mid femur). Uncertainty about the effect of teriparatide on cortical thickness is corroborated by the morphometric data on a delayed decrease in cortical thickness of the iliac crest.

Withdrawal of teriparatide treatment after 12 months in the monkey had different effects in cancellous and cortical bone. At predominantly cancellous sites (vertebra and femoral neck), bone mass and bone strength were usually decreased after withdrawal, and histomorphometric parameters of bone formation were reduced. At cortical bone sites, strength and thickness were not affected after treatment withdrawal, while porosity and parameters of bone formation were largely reversed. Apparently, teriparatide withdrawal led to a termination of the increase in intracortical remodeling and bone surface mineral apposition reflected by the reduction in cortical bone formation. However, this was not associated with a reversal of the established changes in endocortical and/or periosteal bone perimeter within the time of the withdrawal period (6 months). The different effects of withdrawal may be related to the shorter duration of the bone turnover cycle in cancellous than in cortical bone.

Thus, although the data from the monkey study do not suggest a significant safety issue for cortical bone, some concern about the effects of long term treatment with teriparatide on the quality of cortical bone remains.

In the rabbit tibial and femoral midshaft similar results were obtained as in the monkey humerus. Although at these cortical bone sites porosity was increased bone strength was increased as a result of a compensatory increase in bone thickness. Vertebral BMD and strength in this species were not affected by teriparatide. Although the lack of a vertebral effect suggests that the rabbit is not a good animal model for testing of teriparatide the cortical bone data confirm the conclusion from the monkey study that the compound improves the biomechanical strength of cortical bone.

The data from the various rat studies demonstrated that teriparatide increased cancellous and cortical bone mass and strength. However, unlike rabbit, monkey and human, the rat is a species that lacks Haversian (osteonal) remodeling. Therefore the data on cortical bone in the rat have limited clinical relevance.

The predictive value of the monkey study data was generally supported by the clinical bone mass and fracture data obtained in the large phase III osteoporosis trials (GHAC, GHAI). These studies demonstrated marked efficacy of teriparatide to increase vertebral BMD and reduce vertebral fractures. In addition, the incidence of nonvertebral fragility fractures was reduced at most appendicular skeletal sites although the effect was less than in the axial skeleton and appeared to vary by site.

SAFETY PHARMACOLOGY

Acute cardiovascular effects of teriparatide were evaluated in rats and dogs. In these species, the vasodilatory, hypotensive action of teriparatide was maximal at 2h post dosing. A compensatory increase in heart rate was observed at ≥ 23 $\mu\text{g}/\text{kg}$ and at 6 $\mu\text{g}/\text{kg}$ in the rat and dog, respectively. In a 3-month toxicity study in cynomolgus monkeys increases in R-amplitude in electrocardiograms were observed at 10 $\mu\text{g}/\text{kg}$ in males, and in a 12-month monkey toxicity study PQ interval decreases were seen at 10 $\mu\text{g}/\text{kg}$ in males. Slight increases in heart rate were observed in the 12-month study at 2 $\mu\text{g}/\text{kg}$ and 10 $\mu\text{g}/\text{kg}$ in males only. These preclinical data indicate that teriparatide can cause vasodilation resulting in hypotension and a compensatory heart rate increase. Based on their transient nature the ECG changes observed in the monkey are of unclear significance. Clinical trial data have shown increases in pulse rate within 6 hours after dosing but no clinically significant effects on the rates of syncope, dizziness or vertigo.

Renal pathology with dose-related incidence and severity was observed in cynomolgus monkeys in 3- month and 12-month toxicity studies. The doses used in these studies were 2, 10, 20, 40 $\mu\text{g}/\text{kg}$ and 0.5, 2, 10 $\mu\text{g}/\text{kg}$, respectively. Interstitial medullary expansion was associated with increased deposition of extracellular matrix in the outer medulla of the lowest dose groups (0.5 $\mu\text{g}/\text{kg}$ and 2 $\mu\text{g}/\text{kg}$) but extended into the medullary rays at higher doses. It is unclear if the renal lesions were a direct effect of teriparatide or an indirect consequence of increased calcium reabsorption in the distal nephron or of the observed increase in serum ionized calcium.

In a 4-month reversibility toxicity study in monkeys at 40 $\mu\text{g}/\text{kg}$ nephropathy was evidenced by medullary interstitial expansion with deposition of basophilic material, multifocal interstitial inflammation, multifocal mineralization, multifocal tubular regeneration and multifocal tubular dilatation and inflammation. One animal had acute renal failure, accompanied by declining renal function. Renal function parameters were not affected in the other monkeys. The kidney findings including renal failure appeared partially reversible.

The expanded medullary interstitium following chronic dosing in monkeys was observed at the low doses in both the 3-month and the 12-month toxicity study (0.5 $\mu\text{g}/\text{kg}$ and 2 $\mu\text{g}/\text{kg}$). Since these

doses represent exposures of only 1x-3.5x the human exposure at the 20- μ g dose (based on C_{max}) this may constitute a clinical concern for renal toxicity with long term dosing. Renal findings were, however, not observed in a long-term 18-month monkey bone quality study at doses of 1 and 5 μ g/kg (equivalent to 1x-7x the human AUC at 20 μ g/day). The dietary calcium content in the bone pharmacology study was lower (0.3%) than that used in the toxicity studies (0.7-0.9%), and this difference has been proposed to explain the discrepancy in the study findings. Thus, the histologic renal lesions observed in the toxicity studies were probably related to post-dose hypercalcemia. In the largest phase III clinical trial (GHAC) there were transient and small but statistically significant increases in post-dose serum calcium and in the frequency of hypercalcemia. However, there has been no indication of renal toxicity (serum creatinine, creatinine clearance, serum urea nitrogen).

GENERAL TOXICOLOGY

Chronic toxicity was assessed in 6-month rat and 12-month monkey toxicity studies. The toxicities observed were related to the pharmacologic activity of teriparatide. Target organs were the kidney and bone/marrow.

In the 6-month rat toxicity study (10, 30, 100 μ g/kg) dose-related trabecular and cortical hypertrophy was observed at all doses and was the desired effect of teriparatide. Females were more sensitive than males despite similar exposures. Decreased bone marrow space was secondary to increased bone formation and was the likely cause of the decreased hematology parameters observed in all dose groups, which may have clinical significance when long term treatment is intended. The increase in spleen weight and the splenic and hepatic extramedullary hematopoiesis in all dose groups probably represented compensatory responses. Likewise the hypercalcemia and hyperphosphatemia at 30 and 100 μ g/kg were expected consequences of PTH agonist activity. An increased incidence of glomerular nephritis in males and kidney mineralization in females was observed at 100 μ g/kg/day. The NOAEL in this rat study was <10 μ g/kg based on hematologic effects. This dose is equivalent to 5-10x the human exposure at the 20 μ g dose, based on AUC.

In the 12-month monkey toxicity study (0.5, 2, 10 μ g/kg) diffuse expanded basophilic medullary interstitium (minimal-moderate) was observed in all female dose groups (0.5, 2, 10 μ g/kg) and in high dose males (10 μ g/kg). A similar incidence pattern as for medullary expansion was observed for cortical or medullary tubular/interstitial mineralization. Relative kidney weights were increased significantly in high dose males but not females. Erythrocyte parameters were decreased in males and females at 10 μ g/kg. As in the rat, this was probably related to the dose-related increase in trabecular bone in the femur and sternebra, which was characterized by a denser and thicker trabecular meshwork with a decrease in intertrabecular space. Transient increases in serum ionized calcium in all dose groups, and decreases in total serum calcium and phosphorus at 2 and 10 μ g/kg, were related to the pharmacologic effect of the test compound. A dose- and time-dependent increase in the proportion of animals with measurable anti-teriparatide immunoglobulin (IgG) levels was also observed. The NOAEL in this monkey study was 0.5 μ g/kg, based on renal histopathologic effects. This dose is equivalent to 1x the human exposure at the 20 μ g dose, based on C_{max} .

The toxicities observed at the lowest adverse effect dose levels were due to the expected pharmacological effect of the test compound on calcium metabolism. In clinical trials, related adverse effects have been absent or minor and have not elicited any significant clinical concern.

CARCINOGENICITY

The main result obtained in the two-year rat carcinogenicity study was that teriparatide causes osteosarcomas. Two-year subcutaneous dosing of the rat with 5, 30, or 75 μ g/kg/day revealed cellular proliferative lesions of the bone including malignant osteosarcoma in all dose groups at exposure levels equivalent to 3x-60x the human exposure at a clinical dose of 20 μ g/day. At the

highest dose the incidence of osteosarcoma reached 52% in males and 38% in females (average 45%).

Incidence of osteoblastic neoplasms and osteoblast hyperplasia in rat carcinogenicity study

Group	MALES				FEMALES			
	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined	60	60	60	60	60	60	60	60
Dose (ug/kg/day)	0	5	30	75	0	5	30	75
Human exposure multiple (AUC based)*	-	3x	21x	58x	-	3x	21x	58x
No. of animals with								
Osteosarcoma	0	3	21	31	0	4	12	23
Osteoma	0	0	2	1	0	0	0	1
Osteoblastoma	0	0	2	7	0	1	1	3
Osteoblast hyperplasia	0	1	2	4	0	2	1	3
Historical control data on the incidence of osteosarcoma in the rat (Lilly database)	1/360	-	-	-	0/360	-	-	-

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

The time to osteosarcoma appearance appeared to be dose-related and the earliest time of tumor diagnosis was 21, 19 and 13 months for the 5, 30, 75 $\mu\text{g}/\text{kg}$ groups, respectively. Notably, some tumors may have gone undetected since not all tumors resulted in gross lesions, not all bones were examined histopathologically and examined bones were not completely evaluated. In addition, the exact time of tumor induction is unknown since tumors were detected prior to study termination only by detection of gross lesions or premature death.

Examination of historical control databases revealed that the background incidence of osteosarcoma in rats is extremely low. According to the Sponsor's database (6 studies, 5-year period) there was 1 osteosarcoma in 360 male rats (0.28%) and none in 360 females (0%). The National Toxicology Program historical control database indicates an incidence of 0.22% in males and 0.37% in females. Using an average background incidence of 0.2% it can be calculated that the relative risk of osteosarcoma in the current study varied from 30x in the 5 $\mu\text{g}/\text{kg}$ group to 225x in the 75 $\mu\text{g}/\text{kg}$ groups. Since this is a substantial relative risk increase at near human exposure the finding constitutes a major clinical concern.

The Sponsor has suggested that osteosarcoma formation in the rat is associated with the exaggerated pharmacological response, i.e., the increase in bone mass, of the rat skeleton to teriparatide. Compared to monkeys and humans the increase in bone mass in rats after 24 months of treatment was indeed marked at all doses, particularly at non-vertebral sites. At the end of the study the BMC of the mid-femur in female rats in the 5- and 30- $\mu\text{g}/\text{kg}$ groups (~3x, 20x the human exposure at the 20 μg dose) was increased by 25% and 65%, respectively. By contrast, postmenopausal women treated for ~21 months with 40 $\mu\text{g}/\text{day}$ had an increased midshaft radius BMC of only 2%. On the other hand, the increase in vertebral BMC in the 5 $\mu\text{g}/\text{kg}$ female dose group (3x the human exposure at 20 $\mu\text{g}/\text{day}$) was 50%, which represented a much lower multiple of the increase in humans treated with 20 $\mu\text{g}/\text{day}$ (10%) than at the cortical site. However, vertebral tumors but not femoral tumors were observed in the 5 $\mu\text{g}/\text{kg}$ group. It should also be noted that the tumors in the rat must have been induced at a time when the increase in BMC was far less than the increase measured at the end of study. Moreover, since the NOAEL or threshold for the tumor induction has not been determined osteosarcomas may arise at doses associated with smaller BMC increases.

Although the increase in bone mass in the rats that developed bone tumors was larger than the increase expected in humans, the difference is quantitative rather than qualitative. Therefore, in the opinion of this Reviewer it is not justified to conclude that the tumors have no clinical relevance. In fact, the critical issue is a qualitative one, namely whether the rat osteoblast responds in the same manner to intermittent activation with teriparatide as the human osteoblast. As there is increased osteogenesis in both the rat and the human, there is reason to believe that the osteoblast response to

teriparatide is similar in the two species, and it is an entirely plausible hypothesis that the induction of bone tumors is the result of chronic, intermittent hormonal stimulation of the osteoblast. This chronic stimulation may cause target cell proliferation and confer a selective growth advantage to precancerous or initiated cells, i.e., cause tumor promotion. Thus, the osteosarcoma finding in the rat may very well be relevant for all species that respond to intermittent treatment with an increase in bone formation and apposition, including humans. Support for this hypothesis is provided by data from studies employing intermittent injection of a PTHrP analogue with a similar pharmacodynamic effect as teriparatide, showing that both rats (Sprague-Dawley) and mice (CD-1) develop osteosarcomas.

The molecular and genetic events underlying the bone tumor induction by teriparatide in rats are unknown, and so are the mechanisms underlying the anabolic action of PTH on bone. Based upon nonclinical pharmacology data, osteoblastic gene expression differs upon continuous and intermittent activation of the PTH receptor, possibly due to homologous desensitization of the receptor with continuous activation. Concomitantly with increased bone formation this differential gene expression may be associated with increased osteoblast or osteoprogenitor cell proliferation and osteoblastic tumorigenesis in the case of an intermittent administration regimen. Based on evidence that increased bone formation upon intermittent dosing is due to inhibition of osteoblast apoptosis, it is also possible that an anti-apoptotic action may contribute to an increased chance of neoplastic cell transformation.

No bone tumors have been observed in other animal studies, such as a 6-month chronic rat toxicity study (n=15/sex/group) with doses up to 100 µg/kg/day, and an 18-month female monkey pharmacology study (n=40/group) with doses of 1 and 5 µg/kg. However, negative tumor results from these studies have limited relevance since these studies were not designed to detect carcinogenicity. They were of relatively short duration (rat study), encompassed limited bone evaluations (monkey study) or utilized an insufficiently large sample size (monkey study). In particular, the absence of radiographically detectable tumors in the monkey study was not entirely reassuring, since the bone samples examined by microscopic evaluation were merely sections taken from small specimens intended for histomorphometric evaluation and small neoplastic lesions may have easily escaped detection. Moreover, even if the bone evaluations had been all-inclusive, the number of animals used in the monkey study was too small to detect with reasonable probability even a large increase (10x-100x) in bone tumor risk, assuming a low background incidence in this species of e.g. 1 in 10,000. Therefore, these negative results do not provide sufficient evidence of a lack of tumor risk in humans treated for an extended period of time. Similarly, although in humans treated with teriparatide in the clinical trials no osteosarcomas have been reported, the number of patients treated for >1 year (~1150) was too small to detect even a large increase in the risk of this tumor due to the very low background incidence in the human population (1 in 250,000).

Although Sponsor has argued otherwise, the absence of a clinical association between hyperparathyroidism and osteosarcoma is not entirely reassuring either. The intended therapeutic use of teriparatide is intermittent dosing causing pulsatile increases in serum PTH(1-34) levels while in hyperparathyroidism endogenous PTH levels are chronically elevated. This difference in pharmacokinetics is related to differences in pharmacodynamics at the tissue and cellular level and is potentially related to the bone tumor finding.

In conclusion, the rat osteosarcomas can not be dismissed as clinically irrelevant without knowing the mechanism of tumor formation and without any information about the presence or absence of this mechanism in humans.

In order to address the effects of (1) age/skeletal maturity at treatment onset and (2) treatment duration the Sponsor is currently conducting a follow-up carcinogenicity study in female rats using doses of 5 and 30 µg/kg. In this study, groups of animals are treated starting at two different ages (1.5 or 6 months), and for two different treatment periods (6 months or 20-24 months). Part of the animals treated for 6 months will be followed up for the remainder of the study. The results of this follow-up rat study will be available by mid-2002. Sponsor is also conducting an additional monkey

study in which ovariectomized animals are treated with 5 µg/kg teriparatide for 18 months, and followed up for 3 years. At the end of this study bones will be evaluated for the presence of proliferative lesions. The study is done in order to investigate whether there is a lag time in tumor development.

GENETIC TOXICOLOGY

Teriparatide did not exhibit any genotoxic potential in a standard test battery (bacterial mutagenicity assay, mouse lymphoma assay, chromosomal aberration test in CHO cells, mouse micronucleus test) of *in vitro* (with or without metabolic activation) and *in vivo* genotoxicity assays. These data indicate that teriparatide is not genotoxic.

REPRODUCTIVE TOXICOLOGY

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

In a Segment II developmental toxicity study in pregnant CD rats, there were no significant findings at daily subcutaneous doses of 30 to 1000 µg/kg.

In a Segment II developmental toxicity study in pregnant CD-1 mice, there was an increase in the incidence of one malformation (eyelid open) at a daily subcutaneous dose of 1000 µg/kg. There were also increases in the incidences of a number of skeletal deviations and variations (interrupted rib, extra vertebra, extra rib) at subcutaneous doses \geq 225 µg/kg.

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

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

RECOMMENDATION

The results submitted to NDA 21,318 on the pharmacologic effects of teriparatide (rhPTH(1-34), FORTEO™) support the efficacy and safety of the compound for the treatment of osteoporosis.

The results submitted on the toxicologic effects of teriparatide, excluding those obtained in the rat carcinogenicity study, also support the safety of the compound for the treatment of osteoporosis.

However, the finding of osteosarcoma obtained in the rat carcinogenicity study does not support the safety of teriparatide for long term use in postmenopausal women and men with osteoporosis.

The currently ongoing follow-up rat carcinogenicity study addresses two issues, (1) effect of animal age at treatment onset and (2) effect of treatment duration, that are critically important for further evaluation of the bone tumor finding and its clinical relevance.

This Reviewer feels that the pending results from the follow-up rat carcinogenicity study need to be available to the Agency before a decision to approve the product for marketing in the treatment population can be made.

For that reason, this Reviewer recommends the NDA to be approvable (AE) pending the results from the follow-up rat carcinogenicity study.

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APPENDIX

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**Executive CAC
March 20, 2001**

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Frank Sistare, Ph.D., HFD-910, Alternate Member
Jim Farrelly, Ph.D., HFD-530, Alternate Member
Karen Davis-Bruno, Ph.D., Team Leader
Gemma Kuijpers, Ph.D., Presenting Reviewer

Author of Draft: Gemma Kuijpers

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21,318
Drug Name: Forteo
Sponsor: Eli Lilly

LY333334 (Forteo^R) is the recombinant (1-34)-amino-acid fragment of human parathyroid hormone (rhPTH(1-34)). When administered by daily s.c. injections, this compound causes an increase in osteoblastic bone formation resulting in increased bone mass and bone strength. The compound is under review for marketing for the indication of treatment of osteoporosis in postmenopausal women and men. One 2-year carcinogenicity study in the rat was carried out using doses of **0, 5, 30, 75 ug/kg/day**.

RAT CARCINOGENICITY STUDY:

The main result of the study was a dose-related statistically significant ($p < 0.05$) increase in incidence of osteblastoma and osteosarcoma at various bone sites in males and females in all dose groups. No bone tumors were seen in the control groups. The human exposure multiple in the dose groups varied from 1.6x to 42x. There was also a statistically significant positive dose-response relationship in the incidence of thyroid C-cell adenoma in males.

Executive CAC Recommendations and Conclusions:

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Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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ADVISORY COMMITTEE BRIEFING DOCUMENT
Pharmacology/Toxicology

NDA 21,318
FORTEO™
LY333334

July 27, 2001

INTRODUCTION

The intermittent administration of PTH causes an anabolic bone response in animals and in humans. It is generally thought that the anabolic effect of intermittent PTH is due to stimulation of osteoblastic bone formation preceding and/or dominating osteoclastic bone resorption. However, the precise molecular mechanism of the anabolic action of PTH is unknown.

The osteoblast is the major target cell for PTH in bone. The cell possesses high-affinity surface membrane PTH/PTHrP receptors which are coupled to adenylyl cyclase and phospholipase C. Activation of the two associated signal transduction pathways leads to alterations in osteoblastic function and gene expression. Most likely the anabolic action of PTH on bone is mediated by a combination of intracellular responses triggered specifically by intermittent receptor occupation.

The Sponsor has developed recombinant human PTH(1-34) (rhPTH1-34), or LY333334, as a therapeutic agent for the treatment of osteoporosis in postmenopausal women and men. In order to evaluate preclinical efficacy and safety LY333334 was tested extensively in a variety of pharmacology and toxicology studies. The studies were generally done by daily subcutaneous administration.

In the pharmacological studies carried out in monkeys, rats and rabbits, all species responded to intermittent LY333334 dosing with an increase in bone formation at various skeletal sites. The long term monkey bone quality study was the most clinically relevant study. In this study, a thorough assessment of the efficacy and safety of daily dosing with LY333334 on both cancellous and cortical bone was carried out.

In the toxicity studies in rats, monkeys, rabbits and mice, the effects of LY333334 were frequently related to the pharmacological effect of PTH on bone and mineral homeostasis. The main findings prompting clinical concern were cardiovascular toxicity, nephrotoxicity, and carcinogenicity. LY333334 was not genotoxic.

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BONE EFFICACY/SAFETY

Preclinical and clinical studies have shown that intermittent treatment with PTH injections increases vertebral bone mass and strength. However, the effect of this treatment on non-vertebral, predominantly cortical bone sites is not as clear. Sponsor carried out pharmacology studies with LY333334 in rats, monkeys and rabbits to evaluate bone quality. The monkey and rabbit studies were particularly designed to address the issue of non-vertebral bone efficacy and safety in remodeling species.

Adult skeletally mature ovariectomized (OVX) monkeys were treated with LY333334 at daily dose of 1 or 5 ug/kg (PTH1, PTH5) for 18 months, or for 12 months followed by a withdrawal period of 6 months (PTH1W, PTH5W). Ovary-intact New Zealand rabbits were treated with daily doses of 10 or 40 ug/kg, for up to 5 months. Rat studies were carried out in ovariectomized and intact animals, starting treatment at different ages, for time periods up to 2 years. Bone quality was assessed by X-ray densitometry, histomorphometry and biomechanical testing. Monkey study data described are from end of study.

Monkey study

In the vertebrae, LY333334 dose-dependently increased vertebral cancellous bone mass (BMC and BMD) and bone strength (yield force), mainly due to an increase in trabecular number and connectivity (TABLE 1). Bone formation rate (BFR/BV) was increased without a clear effect on bone resorption parameters. BMD was significantly correlated with yield force in the spine ($r=0.83$).

TABLE 1. Vertebral bone properties

	Sham	OVX				
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
BMD (Change from baseline, %) (0-18mo)	5.3%*	0.6%	9.1%*	15.4%*	7.2%*	7.3%*
Yield force F_y (N)	1738	1499	1915*	2113*	1899*	1792*
BV/TV (%)	26	23	33*	35*	30*	27*
Tb.N (mm^{-1})	2.7	2.5	3.4*	3.6*	2.9*	2.9*
BFR/BV	23	20	36*	47*	26	24

*significantly different from OVX

In proximal tibia and distal radius, pQCT (peripheral quantitative computed tomography) measurements showed that BMD was increased in the middle and/or innermost zones (cancellous bone), whereas significant differences in the outermost zone (cortical bone) could not be resolved. Cross-sectional bone area at these bone sites was not significantly affected.

Histomorphometry of the distal radius indicated that in cancellous bone fractional bone volume (BV/TV) was increased associated with an increase in trabecular number and bone formation (TABLE 2). In cortical bone BV/TV was decreased while bone surface to volume ratio (BS/BV) and bone formation rate (BFR/TV) were increased.

TABLE 2. Distal radius histomorphometry data

	Sham	OVX				
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
CANCELLOUS						
BV/TV (%)	17	16	20	22*	19	19
Tb.N (mm^{-1})	2.3	1.9	2.6*	2.6*	2.4*	2.3
BFR/BV (%/year)	9.0*	26	33	42*	28	32
CORTICAL						
BV/TV (%)	56	62	53*	58	58	57
BS/BV (mm/mm^2)	8.7	8.1	12.4*	11.0*	9.5	9.9
BFR/TV (%/year)	8.8*	42	45	50	42	36

*significantly different from OVX

In the femoral neck, LY333334 significantly increased ultimate bone strength, and increased cancellous BV/TV associated with an increase in bone formation and trabecular number (TABLE 3). In

cortical bone there were no significant dose-dependent effects of LY333334 treatment on BV/TV, BS/BV or BFR/TV. BMD data for this site were not available.

TABLE 3. Femoral neck properties

	Sham		OVX			
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	1738	1499	1915*	2113*	1899*	1792*
CANCELLOUS BONE						
BV/TV (%)	44	39	54*	57*	48*	50*
Tb.N (mm ⁻¹)	2.9	2.6	3.5*	3.4*	3.1*	3.3*
BFR/TV (%/year)	21	25	42	59*	27	26

*significantly different from OVX

In the midshaft humerus bone strength (ultimate force, F_u) was not significantly affected by either dose of LY333334 (TABLE 4). Macroscopic cortical thickness was increased by LY333334, significantly at 5 ug/kg. Histomorphometrically, LY333334 treatment increased cortical porosity (Po), number of mineralizing osteons and activation frequency. Total bone area (B.Ar) and cortical bone area including porosities (Ct.Ar) were increased, and medullary cavity area (Me.Ar) was slightly decreased. Endocortical, but not periosteal, mineralizing surface (MS/BS) and BFR were increased. All effects were more pronounced at the high dose. There were no significant effects on osteoid or wall width. There were no data on bone perimeters. BMC and BMD of the humerus were not determined.

TABLE 4. Humerus midshaft properties

	Sham		OVX			
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
Ultimate force F_u (N)	725	636	654	680	689	707
Cortical thickness (mm)	1.74	1.63	1.68	1.80*	1.66	1.72
Po (%)	1.3*	2.6	4.7	6.7*	2.3	6.5*
B.Ar (mm ²)	53	53	54	55	55	58
Ct.Ar (mm ²)	37	35	38	41*	38	41*
Me.Ar (mm ²)	16	18	17	15	17	17
MS/BS.Ec (%)	3.1	21	25	42*	12	9.2
BFR/BS.Ec (um/day)	7.1	21	18	34	15	14

*significantly different from OVX

Although cortical porosity was increased, the mechanical strength of the mid humerus was not significantly affected or slightly increased since the LY333334-induced increase in porosity was concentrated near the inner endocortical surface zone and since LY333334 increased cortical bone thickness. The latter two phenomena lead to a (relative) increase in the moment of inertia, which caused an increase in bone strength.

In the midshaft radius, pQCT measurement showed a significant increase in cross-sectional area, concomitant with a slight non-statistically significant decrease in BMD in LY333334-treated groups. As in the humerus, cortical porosity was significantly increased. Moreover, bone area and medullary area were increased, but mineralized area (excluding porosities) was not significantly affected. Endocortical perimeter was slightly increased, and endocortical bone formation was increased. Periosteal perimeter was slightly but not significantly increased.

In the midshaft femur porosity was increased, while bone area, medullary area, mineralized area (excluding porosities), and periosteal and endocortical perimeters were not significantly affected.

Biomechanical testing of femoral diaphyseal beam specimens containing both endocortical and periosteal bone showed that the material properties of this bone site (ultimate stress, Young's modulus) were slightly decreased at the high dose of LY333334 (TABLE 5). Dose effects for both parameters were significant according to a two-way ANOVA model. The effects might be due to an increased endocortical porosity.

TABLE 5. Femoral diaphyseal beam specimen properties

	Sham	OVX				
	Vehicle	Vehicle	PTH1	PTH5	PTH1W	PTH5W
σ_u (Mpa)	222	216	222	206	214	208
E (Gpa)	17.2	16.4	17.1	15.4*	16.6	15.3*

* significantly different from sham

In the cancellous bone of the iliac crest, LY333334 caused a dose-dependent increase in bone formation at 6 months followed by an increase in bone formation and resorption at 15 months, and increases in trabecular parameters (density, thickness) at both time points. In the cortical bone both doses of LY333334 caused increased cortical thickness at 6 months. However, this effect was reversed after 15 months at which time thickness was lower than in the sham or OVX groups. Porosity was not significantly affected by LY333334 at either time point.

The incidence and degree of tunneling (intratrabecular remodeling) was dose-dependently and reversibly increased in vertebrae and iliac crest by LY333334. The incidence of woven in iliac crest biopsies bone was also increased at 15 months.

Rabbit study

In the tibial midshaft, treatment with LY333334 (10 or 40 ug/kg/day) for 20 weeks (2 remodeling cycles) increased intracortical bone remodeling and cortical porosity, particularly in the endocortical zone. At this site, LY333334 also increased cortical bone area, and increased both endocortical and periosteal bone formation. In the femoral midshaft, LY333334 caused a dose-dependent increase in X-area and apparent BMD, and an increase in strength in parallel with an increase in cross-sectional moment of inertia (CSMI). In the vertebrae, resorption and formation parameters were increased, but BMD, strength and cortical thickness were not affected.

In the tibial midshaft, treatment with LY333334 (10 ug/kg) for 5 or 10 weeks (1/2-1 remodeling cycle) increased endocortical, periosteal and intracortical bone formation without a significant increase in porosity. The net result was increased bone thickness and strength. Femoral midshaft strength was also increased by LY333334.

Rat studies

In rats, LY333334 generally increased BMC, BMD and projected or cross-sectional area of proximal tibia, femur (midshaft, neck, distal end) and vertebrae. Bone strength of lumbar vertebrae, femoral neck and midshaft were increased with a significant correlation between femoral midshaft BMC and ultimate load to failure (Fu). However, the biomechanical characteristics of these three sites were affected differently by the treatment with LY333334. Notably, brittleness was decreased in the vertebrae, unaffected in the femoral neck, but increased in the femoral diaphysis.

Histomorphometry data indicated that in cancellous bone LY333334 increased bone formation and trabecular morphometric parameters (density, thickness, connectivity). In cortical bone LY333334 increased periosteal and endocortical bone formation, and increased bone thickness and moment of inertia.

Conclusions

The data from the monkey study suggest that LY333334 at doses of 1 and 5 ug/kg, equivalent to approximately 1x and 8x the human exposure at 20 ug per day, increases bone mass in the vertebrae and in the cancellous bone of the distal radius and femoral neck. This increase is associated with increased bone formation and improved trabecular architecture and leads to increased bone strength of the lumbar spine and the femoral neck.

The effects of LY333334 on cortical bone are not as clear. The data suggest that LY333334 dose-dependently stimulates intracortical (osteonal) bone remodeling and porosity, while simultaneously increasing endocortical bone formation with unclear effects on resorption. At the humerus midshaft these effects were accompanied by a slight increase in bone thickness and morphometric cortical area and a slight decrease in medullary area. The location of the increased porosity to the endocortical bone zone in combination with the increased bone thickness presumably caused a

preservation of the cross sectional moment of inertia (CSMI) which could explain why bone strength at this site was not significantly affected.

At other cortical bone sites, there were inconsistent effects on BMD and fractional bone mass (BV/TV), and on histomorphometrically determined parameters of total bone tissue area, cortical mineralized area, medullary area, periosteal and endocortical perimeter (midshaft radius, radial cortex, mid femur, femoral neck cortex). However, at all cortical bone sites evaluated, porosity was increased. Bone strength was not measured at sites other than the humerus.

At the high dose of LY333334 there was a slight deterioration in the intrinsic or material properties of mid femoral bone, σ_u (ultimate stress) and E (Young's modulus), which may be due to increased porosity in the endocortical bone zone.

Taken together, these data suggest that treatment with LY333334 can cause two different effects in cortical bone: (1) increased endocortical porosity due to increased intracortical remodeling and (2) altered bone thickness. In the mid humerus, this led to a maintenance of bone strength due to the dominating effect of an increase in cortical thickness and area. However, it is not clear whether this result can be generalized for all cortical bone sites. The increased porosity was observed at all evaluated cortical bone sites but the effects on bone thickness and bone area were not evident at all sites. As bone strength is determined by the balance between the two opposing effects it is conceivable that strength is maintained or increased at sites where bone thickness and area are sufficiently increased (e.g. humerus, mid radius) but impaired at sites where increased porosity is not counterbalanced by increased cortical bone thickness and/or area (e.g. midfemur). Uncertainty about the effects of LY333334 on cortical thickness is corroborated by the data on cortical thickness of the iliac crest.

Similar data as in the monkey humerus were obtained in the rabbit tibial and femoral midshaft, at which sites porosity was increased but bone strength was increased due to a compensating increase in bone thickness apparently preceding the increase in porosity. However, the clinical relevance of the data obtained in the intact rabbit model is not clear since vertebral BMD and strength in this species were not affected by LY333334.

The data from the rat studies showed that in this species LY333334 causes increases in cancellous and cortical bone mass and strength. However, unlike rabbits, monkey and humans, the rat lacks Haversian (osteonal) remodeling. Therefore, the data on cortical bone in the rat have limited clinical relevance.

The monkey study results and the BMD and fracture data obtained in clinical trial GHAC (postmenopausal women) generally support the predictive value of the preclinical monkey study data. In trial GHAC, after ca. 21 months of treatment with 20 ug/day, BMD was markedly increased at the lumbar spine (9% as compared to placebo), and the risk of one or more new vertebral fractures was significantly decreased (by 65%). The BMD was also increased albeit less pronounced at sites with a relative larger contribution of cortical bone (hip, femoral neck, intertrochanter area and Ward's triangle). However, in the distal radius BMD was decreased as compared to placebo (average -1% at 20 ug, -2% at 40 ug). Further pQCT evaluation of the radius (proximal area) indicated that periosteal and endosteal circumferences and total bone area were increased while BMC was unchanged, which would reduce BMD but increase computed measures of bone strength. The latter appears to bear some analogy to the effects of LY333334 in the monkey humerus and radius. The incidence of clinical fragility fractures at nonvertebral sites (hip, wrist, ankle, humerus, rib, foot, pelvis) was either unaffected (humerus, foot) or decreased (other sites). However, the number of fractures was too small to demonstrate a statistically significant fracture risk reduction at individual nonvertebral sites. Transiently increased cortical porosity in the human iliac crest confirms that this phenomenon also takes place in humans.

The possible implication of the preclinical data of a decrease in cortical bone strength so far has not been borne out by clinical data. This may be due to the positive effect of LY333334 on cancellous bone which is present at most skeletal bone sites (e.g. femoral neck/hip), combined with a generally

positive effect on cortical bone thickness or area and a relatively small adverse effect of an increase in porosity. Thus, although the small numbers of non-vertebral fractures preclude a solid conclusion, the clinical data suggest that there is no major safety problem in cortical bone.

CARDIOVASCULAR TOXICITY

The acute cardiovascular effects of LY333334 were evaluated in rats and dogs. Electrocardiography was carried out in the acute toxicity study in dogs, and in 3-month and 1-year repeated-dose studies in monkeys. All doses were administered subcutaneously. Results are shown in TABLE 1.

TABLE 1. Cardiovascular effects of LY333334 in rats, dogs, and monkeys

Species	Study type	Doses (ug/kg/day)	EFFECTS			
			Heart rate	Arterial blood pressure	Other findings	ECG findings
RAT (Sprague-Dawley), male	Single dose	0, 0.5, 4, 23, 100, 300, 1000	Increased at doses \geq 23 ug/kg	Decreased at doses \geq 23 ug/kg	Transient redness of extremities	-
DOG (Beagle), female	Single dose	0, 6	Increased	Decreased	Left ventricular inotropic state increased; Serum ionized Ca level elevated postdosing	No abnormalities
MONKEY (Cynomolgus), male and female	3-month, repeated dose	0, 2, 10, 20, 40	No effect	Not determined	Serum ionized Ca level increased post dosing	R-amplitude increased at doses \geq 10 ug/kg, 1.5h post dosing (Weeks 3-11, males only)
	1-year, repeated dose	0, 0.5, 2, 10	Increased at doses \geq 2 ug/kg, 1.5h post dosing (Week 25, males only)	Not determined	Serum ionized Ca level increased post dosing	PQ-interval decreased at 10 ug/kg, 1.5h post dosing (Week 25, males only); QT-interval decreased at all doses, 1.5h post dosing (Week 25, males only) No effects on QTc-interval

In rats and dogs, effects on heart rate and blood pressure were maximal during first 2 hours after dosing. The effects in these species are consistent with drug-induced vasodilation and a compensatory cardiac response.

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

In clinical trials orthostatic hypotension and a decrease in QTc interval upon dosing with LY333334 have been observed.

NEPHROTOXICITY

Renal pathology was observed in male and female cynomolgus monkeys (age 2-2.5 years) treated daily with subcutaneous doses of LY333334 for 3 months or 1 year. In a special 4-month toxicity study in female monkeys with a 3-month reversibility period, renal function was further evaluated. The data from these studies are summarized in TABLE 1.

TABLE 1. Clinical pathology and histopathology findings in monkey toxicity studies

	3-month toxicity study	1-year toxicity study	4-month reversibility study
Doses (ug/kg/day)	0, 2, 10, 20, 40	0, 0.5, 2, 10	0, 40
N/sex/group	3 or 4	4	4 (control), 8 (treated) (females only)
Ionized serum calcium	Increased at all doses at 4-8h post-dosing	Increased at all doses at 4-8h post-dosing	Increased at 40 ug/kg at 4-8h post-dosing
Serum urea nitrogen	Increased in some animals at 20 and 40 ug/kg	Increased in two males at 10 ug/kg, and in one female at 2 ug/kg	Increased in one animal with moderate nephropathy
Urinalysis	No effects on measured parameters	Increase in volume at 10 ug/kg in males	No effects on measured parameters
Kidney weight (relative to body)	Increased at 20 and 40 ug/kg in females	Increased at 10 ug/kg in males	Increased at end of treatment or reversibility period
Renal histopathology	<ul style="list-style-type: none"> Expanded basophilic medullary interstitium at ≥ 2 ug/kg in females, and at doses ≥ 10 ug/kg in males Cellular basophilia and epithelial regeneration at ≥ 10 ug/kg in females and at ≥ 20 ug/kg in males Tubular dilation and medullary mineralization at ≥ 20 ug/kg in both sexes 	<ul style="list-style-type: none"> Expanded basophilic medullary interstitium at ≥ 0.5 ug/kg in females, and at 10 ug/kg in males Tubular/interstitial mineralization at ≥ 0.5 ug/kg in females and at 10 ug/kg in males 	<ul style="list-style-type: none"> Slight to marked nephropathy in 4/5 animals at end of treatment phase (Marked lesions in animal with renal failure) Minimal nephropathy in 3/3 animals at end of reversibility phase
Renal function	<ul style="list-style-type: none"> No specific tests don 	<ul style="list-style-type: none"> Increase in Ca excretion at ≥ 2 ug/kg in both sexes Slight decreases in creatinine and osmolal clearance at 10 ug/kg in males, after 3-6 months 	<ul style="list-style-type: none"> Renal failure in 1/8 animals treated with 40 ukd (Day 78). The animal was sacrificed prematurely No remarkable effects on urine acidification or concentration ability in surviving animals No significant effects on general renal function (creatinine or osmolality clearance, or fractional electrolyte excretion) in surviving animals

Dose (ug/kg)	Human C _{max} multiples*	
	3-mo study	12-mo study
0.5	-	0.94x
2	3.4x	4.5x
10	27x	28x
20	73x	-
40	120x	-

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

The incidence and degree of the renal lesions in the 3-month and 1-year study were dose-related. The medullary interstitial expansion was associated with increased deposition of extracellular matrix. This lesion was limited to the outer medulla in the lowest dose groups, but extended into the

medullary rays at higher doses. It is unclear if (and which) renal lesions were indirectly due to a stimulation of calcium reabsorption in the distal nephron by LY333334, underlying the observed increase in serum ionized calcium, or if the lesions were due to a direct effect of LY333334 on the renal tissue.

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

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

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

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

CARCINOGENICITY

A two-year subcutaneous carcinogenicity study in Fisher 344 rats was carried out with LY333334 doses of 5, 30, and 75 ug/kg/day (low dose, mid dose and high dose). The number of animals treated was 60/sex/group. The animals were treated from an age of 6-7 weeks for the duration of 24 months. Histologic evaluation of tissues was carried out of all animals that were euthanized at the end of the study and all animals that died or were killed prematurely.

Bone tumor findings

LY333334 caused an increase in the incidence of osteosarcoma in all dose groups in a dose-dependent manner. LY333334 also caused an increase in the incidence of other osteoblast neoplasms (osteoblastoma and osteoma), and an increase in the incidence of osteoblast hyperplasia. In some animals multiple neoplasms occurred (TABLE 1). The increased incidences of osteosarcoma and osteoblastoma were statistically significant (trend test).

TABLE 1. Incidence of osteoblastic neoplasms and osteoblast hyperplasia at all bone sites

Group	Males				Females			
	Contr	LD	MD	HD	Contr	LD	MD	HD
N examined	60	60	60	60	60	60	60	60
No. of animals with								
Osteosarcoma	0	3	21	31	0	4	12	23
Osteoma	0	0	2	1	0	0	0	1
Osteoblastoma	0	0	2	7	0	1	1	3
Osteoblast hyperplasia	0	1	2	4	0	2	1	3
No. of animals with bone neoplasm(s)	0	3	24	36	0	5	13	25
Osteosarcoma: Historical control incidence	1/360				0/360			

In several, but not in all cases, the osteosarcomas presented as clinically palpable bone nodules. These lesions were first detected in the high dose groups after 17 months in the study. Metastatic tumors were detected predominantly in males at soft tissue sites such as lung, liver, kidney and spleen. In a subset of animals the osteosarcoma was fatal and lead to early death of the animal (TABLE 2).

TABLE 2. Incidence of gross lesions, metastasis, and fatal osteosarcoma

Group	Males				Females			
	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined	60	60	60	60	60	60	60	60
Osteosarcoma characteristics								
Total incidence	0	3	21	31	0	4	12	23
Gross bone nodule/lesion	0	1	17	24	0	3	7	13
Soft tissue metastasis	0	0	10	17	0	1	2	4
Fatal	0	1	12	22	0	3	6	8

Osteosarcomas were detected at various bone sites. Identification occurred either upon gross observation of a bone nodule, or upon microscopic evaluation of four routinely examined bone sites: femur (distal end), tibia (proximal end), sternum (one or two sternbrae), and vertebrae (one lumbar vertebra).

The most common site for osteosarcoma in males was the tibia and in females the vertebra (TABLE 3). The larger incidence in the mid dose and high dose males as compared to females was mainly due to the incidence of tibial tumors in the male dose groups. The increased incidences of osteosarcomas were statistically significant in tibia, femur, vertebra, rib and sternum in both males and females (trend test).

TABLE 3. Incidence of osteosarcoma by bone site

Group	Males				Females			
	Ctrl	LD	MD	HD	Ctrl	LD	MD	HD
N examined	60	60	60	60	60	60	60	60
Bone site								
Tibia	0	2	12	14	0	0	0	5
Femur	0	1	3	5	0	0	2	7
Vertebra	0	0	3	6	0	2	5	5
Rib	0	0	2	4	0	1	2	4
Sternum	0	0	3	5	0	0	3	2
Other (pelvis, skull, humerus)	0	0	2	3	0	1	2	1
Single site	0	3	16	22	0	4	10	22
Multiple sites	0	0	3	7	0	0	2	1

Time-to-death of tumor-positive animals

In addition to the osteosarcoma incidence, the time-to-death of animals with osteosarcoma appeared to be dose-dependent (Figure 1A,B). The data points represent all animals that were diagnosed with osteosarcoma, i.e. animals that died or were euthanized prematurely due to any event, and animals that were sacrificed at study termination. Most animals that died early did so because the osteosarcoma was fatal, although some died early for another cause and the osteosarcoma was detected as a coincidence. The earliest bone tumor diagnosed was a vertebral osteosarcoma in a high dose male which died after 13 months of treatment. The tumor was fatal but was only detected upon microscopic examination. The data suggest that osteosarcomas in the high dose groups either originated earlier or grew faster than the tumors in the low dose groups. This dose effect was more pronounced in males than in females.

Figure 1A. Time-to-death of animals diagnosed with osteosarcoma (males)

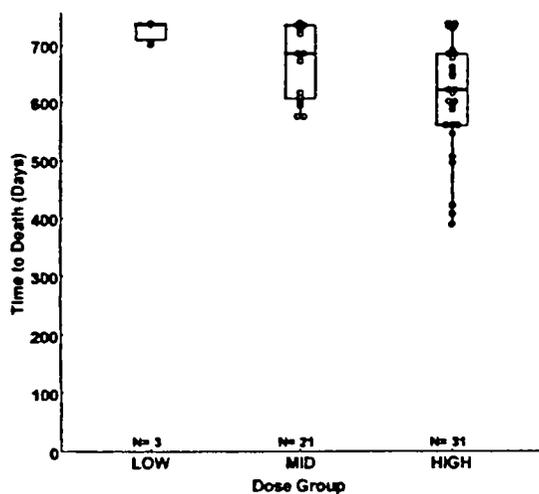
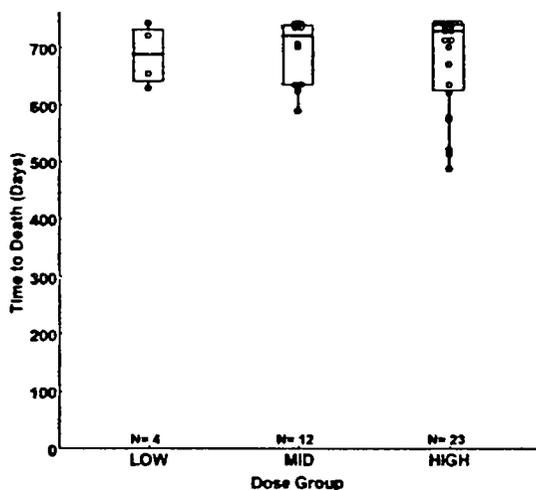


Figure 1B. Time-to-death of animals diagnosed with osteosarcoma (females)



Bone tumor location

Osteosarcomas that were associated with gross bone nodules were often several mm in diameter and invasive in character, and the anatomical site of origin of these tumors could not be established. However, for those tumors for which a site of origin could be determined the most common anatomical location was intramedullary. The most common bone site was the metaphysis. Rarely, tumors were located in the diaphysis and one tumor was limited to the epiphysis. In contrast to intramedullary neoplasms, periosteal or parosteal surface osteosarcomas were only seen in two cases in the tibia.

Pharmacodynamic effect of LY333334

It is of interest to consider the association between bone tumor incidence and the pharmacodynamic effect of LY333334. As expected LY333334 induced a change in bone architecture through increased bone formation associated with an increase in BMC and BMD in all dose groups.

In the femoral midshaft, QCT scanning showed that LY333334 caused a dose-dependent increase in endocortical bone apposition, resulting in a reduction of the marrow cavity, and periosteal bone expansion (Figure 2).

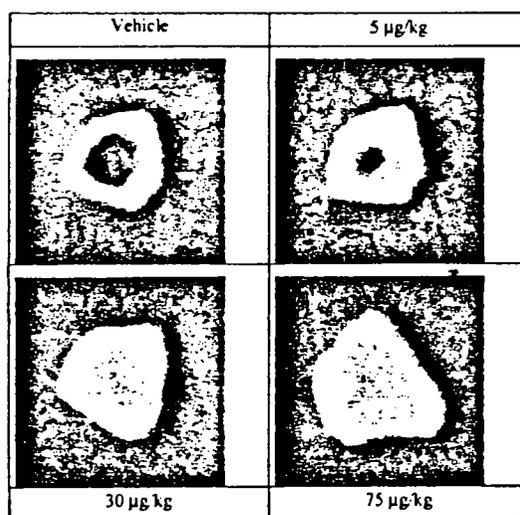


Figure 2

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

In the proximal femur and vertebrae the morphologic effect of LY333334 were also dose-dependent and consisted of an increase in trabecular thickness and number, an increase in cortical and bone thickness, and a decrease in marrow area (Figure 11).

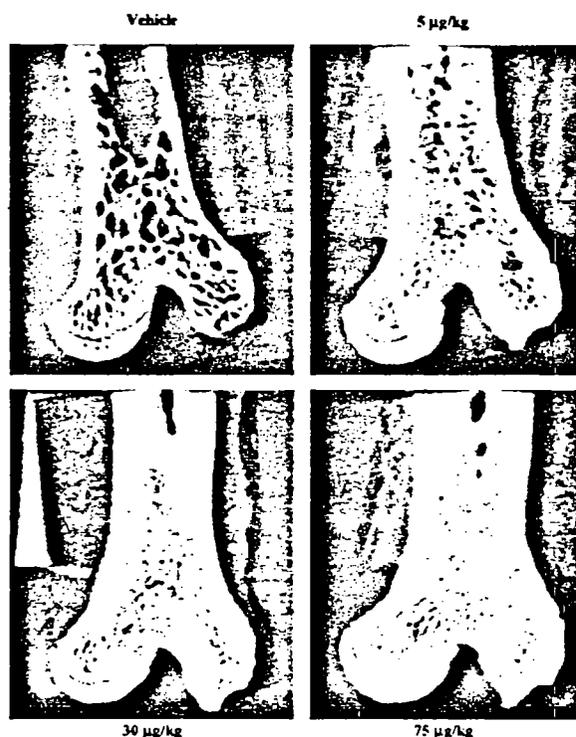


Figure 11 High Resolution OCT Images of the Proximal Femur. Representative coronal images at 24x24x24 µm resolution are shown of the vehicle, 5, 30, and 75 µg/kg groups of females. Images show significant loss of marrow space due to increased cortical thickness, trabecular number, trabecular thickness, and trabecular connectivity.

In femur (midshaft and distal part) and vertebrae, there was a dose-dependent increase in BMC, X-sectional area and BMD (TABLE 4). Bone strength was increased accordingly.

TABLE 4. BMC and BMD of femur and vertebrae at end of study (approximate values for females)

	Femur midshaft		Distal femur		L-6 Vertebra	
	BMC % increase	BMD % increase	BMC % increase	BMD % increase	BMC % increase	BMD % increase
Control	-	-	-	-	-	-
LD	28%	27%	75%	48%	48%	23%
MD	68%	38%	140%	76%	91%	29%
HD	100%	41%	170%	85%	140%	34%

LY333334 Toxicokinetics

The serum levels of LY333334 in the rats were used to calculate the ratio of C_{max} and AUC between the rats and humans treated with 20 µg LY333334, the intended clinical dose of PTH1-34. LY333334 serum levels were clearly dose-related but there were inconsistent differences between males and females and inconsistent changes over time. Levels appeared to decrease, in males after 6 months and in females after 12 months, and lowest levels were seen in both sexes at 18 months. The reason for these inconsistencies is not known. Therefore, the C_{max} and AUC multiples should be considered mere approximations.

TABLE 5. Human C_{max} and AUC multiplesRat:human C_{max} and AUC multiples based on average values for males and females, at 12 and 18 months

Group	Dose (ug/kg/day)	Cmax multiples (rat:human)		AUC multiples (rat:human)	
		Month 12	Month 18	Month 12	Month 18
LD	5	7x	5x	3.6x	1.6x
MD	30	57x	31x	25x	14x
HD	75	138x	94x	68x	42x

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

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

Conclusions

Long term treatment of the rat with LY333334 caused bone tumors at all doses tested. The majority of tumors observed were osteosarcomas. The tumor incidence was dependent on the dose, and tumors appeared to occur earlier in the higher dose groups. An NOAEL for the tumorigenesis was not established since tumors occurred in all dose groups. It is possible that some tumors remained undetected since (1) not all tumors resulted in gross bone lesions and not all bones were microscopically examined, and (2) microscopically examined bones were not evaluated completely.

The fact that the animals were treated from a young age and for a large part of their life time might be taken to suggest that relatively short term treatment of an adult human population is unlikely to carry an increased risk for bone tumor formation. However, long term rodent carcinogenicity studies are carried out with relatively large numbers of animals over an extended period of time specifically for the purpose of identifying a potential risk. Therefore, the positive finding in the current carcinogenicity study coupled with the fact that the tumors were seen at very small human exposure multiples and the fact that an NOAEL was not established raises considerable concern.

It should also be noted that, although near-lifetime treatment was employed in the rodent study, tumor initiation was not systematically evaluated and tumors were only detected when the animal died prematurely or had evidence of a gross abnormality or was sacrificed at study termination. However, from the data on time-to-death due to fatal bone tumors it can be concluded that the earliest time of tumor initiation at the cellular level in the low, mid and high dose groups was less than 21, 19 and 13 months.

In contrast to the 2-year rat carcinogenicity study, bone tumors were not detected in femur or sternum of rats treated with LY333334 for 6 months at doses up to 100 ug/kg/day, or in the proximal tibia of male or ovariectomized female animals treated for 1 year with 8 or 40 ug/kg. Moreover, in skeletally mature ovariectomized monkeys treated for 18 months with 1-5 ug/kg/day (1-8x AUC at 20 ug human dose) no bone neoplasms were observed by X-ray radiography or histology evaluation of various bone sites. However, these findings were obtained in studies with relatively small number of animals (15-20/dose group) using evaluation of a limited number of bone sites (rat) or relatively small histologic bone samples (monkey), uncompensated for by a prolonged treatment duration. Thus, the negative results from shorter term rodent or intermediate term primate studies, although somewhat reassuring, are from studies that were not designed to detect potential carcinogenicity. Therefore they do not constitute sufficient evidence that there is no increased risk of tumor formation in humans treated for e.g. 2 years.

The Sponsor has argued that the tumor formation in the rat is associated with the exaggerated response of the rat skeleton to LY333334 in terms of its pharmacodynamic effect, i.e., bone mass increase. This is consistent with the data submitted. However, it is unclear where the threshold for tumor development is regarding dose and treatment duration.

The increase in bone mass in the rat study was marked at all doses tested. Particularly for the nonvertebral sites the effect appeared to be very large in comparison to what has been observed in monkeys and humans. For example, after 24 months of dosing the BMC of the mid femur in the

females at the low and mid dose (approximately 2x and 20x the 20 ug/day human exposure) was increased by 30% and 70%. Femoral tumor incidence in these dose groups was 0/60 and 2/60. By contrast, in monkeys treated for 18 months with doses up to 5 ug/kg (8x the 20 ug/day human exposure), the midshaft radius BMC was not increased, while in humans treated for an average of 21 months with a dose of 40 ug/day the mid radius BMC was increased by only 2%.

However, at the vertebral site in the female rat the increase in BMC at the low dose of 5 ug/kg (2x the 20 ug/day human exposure) was ca. 50% which was only 5-fold the increase in humans treated with 20 ug/day (10%). In this dose group, vertebral tumors were observed in 2/60 animals whereas none occurred in 60 concurrent controls or in 360 historical control animals. However, vertebrae were not routinely evaluated in the historical control animals.

It should be noted that the increases in BMC for the animals are increases at the end of the study. In the female low dose group mentioned above one of the vertebral tumors was fatal at ca. 21 months in the study, and was evident as a 0.5 cm nodule in the spinal canal. Initiation of the tumor must have occurred before 21 months at which time the increase in BMC was probably less than 48%, since it has been shown that treatment of the intact female rat for 9 months with 5 ug/kg leads to an increase in vertebral BMC of 10%.

Although the rat osteosarcomas occurred at sites where the increase in bone mass was larger than observed in humans or monkeys, the NOAEL or threshold for the tumor induction has not been determined. Thus it can not be excluded that tumors would arise at doses leading to a smaller BMC increase. Moreover, it is also possible that the bone mass effect is not related to the tumor induction since the two events may be mediated by intracellular events that are at least partially uncoupled. In that case, the large extent of the bone mass effect in the rat would not be relevant.

The absence of a clinical association between hyperparathyroidism and osteosarcoma in humans is no reassurance that the intended treatment, i.e., intermittent dosing with LY333334, is not associated with an increased human tumor risk. The pharmacokinetics and the pharmacodynamic effects of PTH in hyperparathyroidism, a condition with chronically elevated PTH levels, are different from those upon intermittent dosing with exogenous PTH(1-34). Preclinical data are available which indicate that intermittent PTH receptor activation can induce a differential effect on osteoblastic gene expression that is distinct from the effect of continuous activation, possibly due to the lack of homologous receptor desensitization in the case of intermittent receptor activation. This differential genetic response may be linked to the tumor formation, and the similarly increased osteoblastic bone formation activity that occurs in both cases may therefore be irrelevant.

The mechanism responsible for the osteosarcoma formation is not known. Likewise, the mechanism of the anabolic effect of intermittent PTH on bone has not been elucidated. Possibly, in conjunction with the increased osteogenesis, the repeated stimulation of the osteoblast by PTH increases cell proliferation and the increased cell division drives the accumulation of genetic errors leading to neoplastic transformation. Signal transduction following PTH receptor activation is mediated by adenylyl cyclase/cAMP/PKA and phospholipase-c/PKC activation, and PKC has been associated with cell proliferation and the promotion of tumor formation.

Hormonal agents have elicited positive responses in rodent carcinogenicity studies without parallel increases in human tumor incidences. For example, chronically elevated TSH levels cause thyroid follicular hyperplasia and neoplasia in rats but not in humans. By contrast, however, elevated peptide hormone levels can induce tumor formation in the rat and increase tumor risk in humans. Specifically, there is evidence that chronically increased gastrin levels, which cause tumors of ECL (enterochromaffin-like) cells in the gastric corpus in rats, increase the incidence of ECL-cell carcinoid tumors in hypergastrinemic patients with Zollinger-Ellison syndrome, or in humans with sustained hypergastrinemia following antisecretory therapy with proton pump inhibitors.

With the currently available data it is not justified to conclude that it is unlikely that the bone tumors in rats predict an increased tumor risk in humans. Ultimately, the animal findings can not be dismissed

without knowledge of the mechanism of the tumor formation and without information as to whether or not this mechanism also occurs in humans. Therefore, the animal osteosarcoma finding causes a concern for an increased risk of bone neoplasms in humans treated with LY333334. The data, however, can not predict the extent of the human risk.

To address the issues of animal age at treatment onset and length of treatment the Sponsor is currently conducting an extensive follow-up carcinogenicity study in female rats using doses of 5 and 30 ug/kg. The study includes dose groups that are treated either from 2 months or 6 months of age, either for 6 months or for 24 or 20 months. In order to uncover a possible lag time in the neoplastic transformation of the osteoblast animals treated for 6 months are followed up by a withdrawal period of 18 or 14 months. The results of this study will be available in 2002.

In conclusion, although the relevance of the rodent osteosarcoma finding for humans remains unclear, the data suggest a carcinogenic potential of intermittent LY333334 injection.

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