

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.

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						
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 $\mu\text{g}/\text{kg}$. 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 ($\mu\text{m}/\text{day}$)	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: increased endocortical porosity due to increased intracortical remodeling and altered bone thickness. In the mid humerus, this lead to a maintenance of bone strength due to the dominating effect of an increase in cortical thickness and area. However, it is not clear whether this result can be generalized for all cortical bone sites. The increased porosity was observed at all evaluated cortical bone sites but the effects on bone thickness and bone area were not evident at all sites. As bone strength is determined by the balance between the two opposing effects it is conceivable that strength is maintained or increased at sites where bone thickness and area are sufficiently increased (e.g. humerus, mid radius) but impaired at sites where increased porosity is not counterbalanced by increased cortical bone thickness and/or area (e.g. midfemur). Uncertainty about the effects of LY333334 on cortical thickness is corroborated by the data on cortical thickness of the iliac crest.

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

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

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

The possible implication of the preclinical data of a decrease in cortical bone strength so far has not been borne out by clinical data. This may be due to the positive effect of LY333334 on cancellous bone which is present at most skeletal bone sites (e.g. femoral neck/hip), combined with a generally positive effect on cortical bone thickness or area and a relatively small adverse effect of an increase in porosity. Thus, although the small numbers of non-vertebral fractures preclude a solid conclusion, the clinical data suggest that there is no major safety problem in cortical bone.

CARDIOVASCULAR TOXICITY

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

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

Species	Study type	Doses (ug/kg/day)	EFFECTS			
			Heart rate	Arterial blood pressure	Other findings	ECG findings
RAT (Sprague-Dawley), male	Single dose	0, 0.5, 4, 23, 100, 300, 1000	Increased at doses \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 (>100x human C_{max}) did not indicate renal function impairment, there is a potential clinical concern for renal toxicity after long treatment with LY333334.

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

CARCINOGENICITY

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

Bone tumor findings

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

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

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)

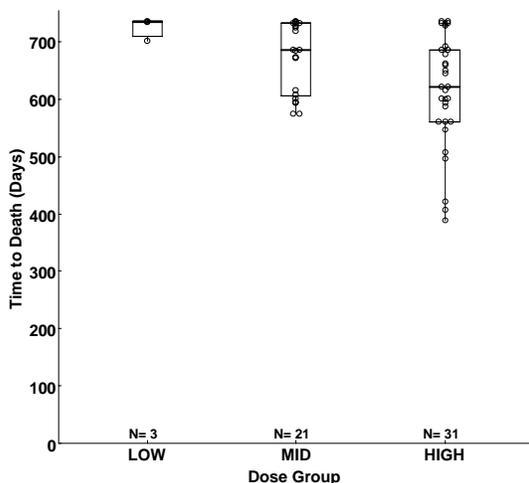
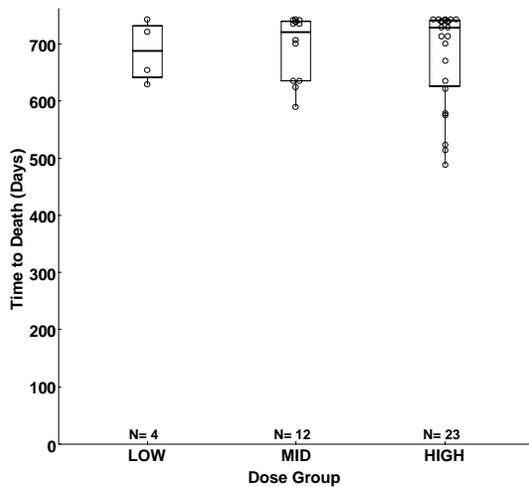


Figure 1B. Time-to-death of animals diagnosed with osteosarcoma (females)



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).

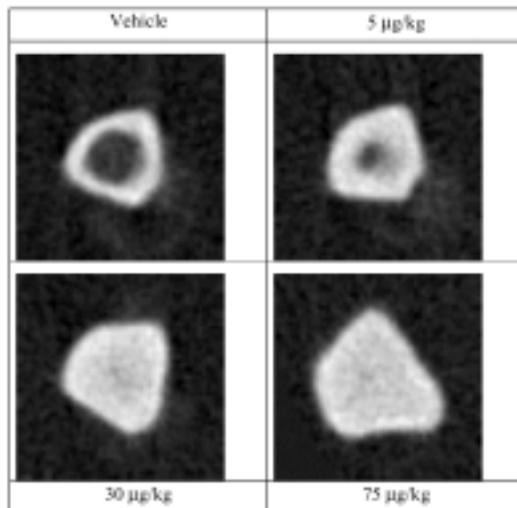


Figure 2 QCT images of the Femoral Midshaft from Females Treated for Two Years. The midshaft of left femora were analyzed in cross-section by QCT, using voxel dimensions of 156x158x1200 µm. Images show reduction of the marrow cavity at 5 µg/kg, loss of marrow space and expansion of bone at 30 µg/kg, and further expansion of bone area with altered geometry at 75 µg/kg.

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

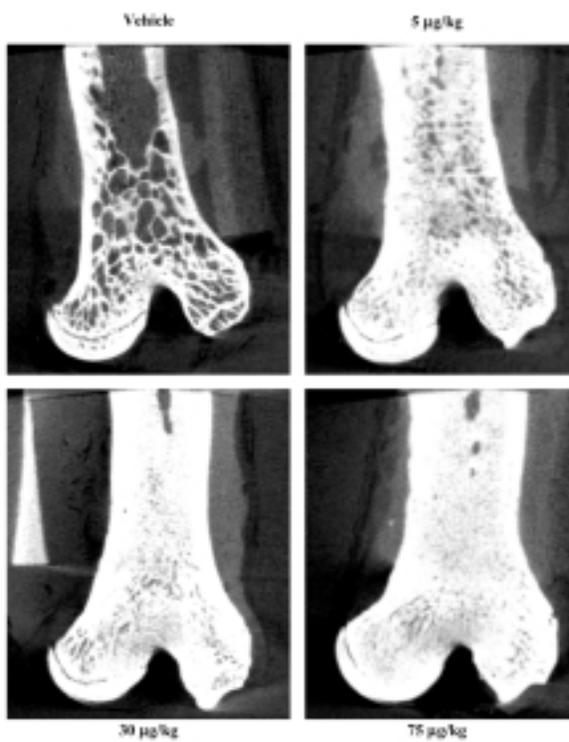


Figure 11 High Resolution QCT Images of the Proximal Femur. Representative coronal images at 24x24x24 µm resolution are shown of the vehicle, 5, 30, and 75 µg/kg groups of females. Images show significant loss of marrow space due to increased cortical thickness, trabecular number, trabecular thickness,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