CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208743Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA # 208743
Supporting document/s:	SD 1 (SN 0001)
Applicant's letter date:	03/30/2016
CDER stamp date:	03/30/2016

Product:	Abaloparatide - SC
Indication:	Treatment of postmenopausal women with osteoporosis
Applicant:	Radius Health Inc., Waltham, MA
Review Division:	Division of Bone, Reproductive and Urologic Products
Reviewer:	Gemma Kuijpers, Ph.D.
Supervisor/Team Leader:	Mukesh Summan, Ph.D., DABT
Division Director:	Hylton Joffe, M.D., M.M.Sc.
Project Manager:	Samantha Bell, B.S., B.A.

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208743 are owned by Radius or are data for which Radius has obtained a written right of reference. Any information or data necessary for approval of NDA 208743 that Radius does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of 208743.

ABBREVIATIONS

Abl= abaloparatide
ABL= abaloparatide
APA= action potential amplitude
APD= action potential duration
AUC= Area-under-the-curve
BAP= bone specific alkaline phosphatase
BMC= bone mineral content
BMD= bone mineral density
BV/TV= bone volume to total volume
cAMP= cyclic adenosine monophosphate
DPD= deoxypyridinoline
EAD= early after depolarization
EC50= median effective concentration
ED50= median effective dose
EIA= enzyme immunoassay
ELISA= enzyme-linked immunosorbent assay
ERK= extracellular signal-regulated kinase
F= female
FPRL1= N-Formyl Peptide Receptor- Like
HEK= human embryo kidney
hERG= human ether-a-go-go-related gene
HPLC= high-performance liquid chromatography
HMM= histomorphometrry
hPTH= human parathyroid hormone
hPTHrP= human parathyroid hormone-related protein
IC50= half-maximal inhibitory concentration
LA-PTH=long-acting PTH analog
M= male
N/A= not applicable
NCE= normochromatic erythrocytes
NET= transporter norepinephrine
nNOS= Nitric Oxide Synthase, neuronal
OVX= ovariectomized
NTX= urinary collagen type 1 cross-linked N-telopeptide
OC= osteocalcin
PCE= polychromatic erythrocytes
PTHR= parathyroid hormone receptor
PTX= parathyroidectomized
PTH1R= parathyroid hormone receptor 1
PTH2R= parathyroid hormone receptor 2
RIA= radioimmunoassay
SC= subcutaneous
s-CTX= serum collagen type 1 cross-linked C-telopeptide
s-P1NP= serum type 1 procollagen N-terminal;
sst2= somatostatin

TPR= total peripheral resistance
VIP1= Vasoactive Intestinal Peptide
Vmax= maximal upstroke velocity.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Radius Health Inc. seeks to market TYMLOS (abaloparatide), a parathyroid hormone related peptide (PTHrP) analog, for the treatment of osteoporosis in postmenopausal women.

Abaloparatide is a synthetic 34 amino acid analog of the endogenous hormone PTHrP(1-34), with 8 amino acids substituted in C-terminal region 22-31. Full length PTHrP is a 139-174 amino acid peptide that shares its N-terminal end with parathyroid hormone (PTH) and can activate the PTH1 receptor (PTH1R).

PTHrP can simulate most of the actions of PTH including increases in bone resorption and formation, distal tubular calcium reabsorption, and inhibition of proximal tubular phosphate transport.

Abaloparatide has an anabolic effect on bone. The mechanism of action is not entirely clear, but is based on the fact that, like others in this class, it stimulates bone formation more than bone resorption when administered intermittently.

.

1.2 Brief Discussion of Nonclinical Findings

The main animal findings, occurring at relatively low human exposure multiples, included (1) osteosarcoma and osteoblastoma in rats, (2) hemodynamic effects, (3) hypercalcemia and hypercalciuria, (4) mineralization of soft tissues, (5) hematology changes, and (6) renal function impairment.

The above mentioned findings were observed in up to 9-month animal studies at <5x the proposed human dose of 80ug/day. They are expected to be clinically relevant due to their occurrence in multiple animal species (rats, monkeys and/or dogs), and they are listed according to projected level of concern. Findings at large human exposure multiples and those of minor significance at small multiples are not discussed here. Exposure multiples were calculated based on the observed human AUC of 1546 pgxh/mL at the daily dose of 80µg.

The major nonclinical finding suggestive of potential human risk was the incidence of bone tumors in a 2-year carcinogenicity study in rats conducted at doses of 10, 25, and 50µg/kg/day. Abaloparatide caused a marked dose-related increase in osteosarcomas and osteoblastomas in all dose groups. Tumors were seen in both sexes, but more frequently in males. Tumor incidences in low, mid and high dose groups were 35%, 57% and 74%, at doses equivalent to 4x, 14x, and 25x human exposure. The tumors were also observed in a 30 ug/kg PTH1-34 positive control group. The osteosarcomas were often fatal and led to early dose discontinuation and sacrifice of the mid, high dose and PTH1-34 groups.

The osteosarcomas occurred concomitantly with relatively large increases in bone mass. In the low dose females (3x human exposure), the vertebral BMC change was approx. 4 times the 14% BMC change observed at the lumbar spine in postmenopausal women in Phase 3 Study BA058-

05-003 after 18 months of daily dosing with 80 ug. Correlation of osteosarcoma incidence with rat vs human BMC increase suggested that abaloparatide may pose a similar or larger tumor risk to humans than PTH1-34. However, it is unclear whether the relative tumor response to different PTH receptor agonists in rats, no matter how normalized, has any predictive value for relative risk in humans. Nevertheless, the potential for an increase in human risk of osteosarcoma with PTH and PTHrP analogs remains uncertain and continues to be a serious safety concern.

Osteosarcomas and other bone tumors have previously been observed at low human exposure multiples in rat carcinogenicity studies with rhPTH1-34 (Forteo) and hPTH1-84 (Natpara). Their development is probably associated with the pharmacologic effect of these products to stimulate osteoblast activity and recruitment or inhibit osteoblast apoptosis, which confers potential clinical relevance to the finding. However, the probable dependence of tumor development on treatment duration may serve as mitigating factor, and prolonged treatment would likely be needed to put a patient at significant risk of bone tumor formation.

As appropriate, the applicant included the osteosarcoma finding in a Black Box Warning in the product label. Pertinent warnings and precautions were also articulated. The Division is recommending limiting cumulative treatment duration with abaloparatide and other parathyroid hormone analogs to 2 years. This Reviewer strongly concurs with that recommendation.

Clinical and regulatory judgment is needed to decide about further risk evaluation and mitigation strategies. Labeling and pharmacovigilance may be adequate. The postmarketing experience with Forteo suggests that osteosarcoma is not a significant clinical concern with that product, but the conclusion is not definitive. Additional nonclinical studies with abaloparatide are not recommended since they are unlikely to contribute any more critical or clinically relevant information.

The other nonclinical toxicity findings with abaloparatide were primarily related to the pharmacologic action of this PTHrP1-34 analog to modify intercompartmental calcium and phosphorus fluxes and cause hypercalcemia. PTH receptor agonists are also known to induce peripheral vasodilation and exert inotropic and chronotropic effects on the heart, which was confirmed in a dog study with abaloparatide. The safety assessments in the clinical studies addressed the main nonclinical toxicity findings. They included vital signs (HR, BP), ECGs, routine lab testing (hematology, chemistry, urinalysis), serum and urine Ca and P, and various organ system adverse events. The potential for kidney mineralization (urolithiasis) was addressed by performing renal CT scans in a subset of Phase 3 subjects, and reporting of urinary system AEs. Specific cardiovascular or pulmonary evaluations to detect tissue mineralizations were not performed. The nonclinical safety concerns appear to have been adequately addressed during clinical drug development.

Long term bone pharmacology studies in ovariectomized (OVX) rats and monkeys showed that abaloparatide increases BMD, BMC and/or Bone Area at various skeletal sites, including vertebrae, tibia, radius and femur. The effects were mainly due to increases in trabecular thickness in vertebrae and long bone metaphyses, and increases in cortical thickness in long bone diaphyses and/or metaphyses. The increase in cortical thickness was generally due to endosteal bone apposition. Changes in total bone area and width were also observed. Abaloparatide was

able to activate bone formation at trabecular, periosteal and/or endosteal surfaces. However, at peri- and/or endosteal surfaces bone resorption appeared to be enhanced as well. Overall, the effects of abaloparatide varied with bone site, region and surface. The data confirmed that bone quality, comprised of microscopic bone structural and architectural properties, was not negatively affected. The studies did not include teriparatide comparator groups.

The applicant has proposed that abaloparatide potently activates bone formation with little effect on resorption. It was postulated that this is due to selective binding of abaloparatide to the RG conformation of the PTH1 receptor and a comparatively transient 'residual' in vitro cAMP response. Circumstantial evidence for this hypothesis was provided, but was not sufficiently convincing. Importantly, stimulation of bone resorption by abaloparatide, albeit transient, was evident in clinical and nonclinical studies. In this Reviewers' opinion, it is not clear how bone tissue responses are related to specific intracellular events and how these relationships evolve over the course of therapy.

Edits and recommendations for the product label have been proposed by this Reviewer for the Highlights (Black Box warning), Section 5.1 (Potential Risk for Osteosarcoma), Section 12.1 (Mechanism of Action), Section 13.1 (Carcinogenesis), and Section 13.2 (Animal Toxicology and Pharmacology.

The submitted nonclinical data support the marketing of abaloparatide. The basis of the recommendation to approve is briefly outlined in section 1.3.

1.3 Recommendations

1.3.1 Approvability

Given that (1) clinical safety evaluations based on animal findings were performed in an adequate manner, (2) the rat osteosarcoma finding is appropriately communicated in the product label and (3) additional strategies deemed necessary to evaluate or mitigate tumor risk will be put in place, the submitted nonclinical data support the safe use of abaloparatide in humans at the recommended dose of 80µg/day.

1.3.2 Additional Non-Clinical Recommendations

No additional nonclinical assessments are needed for approval of the application.

1.3.3 Labeling

Proposed by sponsor:

12.1 Mechanism of Action

(b) (4)

(b) (4)

Recommended by Pharmacology/Toxicology Reviewer (draft):

12.1 Mechanism of Action

Abaloparatide is a PTH1 receptor (PTH1R) agonist that activates the cAMP signaling pathway in target cells. In rats and monkeys, abaloparatide had an anabolic effect on bone, demonstrated by increases in BMD and bone mineral content (BMC) that correlated with increases in bone strength at vertebral and/or nonvertebral sites *[see NonClinical Toxicology (13.2)]*.

Proposed by sponsor:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

(b) (4)

Abaloparatide was not genotoxic or mutagenic in a standard battery of tests including the Ames test for bacterial mutagenesis, the chromosome aberration test using human peripheral lymphocytes, and the mouse micronucleus test.

(b) (4)

Recommended by Pharmacology/Toxicology Reviewer (draft):

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 2-year carcinogenicity study, abaloparatide was administered once daily to Fischer rats by subcutaneous injection at doses of 10, 25, and 50 mcg/kg. These doses resulted in systemic exposures to abaloparatide that were 4, (b) (4), and (b) (4) times, respectively, the systemic exposure observed in humans following the recommended subcutaneous dose of 80 mcg (based on AUC comparisons). Neoplastic changes related to the treatment with abaloparatide consisted of marked dose-dependent increases in osteosarcoma and osteoblastoma incidence in all male and female dose groups. The incidence of osteosarcoma was 0-2% in untreated controls and reached 87% and 62% in male and female high dose groups, respectively. The bone neoplasms were accompanied by marked increases in bone mass.

The relevance of the rat findings to humans is uncertain. The use of TYMLOS is not recommended in patients at increased risk of osteosarcoma *[see Warnings and Precautions]*.

Abaloparatide was not genotoxic or mutagenic in a standard battery of tests including the Ames test for bacterial mutagenesis, the chromosome aberration test using human peripheral lymphocytes, and the mouse micronucleus test.

(b) (4)

Proposed by sponsor:

13.2 Animal Toxicology

Recommended by Pharmacology/Toxicology Reviewer (draft):

13.2 Animal Toxicology and Pharmacology

In toxicity studies in rats and monkeys of up to 26-week and 39-week duration, respectively, findings included vasodilation, increases in serum calcium, decreases in serum phosphorus, and soft tissue mineralizations at doses ≥10 mcg/kg/day. The 10 mcg/kg/day dose resulted in systemic exposures to abaloparatide in rats and monkeys that were 2 and (b) (4) times, respectively, the exposure in humans at daily subcutaneous doses of 80 mcg.

Pharmacologic effects of abaloparatide on the skeleton were assessed in 12- and 16-month studies in ovariectomized (OVX) rats and monkeys, at doses up to (b) (4) and 1-times human exposure at the recommended subcutaneous dose of 80 mcg, respectively (based on AUC comparisons). In these animal models of osteoporosis, treatment with abaloparatide resulted in a dose-dependent (b) (4) at vertebral and/or nonvertebral bone sites, correlating with increases in bone strength. The anabolic effect of abaloparatide was due to (b) (4) increase in osteoblastic bone formation and was evidenced by trabecular and/or cortical bone thickening due to endosteal bone apposition. Abaloparatide maintained or improved bone quality at all skeletal sites evaluated.

2 Drug information

2.1 Drug

CAS Registry Number (Optional)

- N/A

Generic Name

- Abaloparatide-SC

Code Names

- BA058, BIM44058, BIM44058C

Chemical Name

- H-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Glu-Leu-Leu-Glu-Lys-Leu-Leu-Aib-Lys-Leu-His-Thr-Ala-NH2

Molecular Formula

- $C_{174}\ H_{300}\ N_{56}\ O_{49}$

Molecular Weight

- 3961 Daltons

Amino acid sequence:

- H-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Glu-Leu-Leu-Glu-Lys-Leu-Leu-Aib-Lys-Leu-His-Thr-Ala-NH2

Structure or Biochemical Description

- Abaloparatide is PTHrP(1-34) with 8 amino acid substitutions at residues 22, 23, 25, 26, 28, 29, 30 and 31, or [$Glu_{22,25}$, $Leu_{23,28,31}$, Aib_{29}, $Lys_{26,30}$] hPTHrP(1-34)-NH_2. Abaloparatide has 76% homology to PTHrP (1-34) and 41% homology to PTH(1-34).

Pharmacologic Class

- Para-Thyroid Hormone related Peptide (1-34) (PTHrP1-34) analog

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 73176 (hPTHrP1-34 analog; Abaloparatide; Radius)
NDA 21318 (rhPTH1-34; Teriparatide; Forteo®; Eli Lilly)
BLA 125551 (hPTH1-84; Parathyroid Hormone; Natpara®; NPS Pharmaceuticals)

Data included in the review of NDA 21-318 were used to estimate the AUC of PTH(1-34) achieved in the abaloparatide rat carcinogenicity study in order to calculate approximate [rat:human] PTH(1-34) exposure multiples.

2.3 Drug Formulation

The peptide is provided in a sterile solution for disposable pen injection system, at strength of 2 mg/mL. The product contains sodium acetate trihydrate and acetic acid (b) (4) and phenol (b) (4) as excipients.

2.4 Comments on Novel Excipients

N/A

2.5 Comments on Impurities/Degradants of Concern

Drug substance and drug product contain the degradant (b) (4) The specified levels in drug substance and product exceed the qualification thresholds in ICH Guidances Q3A and Q3B(R2). Nonclinical studies were conducted to qualify the degradant.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is postmenopausal women with osteoporosis. The proposed dosing regimen is 80 µg once daily administered by subcutaneous injection.

2.7 Regulatory Background

Abaloparatide (BA058) was originally discovered and developed by the Beaufour Ipsen Pharma Group (Ipsen) under the name BIM44058 or BIM44058C. The license was acquired by Nuvios Inc., which renamed the product BA058, and conducted a first in human phase 1a single dose study in Germany before opening a US IND. IND 073176 was submitted on December 8, 2005 by Nuvios whose name later changed to Radius Health Inc.

At the EOP2 meeting on January 21, 2010, DBRUP expressed concern about the findings of tissue mineralization in a 39-week monkey study, possibly in the absence of hypercalcemia, and recommended that the Sponsor submit a proposal to adequately evaluate potential mineralization and renal function in the phase 3 study.

At the pre-NDA meeting (5/28/15), the Sponsor was advised that the potential risk of osteosarcoma would require (at minimum) a labeled boxed warning, Medication Guide and plan to mitigate the risk post-marketing. Sponsor was encouraged to submit a pharmacovigilance plan.

The carcinogenicity study protocol was reviewed by the Executive Carcinogenicity Assessment Committee (ECAC) on July 14, 2009. ECAC occurred with the dosing termination and early animal sacrifice (communication to Sponsor, IND 73176, August 28, 2012). The rat carcinogenicity study data were reviewed by ECAC on September 6, 2016.

3 Studies submitted

3.1 Studies Reviewed

All pivotal pharmacology and toxicology studies submitted to the NDA were reviewed. Studies reviewed in detail included most in vitro and in vivo pharmacology studies (including bone quality studies in ovariectomized rats and monkeys), repeat dose toxicity studies in rats and monkeys, and carcinogenicity and reproductive toxicity studies in rats. The results of the rat and monkey toxicity studies, several PK/ADME studies, and genotoxicity studies are presented in tabular format.

3.2 Studies Not Reviewed

The assay validation studies were not reviewed. A number of safety pharmacology, PK/ADME and genotoxicity studies were not reviewed in detail, but the results of those studies are nevertheless summarized in tabular format in this review.

3.3 Previous Reviews Referenced

Several studies were reviewed in summary format in reviews for IND 73,176.

4 Pharmacology

4.1 Primary Pharmacology

Background

Abaloparatide (Abl) is a synthetic 34 amino acid peptide that binds to the parathyroid hormone receptor 1 (PTH1 receptor, or PTH1R, also called classical PTH/PTHrP receptor). It has 41% homology to human PTH(1-34) and 76% homology to human PTHrP(1-34). The difference between Abl and PTHrP1-34 is the replacement of 8 amino acids residues between 22 and 34. In part, this was aimed at stabilizing the C-terminal helix portion of the molecule which is responsible for binding to the PTH1R.

PTHrP is a member of the PTH family and is secreted endogenously. It generally acts in para-, auto-, or intracrine fashion and among other functions it plays an important role in the maintenance of the epiphyseal growth plate. Six of the first seven N-terminal amino acids and 8 of the first 13 terminal amino acids are identical between PTH and PTHrP. Therefore, PTHrP can mimic nearly all effects of PTH in classical PTH target cells (kidney, bone) which are mediated by the NH_2-terminally located adenylate cyclase-activating domain. PTHrP is widely expressed in normal tissues. It can have effects on cells of the cardiovascular system that it may or may not share with PTH. PTHrP improves cardiomyocyte contractility via PKA actication, while PTH does not, but it can also activate PKC. In smooth muscle cells of the CV system, both PTHrP and PTH cause PKA-mediated vasorelaxation.

The sponsor conducted in vitro and in vivo pharmacology studies with abaloparatide. Single dose and repeat dose studies of 14-day to 12-month duration were conducted in rats. Single and repeat dose studies of 45-week to 16-month duration were conducted in monkeys. The studies were not GLP-compliant.

Primary pharmacology studies

Tabular summary

In vitro studies

Study Nr	Type of Study	Methods	Findings
BIM-44058/001, BA058-152	Binding and activation of PTH1R and PTH2R HEK-293 cells, containing either human PTH1R or PTH2R	Abaloparatide, PTH(1-34), PTHrP(1-34): 0.1 to 100 nM Acidification (cell metabolism) cAMP production: over 30 min	EC_{50} (nM) of cell response through PTH1R activation (HEK-C21 cells): Ligand — Extracellular acidification route (nM) — cAMP production (nM) Abaloparatide — 0.17±0.05 — 0.17±0.06 hPTH(1-34) — 0.26±0.08 — 0.40±0.16 hPTHrP(1-34) — 0.21±0.05 — 0.48±0.13 EC_{50} (nM) of cell response through PTH2R activation (HEK-BP16 cell): Ligand — Extracellular — cAMP

			acidification route (nM) / production (nM) hPTH(1-34) 4.32±0.56 / 1.41±0.24 Abaloparatide > 100 / > 100 hPTHrP(1-34) > 100 / > 100 Conclusions: o At the PTH1R, abaloparatide was approx. 2 times as potent as hPTH1-34 and hPTHrP1-34 to produce a cAMP response. o At the PTH2R, abaloparatide (and PTHrP1-34) was <1/70 times as potent as hPTH1-34 to elicit a cAMP response. o Data confirmed that abaloparatide is a selective PTH1R agonist and does not bind or activate the PTH2R.
14RAD029	GP-2.3 cells (HEK-293 derived cells with PTH1R and cAMP reporter plasmid)	0.003 to 3000 nM range	Binding affinity IC_{50} (nM) Peptide RG R^0 Abaloparatide 0.20 316 hPTHrP(1-36) 0.32 35 hPTH(1-34) 0.33 3.9 LA-PTH 0.38 0.83 Sponsor believes that the data support the theory that the RG (vs R0) -selective binding of abaloparatide is associated with relatively short duration of cAMP signaling, and therefore with a relatively small calcemic effect and minor enhancement of bone resorption. This Reviewer does not agree with that conclusion. While the extent and duration of the wash-out cAMP response appeared positively related to R0-affinity, the relationship of the in vitro washout response with in vivo bone resorption and serum Ca regulation has not been clearly established. *Study reviewed in detail.*

In vivo studies

Study Nr	Type of Study	Species	Methods/ Doses	N/s/g	Findings
BIM-44058/002, BA058-153	Calcium mobilization in TPTX rat	Rats, PTXed	Abl, PTH(1-34), PTHrP(1-34): 0, 5, 20, 80, 320 µg/kg (SC)	M/2	SC injection of PTH receptor agonists immediately following PTX significantly increased plasma the low Ca levels in these PTX animals at 6h after surgery. *Study reviewed in detail*
BIM-44058/003 BA058-154	Effect on OVX-induced bone loss in rats 4-week study	Rats/SD/OVX	Sham: 0 ug/kg OVX: Abl and PTH(1-34): 0 (control), 0.04-40 µg/kg, 5 days/wk (SC)	F/8	PTH(1-34) (5 µg/kg) and abaloparatide (2.5 µg/kg) restored femoral BMD after a 4-week treatment period of OVX rats. Within the tested dose range, abaloparatide was approximately 2-fold more potent than hPTH(1-34). *Study reviewed in detail*

10RAD 008	Effect on OVX-induced bone loss in rats (42-day study)	Rats/SD/ OVX	Rats were sham-operated or OVXed, 5-wk bone depletion, 6 week treatment Sham controls: 0 ug/kg (SC) OVX rats: Abaloparatide: 0 (control), 5 and 20 µg/kg/day (SC) DXA, uCT and biomechanical testing at end of treatment	F/20-24	Abaloparatide increased BMD in femur (by 17-24% at 5-20 µg/kg), and in vertebrae (by 30-42% at 5-20 µg/kg). Abaloparatide increased femur BV/TV and mean bone density at both doses, reaching sham levels at 20 µg/kg, and improved bone structure by increasing Tb.Th and Tb.N. while decreasing Tb.Sp. Abaloparatide significantly enhanced bone strength in a femur 3-point bending test, a femur neck cantilever compression test, and a vertebral compression test. Conclusion: Abaloparatide had anabolic bone effect. At 5 and 20 ug/kg/day, it dose-dependently restored bone loss and improved bone strength in OVX animals: *Study NOT reviewed in detail*
10RAD 029	Effect on OVX-induced bone loss in rats (12-month efficacy/safety study)	Rats/SD/ OVX	Rats sham-operated or OVXed, 3-mo bone depletion, 12 mo Tx. Sham control: 0 ug/kg/day (SC) OVX: 0 (control), 1, 5, or 25 µg/kg/day (SC) Bone turnover, DXA, pQCT, HMM, mechanical testing, histology	F/18	Abaloparatide increased bone formation markers (s-P1NP and OC). Bone resorption markers (s-CTX and DPD) were transiently increased (i.e., at Wk 11/12). Abaloparatide increased DXA and pQCT BMC and BMD relative to OVX vehicle. This resulted in restoration of bone mass to sham or above-sham levels. Abaloparatide increased trabecular number and thickness, cortical thickness, and reduced endocortical perimeter and medullary area. Abaloparatide increased bone strength at vertebral and nonvertebral sites: lumbar spine, femoral neck and femur diaphysis. Bone mass were positively and significantly correlated bone strength parameters at lumbar spine, femur neck, and femur in all groups. There was no deterioration in bone quality, when defined as mass/strength ratio. *Study reviewed in detail*
BA058-109	Effect on OVX-induced bone loss in cynomolgus monkeys (10-month study)	Monkeys/ cynomolgus/ OVX	Monkeys sham-operated or OVX-ed, 10-mo bone depletion, 44-wk treatment Sham control: 0 µg/kg/day (SC) OVX: 0 (control), 0.1 µg/kg/day for 7 months, then 10 µg/kg/week, and 1 or 10 µg/kg/day (SC) Bone turnover, DXA, pQCT, uCT, HMM,	F/9-10 (F/15 sham)	Abaloparatide increased spine DXA-BMD to sham levels within 6 months of treatment at 1-10 ug/kg. OVX had no effect on femur DXA-BMD, or tibia/radius pQCT-BMD. Bone formation and resorption markers (OC, PICP, DPD) transiently increased. HMM data showed no effect of OVX on static parameters, but showed Abl-related increases in osteoblast parameters in vertebrae (ObS/BS and OS/BS). Micro-CT mechanical testing showed no effects of OVX or Tx on trabecular microarchitecture. It is not clear whether this study served as dose range finding study, since it suggested that 10 ug/kg/day was sub-optimal, but a lower dose (5 ug/kg) was used in the 16-mo study.

			strength testing		*Study NOT reviewed in detail*
10RAD 030	Effect on OVX-induced bone loss in cynomolgus monkeys (16-month efficacy/safefy study)	Monkeys/ cynomolgus/ OVX	Sham controls: 0 µg/kg/day (SC) OVX rats: Abaloparatide: 0 (control), 0.2, 1 or 5 µg/kg/day (SC)	F/16-17	Abaloparatide increased bone formation marker PINP; and caused a smaller increase in resorption marker NTx. Abaloparatide increased DXA and pQCT BMC and BMD at most bone sites to sham control levels. There were increases in cortical thickness in the proximal tibia diaphysis at end of the study in all ABL dose groups. Also observed were increases in Tb.Th and BV/TV at 1 and 5 µg /kg/day, and increases in wall thickness at all doses in spine and femur neck. BFR increases were not observed in cancellous bone sites after 16 months, but were seen in cortical bone at peri- and endosteal bone surfaces. Abaloparatide increased bone strength of vertebrae to Sham control levels at 1 and 5 µg/kg/day. Effect on femoral neck strength was not significant. There were no clear increases in strength parameters of femur diaphysis. Bone mass and bone strength positively and significantly correlated at all doses. Slope of regression lines slightly decreased in OVX vs sham controls, and reversed to sham control level in 1 and 5 ug/kg/day dose groups. *Study reviewed in detail*

Review of selected studies

In vitro studies

Binding affinity and selectivity to PTHR1 Conformations and Effects on Downstream Signaling
(Study 14RAD029)

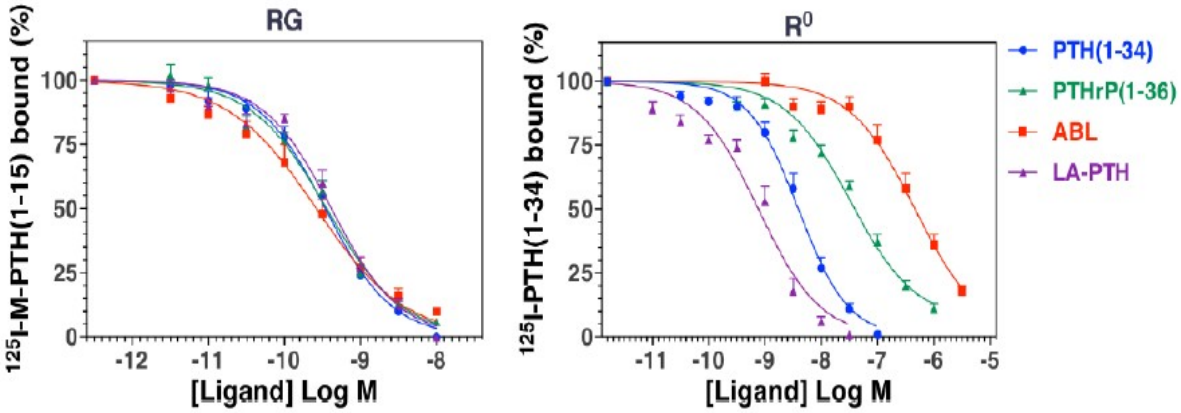
The ability to bind to 2 different PTH1R conformations (RG and R^0) was determined for 4 different peptide receptor ligands (ABL, PTHrP1-36, LA-PTH, PTH1-34). RG is the conformation that is G-protein dependent (GTPy-sensitive), while R0 is G-protein independent (GTPy insensitive). The GP-2.3 cells used in this study are HEK293-derived cells that express 'glosensor' cAMP reporter plasmid, and PTH1R.

Methods
Binding to the RG conformation was determined in GP-2.3 cell membranes by measuring displacement of tracer radioligand ^{125}I-M-PTH(1-15) (which binds only to receptors in the RG conformation) in the absence of GTPyS (a compound that dissociates receptor-G-protein complexes). Binding to the R0 conformation was determined in GP-2.3 membranes by measuring displacement of tracer radioligand ^{125}I-PTH(1-34) in the presence of 10 uM GTPyS (which causes receptor-G-protein dissociation and make PTH1-34 only bind to the R^0 receptor conformation). The study also assessed cAMP production and phosphorylated ERK-1/2 proteins. Ligand LA-PTH (long acting PTH) is known to have enhanced bone resorptive and prolonged calcium mobilizing activity which is not due to prolonged receptor binding.

Results

Figure 1 shows the binding affinities for the RG and R0 receptor conformations. The affinities were similar for the 4 ligands (0.20-0.38nM), although slightly higher for ABL than the 3 other compounds. The affinity for the R^0 isoform was different for all 4 ligands, with binding affinity sequence (high to low): LA-PTH > PTH1-34 > PTHrP(1-36) > ABL. ABL's affinity for the R0 conformation was lowest (80-fold lower than of PTH1-34).

Figure 1: Binding of Abaloparatide, PTH(1-34), PTHrP(1-36) and LA-PTH to Different Conformations of the PTH1R in GP-2.3 Cell Membranes



ABL = abaloparatide; PTH = parathyroid hormone; PTHrP = parathyroid hormone related peptide; LA-PTH = long acting PTH

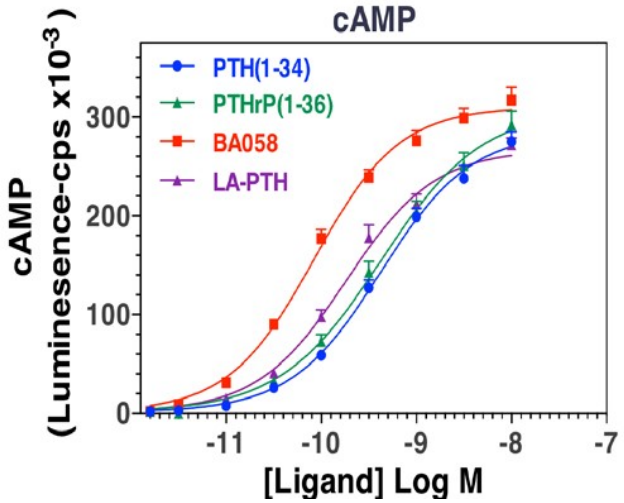
Ligand	RG IC50 (nM)	R^0 IC50 (nM)	IC50 ratio (RG vs. R^0)
ABL	0.2	316	1600
PTHrP	0.32	35	110
PTH1-34	0.33	3.9	12
LA-PTH	0.38	0.83	2.2

Figure 2 (below) shows the dose-dependence of cAMP signaling. The EC50 was lower for ABL than for the other 3 ligands compounds, as was binding affinity to the RG conformation.

Figure 3 (below) shows the cAMP signaling response upon initial ligand binding (for 14 mins) and after washout of unbound ligand ("residual response"). The initial response was similar for the 4 ligands at the concentrations used, which were selected so as to produce a similar maximum response (ABL 0.1 nM, PTH1-34 0.3nM, LA-PTH 0.3 nM, PTHrP 1 nM). The order of magnitude for both extent and duration of the washout response was: LA-PTH > PTH1-34 > PTH1-36 > ABL. AUC of cAMP for that order of ligands was 14682, 8664(PTH1-34), 4953 and 4298(ABL) cps$x10^3$xmins.

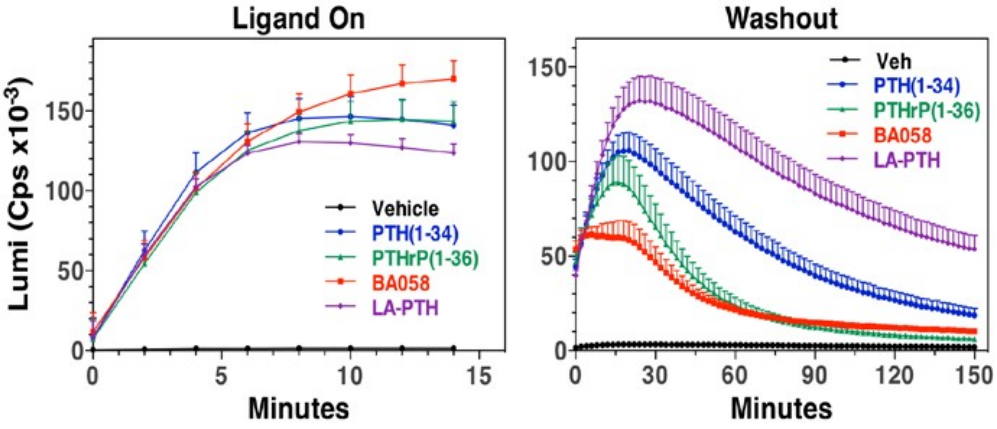
Figure 2: Effect of Abaloparatide, PTH(1-34), PTHrP(1-36) and LA-PTH

on cAMP-signaling in GP-2.3 Cells



BA058 = abaloparatide; PTH = parathyroid hormone; PTHrP = parathyroid hormone related peptide; LA-PTH = long acting PTH

Figure 3: Effect of Abaloparatide, PTH(1-34), PTHrP(1-36) and LA-PTH on cAMP-signaling upon Ligand On and Washout GP-2.3 Cells



BA058 = abaloparatide; PTH = parathyroid hormone; PTHrP = parathyroid hormone related peptide; LA-PTH = long acting PTH

The Sponsor concluded that abaloparatide binds differentially with high affinity to the RG isoform of the PTH1R, resulting in a more transient intracellular cAMP response. It was suggested that the effect of 'more transient' cAMP signaling, such as with abaloparatide, likely results in differential downstream effects which **'strongly favors increased bone formation with a more limited effect on bone resorption'**. Sponsor referred to data published by Makino et al (ASBMR, 2015), which showed that, upon short term in vitro cell exposure, the expression (mRNA) of osteoblast-derived resorption-stimulating factors (such as RANK-L) was more transient with Abl than with teriparatide, while the expression of osteoblastic formation-related

factors (such as SOST) was affected similarly and continuously by both ligands even after ligand removal.

The 4 ligands also activated downstream ERK-1/2 phosphorylation in intact GP-2.3 cells. Sponsor stated there were no potency differences between the ligands. However, the data suggested that the potency to activate this arrestin-mediated signaling pathway was larger for PTHrP1-36 and ABL than for PTH1-34 and LA-PTH. Thus, ligand selectivity may not have been limited to the PTH1R-mediated AC/PKA signaling pathways alone.

Reviewer comments:

- *Differential selectivity of RG vs R0 binding of different PTH1R receptor ligands was clearly demonstrated. ABL was relatively more RG selective than the other three ligands.*
- *Binding to the R0 isoform is thought to be associated with prolonged cAMP signaling, and binding to the RG form with more transient cAMP signaling. The latter is believed to be due to a more rapid dissociation of RG-receptor-ligand complex upon G protein activation. The Fig. 3 data showed that, at the concentrations selected, this difference in duration of residual cAMP response was confirmed in the washout experiment.*
- *However, the cAMP data raise many questions and their biological and clinical significance is unclear. Reviewer believes that there is only limited and indirect evidence for the Sponsor's suggested MOA.*
    - *Sponsor's hypothesis that the different cAMP responses with ABL and PTH1-34 are related to relatively small resorption activation is not supported by animal bone pharmacology data and clinical data from Phase 2 Study 002 and Phase 3 Study 003. The clinical studies showed similar resorption marker changes with 20ug/day teriparatide vs. 80 ug/day abaloparatide in the first 1-3 months of treatment. Resorption markers declined in the Abl group after 3 months, while they remained increased with teriparatide. Bone formation markers were increased by abaloparatide to a lesser extent than by teriparatide. The data from Makino et al (2015) also appear to conflict with the clinical study data.*
        - *Comment: <u>The slightly larger bone efficacy of abaloparatide vs teriparatide in Study 003, in terms of BMD increase and fracture reduction, may be due to induction of smaller turnover response and thus a lesser increase in remodeling space with Abl treatment</u>.*
    - *Considering the clinical PK profile upon SC dosing of PTH peptides, it is unclear whether the residual cAMP responses observed under artificial in vitro washout conditions would be relevant. It would depend on the characteristics of the residual cAMP response. If it's only quantitatively different form the ligan-on response, its relevance would depend on the relative T1/2 of plasma exposure (ca. 1.2h for PTH1-34 and 1.7h for Abl) vs the T1/2 of the residual cAMP response (approx. 0.4h for Abl and 1h for PTH1-34). However, if it is qualitatively different (e.g. endosomal vs membranous), it may have significance for biological outcome. However, sponsor did not expand on this, and the issue remains unclear.*
    - *The data from the referenced study by Okazaki et al ((2008) using PTH receptor ligands with differential RG selectivities are in agreement with Sponsor's in vitro*

results. However, the Okazaki study showed that in mice the less RG selective ligand (M-PTH-34) caused a large increase in bone mass while a similar dose of the more RG selective ligand PTH1-34 had no effect on bone mass. However, bone resorption was similarly activated by M-PTH1-34 vs PTH1-34. The decrease in cortical thickness and endocortical bone resorption by M-PTH1-34 may have been due to the use of a relatively large dose of this compound.

- *In conclusion, the nature of the relationship between PTH1R mediated cAMP signaling in vitro - including its duration and intracellular characteristics - and the response of bone tissue in vivo has not been established. More studies are needed to gain information. Sponsor's general conclusion that abaloparatide increases bone formation with a more limited effect on bone resorption due to its RG selectivity and the relatively short residual cAMP response observed in vitro is not sufficiently supported by the data provided.*
- *While the exact in vivo mechanisms of action of Abl and teriparatide are still unclear, this does not eradicate the finding that abaloparatide has a clear anabolic effect on bone and that, integrated over time, it stimulates bone formation more than bone resorption.*

In vivo studies

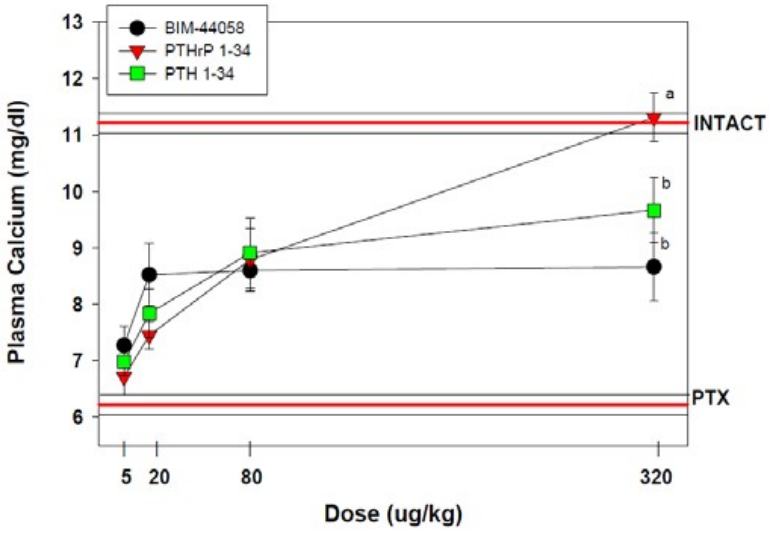
Ability of BIM-44058 to Mobilize Calcium in the Rat
(Study BIM44058/002 (BA058-153)

Male CD rats were parathyroidectomized (PTX) and immediately injected subcutaneously with a single dose of PTH1-34, PTHrP1-34 or BA-058. Serum Ca was determined 6 hours later. The time course of the serum Ca effect of the PTH ligands was not determined.

Data (Figure 1) showed increases in plasma calcium with all 3 ligands. Shapes of the dose response curves appeared different. In the biologically most relevant 5-20 ug/kg dose range, the increase in plasma Ca was largest with BA-058, then PTH1-34 and then PTHrP1-34. At high doses, PTHrP1-34 achieved plasma Ca concentrations similar to those in intact non-PTX rats.

Sponsor concluded that "abaloparatide has significantly less calcium mobilizing activity at higher doses than hPTH(1-34)". However, this was only true for the supra-pharmacological high dose of 320 ug/kg.

Figure 1: Effect of Abaloparatide, hPTH(1-34), and hPTHrP(1-34) on Plasma Calcium in Parathyroidectomized, Hypocalcemic Rats (data from N=2)



BIM-44058=abaloparatide; PTX=parathyroidectomized
Note: Results are combined from 2 experiments.

Reviewer comments:

- *This was one of two short term rat pharmacology studies in which the action of abaloparatide was compared to that of teriparatide (PTH1-34)*
- *The PTX rat data from this study are of questionable significance. They do not clearly support the theory that abaloparatide would cause less bone resorption and/or hypercalcemia, even at similar doses of PTH1-34. There are no adequate nonclinical data on comparative effects of efficacious (single or repeat) doses of abaloparatide or teriparatide on plasma Ca concentrations.*
- *In Phase 3 study 003, the evaluated doses of 80 ug/day abaloparatide and 20 ug/day teriparatide were similarly efficacious with regard to the change in spine BMD. However, abaloparatide caused less hypercalcemia and smaller total serum Ca changes than teriparatide. The available clinical data are most relevant.*

Restoration of Bone Mineral Density in Ovariectomized, Osteopenic Rats - 4-Week Study
Study BIM-44058/003 (BA058-154)

Female SD rats were OVX-ed or sham-operated at 8 weeks of age and maintained for 5 weeks to allow for development of osteopenia in OVX rats. Rats (N=8/grp) were then injected SC with BA-058 (abaloparatide) or PTH1-34, 5 days/week, for 4 weeks, at doses between 0.04 and 40 ug/kg. BMD of femur regions was determined by DXA.

Fig. 1 shows data for total femur, and Fig. 2 for femur diaphysis, BMD increases were observed in total, proximal, metaphysis femur regions at doses of 1.25-40 ug/kg abaloparatide, and 2.5-40 ug/kg PTH1-34.

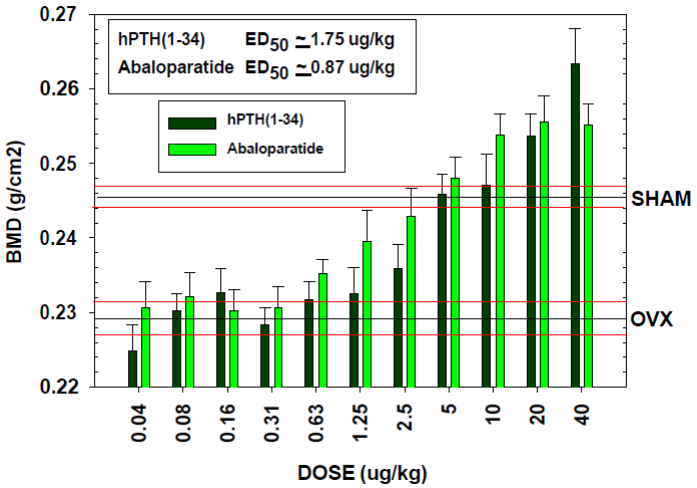
Data showed BMD decreases in OVX rats. Calculated ED50 values for the "restorative range" (between sham and OVX) were 1.8 ug/kg for PTH1-34, and 0.9 ug/kg for abaloparatide for total femur. BMD effect increased to above-sham level at doses >2.5-5 ug/kg, especially for PTH1-34. Data were similar for total, proximal and metaphysis femur, all mainly cancellous bone regions. BMD of femur diaphysis was not significantly decreased in OVX rats. Treatment with abaloparatide or PTH1-34 increased diaphyseal BMD at 10-40 ug/kg abaloparatode, and 20-40 ug/kg PTH1-34.

Sponsor concluded that relatively low doses of abaloparatide and PTH1-34 are needed to restore BMD to sham levels in young osteopenic rats. They also concluded that BA-058 was 2-fold more potent in restoring BMD.

Reviewer comments:

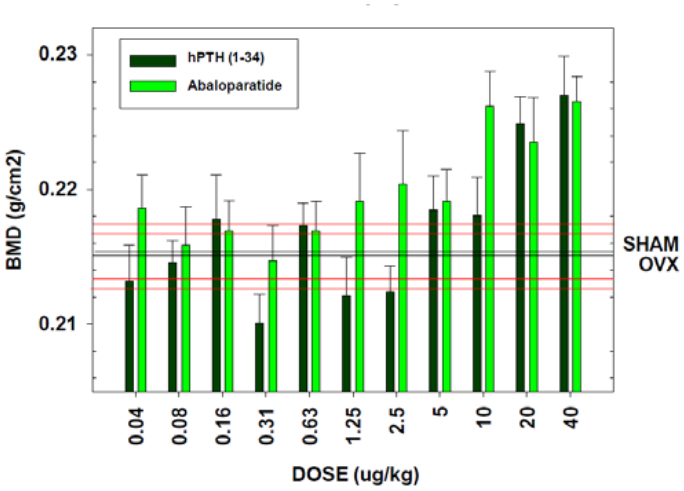
- *This was the second of two rat pharmacology study in which the action of abaloparatide was compared to that of teriparatide (PTH1-34)*
- *The 2-fold larger potency of abaloparatide in rats was not reproduced in human studies. Clinical studies showed lower potency of abaloparatide to increase BMD.*

Figure 1: Effect of Abaloparatide and hPTH(1-34) on BMD in Ovariectomized, Osteopenic Rats – Total Femur



SHAM=sham-ovariectomized; OVX=ovariectomized
Each bar represents the mean±SEM of 8 animals.

Figure 2: Effect of Abaloparatide and hPTH(1-34) on BMD in Ovariectomized, Osteopenic Rats - Femur Diaphysis



SHAM=sham-ovariectomized; OVX=ovariectomized
Each bar represents the mean±SEM of 8 animals.

Bone quality studies

A 12-Month Osteoporosis Intervention Study of BA058 by Subcutaneous Injection in the Ovariectomized Sprague Dawley Rats
(Study 10RAD029)
(b) (4)
(GLP/QA) (Jan 21, 2015)
Batch 4AI1

SUMMARY

In a long term rat bone quality study, abaloparatide was given once daily to ovariectomized (OVX) Sprague Dawley rats, at subcutaneous doses of 1, 5, 25 µg/kg/day for 12 months (N=18F/grp). Systemic exposure (AUC) at these doses was equivalent to 0.3, 1.2 and 12 times the AUC at the human SC dose of 80µg/day.

Abaloparatide caused a sustained increase in bone formation, while resorption was increased to a lesser extent and/or for a shorter time. Osteoblastic bone formation was stimulated probably due to increases in both osteoblast number and activity.

Abaloparatide caused dose-dependent increases in BMD, BMC and Area (DXA), and increases in BMD and BMC with changes in bone surface circumferences (pQCT) at the evaluated bone sites (spine, femur, proximal tibia). Effects were the result of increases in trabecular thickness and number in cancellous bone, and increases in cortical thickness in meta- and diaphysis of proximal tibia. The increase in diaphyseal cortical thickness in proximal tibia was caused by some deposition of new bone at the periosteal surface, but mainly by apposition at the endosteal surface causing a reduction in medullary area. In the metaphysis, cortical bone thickness was also slightly increased, concomitant with some slight resorption of (sub)cortical bone ('trabecularization'). Treatment also increased cortical volumetric BMD in proximal tibia meta- and diaphysis.

Histomorphometry (HMM) at end of treatment showed marked increases in BV/TV, Tb.Th, Tb.N, BFR/BS and % osteoblast surface in cancellous bone of vertebrae and proximal tibia. In the distal tibia diaphysis, cortical thickness was increased along with a decrease in endocortical perimeter, consistent with the proximal tibia pQCT findings. Periosteal bone perimeter of the distal tibial diaphysis was only very slightly increased. However, periosteal BFR/BS was markedly increased, and periosteal resorption may have been increased along with formation. The slight increase in % eroded perimeter (i.e. resorption) at the endocortical surface, coincident with a slight increase in endocortical BFR/BS, was consistent with the fact that continued endosteal bone accretion was no longer observed at the end of the treatment period.

Biomechanical strength testing showed increases in peak load, yield load, stiffness and AUC in vertebral bodies and femur (shaft and neck). The correlation between BMC and strength parameters (peak load) was very good in vertebrae, reasonable in diaphysis, and moderate in femoral neck. Abaloparatide maintained 'bone quality' based on similar correlations between bone mass and strength in all OVX groups.

Overall, the data showed that, in the OVX rat, at doses ranging from 0.3x to 12x the human AUC at the 80 µg/day dose, abaloparatide has marked anabolic effects in bone tissue, consisting of increases in amount and size of trabeculae and cortical bone thickening in the axial and appendicular skeleton. The data provided a detailed picture of the bone changes induced by abaloparatide at various skeletal sites. Also, it revealed information on osteoblast and osteoclast activity at different bone surfaces that are not evident from bone biomarker analysis or clinical evaluations.

This study was an adequate nonclinical bone efficacy/safety assessment.

METHODS

SD rats (N=18F/grp) were sham-operated or ovariectomized (OVX-ed) at 6 months of age. Treatment with SC abaloparatide (Abl) doses of **0, 1, 5, 25 µg/kg/day** (0.1 mL/kg) was started after a 13-week bone depletion period, and continued for 12 months. Animals were assigned to Sham, OVX, OVX + 1, 5, or 25 ug/kg/day Abl, or baseline groups. Baseline animals (N=10F) were euthanized at end of bone depletion period.

Text Table 2
Experimental Design

Group Number	Dose Level (µg/kg/day)	Dose Concentration (µg/mL)	No. of Animals
1/ Sham Vehicle Control	0	0	18
2/ OVX Vehicle Control	0	0	18
3/ BA058 (OVX)	1	10	18
4/ BA058 (OVX)	5	50	18
5/ BA058 (OVX)	25	250	18
6/ Baseline (OVX)	a	a	10

a Animals were euthanized at the end of the bone depletion period and used for biomechanics and histomorphometry.

In-life observations/measurements in main study animals:

- Mortality (2x/day), clinical observations (weekly)
- Body weight (weekly) and food consumption (weekly, then monthly)
- Clinical and urine chemistry (Ca, P, creatinine)
- Biochemical markers of bone turnover CTx (serum), DPD (urine), OC and PINP (serum) (prior to sham/OVX, end of bone depletion, Wks 11-12, 30-31, 47-48) (Data in Appendix 12, which was erroneously entitled "Immunology Report")
- Toxicokinetics (RIA) (Day 1, Wk 26, Wk 52): samples from N=3F/time point, for 6 time points up to 3h post-dosing
- ADAs (RIA) (during bone depletion, at end of bone depletion, and pre-dosing at Wks 24 and 51)
- Labeling for histomorphometry (8 and 3 days pre-sacrifice).

Densitometry (in vivo, N=18F/grp)

- DXA of whole body, right femur and lumbar spine L1-L4 (prior to sham/OVX, end of bone depletion, Wks 12-13, 25-26, 51-52); Parameters: Area, BMD, BMC

- PQCT of proximal tibia (same time points as DXA); Parameters: metaphyseal Area, BMC, BMD; diaphyseal total Area, Ct.Th., and periosteal and endosteal circumference; and diaphyseal cortical Area, BMC, BMD
- Data in Text Tables are expressed as "total across time", which means that the values represent the additive values at the 3 measuring time points (this results in a larger effect vs OVX than that seen at single time points)

Terminal procedures

- Necropsy, including organ weight and tissue collection, histopathology, bone marrow smears

Histomorphometry (static and dynamic parameters, N=10F/grp)

- Proximal tibia (right), cancellous bone of metaphysis
- Vertebrae (L3), cancellous bone
- Tibia (right), tibio-fibular junction (ie distal tibia), cortical bone of diaphysis

Biomechanical testing (N=10F/grp)

- Femur (right): 3-point bending test
- Femoral neck (right): shear test
- Lumbar vertebrae (L4 and L5): compression test
- Ex vivo DXA (right femur) and/or pQCT (right femur, L4 and L5) of to-be-tested bones at end of treatment and end of bone depletion (i.e. baseline animals) (10F/grp)

Bone	Test	Parameter	Unit
Right Femur	3-Point Bending	Peak load Ultimate stress Stiffness Modulus Area under the curve (AUC) Toughness	N MPa N/mm MPa N-mm MPa
Right Femur	Femoral Neck Shear	Peak load Stiffness Area under the curve (AUC)	N N/mm MPa
Lumbar Vertebra (L4, L5, L4 and L5)	Compression Caliper	Peak load Apparent Strength Yield Load Yield Stress Stiffness Modulus Area under the curve (AUC) Toughness Height	N MPa N MPa N/mm MPa N-mm MPa mm

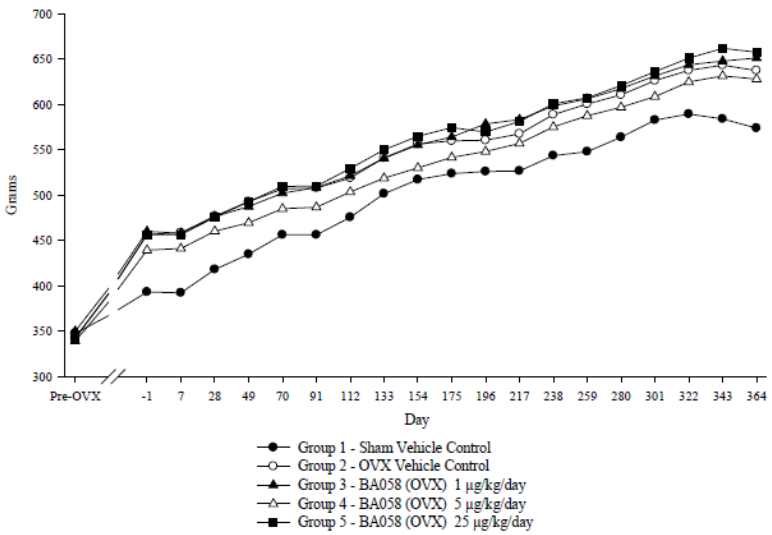
MPa = N/m^2 (measure of "stress")

RESULTS

No remarkable effects on mortality, clinical observations, food consumption

Increase in BW in OVX groups, but no dose-dependent Abaloparatide treatment effects.

Figure 1 Summary of Body Weights - Females



Serum Ca

Slight decrease vs control in OVX animals at all time points; and slight increase in Abaloparatide Grp 5 (25 ug/kg/day) at Wks 30/31 and 47/48 (possibly due to renal or gut effect)

Urine Ca

Minimal decrease in OVX animals, at Wks 11/12 and 30/31, but no Abaloparatide effects

Bone markers

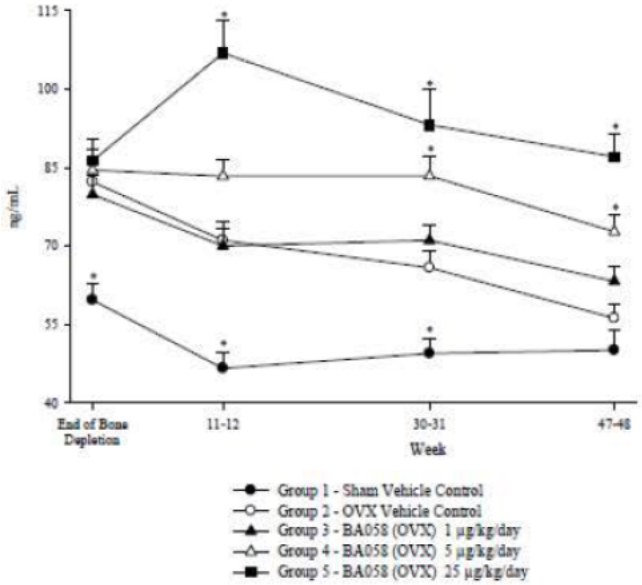
OVX:

- OC, DPD and Ctx (but not PINP) increased (all time points)

Abaloparatide:

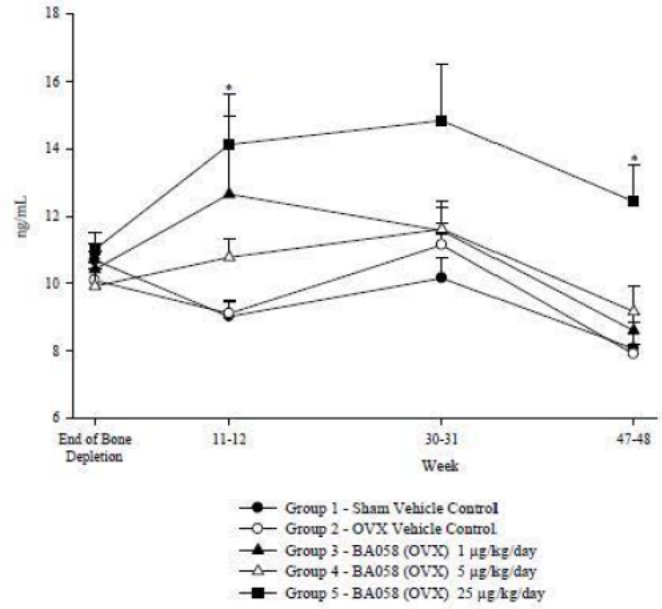
- OC increased in dose-dependent manner (all time points) (Text Fig.2)
- PINP increased (less than OC) at 1 and 5 ug/kg (Wk 11-12) and at 25 ug/kg (all time points)
- CTx (and DPD) increased at 25 ug/kg only (Wk 11-12 only) (Text Fig.3)
- When added together (for the 3 Tx time points), marker values were increased vs OVX for OC and PINP at 5 and/or 25 ug/kg, but not significantly for CTx and DPD (Text Table 18)

Text Figure 1
OC - Treatment Period - Mean (SEM)



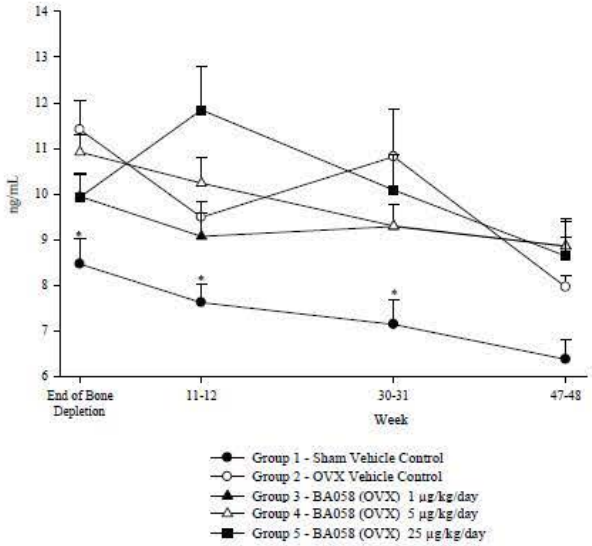
Significantly different from control group (Group 2) value: *-p≤0.05

Text Figure 2
PINP - Treatment Period - Mean (SEM)



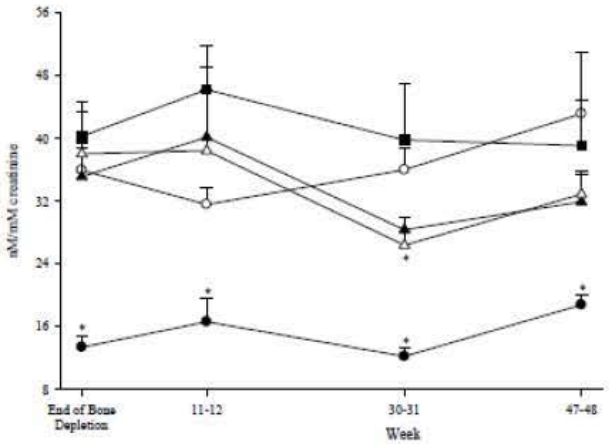
Significantly different from control group (Group 2) value: *-p≤0.05

Text Figure 3
CTx - Treatment Period - Mean (SEM)



Significantly different from control group (Group 2) value: *-p≤0.05

Text Figure 4
DPD - Treatment Period - Mean (SEM)



Significantly different from control group (Group 2) value: *-p≤0.05

Text Table 18
Effect of OVX and BA058 Treatment on Biochemical Markers of Bone Turnover
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 1 µg/kg/day	BA058 5 µg/kg/day	BA058 25 µg/kg/day
OC ng/mL	**143.88**	193.12	205.91	**237.53**	**287.11**
PINP ng/mL	26.688	28.164	34.184	31.441	**41.424**
CTx mg/mL	**20.874**	28.262	27.370	28.058	30.566
DPD nM/mM creatinine	**48.689**	110.782	103.422	98.733	125.171

Values in bold are significantly different from OVX vehicle controls.

NOTE: "Total across Time" indicates that the difference in marker values between treated and OVX groups were added together for the 3 sampling time points

Sponsor depicted the "Bone Marker Anabolic Window" by plotting PINP and DPD (as % of change from OVX) over time (Text Fig. 5).

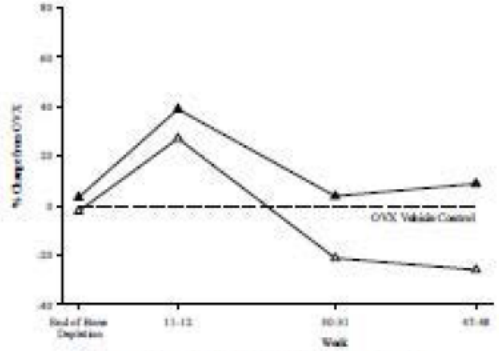
> *Reviewer Comment:*
> *In published literature, the difference between the effects on formation vs. resorption (markers) is referred to as the anabolic window.*

Abaloparatide effects on bone markers (PINP vs DPD):

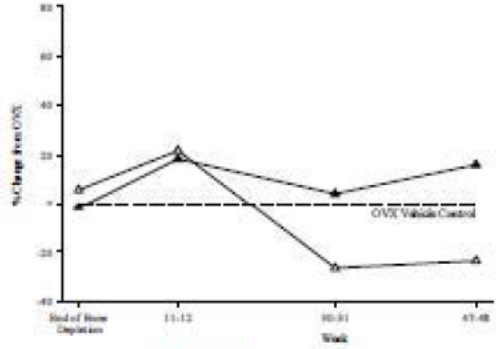
- Both formation and resorption were increased at Wk 11/12, at ALL doses
- Formation was also increased at later time points, especially at 25 ug/kg
- Resorption was DECREASED vs OVX at 1 and 5 ug/kg, while it was unaffected by 25 ug/kg at the 2 later time points. The decrease in resorption at the lower doses was an unexpected effect of a PTH1R ligand, suggesting an anti-resorptive effect of Abaloparatide. *NOTE: This has not been observed in monkeys or humans. The rat data appear unreliable.*
- Sponsor concluded that there was a positive bone anabolic window at all doses. The window appeared to widen with time. *NOTE: In Abaloparatide studies in humans and monkeys this window appears to decrease, not increase, with time.*
- Reviewer concludes that bone formation was increased for a longer time and to a larger extent than bone resorption.
- Data for PINP vs CTx were similar (Reviewer's analysis, not shown).

Text Figure 5
Bone Marker Anabolic Window - PINP vs. DPD - Percent vs. O

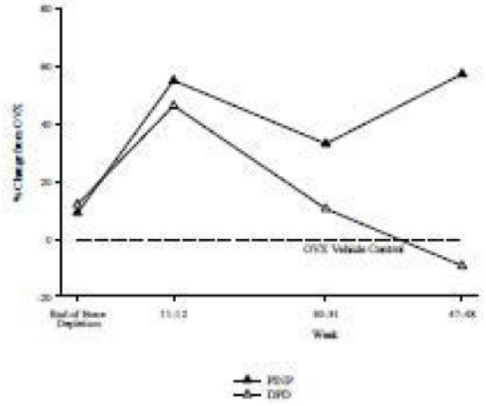
Group 3 - BA058 (OVX) 1 µg/kg/day



Group 4 - BA058 (OVX) 5 µg/kg/day



Group 5 - BA058 (OVX) 25 µg/kg/day



Densitometry in vivo

Data represented as % change from acclimation (pre-bone depletion) or end-of-bone-depletion period

DXA whole body, spine L1-L4, femur (in vivo)

DXA Spine:

OVX:

- Decreases in BMC and BMD during the 13-week bone depletion period, as expected (Text Figure 9 and Text Table 20)

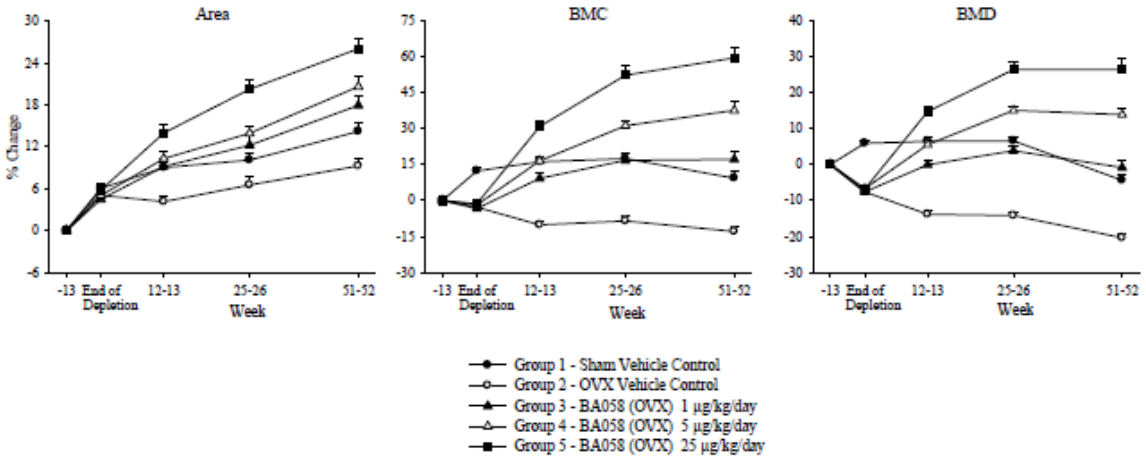
Abaloparatide:

- Dose-dependent increases in Area, BMC and BMD, at 1, 5 and 25 ug/kg
- Increases in BMD occurred mainly in first 6 months of Tx, while increases in Area continued throughout the Tx period
- Spine DXA parameters reached levels above sham in the 5 and 25 ug/kg groups

Reviewer Comment:

The increase in vertebral BMD was 125% at the 25 ug/kg dose, a dose equivalent to the human 80 ug dose based on AUC (Table 20). However this % was "total across time", and corresponds to an approx. 45% increase in BMD (60% increase in BMC) vs OVX control at the 12-month time point (data in Fig 9). This increase was much larger than the spine BMD change from baseline vs pbo in the 18-month Phase 3 Study 003 at the 80 ug daily dose (ca. 9%), which may have been due in part to higher animal exposure (11x human AUC at 25 ug/kg/day). However, at 1 and 5 ug/kg ug/kg (0.3 and 1.2x human AUC), BMD increases were still 25% and 33%, much larger than the 9% increase in humans. This suggests that rat bone is more sensitive than human bone to the effects of abaloparatide.

Text Figure 9
Bone Densitometry Values by DXA as Percent Change from Acclimation Period
Lumbar Spine - Mean (SEM)



Note: X-axis not linear in Text Fig. 9

Text Table 20
Effect of OVX and BA058 Treatment on L1-L4 DXA
as Percent Change from End of Bone Depletion Period
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 1 µg/kg/day	BA058 5 µg/kg/day	BA058 25 µg/kg/day
BMD	-9.8	-26.6	30.0	58.0	94.1
BMC	3.4	-21.4	55.1	91.9	146.2
Area	13.8	5.7	22.5	27.9	39.1

Values in bold are significantly different from OVX vehicle controls.

NOTE: Percent changes are "total across time", ie values for 3 evaluation time points were added together Values in sham and Tx groups should be bolded

DXA Femur:

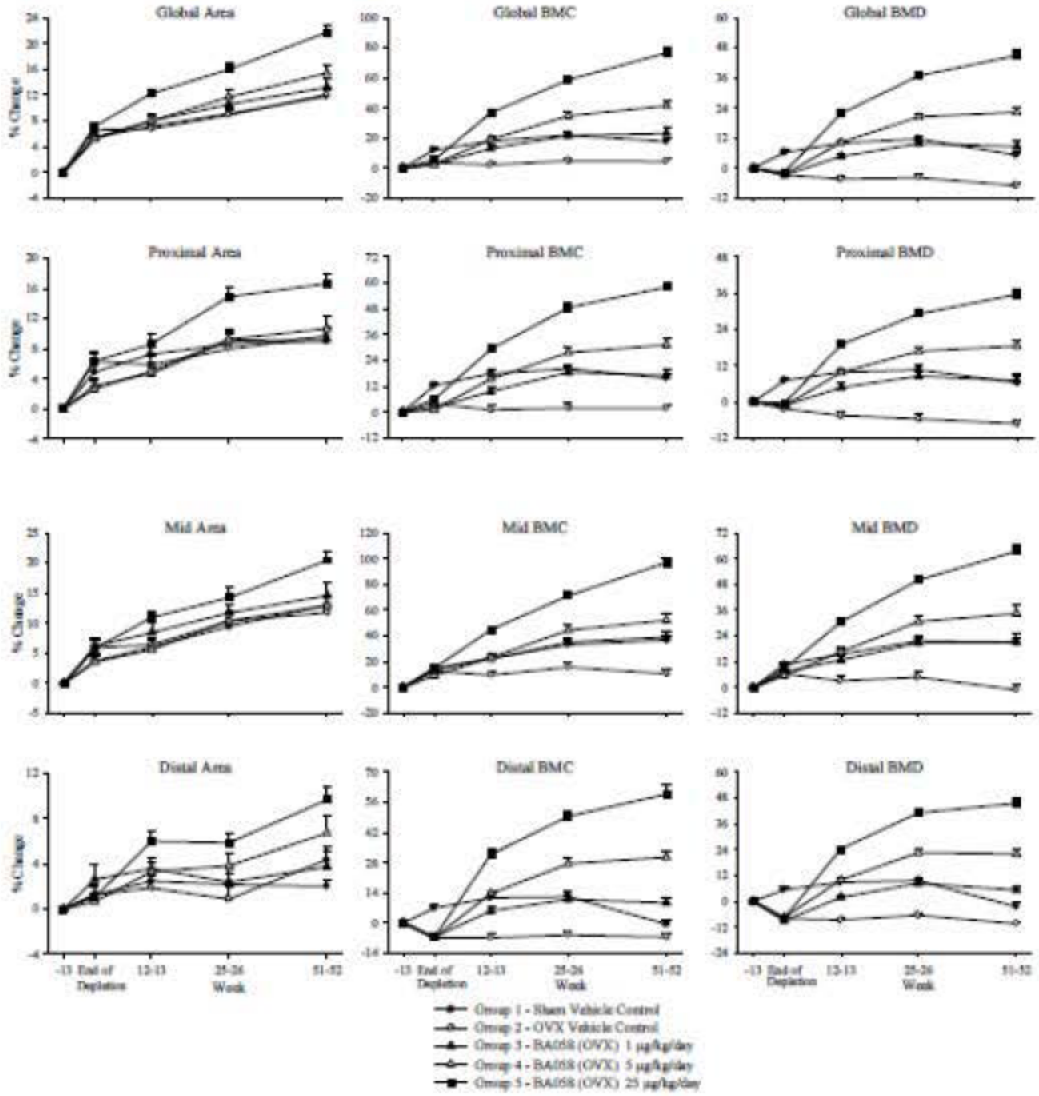
OVX:

- Decreases in BMC, BMD and Area in OVX animals during 13-week bone depletion period, as expected (Text Figure 11 and Text Table 21)

Abaloparatide:

- Dose-dependent increases in Area, BMC and BMD of proximal, mid and distal femur, at 1, 5 and 25 ug/kg
- Increases in Area and BMD (and thus BMC) continued throughout Tx period
- Femur DXA parameters reached levels above sham in the 5 and 25 ug/kg groups

Text Figure 11
Bone Densitometry Values by DXA as Percent Change from Acclimation Period
Right Femur - Mean (SEM)



Text Table 21
Effect of OVX and BA058 Treatment on Global Femur DXA
as Percent Change from End of Bone Depletion Period
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 1 µg/kg/day	BA058 5 µg/kg/day	BA058 25 µg/kg/day
BMD	7.8	-6.0	**33.6**	**61.7**	**111.2**
BMC	20.4	1.3	**51.9**	**85.1**	**149.0**
Area	12.4	7.4	**16.4**	**19.0**	**26.7**

Values in bold are significantly different from OVX vehicle controls.

DXA Whole Body:
Results similar to those for collective bones; effects continued throughout treatment period.

pQCT proximal tibia (in vivo)
Metaphysis

OVX:

- Decreases in Total BMD, BMC and Area during 13-week bone depletion period (Text Fig. 13)

Abaloparatide:

- Dose-dependent increases in TOTAL slice Area, BMC and BMD, at 1, 5 and 25 ug/kg (Text Table 22)
- Increase in trabecular and (sub) cortical BMD and BMC.
- Trabecular and Cortical Area were not measured, but data suggested increases in both of them, since changes in BMC were larger than changes in BMD. Calculations based on data from Text Fig. 12 (changes in BMC and BMD from end of bone depletion, not shown) indicated that Total, Trabecular and Cortical diameters were proportionally increased, and thus that all bone areas expanded by a similar factor.
- The calculated increase in trabecular area/diameter suggested an increase in 'endosteal' circumference through trabecularization, i.e. resorption, of (sub)cortical bone
- Increases continued throughout Tx period
- pQCT parameters reached above-sham levels in 1, 5 and 25 ug/kg groups

Diaphysis

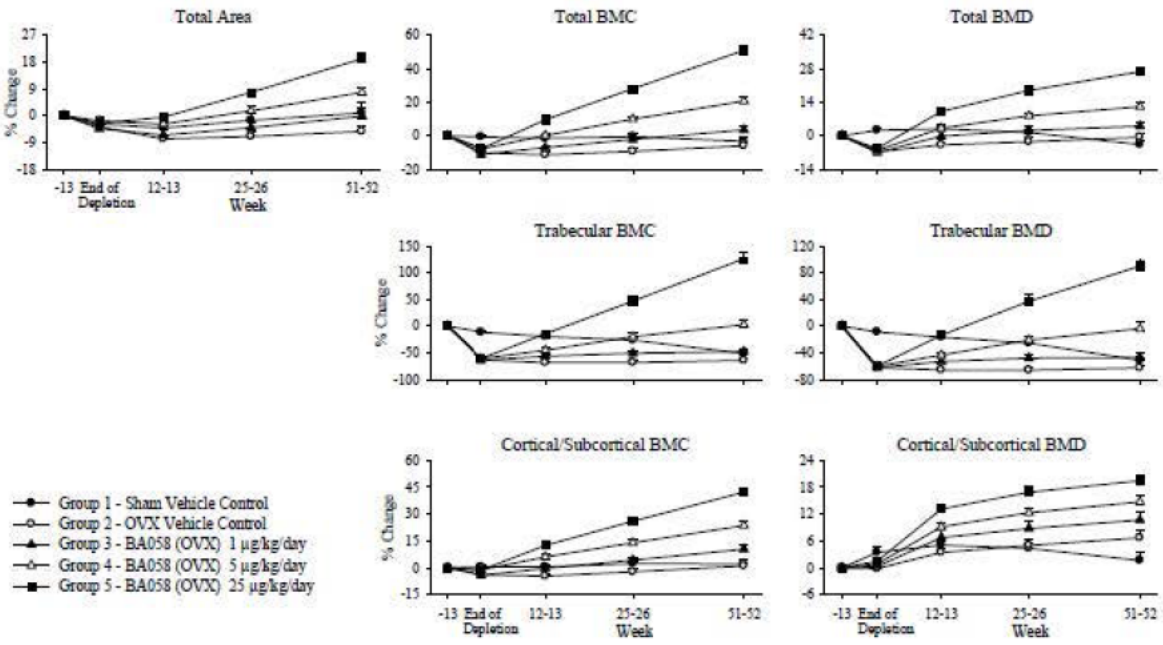
OVX:

- Increases in peri- and endosteal circumference and Total Area during 13-week bone depletion period (Text Fig. 13 and Text Table 22) (*indicating widening of bone in this region*)

Abaloparatide:

- Dose-dependent increases in Total Area and in Cortical Area, BMC, BMD, and Cortical Thickness, at doses of 1, 5 and 25 ug/kg (Text Figure 15, and Text Table 23).
- Dose-dependent marked decrease in endosteal circumference and slight increase in periosteal circumference, underlying observed increase in Cortical Thickness
- Increases continued throughout Tx period
- pQCT parameters reached beyond sham levels in 5 and 25 ug/kg groups

Text Figure 13
Bone Densitometry Values by pQCT as Percent Change from Acclimation Period
Right Proximal Tibia - Metaphysis - Mean (SEM)

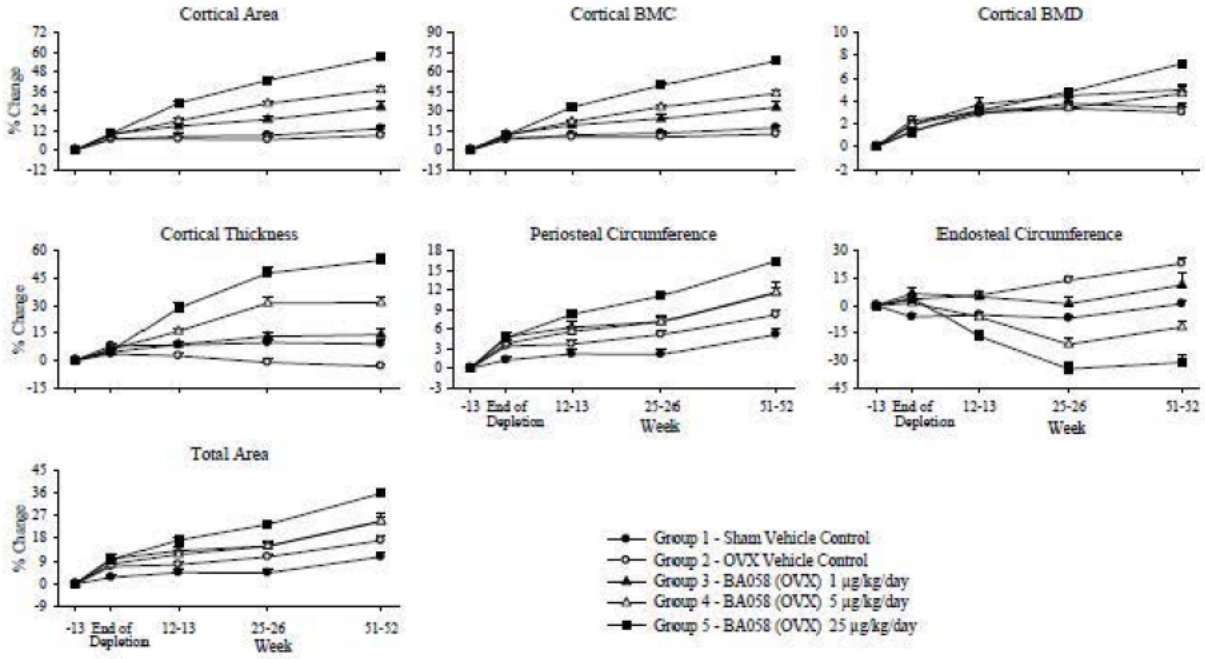


Text Table 22
Effect of OVX and BA058 Treatment on Proximal Tibia Metaphysis pQCT Parameters
as Percent Change from End of Bone Depletion Period - Total Across Time

Parameters		Sham Control	OVX Vehicle	BA058 1 µg/kg/day	BA058 5 µg/kg/day	BA058 25 µg/kg/day
Total slice	BMD	-6.9	14.3	31.9	45.1	76.9
	BMC	-3.9	5.5	32.7	58.8	121.0
	Area	3.8	-7.9	1.1	11.8	34.0
Trabecular	BMD	-75.0	-14.8	113.8	278.8	762.4
	BMC	-72.8	-23.6	113.6	301.7	906.6
Cortical/Subcortical	BMD	0.0	15.4	27.8	33.6	45.8
	BMC	2.9	7.1	28.9	47.3	85.9

Values in bold are significantly different from OVX vehicle controls.

Text Figure 15
Bone Densitometry Values by pQCT as Percent Change from Acclimation Period
Right Proximal Tibia - Diaphysis - Mean (SEM)



Text Table 23
Effect of OVX and BA058 Treatment on Proximal Tibia Diaphysis pQCT Parameters
as Percent Change from End of Bone Depletion Period - Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 1 µg/kg/day	BA058 5 µg/kg/day	BA058 25 µg/kg/day
Total slice area	10.4	14.1	20.1	**26.1**	**43.8**
Periosteal Circumference	5.0	6.9	9.8	**12.7**	**21.0**
Cortical BMD	5.3	2.4	**7.3**	**5.4**	**11.4**
Cortical BMC	**14.1**	3.1	**35.5**	**57.0**	**104.9**
Cortical area	8.7	0.7	**27.6**	**50.7**	**89.6**
Endosteal Circumference	7.9	33.7	**-5.4**	**-42.5**	**-91.2**
Cortical Thickness	**2.8**	-13.1	**22.3**	**57.0**	**109.3**

Values in bold are significantly different from OVX vehicle controls

Summary of selected DXA and pQCT data:

Text Table 24
Effect of OVX and BA058 Treatment on Selected Bone Densitometry Parameters
as Percent Change from Acclimation Period

Parameters	Groups	End of Bone Depletion	Week 12-13	Week 25-26	Week 51-52
Lumbar Spine (L1-L4) BMD (DXA)	Sham	5.9	6.5	6.6	-4.4
	OVX	-7.6	-13.8	-14.2	-20.3
	BA058 1 µg/kg/day	-7.6	0.0	3.8	-0.8
	BA058 5 µg/kg/day	-6.7	5.6	15.1	13.9
	BA058 25 µg/kg/day	-6.8	14.8	26.4	26.5
Proximal Femur BMD (DXA)	Sham	7.0	9.8	10.6	6.3
	OVX	-2.3	-4.6	-5.7	-7.3
	BA058 1 µg/kg/day	-1.3	4.7	8.5	7.0
	BA058 5 µg/kg/day	-1.5	9.8	16.8	18.5
	BA058 25 µg/kg/day	-0.5	19.0	29.2	35.6
Proximal Tibia Trabecular BMD (pQCT)	Sham	-8.9	-16.5	-25.3	-51.0
	OVX	-61.7	-65.3	-65.6	-62.3
	BA058 1 µg/kg/day	-61.9	-52.6	-47.8	-46.7
	BA058 5 µg/kg/day	-60.1	-43.2	-20.9	-4.0
	BA058 25 µg/kg/day	-59.7	-13.5	36.9	89.3
Proximal Tibia Diapahysis Cortical Thickness (pQCT)	Sham	7.8	8.3	9.6	8.9
	OVX	3.5	2.4	-1.2	-3.2
	BA058 1 µg/kg/day	4.5	8.7	13.0	14.0
	BA058 5 µg/kg/day	6.0	15.9	31.3	31.5
	BA058 25 µg/kg/day	5.3	28.5	47.7	54.7

Reviewer Comments:

- *The in vivo bone densitometry showed marked increases in DXA bone parameters indicating increases in bone mass (BMD, BMC, and Area) in both cancellous and cortical bone, usually at all doses employed. PQCT data from proximal tibia were consistent with the DXA findings, showing increases in Trabecular BMD and BMC, and in Cortical Thickness.*
- *The tibial diaphyseal Cortical Thickness increase was mainly due to endosteal bone apposition, but also to periosteal bone apposition. In the metaphysis, periosteal apposition also occurred, but (sub)cortical bone was resorbed in the process of trabecularization leading to an enlarged trabecular area.*
- *The data are in accordance with the bone marker data and the anabolic window at all doses.*
- *Sponsor suggested that the bone effect was due to a stimulation of bone formation with very little effect on resorption and stated that bone resorption markers were 'essentially unaffected'. However, the data showed transient increases in resorption markers at all doses at the earliest time point (Wk 11/12). Bone formation markers were also increased at all doses and, like resorption markers, they returned towards control levels at later times. Formation was increased more than resorption, and although the difference between the two was not clearly dose-dependent, the data support the notion of an 'anabolic window' at all doses.*

Densitometry ex vivo

DXA (femur, total) and pQCT (femur, vertebral bodies) of bones to be used for biomechanical testing showed similar results as the in vivo measurements at end of treatment:

Abaloparatide effects:

- Increases in cancellous and cortical region BMD/BMC, and smaller increases in area (femur, DXA)
- Increases in cortical thickness and BMC, due to a decrease in endosteal circumference and slight increase in CSMI (femur, pQCT)
- Increases in BMC and BMD of lumbar vertebral bodies (pQCT)

Biomechanical testing

Testing of femur and lumbar vertebral bodies

Femur Diaphysis (bending test):

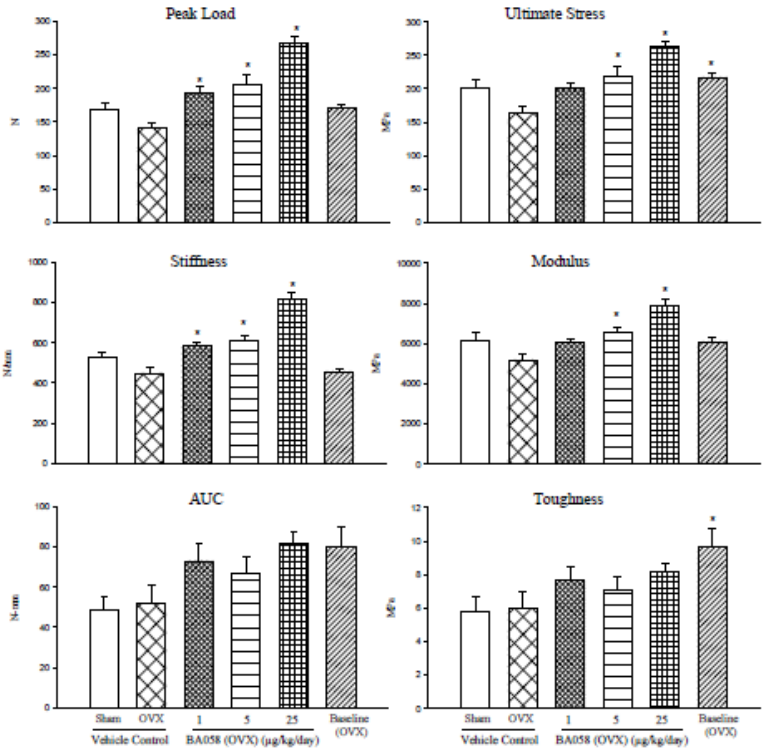
OVX:

- Decreases in peak load, ultimate stress, stiffness, modulus (Text Fig. 19)

Abaloparatide:

- Dose-dependent increases in peak load, ultimate stress, stiffness, modulus (Text Fig. 19), significant at (1), 5 and 25 ug/kg
- Not clearly dose-related increases in AUC (work to failure) and toughness
- All parameters reached beyond-sham levels in (1), 5 and 25 ug/kg groups

Text Figure 19
Biomechanical Parameters – Right Femur – 3-point Bending – Mean (SEM)



Significantly different from OVX Vehicle Control group (group 2) value: *-p≤0.05

Femoral Neck (shear test)

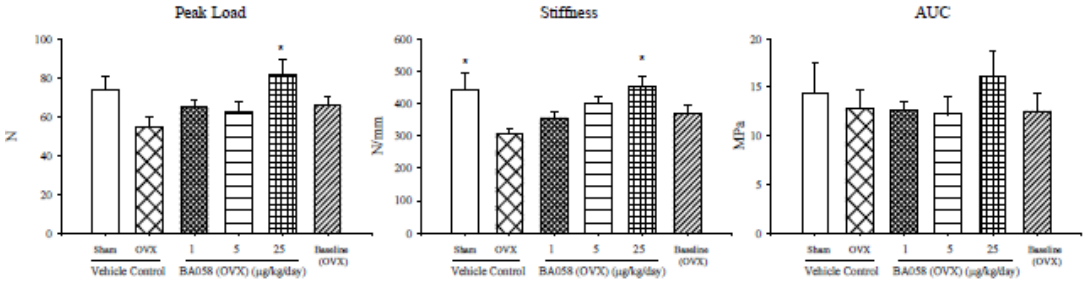
OVX:

- Decreases in peak load, stiffness, AUC (Text Fig. 21)

Abaloparatide:

- Dose-dependent increases in peak load, stiffness, AUC, stat significant only at 25 ug/kg (Text Fig. 21).
- Parameters reached beyond-sham levels in 25 ug/kg group only

Text Figure 21
Biomechanical Parameters – Right Femur – Femoral Neck – Mean (SEM)



Significantly different from OVX Vehicle Control group (group 2) value: *-p≤0.05

Vertebral bodies (compression test)

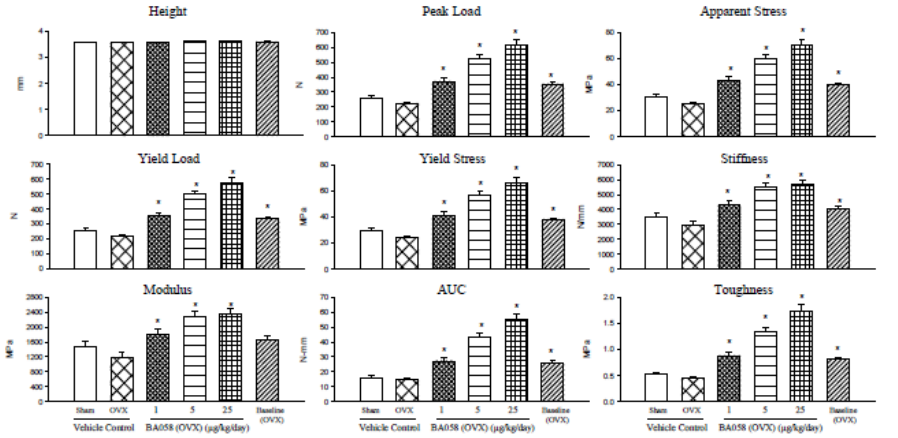
OVX:

- Decreases in peak and yield load, apparent and yield stress, stiffness (Text Fig. 23)

Abaloparatide:

- Dose-dependent increases in peak and yield load, apparent and yield ultimate stress, stiffness, modulus, AUC and toughness (Text Fig. 23). Increases were statistically significant in all dose groups
- All parameters reached beyond-sham levels in all 1, 5 and 25 ug/kg groups

Text Figure 23
Biomechanical Parameters – L4+L5 Vertebral Bodies (Averaged) – Mean (SEM)

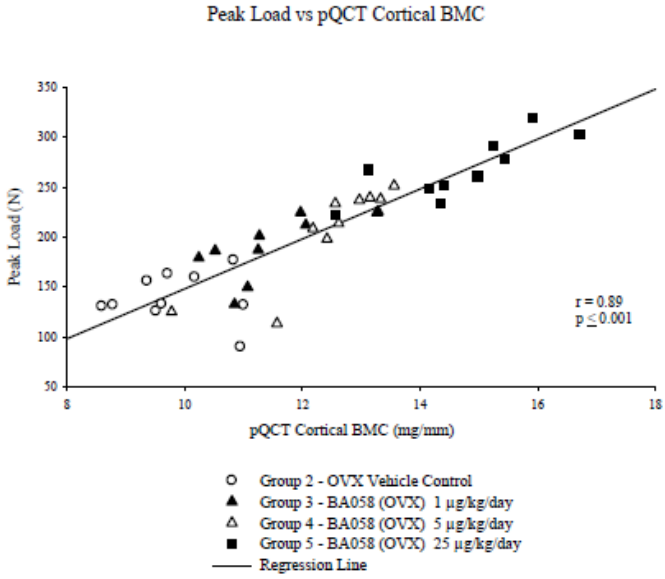


Significantly different from OVX Vehicle Control group (group 2) value: *-p≤0.05

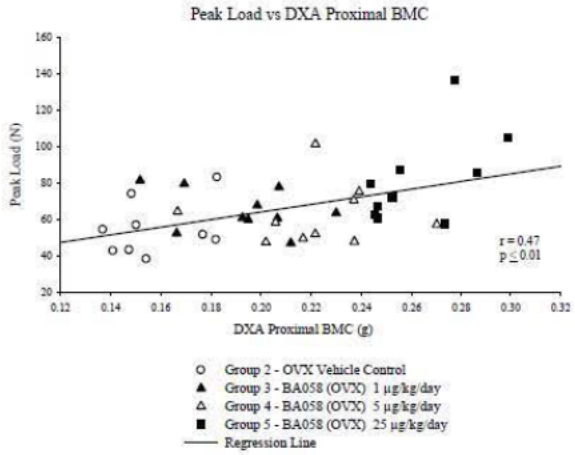
Correlation analysis

- Analysis was carried out between both ex vivo and in vivo densitometry parameters and biomechanical parameters.
- For the ex vivo BMD/BMC measures (Text Figures 20, 22, 24):
  - There was a good correlation between femur cortical BMC and peak load (Text Figure 20), moderately good correlation between proximal femoral BMC and femoral neck Peak Load (Text Figure 22), and very good correlation between vertebral BMC and Peak Load as well as between vertebral BMD and Apparent Strength (Text Figure 24).
  - The correlation coefficients had the expected values for the different types of bone.
  - The positive slopes of the regression lines appeared to be similar for the OVX and Abaloparatide treatment groups, indicating no significant changes in the material or structural quality of bone
- For the in vivo BMD/BMC measures (not shown):
  - Correlations between densitometric parameters (BMD or BMC) and strength (peak load or stiffness) were generally less good for femoral shaft and neck than when based on ex vivo parameters. In some cases they were negative (!)
  - Correlations for vertebral bodies were good and similar to the ones constructed with ex vivo densitometry data

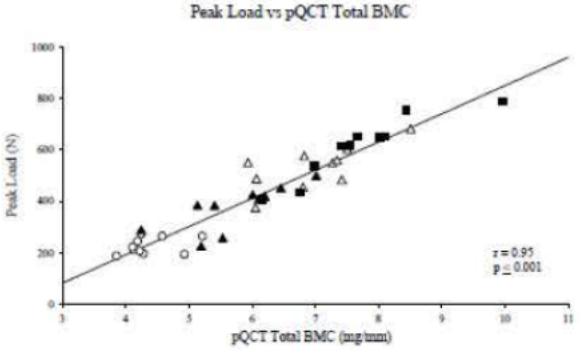
Text Figure 20
Regression Plot of Biomechanics - Femur 3-point Bending vs. Femur pQCT

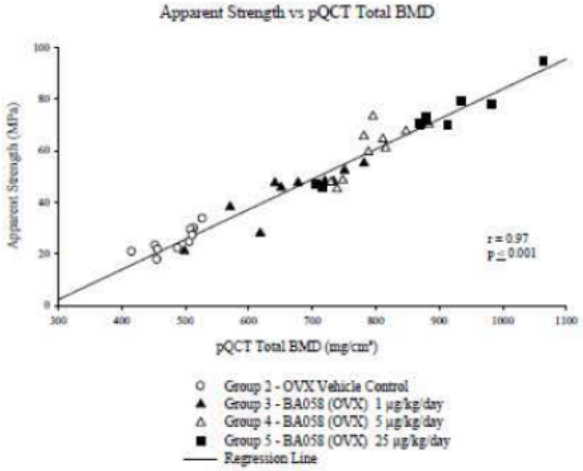


Text Figure 22
Regression Plot of Biomechanics - Femoral Neck Shear vs. Proximal Femur DXA



Text Figure 24
Regression Plot of Biomechanics - L4+L5 Average Vertebral Body Compression vs. pQCT





Histomorphometry (HMM)
Right tibia and L3 Vertebrae

Cancellous bone (L3 Vertebral Body and Proximal Tibia Metaphysis)

OVX:

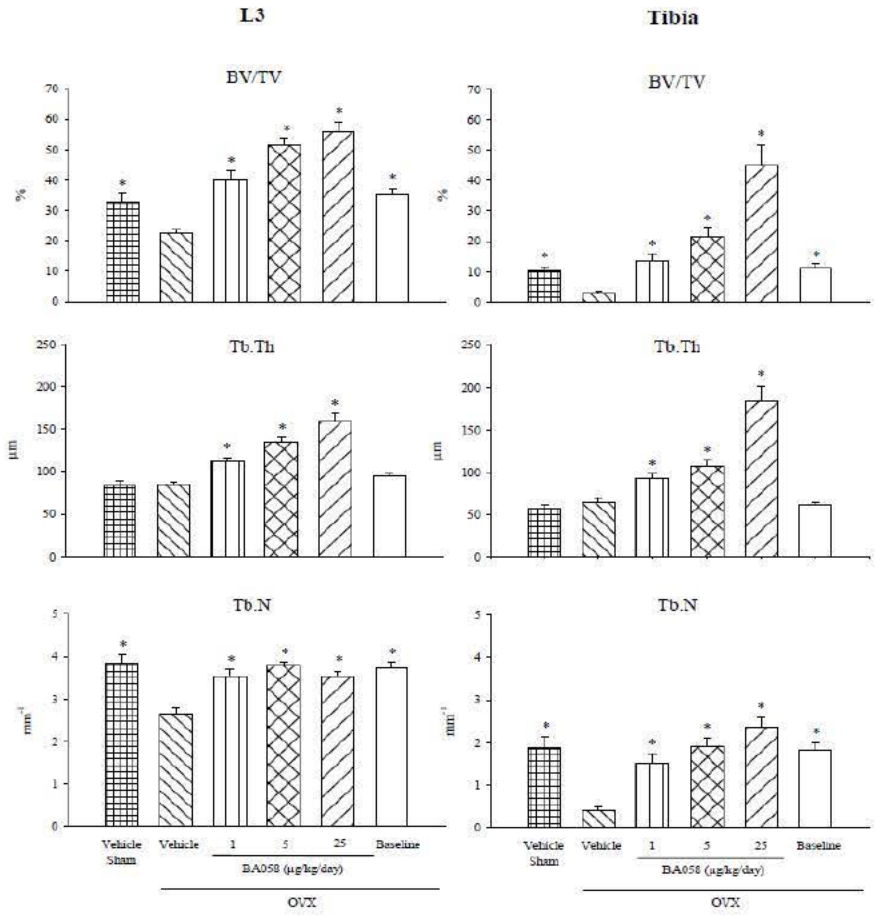
- Decreased BV/TV and Tb.N, but no change in Tb.Th vs sham controls
- Slight decreases in MS/BS and BFR/BS vs sham

Abaloparatide:

- Increased BV/TV, Tb.Th and Tb.N, dose-dependent effect
- Increased OS/BS and ObS/BS significantly at all doses, but no effect on Oc.S/BS.
- Increased MS/BS and BFR/BS (not BFR/BV) at all doses, significantly, Increase in Ac.F (BMU generation) at 25 ug/kg
- Wall thickness not significantly affected in any group in L3 vertebrae
- Data indicate abaloparatide-related increase in osteoblastic bone formation on trabecular surfaces, and possibly trabecular tunneling

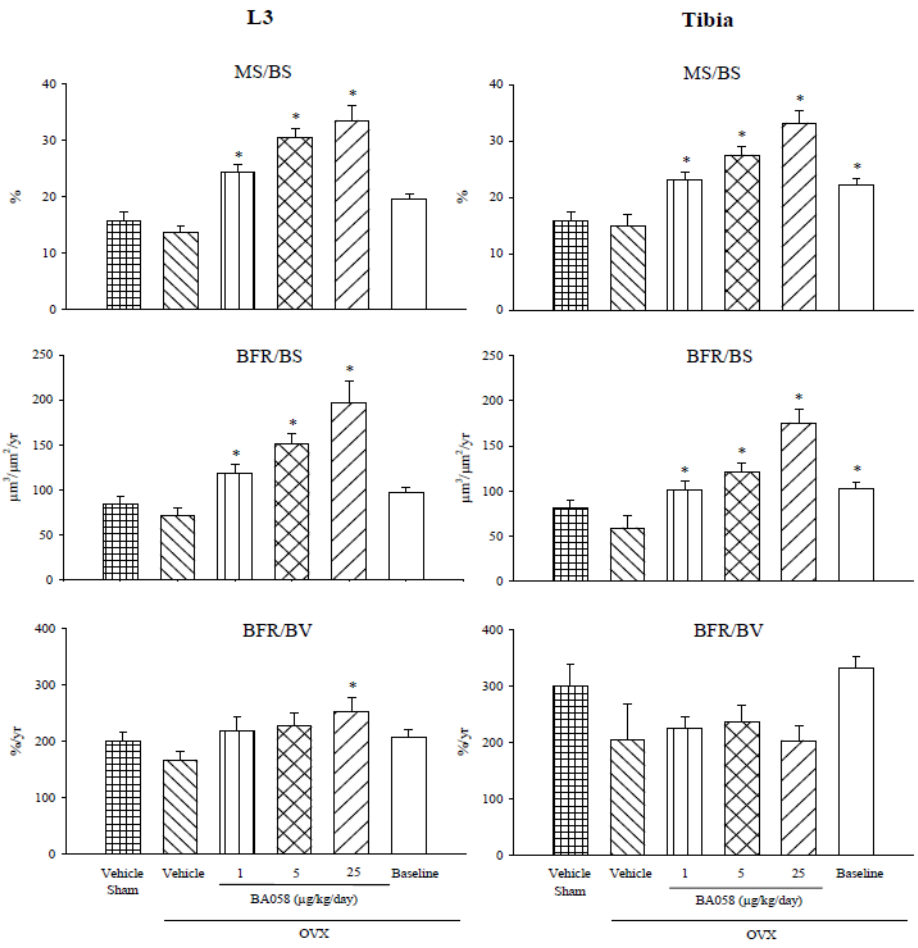
Text Figure 3
Cancellous Bone Histomorphometry - Structural Variables

Mean (SEM)



Text Figure 5
Cancellous Bone Histomorphometry - Dynamic Variables

Mean (SEM)



Significantly different from OVX Vehicle Control group (group 2) value: * -$p \leq 0.05$

Cortical Bone (Tibia diaphysis, tibia/fibula junction, i.e. distal tibial region)

OVX:

- Decrease in cortical width and % cortical area, increased endocortical perimeter, decreased medullary area, no effect on periosteal perimeter
- No clear effects on bone formation rate (BFR) at either bone surface

Abaloparatide

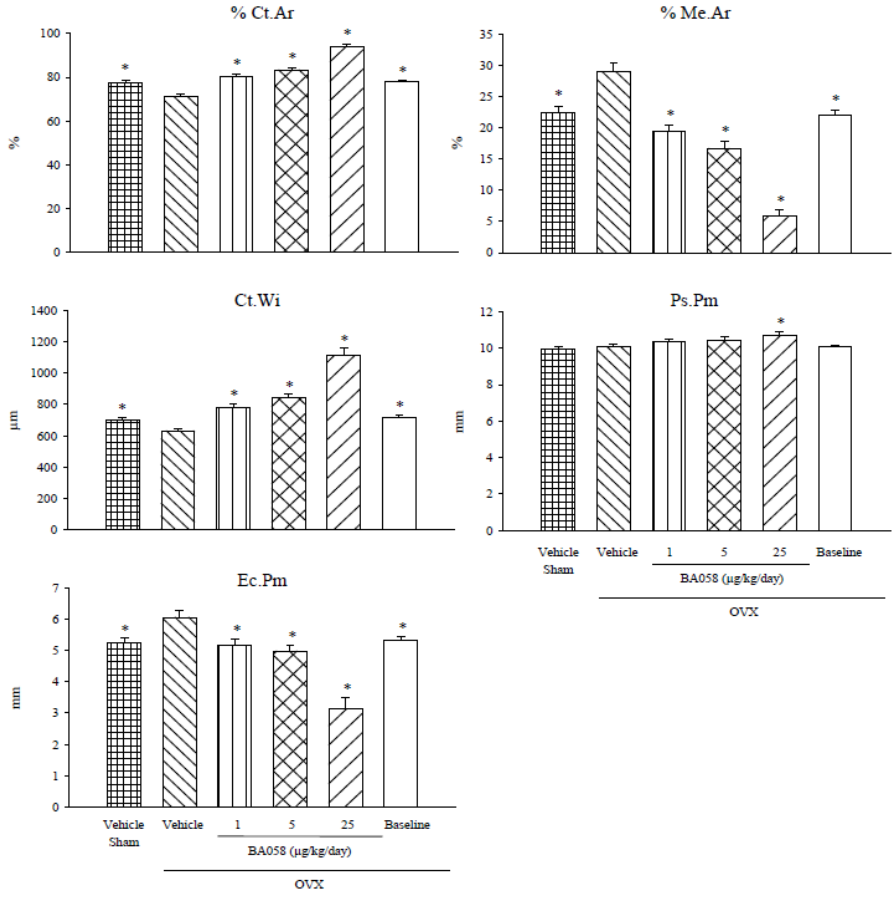
- Cortical width (i.e. thickness) and % cortical area increased, mainly due to decreased endocortical perimeter (and decreased medullary area). Minimal but significant increase in periosteal perimeter in tibia diaphysis at 25 ug/kg. These were all reversals of OVX effects.
- Surprisingly, periosteal BFR/BS was markedly and significantly increased (by 170-250%) at all doses, even though the periosteal perimeter was only very slightly increased. This suggested concomitantly enhanced periosteal resorption (*Note: This*

was also observed in the monkey study). Data on % Periosteal Eroded Perimeter were not shown to confirm or contradict.

- Both Ec.BFR and Ec.E.Pm were non-significantly increased at 25 ug/kg (both by ca. 50%), suggesting that formation and resorption were slightly increased at that surface. (*Note: HMM data are from the 12-month sampling time point, while the major part of the marked endocortical bone apposition took place during the first 26 weeks of treatment).*
- No cortical porosity data

Text Figure 1
Tibial Cortical Bone Histomorphometry - Structural Variables

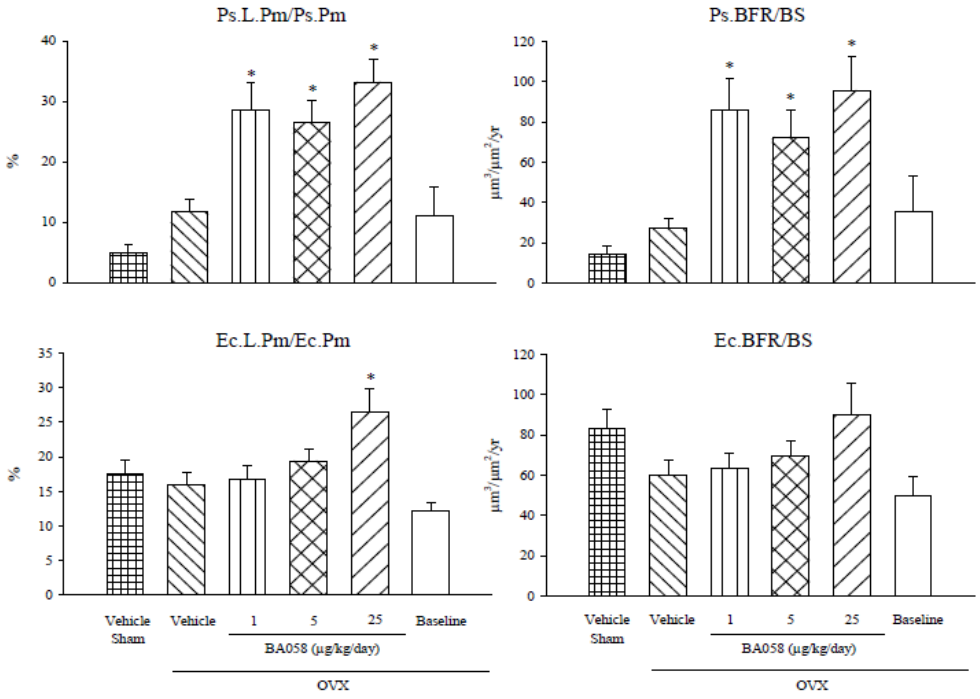
Mean (SEM)



Significantly different from OVX Vehicle Control group (group 2) value: * -$p \leq 0.05$

Text Figure 2
Tibial Cortical Bone Histomorphometry - Dynamic Variables

Mean (SEM)



Significantly different from OVX Vehicle Control group (group 2) value: * -$p \leq 0.05$

Reviewer comment:

The histomorphometry data (vertebrae, tibia) are consistent with the densitometry and biomechanical data and the bone formation marker increases at 12 months. They showed increases in vertebral and tibial cancellous bone volume, and in trabecular thickness and number. At the 12-month time point, in cancellous bone, osteoblast but not osteoclast number was increased. Increases in bone formation rate were seen at the distal tibia diaphyseal endosteal surface but particularly at the periosteal surface. The latter suggests a concomitant increase in periosteal resorption since bone perimeter was only minimally increased in this tibial bone region (near TF junction). By contrast, densitometry showed slight increases in total bone area (ie diameter/perimeter) in femur (total, proximal, mid) (by DXA) and proximal tibial meta- and diaphysis (by pQCT). The pQCT analysis of the proximal tibia also showed slight thickening of the metaphyseal bone cortex due to periosteal bone apposition along with slight endocortical resorption, and marked thickening of the diaphyseal bone cortex primarily due to endosteal bone apposition, similar to what was observed in distal tibia diaphysis using histomorphometry. Hence, the rat study showed region-specific (proximal vs distal) and bone surface-specific (periosteal vs endosteal) effects of abaloparatide.

Toxicokinetics

- AUC was increased more than dose-proportionally
- AUC was increased over time, possibly due to decrease in renal clearance due to Abaloparatide treatment-related renal toxicity
- The TK data explain the relatively large bone effects of Abaloparatide in the 25 ug/kg dose group

Table 2.1

Summary Mean (± SE) BA058 Pharmacokinetic Parameters in Female Sprague-Dawley Rat Plasma Following 1 µg/kg, 5 µg/kg, and 25 µg/kg Subcutaneous Administration on Day 1

Dose (µg/kg)	T_{max} (hr)	C_{max} (pg/mL)	C_{max}/D (pg•kg/mL/µg)	$AUC_{(0-t)}$ (pg•hr/mL)	$AUC_{(0-t)}/D$ (pg•hr/mL/µg/kg)	$T_{1/2}$ (hr)
1	0.25	396 ± 206	396	219 ± 59.9	219	0.209
5	0.25	2510 ± 211	501	1430 ± 167	287	0.287
25	0.25	5620 ± 458	225	4310 ± 420	172	0.389

Table 2.2

Summary Mean (± SE) BA058 Pharmacokinetic Parameters in Female Sprague-Dawley Rat Plasma Following 1 µg/kg, 5 µg/kg, and 25 µg/kg Subcutaneous Administration on Day 176

Dose (µg/kg)	T_{max} (hr)	C_{max} (pg/mL)	C_{max}/D (pg•kg/mL/µg)	$AUC_{(0-t)}$ (pg•hr/mL)	$AUC_{(0-t)}/D$ (pg•hr/mL/µg/kg)	$T_{1/2}$ (hr)	R_{AUC}[a]
1	0.25	828 ± 47.0	828	353 ± 41.4	353	0.214	1.61
5	0.25	3940 ± 887	787	1590 ± 287	319	0.224	1.11
25	0.50	22,800 ± 12,600	910	21,000 ± 6130	839	0.586	4.87

[a] R_{AUC} = Day 176 $AUC_{(0-t)}$/ Day 1 $AUC_{(0-t)}$.

Table 2.3

Summary Mean (± SE) BA058 Pharmacokinetic Parameters in Female Sprague-Dawley Rat Plasma Following 1 µg/kg, 5 µg/kg, and 25 µg/kg Subcutaneous Administration on Day 358

Dose (µg/kg)	T_{max} (hr)	C_{max} (pg/mL)	C_{max}/D (pg•kg/mL/µg)	$AUC_{(0-t)}$ (pg•hr/mL)	$AUC_{(0-t)}/D$ (pg•hr/mL/µg/kg)	$T_{1/2}$ (hr)	R_{AUC}[a]
1	0.25	884 ± 4.50	884	515 ± 79.4	515	0.265	2.36
5	0.25	4110 ± 733	821	2080 ± 267	416	0.289	1.45
25	0.25	13,700 ± 644	547	14,400 ± 2200	575	0.565	3.34

[a] R_{AUC} = Day 358 $AUC_{(0-t)}$/ Day 1 $AUC_{(0-t)}$.

Rat AUC(0-t): Multiples of Human AUC

Dose	Month 6	Month 12	Comments
1 ug kg	0.23x	0.33x	
5 ug kg	1x	1.34x	Human Equivalent Dose (HED)

25 ug kg	13.6x	9.3x	

Human AUC = 1546 pgxh/mL (Study BA058-05-001b)

Anti-therapeutic Antibodies

- Abaloparatide-antibodies were present in Animals No. 3502 and 5505 (Month 6), Animal No. 5516 (Month 12) and Animal No. 5503 (Months 6 and 12).
- Animals No. 3502 and No. 5516 had comparable Abaloparatide plasma concentrations to those of their cohorts.
- Animal No. 5503 (at months 6 and 12) and No. 5505 (at month 6) had much higher BA058 plasma concentrations than those of their cohorts.
- For Animal No. 5503, BA058 plasma concentrations were up to 5-fold higher than the mean concentrations of the other two animals in its cohort. For Animal No. 5505, the BA058 plasma concentration was 56-fold higher at month 6, and 7-fold higher at Month 12. The report did not mention whether total and free BA058 were distinguished in the measurement.
- Upon review of the densitometry and TK data, Sponsor considered these animals exposed to BA058 and included their data in the result interpretation. Reviewer agrees with this approach, based on individual animal data inspection.

Histopathology

These were expected effects of OVX and Abaloparatide treatment.

- OVX caused decreases in uterus and vagina size and weight, atrophy of cervix, uterus and vagina. Since 3 animals in the 1 ug/kg group were estrogen replete, i.e. like sham animals, the 1 ug/kg bone data from this group may have been slighty overestimated. This did not affect the study conclusions.
- Abaloparatide caused hyperostosis (bone thicker and less porous) in frontal/nasal bone, sternebrae and calvarium, and reduced bone marrow space at all doses. Firm bone marrow was seen at 25 ug/kg, and increased spleen and liver hematopoiesis occurred at all doses.

CONCLUSIONS

Bone markers

- Abaloparatide induced a sustained increase in bone formation. Bone resorption was increased to a lesser extent and/or for a shorter time.

Densitometry:

- Abaloparatide markedly increased DXA BMD and BMC at cancellous and/or cortical bone sites (spine, femur, whole body), to above-sham levels at 5 and 25 ug/kg
- DXA and pQCT measurements suggested widening (i.e., area and diameter increase) of femoral bone and proximal tibia in both metaphysis and diaphysis regions
- pQCT analysis showed that abaloparatide increased proximal tibia metaphyseal trabecular BMC and BMD, and slightly increased trabecular Area. Data or data analysis showed proportional increases in Total, Cortical and Trabecular Areas and Diameters. Widening of the bone in the diaphyseal area was accompanied by thickening of the cortex through endosteal apposition. In the metaphysis, cortical bone at the endosteal surface was resorbed (trabecularization). (Sub)cortical vBMD was increased by Abaloparatide.

Bone strength

- Abaloparatide increased strength of femur shaft, neck and vertebral bodies, reflected by increases in peak load, yield load, stress, stiffness, modulus and/or AUC and toughness.
- Abaloparatide maintained 'bone quality', since the compound did not affect the releationship between bone mass and bone strength
- Abaloparatide caused strength to be increased to above-sham levels at all doses (vertebrae), or at 5 and/or 25 ug/kg (femur)

Histomorphometry

- Abaloparatide increased BV/TV in vertebrae and proximal tibial metaphysis due to increases in Tb.Th. and Tb.N. There was also an increase in % osteoblast but not % osteoclast surface. Abaloparatide increased Ac.F at the high dose only.
- In vertebral and tibial metaphysis, abaloparatide increased BFR/BS and related parameters, indicating enhanced trabecular bone apposition
- Abaloparatide caused a slight increase in bone perimeter (i.e. increased bone width) at the distal tibia diaphysis. However, cortical thickness was mainly increased due to a decrease in endosteal perimeter along with a reduction in medullary area.
- BFR/BS was increased at the periosteal more than at the endosteal surface of the distal tibia diaphysis. Probably, increased resorption at the periosteal surface kept total bone width 'in check'. Total bone width had only very slightly increased during the 12-month treatment period.
- Histomorphometry revealed information on osteoblastic formation and osteoclastic resorption activity at different bone surfaces that cannot be obtained from systemic bone biomarker data.

Histopathology

- Data showed uterine atrophy in OVX rats and hyperostosis in Abaloparatide treated.

Systemic Exposure

- AUC at Month 6-12 was equivalent to 0.3, 1.2 and 12 times human exposure at the 80 ug daily dose

General

- The data provided a detailed picture of the effects of abaloparatide at various bone sites.
- The study revealed bone region-specific (proximal vs distal) and surface-specific (periosteal vs endosteal) effects of abaloparatide on osteoblastic bone formation and osteoclastic bone resorption.

A 16-Month Intervention Osteoporosis Study to Determine the Effects of Daily Subcutaneous Injection of BA058 in the Aged Ovariectomized Cynomolgus Monkey
(Study 10RAD030)
(b) (4)
(GLP/QA) (June 12, 2015)
Batch 4AI1

SUMMARY

Abaloparatide (Abl) was given to osteopenic ovariectomized cynomolgus monkeys at SC doses of **0 (vehicle), 0.2, 1, 5 µg/kg/day** for 16 months. The study also included a sham-operated group which received vehicle only. Systemic exposures (AUCs) at these doses were equivalent to **0.1x, 0.13x and 0.7x** the human dose of 80 mcg/day.

Bone turnover marker data indicated that Abl caused an increase in osteoblastic bone formation and was associated with a positive anabolic window. Abl also caused VitD(OH)2 increases in all Abl treatment groups, suggesting enhanced Ca absorption.

Abl caused increases in DXA-BMD, BMC and/or Area in spine, whole body, proximal tibia, and proximal and distal femur. However, in 1/3 distal radius, it decreased BMC due to a decrease in bone area. In tibia and radius metaphyses, pQCT-determined cortical thickness/area was increased due to endosteal bone apposition in the absence of significant changes in total bone thickness. In proximal tibia, trabecular BMD and area were increased, but in distal radius they were unaffected. In proximal tibia diaphysis there was a slight decrease in periosteal circumference, but an increase in cortical thickness was maintained due to endosteal apposition. In distal radius diaphysis, the bone was slightly enlarged (inconsistent with DXA) without a change in cortical thickness, suggesting net periosteal apposition and endosteal resorption. Cortical volumetric BMD was unaffected in long bones. Most of the effects occurred within the first 8-12 treatment months. The densitometry data suggested differential sensitivities of different bone sites and/or regions to Abl treatment. Bone density changes induced by OVX were generally increased by Abl to levels lower than those observed in vehicle-treated Sham animals. Ex vivo DXA data from samples used for mechanical testing were partly inconsistent with in vivo data, particularly for the femur.

Histomorphometry at the end of the 16-month treatment period showed Abl-related increases in bone volume, trabecular thickness and number in vertebrae and femoral neck. Wall thickness was increased in vertebrae. There were no increases in bone formation parameters at these sites. Surprisingly, there were marked increases in bone formation at peri-and endosteal surfaces of the femoral shaft, a site at which bone and cortical width had not been affected by Abl (ex vivo densitometry), suggesting concomitant stimulation of bone resorption. Cortical porosity was slightly but not significantly increased by Abl treatment.

MicroCT showed increased bone volume and increases in trabecular thickness and number in vertebral and distal femoral bone. In distal femur, cortical thickness was not affected. The data again suggested bone-site dependence of the response to Abl.

Biomechanical testing showed increases in several strength parameters in the vertebrae and positive effects on peak load and stiffness of the femoral neck. No significant effects on the strength of the femoral shaft or humeral cortical bone were observed. The correlation between mass and strength was good in vertebrae, reasonable in femur diaphysis, and modest in femoral neck. Restoration by Abl of the OVX-induced impairment of vertebral bone quality, defined as the slope of the mass-strength regression line, was demonstrated.

The dose selection for this bone quality study was less than adequate (doses up to 1x human exposure only) and use of higher doses would have been more informative. However, the data did not warrant a concern about bone quality. By contrast, they suggested improved vertebral quality at 0.13x and 0.7x human exposure. Some lack of data consistency, significance or dose-relatedness may have been due to low Abl exposures.

Overall, the data showed that in OVX monkeys, at doses ranging from approx. 0.1-0.7 times human exposure at 80 µg/day, abaloparatide's anabolic effects consisted of trabecular and cortical bone thickening in the axial and appendicular skeleton due to net increases in bone formation, resulting in increases in bone strength. However, there appeared to be differences in Abl sensitivity and/or regional differences in the stimulation of formation vs. resorption, reflected by lack of effects in cancellous and/or cortical bone at some of the evaluated bone sites.

The data from this animal study provide insight in the mechanisms of the anabolic effect of abaloparatide at the tissue level and may help with the interpretation of the clinical efficacy data. In the pivotal Phase 3 Study 003, spine BMD was increased by 8.7% vs. placebo and the reduction in the incidence of new vertebral fractures was 86%, consistent with monkey data. In the hip, BMD was increased by 3.4%. Nonvertebral fracture incidence was decreased by 45%. Hip fracture reduction appeared substantial but could not be accurately quantified due to small event incidence. In monkeys, femoral neck BMD (ex vivo) and femoral neck strength were not significantly affected. At the 1/3 distal radius in humans, there was a small decrease in BMC and BMD, a bone site that was similarly affected in the monkey. The nonclinical data suggest that the clinical, vertebral and non-vertebral (i.e. wrist, arm, shoulder, hand, clavicle, rib, pelvis, hip, leg, knee, ankle/foot) fracture risk reductions may be mostlyy due to cancellous bone effects.

METHODS

Cynomolgus monkeys were received from Mauritius at ages 9-18 years (weight 3-7kg at end of baseline assessment). Monkeys were acclimatized for up to 8 weeks and underwent a 2-month baseline period. Subsequently, they were sham-operated or ovariectomized (OVX-ed) and Abl (BA058) treatment was started after a 9-month bone depletion period. OVX groups were treated with subcutaneous abaloparatide (Abl) at doses of **0, 0.2, 1, 5 ug/kg/day** (0.02 mL/kg) for 16 months. Sham-operated animals were dosed with vehicle only.

Text Table 1
Experimental Design

Group Number	Dose Level (µg/kg/day)	Dose Concentration (µg/mL)	Number of Females
1/ Sham Vehicle Control	0	0	16
2/ OVX Vehicle Control	0	0	17
3/ BA058 (OVX)	0.2	10	16
4/ BA058 (OVX)	1	50	16
5/ BA058 (OVX)	5	250	16

In-life observations/measurements in main study animals:

- Mortality (2x/day)
- Clinical observations (menses)
- Detailed clinical observations (weekly)
- Body weight (weekly) and food consumption (weekly, then monthly)
- Clinical pathology (hematology, coagulation, clinical chemistry, urine volume and SG) (baseline, end of bone depletion, Wk 51/52, end of Tx)
- Biochemical markers of bone turnover CTx (serum), NTx (urine), BAP and PINP (serum) (during baseline, at end of bone depletion, Wks 14/15, 32/22, 50/1, an 67/68); urine samples taken during 2hrs pre-dosing
- Hormones (estradiol, PTH, VitDOH2)
- Radiography in vivo
- Bone densitometry in vivo (DXA or pQCT)
- PK (RIA) (Day 1, Wk 39, and end of treatment): samples taken at 6 time points up to 3h post-dosing
- ADAs (RIA) (once during bone depletion, and pre-dosing at Wks 39 and during last week of Tx)
- Labeling for histomorphometry (15 and 5 days pre-sacrifice, calcein)

Densitometry (in vivo)

- DXA of whole body, lumbar (L1-L4) and thoracic (T9-T12) spine, proximal femur, distal femur, proximal tibia diaphysis, right distal radius (2x during acclimation/baseline, at end of bone depletion, and Wks 16/17, 33/34, 51,52, 68/69 of treatment); Parameters: Area, BMD, BMC (N=16-17/grp)
- PQCT of proximal tibia (1x during baseline, end of bone depletion, and Wks 16/17, 33/34, 51,52, 68/69 of treatment); Parameters:
  - Metaphysis (distal radius and proximal tibia): Area (total, trabecular, cortical/subcortical), BMC, BMD
  - Diaphysis: Area (Total, cortical), Ct.Th., and periosteal and endosteal circumference; BMC, BMD)

Terminal procedures

- Necropsy, including organ weights (uterus), tissue collection, histology and histopathology (bone, lesions, heart, kidney, lung, ovary, uterus)

Histology (undecalcified) and histomorphometry (static and dynamic parameters)

- Vertebrae (L2), cancellous bone

- Femur (neck, 2 levels), cancellous bone
- Femur (midshaft, 2 sections): cortical bone

Densitometry (ex vivo)

- DXA: Excised femur, lumbar vertebrae (L3 and L4) and vertebral cores (from L5 and L6) for each specimen destined for biomechanical strength testing
- Peripheral QCT: Femur, L3 and L4 vertebrae, and L5 and L6 vertebral cores, all specimens destined for biomechanical testing

Micro CT scanning

- Micro-CT (ex vivo) of T12 body, L6 core (pre-compression).
- Micro-CT of distal femur and humeral cortical beam, of fragments obtained after mechanical bending tests
- Parameters: vBMD mgHA/cm3, BMC mg HA, Tissue BMD mg HA/cm3, BV/TV (%), Ct.Th (mm), Tb parameters, SMI (structure model index, reflecting rod vs plate structure), DA (degree of anisotropy), porosity

Biomechanical testing

- Vertebral body (L3, L4) and core (L5, L6): compression
- Femur (right): 3-point bending test
- Femoral neck (right) shear test
- Humerus (cortical beams) 3-point bending test
- Ex vivo DXA (femur, lumbar vertebra L3 L4, vertebral cores L5 L6) (Area, BMC, BMD) and/or pQCT (Area, BMC, BMD, CtTh, endo and periosteal circumference, CSMI) (femur, vertebrae L3 L4, vertebral cores L5 L6) of to-be-tested bones at end of treatment
- Correlation analysis between densitometry and ex-vivo or in-vivo densitometry parameters

Text Table 15
Biomechanical Tests

Bone Specimen	Test Type	Test Rate	Results Reported *
Right femur (shaft) (left for Animal Nos. 261, 360 and 568)	3-point bending pQCT (at the breaking point) DXA	1 mm/sec	Peak load, Ultimate Stress, Stiffness, Modulus, Work to Failure (area under the curve - AUC), Toughness, Displacement to yield, post-yield displacement and post-yield toughness Total area, BMC and BMD Moment of Inertia (CSMI), Cortical BMC, BMD, Area and Thickness, Periosteal and Endosteal Circumference Area, BMC, BMD
Right proximal femur (left for Animal Nos. 261, 360 and 568)	Femoral neck shear DXA	1 mm/sec	Peak load, Stiffness, Work to Failure (area under the curve - AUC), Maximum Displacement Area, BMC, BMD
L3, L4 vertebrae	Compression Measurement by caliper DXA pQCT	20 mm/min	Peak Load, Apparent Strength , Yield Load (off-set: 0.5%), Yield Stress, Stiffness, Modulus, Work to Failure(area under the curve - AUC), Toughness, Displacement to yield, post-yield displacement and post-yield toughness Height Area, BMC, BMD Total Area, BMC, BMD and trabecular BMC, BMD
L5, L6 vertebral cores (cranial, caudal)	Trabecular Core Compression Measurement by caliper DXA pQCT	20 mm/min	2 % off-set Load, Apparent Strength , Yield Load (off set 0.5%), Yield Stress, Stiffness, Modulus, Work to Failure (at 2 % offset load) (area under the curve - AUC), Toughness Height Area, BMC, BMD Trabecular Area, BMC, BMD
Right humerus	3-point bending of cortical beam Measurement by caliper	20 mm/min	Peak load, Ultimate Stress, Stiffness, Modulus, Work to Failure (area under the curve - AUC), Toughness, Displacement to yield, post-yield displacement and post-yield toughness Width, thickness

* Some individual result or parameter were excluded with the appropriate justification at the discretion of the responsible PI/IS if the curve appeared to be abnormal.

RESULTS

Toxicokinetics

- AUC was similar at 0.2 and 1 ug/kg, but increased with dose at 5 ug/kg
- AUC was increased over time in the 5 ug/kg group

Text Table 24
Mean (SD) Pharmacokinetic Parameters Following SC Administration of BA058 at 0.2, 1 and 5 µg/kg to OVX Cynomolgus Monkeys

Dose (µg/kg)	Days	T_{max}[a] (hr)	C_{max} (pg/mL)	AUC_{0-t} (pg*hr/mL)	$T_{1/2}$ (hr)
0.2	1	0.500 (0.250 – 0.500)	98.6 (26.6)	80.3 (39.0)	0.314 (ID)
	267	0.500 (0.250 – 0.500)	130 (126)	135 (239)	NC
	483	0.500 (0.250 – 1.00)	155 (205)	220 (563)	0.376 (ID)
1	1	0.250 (0.250 – 0.300)	422 (162)	261 (99.8)	0.374 (0.0602)
	267	0.250 (0.250 – 0.500)	232 (113)	183 (125)	0.475 (0.258)
	483	0.250 (0.250 – 0.250)	207 (79.2)	150 (101)	0.343 (0.0515)
5	1	0.250 (0.250 – 0.300)	1390 (668)	841 (551)	0.354 (0.0794)
	267	0.250 (0.250 – 0.317)	1020 (1330)	1360 (2900)	0.531 (0.378)
	483	0.250 (0.250 – 0.500)	851 (771)	946 (1620)	0.415 (0.259)

[a] Median (Min – Max), ID: Insufficient Data, NC: Not calculated

AUC multiples (avg of 3 time points):
0.2 ug/kg 0.1x
1 ug/kg 0.13x
5 ug/kg 0.7x (Human Equivalent Dose)
(Human AUC = 1546 pgxh/mL, Study BA058-05-001B)

Anti-therapeutic Antibodies
No samples positive for anti-Abl

Mortality and Clinical Observations
Three animal died (Tx unrelated);
No remarkable clinical observations.

Body Weight and Food Consumption
No effects

Hematology
Reticulocyte decrease in OVX treated
Abl: Small decrease in RBC at 5 ug/kg, from Month 12-16.

Coagulation
No effects of OVX or Abl treatment.

Clinical Chemistry

- Serum ALP
  Moderate/marked increases in OVX groups due to increased bone turnover.
  Small, non-significant dose-related increase vs OVX in Abl-treated females, at Wk 51/52 and end-of-Tx.

- Serum Ca and P
  Slight increases in OVX animals. No effects of Abl treatment. Samples probably taken pre-dosing (ie at Cmin) so no changes in Ca expected

- Serum Albumin/Globulin
  Increase in Albumin, decrease in Globulin in OVX groups. No effects of Abl.

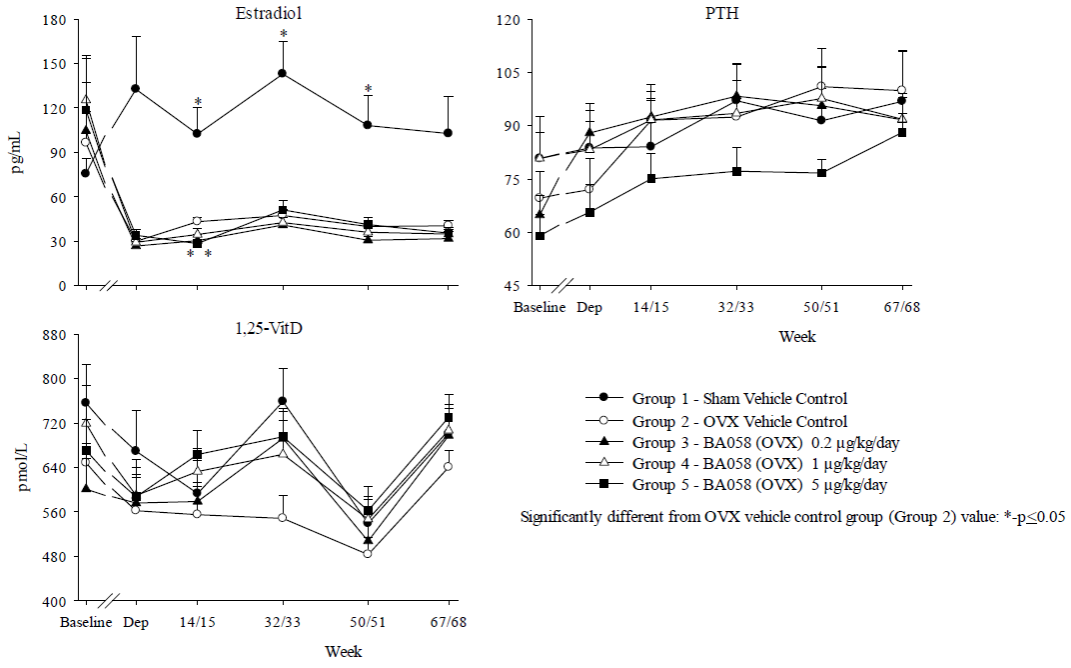
Urinalysis

No effects of OVX or Abl

Hormones

Decrease in estradiol in OVX animals. Text stated that PTH and VitD were not affected. However Text Fig 3 showed decrease in PTH at 5 ug/kg and increases in 1,25 VitD at all doses, which could be expected with PTHrP analog although conflicting results have been published on this in the literature.

Text Figure 3
Hormones - Mean (SEM)



Radiography
Unremarkable

Gross pathology
No findings

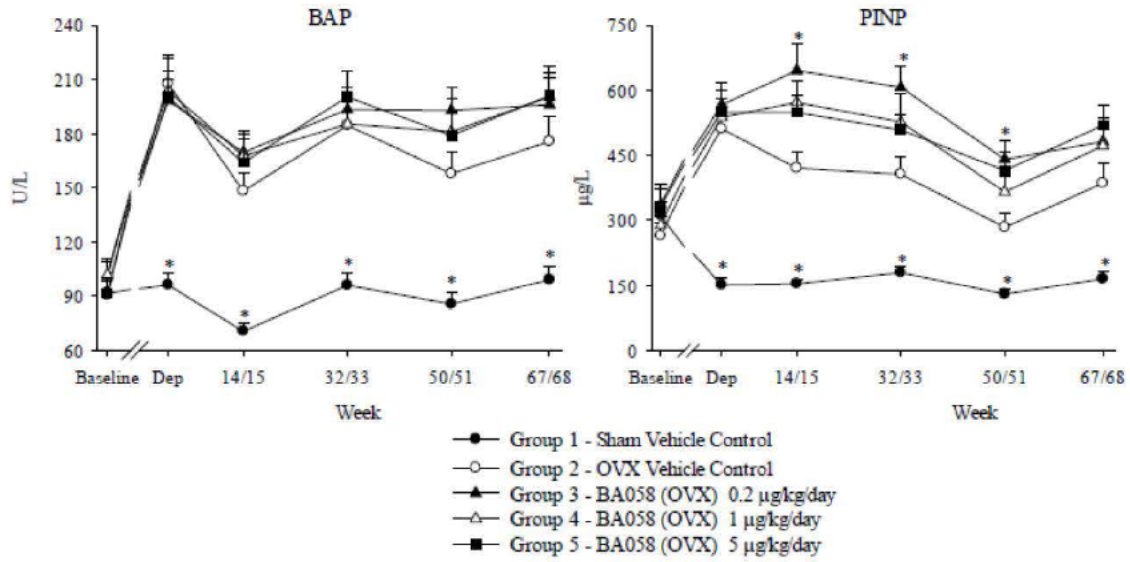
Organ weights
Reduced uterus weight (2.6x) in OVX vs Sham, as expected

Histopathology
- Decrease in cancellous/cortical bone in OVX animals (vertebrae, sternum), at least partly reversed by Abl treatment

Bone markers
OVX:
- BAP, PINP, NTx and Ctx increased vs OVX (2-4x) (all time points)

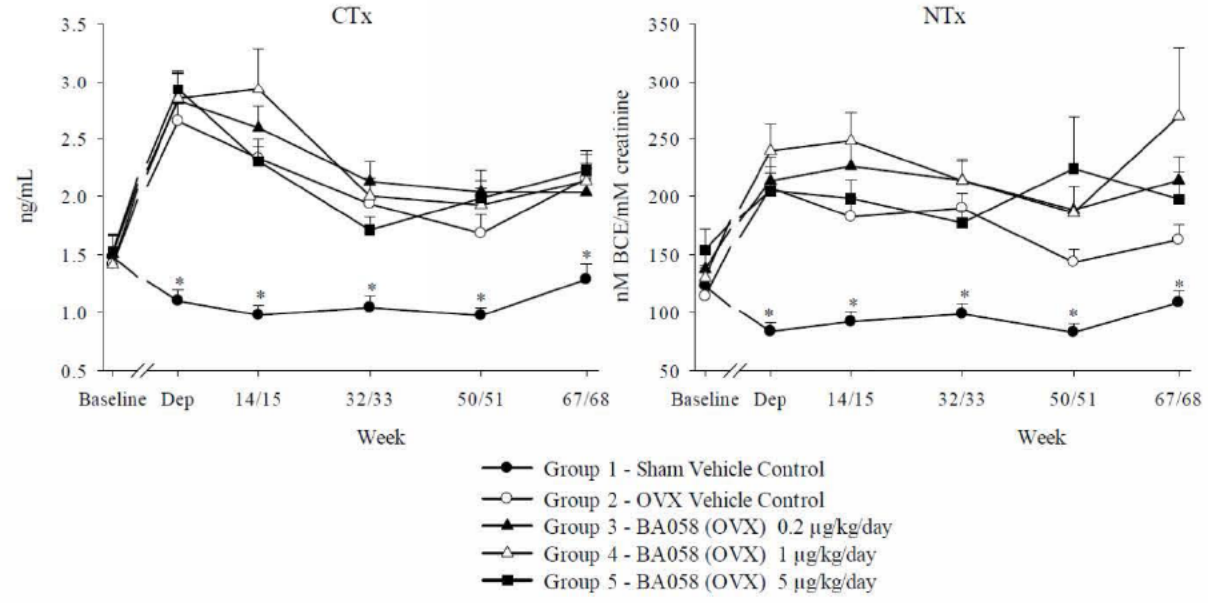
Abl Tx:
- PINP (bone formation marker) increased by Abl at all the time points, but NOT in dose-dependent manner (Text Fig.1)
- BAP (bone formation marker) slightly increased by Abl, not dose-dependently (Text Fig.1, 2).
- CTx (bone resorption marker) not clearly affected
- NTx (bone resorption marker) increased by all doses of Abl at later time points
- When added together (for the 3 time points), PINP was were clearly increased at all 3 doses; BAP was slightly increased; CTx and NTx slightly increased at 0.2 and 1 ug/kg (Text Table 17)

Text Figure 1
Bone Formation Markers - Mean (SEM)



Significantly different from OVX vehicle control group (Group 2) value: *-p≤0.05

Text Figure 2
Bone Resorption Markers - Mean (SEM)



Significantly different from OVX vehicle control group (Group 2) value: *-p≤0.05

Text Table 17
Effect of OVX and BA058 Treatment on Biochemical Markers of Bone Turnover
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
BAP U/L	346	675	752	735	752
PINP µg/L	626	1508	2177	1938	1980
CTx ng/mL	4.30	8.23	8.79	8.99	8.07
NTx nM BCE/mM creatinine	379	664	842	898	803

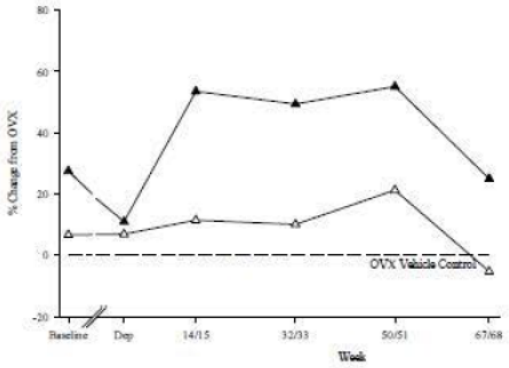
Values in bold are significantly different from OVX vehicle controls

Sponsor depicted the "Bone Marker Anabolic Window" by plotting PINP and CTx (as % of change from OVX) over time (Text Fig. 4). Data were not discussed in the study report.
Data showed:

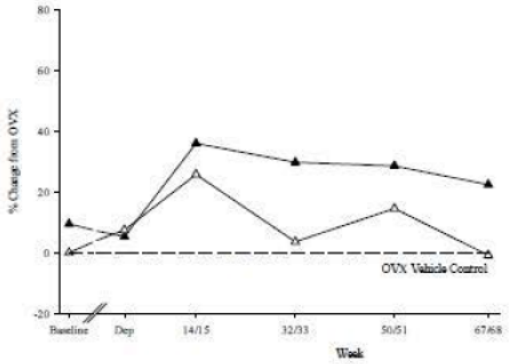
- PINP increased at all doses and time points; however, effect was NOT dose dependent
- CTx slightly increased at earlier time points and mostly at 0.2 and 1 ug/kg (Tx-relatedness questionable)
- NTx increased in 1 and 5 ug/kg groups at later time points (≥Wk 50) (Table 7)

Text Figure 4
Bone Marker Anabolic Window - PINP vs. CTx - Percent change from OVX Control

Group 3 - BA058 (OVX) 0.2 µg/kg/day



Group 4 - BA058 (OVX) 1 µg/kg/day



Group 5 - BA058 (OVX) 5 µg/kg/day

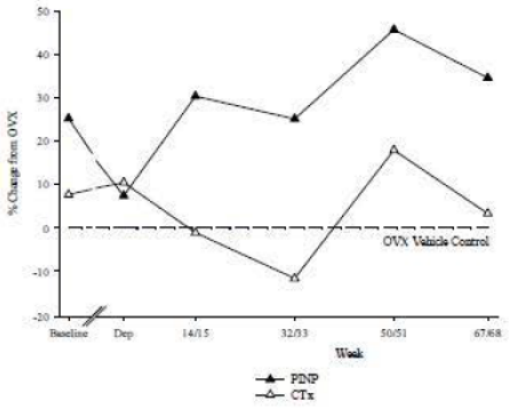


Table 7 Summary of Cross-Linked N-Telopeptides of Type I Collagen Values (nM BCE/mM creatinine)

Females

Group 1 - Sham Vehicle Control
Group 2 - OVX Vehicle Control
Group 3 - BA058 (OVX) 0.2 µg/kg/day

Group	Summary Information	Baseline Period	End of Bone Depletion Period ε	Week 14/15 ε	Week 32/33 ε	Week 50/51 ε	Week 67/68 ε
1	Mean	122.77	83.65 c	92.09 c	98.71 c	82.86 c	108.65 b
	SD	62.12	28.80	34.11	33.60	30.66	37.80
	N	15	15	15	15	15	14
2	Mean	113.93	207.28	182.48	189.72	143.40	162.63
	SD	45.09	75.36	68.14	53.73	45.94	51.83
	N	17	17	17	17	17	16
3	Mean	137.49	213.39	226.05	213.86	188.45	213.89
	SD	53.42	77.74	78.00	63.78	78.43	79.02
	N	15	15	15	15	15	15

Rank transformed: $\mathcal{R}$
Heteroscedastic: ε
Significant linear dose effect: λ^A - P ≤ 0.05 λ^B - P ≤ 0.01 λ^C - P ≤ 0.001
Significant quadratic dose effect: φ^A - P ≤ 0.05 φ^B - P ≤ 0.01 φ^C - P ≤ 0.001
Significantly different from OVX vehicle control group (Group 2) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Dunnett)

Group 4 - BA058 (OVX) 1 µg/kg/day
Group 5 - BA058 (OVX) 5 µg/kg/day

Group	Summary Information	Baseline Period	End of Bone Depletion Period ε	Week 14/15 ε	Week 32/33 ε	Week 50/51 ε	Week 67/68 ε
4	Mean	130.17	239.43	247.94	213.62	185.95	269.05
	SD	41.65	90.83	96.02	72.96	89.23	231.90
	N	16	16	16	16	16	15
5	Mean	154.09	205.00	198.27	177.35	223.91	197.79
	SD	72.78	60.32	65.10	40.44	172.45	92.38
	N	16	16	16	16	15	16

Rank transformed: $\mathcal{R}$
Heteroscedastic: ε
Significant linear dose effect: λ^A - P ≤ 0.05 λ^B - P ≤ 0.01 λ^C - P ≤ 0.001
Significant quadratic dose effect: φ^A - P ≤ 0.05 φ^B - P ≤ 0.01 φ^C - P ≤ 0.001
Significantly different from OVX vehicle control group (Group 2) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Dunnett)

Reviewer Comment:
Reviewer concludes that there is an anabolic window at all doses due to increases in bone formation and smaller increases in bone resorption. However, the marker values fluctuated over time and did not follow a specific pattern and were not clearly dose-related. The size of the anabolic window was also not dose-dependent, with effects apparently larger at 0.2 than at 1 ug/kg. At end of study, formation appeared still increased. The marker data are <u>not consistent</u> with the time profile of the BMD and BMC effects, which occurred mainly in the first 33 weeks of dosing.

Hormones

VitD(OH2) appeared decreased on OVX animals, and increased in all Abl-treated groups

Densitometry (in vivo)

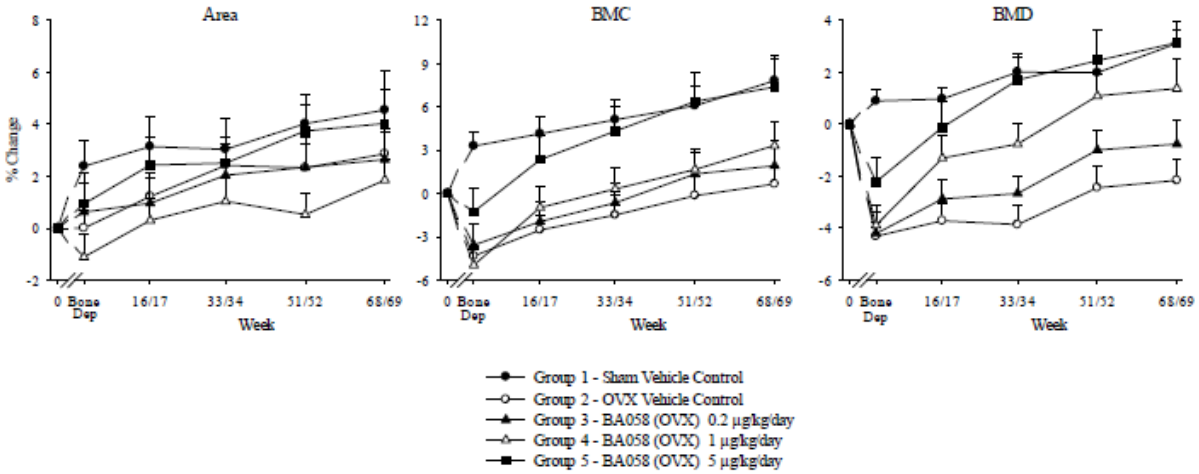
- *Data represented as % change from acclimation (pre-bone depletion) or end-of-bone-depletion period*
- *Data in Text Tables show % changes in OVX vs sham control, and changes in BA058 treated vs OVX as "total across time", ie, results at 4 evaluation time points were added together.*

DXA whole body, spine, radius, femur, tibia (in vivo)

DXA Whole Body (Text Fig 5):

- OVX:
  - decrease in BMD, but not Area
- Abl treatment:
  - dose-related increases in BMD and BMC (similar at 1 and 5 ug/kg), but not any clear increases in Area
- BMD and BMC effects were largest at beginning of treatment but continued throughout 51/52 wks

Text Figure 5
Bone Densitometry Values by DXA as Percent Change from Baseline Period
Whole Body - Mean (SEM)

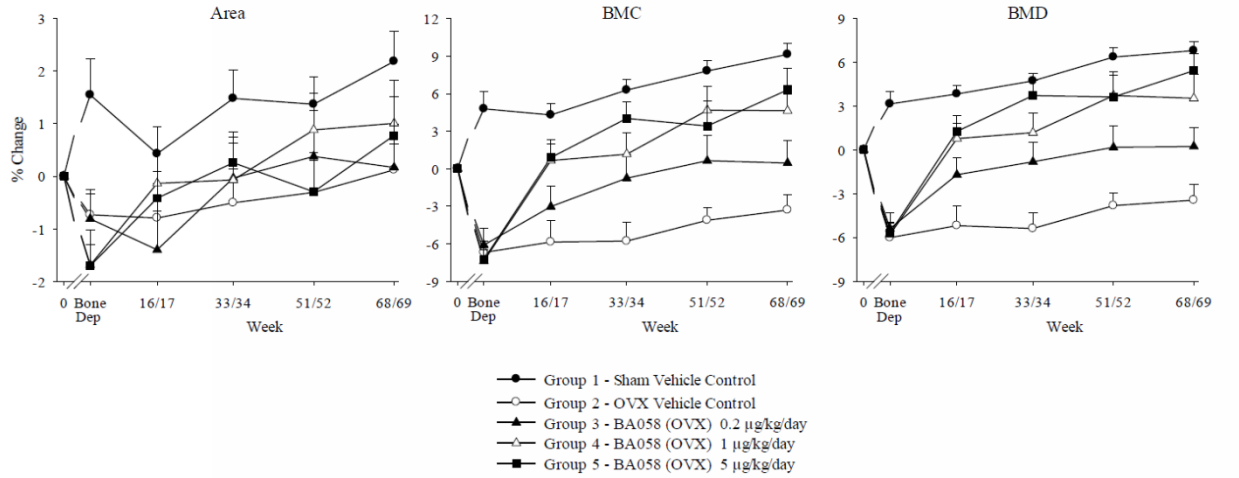


DXA Lumbar Spine L1-L4 (Text Fig 7 and Text Table 19):

- OVX:
  - Decreases in BMC and BMD during 9-month bone depletion period
- Abl-treatment:
  - Dose-dependent increases in BMC and BMD (and Area), similar at 1 and 5 ug/kg
  - Dose-dependence was not consistent with dose-dependence of AUC exposure (0.1x, 0.13x, 0.7x human exposure)
  - Increases in BMD occurred mainly in first 33/34 wks (8 months) of Tx
  - BMD parameters in Abl-treated did not reach sham levels

Reviewer Comment:
The increase in vertebral BMD was 39% at the 5 ug/kg dose, a dose equivalent to the human 80 ug dose based on AUC (Table 19). This % was "total across time", and translates to an approximately 10% change in BMD vs OVX control at 16 months (see Fig 7). This was very similar to the spine BMD change vs pbo at end of Tx in the clinical 18-month Phase 3 Study 003 (ca. 9%) suggesting similar bone efficacy of abaloparatide in monkeys and humans.

Text Figure 7
Bone Densitometry Values by DXA as Percent Change from Baseline Period
Lumbar Spine - Mean (SEM)



Text Table 19
Effect of OVX and BA058 Treatment on L1-L4 DXA
as Percent Change from End of Bone Depletion Period
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
BMD	9.0	6.7	**20.6**	**34.4**	**39.1**
BMC	8.6	8.3	23.3	**44.1**	**47.2**
Area	-0.5	1.5	2.4	8.6	7.3

Values in bold are significantly different from OVX vehicle controls.

Tables show % changes in OVX vs sham control, and changes in BA058 treated vs OVX as "total across time, ie, results at 4 time points were added together! This greatly overestimates the change at end of study

DXA Distal Radius/Proximal Femur/Distal Femur (Text Figs 11, 13, and Text Tables 21, 22, 23):

- OVX:
  - Decreases in BMC, BMD and Area during 9-month bone depletion period at all 3 bone sites
- Abl treatment:

  Distal Radius
  - No clear effects on Area, BMD or BMC in total distal radius (Text Fig 11)
  - In 1/3 distal radius, Abl appeared to DECREASE Area, BMD and BMC (Text Fig 11, Text Table 21)
  - In ultradistal radius, Abl did not dose-dependently affect Area, BMD or BMC (Text Table 21)

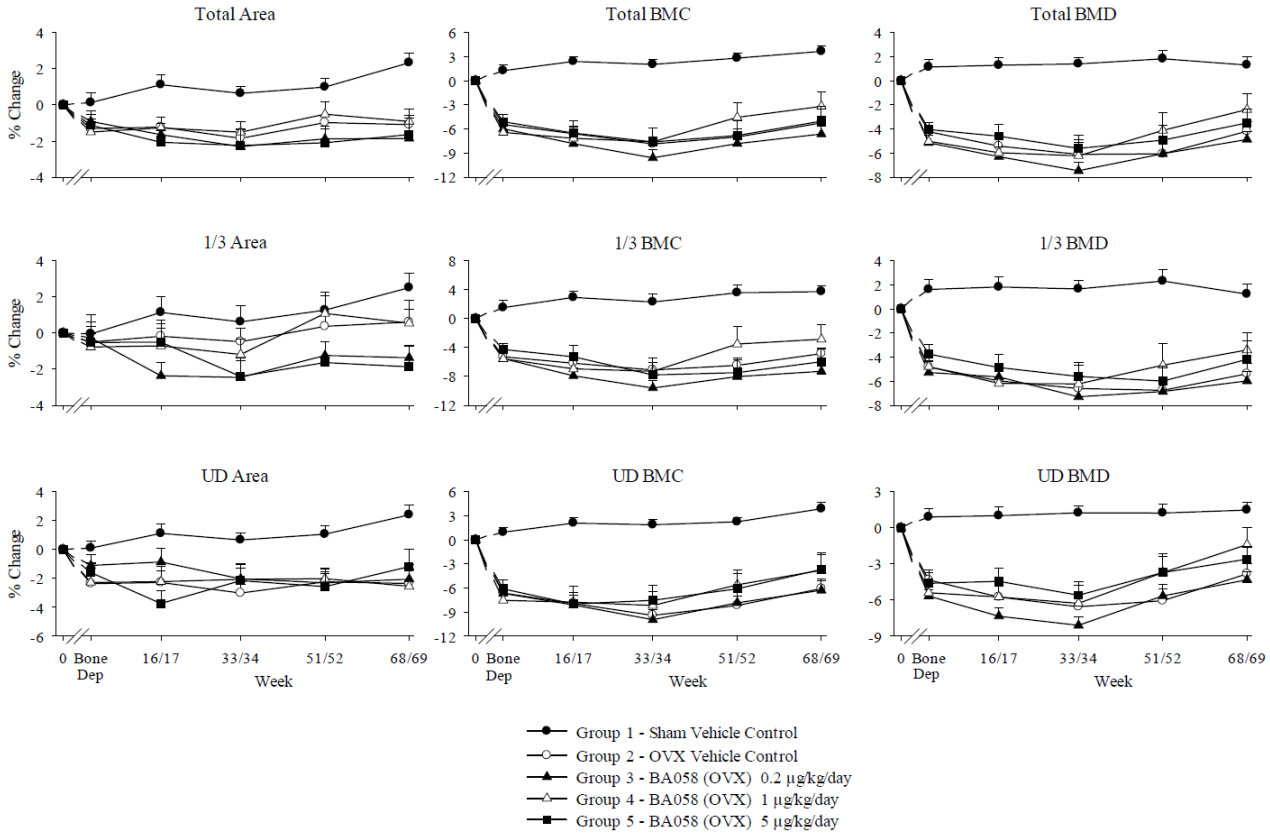
  Prox/Dist Femur
  - Dose-dependent increases in BMC and BMD in proximal femur (global, trochanter, and femoral neck) and distal femur, with parameters reaching levels at

or above-sham in proximal (Text Fig 13) but not distal femur (not shown). No clear effects on Area.

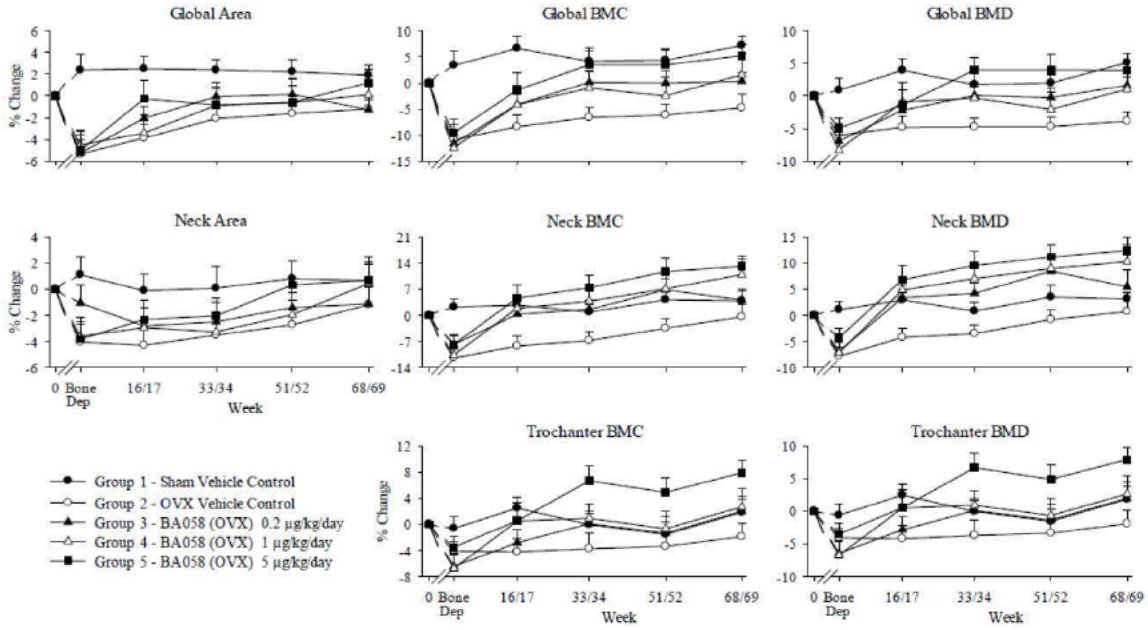
- Effects occurred for the most part in initial 33 weeks following start of treatment

Reviewer Comment:
In Phase 3 Study 003, in 1/3 radius, Abl caused a decrease in Area, BMC and BMD thus mimicking the 1/3 distal radius findings in the OVXed monkeys.

Text Figure 11
Bone Densitometry Values by DXA as Percent Change from Baseline Period
Right Distal Radius - Mean (SEM)



Text Figure 13
Bone Densitometry Values by DXA as Percent Change from Baseline Period
Proximal Femur - Mean (SEM)



Text Table 21
Effect of OVX and BA058 Treatment on Distal Radius DXA
as Percent Change from End of Bone Depletion Period
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
1/3 BMD	0.74	-5.68	-4.60	-1.51	-5.69
1/3 BMC	6.62	-3.32	-10.6	1.58	-9.98
1/3 area	6.18	2.60	-6.19	2.91	-4.19
UD BMD	1.4	-4.9	-2.7	4.9	2.5
UD BMC	**6.3**	-5.3	-5.6	5.6	-0.7
UD Area	4.9	-0.4	-2.8	0.8	-3.3

Values in bold are significantly different from OVX vehicle controls.

Text Table 22
Effect of OVX and BA058 Treatment on Proximal Femur DXA
as Percent Change from End of Bone Depletion Period
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
Global BMD	10.0	7.8	29.9	34.4	32.4
Global BMC	10.2	23.1	51.6	50.1	56.1
Global Area	0.1	14.7	19.4	14.6	21.8
Femoral neck BMD	6.8	26.5	55.1	65.6	60.1

Values in bold are significantly different from OVX vehicle controls.

Reviewer comment (DXA femur):
Results for global BMD suggested increases vs OVX control at 16 months of approx. 7-8% (total hip) and 9-10% (femoral neck). This was larger than the BMD changes in the spine and also larger than the change vs pbo in Phase 3 study 003 (3.5% and 3.4%). BMD and BMC of distal radius were slightly decreased in Study 003, as seen in this monkey study.

Text Table 23
Effect of OVX and BA058 Treatment on Distal Femur DXA
as Percent Change from End of Bone Depletion Period
Total Across Time

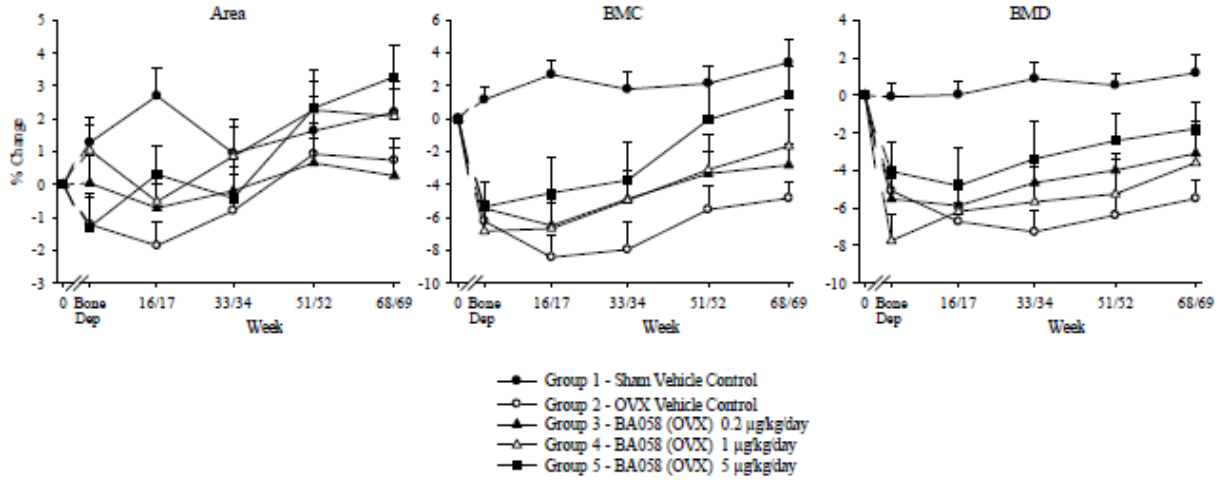
Parameters	Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
BMD	1.8	3.8	7.0	15.0	12.4
BMC	-3.7	1.6	5.6	26.7	16.8
Area	-5.4	-2.0	-1.5	11.0	3.8

Values in bold are significantly different from OVX vehicle controls.

DXA Proximal Tibia Diaphysis (Text Fig 17 and Text Table 24):

- OVX:
  - Decreases in BMC, BMD and Area in OVX animals during 9-month bone depletion period
- Abl treatment:
  - Increase in BMD at all doses, maximal at 1 ug/kg, and increase in Area at 5 ug/kg only; resulting in dose-related increase in BMC
  - Effects occurred mainly in first 33 weeks of Tx
  - BMD and BMC in Abl-treated animals did not reach sham levels, but Area did at 5 ug/kg

Text Figure 17
Bone Densitometry Values by DXA as Percent Change from Baseline Period
Right Proximal Tibia Diaphysis - Mean (SEM)



Text Table 24
Effect of OVX and BA058 Treatment on Proximal Tibia DXA
as Percent Change from End of Bone Depletion Period
Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
BMD	3.1	-5.7	5.2	11.0	4.3
BMC	5.5	-1.7	5.2	11.6	15.3
Area	2.4	4.2	0.1	0.6	11.0

Values in bold are significantly different from OVX vehicle controls.

<u>pQCT tibia and radius (in vivo)</u>

pQCT METAPHYSIS (proximal tibia and distal radius) (Text Fig. 21, 22; Text Table 25, 26)

- OVX:
  - Decrease in Cortical Area and increase in Trabecular Area in OVX animals (trabecularization) during bone depletion period
  - Clear decreases in all BMC and BMD values (total, cort, trab), except Trabecular BMC
- Abl-treatment:
  Proximal Tibia
  - Treatment-related increases in Total BMC and BMD at all doses, but no clear effect on Total Area
  - Increases in (Sub)Cortical Area, and increases in Cortical BMC at all doses, but no effect on Cortical BMD
  - Dose-related significant decreases in Trabecular Area, non-dose-related increase in Trabecular BMD, and no clear effect on Trabecular BMC (probably due to reduced Area plus increased BMD)

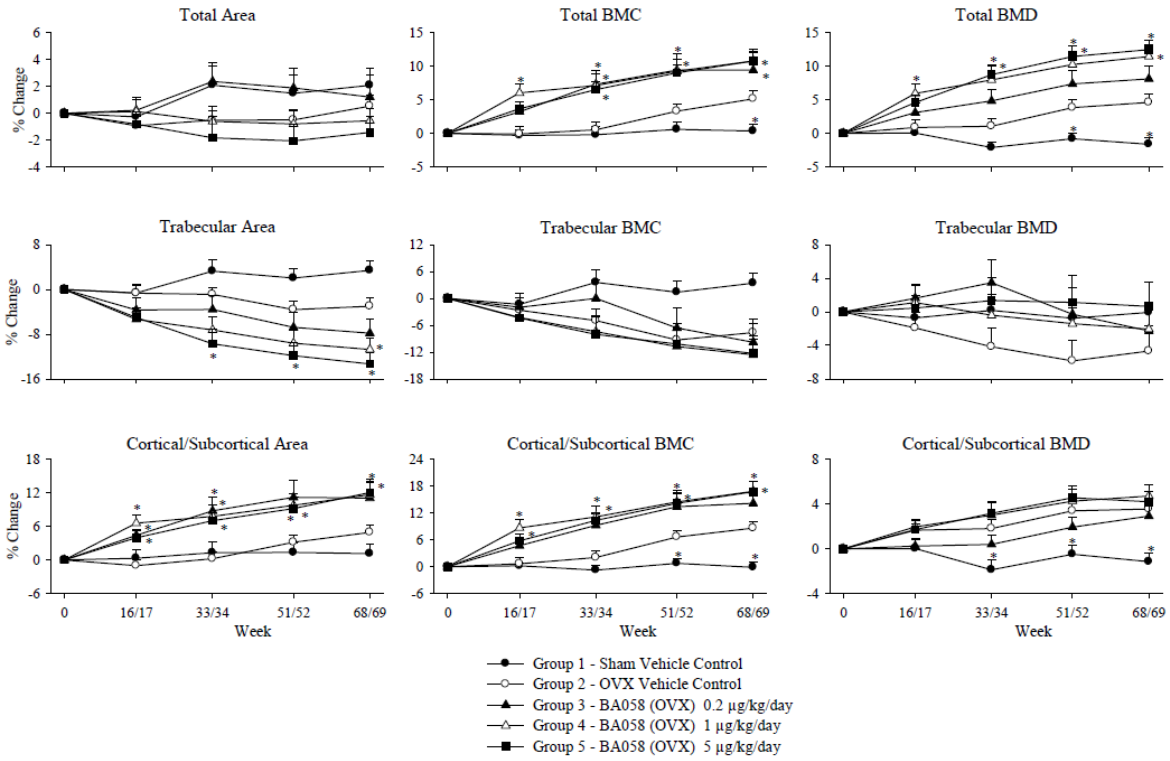
- Several pQCT changes were not clearly dose-related
- Most OVX-induced changes that were reversed by Abl treatment reached or surpassed sham levels in 0.2, 1 and/or 5 ug/kg groups

Distal Radius

- Non-dose-related increases in Total BMD and Total BMC at all doses, but no clear effect on Total Area
- Treatment-related increase in (Sub)Cortical Area, Cortical BMC increased dose-dependently, (sub)cortical BMD not clearly affected
- Tx-related decrease in Trabecular Area, and thus a decrease in Trabecular BMC; Trabecular BMD not clearly affected (different from prox tibia result)
- Most OVX-induced changes that were reversed by Abl did not reach sham levels

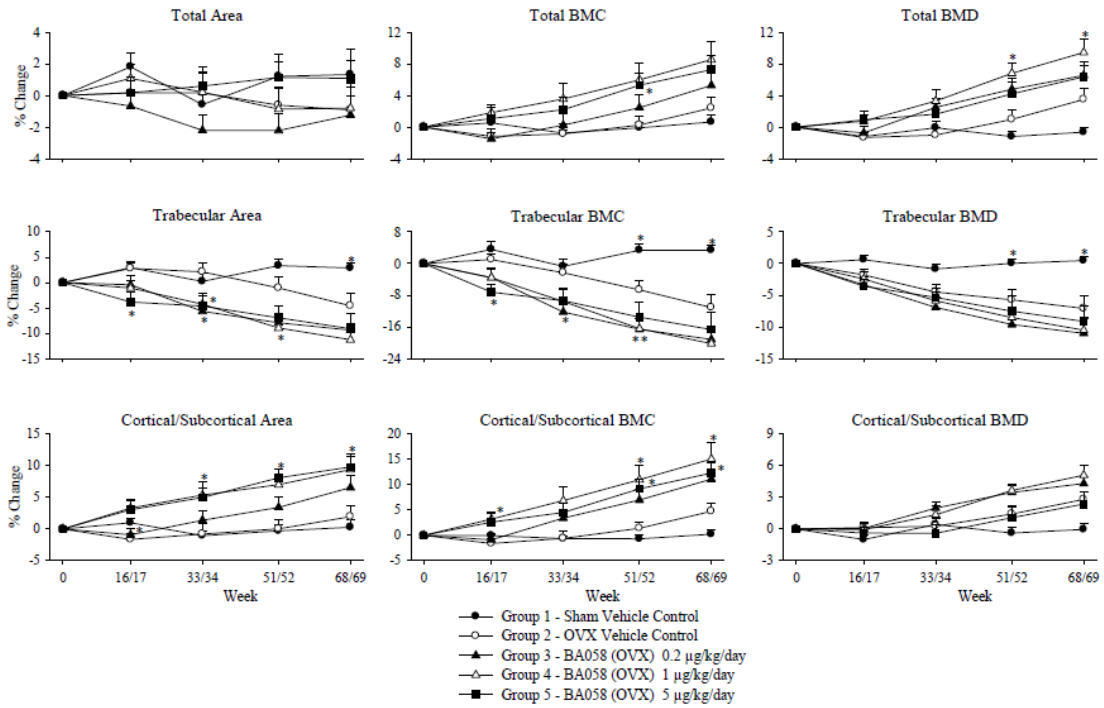
Reviewer Comment (pQCT):
Results were similar for the two metaphyseal bone sites. Abl had no effect on bone size, but total BMD and BMC were increased. This was due to increases in cortical area (ie thickness) and BMC, accompanied by decreases in trabecular/medullary area. Trabecular BMD was increased in tibia but not affected in radius.
Effects were different in the rat, where Abl causes net periosteal bone apposition in the proximal tibia metaphysis (at 0.3-12x human AUC).

Text Figure 21
Bone Densitometry Values by pQCT as Percent Change from End of Bone Depletion Period
Right Proximal Tibia - Metaphysis - Mean (SEM)



(Changes from end of bone depletion)

Text Figure 22
Bone Densitometry Values by pQCT as Percent Change from End of Bone Depletion Period
Right Distal Radius - Metaphysis - Mean (SEM)



Significantly different from OVX vehicle control group (Group 2) value: *-p≤0.05

(Changes from end of bone depletion)

Text Table 25
Effect of OVX and BA058 Treatment on Proximal Tibia Metaphysis pQCT Parameters
as Percent Change from End of Bone Depletion Period - Total Across Time

Parameters		Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
Total slice	BMD	**-4.5**	10.4	23.4	**35.6**	**37.2**
	BMC	0.4	8.9	29.4	**33.2**	**30.0**
	Area	5.4	-1.4	5.7	-1.8	-6.2

Values in bold are significantly different from OVX vehicle controls.

Text Table 26
Effect of OVX and BA058 Treatment on Distal Radius Metaphysis pQCT Parameters
as Percent Change from End of Bone Depletion Period - Total Across Time

Parameters		Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
Total slice	BMD	-3.15	2.09	13.04	**20.26**	13.10
	BMC	0.32	0.61	6.48	19.9	15.81
	Area	3.75	-1.23	-6.34	-0.39	2.97

Values in bold are significantly different from OVX vehicle controls.

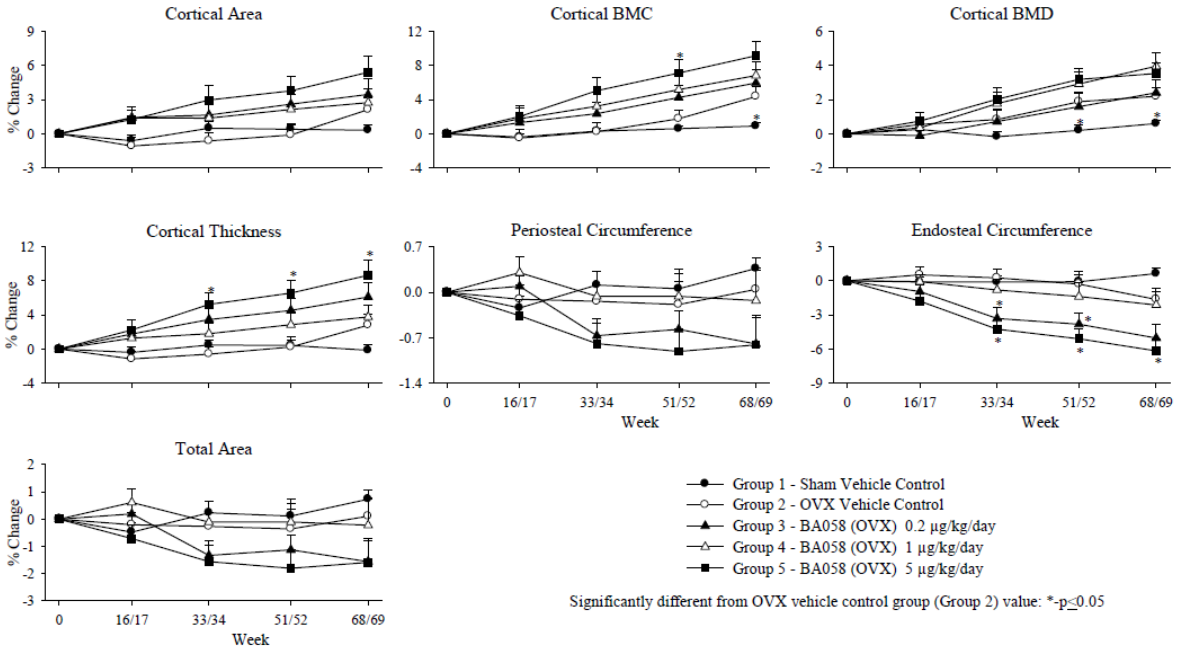
pQCT DIAPHYSIS (proximal tibia and distal radius) (Text Fig 25, 26; Text Table 27)

- OVX:
  - Decrease in Total Area and periosteal circumference
  - Decrease in Cortical BMD, BMC, Area and Thickness
  - No clear effect on endosteal circumference
- Abl-treatment:

  Proximal Tibia Diaphysis
  - Slight decrease in Total Bone Slice Area and periosteal circumference (contrast to proximal tibia shaft DXA-Area increase)
  - Increase in Cortical Thickness, Area at all doses, resulting in dose-related increase in Cortical BMC. BMD was slightly increased at 1 and 5 ug/kg.
  - Decrease in Endosteal Circumference with increased Cortical Thickness, not clearly dose-related (1 ug/kg group often showed outlying results)
  - Effect of Abl was to thicken the cortex that had been thinned by OVX (due to decreased periosteal circumference) by endosteal bone apposition

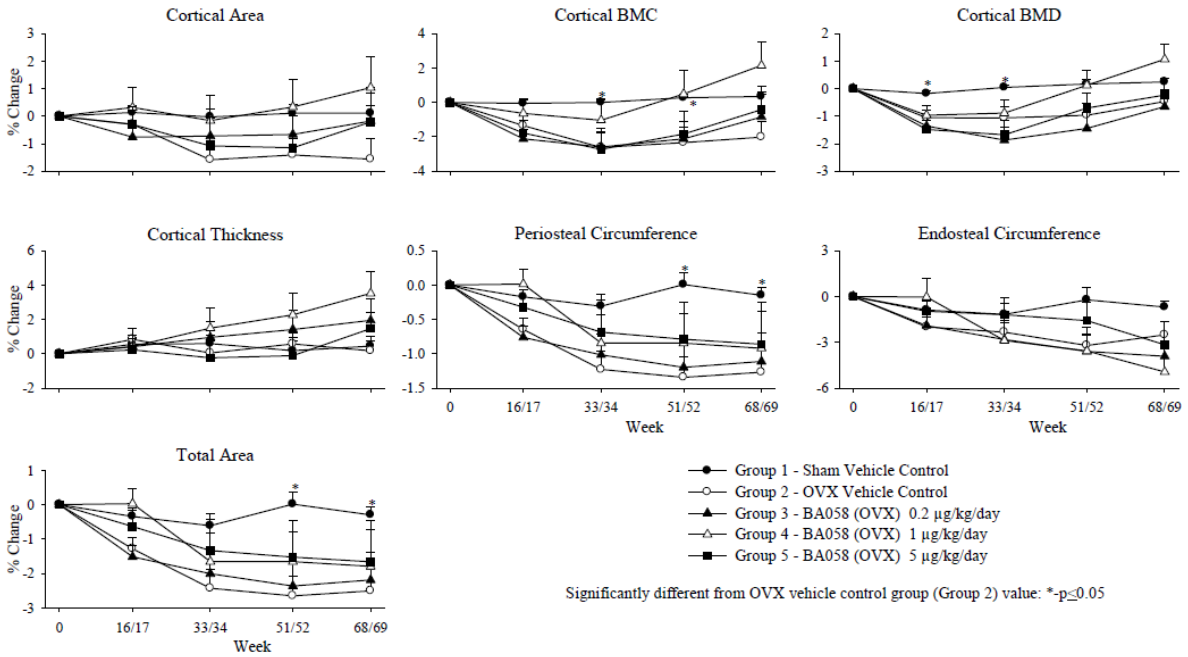
  Distal Radius Diaphysis
  - Abl effects in radius were different from those in proximal tibia diaphysis. Periosteal circumference and Total Area were slightly increased, while endosteal circumference was also slightly increased at 5 ug/kg. Cortical thickness, BMC and BMD were not significantly changed.

Text Figure 25
Bone Densitometry Values by pQCT as Percent Change from End of Bone Depletion Period
Right Proximal Tibia - Diaphysis - Mean
(SEM)



(Changes from end of bone depletion)

Text Figure 26
Bone Densitometry Values by pQCT as Percent Change from End of Bone Depletion Period
Right Distal Radius - Diaphysis - Mean (SEM)



(Changes from end of bone depletion)

Proximal Tibia Diaphysis

Text Table 27
Effect of OVX and BA058 Treatment on Proximal Tibia Diaphysis pQCT Parameters
as Percent Change from End of Bone Depletion Period - Total Across Time

Parameters	Sham Control	OVX Vehicle	BA058 0.2 µg/kg/day	BA058 1 µg/kg/day	BA058 5 µg/kg/day
Total slice area	0.6	-0.7	-3.8	0.2	-5.7
Periosteal Circumference	0.3	-0.4	-1.9	0.0	-2.9
Cortical BMD	0.9	5.5	4.6	9.0	9.5
Cortical BMC	1.4	5.9	13.9	17.0	23.4
Cortical area	0.6	0.3	9.2	7.7	13.4
Endosteal Circumference	0.4	-1.1	-13.1	-4.4	-17.3
Cortical Thickness	0.3	1.3	15.8	9.7	22.7

Values in bold are significantly different from OVX vehicle controls

Selected data summary:

Text Table 28
Effect of OVX and BA058 Treatment on Selected Bone Densitometry Parameters
as Percent Change from Baseline Period*

Parameters	Groups	End of Bone Depletion	Week 16/17	Week 33/34	Week 51/52	Week 68/69
Lumbar Spine (L1-L4) BMD (DXA)	Sham	3.15	3.83	4.73	6.36	6.80
	OVX	-6.03	-5.19	-5.38	-3.82	-3.44
	BA058 0.2 µg/kg/day	-5.38	-1.71	-0.81	0.18	0.24
	BA058 1 µg/kg/day	-5.79	0.77	1.19	3.72	3.54
	BA058 5 µg/kg/day	-5.69	1.27	3.73	3.62	5.43
Proximal Femur Global BMD (DXA)	Sham	0.89	3.94	1.74	2.01	5.10
	OVX	-6.00	-4.77	-4.69	-4.69	-3.83
	BA058 0.2 µg/kg/day	-6.88	-2.15	0.09	-0.24	1.63
	BA058 1 µg/kg/day	-8.25	-0.91	-0.30	-2.02	1.04
	BA058 5 µg/kg/day	-4.92	-1.34	3.98	3.95	3.93
Proximal Tibia Total BMD (pQCT)	Sham	1.73	1.75	-0.46	0.88	0.06
	OVX	-8.28	-7.49	-7.31	-4.79	-4.08
	BA058 0.2 µg/kg/day	-6.02	-3.24	-1.68	0.64	1.36
	BA058 1 µg/kg/day	-7.81	-2.53	-0.68	1.48	2.61
	BA058 5 µg/kg/day	-6.33	-2.07	1.77	4.27	5.20
Proximal Tibia Diaphysis Cortical Thickness (pQCT)	Sham	1.41	0.99	1.86	1.77	1.23
	OVX	-3.79	-4.92	-4.37	-3.53	-1.06
	BA058 0.2 µg/kg/day	-0.78	0.94	2.52	3.60	5.17
	BA058 1 µg/kg/day	-1.74	-0.42	0.08	1.12	2.02
	BA058 5 µg/kg/day	-0.07	2.19	5.11	6.36	8.41

* Expressed as percent difference of group means.

Reviewer Comments:
Summary of in vivo data (spine, femur, tibia, radius)

- *DXA*
  - *OVX led to expected decreases in DXA parameters*
  - *Spine: Abl caused dose-related increases in bone Area, BMD and BMC (mainly cancellous bone)*
  - *Femur: Abl also caused increases in BMC and BMD in proximal and distal femur (mixed cancellous and cortical bone). Abl-induced changes were largest in proximal femur where DXA parameters reached above-sham levels.*
  - *Tibia, Radius: In proximal tibia diaphysis (cortical bone), Abl caused increases in BMD and BMC. However, in 1/3 distal radius (cortical bone), DXA showed Abl-related decreases in DXA parameters (Area, BMD, BMC), suggesting bone site dependence of cortical Abl effects. In ultradistal radius (cancellous bone), BMD and BMC were not significantly affected.*
  - *Both DXA and pQCT changes occurred mainly in first 32/33 weeks of dosing*
- *pQCT*
  - *OVX caused thinning of long bone (periosteal circumference decrease), with no change in medullary area. OVX also reduced cortical thickness and BMD.*
  - *Proximal tibia*
    - *In metaphysis, Abl caused increases in cortical bone area (ie thickness since total slice area was unaffected) and cortical BMC. Abl caused decreases in trabecular, ie, medullary bone area (no clear dose-dependence). Total slice area was unaffected.*
    - *For the diaphysis pQCT data were consistent with DXA and showed decreases in endosteal circumference, with slight decreases in periosteal circumference, resulting in increases in cortical thickness, area, BMC (all up to 20%) and possibly BMD. Most changes were not clearly dose-dependent.*
  - *Distal radius*
    - *In metaphysis, Abl increased total BMD and BMC and cortical thickness, and trabecular/medullary area was decreased without an effect on trabecular BMD.*
    - *In diaphysis, Abl slightly increased periosteal circumference and Total Slice Area, as well as endosteal circumference (at 5 ug/kg). Cortical thickness, BMC and BMD were not significantly changed.*
      - *Note: Femur diaphysis histomorphometry also showed only a very small increase in cortical width (2%). This suggests bone site dependence of Abl-effects.*
  - *Both DXA and pQCT changes induced by Abl occurred mainly in first 32/33 weeks of dosing.*
  - *pQCT of proximal or mid femur was not performed*

Densitometry – ex vivo

Ex vivo measurements were done on bones to be used for biomechanical testing, including:

- DXA (femur: global, proximal, mid; L3+L4 vertebral bodies; L5+L6 vertebral cores)
- pQCT (femur diaphysis; L3+L4 vertebral bodies, L5+L6 vertebral cores).

DXA:

- Femur (Text Fig 27): Very small decreases in OVX BMC and BMD, and no significant Abl-related increases in BMC and BMD. This was inconsistent with *in vivo* DXA showing clear dose-related proximal femur effects of Abl.
- Vertebrae (Text Fig 29): OVX-induced decrease in BMD and BMC in L3+L4 vertebral bodies and L5+L6 cores, and dose-related increase in BMD/BMC in Abl treated (similar but smaller effect than observed by in vivo densitometry except no increase in Area in ex vivo test)

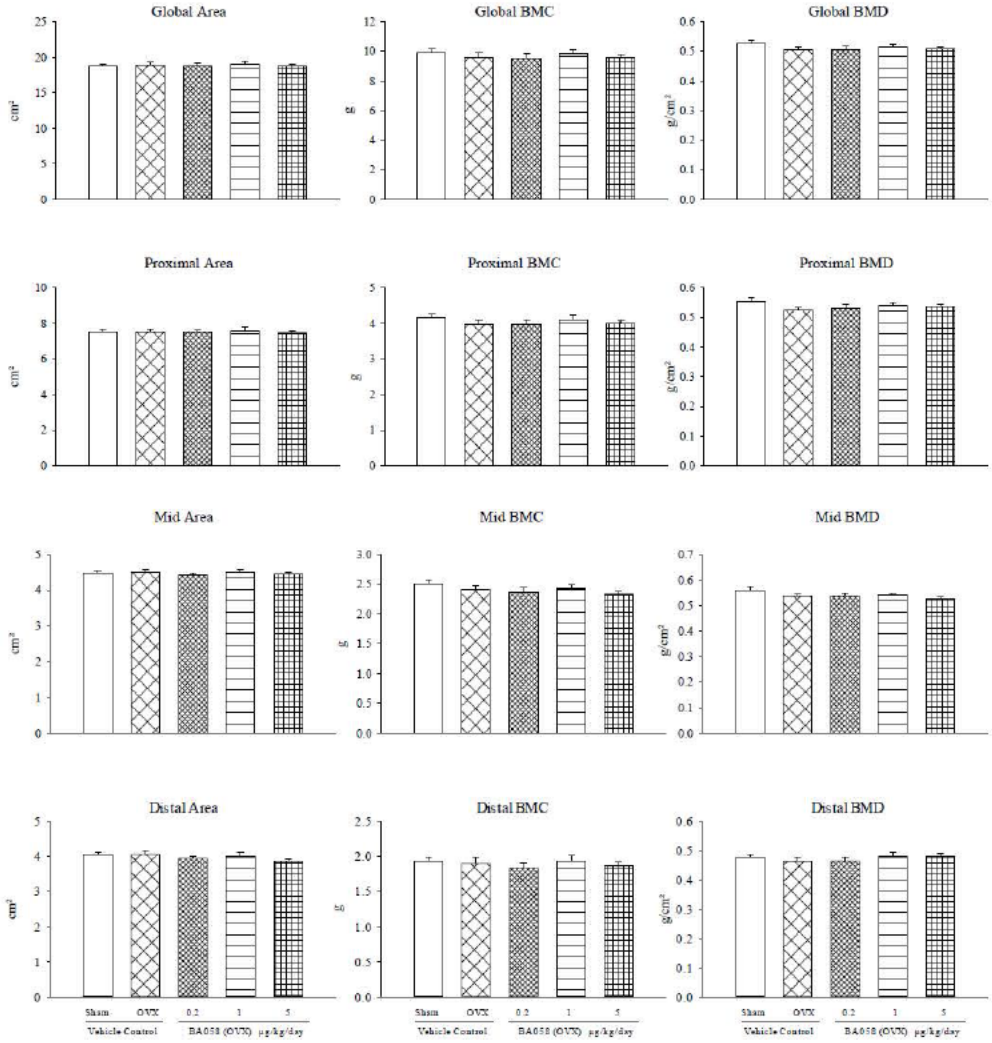
pQCT:

- Femur shaft: No effect of OVX or Abl on Total Area BMC or BMD, cortical parameters (area, BMC, thickness) or peri/endosteal circumferences. This is in contrast to vivo pQCT data from proximal femur and proximal tibia diaphysis showing increases in cortical BMC and thickness vs OVX controls, but consistent with lack of effect on femur bending strength (see below)!
- Vertebrae: Decrease in total and trabecular BMD and BMC in L3+L4 vertebral bodies and L5+L6 cores in OVX group, and dose-related increase in BMD/BMC in Abl-treated (data similar to those obtained by in vivo DXA data except no effects on L3+L4 Area in ex vivo test)

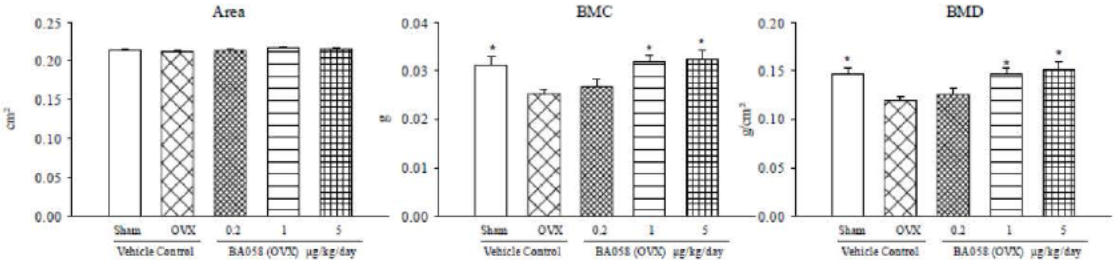
Conclusion:

- The ex vivo data were partly consistent with the in vivo end-of-treatment DXA and pQCT data.

Text Figure 27
Bone Densitometry Values by DXA Parameters – Right Femur – Mean (SEM)



Text Figure 29
Bone Densitometry Values by DXA Parameters – L5+L6 Lumbar Vertebral Cores – Mean (SEM)



Significantly different from OVX vehicle control group value: *-p≤0.05

Micro-CT – ex vivo

(Measurements made before or after mechanical testing)
(NOTE: Several effects of OVX and Abl were not statistically significant)

- Distal Femur Metaphysis (cortical and trabecular regions) (after femur bending test):
  - Trabecular: OVX decreased Tissue BMD; Abl did not affect this, but increased trabecular parameters BV/TV and vBMD. However, Abl had no clear effects on Tb.Th or Tb.N or Tb.Sp.
  - Cortical: OVX decreased some cortical parameters (vBMD, tBMD, BV/TV); Abl had no significant effects on any of these cortical parameters or on cortical BMC or Thickness)
  - Abl caused dose-related decrease in trabecular SMI, indicating a more plate-like structure
- T12 body:
  - OVX caused decreases in trabecular vBMD and tBMD, BV/TV, and Tb.Th and Tb.N. ABL reversed or increased trabecular vBMD, BMC, BV/TV, Tb.Th. and Tb.N. OVX also caused decreases in T12 cortical vBMD, BMC, tBMD, and thickness. Abl increased cortical BMC and thickness only
  - Abl caused dose-related decrease in trabecular SMI, indicating a more plate-like structure
- L6 vertebral core (before biomechanical test)
  - Decreases in trabecular vBMD and BMC parameters , BV/TV, TbTh and TbN in OVX vs Sham; Reversal of all these parameters by Abl Tx, and was dose-related.
  - Increased trabecular SMI in OVX, and Abl dose-related decrease in SMI beyond sham level, may be indicating a more plate-like structure
- Humeral cortical beam (after bending test):
  - Increase in porosity in OVX vs Sham group, no significant effect on porosity by Abl

Reviewer Comments/Summary:

- *Micro-CT data showed that Abl generally reversed OVX-induced decreases in vertebral trabecular parameters (BV/TV, vBMD, Tb.Th and/or Tb.N) in dose-related manner. OVX-induced decreases in vertebral cortical thickness and BMC were also restored by Abl.*
- *In distal femur trabecular bone, Abl had no clear effects onTb.Th or Tb.N. although it increased trab BV/TV. In distal femur cortical bone, Abl had no effect on OVX-induced decreases in BMD and BV/TV, nor on cortical BMC or thickness*
- *Abl did not significantly affect the increase in cortical porosity caused by OVX.*
- *Decreased SMI in Abl-treated may have indicated a more plate-like trabecular structure.*

Biomechanical testing

(Femur shaft and neck, humerus beam, and lumbar vertebral bodies and cores)

Femur 3-point bending (Text Fig. 41):

- No significant effect of OVX or Abl. However, both OVX and Abl appeared to increase AUC and toughness (including post-yield toughness) slightly but not significantly. Peak load correlated well with (particularly ex-vivo) femur pQCT cortical BMC (not shown).

Humerus beam 3-point bending (Text Fig 44):

- No meaningful effects of OVX or Abl-treatment on peak load, stiffness or AUC/toughness. This measurement yields intrinsic (material) cortical bone parameters only. No correlation analysis.

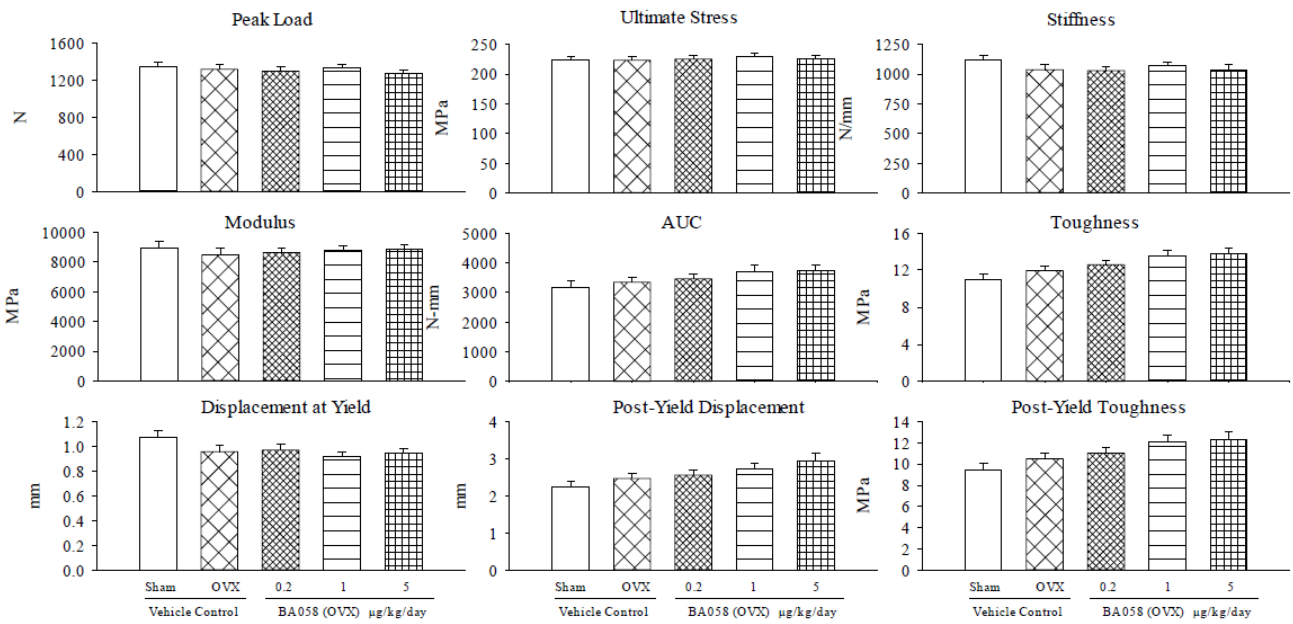
Femoral neck shear:

- No meaningful effects of OVX, consistent with lack of ex vivo (not in vivo) DXA changes. Abl had a small positive effect on peak load at 1 and 5 ug/kg. Peak load correlated moderately well with proximal femur DXA-BMC

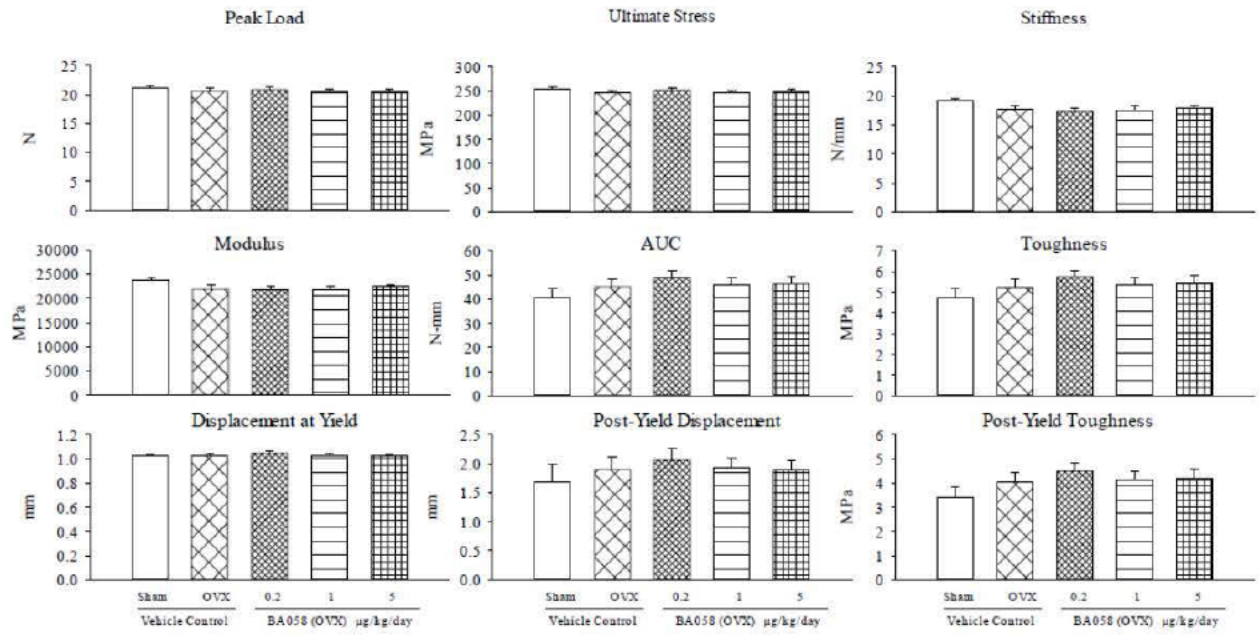
Vertebral body L3+L4 compression (Text Fig. 48):

- OVX: Decreases in Peak Load, App Strength, Yield Load and Stress, Stiffness and Modulus, AUC and Toughness. Post-yield displacement and toughness also decreased by OVX. Most changes were significant (vs sham controls). Bone quality slightly deteriorated (see below).
- Abl reversed ALL OVX-induced decreases in bone strength parameters, although statistical significance was only shown for Peak Load and Apparent Strength.
- Correlation of Peak Load vs. Total BMC was good for all groups (Text Fig 49).
- Sponsor suggested that bone quality was improved (ie reversed from OVX to sham levels) in 1 and 5 ug/kg groups based on a "positive shift" in these groups ('positive shift' meaning steeper slopes of regression lines).
- Correlation was good/similar for all groups (r=0.9) for Total BMD vs Apparent Strength (N/mm2).
- Reviewer agrees with sponsor about improved quality when defined as slope of BMC-strength regression line.

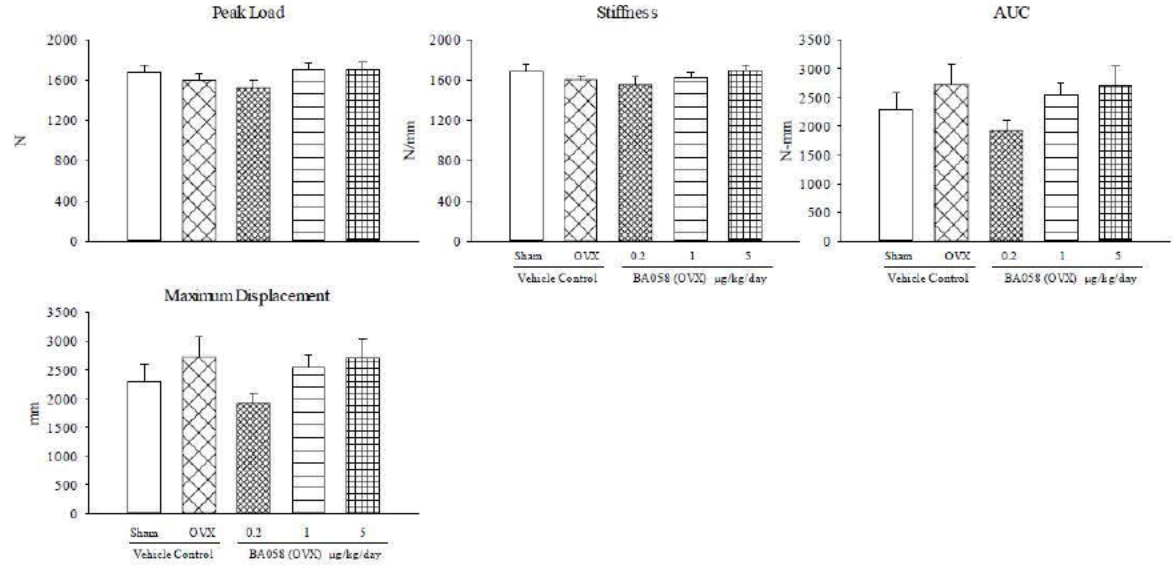
Text Figure 41
Biomechanics Parameters – Femur – 3-point Bending – Mean (SEM)



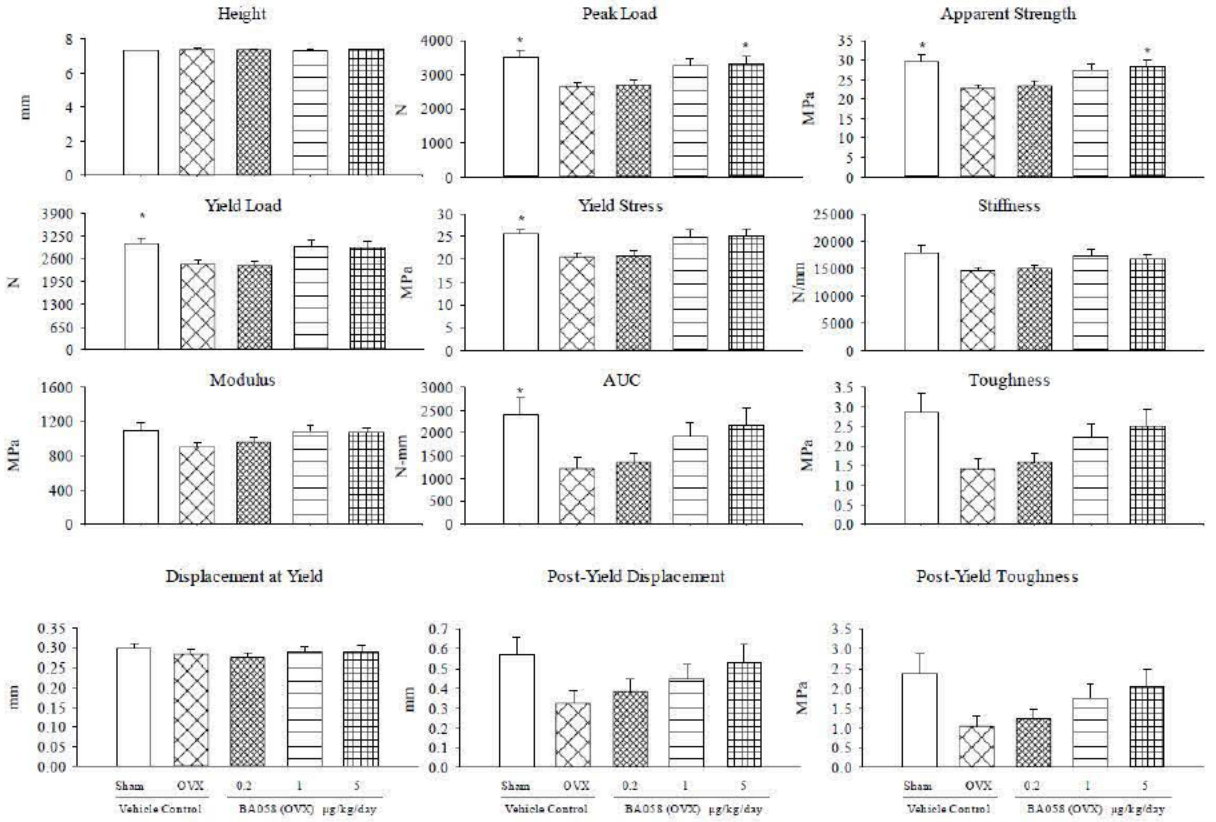
Text Figure 44
Biomechanics Parameters – Humerus – 3-point Bending– Mean (SEM)



Text Figure 45
Biomechanics Parameters – Femur – Femoral Neck – Mean (SEM)

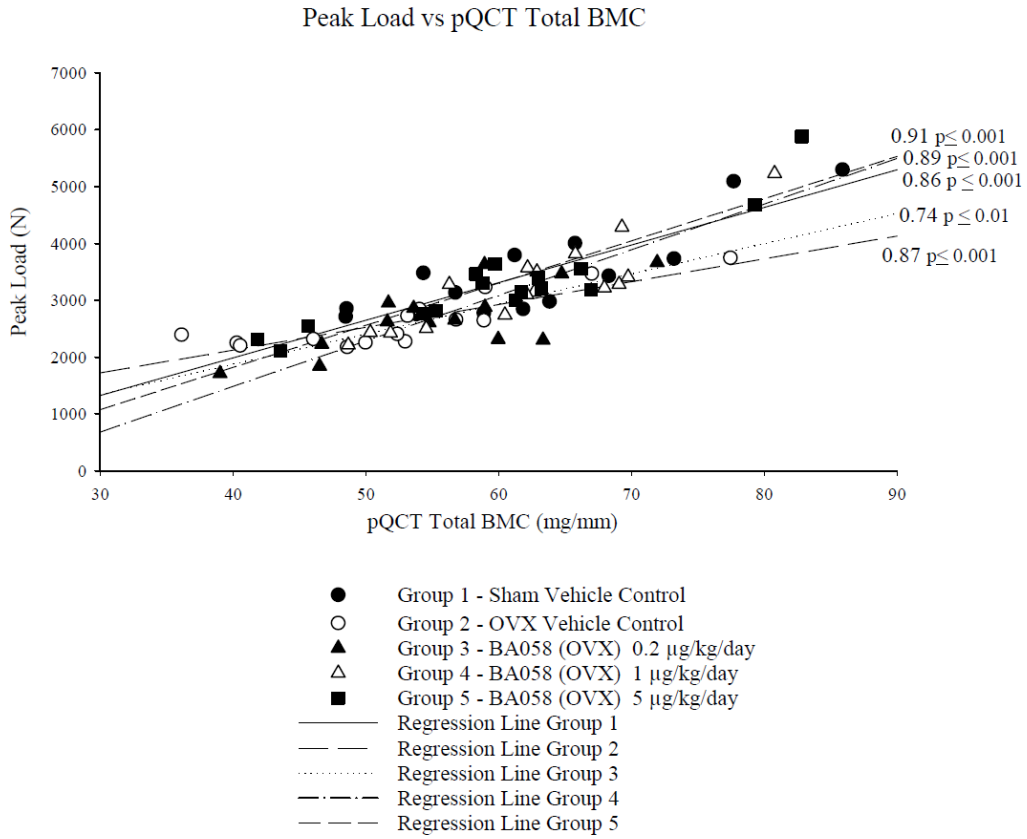


Text Figure 48
Biomechanics Parameters – Lumbar Vertebral Bodies L3+L4 Averaged – Mean (SEM)



Significantly different from OVX vehicle control group value: *-p≤0.05

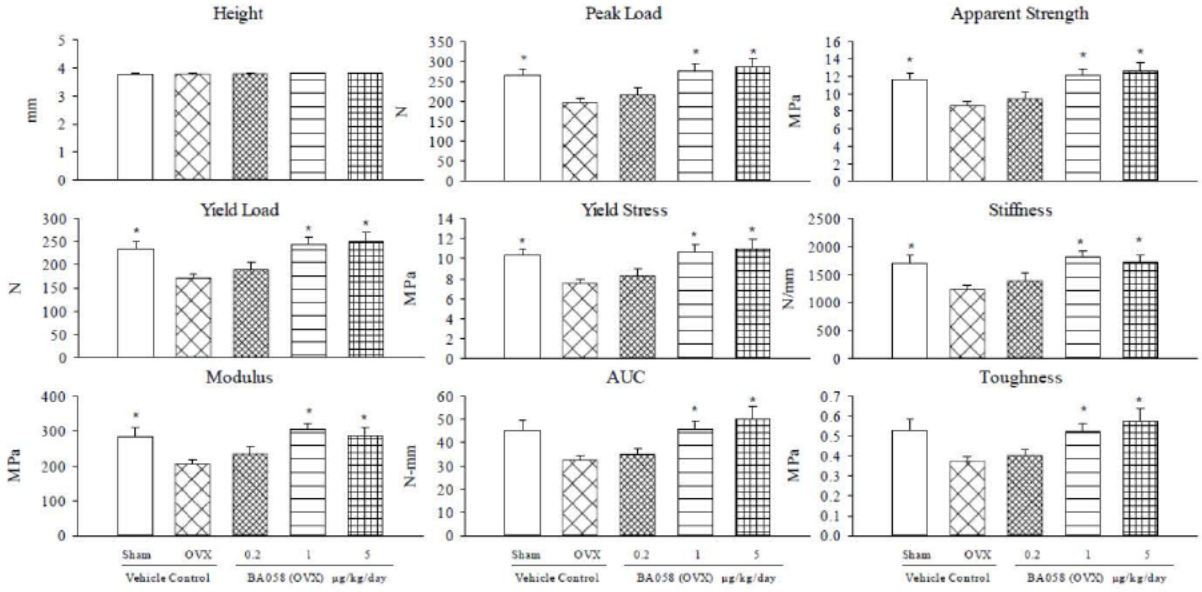
Vertebral bodies:



Vertebral core L5+L6 compression (Text Fig. 51, 52):

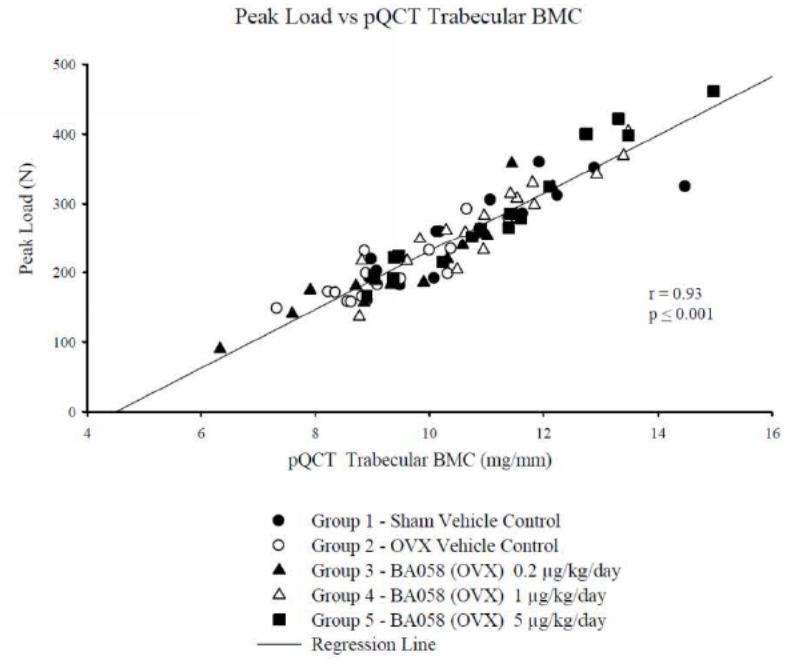
- Similar results as those for vertebral bodies (Fig. 51)
- Correlation of Peak Load with pQCT Trabecular BMC was good and similar for all groups (r=0.93) (Fig 52)
- Correlation was also good (r = 0.93) between App Strength and pQCT BMD for cores (peak load normalized per unit area, N/mm2).

Text Figure 51
Biomechanics Parameters – Lumbar Vertebra Cores L5+L6 Caudal and Cranial Averaged – Mean (SEM)



Significantly different from OVX vehicle control group value: *-p≤0.05

Text Figure 52
Regression Plot of Biomechanics – L5+L6 Average Vertebral Cores vs. ex vivo pQCT Trabecular BMC



Reviewer Comments/Summary:

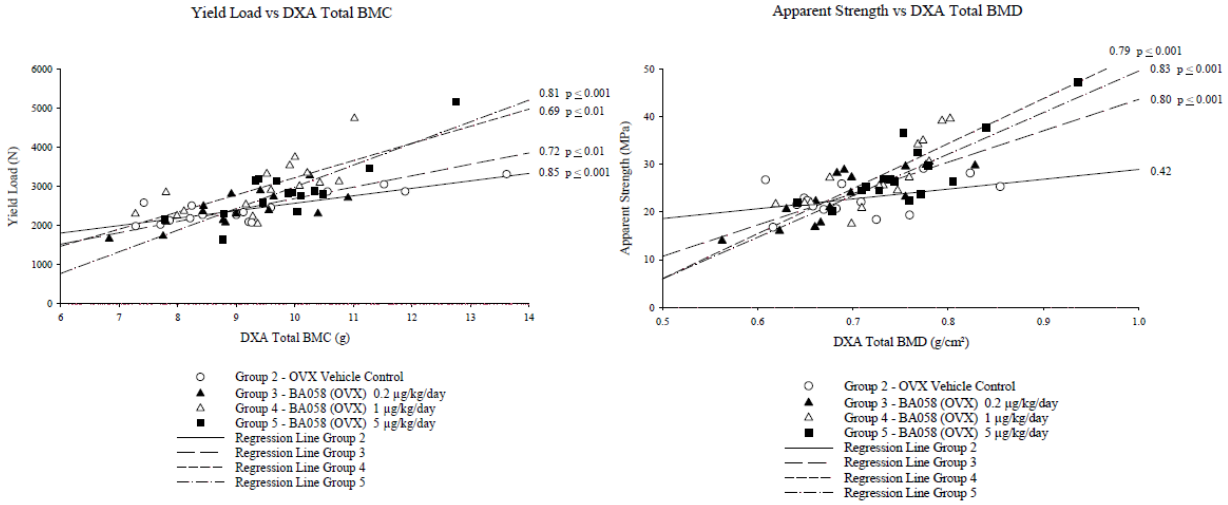
- *Abl increased bone strength parameters in lumbar vertebrae cores (and bodies) in statistically significant manner. However, there were no clear effects in femoral neck, which was consistent with lack of significant increase in ex vivo DXA BMD and BMC.*

- *Abl did not significantly affect femoral diaphysis strength, although AUC and toughness appeared slightly increased*
- *For femur and vertebrae, data suggested good correlations between measures of bone mass (BMC ore BMD) and bone strength (peak load or apparent strength), and they were generally similar for all groups*
- *In vertebral bodies, ABL appeared to restore the OVX-induced impairment in bone quality (i.e. slope of ex vivo BMC-strength regression line) to sham level*

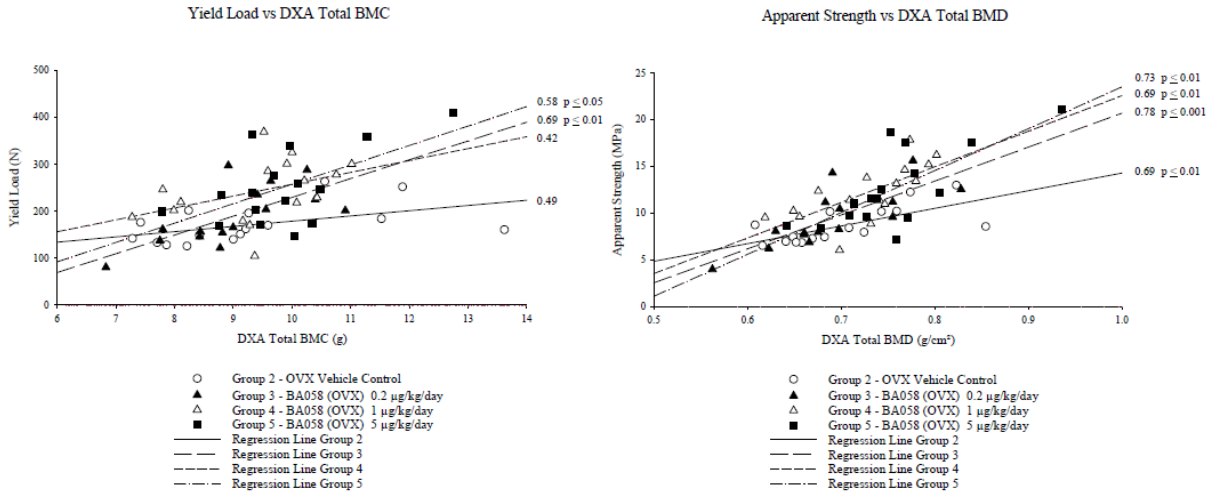
Correlation analysis was also carried out using <u>in vivo</u> densitometry parameters (Text Figs 60, 64).

- Data yielded moderately good correlations between BMC or BMD and several strength parameters for femur and vertebrae.
- Correlation data for vertebral bodies and cores again suggested better bone "quality" in low, mid and/or high dose Abl-treated vs. OVX, based on slope of regression lines (Text Figs 60, 64). It is not clear why Sponsor used yield and not peak load for comparisons.
- Sham was not included in the correlation analysis.

Text Figure 60
Regression Plot of Biomechanics L3+L4 Lumbar Vertebral Bodies - Compression vs. DXA Lumbar Spine (L1-L4)
Week 68/69



Text Figure 64
Regression Plot of Biomechanics L5+L6 Lumbar Vertebral Cores - Caudal + Cranial - Compression vs. DXA Lumbar Spine (L1-L4) Week 68/69



Histomorphometry

Vertebrae, femur neck (Text Table 3 and Fig. 3):

- In vertebrae and femoral neck, OVX caused decreased BV/TV, which was reversed by Abl doses of 1 and 5 ug/kg (Text Table 3)
- Vertebral wall thickness was increased by OVX and also increased non-dose-dependently by Abl in vertebrae, but this was not observed in femur neck. This effect was the only statistically significant effect of Abl. An increase in wall thickness indicates more bone deposition in a bone remodeling or formation unit.
- Tb.Th was increased and Tb.Sp was decreased slightly by Abl (reversal of OVX effect), but not in dose-related manner (restoring effect of OVX). Conversely, Tb.N was increased dose-dependently by Abl treatment.
- OVX caused increases in bone formation measures (eg BFR/BV or BFR/BS, OS/BS or OV/BV, or MS/BS, OS/BS or Ob.S/BS) and Ac.F (bone turnover), some of which were significant. However, Abl did NOT have a clear effect on any of these bone parameters in vertebrae or femoral neck, at odds with the densitometry and bone marker data.
- Cancellous ES/BS (eroded surface %) was not affected by OVX, but markedly increased in vertebrae without clear dose-dependence. This is consistent with increases in NTx at all doses at study end (Wk 67/68) and suggests that cancellous bone resorption may have been increased in addition to formation.

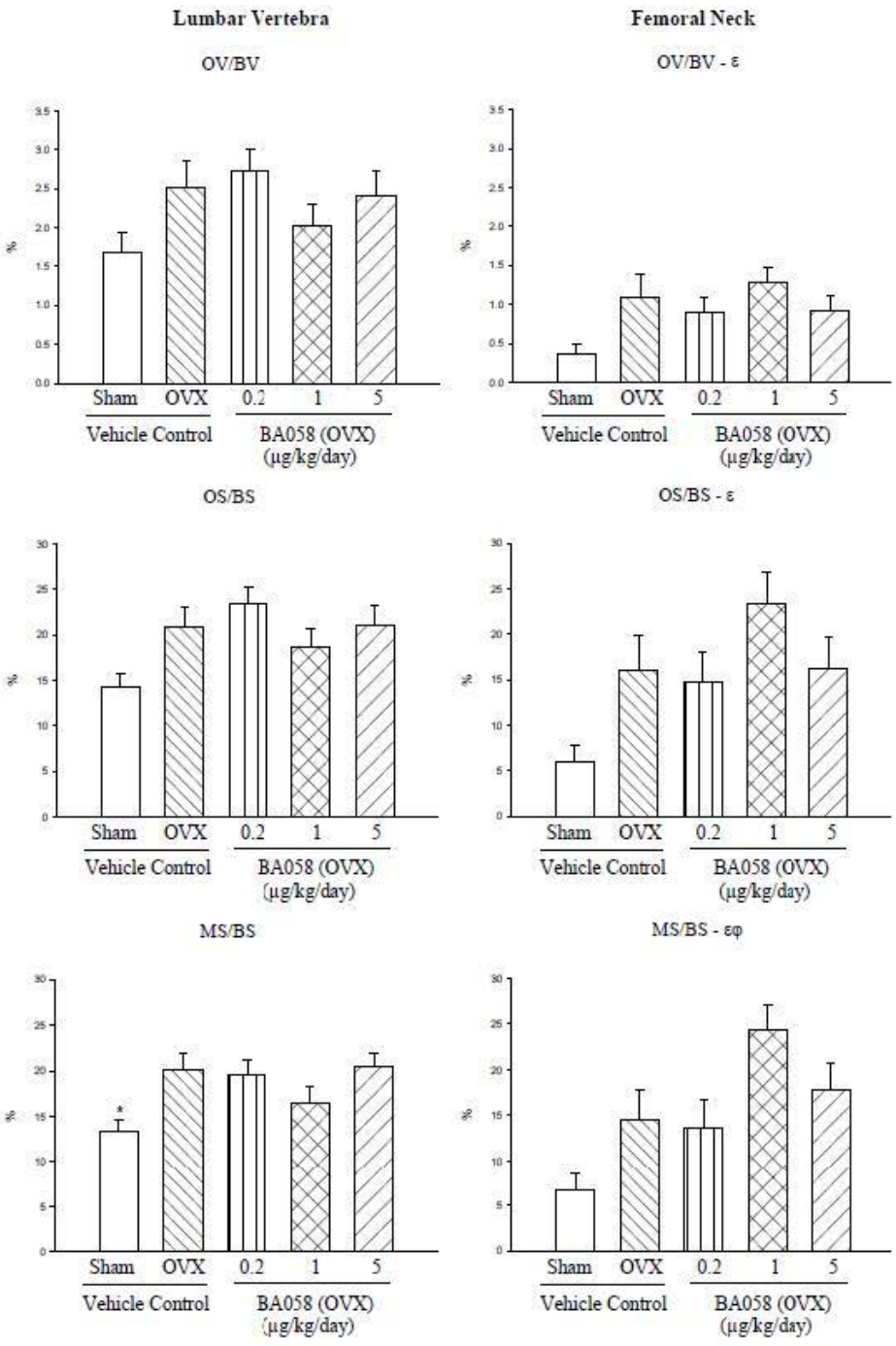
Text Table 3
Differences in Histomorphometric Variables of Cancellous Bone (Lumbar Vertebra and Femoral Neck)*

No. Of Animals	16	15	16	16	16	16	15	16
Operation/Bone	**OVX - Lumbar Vertebra**				**OVX - Femoral Neck**			
BA058 (µg /kg/day)	0	0.2	1	5	0	0.2	1	5
Effects of	**OVX**		**BA058**		**OVX**		**BA058**	
Structural Parameters								
BV/TV	-11.93	0.78	15.49	13.72	0.87	6.79	21.72	24.05
Tb.Sp	15.83	4.25	-10.12	-11.59	-5.56	-2.76	-11.70	-17.94
Tb.N	-9.21	-3.54	4.37	7.85	-10.68	4.93	9.54	15.24
Tb.Th	-4.53	4.36	10.58	5.70	12.96	1.38	12.85	9.67
W.Th	**16.56**	**32.93**	**30.40**	**27.49**	**38.68**	9.43	-0.30	10.42
Ob.S/BS	**105.37**	5.42	-8.85	4.53	**233.19**	-8.15	47.21	3.32
Oc.S/BS	10.59	4.33	-7.99	6.35	38.50	26.22	29.80	-3.53
OV/BV	48.54	8.65	-19.62	-4.24	202.05	-17.64	17.56	-16.23
OS/BS	46.85	12.35	-10.20	0.43	169.18	-8.18	44.92	1.46
ES/BS	-0.54	44.62	25.12	52.36	-7.26	24.04	38.56	-9.80
Dynamic parameters								
MS/BS	**50.87**	-2.85	-18.08	1.92	114.48	-5.61	69.22	22.93
MAR	0.41	8.04	1.64	0.24	10.66	8.86	9.43	6.10
sLS/BS	16.67	-4.74	-19.44	5.01	74.14	7.18	**80.49**	51.49
dLS/BS	**92.40**	-1.45	-17.08	-0.36	146.37	-12.76	62.92	6.98
BFR/BS (µm3/µm2/yr)	55.50	0.95	-18.75	-1.63	151.02	-10.24	49.22	7.14
BFR/BV	50.91	1.24	-22.33	-4.41	121.34	-4.40	43.59	0.41
Aj.AR	4.30	-2.67	-4.60	0.58	12.69	8.97	23.98	11.51
Omt	6.09	**-18.68**	-5.21	-6.62	-2.66	-9.85	-8.40	-3.76
Mlt	5.20	-11.89	-0.35	-8.79	-17.47	-5.29	-19.93	-5.53
Ac.f	33.49	-21.98	-33.33	-26.02	134.70	-15.74	39.47	-15.75
FP	14.68	**33.80**	**31.80**	21.42	-4.99	3.69	-22.87	-1.26
Rs.P	-40.08	40.99	48.98	49.79	54.88	-63.57	-89.14	-67.22

* Expressed as percent difference of group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used

Data are expressed as % difference between (1) OVX and sham controls, and (2) Abl and OVX vehicle controls

Text Figure 3
Cancellous Bone Histomorphometry – OB/BV, OS/BS, MS/BS – Mean (SEM)



Significantly different from OVX vehicle control group value: *-p<0.05
Statistical Analysis Using Heteroscedastic ANOVA Model: ε
Significant quadratic dose effect: φ -p<0.05

Femur shaft (Text Table 4 and Text Figures):

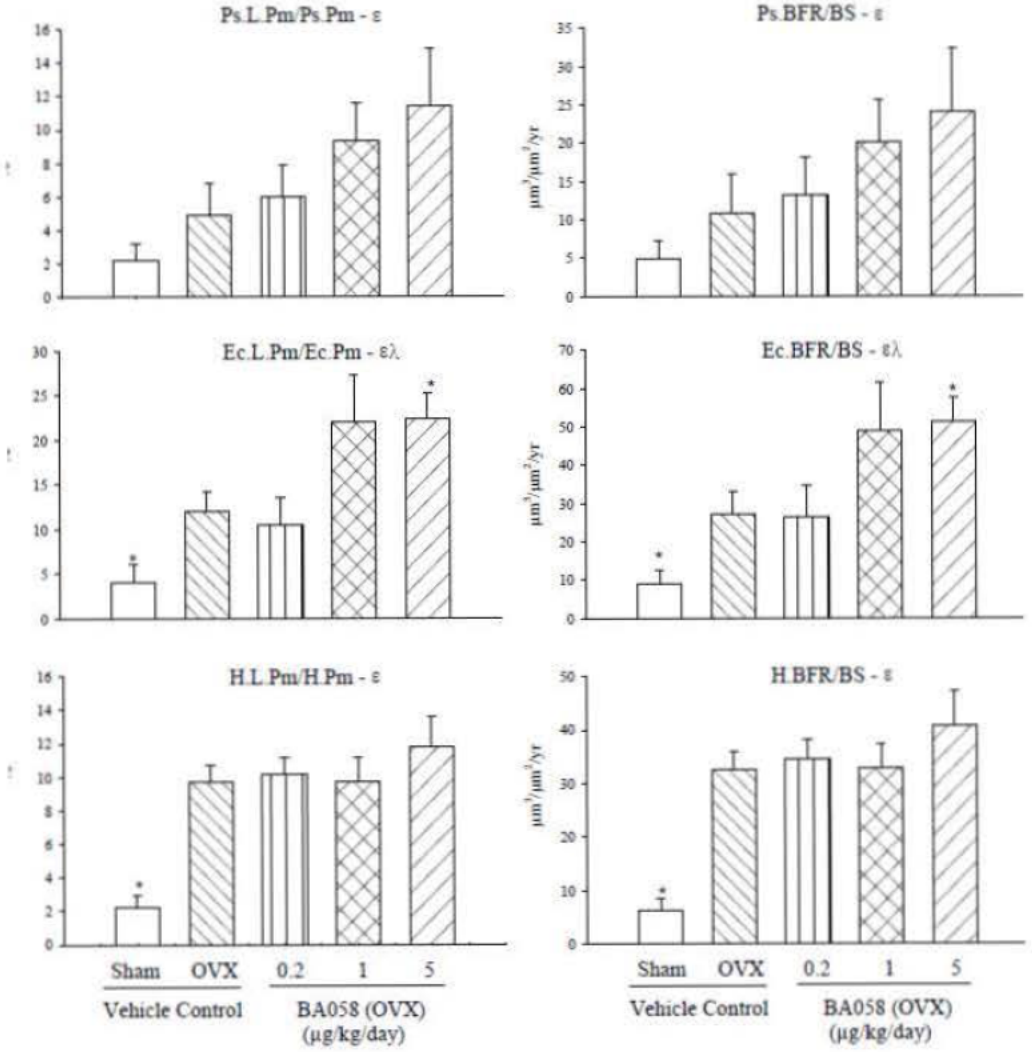
- Total bone area (ie bone thickness) not clearly affected by OVX or Abl.
- Cortical area minimally decreased and Me.Ar minimally increased by OVX. There were no significant dose-related changes related to Abl Tx
- OVX decreased and Abl minimally increased Ct.Wi (consistent with DXA/pQCT data)
- Cortical porosity (Po.Ar) not significantly affected by OVX or Abl
- Bone formation measures (periosteal, endocortical and Haversian BFR/BS) increased by OVX. Abl increased endocortical BFR dose-dependently and significantly and also increased perisotela BFR dose-relatedly. Since there was no net bone deposition at either surface according to ex vivo DXA/pQCT, this suggested increased bone turnover at both surfaces, i.e., increases in peri- and endosteal resorption and formation.
- % Endocortical eroded perimeter increased by OVX, but not affected by Abl Tx. There were no data for erosion at periosteal surface.

Text Table 4
Differences in Histomorphometric Variables of Cortical Bone (Femur Shaft)*

No. Of Animals	16	15	16	16
Treatment/Bone		**OVX - Femur Midshaft**		
BA058 (µg /kg/day)	0	0.2	1	5
Effects of	**OVX**		**BA058**	
Structural parameters				
Tt.T.Ar	-0.85	-2.28	-0.67	-0.28
Ct.Ar	-2.19	-0.34	1.19	0.43
Me.Ar	1.96	-6.24	-4.45	-1.73
Ct.Wi	-3.72	1.70	3.34	1.36
% Po.Ar	6.50	13.60	8.34	26.07
Ec.E.Pm/Ec.Pm	339.07	-65.93	-50.62	6.86
Endocortical dynamic parameters				
Ec.sL.Pm/Ec.Pm	232.97	-20.65	43.20	**70.80**
Ec.dL.Pm/Ec.Pm	154.70	-1.72	131.60	105.61
Ec.L.Pm/Ec.Pm	**191.92**	-11.99	83.65	**86.73**
Ec.MAR	53.95	-2.78	-1.41	17.78
Ec.BFR/BS (µm3/µm2/yr)	**209.79**	-3.00	80.26	**88.89**
Periosteal dynamic parameters				
Ps.sL.Pm/Ps.Pm	158.64	8.70	75.09	82.19
Ps.dL.Pm/Ps.Pm	70.27	58.84	132.62	262.04
Ps.L.Pm/Ps.Pm	124.65	23.32	91.86	134.62
Ps.MAR	-1.43	12.27	41.34	34.86
Ps.BFR/BS (µm3/µm2/yr)	124.16	22.46	86.75	123.21
Haversian dynamic parameters				
H.sL.Pm/H.Pm	**255.44**	4.99	-12.97	18.74
H.dL.Pm/H.Pm	**409.79**	4.70	9.90	24.54
H.L.Pm/H.Pm	**333.88**	4.81	0.68	22.20
H.MAR	**62.87**	-0.71	0.64	1.02
H.BFR/BS (µm3/µm2/yr)	**426.03**	5.89	0.84	25.39
H.BFR/BV	**380.90**	13.63	9.21	39.28

* Expressed as percent difference of group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used

Text Figure 4
Cortical Bone Histomorphometry – Mean (SEM)



Significantly different from OVX vehicle control group value: *-p<0.05
Statistical Analysis Using Heteroscedastic ANOVA Model: ε
Significant linear dose effect: λ -p<0.05

CONCLUSIONS

Exposure:

- AUC at 0.2, 1, 5 ug/kg/day was **0.1x, 0.13x, 0.7x** human AUC of 1546 pg.h/mL. AUC was not dose-proportional.

Hormones:

- Abl increased ViT(OH2) production

Bone resorption and formation:

- Abl increased overall skeletal bone formation by the osteoblast, with a less consistent/smaller increase in osteoclastic bone resorption.
- Based on DXA, pQCT, uCT and HMM, this was due to net increases in bone formation in trabecular and/or cortical bone.

Densitometry:

- Cancellous bone:
  - DXA showed that Abl increased cancellous bone mass, i.e., BMC, BMD and/or Area in vertebrae and long bone ends (proximal tibia, and proximal and distal femur). In 1/3 distal radius, Abl decreased DXA-BMC due to a decrease in bone area.
  - PQCT confirmed increase in trabecular BMD (with decrease in Trab Area) in proximal tibia metaphysis, but had no effect on trabecular BMD (with decrease in Trab Area) in distal radius metaphysis
- Cortical bone:
  - DXA of proximal tibia diaphysis showed that Abl increased total bone area suggesting periosteal (cortical) bone apposition. PQCT did not confirm this for proximal tibia diaphysis, but did show a slight non-dose-related increase in total area of distal radius diaphysis.
  - In proximal tibia meta- and diaphyseal regions there were increases in cortical thickness and decreases in medullary/trabecular area.
  - In distal radius metaphysis, Abl also increased cortical thickness and decreased medullary area
  - In proximal tibia diaphysis, periosteal circumference was slightly decreased, endosteal circumference decreased and cortical thickness increased.
  - In distal radius diaphyses, Abl slightly increased Total Area, periosteal circumference, and endosteal circumference (at 5 ug/kg), while cortical thickness, BMC and BMD were not significantly affected.
- Data from ex vivo DXA/pQCT (whole femur, used for mechanical testing) and micro-CT (distal femur) suggested that significant cortical thickening did not occur in proximal, mid or distal femur.
- DXA showed that for most bones Abl at ca. 1x human AUC increased bone density/content to levels between OVX and sham

Reviewer comments:

- *Data suggested there may be bone site and/or regional differences in the response to Abl. For example, the positive effects of Abl on trabecular and cortical bone mass and/or*

thickness were seen in some bones but not others. This may be due to different sensitivities of different bones to the formation and resorption effects of Abl, OR to differences in the balance between formation and resorption at different sites.

- *Monkey data (proximal tibia) were consistent with rat data showing thickening of bone cortex, although in rats, at a 1-12x multiple of human AUC, the increased cortical thickness was due to both endosteal and periosteal apposition. This may be due to difference in doses or difference in species sensitivities.*

Micro-CT (ex vivo)

- Micro-CT showed that Abl generally reversed OVX-induced decreases in vertebral trabecular parameters (BV/TV, vBMD, Tb.Th and/or Tb.N). Abl also restored OVX-induced decreases in vertebral cortical thickness and BMC.
- In distal femur trabecular bone, Abl also reversed the OVX-induced decreases except for Tb.N. In distal femur cortical bone, Abl had no effect on the OVX-induced decreases in vBMD, tBMD or BV/TV, and did not affect Cortical Thickness or BMC.
- Abl did not significantly affect the increase in humerus cortical porosity caused by OVX.
- Decreased SMI in Abl-treated may have indicated a more plate-like trabecular structure.

Strength:

- Abl clearly increased vertebral bone strength (bodies and cores), reflected by increases in peak load, yield load, stiffness, AUC and toughness.
- Femoral neck peak load was not clearly affected in any group. Unlike in vivo results, ex vivo DXA of the mechanically tested proximal femur (including neck), had also shown no significant effects.
- Femur shaft bending strength was not significantly affected. This was not in line with the proximal tibia cortical bone thickness increase. However, it was consistent with the lack of ex vivo pQCT changes in the tested femur.
- Intrinsic cortical bone strength of the humerus cortical beam was not significantly affected.
- When bone quality (BQ) was defined as slope of regression line between ex vivo BMC and peak load, OVX slightly impaired BQ of vertebral bodies, while Abl at 1 and 5 ukd restored BQ to sham levels. This was not shown for vertebral cores, even though ex vivo trabecular BMC and peak load were better correlated in vertebral cores than bodies. This weakens the significance of the vertebral body finding, unless the vertebral body quality increase is related to vertebral cortex rather than trabecular effects.
- Positive shifts in correlations between in vivo vertebral core BMC or BMD and yield load or apparent strength, respectively, were observed in (0.2), 1 and 5 ug/kg groups.

Histomorphometry (HMM):

- In cancellous bone of vertebrae and femoral neck, Abl caused increases in BV/TV, Tb.Th and Tb.N., consistent w densitometry and uCT. Increased Tb.N. may indicate trabecular tunneling occurred.
- Abl increased Wall Thickness in vertebrae indicating positive bone balance at BMU level
- BFR and related parameters were NOT significantly increased in vertebrae and femoral neck

- There was no effect on Ac.F, suggesting lack of stimulation of remodeling at 16 months
- Cortical width of femur shaft was minimally increased and measures of bone formation eg BFR were increased at BOTH periosteal (largest effect) and endosteal surfaces. The latter increases were expected for the endosteal surface. However, since bone width was unaffected based on various evaluation methods, while BFR was increased, it may suggest a parallel increase in resorption at the periosteal surface. Abl had no effect on % endocortical eroded surface, which may suggest increased osteclast activity in the BRU (bone remodeling unit) rather than an increase in BRU number.
- Cortical porosity was slightly but not significantly affected. Teriparatide and PTH1-84 have large effects on cortical porosity (2x-5x OVX control), particularly near the endocortical surface (Burr et al 2001; Fox et al 2007)

  NOTE: HMM data are from end of study and don't provide information on the events that led to the bone changes observed at this sampling time.

4.2 Secondary Pharmacology

Sponsor conducted 4 in vitro secondary pharmacology studies.

Tabular summary

In vitro studies

Study Nr	Type of Study	Methods	Findings
14RAD005	Target selectivity (abaloparatide)	Binding assay, Enzyme assay Screening: 10 µM (≥50% inhibition criterion) IC50 and Ki (inhib constant) determined	Total of 4 targets identified: Bombesin BB1 Receptor: IC50= 3.29 µM; Ki= 1.56 µM N-Formyl Peptide Receptor-Like FPRL1: IC50= 5.56 µM; Ki= 1.59 µM Orexin OX1 Receptor: IC50= 4.89 µM; Ki= 3.43 µM Vasoactive Intestinal Polypeptide Receptor: IC50= 8.68 µM; Ki= 6.13 µM Conclusion: Safety margins for interaction of abaloparatide with these receptor is >7000-fold, based on Cmax of 812 pg/mL (=0.205nM) at 80 ug clinical dose (Study BA058-05- 001B)
(b) (4)	Potency of degradant (b) (4)	UMR-106 cells exposed to Abl and (b) (4) -degradant and cAMP measured. Doses: 0 (control) (b) (4) pg/ml	(b) (4) compared to abaloparatide to elicit cAMP response EC50 of (b) (4) was (b) (4) ng/ml (EC50 of abaloparatide 0.325 ng/mL) Degradant has (b) (4) compared to product
(b) (4)	Target selectivity for (b) (4)	Binding assay, Enzyme assay (b) (4) (criterion > (b) (4) % inhibition)	Total of 8 targets identified: FPRL1, Orexin OX1, VIP1 receptor, nNOS , Gabapentin (gabapentin- binding protein), Orexin OX2, and sst2 receptors, and NET Conclusion: Safety margins for interaction of (b) (4) with these receptors is (b) (4) -fold, based on maximum Cmax of (b) (4) pg/mL (= (b) (4) nM) at 80 ug clinical dose (Study (b) (4)
09RAD060	Functional activity of abaloparatide fragments on PTH1 receptor	UMR-106 cells Agonist activity: XXpM-100 nM of abaloparatide fragments Antagonist activity: 46 pM-100 nM of abaloparatide fragments in combination of 1 nM abaloparatide	Agonist activity: At concentrations ≥ 200 pM, BM1 (abaloparatide fragment 1-31) stimulated cAMP production in a dose-dependent manner (~ 6× less potent than abaloparatide). Remaining 9 fragments did not significantly increase cAMP production. Agonist activity: The abaloparatide fragments did not antagonize cAMP production induced by 1 nM abaloparatide.

cAMP= cyclic adenosine monophosphate; EC_{50}=median effective concentration; FPRL1=N-Formyl Peptide Receptor- Like;IC_{50}=half-maximal inhibitory concentration; NET=transporter norepinephrine; nNOS=Nitric Oxide Synthase, neuronal; sst2=somatostatin; VIP1=Vasoactive Intestinal Peptide

4.3 Safety Pharmacology

Sponsor conducted in vitro and in vivo safety pharmacology studies. Single dose CNS studies were conducted in rats. Cardiovascular safety was evaluated in in vitro and single and short term repeat dose studies in dogs. Single dose respiratory, renal and hematological studies were

conducted in rats. Single dose gastrointestinal studies were conducted in rats. All studies were GLP compliant except Study BA058-133 (hERG channel study).

Tabular summaries

In vitro and in vivo studies

CNS

Study Number	Organ System	Species / Strain	Route	Doses* (ug/kg)	N/s/g	Findings
BA058-125	CNS (Irwin test)	Rat/Wistar	SC, IV	0.2, 1, 5, 25, 125, 625	F/4	SC: No observable CNS effects at the doses tested. IV: Non-dose-dependent signs of excitation and stereotypies (30 min post dose) at all doses. No signs of neurobehavioral effects up to 625 µg/kg.
BA058-126	CNS	Rat/Wistar	SC	1, 5, 25, 125	F/10	Slight decrease (15%) in number of photocell crossings at 125 µg/kg. No significant effect on spontaneous locomotor activity at 1-25 ug/kg.
BA058-127	CNS	Rat/Wistar	SC	1, 5, 25, 125	F/10	No effect on the sleep-inducing properties of barbital at any of the tested doses.
BA058-128	CNS	Rat/Wistar	SC	1, 5, 25, 125	F/15	No pro- or anticonvulsant effects at any dose tested.
BA058-129	CNS	Rat/Wistar	SC	1, 5, 25, 125	F/10	No significant effect on convulsive and lethal effects of pentylenetetrazole at the doses tested.

**All single dose studies*

Cardiovascular system

Study Number	Organ System	Species / Strain	Method/ Route	Doses/ Concentrations	N/s/g	Findings
BA058-133	Cardiovascular system	HEK-293 cells	*In vitro*	0.1, 0.3, 1, 3, 10, and 30 µM	N/A	Slight, but statistically significant inhibition of hERG current at 1-30 µM. Effect was not clearly dose-related, but maximal inhibition of 14% was achieved at 30 µM (0.1Hz). Positive control E-4031 had IC50 of 32nM. Data indicate weak-moderate concern for QT prolongation. *Reviewer comments:* • *This in vitro study was not GLP-compliant* • *Clinical data have not shown QTc prolongation*
BA058-132	Cardiovascular system	Purkinje fibers from NZ Albino Rabbits	*In vitro*	Prepared conc's: 0.3, 3, 10 µM Achieved conc's: 0.08, 2.16, 7.5 µM	M/6	No effect on RMP, V_{max}, APA, or APD_{30} at all doses. Slight prolongation of APD_{60} and APD_{90} at 2.16 and 7.5 µM, possibly indicating weak blockade of delayed rectifier K channels, or activating effect on Ca channels. One occurrence of EAD at 7.5 µM at 12 pulses/min, but not at 20 pulses/min.

BA058-130	Systemic, cardiac, renal, and pulmonary hemodynamics	Dog/Beagle (anesthetized)	IV	0.03, 0.1, 0.3, 1, 3 µg/kg ascending doses at intervals of 20 minutes	M/3, F/3	Dose-dependent decrease in aortic BP and TPR at ≥0.1 µg/kg. Dose-dependent increase in HR and dP/dt max at ≥0.1 µg/kg. Increase in cardiac output secondary to changes in aortic BP, TPR, and HR. Decrease in stroke volume at ≥0.3 µg/kg. Increase in tension time index (TTI) and left cardiac work (LCW) at 0.3 and 1 µg/kg, and decrease at 3 ug/kg/day. No significant effect on pulmonary artery BP and pulmonary capillary wedge pressure. Increase in renal blood flow at 0.03, 0.1, and 0.3 µg/kg; no effect at 1 µg/kg, and decrease at 3 µg/kg. Dose-dependent shortening of PR and QT intervals at ≥0.3 µg/kg. Marginal prolongation of QTc at 0.03 ug/kg, and QTc shortening at 3 ug/kg.
BA058-131	Cardiovascular system	Dog/Beagle (conscious)	SC	0, 1, 3, 10 µg/kg; total of 4 different doses with at least 48 hr washout between doses	M/2, F/2	Decrease in arterial BP at 3-10 µg/kg. Dose-dependent increase in HR at ≥1 µg/kg. Shortened PR interval at 3-10 µg/kg, and shortened QT interval at all doses, most likely secondary to increases in heart rate. No statistically significant effect on QTc interval. No ABL-related arrhythmia or other changes in ECG profile.

Respiratory system

Study Number	Organ System	Species / Strain	Route	Doses	N/s/g	Findings
BA058-134	Respiratory system	Rat/Wistar	SC	5, 25, and 125 µg/kg (single dose)	F/8	No effect on respiratory parameters.

Renal system

Study Number	Organ System	Species / Strain	Route	Doses (ug/kg)	N/s/g	Findings
BA058-138	Renal system	Rat/Wistar	SC	5, 25, 125 ug/kg (single dose)	F/11-12	Slight increase in urinary volume at 25-125 ug/kg. No effect on urinary pH, or on potassium, creatinine or phosphate excretion. Statistically significant increase in Ca excretion at 25 and 125 µg/kg, with unclear dose-relatedness. Non-dose-related increase in Na excretion, statistically significant at mid dose of 25 µg/kg.

Gastrointestinal system

Study Number	Organ System	Species / Strain	Route	Doses (ug/kg)	N/s/g	Findings

BA058-135	Gastrointestinal system	Rat/Wistar	SC	5, 25, 125 ug/kg (single dose)	F/8	No statistically significant effect on gastrointestinal transit. Slight (12%) non-statistically significant decrease at the highest dose of 125 µg/kg.
BA058-136	Gastrointestinal system	Rat/Wistar	SC	5, 25, 125 µg/kg Daily for 1 or 4 doses	F/8	No ulcerogenic activity after single or 4-day repeat dose treatment at the doses tested.
BA058-137	Gastrointestinal system	Rat/Wistar	SC	5, 25, 125 µg/kg (single dose)	F/8	Slight non-statistically significant reduction (10-15%) in gastric fluid volume and gastric acid secretion at 125 ug/kg. No effects at 5 and 25 ug/kg.

Hematologic system

Study Number	Organ System	Species / Strain	Route	Doses (ug/kg)	N/s/g	Findings
BA058-139	Hematological system	Rat/Wistar	SC	5, 25, 125 µg/kg (single dose)	F/8	No statistically significant effect on bleeding time at any of the tested doses.

APA=action potential amplitude; APD=action potential duration; BP=blood pressure; CNS=central nervous system; conc.=concentration; EAD=early after depolarisation; ECG=electrocardiogram; GI=gastrointestinal; HEK=human embryonic kidney; hERG=human ether-a-go-go-related gene; HR=heart rate; IV=intravenous; N/A=not applicable; PR=time from the onset of the P wave to the start of the QRS complex; QT=depolarisation and repolarisation interval; QTc=depolarisation and repolarisation interval corrected for heart rate; RMP=resting membrane potential; SC=subcutaneous; TPR=total peripheral resistance; Vmax=maximal upstroke velocity.

Review of selected studies

In vivo studies

Hemodynamic Effects of Intravenous Abaloparatide in the Anesthetized Dog
(Study BA058-130, GLP)

A systemic, cardiac, renal, and pulmonary hemodynamics study was conducted in Beagle dogs. Animals were anesthetized, and given ascending IV doses (bolus of 30 sec) of **0.03, 0.1, 0.3, 1, 3 µg/kg** at intervals of 20 minutes (N=3/s/g). Evaluations were performed after 1, 2, 5, 10, 15, 20 minutes post dosing.

Results:

- Dose-dependent decrease in aortic BP (ABP), total peripheral resistance (TPR) and increase in heart rate (HR) at doses of 0.1, 0.3, 1, and 3 µg/kg. Mean aortic BP decreased from baseline by 7, 13, 21 and 45%, respectively, within 1-2 minutes. Mean HR increased by 21, 48, 86 and 48%, respectively, within 1-5 minutes.
- The HR increase/tachycardia was possibly a reflex response to hypotension, but it was suggested that abaloparatidealso induced a positive chronotropic effect.
- Abaloparatide caused a dose-dependent increase dP/dt max (index of myocardial contractility) at doses ≥0.1 µg/kg, indicating also a positive inotropic effect.

- Renal blood flow was increased at 0.03, 0.1, and 0.3 µg/kg, but there was no effect at 1 µg/kg, and a decrease at 3 µg/kg probably due to the decrease in ABP.
- Cardiac output was increased and stroke volume decreased at doses ≥0.3 ug/kg, probably secondary to changes in aortic BP, TPR, and HR.
- Tension time index (TTI) (index of external cardiac work) and left cardiac work (LCW) (index of myocardial work) were increased at 0.3 and 1 µg/kg, but decreased at 3 ug/kg/day. The decrease in TTI and LCW at the highest dose of 3 ug/kg was probably due to the peripheral arterial vasodilatation and hypotension, rather than myocardial depression, although some signs of depression at 3 ug/kg included reduction in dP/dt max and increase in pulmonary capillary wedge pressure at 3 ug/kg.
- No significant effect on pulmonary artery BP (PAP), and left ventricular end diastolic BP (LVEDP) decrease at 0.3-3 ug/kg, suggesting no significant effect on pulmonary circulation or preload.
- Dose-dependent shortening of PR and QT intervals at ≥0.3 µg/kg in response to incraeed HR, without significant prolongation of QTc.
- No arrhythmias or changes in ECG shape.
- It was concluded that abaloparatide exerted peripheral arteriolar vasodilatory effects as well as direct positive chronotropic and inotropic effects on the heart.

Reviewer comments:

- *The data from this anesthetized dog study confirm the expected increase in HR and other cardiovascular parameters due to the known vasodilatation and hypotensive effects of PTH receptor ligands (Pang et al, PNAS, 1980). The study also suggests potential direct positive chronotropic and inotropic effects of abaloparatide on the heart.*
- *Reviewer agrees with Sponsor that the decrease in TTI and LCW at 3 ug/kg/day was likely due to hypotension.*
- *PTHrP has been found to be more potent and more effective than PTH in producing positive inotropic and chronotropic effects in an isolated rat heart preparation (Nickols et al, 1989). This larger potency/effectiveness may also apply to abaloparatide. Part of the increases in heart rate, contractility and cardiac output observed in the abaloparatide dog study may reflect these potent direct effects on the heart.*
- *In Phase 3 Study 003, treatment emergent AEs occurring in >1% of abaloparatide patients and more frequent than placebo included palpitations, dizziness and nausea, and tachycardia, which may have been related to hypotensive and/or cardiac effects of the product.*
- *The adverse findings of orthostatic hypotension related effects including palpitations, dizziness and nausea, and tachycardia are mentioned in Labe Sections 5 (W&P) and 6 (AEs). Syncope was not observed in abaloparatide treated subjects.*
- *The incidence of cardiac events did not appear to be increased vs. placebo in the Phase 3 study.*

Safety pharmacology assessments in toxicity studies:

Evaluation of ECG's was performed in repeat dose monkey toxicity studies. In the 4-week, 13-week or 39-week study, there were no effects on HR or wave intervals at evaluation times of ≥2h after dosing.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The nonclinical ADME/PK study program included single and repeat-dose studies in rats, repeat dose studies in monkeys; and evaluations of tissue distribution and excretion in rats, in vitro and in vivo metabolite profiles, and enzyme and drug transporter induction and/or inhibition. In plasma and serum of rats and monkeys, abaloparatide was measured using validated radioimmunoassay (RIA) methods, while it was measured by HPLC in dose formulations. Biochemical markers of bone turnover in rats and monkeys were measured by validated RIA, ELISA, or EIA methods.

Absorption

Absorption of abaloparatide was evaluated in in single and repeat-dose studies in male and female Sprague Dawley rats (IV, SC) and cynomolgus monkeys (SC). TK data from repeat dose studies are summarized in Section 5.2 ('Toxicokinetics') of this review.

Absorption and bioavailability studies

Study Nr.	Study Type	Species/strain	Route	GLP
Single Dose				
BA058-142	Single-dose pharmacokinetics and bioavailability	Rat/Sprague-Dawley	SC, IV	Y
BA058-143	Preliminary single-dose pharmacokinetics	Rat/Sprague-Dawley	SC	N
Repeat-Dose				
BA058-114	Repeat-dose toxicokinetics from 4-week toxicity study	Rat/Sprague-Dawley	SC	Y
BA058-115	Repeat-dose toxicokinetics from 13-week toxicity study	Rat/Sprague-Dawley	SC	Y
7801-124	Repeat-dose toxicokinetics from 26-week toxicity study	Rat/Sprague-Dawley	SC	Y
10RAD032	Repeat-dose toxicokinetics from 24-month carcinogenicity study	Rat/Fischer 344 Albino	SC	Y
10RAD008	Repeat-dose pharnacokinetics from 42-day pharmacology study	Rat/Sprague-Dawley	SC	N
10RAD029	Repeat-dose pharmacokinetics from 12-month pharmacology study	Rat/Sprague-Dawley	SC	Y
(b) (4)	Repeat dose toxicokinetics from 2-Week Toxicity Study of Abaloparatide spiked with (b) (4)	Rat/Sprague-Dawley	SC	Y
(b) (4)	Repeat dose toxicokinetics from 4-week toxicity study of Abaloparatide spiked with (b) (4)	Rat/Sprague-Dawley	SC	Y

BA058-118	Repeat-dose toxicokinetics from 4-week toxicity study	Monkey/ Cynomolgus	SC	Y
BA058-119	Repeat-dose pharmacokinetics from 13-week toxicity study	Monkey/ Cynomolgus	SC	Y
7801-125	Repeat-dose pharmacokinetics from 39-week toxicity study	Monkey/ Cynomolgus	SC	Y
BA058-109	Repeat-dose pharmacokinetics from 10-month pharmacology study	Monkey/ Cynomolgus	SC	N
10RAD030	Repeat-dose pharmacokinetics from 16-month pharmacology study	Monkey/ Cynomolgus	SC	Y

Bioavailability study of Abaloparatide (10 ug/kg) in Sprague-Sawley Rats
(Study BA058-142)

Rats were given a single 10 ug/kg bolus dose (IV or SC) in 0.2 mL/kg. Blood samples were collected up to 3h post dose. Data are shown in Figure 1, and Tables 1 and 2 below. Upon SC dosing, Cmax was reached by 15 min and declined slowly after 45 min. Serum concentrations were well above LLOQ (0.08 ng/mL) at the last 3h sampling point, with IV and SC dosing. Exposure was moderately higher in males with SC dosing.

MRT (mean residence time) was up to 4 times higher after SC than after IV dosing. AUC values were significantly lower after SC administration, and SC bioavailability was 38.9% in male rats and 27.3% in female rats.

Reviewer comments:
The BA values are similar to the absolute SC bioavailability of abaloparatide in humans (F = 39%) determined in clinical Study BA058-05-010. The reason for the relatively low bioavailability is not known. By comparison, the absolute SC bioavailability of rhPTH1-34 in humans is 95%.

Comparison of T1/2 upon IV and SC dosing with abaloparatide vs teriparatide reveals an interesting result. The T1/2 of IV or SC dosing of abaloparatide in rats is 0.63h and 0.91h, respectively. Similarly, the T1/2 of IV or SC dosing of abaloparatide in humans is 0.87h and 1.7h, respectively. By contrast, the T1/2 of IV dosing of teriparatide in humans is only 8 mins, while the T1/2 of SC dosing is approximately 1.1h.

This explains the relatively large human AUC (1546 pgxh/mL at 80 ug/day) upon SC dosing with 80 ug/day abaloparatide in humans despite its low absolute SC bioavailability (BA) of 39%. By comparison, a human SC dose of 20 ug/day teriparatide, with an SC BA of 95% and a T1/2 of ca. 1.1 hr yields an AUC of approx.. 300 pgxh/mL.

As a result, the AUC in humans at 80 ug/day abaloparatide (1546 pgxh/mL) is ca. 4x larger than the AUC of 20 ug/day teriparatide. However, this is only coincidentally related to the 4-fold difference in doses.

The relatively large T1/2 of IV abaloparatide may be due to relative slow metabolism of this non-endogenous peptide. The T1/2 upon SC dosing of either peptide is 'confined' to relatively large values due to relatively slow absorption from the injection site.

Figure 1: Mean (±SD) Abaloparatide Concentration Following a Single 10 µg/kg IV Bolus or SC Administration to Sprague-Dawley Rats

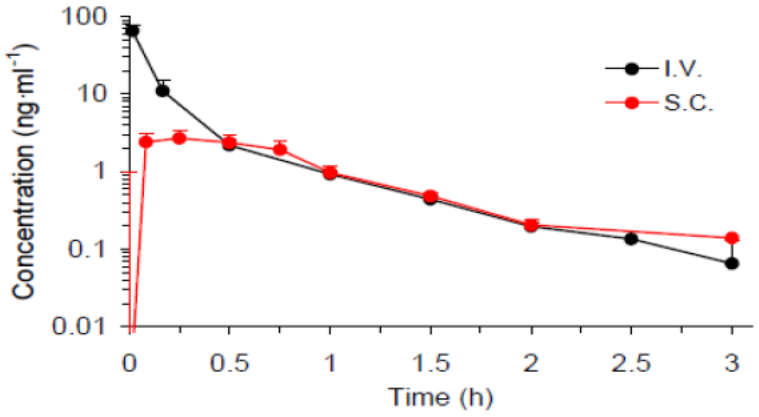


Table 1: PK Parameters in Rats upon IV Dosing

Abaloparatide, 10 µg/kg, i.v.				
		Females	Males	Females + Males
Dose	µg/kg	9 569	9.446	9.507
C0	ng/ml	83 338	73.836	78.579
AUC	ng.h/mL	9 23	8.63	8.92
CL	l/h/kg	1 04	1.09	1.07
Vss	l/kg	0 28	0.35	0.31
Vz	l/kg	0 78	1.32	1.32
T½	h	0 52	0.84	0.63
MRT	h	0 27	0.32	0.29

Table 2: PK Parameters in Rats upon SC Dosing

Abaloparatide, 10 µg/kg, s.c.				
		Females	Males	Females + Males
Dose	µg/kg	9 645	9.650	9.648
Tmax	h	0 250	0.249	0.249
Cmax	ng/ml	2 389	2.992	2.691
AUC	ng.h/ml	2 54	3.44	2.97
T½ gz app	h	1 27	0.75	0.91
MRT	h	1 13	0.90	0.96
Cmax/AUC	1/h	0 941	0.871	0.906
F	%	27 27	38.94	32.81

MRT=mean residence time

Preliminary PK study of abaloparatide (20 ug, SC) in male SC rats
(Study BA058-143)

Male rats were given an SC dose of 20 ug (ca. 58 ug/kg) and PK samples were taken up to 12h post dose. Data are in Table 3, below. Tmax was 30 mins. AUC was 22 ngxh/mL, ca. 7-fold the AUC at the 10 ug/kg SC dose.

Figure 2: PK Profile after a Single SC Dose to Male SD Rats (20 ug/rat)

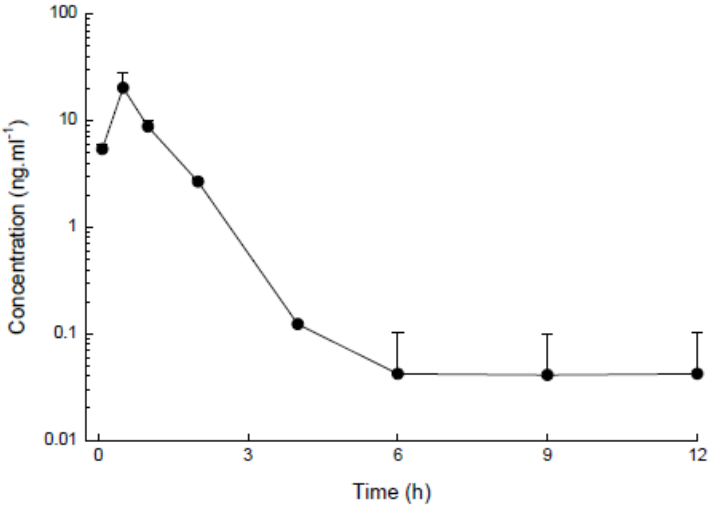


Table 3: Preliminary PK Parameters in Rats (20 ug SC dose/rat)

Abaloparatide, 20 ug SC		
		Values (males)
Dose	µg/kg	57 5
Tmax	H	0 5
Cmax	ng/ml	20 4
AUC(t)	ng·h/ml	21 9
MRT(t)	H	1 05
Cmax/AUC(t)	1/h	0 93
Cmax/D	-	0 36
AUC(t)/D	-	0 38

Single dose PK parameters
Table below shows Cmax, Tmax and AUC values from Day 1, i.e. after a single dose administration, determined mostly in repeat dose studies. Cmax was achieved within 15-30 min post dose in rats, and after 1h in monkeys. AUC0-t exposure increased with dose and T1/2 was generally between 0.5-1hr.

Comparative PK after Single SC dose administration

Species	Dose (µg/kg)	Cmax (ng/mL) [M/F]	Tmax (h) [M/F]	AUC0-t (ng·h/ mL) [M/F]	T½ (h) [M/F]	Study
Rat	1[a]	- / 0.4	- / 0.25	- / 0.2	- / 0.2	12-mo OVX
	5[a]	- / 2.5	- / 0.25	- / 1.4	- / 0.3	Id
	10	3.0 / 2.4	0.3 / 0.3	3.4 / 2.5	0.8 / 1.3	Single dose
	10	4.3 / 3.3	0.3 / 0.3	1.8 / 1.1	0.3 / 0.4	13-wk tox
	10	1.9 / 2.9	0.3 / 0.3	1.2 / 1.5	- / -	26-wk tox
	10	4.1 / 3.4	0.3 / 0.3	2.5 / 2.1	0.3 / 0.3	24-mo carci
	15	7.1 / 5.2	0.5 / 0.5	5.7 / 4.4	0.5 / 0.5	4-wk tox
	25	7.0 / 8.9	0.5 / 0.3	4.1 / 5.0	0.3 / 0.3	13-wk tox
	25	5.7 / 4.9	0.3 / 0.3	3.3 / 2.8	- / -	26-wk tox
	25	17.2 / 19.4	0.3 / 0.3	9.2 / 7.0	0.3 / -	24-mo carci
	25[a]	- /5.6	- / 0.25	- / 4.3	- / 0.4	12-mo OVX
	50	16.8 / 31	0.3 / 0.3	9.6 / 11.2	- / 0.2	24-mo carci
	57	20 / -	0.5 / -	22 / -	- / -	Single dose
	70	21 / 12	0.5 / 0.5	20 / 12	0.5 / 0.4	4-wk tox
	70	17 / 20	0.3 / 0.3	9.3 / 13	0.5 / 0.7	13-wk tox
	70	13 / 11	0.5 / 0.3	9.5 / 8.7	0.3 / -	26-wk tox
	300	62 / 38	0.5 / 0.5	59 / 36	0.6 / 0.6	4-wk tox

[a] Study conducted in ovariectomized females

Species	Dose (µg/kg)	Cmax (ng/mL) [M/F]	Tmax (h) [M/F]	AUC0-t (ng·h/ mL) [M/F]	T½ (h) [M/F]	Study
Monkey	0.2[a]	- / 0.1	- / 0.5	- / 0.08	- / 0.3	16-mo OVX
	1[a]	- / 0.4	- / 0.25	- / 0.3	- / 0.4	16-mo OVX
	5[a]	- / 1.4	- / 0.25	- / 0.8	- / 0.4	16-mo OVX
	10	2.2 / 3.4	0.6 / 0.4	3.5 / 4.4	- / 0.6	39-wk tox
	10	5.4 / 2.4	0.6 / 0.5	9.1 / 4.3	0.9 / 1.1	13-wk tox
	20	11.2 / 8.2	0.7 / 0.4	16.9 / 10.4	0.7 / 0.9	39-wk tox
	50	30 / 11.5	0.6 / 0.4	49 / 14.6	0.8 / 0.9	13-wk tox
	70	24 / 35	0.4 / 0.3	32 / 41	0.6 / 0.5	39-wk tox
	100	68 / 59	0.5 / 0.5	115 / 83	1.1 / 1.1	4-wk tox
	200	87 / 81	0.5 / 0.5	207 / 226	1.1 / 1.4	4-wk tox
	200	71 / 21	0.4 / 0.3	100 / 21	0.7 / 0.8	13-wk tox
	450	161 / 203	0.3 / 0.5	240 / 423	0.9 / 1.3	4-wk tox

[a] Study conducted in ovariectomized females

Species	Dose (µg/kg)	Cmax (ng/mL) [M/F]	Tmax (h) [M/F]	AUC0-t (ng·h/ mL) [M/F]	T½ (h) [M/F]	Study Nr.	Study Type
Human (postmenopausal women)	80 µg /day	0.81	0.41	1.546	1.65	BA058-05-001B	7-day PK, PD, safety

Data are Day 7 parameters from this 7-day repeat dose study (Study BA058-05-001B)

Repeat dose PK parameters

Rat

In rats, there were no consistent differences in PK/TK between males and females. Cmax was observed at 15-30 min. Upon repeat dosing, exposure increased over time, and this was more pronounced at higher dose levels (up to 5.6-fold at 300 µg/kg). Thus, exposures were generally more than dose-proportional after repeat-dosing, and this suggestive of abaloparatide accumulation.

The following TK and PK tables with data from the 13-week rat toxicity study illustrate this. Cmax and AUC increased from Day 1 to 86, particularly in the higher dose groups of 25-70 ug/kg. This corresponded with a decrease in clearance (CL/F). The increased exposure may have been due to kidney toxicity. The 4-week and 26-week rat studies showed the same phenomenon.

TK Parameters in 13-week rat study (Day 1 and 86)

Dose (ug/kg/day)	Parameter	Units	Males		Females	
			Day 1	Day 86	Day 1	Day 86
10	Tmax	hr	0.25	0.25	0.25	0.25
	Cmax	ng/ml	4.262	5.452	3.277	8.825
	AUC0-τ	ng hr/mL	1.785	6.21	1.082	3.317
25	Tmax	hr	0.5	0.5	0.25	0.25
	Cmax	ng/ml	6.980	18.932	8.854	22.330
	AUCτ	ng hr/mL	4.142	19.848	4.967	13.205
70	Tmax	hr	0.25	0.5	0.25	0.25
	Cmax	ng/ml	17.104	104.984	19.981	74.676
	AUCτ	ng hr/mL	9.305	96.540	12.590	68.208

PK Parameters in 13-week rat study (Day 1 and 86)

Dose (ug/kg/day)	Parameter	Units	Males		Females	
			Day 1	Day 86	Day 1	Day 86
10	t½λz	hr	0.29	0.49	0.42	0.31
	Cl/F	(l/hr)/kg	4.772	1.656	4.058	2.596
	MRTt	hr	0.44	0.92	0.32	0.43
	Vss/F	l/kg	2.701	-	3.079	-
25	t½λz	hr	0.28	1.75	0.34	0.33
	Cl/F	(l/hr)/kg	5.515	1.345	4.314	1.715
	MRTt	hr	0.47	1.09	0.47	0.48
	Vss/F	l/kg	3.227	-	2.807	-
70	t½λz	hr	0.46	0.42	0.71	0.47
	Cl/F	(l/hr)/kg	5.700	0.766	5.679	1.082
	MRTt	hr	0.48	0.78	0.83	0.85
	Vss/F	l/kg	4.594	-	5.181	-

Monkey

In monkeys, there were no consistent differences in PK/TK between males and females. Tmax was usually 30 mins, and was not dose-related. After a single dose, T1/2 was 30-50 mins, but was slightly larger after repeat-dosing. As in rats, repeat dosing was generally associated with an increase in exposure, correlated with small increases in T1/2 (increase) and decreases in clearance.

In the 4-week and 13-week monkey studies, some animals had positive pre-dose levels (24h post dose) at Day 28 or 88, while others did not. This appeared to be related to anti-abaloparatide antibody (ADA) development. All animals that developed ADAs had detectable pre-dose abaloparatide levels after 4 or 13 weeks of dosing. However, not all animals with detectable pre-dose levels had developed antibodies. Animals without pre-dose levels had no antibodies.

Anti-abaloparatide antibody development in monkeys appeared to be dose- and/or time-related. This conclusion was supported by the higher incidence of ADA-positive animals in the shorter term toxicity studies at higher dose levels (4- and 13-week studies, doses up to 450 and 200 ug/kg/day, respectively); and the lack of detectable pre-dose levels and a low frequency of ADAs in longer term monkey studies with lower dose levels, including doses of 10, 25, 70/50 µg/kg for 39 weeks (toxicity study; N = 1/28 positive), and doses of 0.2, 1, and 5 µg/kg for 16 months (bone quality study) (N=0 positive).

In the 4- and 13-week studies, exposure (Cmax and AUC) at Day 28 or 88 was higher in animals with pre-dose levels (i.e. antibodies), corresponding with decreased clearance and increased terminal T1/2. By contrast, in animals without pre-dose levels exposure was similar or lower on Day 28 or 88 vs. Day 1. Tk data from the 13-week study are shown below.

Cmax and AUC in monkeys with or without detectable antibody levels in 13-week monkey study

Dose µg/kg/day	Day (antibody status)	Cmax ng/ml	Cmax ratio (D88 vs. D1)	AUCt (ng/ml).h	AUC ratio (D88 vs. D1)
Males					
10	1	5.37	-	9.62	-
	88 (Non-Detectable)	2.44	0.5	4.59	0.5
	88 (Detectable)	6.06	1.1	37.15	3.9
50	1	30.27	-	51.60	-
	88 (Non-Detectable)	13.51	0.4	15.64	0.3
	88 (Detectable)	14.17	0.5	35.90	0.7
200	1	71.37	-	102.79	-
	88 (Non-Detectable)	-	-	-	-
	88 (Detectable)	75.75	1.1	278.20	2.7
Females					
10	1	2.43	-	4.78	-
	88 (Non-Detectable)	2.71	1.1	4.02	0.8
	88 (Detectable)	4.70	1.9	48.15	10.1
50	1	11.52	-	15.59	-
	88 (Non-Detectable)	-	-	-	-
	88 (Detectable)	32.52	2.8	207.22	13.3
200	1	21.14	-	21.73*	-
	88 (Non-Detectable)	-	-	-	-
	88 (Detectable)	48.25	2.3	305.79	14.1

*Value appears to be 10x lower based on results from study BA058-118

In the 39-week study, exposure was similar on Days 1 and 271 and there was no accumulation. There were no sex differences in exposure. Only 1/28 animals developed antibodies (see below).

Antibody formation

Anti-abaloparatide antibodies were analyzed using a validated method in the 26-week, 12-month, and 2-year rat studies, and in 4-week, 13-week and 39-week monkey studies. They were also determined in the long term rat and monkey bone pharmacology studies.

Anti-abaloparatide antibody formation

Study Nr.	Type of Study	Species/Strain	Daily Dose (SC)	Study Duration	Number of Antibody-positive Animals in TK/PK cohort (%)
Rat					
17348 TSR (BA058-114)	Repeat-Dose Toxicity	Rat/SD	0, 15, 70, 300 µg/kg	4 weeks	0
BA058-115	Repeat-Dose Toxicity	Rat/SD	0, 10, 25, 70 µg/kg	13 weeks	0
10RAD029	Pharmacology	Rat/SD	0, 1, 5, 25 µg/kg	12 months	3/54 (5.6%) at 6 months 2/50 (4%) at 12 months
10RAD032	Carcinogenicity	Rat/F344	0, 10, 25, 50 µg/kg	24 months	2/72 (2%)
Monkey					
BA058-118	Repeat-Dose Toxicity	Monkey/ Cynomolgus	0, 100, 200, 450 µg/kg	4 weeks	3/18 (16%)
BA058-119	Repeat-Dose Toxicity	Monkey/ Cynomolgus	0, 10, 50, 200 µg/kg	13 weeks	13/36 (36%)
7801-125	Repeat-Dose Toxicity	Monkey/ Cynomolgus	0, 10, 25, 70 µg/kg	39 weeks	1/28 (4%)
BA058-109	Pharmacology	Monkey/ Cynomolgus	0, 0.1 (/10 µg/kg/week), 1, 10 µg/kg	10 months	4/28 (14%)
10RAD030	Pharmacology	Monkey/ Cynomolgus	0, 0.2, 1, 5 µg/kg	16 months	0

Development of anti-abaloparatide antibodies did not occur frequently in the rat. In rats, antibodies were observed only in the long term 12-month bone quality study and the 24-month carcinogenicity study. Antibody development and/or predose levels of abaloparatide in 4-week and 13-week monkey studies was associated with higher exposures (except in the 4-week study females). This may have been due to reduced elimination of ABL-Ab complexes. Neutralizing activity of ADAs was not determined.

Distribution and excretion

Tissue distribution and excretion studies were conducted in Sprague Dawley rats.

Distribution and excretion studies

Study Nr.	Type of Study	Test System	Route
BA058-144	Preliminary ADME study of	Rat/Sprague-Dawley	SC, IV

	^{125}I-Tyr-abaloparatide		
15RAD103	Distribution and excretion of ^{125}I-abaloparatide after a single SC dose in rats	Rat/Sprague-Dawley	SC
14RAD024	In vitro protein binding of abaloparatide in rat, dog, monkey, and human plasma	Plasma centrifugate from Rat/Sprague-Dawley, Dog/Beagle, Monkey/ Cynomolgus, Human	In vitro

Study BA058-144 was conducted to evaluate distribution with ^{125}I-Tyr-abaloparatide. This study was also conducted to provide data on metabolism profile. However, the tyrosine labelling was unstable leading to fast dehalogenation of a fraction of the I-labeled compound and thus a distribution pattern mirroring that of free iodine.

Study 15RAD103 evaluated the distribution of ^{125}I-abaloparatide, which was labelled at histidine residues 5, 9 and 30.

- Rat (M/F) received a single SC dose of 100 ug/kg ^{125}I-abaloparatide (110 µCi/kg, or 4.1 MBq/kg). Whole body autoradiography was performed through 168 h post-dose (N=1 male each for 8 time points). Blood samples were collected up to 120 h post dosing and mass balance was determined.
- In the blood compartment, the highest radioactive concentrations were observed at 1/4-1/2 h post-dosing (peak concentrations 84 and 104 ng.eq./g, mean for M and F). In blood and plasma radioactivity declined with T1/2 of 6.6-8.3 hrs. Ratio of blood to plasma radioactivity was <1, suggesting little association of abaloparatide with the cellular blood compartment.
- Radioactivity was rapidly distributed to tissues. Tmax in most tissues was 0.5 or 2h. The tissues with the highest peak concentrations (in ng.eq. ^{125}I-Abl/g), were *renal cortex (872), kidney (808), renal medulla 570), pancreas (308), liver (152), and skeletal muscle (144)*. Lowest levels were found in testes (39), abdominal fat (31) and seminal vesicle (29). Bone and bone marrow attained relatively low peak values of 49 and 73 ng.eq./g. Radioactivity at the dosing site declined with similar rate as in other tissues. Elimination from most tissues was indicated by marked decreases in tissue concentrations at 24-48h post-dosing.
- Peak radioactivity in CNS was lower than peak blood concentration. Tmax was 2h post dose and activity declined to BLQ by 48hrs, suggesting low levels of transport across the blood-brain barrier.
- Approx. 95% of the administered dose was excreted in rat urine in the first 48h post-dosing. At 7 days post-dosing, ca. 90% of the dose was excreted in urine, and 4.7% in feces (males and females). Thus, renal clearance is the primary route of elimination.
- ^{125}I-labelled abaloparatide had similar potency as unlabeled parent (Study 15RAD113).

Plasma protein binding was evaluated in Study 14RAD024 in dog, monkey and human plasma. The fraction that was not bound to plasma proteins ('free' fraction) was similar in dogs (26% in males, 29% in females) and humans (26% in males, 30% in females), but

slightly higher in monkeys (43% in males, 53% in females). There were no protein binding data for rats.

Reviewer comment:
For calculation of exposure multiple total concentrations are used. For monkey exposure multiples, correction would lead to slightly larger multiple values. Thus the multiples that were calculated in this review based upon nominal doses are conservative values.

Metabolism

Abaloparatide is a 34 amino acid peptide, and is predicted to be rapidly degraded into fragments by multiple proteases.

Metabolism studies

Study Nr.	Type of Study	Test System	Route	GLP
02/PKE/034 (BA058-144)	Preliminary ADME study of ^{125}I-Tyrosine-abaloparatide	Rat/Sprague-Dawley	SC, IV	N
BA058-145	Preliminary study - metabolism of ^{125}I-Tyrosine- abaloparatide in rat tissue homogenates and purified enzymes	Rat liver and kidney homogenates, cathepsin B, chymotrypsin	In vitro	N
TNED-09-0198	Peptide fragment confirmation	Human liver and kidney homogenates	In vitro	N
15RAD105	Metabolite profiling and identification	Rat plasma, urine, and feces	SC	N
14RAD007	CYP induction in human hepatocytes	Human hepatocytes	In vitro	N
14RAD023	CYP inhibition in human hepatic microsomes	Human hepatic microsomes	In vitro	N
15RAD106	Inhibition of a panel of human drug transporters	HEK293 cells Caco-2 cells	In vitro	N

Study BA058-145 (using ^{125}I-Tyr-ABL) was conducted to evaluate in vitro metabolism using rat liver and kidney homogenates and Study TNED-09-0198 evaluated in vitro metabolism in human liver and kidney homogenates using LC-TOF MS. Study BA058-144 (with ^{125}I-Tyr-ABL) in male SD rats was conducted to obtain data on in vivo metabolism in addition to the distribution/excretion data mentioned above. Study 15RAD105 (using ^{125}I-ABL) evaluated the in vivo metabolite profile in rat plasma, urine and feces.

Study BA058-144 was not informative due to dehalogenation of the tyrosine group.

Study BA058-145, using HPLC analysis of metabolites in the protease-treated rat homogenates suggested that abaloparatide can be degraded in multiple tissues by non-specific proteolytic mechanisms. Three main metabolites were detected, two of which were formed in liver and kidney, and one in kidney only. Data suggested rapid metabolism into smaller peptides. Data were confounded by the use of the 125I-Tyr-labeled compound.

In human kidney and liver homogenates (3 donors) incubated with abaloparatide (Study TNED-09-0198), 15 peptide fragments were found in total. M1 (Mw 3650 amu) was the major metabolite (7-24%). A large fraction of parent remained in kidney and liver. Data again indicated rapid degradation by proteolytic mechanisms.

Pharmacology Study 09RAD60 tested the 10 most prevalent fragments from Study TNED-09-0198 for activity in rat cell line UMR-106 at concentration up to 100 nM. Only one of these had agonist activity, but it was ca. 6 times less potent than parent compound (active at 1nM). The tested fragments were unable to antagonize cAMP production by 1 nM abaloparatide.

The calculated in vitro metabolic clearance of abaloparatide for both rat and human tisuues suggested that metabolic elimination was several-fold higher in kidney than in liver homogenates.

Data from Study 105 on plasma, urine, feces, and cage wash (LC-MS analysis) from 100 ug/kg SC-dosed rats showed mainly metabolites in 0-24h pooled plasma sample. Abaloparatide was not detected in urine but there were 18 metabolites. Feces contained 3 metabolites. Data suggested rapid degradation into multiple peptide fragments, with renal elimination as the major route of excretion.

Abaloparatide did not inhibit or induce CYP enzymes in human hepatocyte cultures and microsomes (Studies 14RAD007 and 14RAD023).

The results of all studies indicate that the metabolic pathway is consistent with non-specific proteolytic cleavage, in both rat tissues and in human liver and kidney preparations.

The drug transporter phospho-glycoprotein (P-gp) in HEK293 cells and the breast cancer resistant protein (BCRP) in Caco-2 cells were not inhibited by abaloparatide (Study 15RAD106).

No nonclinical drug interaction studies were conducted.

5.2 Toxicokinetics

For rats, TK data were derived from 4-week, 13-week, and 26-week SC toxicity studies, and a 2-year SC carcinogenicity study. For monkeys, TK data were derived from 4-week, 13-week, and 39-week SC toxicity studies. Only AUC data from the toxicity studies are given in these toxicokinetics tables. Data on Cmax and Tmax are provided in the single dose and 13-week study PK/TK tables above. The AUC values in these tables were used to calculate [animal:human] exposure multiples for the toxicity findings.

Rat

Rat Toxicokinetics (repeat dose toxicity studies)

Study Number	Species	Study Duration, Dose Frequency	Time Points (TK samples)	Dose (µg/kg)	Male AUC (pg·hr/mL)		Female AUC (pg·hr/mL)	
BA058-114	Rat/Sprague-Dawley	4 weeks, daily	Day 1 and Day 28: 30min, 1h, 3h, 6h, 12h and 24h post dosing		Day 1	Day 28	Day 1	Day 28
				0	-	-	-	-
				15	5815	6995	4448	4516
				70	20752	43172	11648	29641
				300	58994	328820	37020	198370
BA058-115	Rat/Sprague-Dawley	13 weeks, daily	Day 1 and Week 13: 15 min, 1h, 4h, 12h and 24h post dosing		Day 1	Week 13	Day 1	Week 13
				0	-	-	-	-
				10	2056	6240	2417	3979
				25	4706	19957	6016	15647
				70	12791	96718	12838	68451
7801-124	Rat/Sprague-Dawley	26 weeks, daily	Day 1 and Weeks 13 and 26: 5min, 15min, and 30 min post dosing Weeks 13 and 26: 1h, 2h and 4h post dosing		Day 1	Week 13 / Week 26	Day 1	Week 13 / Week 26
				0	-	- / -	-	- / -
				10	1221	5141 / 2598	1468	3056 / 2783
				25	3267	11962 / 12315	2752	10117 / 10472
				70	9469	48506 / 44228	8683	38628 / 29234

Rat toxicokinetics (2-year carcinogenicity study)

Study Number	Species	Study Duration, Dose Frequency	Time Points (TK samples)	Dose (µg/kg)	Male AUC (pg·hr/mL)		Female AUC (pg·hr/mL)	
10RAD 032	Rat/Sprague-Dawley	104 weeks, daily	Wk 26 and Wk 52		Wk 26	Wk 52*	Wk 26	Wk 52*
				0	-	-	-	-
				10	4940	7440	3260	6290
				25	19858(c)	**24000**	11021(c)	**27300**
				50	31300	**42700**	30500	**46500**

* By Wk 52, at 25 and 50 ug/kg/day, values were no longer higher in males than females (**bolded**)
(c) corrected for missing post-dose values, for the 3h samples (M) or the 2h and 3h samples (F)

Monkey

Monkey Toxicokinetics (repeat dose toxicity studies)

Study Number	Species	Study Duration, Dose Frequency	Time Points (TK samples)	Dose (g/kg)	Male AUC (pg·hr/ml)		Female AUC (pg·hr/mL)	
BA058-118	Monkey/ Cynomolgus (3/s/g)	4 weeks, daily	Day 1 and Day 28: pre-dosing and 0.25h, 0.5h, 1h, 4h, 12h and 24h after dosing		Day 1	Day 28	Day 1	Day 28
				0	-	-	-	-
				100	119990	61090(1)	91700	72880(1)
				200	213040	146690(2)		178770(2)
				450	243510	238290(1)	246740	320470(1)
				100		642200(2)		144242(1)
				200		659460(1)	446570	-
				450		420250(2)		197520(1)
BA058-119	Monkey/ Cynomolgus (6/s/g)	13 weeks, daily	Day 1 and Week 13: pre- dosing and 0.25h, 0.5h, 1h, 4h, 12h and 24h after dosing		Day 1	Week 13	Day 1	Week 13
				0	-	-	-	-
				10	9620	4590(3)	4780	4020(3)
				50	51600	15630(3)	15590	-
				200	102790	-	21730	-
				10		41830(4)		21090(4)
				50		39050(4)		293310(4)
				200		321230(4)		283190(4)
7801-125	Monkey/ Cynomolgus (6 or 4/s/g)	39 weeks, daily	Day 1 and Week 39: pre-dosing and 5min, 15min, 30min, 1h, 2h, and 4h		Day 1	Week 39	Day 1	Week 39
				0	-	-	-	-
				10	3493	4147	4421	5342
				25	16897	15581	10422	11661

			after dosing	70 50	32282	- 28578	40852	- 27728

1 Values from only 1 animal
2 Values from 2 animals
3 Values from animals with non-detectabel antibodies
4 Values form animals with detectable antibodies

Written summary

Abaloparatide is absorbed quickly, reaching C_{max} within 30-40 minutes in rats and monkeys. The plasma $T_{1/2}$ is approximately 30 min (rat) to 60 min (monkey). In the rat, it is rapidly distributed to tissues and mainly excreted in the urine (95% within 2 days). Protein binding was similar in dogs and humans (25-30%), but slightly higher in monkeys (ca. 50%). Rat protein binding data were not available. Studies with radioactively labelled abaloparatide suggested that its metabolism consists of rapid degradation by non-specific proteolytic mechanisms, in both rat and human liver and kidney preparations. The degradation results in the formation of mostly inactive peptide fragments. Elimination of abaloparatide via proteolytic degradation appeared faster in the kidney than in the liver. No nonclinical PK studies were conducted to assess the potential of abaloparatide drug interactions. Abaloparatide did not inhibit or induce CYP enzymes in human liver preparations. TK data were available from all pivotal repeat dose toxicity studies. Antibody formation was observed at relatively high doses in monkeys, and was associated with higher exposure. However, ADA formation did not appear to affect toxicity.

General Toxicology

General Toxicity

6.1 Single-Dose Toxicity

Tabular Summary

Single dose toxicity studies in mice and rats (SC dosing)

Study Number	Species/ Strain	Route/ Vehicle	Dose	N/s/ g	Necro psy	Maximum Non-lethal Dose	Findings
BA058-110	Mouse/OF1	IV (saline[a])	0, 42mg/kg	5	Day 15	42 mg/kg	No mortality Hypoactivity on D1 and D2 LD_{50} >42 mg/kg
BA058-111	Mouse/OF1	SC (saline[a])	0, 42mg/kg	5	Day 15	42 mg/kg	No mortality LD_{50} >42 mg/kg
BA058-112	Rat/SD	IV (saline[a])	0, 42mg/kg	5	Day 15	42 mg/kg	No mortality Transient hypoactivity or sedation, unsteady gait, lateral recumbency and dyspnea after treatment on Day 1 LD_{50} >42 mg/kg
BA058-113	Rat/SD	SC (saline[a])	0, 42mg/kg	5	Day 15	42 mg/kg	No mortality LD_{50} >42 mg/kg

[a] 0.9% NaCl, pH 4.0 with 0.01N HCl
IV=intravenous; SC=subcutaneous

Written Summary

Studies in mice and rats showed transient hypoactivity or sedation, unsteady gait, lateral recumbency and dyspnea upon single IV dosing in mice and rats. There were no effects on body weight gain and no necropsy findings. The peptide was well tolerated and caused no mortality upon acute dosing with up to 42 mg/kg.

6.2 Repeat-Dose Toxicity

Repeat dose toxicity studies in rats

Tabular Summaries

Rat, 4-week study (SC, daily dosing)

Study Number	Species, Strain	Study Duration	Doses (ug/kg/ day)	N/s/g	Findings	NOAEL LOAEL
BA058-114	Rat, SD [9 wks old]	4 weeks No recovery period	0, 15, 70, 300 ukd (in saline)	10	**15, 70, 300 ug/kg/day** Reddish color, extremities (M and F) and testis RBC/Hb/MCV/MCHC decreases (M and F) Thrombocyte decrease (M and F) Eosinophil decrease (M and F)	**NOAEL <15 µg/kg/day (AUC multiple < 3.7x)**

		Test facility: CIT, Evreux,France GLP			PT/APTT decrease (M) Alb/Glob ratio decrease (M and F) ALT decrease (M) Urine Ca increase @3h post-dose (M and F) Urine Ca/creat ratio increase (M and F) Bone (femur): Subendosteal fibrobl proliferation, osteoblasts and osteoclasts (not seen in controls, no clear dose-dependence) Histomorphometric increase in trabecular number and thickness, and decrease in marrow space Liver and Spleen: Increased hematopoiesis (M and F) NOTE: No effect on epididymal sperm count, viability, or morphology **70, 300 µg/kg/day** Fibrinogen increase (M and F) Serum Ca increase @3h post-dose (M and F) Glucose decrease (M and F) Urea, creatinine increase (M and F) Total protein increase (M) Cholesterol increase (M) Adrenal: rel weight increase (F) Spleen: rel weight increase (M and F) Kidney: rel weight increase (F) Spleen: enlarged (M and F) Bone (femur): Woven bone formation (M and F) **300 µg/kg/day** Urine P/creat ratio increase	**LOAEL = 15 ug/kg/day (AUC multiple = 3.7x)** Sponsor NOAEL = 15 ukd)

Rat, 13-week study (SC, daily dosing)

Study Number	Species, Strain	Study Duration	Doses (ug/kg/day)	N/s/g	Findings	NOAEL LOAEL
BA058-115	Rat, SD [5 wks old]	13 weeks No recovery period Test facility: CIT, Evreux,France GLP	0, 10, 25, 70 ukd (in saline)	10	**10, 25, 70 ug/kg/day** Reddish color, extremities (M and F) and testis RBC/Hb/MCV/MCH decreases (M and F) Eosinophil decrease (F) APTT decrease (M) Serum P decrease @3h post dose (M and F) Fibrinogen increase (M and F) Trig decrease (M and F) Alb/Glob ratio decrease (M and F) AST, ALT decrease (M and F) ALT decrease (M) Urine Ca increase (M and F) Urine Ca/creat ratio increase (M and F) Spleen: Increased hematopoiesis and megakaryocytosis (M and F) Bone (femur):	**NOAEL < 10µg/kg/day (AUC multiple < 3.3x)** **LOAEL = 10 ug/kg/day (AUC multiple = 3.3x)** Sponsor NOAEL = 25 ug/kg/day

					Histomorphometric increase in trabecular number and thickness (femur, vertebrae), and decrease in marrow space Subendosteal fibroblast proliferation, osteoblasts and osteoclasts (not observed in controls, but no clear dose-dependence) Woven bone formation **25, 70 µg/kg/day** Serum Ca DECREASE at 4 wk time point @24h post dose, in M and F Serum P decrease (M and F) Glucose decrease (M and F) Urea increase (M and F) Total protein increase (M) Urine Ca increase (M and F) Urine P decrease (F) TRAP increase (M and F) Spleen: abs and rel weight increase (M and F) Kidney: rel weight increase (M) Spleen: enlarged (M and F) Bone (femur): Woven bone formation (M and F?) **70 µg/kg/day** Serum Ca INCREASE at 4, 8, 13 wks @3h post-dose and at 13 wks @24h post-dose (M only) Cholesterol increase (M) ALKP increase (M and F) Urine P/creat ratio increase (F only) Heart: abs and rel weight increase (M and F) Bone (vertebrae): Subendosteal fibrobl proliferation, osteoblasts and osteoclasts *Reviewer comment:* *Data on 3h post dose serum Ca at 4, 8, 13 wks shown in Table below*	

Rat (13-week study): Plasma calcium @ 3h and 24h post-dose

Dose (µg/kg/day)	Gender	Plasma Ca (mmol/L) (3h postdose)			Plasma Ca (mmol/L) (24h postdose)		
		Wk4	Wk8	Wk13	Wk4	Wk8	Wk13
0	Males	2.86	2.80	2.88	2.90	2.84	2.74
	Females	2.85	2.82	2.91	2.92	2.90	2.82
10	Males	2.92	2.84	2.85	2.82	2.78	2.71
	Females	2.92	2.88	2.88	2.82	2.84	2.72
25	Males	2.97	2.85	2.88	**2.79***	2.79	2.70
	Females	2.91	2.87	2.92	**2.77****	2.80	2.72
70	Males	**3.13****	**3.03****	**3.05****	2.85	2.92	**2.84***
	Females	2.94	2.89	2.91	**2.81***	2.90	2.75

* $p<0.05$; statistically significant from controls
** $p<0.01$; statistically significant from controls

Rat, 26-week study (SC, daily dosing)

Study Number	Species, Strain	Study Duration	Doses (ug/kg/day)	N/s/g	Findings	NOAEL LOAEL
7801-124	Rat, SD [7 wks old]	26 weeks No recovery period Covance Labs, VA, USA GLP	0, 10, 25, 70 ukd (in saline)		**10, 25, 70 µg/kg/day** Red skin (M>F), dose-related RBC/MCV/MCH decreases (M and F), dose-related Thrombocyte decrease (F) Eosinophil decrease (M and F) Monocyte decrease (M and F) No serum Ca effect, at 13 or 26 wks (@pre-dose – not specified) Lung: Abs weight increase (M and F) Spleen: Extramedullary hematopoiesis (M and F), dose-related Adrenal cortex: Vacuolation of zona glomerulosa (M) Renal pelvis: Mineral (F), dose-related Bone (femur, sternum): Bone and marrow: Hyperostosis (M and F), dose-related **25, 70 µg/kg/day** BW, FC increase (M and F) Hb decrease (M and F), dose-related Reticulocyte increase (M and F), dose-related PT decrease (D184) (M and F) APTT decrease (D86/184) (M and F) ALKP increase (M and F), dose-related Alb/Glob ratio decrease (M and F), dose-related Serum P decrease (M and F) Spleen: abs and rel weight increase (M and F); and adrenal weight increase, dose-related Adrenal cortex: Vacuolation of zona glomerulosa (M,F), dose-related Renal pelvis: Mineral (M and F), dose-related **70 µg/kg/day** Mortality: 1F sacrificed on D133 with hindlimb paralysis (lymphosarcoma) Serum BUN increase (M and F) Albumin decrease (M and F) Globulin increase (M and F) Alb/Glob decrease (M, F) Kidney: abs weight increase (M and F) Uterus: abs weight increase (F)	**NOAEL < 10 µg/kg/day (AUC multiple < 1.7x)** **LOAEL = 10 ug/kg/day (AUC multiple = 1.7x)** Sponsor NOAEL = 25ug/kg/day

Toxicokinetics and exposure multiples

Rat Toxicokinetics

Study Number	Species/ Strain	Study Duration, Dose Frequency	Time Points (TK samples)	Dose (µg/kg)	Male AUC (pg·hr/mL)		Female AUC (pg·hr/mL)	
BA058-114	Rat/Sprague-Dawley	4 weeks, daily	Day 1 and Day 28: 30min, 1h, 3h, 6h, 12h and 24h post dosing		Day 1	Day 28	Day 1	Day 28
				0	-	-	-	-
				15	5815	6995	4448	4516
				70	20752	43172	11648	29641
				300	58994	328820	37020	198370
BA058-115	Rat/Sprague-Dawley	13 weeks, daily	Day 1 and Week 13: 15 min, 1h, 4h, 12h and 24h post dosing		Day 1	Week 13	Day 1	Week 13
				0	-	-	-	-
				10	2056	6240	2417	3979
				25	4706	19957	6016	15647
				70	12791	96718	12838	68451
7801-124	Rat/Sprague-Dawley	26 weeks, daily	Day 1 and Weeks 13 and 26: 5min, 15min, and 30 min post dosing Weeks 13 and 26: 1h, 2h and 4h post dosing		Day 1	Week 13 / Week 26	Day 1	Week 13 / Week 26
				0	-	- / -	-	- / -
				10	1221	5141 / 2598	1468	3056 / 2783
				25	3267	11962 / 12315	2752	10117 / 10472
				70	9469	48506 / 44228	8683	38628 / 29234

Rat exposure multiples (Females only)[1]

Study Nr.	Study Duration	Dose (µg/kg)	NOAEL	End of Study	
				AUC (pg h/mL)	**AUC Multiple***
BA058-114	4 weeks	15	<15	4516	**2.9x**
		70		29641	**19.2x**
		300		198370	**128x**
BA058-115	13 weeks	10	<10	3979	**2.6x**
		25		15647	**10.1x**
		70		68451	**44.3x**
7801-124	26 weeks	10	<10	2783	**1.8x**
		25		10472	**6.8x**
		70		29234	**18.9x**

[1] Female AUC values were used for these calculations since they were usually lower than male values
*Human AUC = 1546 pg h/mL (7-day Study BA058-05-001B)

Written summary
(Rat toxicity studies)

The test compound was well tolerated by rats. There was no mortality at doses up to 128x and 44x human AUC at the clinical 80 ug/day dose in the 4-week and 13-week toxicity studies. One 70 ug/kg/day female was sacrificed moribund on Day 133 (4 months) with hindlimb paralysis and had lymphosarcoma which was most likely not treatment-related.

A major part of the findings in all three toxicity studies were related to the pharmacodynamic activity of abaloparatide, i.e., to increase serum calcium through effects on bone and/or kidney and to exert a net anabolic effect on bone. However, the pharmacologic or toxicologic mechanism of some findings was unclear.

Clinical sign in all rat studies was reddening of skin and tesis. This is related to peripheral dilation by PTH and PTHrP peptides. The vasodilation is accompanied by compensatory heart rate increases (not measured in rats). PTH and particularly PTHrP, and thus also PTHrP-analog abaloparatide, have direct positive inotropic and chronotropic effects on the heart.

The hematologic effects, e.g. RBC decrease (anemia) and possibly the other blood cell changes (thrombocyte, eosinophil) were either an indirect effect of increased cortical bone formation and narrowing of the hematopoietic bone marrow space, or a direct effect on hematopietic cells. A compensatory increase in spleen extramedullary hematopoiesis was an adaptive response to the anemia.

Plasma Ca was slightly increased, generally @ 3h post-dose, at doses 70 or 300 µg/kg/day, as measured in the 4- and 13-week studies. Serum Ca levels declined at time points after 3 hours post-dose and returned to baseline or below baseline at 24h post-dose (pre-dose time). There was a parallel increase in urinary Ca excretion at lower doses of ≥10 µg/kg/day indicating that the serum Ca increase was at least partly due to increased bone resorption. However, it is possible that renal Ca reabsorption may have been increased too. Not all Ca fluxes were examined.

Plasma phosphorus levels were reduced at doses of 25 and 70 ug/gk/day @3 hours post-dose in 13- and 26-week studies, but were unaffected at later times. Urine P excretion was increased at higher doses of 300 and 70 ug/kg/day in 4-, 13- and 26-week studies.

ALKP was elevated in 13 and 26-wk studies, at doses of 25 and/or 70 ug/kg/day. This was probably due to increases in bone-specific bone ALKP reflecting increased osteoblast activity.

Albumin/globulin ratios were decreased at ≥10 µg/kg/day in all three studies and total protein increases were seen at doses ≥25 ug/kg/day in 4- and 13-week studies. The cause of these effects is unclear.

Histologic effects related to the expected PD effect of abaloparatide included bone effects, such as subendosteal fibroblast proliferation, appearance of osteoblasts and osteoclasts in treated groups, sometimes with dose-related severity and woven bone formation. Histomorphometric measurements in 4- and 13-week studies revealed increase in trabecular number and thickness,

and decrease in marrow space. Hyperostosis of bone and marrow was observed in the 26-week study. The bone effects were seen at all doses in all three rat studies. The compensatory increase in urinary Ca excretion prevented serum Ca increases at the lower doses.

Kidney toxicity was evidenced by increases in serum urea and creatinine and increased kidney weight at doses ≥25 ug/kg/day in all three rat studies. Renal pelvis mineral was also observed at 70 ug/kg/day in the 26-week study.

In rats, accumulation occurred, so that the end-of-study AUC values were always higher than those at Day 1. PK data indicated concomitant decrease in clearance (see PK/ADME section of review) and slight increase in T1/2. The reason for this TK change over time is not entirely clear. It may have been related to kidney toxicity causing reduced efficiency of renal peptide (fragment) excretion. Saturation of renal elimination/transport mechanisms responsible for excreting the peptide and its fragments leading to nonlinear plasma PK might have caused the larger than dose proportional AUC increases in 4- and 13-week studies, however, such an increase was not obvious in the 26-week study.

The toxicity studies were generally adequate and the PD and/or toxic effects were dose-related.

Repeat dose toxicity studies in monkeys

Tabular Summaries

Monkey, 3-day study, (SC, daily dosing)

Study Report	Species, Strain	Study Duration	Doses (ug/kg/day)	N/s/g	Findings	NOAEL LOAEL
BA058-116	Monkey, Cynomolgus	3 days, DRF study	Abaloparatide: 0.75, 7.0, 17.5 hPTH(1-34): 0.75	2	Abaloparatide: **≥0.75 µg/kg/day:** Total blood Ca increased, but levels remained within physiological range. hPTH(1-34) **≥0.75 µg/kg/day:** Total blood Ca increased	NA (limited toxicological assessment performed in this study)

Data from 14-day study in monkeys not included (BA-058-117)

Monkey, 4-week study, (SC, daily dosing)

Study Report	Species, Strain	Study Duration	Doses (ug/kg/day)	N/s/g	Findings	NOAEL LOAEL
BA058-118	Monkey, Cynomolgus [≥24 mo old]	4 weeks No recovery period Test facility:	0, 100, 200, 450 (saline vehicle)	3	**100, 200, 450 µg/kg/day** No ECG abnormalities No ophthalmology abnormalities Slight RBC decreases ALKP increase, dose-related Antibodies in 1/3M Bone (sternum):	**NOAEL = 100 µg/kg/day (AUC multiple = 43x)**

		CIT, Evreux,France GLP			Subendosteal osteoblasts, osteoclasts, fibroblast proliferation, woven bone formation (M and F) (not seen in controls, dose-dependent severity) **200, 450 µg/kg/day** Slight decreases in BW (M and F) PT increase (M) Antibodies in 1/3M Kidney: rel weight increase (M>F) Bone (tibia, morphometry): Increase in BV/TV, TbTh, TbN, Ct.Th Increases in ObS/BS and OS/BS, not ES/BS Increase in Ct.Th and Co.Porosity **450 µg/kg/day** Fibrinogen increase (M) Myeloid/erythroid ratio increase (F) PolyChromPhilic Erythroblasts % decrease (F) Serum Total Ca DE-crease (F) Alb/Glob ratio decrease (M) Urine Ca/creat increase (M only) Antibodies in 1/3F Kidney abs weight increase (M) Kidney: peritubular fibrosis (1/3M, 1/3F) Kidney: mineralization, tubular dilatation (1/3M) *Comment: Antibody formation in 1/3 HD-F, 1/3LD-M and 1/3MD-M did not appear to correlate with safety findings*	**LOAEL = 200 ug/kg/day (AUC multiple = 105x)** Sponsor NOAEL = 100ug/kg/day

AUC Multiples based on Human AUCss = 1546 pgxh/mL (7-day Study BA058-05-001B)

Monkey, 13-week study, (SC, daily dosing)

Study Report	Species, Strain	Study Duration	Doses (ug/kg/day)	N/s/g	Findings	NOAEL LOAEL
BA058-119	Monkey, Cynomolgus [≥24 mo old]	13 weeks 4-week recovery period Test facility: CIT, Evreux,France GLP	0, 10, 50, 200	6 (N=2/s/gf or recovery)	**10, 50, 200 µg/kg/day** Mortality: 1/6 F (10 ukd) died w pneumonia No ECG or ophthalmology abnormalities No hematology, serum chemistry, urinalysis findings Serum Ca: increased @3h but not @24h post dose (M, F) Serum P: increased @3h but not @24h post dose (M only) (not in 200 ukd group) Kidney: abs and rel weight increase, dose-related (F) **50, 200 µg/kg/day** Bone (femur): Subendosteal osteoblasts, osteoclasts, and fibroblast proliferation, and woven bone formation (M and F) (dose-related incidence and	NOAEL = 50 µg/kg/day **(AUC multiple= 10x)** LOAEL = 200 ug/kg/day **(*AUC multiple* = *50x, extrapolated*)** Sponsor NOAEL = 50ug/kg/day**)**

					severity) No effect on sternum marrow volume (histomorphometry) **200 µg/kg/day** Mortality (Tx-related): 1/6 M and 1/6 F (200 ukd) found dead (Days 67, 56); death was due to cardiac degeneration/necrosis/mineralization and nephropathy Heart: thickened pericardium (1/4M), irregular color (1/4F) Kidney: abs and rel weight increase (M) Kidney: enlarged, pale (1/4M) Heart: Myocardial degeneration/necrosis, myocarditis, mineralization in 1M and 1F (both found dead) Kidney: Tubulo-interstitial nephropathy (1M, 1F that died; and in 1F that survived); nephropathy included basophilia, tubular degen/regen, peritubular fibrosis, mineralization) *RECOVERY:* *Animals did not clearly show residual kidney, cardiac or bone effects in any group* *Comment:* *Antibody formation occurred in 1/6 MD-M, 4/5 HD-M, 1/6 LD-F, 4/5 MD-F, 3/5 HD-F. ADAs did not have a clear effect on toxicity findings.* *No urine Ca changes.*	

AUC Multiples based on Human AUCss = 1546 pgxh/mL (7-day Study BA058-05-001B)

Monkey, 39-week study, (SC, daily dosing)

Study Report	Species, Strain	Study Duration	Doses (ug/kg/day)	N/s/g	Findings	NOAEL LOAEL
7801-125	Monkey, Cynomolgus [≥33 mo old]	39 weeks 4-week recovery period Covance Labs, VA, USA GLP	0, 10, 25, 70/50 (High dose reduced to 50 µg/kg/d in Wk 21) (saline vehicle)	6 (0, 70 µg/kg) 4 (10, 25 µg/kg) N=2/s/g for recovery (control and high dose)	**10, 25, 70/50 µg/kg/day** Mortality in N=1 (Tx-related): **1/6M** (10 ukd) sacrificed moribund on D144, with increased serum Ca (11mg/dL baseline to 15-14mg/dL on D89-D144) and kidney and mandibular gland mineralization at necropsy No ECG abnormalities or changes Body weight: slight decrease (M) RBC decrease (F) (D89) Hct decrease (M and F) (D89) Increase in BUN (M, F) No effect on serum Ca (sample time not specified, likely 24h post dose) Serum P **decrease** (M only) Kidney: Rel weight increase (M)	NOAEL < 10 µg/kg/day **(AUC multiple < 3.1x)** LOAEL = 10 ug/kg/day **(AUC multiple = 3.1x)** Sponsor NOAEL ≤ 10ug/kg/day**)**

					Lung, infiltrate (M and F) Kidney: tubule mineralization (M and F) (dose-related), papilla mineralization (M and F) (dose-related), Adrenal, cortex: mineralization (M only, at 10 and 25 ukd) Lung: mineralization (M and F) (dose-related) **25, 70/50 µg/kg/day** RBC decrease (F) (D89, D270) Hct decrease (M and F) (D89, D270) ALKP increase, dose-related Kidney: rel weight increase (M and F) Lung: mineralization (M) Mandibular gland: mineralization (M and F) (dose-related) **70/50 µg/kg/day** Mortality in N=3 (Tx-related): **1/6M** sacrificed moribund on D54, with increased serum Ca (16.2mg/dL on D54) and myocardial and kidney tubule mineralization at necropsy **2/6F** sacrificed moribund on D55 and D110, with increased serum Ca (25 and 18mg/dL) and myocardial and kidney tubule mineralization at necropsy Food consumption: low (M and F) RBC, Hb decrease (M and F) (D69, D89, D270) Reticulocyte increase (M) (D69, D89, D270) Hct decrease (M and F) (D69, D89/D270) MCHC increase (M) (D89) ALKP increase (M and F) Heart: mineralization, vascular media (1F) Lung mineralization (F) Kidney: Artery intimal thickening, arteriole fibrosis (1F) Urine bladder: muscle mineralization (1F) Bone (femur, sternum): No effects at any dose *RECOVERY (N=2/s/g) (controls and HD):* *Kidney: tubule mineralization(1/1M, 1/1F)* *Lung: lung mineralization (1/1M)* *Mandibular gland: mineralization(1/1M, 1/1F)* *Spleen: increased pigment (1/1M, 1/1F)* *Comments:* *Antibody formation in 1/6 HD-F did not correlate to toxicity findings; animal had kidney mineralization at scheduled sacrifice.* *No serum or urine Ca changes*	

AUC Multiples based on Human AUCss = 1546 pgxh/mL (7-day Study BA058-05-001B)

Monkey ADA formation

Study	Duration	Doses	ADA-positive animals
BA058-118	4 weeks	0, 100, 200, 450 µg/kg	3/18 (16%)
BA058-119	13 weeks	0, 10, 50, 200 µg/kg	13/36 (36%)
7801-125	39 weeks	0, 10, 25, 70 µg/kg	1/28 (4%)

Monkey (4-week study): Total Calcium Plasma Level (mmol/L)

	Males				**Females**			
Dose (µg/kg/day)	**0**	**100**	**200**	**450**	**0**	**100**	**200**	**450**
Day	@3 hrs post-dose							
1	2.77	2.92	2.86	2.95	2.75	2.99	**3.11***	2.90
15	2.79	2.59	2.61	2.79	2.62	2.60	2.77	2.76
28	2.74	2.94	2.75	**3.19 (n.s.)**	2.67	2.97	2.89	2.68
Day	@ 24 hrs post-dose							
2	2.62	2.57	2.59	2.53	2.56	2.48	2.51	2.44
16	2.83	2.42	2.44	2.61	2.67	2.31	2.46	2.45
29	2.71	2.46	**2.20***	2.73	2.61	2.40	2.44	2.24

* p<0.05, statistically significant from controls

Monkey (13-week study): Total Calcium Plasma Level (mmol/L)

	Males				**Females**			
Dose (µg/kg/day)	**0**	**10**	**50**	**200**	**0**	**10**	**50**	**200**
Weeks	@3 hrs post-dose							
4	2.60	**2.84***	2.77	**2.83***	2.62	**2.96****	**2.86****	**2.80****
8	2.55	**2.81***	**2.83***	**3.22****	2.58	**2.92***	2.94	**3.32****
13	2.52	**2.83***	2.72	**2.92***	2.54	2.91	**3.01***	**3.24****
Weeks	@ 24 hrs post-dose							
4	2.58	2.55	2.51	2.47	2.49	2.43	2.43	2.39
8	2.52	2.49	2.39	2.59	2.56	2.50	2.56	2.95
13	2.60	2.58	2.49	2.49	2.49	2.51	2.59	2.72

* p<0.05, ** p<0.01, statistically significant from controls

Monkey (39-week study): Incidence/severity of soft tissue mineralizations (scheduled sacrifices)

	Males					Female			
N	4	3	4	4		4	4	4	3
Dose (ug/kg/day)	0	10	25	70/50		0	10	25	70/50
AUC multiple (M+F avg)		**3.1x**	**8.8x**	**18x**			**3.1**	**8.8x**	**18x**
Kidney tubule mineralization									
Minimal			1				1	1	1
Slight		1	1	2					
Moderate				1				1	1
Kidney papilla mineralization		2	2	3		1		2	2
Lung mineralization									
Minimal			1	1					

Moderate									1
Adrenal cortex, mineralization		1	2						
Mandibular gland, mineralization			1						2
Heart mineralization, vascular media									1

Monkey Toxicokinetics

Study Number	**Species**	**Study Duration, Dose Frequency**	**Time Points (TK samples)**	**Dose (g/kg)**	**Male AUC (pg·hr/ml)**		**Female AUC (pg·hr/mL)**	
BA058-118	Monkey/ Cynomolgus (3/s/g)	4 weeks, daily	Day 1 and Day 28: pre-dosing and 0.25h, 0.5h, 1h, 4h, 12h and 24h after dosing	0 100 200 450 100 200 450	Day 1 - 119990 213040 243510	Day 28 - 61090(1) 146690(2) 238290(1) 642200(2) 659460(1) 420250(2)	Day 1 - 91700 246740 446570	Day 28 - 72880(1) 178770(2) 320470(1) 144242(1) - 197520(1)
BA058-119	Monkey/ Cynomolgus (6/s/g)	13 weeks, daily	Day 1 and Week 13: pre- dosing and 0.25h, 0.5h, 1h, 4h, 12h and 24h after dosing	0 10 50 200 10 50 200	Day 1 - 9620 51600 102790	Week 13 - 4590(3) 15630(3) - 41830(4) 39050(4) 321230(4)	Day 1 - 4780 15590 21730	Week 13 - 4020(3) - - 21090(4) 293310(4) 283190(4)
7801-125	Monkey/ Cynomolgus (6 or 4/s/g)	39 weeks, daily	Day 1 and Week 39: pre-dosing and 5min, 15min, 30min, 1h, 2h, and 4h after dosing	0 10 25 70/ 50	Day 1 - 3493 16897 32282	Week 39 - 4147 15581 - 28578	Day 1 - 4421 10422 40852	Week 39 - 5342 11661 - 27728

1 Values from only 1 animal
2 Values from 2 animals
3 Values from animals with non-detectable antibodies
4 Values form animals with detectable antibodies

Monkey exposure multiples (Males and Females, average)

Study Nr.	Study Duration	Dose (µg/kg/d)	NOAEL (µg/kg/d)	End of Study(1)	
				AUC (pg.h/mL)	**AUC Multiple***
BA058-118	4 weeks	100 200 450	100	66985 (n=1) 162725 (n=2) 279380 (n=1)	**43x** **105x** **181x**
BA058-119	13 weeks	10 50 200	10	4305 15630 *(approx.75000)*	**2.8x** **10x** ***(50x)***
7801-125	39 weeks	10 25 70/50	<10	4745 13621 28153	**3.1x** **8.8x** **18x**

(1)Values from animals without pre-dose levels (no ADA development)
*Human AUC = 1546 pg h/mL (7-day Study BA058-05-001B)

Monkey exposure multiples (Females only) (39-week study)

Study Nr.	Study Duration	Dose (µg/kg/d)	NOAEL (µg/kg/d)	End of Study	
				AUC (pg.h/mL)	**AUC Multiple***
7801-125	39 weeks	10 25 70/50	<10	5342 11661 27728	**3.46x** **7.5x** **18x**

*Human AUC = 1546 pg h/mL (7-day Study BA058-05-001B)

Written summary
(Monkey toxicity studies)

As in the rat, a major part of the findings in the monkey toxicity studies were related to the pharmacodynamic activity of abaloparatide, i.e., to increase serum calcium through effects on bone and/or kidney and to exert an anabolic effect on bone. However, the pharmacologic or toxicologic mechanism underlying some of the findings was unclear.

Mortality was observed at doses of 1000 µg/kg in the 14-day, 200 µg/kg in the 13-week, and 10 and 70 ug/kg in the 39-week studies, respectively. The deaths or early sacrifices were attributed to myocardial degeneration/necrosis and/or nephropathy/renal tubular necrosis, and were often associated with tissue mineralization in the heart and/or kidneys. Somewhat concerning was the early sacrifice of a 10 ug/kg/day male in the 39-wk study on Day 144, at low AUC multiple (2.7x). However, this animal had high serum Ca levels (14-15 mg/dL) and kidney mineralization at necropsy, probably explaining its health condition.

There were no toxicologically relevant clinical signs in monkeys that survived until scheduled sacrifice. Body weight loss was observed in the 4-week study at 200-450 ug/kg/day in both sexes.

A large part of the changes in clinical pathology parameters were attributable to the pharmacological effects of abaloparatide to increase blood calcium levels. The transient hypercalcemia was probably the net result of increases in bone resorption and formation and changes, combined with changes in kidney handling of calcium (increase in Ca reabsorption) and phosphate (inhibition of P reabsorption).

Slight, dose-related decreases in RBC parameters were observed in male and female monkeys treated at doses ≥10 µg/kg/day in the 39-week study on Days 69-270. Since bone effects were not noted qualitatively in this study, this RBC decrease may have been due to increases in trabecular and cortical bone or direct effects on the bone marrow cells. Bone mass and %Obl surface increases were noted in the 4-week study in the 200 ug/kg dose group by quantitative histomorphometry. Percent osteoblast surface was also increased in this group. However, sternum marrow volume was not decreased histomorphometrically in the 13-week study at 50-200 ug/kg/day. Also, there was no increase in extramedullary hematopoiesis in any of the monkey studies.

Increased ALKP was noted after 4-weeks of dosing at 100-450 µg/kg/day, and by Day 69 after daily dosing at 25-70 µg/kg/day, likely due to increases in bone-specific ALKP, indicating enhanced bone formation.

Abaloparatide caused transient, slight increases in blood calcium levels compared to pre-dose values, at all doses used in the toxicity studies (≥0.75 µg/kg/day), generally @2-4h post-dose. Increases were most clearly demonstrated in the 13-week study at 4, 8 and 13-week time points. The increases in calcium levels were not always dose-dependent, and may have been affected by normal variations between monkeys. Blood calcium levels were generally comparable to pre-dose or vehicle control values when measured at >4h post-dose, at any dose level. No effect on serum Ca was seen in the 39-week study since it was most likely measured pre-dose only. Higher urinary calcium excretion was detected only in the 4-week study at 450 ug/k/gday.

Changes in plasma phosphorus levels were not consistent. In the 3-day study, phosphorus levels were unaffected over a 24 hour period after dosing with 0.75-17.5 µg/kg/day. In the 4-week study, slightly lower phosphorus levels were observed @3h post-dose at 450 µg/kg/day, at various times during the treatment period. In the 13-week study, plasma phosphorus levels were generally reduced @3h and 24h post-dosing in female monkeys at 10-200 µg/kg/day, but the change was variable. The changes were probably related to abaloparatide's PTHrP-like inhibitory effect on renal P reabsorption.

Changes in serum protein levels (decreases in albumin and A/G ratio) of unclear origin were only detected in the 4-week study at doses of 100-450 µg/kg. Slight increase in fibrinogen was mainly seen at the 450 µg/kg/day in the 4-week study. There were no noteworthy PT or APTT changes.

Abaloparatide caused no ophthalmology findings in monkeys. Electrocardiogram parameters (wave forms and interval times) were not adversely affected in any of the three monkey toxicity studies. Blood pressure was not measured.

Apart from the histomorphometric bone effects mentioned above, there were histologically observed subendosteal fibroblasts, osteoblasts and osteoclasts at 100-450 µg/kg/day in the 4-week study, and at 50-200 µg/kg/day in the 13-week study. These cells were not noted in control animals, and suggested increases in bone formation and resorption. Incidence and severity of the findings were generally dose-related. Woven bone formation was observed in sternum and femur of animals given 50-450 µg/kg/day in 4- and 13-week studies. No bone changes, bone cell changes or woven bone formation were detected at the end of the 39-week study.

Kidney changes were observed in 4-week, 13-week and 39-week studies. Tubular dilatation with flattened epithelium was noted in a male monkey after 4-weeks of dosing at 450 µg/kg/day. After 13 weeks of dosing, marked tubulo-interstitial nephropathy was found in 1/4 males and 2/4 females at 200 µg/kg/day. Two fo the three affected animals were found dead during the study. These animals also had cardiac degeneration/necrosis/mineralization. Nephropathy was not observed after 39 weeks of dosing up to 70/50 µg/kg/day. However, the animals that were sacrificed early in the 39-week study (one 10 ug/kg male and two 70 ug/kg animals) had kidney and myocardial mineralizations.

In the 39-week study, mineralization was observed in the kidney, lung, adrenal, mandibular gland, urinary bladder and/or heart at all doses of 10, 25 and 70/50 ug/kg/day in animals that survived until scheduled sacrifice. The soft tissue mineralization was probably related to the transient post-dose increases in serum Ca taking place over the course of this 9-month study. The increased treatment time exacerbated the mineralization finding.

The toxicity studies were generally adequate and the PD and/or toxic effects were dose-related. AUC was generally dose-proportional and the study findings were often dose-related. AUC in monkeys was similar in males and females. Anti-abaloparatide antibodies were detected in monkeys, especially after short term dosing at higher dose levels. Animals with detectable antibodies had clearly higher exposures. However, ADA development was not obviously associated with differences in toxicity.

Local Tolerance

Tabular summary

Local tolerance studies (SC dosing)

Study Report	Species, Strain	Route	Doses (ug/kg/day)	GLP
BA058-124	Rabbit/NZW	IV bolus, PV	50.6 µg/mL, single dose	Yes
BA058-123	Rabbit/NZW	SC	100 µg/mL, single dose	Yes
BA058-114	Rat/Sprague Dawley	SC	0, 15, 70, 300 µg/kg, daily × 4 weeks, 1 mL/kg (up to 300 ug/mL)	Yes

			(up to 300 ug/mL)	
BA058-115	Rat/Sprague Dawley	SC	0, 10, 25, 70 µg/kg, daily × 13 weeks, 1 mL/kg (up to 70 ug/mL)	Yes
BA058-118	Monkey/Cynomolgus	SC	0, 100, 200, 450 µg/kg, daily × 4 weeks, 1 mL/kg (up to 450 ug/mL)	Yes
BA058-119	Monkey/Cynomolgus	SC	0, 10, 50, 200 µg/kg, daily × 13 weeks, 0.5 mL/kg, (up to 400 ug/mL)	Yes

Written summary

Local tolerance was evaluated in two studies in NZW rabbits using single dose IV, perivenous, and SC administrations at a concentration of 51-100 µg/mL. There were no local reactions and no microscopic findings at the injection site attributed to abaloparatide treatment.

The SC injection site was also assessed in 4- and 13-week repeat-dose toxicity studies in rats and monkeys. There were no lesions at the injection site that were related to treatment with abaloparatide.

Other Toxicity Studies (Impurity)

Tabular summary

Repeat-dose toxicity studies with (b) (4) -degradant in rats (SC dosing)

Study Report	Species, Strain [Age]	Study Duration	Doses (ug/kg/day) Abl/Deg)	N/s/g	Findings	NOAEL
(b) (4)	Rat, SD	2 weeks	Grps 1,2,3,4: 0/(b) (4) 18.5/(b) (4) 16.9/(b) (4) 5.3 (b) (4) (100%, 93%, 30% Abl)	10 (main) 3 or 9 (TK)	**Grps 2, 3**: Slight increase in ALKP in M **Grps 2, 3, 4**: Slight increase in fibrinogen and decrease in albumin:globulin ratio. No changes in micronucleated PCEs in any of the treated groups **TK:** Cmax and AUC were increased in Grp 4 vs Grps 2 and 3, up to ca. 2-fold	N/A
(b) (4)	Rat, SD	4 weeks	Grps 1,2, 3,4: 0/(b) (4) 70/(b) (4) 65 (b) (4) 21/(b) (4) (100%, 93%, 30% Abl)	10 (main) 3 or 9 (TK)	**Grps 2, 3 (mostly) (and 4)** Increase in trabecular bone (femur and sternum), woven bone (sternum), osteoblasts (femur) Increase in spleen and liver extramedullary hematopoiesis Increased adrenal cortical vacuolation **Grps 2, 3, 4**: ***Males and females:*** Slight decrease in albumin:globulin ratio, Alb, and RBC Slight increase in fibrinogen and ionized Ca ***Males only***: Slight increase in ALKP and reticulocyte ct	N/A

					Slight decrease in PT and APTT ***Grp 4*** ***Females only:*** Slight decreases in neutrophil ct and APTT No findings in Grps 3 and 4 that were not observed in Grp 2 (at similar or lower frequency/magnitude) **TK:** Cmax and AUC were increased in Grp 4 vs Grps 2 and 3, up to 2-fold	

Toxicokinetics in 4-wk rat study with Abl and [redacted] (Study [redacted])

Day	Group	Abaloparatide (µg/kg/day)	[redacted] (µg/kg/day)	Sex	Cmax (ng/mL)	AUC0-4h (ng.hr/mL)
1	2	70	[redacted]	M	38.7	30.2
				F	27.6	22.2
	3	65		M	42.4	22.9
				F	25.2	19.9
	4	21		M	48.0	42.1
				F	41.8	39.1
28	2	70		M	48.9	73.9
				F	42.1	42.6
	3	65		M	48.7	53.2
				F	55.8	52.0
	4	21		M	75.9	97.9
				F	60.5	63.9

Written summary

[redacted], is a degradation product of abaloparatide and has been detected upon storage of liquid formulations. The levels of this impurity were not measured in batches used for toxicology studies, but are likely to have been [redacted]%. The release specification in the drug substance is [redacted]%, and in the drug product it is [redacted]% (24 month shelf life). As the ICH impurity threshold levels [redacted] the degradant was qualified in general toxicity and genotoxicity studies. Regulatory requirements for the potency of the drug product [redacted]% range within the 24-mo shelf life) were met, since the product contains a maximum of [redacted] of the parent's potency.

The degradant was qualified in 2- and 4-week studies in rats by dosing rats with abaloparatide/degradant mixtures with the same total dose levels. Pharmacology Study [redacted] showed that [redacted] to elicit a cAMP response in osteoblastic cells than abaloparatide.

In the 2-week study actual total dose levels were lower than the targeted 25 ug/kg/day (ca. [redacted] ug/kg). More toxicities were observed in Grps 2 and 3 than in Grp 4. The study also served as in

vivo micronucleus study for the degradant. No increase in micronucleated PCEs was detected in any of the dose groups (2,3,4).

In both the 2-week and 4-week studies, the substitution of abaloparatide with degradant in Grps 3 and 4 did not have a clear and consistent effect on the toxicity findings. Importantly, it did not cause any new toxicities. Toxicities were similar in all groups, and some were more pronounced in Grps 2 and 3 vs. Grps 4, e.g., bone, spleen and liver, and adrenal effects. However, some findings occurred only in Grp 4, or in Grp 4 as much as in Grps 2 and 3. Possibly, accumulation of (b) (4) occurred in Grps 3 and 4, since it may be (b) (4) than abaloparatide due to the (b) (4) This would explain the increases in RIA-measured plasma concentrations in Grps 3 and 4, since this assay probably detected both Abl and degradant. While the osteoblast signaling potency of (b) (4) that of abaloparatide, accumulation may have caused some of the larger than expected toxicities in Grps 4 vs. Grps 2 and 3. Alternatively, the presence of the degradant may have increased exposure to abaloparatide (b) (4)

The 2-week and 4-week toxicity studies were adequate to qualify the toxicity of the (b) (4) degradant.

Exposure multiples (rat and monkey)

Exposure multiples based on AUC at NOAEL in repeat-dose toxicity studies

Study Nr.	Species Study duration Doses	NOAEL (µg/kg/day)	LOAEL Findings @ LOAEL	AUC at NOAEL* (Females) (pg·h/mL)	Exposure Multiple at NOAEL**
BA058-05-001B	Human 7 Days	N/A		1546 (AUC_{0-t})	N/A
BA058-114	Rat 4 weeks 15, 70, 300 ug/kg/d	<15 µg/kg/day	15 µg/kg/day Skin reddening RBC decrease Thrombocyte and eosinophil decreases PT/APTT changes Alb/Glob decrease Urine Ca and Ca/creat increases Bone subendosteal cell proliferation, trabecular increases, marrow space decrease Liver, spleen: increased hematopoiesis	<4516	<2.9x

BA058-115	Rat 13 weeks 10, 25, 70 ug/kg/d	<10 ug/kg/day	10 ug/kg/day Skin reddening RBC, eosinophil decreases APTT, fibrinogen changes Alb/Glob decrease Urine Ca and Ca/creat increases Bone: subendosteal cell proliferation, trabecular increase, marrow space decrease, woven bone formation Spleen: Increased hematopoiesis and megakaryocytosis	3979	2.6x
7801-124	Rat 26 weeks 10, 25, 70 ug/kg/d	<10 µg/kg/day	10 µg/kg/day Skin reddening RBC, thrombocyte, eosinophil, monocyte decreases Lung weight increase Adrenal cortex vacuolation (ZG) Renal pelvis mineral No serum Ca effect (pre-dose) (?) Bone and marrow: Hyperostosis Spleen: Extramedullary hematopoiesis	2783	1.8x
10RAD032	Rat 104 weeks 10, 25, 50 ug/kg/d	<10 µg/kg/day	10 µg/kg/day Osteosarcoma, osteoblastoma	5483 (M,F avg)	<3.5x
BA058-118	Monkey*** 4 weeks 100, 200, 450 ug/kg/d	100 µg/kg/day	200 µg/kg/day BW decrease RBC decrease PT increase Kidney rel. weight increase Bone subendosteal cell proliferation, woven bone formation, trabecular and cortical bone increases	66985	43x

BA058-119	Monkey 13 weeks 10, 50, 200 ug/kg/d	50 µg/kg/day	200 µg/kg/day Mortality in 1/6 M and 1/6 F due to cardiac degeneration/ necrosis/ mineralization and nephropathy Bone subendosteal cell proliferation, woven bone formation, woven bone formation, no effect on sternum marrow volume Heart, thickened pericardium Kidney weight increase, enlarged Kidney: Tubulo-interstitial nephropathy (basophilia, tubular degen/regen, peritubular fibrosis, mineralization)	15630	10x
7801-125	Monkey 39 weeks 10, 25, 70/50 ug/kg/d	<10 µg/kg/day	10 µg/kg/day Mortality (N with Ca 15 mg/dL, kidney mineralization) BW decrease RBC, Hct decreases No serum Ca effect (pre-dose) (?) Serum P decrease BUN increase Kidney rel weight increase Mineralization in kidney (tubules and papilla), adrenal cortex, lung, mandibular gland	<4745	< 3.1

* The animal AUC values are from the last sampling time point in the toxicology studies
** Exposure multiple = [Animal AUC @ NOAEL] / [Human AUC_{ss} of 1546 pg·h/mL] (Study BA058-05-001b)
***Multiples not corrected for differences in protein binding (ie conservative estimates)
NOTE: Rat: Female AUC values, Monkey: Male and Female AUC averages

7 Genetic Toxicology

Abaloparatide

Tabular Summary

Genotoxicity studies with abaloparatide

Study Nr.	Study Type	Cells; Species/Strain	Concentrations/Doses	GLP	Findings
BA058-120	*In vitro,* Bacterial reverse mutation assay	S. typhimurium (TA98, 100, 1535, 1537), E. coli (WP2 uvrA)	156-5000 µg/m,L or 78-2500 ug/mL (TA1537), incubation 48-72h (N=2)	Yes	No dose-related or statistically significant increase in number of revertants in any of the tested strains, in absence or presence of S9. Positive and negative controls adequate. Study was valid.
BA058-121	*In vitro,* Chromosomal aberration test	Human peripheral blood lymphocyte cultures	113-5000 µg/mL 3h±S9 plus 17h recovery; 20h-S9 plus 0h recovery, 3h+S9 plus 17h recovery (N=2)	Yes	No significant differences in the structural or numerical aberrations as compared to negative control in any culture. Statistically significant increase in aberrations in positive controls. Study was valid.
BA058-122	*In vivo,* Micronucleus test	Mouse/CD-1, SC dosing, 6M/grp	0, 32.5, 65, 130 mg/kg exposure 48h, sacrifice at 72h	Yes	Doses of 65 and 130 mg/kg caused CNS toxicity and weight loss. PCE:NCE ratios in treated mice similar to negative vehicle control values at all doses. Micronucleated PCE frequency similar in dosed groups vs vehicle control. Statistically significant increase in the number of PCE in the positive controls. Study was valid.

Written summary

The genotoxic potential of abaloparatide was assessed in vitro in a bacterial reverse mutation assay, and in a chromosome aberration assay in human peripheral blood lymphocyte, and an in vivo mouse micronucleas assay.

Abaloparatide did not induce mutations in two bacterial strains (*S. typhimurium* and *E. coli*.) at concentrations up to 5000 µg/mL, in the absence or presence of S9.

Treatment abaloparatide at doses up to 5000 µg/mL did not induce chromosome aberrations in cultured human peripheral blood lymphocytes, in the presence and absence of S9, and was no considered clastogenic.

Abaloparatide did not induce micronuclei in the PCEs of the bone marrow of mice treated with doses up to 130 mg/kg/day (MTD).

In conclusion, there was no evidence of genotoxicity of abaloparatide in a standard battery of genotoxicity tests.

[redacted] (degradant)

Tabular summary

Genotoxicity studies with [redacted]

Study Nr.	Study Type	Cells; Species/Strain	Concentrations/Doses	GLP	Findings
[redacted]	*In vitro,* Bacterial reverse mutation assay	S. typhimurium (TA98, 100, 1535, 1537), E. coli (WP2 uvrA)	5-5000 µg/mL Incubation 52±4h (N=1)	Yes	No increases in number of revertant colonies of ≥2-3-fold at any dose level, in absence or presence of S9. Increases were observed at intermediate dose levels and were not dose-dependent. Positive and negative controls adequate. Study was valid.
	In vitro, Chromosomal aberration test	Human peripheral blood lymphocyte cultures	3.4- 500 µg/mL 3h and 24h-S9, and 3h+S9, harvested ~ 24h after initiation of treatment (N=1)	Yes	No significant increases in cells with chromosomal aberrations, polyploidy, or endoreduplication as compared to negative control in any culture. Statistically significant increase in aberrations in positive controls. Study was valid.
	In vivo, Micronucleus test	Rats/SD (2-week toxicity study with spiked abaloparatide); N=10/s/g	0/0, 18.5/0, 16.9/1.27, 5.3/12.4 ug/kg/day (actual doses) Exposure 2 wks, sacrifice at 2 wks	Yes	Small increase in % micronucleated PCEs (0.12%) in females given 5.3/12.4 ug/kg/day, This was not biologically relevant since the response was not dose-related and remained within the historical control (HC) control range of 0-0.5%. No decreases in PCE:NCE ratio in any dose group, indicating absence of bone marrow cytotoxicity. Study was valid.

Written summary

The genotoxic potential of [redacted] was assessed in vitro in a bacterial reverse mutation assay, and in a chromosome aberration assay in human peripheral blood lymphocytes. The 2-week toxicity study with spiked abaloparatide evaluated the in vivo genotoxic potential of the degradant using the bone marrow micronucleus assay.

[redacted] was not mutagenic in a bacterial reverse mutation assay, did not induce chromosomal aberrations *in vitro* in human peripheral blood lymphocytes, and did not increase micronucleated PCEs in the rat.

In conclusion, there was no evidence of genotoxicity of [redacted] in a standard battery of genotoxicity tests.

Based on the results of the genotoxicity studies and the two general toxicity studies with the degradant [redacted], this impurity is considered qualified.

8 Carcinogenicity

The carcinogenic potential of abaloparatide was evaluated in a 2-year carcinogenicity study in F344 rats. The study utilized doses of 10, 25 and 50 ug/kg/day and included a PTH1-34 (30 ug/kg/day) positive control arm.

A 2-year Subcutaneous Carcinogenicity Study of BA058 in the Fischer 344 Albino Rat
(Study 10RAD032) (Test Facility Study Nr: 670364)

Key study findings:

- A 2-year rat study was conducted with daily subcutaneous doses of **0, 10, 25, 50 ug/kg/day** abaloparatide (ABL), or **30 ug/kg/day** PTH1-34.
- The study was positive for carcinogenicity in males and females. Neoplastic findings included dose-dependent statistically significant and drug-related increases in osteosarcoma (malignant osteoblast tumor) and osteoblastoma in all male and female dose groups. The tumors are considered treatment-related. Bone is a pharmacologic target organ of the product and abaloparatide stimulates the osteoblast.
- Incidence of osteoblast hyperplasia was increased in all male and female dose groups
- Mortality was increased in a dose-related manner in 10, 25 and/or 50 ug/kg ABL-treated males and females, and 30 ug/kg PTH1-34-treated males. Due to early mortality, dosing was discontinued in 10, 25 and 50 ug/kg/day ABL- and 30 ug/kg/day PTH1-34-treated males and in 50 ug/kg/day ABL-treated females, when N reached 20 per groups. The 10, 25, 50 ug/kg/day ABL male groups were sacrificed early in weeks 97, 88, and 99, respectively.
- The main cause of the dose-related mortality was the development of osteosarcoma.
- The onset of osteosarcoma was similar in 10-25 ug/kg ABL and 30 ug/kg PTH1-34 male and female groups.
- Abaloparatide caused an increase in bone mass (BMD and BMC) in all dose groups.
- There were no other statistically significant ABL-related tumor findings.
- NOAEL for osteosarcoma and osteoblastoma was <10 ug/kg/day (low dose) in males and females.

Survival and tumor incidence (2-year rat study)

Group	*1*	*2*	*3*	*4*	*5*
Treatment	*Control (0 µg/kg/day)*	*Low dose (10 µg/kg/day)*	*Mid dose (25 µg/kg/day)*	*High dose (50 µg/kg/day)*	*PTH1-34 (30 µg/kg/day)*
MALES (N)	(60)	(60)	(59)	(60)	(60)
Survival	18/60[b]	19/60	15/59[a]	16/60[d]	16/60[NT]
Osteosarcoma	**1**[d]	**31**[d]	**46**[d]	**52**[d]	**39**[d]
Osteoblastoma	**0**[d]	**1**	**15**[d]	**20**[d]	**10**[c]
Osteosarcoma/Osteoblastoma	**1**[d]	**31**[d]	**48**[d]	**54**[d]	**43**[d]
AUC multiple**	-	4x	14x	24x	68x
FEMALES (N)	(60)	(60)	(61)	(60)	(60)
Survival	26/60[b]	33/60	24/61	14/60[b]	25/60[NT]
Osteosarcoma	**1**[d]	**11**[b]	**22**[d]	**37**[d]	**24**[d]
Osteoblastoma	**0**[b]	**8**[b]	**7**[b]	**9**[c]	4
Osteosarcoma/Osteoblastoma	**1**[d]	**16**[d]	**27**[d]	**40**[d]	**27**[d]
AUC multiple**		3x	12x	25x	43x

[a,b,c,d,e] Statistically significant (trend test, or pairwise comparison vs control) (CDER analysis)

[a] $p \leq 0.05$, [b] $p \leq 0.01$, [c] $p \leq 0.001$, [d] $p \leq 0.0001$

[NT] Not Tested

** AUC(rat)/AUC(human)@80µg/day (1546 pg·h/mL, Study BA058-5-001b). Rat AUCs are averages of Wk 26 and 52.

METHODS

Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 7, 2010
GLP/QA:	Yes/Yes
Doses:	**0, 10, 25, 50 ug/kg/day** (BA058) **30 ug/kg/day** (PTH1-34)
Test items:	BA058: Lonza Braine SA, Batch Nr. 4AI1 PTH1-34: (b) (4) (synthetic hPTH1-34), Batch Nrs. 2501265, FPTH0601AR2, FPTH0903B
Study duration:	104 weeks (planned)
Frequency of dosing:	Daily (Day 1 = first day of dosing)
Dose volume:	1 mL/kg
Route of administration:	Subcutaneous (rotated daily between 7 areas in dorsal region)
Formulation/Vehicle:	0.9% NaCl
Basis of dose selection:	MTD and anticipated AUC ratio
Species/Strain:	Rat/Fisher 344
Number/Sex/Group:	60 (main study); 12 (TK study)
Age:	7-8 weeks
Animal housing:	Group (up to 3 of same sex and dosing grp)

Satellite groups: TK (N=12/s/g)

Text Table 1
Experimental Design

Group No.	Dose Level (µg/kg/day)	No. of Animals			
		Main Study		Toxicokinetic Study	
		Males	Females	Males	Females
1/ Vehicle Control	0	60	60	12	12
2/ BA058	10	60	60	12	12
3/ BA058	25	59[a]	61[a]	12	12
4/ BA058	50	60	60	12	12
5/ PTH (1-34)	30	60	60	12	12

[a] One originally identified male was found to be a female pseudohermaphrodite at necropsy; this animal was reassigned to its true sex and kept on study because of the presence of test item-related finding.

Text Table 3
Experimental Design

Group No.	Dose Level (µg/kg/day)	No. of Animals			
		Main Study		Toxicokinetic Study	
		Males	Females	Males	Females
1/ Reference Item (Control)	0	1001 to 1060	1501 to 1560	1061 to 1072	1561 to 1572
2/ BA058	10	2001 to 2060	2501 to 2560	2061 to 2072	2561 to 2572
3/ BA058	25	3001 to 3060	3501 to 3560	3061 to 3072	3561 to 3572
4/ BA058	50	4001 to 4060	4501 to 4560	4061 to 4072	4561 to 4572
5/ PTH (1-34)	30	5001 to 5060	5501 to 5560	5061 to 5072	5561 to 5572
6/ Health Screen[a]	-	6001 to 6010	6501 to 6510	-	-

[a] 10 animals/sex were euthanized for Health Screen and blood sample collection prior to the initiation of dosing.

Observations:
Mortality/moribundity: Twice daily
Clinical observations (incl. palpable masses): Weekly
Body weight: Weekly
Food consumption: Weekly for 14 weeks, monthly thereafter
Ophthalmology: Prestudy, Wks 52 and 102 (main study animals)
Clinical pathology: Hematology samples at Wks 52, 78, and terminal necropsy. Blood cell morphology evaluated in control and 50 ug/kg ABL animals only.
Toxicokinetics: Samples for TK analysis collected from TK animals (N=12/sex/grp) at 0, 5, 15, 30, 60, 120, 180 mins post dose, on Day 1 and Weeks 26 and 52, Analysis of BA058 by RIA. PK parameters estimated by WinNonlin software.
PTH analysis: Samples collected at 0, 5, 15, 30, 60, 120, 180 mins post dose, on Days 1 and at Wks 26 and 52, from N=12/sex/grp. PK parameters not estimated.
Anti-Abl antibody: Samples collected prestudy (all except TK animals), Week 52 (TK animals), terminal necropsy (surviving main study animals)
Radiography: Main study animals X-rayed 2 weeks pre-scheduled necropsy or when N reached 22/grp (Males Grp 1) or 17/grp (see Text Table 2 below). TK animals: X-rayed pre-necropsy at Wk52. Data for main study animals were presented as Text Table 11 (summary) but not provided as individual data.

Text Table 2
Main Study Pre-Necropsy Radiographs

Group No.	Dose Level (µg/kg/day)	Study Week	
		Males	Females
1/ Vehicle Control	0	104	104
2/ BA058	10	104	104
3/ BA058	25	95/96	104
4/ BA058	50	87	100
5/ PTH (1-34)	30	99	104

Necropsy: Tissue collection (Text Table 7), followed by specimen preparation with H&E stain.
Bone densitometry (ex vivo DXA): Scans of lumbar spine L1-L4 and whole femur taken from all main study animals euthanized at study completion, including groups terminated early, except those with radiographic or macroscopic findings (due to osteosarcoma). Thus, DXA scans were taken at different times for different dose groups and were from different group sizes.
Histopathology: Examination of collected tissues, including selected bones (tibia, femur, vertebrae, sternum, and other bones identified by X-ray finding of localized changes)

Deviations from protocol:
When the number of surviving animals in any dosed group reached 20, the dosing in that group for the affected gender was discontinued. When the number of surviving animals in any treatment group reached 15, the affected gender in the given group was sent to necropsy.

Comments on protocol:
- The protocol, submitted as SPA, was reviewed by ECAC on July 14, 2009 for IND 73,176. The Committee concurred with the design and dose selection of the 2-year rat study.
- The Division made standard recommendations to Sponsor about dose discontinuation and early sacrifice on August 12, 2012.

RESULTS

Mortality
Mortality was increased in the 25 and 50 ug/kg/day male ABL groups and the 30 ug/kg male PTH1-34 group, and the 50 ug/kg/day female ABL group. Increases were statistically significant (trend test and pairwise comparison) according to both Sponsor and CDER analysis, except the increase in the male PTH group which was only significant (pairwise comparison vs control) per Sponsor's analysis.

Mortality and survival data for all groups are summarized below:

	Males		Females	
Group	Mortality	Survival	Mortality	Survival
1	40/60	33%	33/60	45%
2	41/60	32%	27/60	55%
3	45/60	25%	35/60	42%
4	45/60	25%	44/60	27%
5	45/60	25%	34/60	43%

These numbers included animals that were found dead during the necropsy period.
Group 3 Males termination started on Day 675 (Week 97).
Group 4 Males termination started on Day 616 (Week 88).
Group 5 Males termination started on Day 692 (Week 99).

Survival

Grp	Males	Females
1	20/60	27/60
2	19/60	33/60
3	15/59	25/61
4	15/60	16/60
5	15/60	26/60

Figure 1 **Survival Curves (%) - Males**

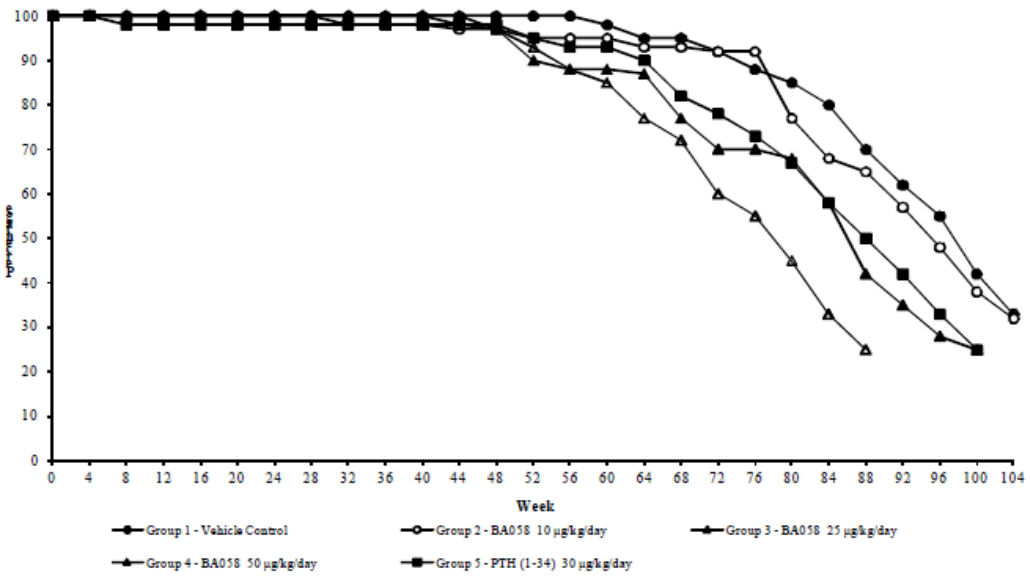
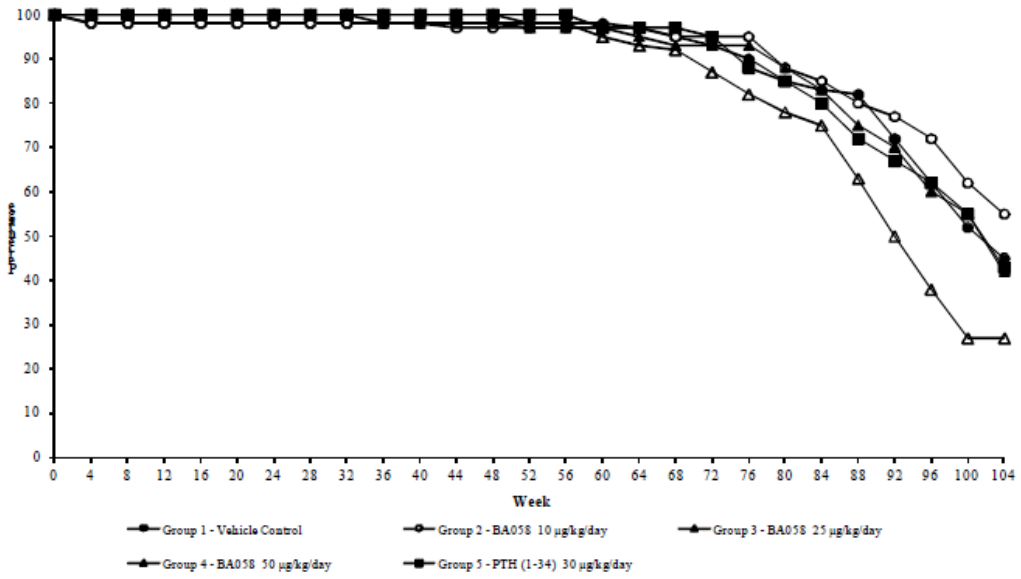


Figure 2 Survival Curves (%) - Females



Cause of death

Most common causes of pre-terminal deaths or euthanasia were osteosarcoma, leukemia and pituitary gland adenoma. Osteosarcoma contributed to morbidity and mortality in a dose-related manner.

The earliest observation of fatal osteosarcoma in males was at necropsy in study Month 11 in a 25 ug/kg ABL-treated male and Month 12 in a 30 ug/kg/day PTH-1-34-treated male (40% of rat life span). The first observation of fatal osteosarcoma in females was in Month 14 in a 50 ug/kg ABL-treated female and Month 14 in a 30 ug/kg/day PTH1-34-treated female. Thus, the onset of osteosarcoma was similar in 25 ug/kg ABL and 30 ug/kg PTH1-34 male and female groups.

In the low dose abaloparatide group (10 ug/kg, 3-4x human AUC), the earliest fatal osteosarcomas were seen in a male in Month 12 and in a female in Month 18. The times of death due to fatal tumors are obviously later than the times of tumor initiation and/or promotion.

The incidences of osteosarcomas and the earliest times of fatal osteosarcomas in the 30 ug/kg/day male and female PTH1-34 groups were shorter than those observed in a previous study conducted with rhPTH1-34 (NDA 21318, Forteo).

Text Table 9
Most Common Causes of Death or Morbidity among Pre-terminal Animals in Main Study

	Males					Females				
Group No.	1	2	3[@]	4[@]	5[@]	1	2	3	4	5
Treatment	Veh*		BA058		PTH	Veh		BA058		PTH
Dose (µg/kg/day)	0	10	25	50	30	0	10	25	50	30
No. of Pre-terminal Rats	42	41	46[a]	45[b]	44[c]	34	27	37	46[d]	35
No. of rats with diagnosis										
Bone (all sites): Osteosarcoma	0	19	32	35	25	0	4	8	24	8
Hemolymphoreticular tissue: Leukemia (LGL)	22	6	2	2	6	14	5	15	10	13
Pituitary gland: Adenoma (pars distalis)	9	11	4	0	6	4	1	6	5	6

Pairwise comparison p-values to Group 1 (two-sided Peto's test): a = 0.0021, b < 0.0001, c = 0.0150, d = 0.0054.
[@] Groups terminated early; Group 4 at study Month 21 and Groups 3 and 5 at study Month 23.
* Veh. = Reference Item (control); LGL = Large granular lymphocytes.

Dose discontinuation/early termination

Towards the end of the dosing period, survival reached N = 20 (33.3%) in 10, 25 and 50 ug/kg/day ABL males and 50 ug/kg/day females; and also in 30 ug/kg/day PTH1-34 males. At that point, dosing in these groups was discontinued.

N/grp reached ≤15 (≤25%) before scheduled termination in the 25 and 50 ug/kg/day ABL males and the 30 ug/kg PTH1-34 males. Early termination of these groups occurred in Weeks 97, 88 and 99, respectively. The Sponsor followed the advice from the Division with regard to discontinuing dosing and terminating dose groups.

Some animals in the TK study, i.e., N=3/24 in the 10 and 50 ug/kg/day ABL male group and N=1/12 in the 50 ug/kg/day female group (N=1/12), also died or were terminated early, most likely due to osteosarcoma.

Clinical Observations

Increase in number of animals with palpable masses in hindlimb, sacral (M), dorsal thoracic (M, F) and urogenital (F) areas, in all ABL and PTH groups. This correlated with macroscopic bone masses and bone thickening. Hindlimb paralysis was observed in all ABL dose groups (dose-related) and in the PTH group.

Body Weights

Males: Decreases, dose-related and statistically significant, at 25 and 50 ug/kg/day ABL (up to -14%) and 30 ug/kg PTH1-34 (up to -5%), starting in the 2nd year of treatment.

Females: Increases, dose-related and statistically significant, at 10, 25 and 50 ug/kg/day ABL and 30 ug/kg/day PTH1-34 (up to +10%), starting after 15 weeks of treatment.

Figure 3 **Summary of Body Weights - Males**

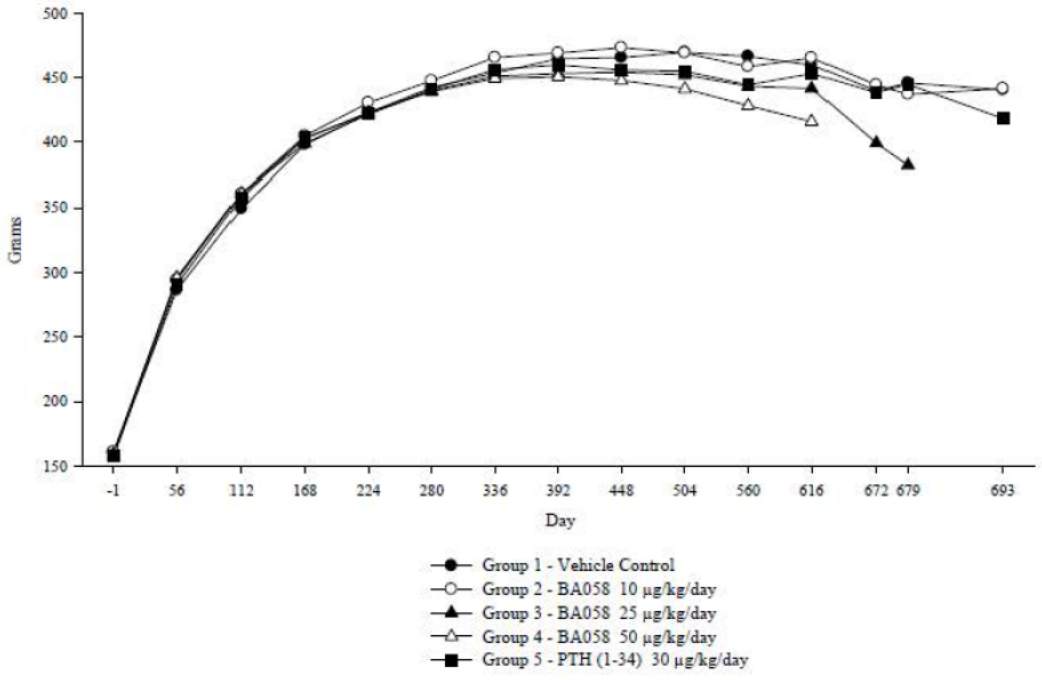
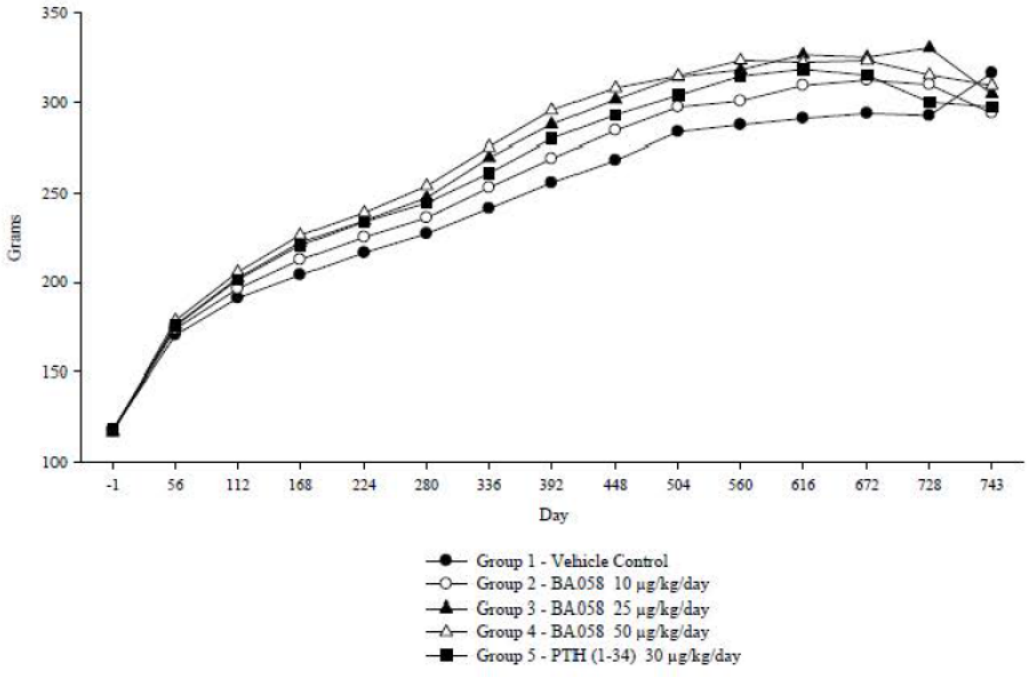


Figure 4 **Summary of Body Weights - Females**



Food Consumption

No effects

Ophthalmology
No effects

Clinical Pathology (hematology)

- Decrease in RBC (up to -18%) in all ABL-treated males (Wks 52, 78) and females (Wks 72, 78, terminal necropsy).
- Decrease in RBC (up to -24%) also seen in PTH1-34-treated males (Wks 52, 78) and females (Wks 52, 78, terminal necropsy).
- Effect was related to bone anabolic effect, i.e., decrease in marrow space.
- Blood cell morphology not affected at ABL 50 ug/kg/day high dose (no other groups examined)

Toxicokinetics:
(Weeks 1, 26, 52)

Abaloparatide
Tmax was 0.25-0.5 hours (15-30 mins) postdose. Peak concentrations were followed by an exponential decline. $T_{1/2,elim}$ was 0.2-0.6h (12-36 mins). No consistent TK differences between sexes.

Exposure (AUC) increased proportionally (Day 1), or more than proportionally (Wks 26, 52) with dose. AUC increased over time, by a factor 2.6x-4.4x from Day 1 to Wk 52. $T_{1/2}$ increased to higher values from Day 1 to Wk 52. However, this did not explain the accumulation ($T_{1/2}$ remained <1h).

In Weeks 1 and 26, in the 25 ug/kg/day group, samples from males were taken only through 2h post-dose (3h samples missing), and from females only through 1h post-dose (2h and 3h samples missing). Reviewer corrected the Week 26 AUC values for these groups, based on the 2h and 3h Cpl concentration data at Week 52, assuming a similar % change at 2h and 3h post-dose vs. 1h post-dose at Weeks 26 and 52. The AUC correction was very small (≤3%).

Time vs. C_{pl} (females and males) at Week 52:

Figure 1.5

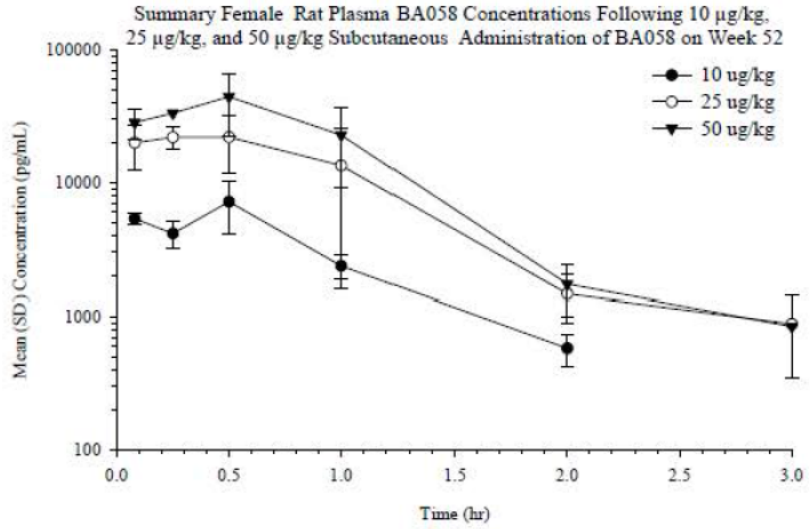
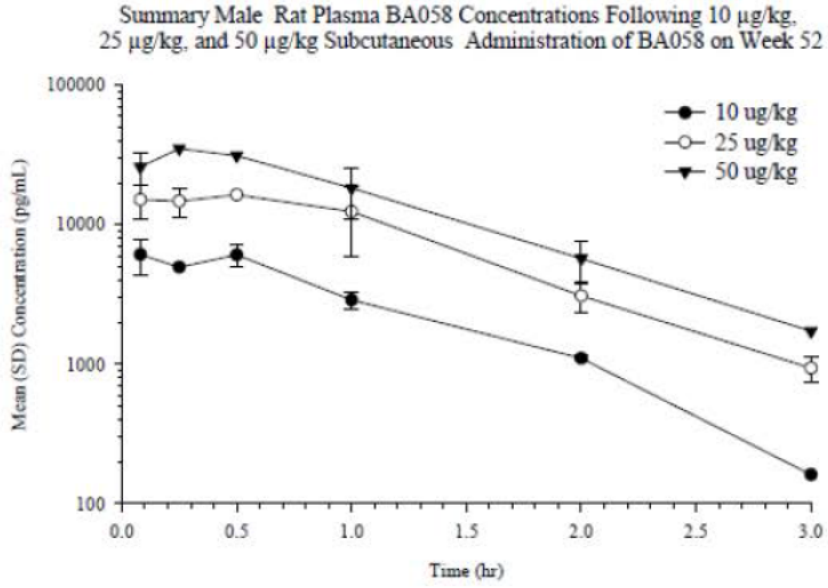


Figure 1.6



TK parameters:

Text Table 10
Summary (Standard Error) BA058 TK Parameters (Combined Males and Females)

Occasion	Dose (µg/kg/day)	T_{max} (Hr)	C_{max} (pg/mL)	$AUC_{(0-t)}$ (pg*hr/mL)
Day 1	10	0.25	3730 (447)	2280 (134)
	25	0.25	18300 (1430)	8740 (602)
	50	0.25	23700 (4470)	11600 (1260)
Week 26	10	0.08	5170 (544)	4580 (190)
	25	0.25	17300 (4100)	17400 (1910)
	50	0.50	31500 (6000)	30900 (2510)
Week 52	10	0.50	6630 (858)	6990 (399)
	25	0.50	19800 (3550)	25900 (2990)
	50	0.50	39200 (7720)	45100 (4570)

(Samples taken in Weeks 1, 26, 52; at 0, 5, 15, 30, 60, 120, 180 mins; two samples from each TK rat)

Cmax and AUC values (Week 26 and 52 averages) (males and females separately)

Dose (ug/kg/day)		Males		Females	
		Cmax (pg/mL)	AUC (pg·h/mL)	Cmax (pg/mL)	(AUC (pg·h/mL)
10	Wk 26 Wk52	5400 6110	4940 7440	4940 7180	3260 6290
	Wk 26+52	**5755**	**6190**	**6060**	**4775**
25	Wk 26 Wk52	25100 16400	19858* 24000	14000 22000	11021* 27300
	Wk 26+52	**20750**	**21959**	**18000**	**19161**
50	Wk 26 Wk52	30700 35200	31300 42700	32300 44400	30500 46500
	Wk 26+52	**32950**	**37000**	**38350**	**38500**

* corrected for missing post-dose Cpl values (3h for males, and 2h and 3h fr females)
NOTE: Sponsor used Wk 52 data only for AUC multiple calculations

PTH(1-34)
(Not included in Results of Study Report; data provided in Appendix 6 of Study Report)
Plasma concentrations of PTH1-34 in Grp 5 (30 ug/kg/day PTH group) were measured up to 3 hrs after dosing on Day 1 and in Weeks 26 and 52. AUC values were not provided. Reviewer calculated AUC values based on AUC/Cmax ratios for rhPTH1-34 derived from the rat carcinogenicity study conducted with 5, 30 and 75 ug/kg/day rhPTH1-34 for NDA 21318 (Forteo). Cmax values for 30 ug/kg/day PTH1-34 in the Radius study were much higher than those for 30 ug/kg/day rhPTH1-34 in the previous study. Thus, AUC values are likely to have been higher too, since the main contribution to AUC are the plasma levels in the first 60-90 min post dosing.

Abaloparatide study: PTH1-34 (30 ug/kg): Cmax and AUC (Wk 26 and 52 averages)

Males		Females	
Cmax (ng/mL)	AUC* (ng·h/mL)	Cmax (ng/mL)	AUC* (ng·h/mL)
24.8	19.9	17.3	12.6

* AUC estimated based on AUC/Cmax ratios for rhPTH1-34 in rat carcinogenicity study conducted with rhPTH1-34 for NDA 21318

rhPTH1-34 study (NDA 21-318): rhPTH(1-34) (30 ug/kg): Cmax and AUC (Wk 26 and 52 averages) (Pharmacology/Toxicology Review; NDA 21-318)

Males		Females	
Cmax (ng/mL)	AUC (ng·h/mL)	Cmax (ng/mL)	AUC (ng·h/mL)
7.6	6.7	12	7.5

AUC multiples in the table at the beginning of this review (under Key Study Findings, pp.138-139) are based on average AUC values at Weeks 26 and 52 in rats vs, human AUC on Day 7 measured in Study BA58-05-001b (1546 pgxh/mL).

Anti-Therapeutic Antibody (ADA)

(Samples at pre-study, Wk 52 necropsy TK animals, and terminal necropsy main study surviving animals) (RIA analysis)

One ADA sample in a 25 µg/kg/day TK female (#3565) was positive for ADA. At Week 52, this animal had a 4-fold higher plasma concentration than the mean plasma concentration in the 25 ug/kg female cohort (1h post-dose).

Four ADA samples from 50 µg/kg/day females were ADA-positive; two from main study animals (#4551, #4553) and two from TK animals (#4564, #4569). The ABL concentrations in the ADA-positive TK animals were 2 to 3 times higher than the mean concentrations in the 50 ug/kg/day female cohort (0.5-1h post-dose).

Apparently, the presence of anti-BA058 antibody was associated with increased exposure.

Radiographic evaluation

(Main study animals: 2 wks pre-scheduled-necropsy or when N reached 17/grp; TK study animals: pre-necropsy at Wk52)

Main study animals

Mono-ostotic (involving one bone) localized radiographic bone changes (bone loss, bone production or mixed reaction) were observed in 10, 25 and 50 ug/kg ABL and 30 ug/kg PTH animals.

Bone changes were correlated with masses found in bones at necropsy and/or by histologic examination identified as primary osteosarcoma. Variability in histologic type of osteosarcoma (see below) correlated with variability in radiographic bone changes.

Localized mineralization, mostly in lungs and abdominal organs, was observed in 10, 25 and 50 ug/kg males (dose-dependent effect) and the 50 ug/kg/day females. Mineralization was also seen in the 30 ug/kg PTH1-34 group (males and females), with similar incidence as in the 25 ug/kg/day ABL groups.

Radiographic mineralization noted in lungs and abdominal organs correlated with osteosarcoma metastases to soft tissues and with nodules in the lungs and masses and/or nodules in liver and spleen.

Increased bone radio-opacity (bilateral) in almost all males and females treated with ≥ 10 µg/kg/day ABL or 30 ug/kg/day PTH1-34.

Abnormal (uni- or bilateral bent or curved) shape of the proximal tibia, upon lateral view, in some females treated with 10,25 or 50 µg/kg ABL (dose-dependent effect) or 30 ug/kg PTH1-34. The abnormality was first noted after 20 months of dosing.

Radiography data were used to decide about additional bone collection from individual animals.

Radiographic Findings:

Text Table 11
Summary of Radiographic Findings - Main Study

	Male					Female				
Group No.	1	2	3	4	5	1	2	3	4	5
Treatment	Veh*		BA058		PTH	Veh		BA058		PTH
Dose (µg/kg/day)	0	10	25	50	30	0	10	25	50	30
No. of Rats Examined	60	60	59	60	60	60	60	61	60	60
Carcass										
Increased bone radiopacity	0	60	58	59	59	0	59	59	60	60
X-ray mineralization*	0	11	16	23	11	0	1	0	9	5
Bone - Femur										
X-ray bone loss	0	2	12	15	8	0	2	6	7	5
X-ray bone production	0	0	2	6	2	0	0	0	0	1
X-ray mixed reaction	0	1	1	2	1	0	0	1	1	0
Bone - Lumbar Vertebrae										
X-ray bone loss	0	0	0	0	0	0	1	0	6	3
X-ray bone production	0	4	1	4	1	0	2	2	3	1
Bone - Sternum										
X-Ray bone loss	0	0	3	3	2	0	2	3	7	1
X-ray bone production	0	1	2	3	1	0	0	0	2	0
X-ray mixed reaction	0	0	0	0	0	0	0	0	1	0
Bone - Tibia										
X-ray abnormal shape	0	0	0	0	0	0	7	6	17	11
X-ray bone loss	0	9	16	30	15	0	4	6	20	6
X-ray bone production	0	3	9	11	4	0	0	0	1	1
X-ray mixed reaction	0	2	5	9	4	0	0	1	2	1
Bone Miscellaneous[@]										
X-ray bone loss	0	13	33	35	26	0	13	17	24	17
X-ray bone production	2	10	21	16	23	0	2	5	10	3
X-ray mixed reaction	0	2	5	5	2	0	3	1	4	3

* Veh. = Reference Item (control); X-Ray = Radiographic; *Mineralization observed in soft tissues.
@ All other bones that were not routinely collected for microscopic evaluation unless a localized macroscopic or radiographic was observed.

Toxicokinetic animals
Localized bone findings were also seen at Wk52 in most TK animals. Detailed data not provided. Based on the correlation between localized bone findings and osteosarcoma, this suggests osteosarcomas were present already in many animals after 52 weeks.

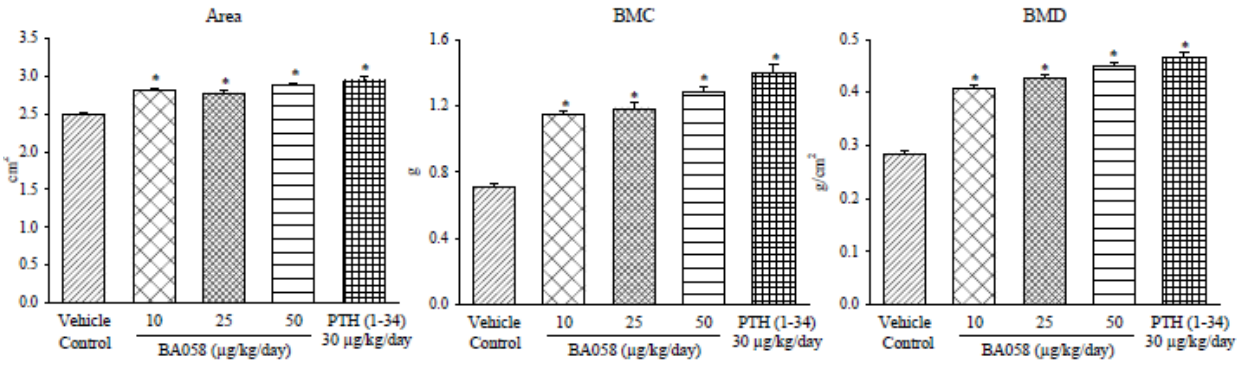
Bone densitometry (ex vivo DXA)
(All main study animals examined)
Abaloparatide caused marked dose-dependent increases in bone area, BMD and BMC of the lumbar spine (L1-L4) and femur (proximal, mid, distal) in all dose groups. This represents the expected pharmacological effect (increase in bone) of BA058 and PTH1-34.

BMD and BMC changes correlated with increased skeletal radio-opacity, widespread macroscopic thickening of the bones, and microscopic hyperostosis in ABL- and PTH(1-34)-treated groups.

Bone changes in 30 ug/kg PTH1-34 groups were similar to those in 25 or 50 ug/kg/day ABL groups.

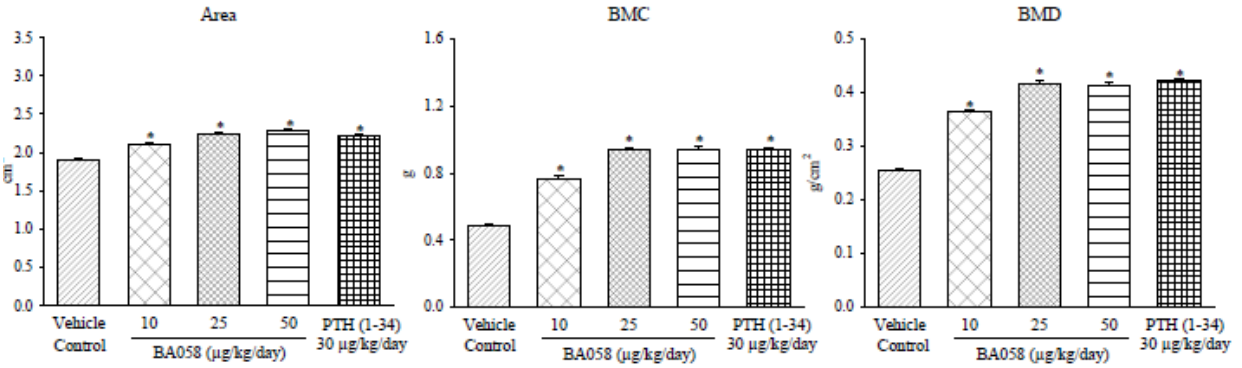
Note that DEXA measurements were performed at earlier times for higher ABL dose groups and PTH1-34 groups that were terminated early.

Text Figure 1
Bone Densitometry Values by DXA Parameters - Ex Vivo - Lumbar Spine (L1-L4) - Males - Mean (SEM)



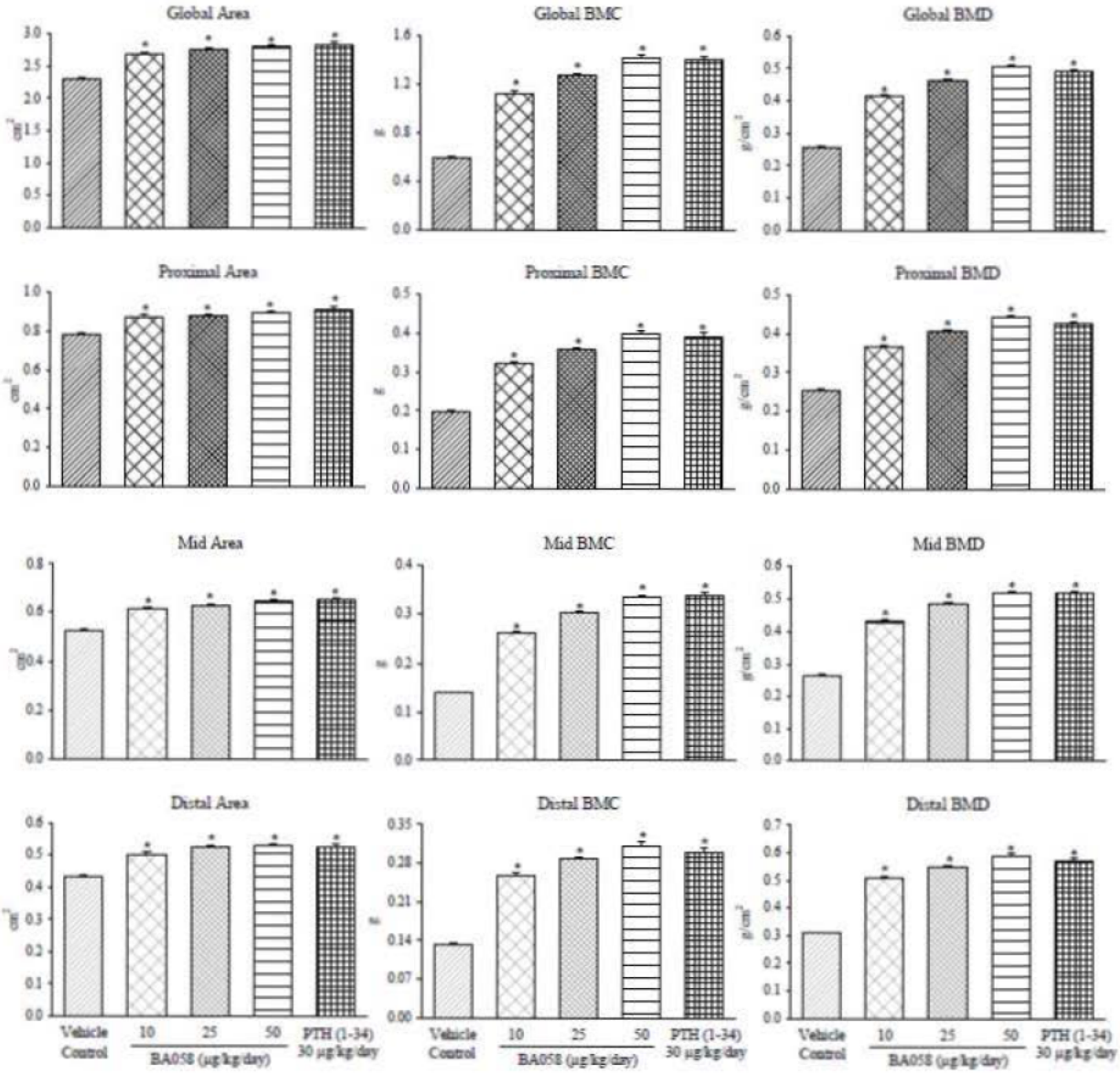
Significantly different from control group (Group 1) value: *-p<0.05

Text Figure 2
Bone Densitometry Values by DXA Parameters - Ex Vivo - Lumbar Spine (L1-L4) - Females - Mean (SEM)



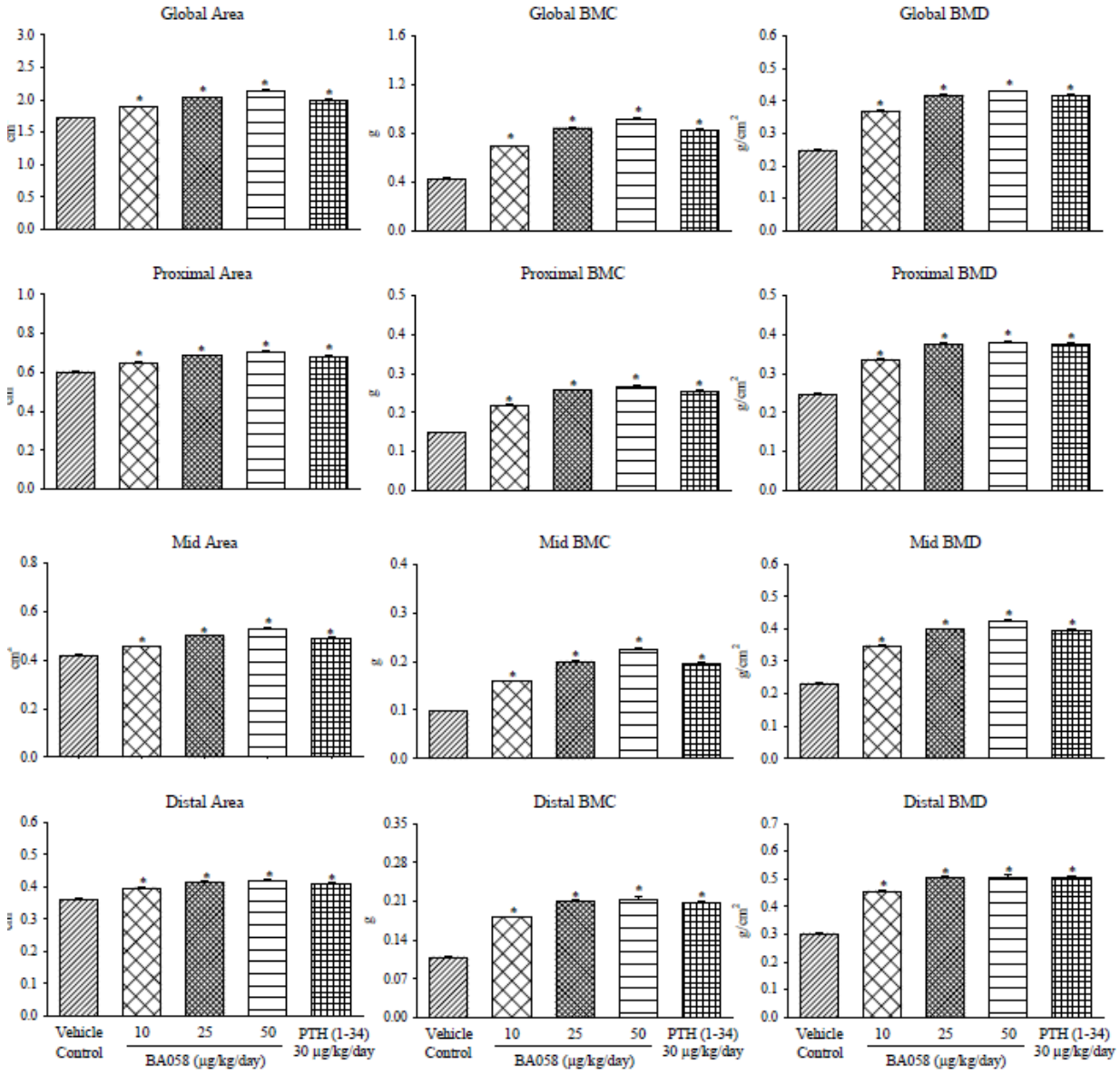
Significantly different from control group (Group 1) value: *-p<0.05

Text Figure 3
Bone Densitometry Values by DXA Parameters - Ex Vivo - Right Femur - Males - Mean (SEM)



Significantly different from control group (Group 1) value: *-p<0.05

Text Figure 4
Bone Densitometry Values by DXA Parameters - Ex Vivo - Right Femur - Females - Mean (SEM)



Significantly different from control group (Group 1) value: *-p<0.05

Gross Pathology

Findings included increase in bone masses, bone thickening, and firm bone marrow.

In soft tissues, findings with increased incidence in ABL- and PTH1-34-treated included irregular kidney surface, pale foci in lacrimal gland, masses or nodules in lung, liver and spleen, thickening and/o dilatation of urinary bladder (correlated often with mass in vertebral column), pale area in lungs.

Text Table 12
Summary of Macroscopic Findings in Bones and Bone Marrow - Main Study

		Male					Female				
Group No.		1	2	3	4	5	1	2	3	4	5
Treatment		Veh*	BA058			PTH	Veh	BA058			PTH
Dose (µg/kg/day)		0	10	25	50	30	0	10	25	50	30
No. of Rats Examined		60	60	59	60	60	60	60	61	60	60
Finding	/ Bone										
Mass	Femur	0	0	3	5	2	0	0	0	1	2
	Sternum	0	0	2	3	2	0	0	2	2	0
	Tibia	0	3	9	13	5	0	0	1	0	0
	Vertebrae L5-L6	0	1	1	5	1	0	0	0	1	1
	Miscellaneous[@]	0	9	15	19	13	0	3	2	11	5
Thickening	Any site[#]	4	56	58	56	59	1	52	60	57	57
Firm	Marrow	0	0	0	4	0	0	4	6	4	14

* Veh. = Reference Item (control).
[#] Incidence listed for the bone site with highest incidence.
[@] All other bones that were not routinely collected for microscopic evaluation unless having a localized macroscopic or radiographic observation.

Text Table 13
Summary of Macroscopic Findings in Soft Tissues - Main Study

		Male					Female				
Group No.		1	2	3	4	5	1	2	3	4	5
Treatment		Veh*	BA058			PTH	Veh	BA058			PTH
Dose (µg/kg/day)		0	10	25	50	30	0	10	25	50	30
No. of Rats Examined		60	60	59	60	60	60	60	61	60	60
Tissue	/ Finding										
Kidney	Surface irregular	13	41	41	47	37	3	2	6	24	6
Lacrimal gland	Foci pale	1	2	0	1	2	26	47	47	43	42
Liver	Mass	3	3	8	10	5	0	0	1	3	2
	Nodule	1	1	7	10	6	0	0	0	1	2
Lung	Area pale	12	30	25	16	27	15	29	37	30	26
	Nodule	1	12	22	23	22	1	1	0	13	8
Spleen	Mass	3	2	5	6	8	1	2	1	3	1
	Nodule	0	0	5	4	2	1	0	0	2	0
Urinary bladder	Dilatation	2	6	10	13	8	3	2	3	8	4
	Thickening	1	4	12	7	10	0	0	1	6	4

* Veh. = Reference Item (control).

Histopathology
(Text Table 14)

Bone: Neoplastic

- Abaloparatide caused dose-dependent increases in bone tumors, mostly osteosarcoma but also osteoblastoma, in both sexes, at all doses.
- Bone tumors were also seen in 30 ug/kg/day PTH1-34 groups, at slightly smaller (males) or similar (females) incidence as in the 25 ug/kg/day ABL groups.
- Percent osteosarcoma occurrences at 10, 25, 50ug/kg/day ABL were **52%, 78%, 87%** in males, and **18%, 36%, 62%** in females. Incidences for 30ug/kg/day PTH-1-34 were **65%** in males and **40%** in females.
- Males were more susceptible to bone tumor development than females.
- Tumors were identified through gross or microscopic pathological examination, or radiological exam followed by histopathology.
- Osteosarcoma was often correlated with localized radiographic changes.
- Bone tumors were observed in several bone sites, particularly tibia, femur and spine
- The time of death of animals that died due to osteosarcoma, either found dead or sacrificed moribund) was decreased with ABL dose.
- Several animals were affected by more than one osteosarcoma (multicentric OS) and several had multiple types of bone tumors
- Osteosarcoma often metastasized to lung, liver and spleen
- Microscopically, osteosarcoma was variable in appearance. It was usually of the osteoplastic, osteoblastic or fibroblastic subtype, giving rise to variable radiographic changes.
- Osteoblastoma was usually seen along trabeculae and was minimally invasive.
- PTH-1-34-induced tumor incidences were larger than those noted in a similar F344 rat study with the same PTH1-34 dose of 30 ug/kg/day (NDA 21318, Forteo)
- According to the CDER statistical review, increases in osteosarcoma in all male and female ABL dose groups were statistically significant (trend and pair-wise test). Increases in male and female PTH1-34 groups were also statistically significant (pairwise comparison). Increases in osteoblastoma were statistically significant in ABL-treated males and females (trend test); and also in male 25 and 50 ug/kg/day ABL groups, female 10, 25 and 50 ug/kg/day ABL groups, and male 30 ug/kg PTH1-34 group (pair-wise test).

Tumor onset:
The earliest observations of fatal osteosarcoma cases were at necropsy in Month 11 in a 25 ug/kg ABL-treated male and Month 12 in a 30 ug/kg/day PTH-1-34-treated male. In females, the earliest fatal cases were in Month 14 in a 50 ug/kg ABL-treated female and also in Month 14 in a 30 ug/kg/day PTH1-34-treated female. Thus, onset of osteosarcoma was similar in 25 ug/kg ABL vs 30 ug/kg PTH1-34 treated males and females.

The earliest observations of fatal osteosarcomas in the low dose Abl group were at necropsy in Month 12 (357 days) in a 10 ug/kg male (#2022), and in Month 18 (549 days) in a 10 ug/kg/day female (#2505). For the 30 ug/kg PTH group (Months 12 and 14), the earliest fatal osteosarcoma appeared at earlier times than in a previous study (Months 19 and 20, data from NDA 21318).

Text Table 14
Noteworthy Neoplastic Findings in All Examined Bones - Main Study

	Male					Female				
Group No.	1	2	3	4	5	1	2	3	4	5
Treatment	Veh*		BA058		PTH	Veh		BA058		PTH
Dose (µg/kg/day)	0	10	25	50	30	0	10	25	50	30
No. of Rats Examined	60	60	59	60	60	60	60	61	60	60
No. of Animals Affected with One or More										
Osteoblastoma	0	1	15[a]	20[a]	10[b]	0	8[c]	7[d]	9[e]	4
Osteosarcoma - primary	1	31[a]	46[a]	52[a]	39[a]	1	11[f]	22[a]	37[a]	24[a]
Osteosarcoma - metastasis	1	14	17	35	21	0	2	3	16	9
No. of Animals With Multicentric Osteosarcoma	0	7	22	34	18	0	1	3	14	4
Tumor Count[#]										
Osteoblastoma	0	1	18	23	11	0	8	7	10	4
Osteosarcoma	1	40	84	131	71	1	12	26	55	30

Pairwise comparison p-values to Group 1 (One-sided Peto's test): a < 0.0001, b = 0.0002, c = 0.0050, d = 0.0058, e = 0.0014, f = 0.0022.

* Veh. = Reference Item (control).

[#] The count is the actual number of distinct non-contiguous neoplasm noted and, consequently, exceeds the sum of tabulated tumors recorded under the entries: bone-femur, bone sternum, bone-tibia, bone-vertebrae L5-L6 and bone miscellaneous. Multiple tumors recorded under bone miscellaneous and/or bilateral tumors recorded as a single neoplasm in the femur or tibia explains the difference.

Bone: Non-Neoplastic

- Findings included tibial deformity (curvature of proximal end), osteofibrous dysplasia (spindled cell elements replacing bone or marrow), osteoblast hyperplasia (proliferation of differentiated osteoblasts) and bone hyperostosis (trabecular hypertrophy in epi-, meta- and diaphyseal regions). Hyperostosis is the expected pharmacological effect of PTH peptide analogues.
- Similar to the bone tumors, the incidences of these findings in the 30 ug/kg/day PTH1-34 groups were generally similar to those in the 10 or 25 ug/kg/day ABL groups.

Text Table 15
Summary of Non-Neoplastic Findings in Bones - Main Study

	Male					Female				
Group No.	1	2	3	4	5	1	2	3	4	5
Treatment	Veh*		BA058		PTH	Veh		BA058		PTH
Dose (µg/kg/day)	0	10	25	50	30	0	10	25	50	30
No. of Rats Examined	60	60	59	60	60	60	60	61	60	60
Finding / Tissue										
Deformity / Tibia	0	1	0	0	0	0	4	5	18	12
Dysplasia: Osteofibrous										
Femur	0	0	2	2	2	0	1	5	2	2
Sternum	0	1	1	0	1	0	2	1	2	1
Tibia	0	1	0	3	0	0	2	1	2	3
Vertebrae L5-L6	0	0	0	0	1	0	1	1	2	0
Miscellaneous[@]	0	0	3	9	2	0	0	2	5	4
All bone sites[#]	0	2	6	14	6	0	6	10	13	10
Hyperplasia: osteoblast										
Femur	0	1	4	1	2	1	2	0	2	0
Sternum	0	0	1	1	1	0	0	0	0	0
Tibia	1	2	2	8	1	0	2	3	4	2
Vertebrae L5-L6	0	2	5	6	4	0	6	1	0	1
Miscellaneous[@]	0	0	3	5	1	0	1	0	0	1
All bone sites[#]	1	5	15	21	9	1	11	4	6	4
Hyperostosis										
Femur	1	59	59	60	60	5	59	61	60	60
Sternum	0	56	58	59	59	9	58	61	60	59
Tibia	3	58	58	59	58	9	59	61	59	60
Vertebrae L5-L6	0	58	58	60	60	5	59	60	60	60
Miscellaneous[@]	0	18	35	38	24	0	9	13	24	11

* Veh. = Reference Item (Control).
[@] Tissue only examined in animals presenting macroscopic and/or radiographic abnormalities.
[#] Number of animals affected with non-neoplastic finding in one or more bones.

Text Table 16
Average Severity Grade[#] of Hyperostosis per Tissue Affected for Routinely Examined Bones - Main Study

	Male					Female				
Group No.	1	2	3	4	5	1	2	3	4	5
Treatment	Veh*		BA058		PTH	Veh		BA058		PTH
Dose (µg/kg/day)	0	10	25	50	30	0	10	25	50	30
No. of Rats Examined	60	60	59	60	60	60	60	61	60	60
Femur	1.0	3.4	4.2	4.3	4.1	1.8	3.7	4.1	4.3	4.3
Sternum	-	2.5	2.4	2.3	2.6	1.9	3.5	3.5	3.3	3.7
Tibia	2.0	3.2	4.3	4.3	4.2	1.9	3.6	4.3	4.3	4.3
Vertebrae L5-L6	-	2.5	2.8	3.1	2.8	1.6	3.3	3.6	3.4	3.5

* Veh. = Reference Item (control).
[#] The maximal severity grade = 5.0.

Soft tissues: Neoplastic

- Increase in adrenal adenoma, cortical, in ABL-treated males (incidence 0-0-0-2).
- Increase in fibroadenoma at injection site, dorsal thoracic left, in ABL treated females (incidence 0-0-2-3).
- Adrenal and injection site tumor increases are not biologically significant.

- Decreases in hemolymphoreticular tissue leukemia, large granular lymphocyte, in males (incidence 36-15-3-2) (significant based on trend and pairwise tests) and in 10 ug/kg females (significant based on pairwise test, 19 vs 12). Relevance of this tumor decrease is not known.

Soft tissues: Non-Neoplastic

- Soft tissue mineralization was found in heart/arteries and kidney/pelvis and was possibly related to the calcemic effect of this PTHrP-analogue. The severity was minimal to mild. This soft tissue mineralization was different from the radiologically observed mineralization. The latter included mineralized nodules and represented metastatic osteosarcoma.

- Other findings included increased splenic extramedullary hematopoiesis (related to the bone effect and the reduced marrow space), lung histiocytosis (minimal, possibly secondary to lung osteosarcoma metastases) and urine bladder changes, possibly related to bone tumors in adjacent spine or pelvis. These findings were also noted in PTH1-34-treated.

Text Table 17
Summary of Soft Tissue Mineralization - Main Study

	Male					Female				
Group No.	1	2	3	4	5	1	2	3	4	5
Treatment	Veh*		BA058		PTH	Veh		BA058		PTH
Dose (µg/kg/day)	0	10	25	50	30	0	10	25	50	30
No. of Rats Examined	60	60	59	60	60	60	60	61	60	60
Tissue / Finding										
Heart										
Mineralization: vascular	4	29	33	35	37	0	6	12	11	5
Kidney										
Increased mineral: pelvis	1	14	27	28	28	1	23	37	41	33

* Veh. = Reference Item (control).

Text Table 18
Summary of Non-Neoplastic Secondary Changes in Soft Tissues - Main Study

	Male					Female				
Group No.	1	2	3	4	5	1	2	3	4	5
Treatment	Veh*		BA058		PTH	Veh		BA058		PTH
Dose (µg/kg/day)	0	10	25	50	30	0	10	25	50	30
No. of Rats Examined	60	60	59	60	60	60	60	61	60	60
Tissue / Finding										
Lung										
Histiocytosis	16	30	28	27	31	23	37	40	38	36
Spleen										
EMH#:increased	5	14	29	39	22	8	29	33	31	30
Urinary bladder										
Dilatation	2	6	9	12	8	2	2	4	6	3
Hemorrhage	1	4	7	9	6	0	1	4	3	0
Inflammation	1	7	6	9	7	0	1	1	4	5

* Veh. = Reference Item (control); # EMH = Hematopoiesis/extramedullary.

Statistical analysis of neoplastic findings

Sponsor

- The Sponsor's statistical analysis is summarized in the CDER Statistical Review (Dr. Hepei Chen).
- Generally, the increases in bone osteosarcoma and osteoblastoma in both males and females were statistically significant according to trend test and pairwise comparison between control and all dose groups, except for osteoblastoma in the male 10 ug/kg group (pairwise test).
- Trend test also revealed a statistically significant increase in adrenal adenoma, cortical in males (incidence 0-0-0-2) and fibroadenoma, injection site dorsal thoracic left (0-0-2-3), if these tumors were classified as rare ($p<0.025$).

CDER

- According to the CDER statistical review, increases in osteosarcoma were statistically significant in ABL males and females (trend test), and also in male 10, 25, 50 ug/kg/day and female 50 ug/kg/day ABL groups, and male and female 30 ug/kg/day PTH1-34 groups (pair-wise comparison vs control). Increases in osteoblastoma were statistically significant in ABL males and females (trend test), and also in male 25 and 50 ug/kg/day and 10, 25 and 50 ug/kg/day female ABL groups, and male 30 ug/kg PTH1-34 group (pair-wise test).
- Increases in combined osteosarcoma and osteoblastoma were statistically significant in ABL males and females (trend test), and in all male and female ABL and PTH groups (pair-wise comparison).
- Increase in adrenal adenoma, cortical in males (incidence 0-0-0-2) and fibroadenoma, injection site dorsal left in females (0-0-2-3) were statistically significant if the tumors were classified as rare ($p<0.05$).
- Decreases in hemolymphoreticular tissue, leukemia, large granular lymphocyte, were significant in males (trend and pairwise) and in 10 ug/kg/day females (pairwise test). Relevance of this tumor decrease is not known.

Dosing Solution Analysis

Samples of dosing formulation samples were analyzed by HPLC for determination of ABL (4AI1) and PTH1-34 (FPTH0903B) at 15 time points during the study.
At a few of the earlier time points (Day 1, Day 3, Week 2), the peptide values (%) for several samples used for various dosing groups were outside acceptance criteria (±10% of theoretical concentration), usually between 75% and 90%.

SUMMARY AND EVALUATION

Abaloparatide given by daily SC injection for 2 years to Fischer 344 rats at doses of 10, 25 and 50 ug/kg/day caused a statistically significant increase in the rate of mortality in animals at all dose levels. Cause of mortality was mainly a dose-related increase in osteosarcoma in males and females.

Dose-related decreases in mean body weight were seen in males given ABL at 25 and 50 µg/kg/day, while increases in body weight were seen in females at all ABL doses. These gender-

specific changes were also seen in the PTH (1-34)-treated group. RBC decreases were seen in ABL and PTH1-34-treated groups.

Tmax of ABL was 1/4 to 1/2 hrs and $T_{1/2}$ was between 0.2 and 0.6 hrs. AUC of ABL (Wk 52) at 10, 25, and 50 ug/kg/day was 6990, 25900, and 45100 pg·hr/mL animals (males and females combined).

Densitometric increases in bone area, mineral density and mineral content were observed in all ABL dose groups and PTH1-34 groups.

Increases in cardiac vascular mineralization, mineral in the renal pelvis, alveolar histiocytosis in the lung and extramedullary hematopoiesis in the spleen were seen at all ABL dose levels.

Urinary bladder changes were also observed in ABL-treated. Generally, these changes were also present in the PTH1-34-treated groups.

In bones, malignant osteosarcoma, correlating with localized radiographic changes (bone loss, bone production or mixed reaction), benign osteoblastoma and focal osteoblast hyperplasia were seen in both sexes in all ABL and PTH1-34 groups. Osteosarcoma metastases in ABL and PTH1-34 groups correlated with radiographic mineralization and macroscopic nodules/masses in lungs, liver and spleen.

The NOAEL for the bone carcinogenic effect of ABl was <10 ug/kg/day.

The study was adequately conducted and the 2-year bioassay was appropriate for the evaluation of the test compound.

Discussion of bone neoplastic findings

The increase in osteosarcomas and osteoblastomas caused by abaloparatide was an expected finding, based on previous bone neoplastic findings in rodent studies conducted with rhPTH1-34 (Eli Lilly, NDA 21-318, Forteo) and hPTH1-84 (NPS Pharmaceuticals, BLA 125551, Natpara®) In the rhPTH1-34 study, a dose-dependent increase in bone tumors (osteosarcoma, osteoblastoma and osteoma) was observed in male and female rats, at doses of 5, 30 and 75 ug/kg/day. The rat osteosarcoma finding prompted a black box warning in the label for Forteo®.

The Sponsor of NDA 21318 showed in a second carcinogenicity study that the increase in rat osteosarcomas caused by rhPTH1-34 was dependent on both dose and treatment duration Dose-dependence was established in the current study with abaloparatide. Although treatment-duration-dependence is also likely for abaloparatide, this was not explicitly demonstrated.

The clinical significance of the bone neoplasm finding is not clear. The tumorigenicity of PTH-receptor agonists may be dependent on species and/or treatment duration relative to life time, which was much larger in rats than humans. The bone BMD or BMC changes observed at all doses in the rat study (≥50%, see Figures 2 and 3 below) were larger than those in Phase 3 at 80 ug/day in humans, in which spine BMC) is increased by approximately 14% over 18 months.

Postmarketing data on Forteo have not given obviousl signal of an increase in osteosarcoma risk in treated patients.

The abaloparatide study included a positive control group of 30 ug/kg PTH1-34, which was the mid-dose in the 2-year F344 rat study conducted by Eli Lilly with rhPTH1-34 (doses 5, 30, 75 ug/kg/day). The bone tumor incidence in the Sponsor's 30 ug/kg/day PTH1-34 group was notably higher than in the 30 ug/kg/day PTH1-34 group in the Lilly study, in both males and females. Sponsor suggested that this could at least partly be due to the additional use of radiography for tumor detection. By comparing the relationship between mortality and tumor incidences between the two studies, this Reviewer concluded that this was probably not a major contributing factor. Another possible cause for the higher tumor incidence with PTH1-34 in the abaloparatide study was the considerably larger Cmax (and thus likely also AUC) of PTH1-34 in the 30 ug/kg PTH1-34 groups in the Sponsor's study as compared to the Lilly study.

Comparison of Abaloparatide and PTH1-34 bone tumorigenicity

Reviewer correlated rat osteosarcoma incidence with ABL and PTH1-34 treated groups using different bases of comparison, i.e., dose, AUC, [rat vs. human]-AUC multiple, and vertebral or distal femoral BMC change. The relationship between dose or AUC and osteosarcoma incidence was similar for both compounds. However, when normalizing tumor incidences based on bone effect, the tumorigenicity of abaloparatide appeared to be either similar (females) or larger (males) than that of PTH1-34.

The outcome of the comparisons was as follows:

Basis	*Osteosarcoma incidence*
Dose, AUC	Similar in ABL vs. PTH1-34-treated males and females
BMC change	Larger in ABL vs. PTH1-34-treated males, but similar in females

BMC change multiple vs osteosarcoma

FIG.1 shows the relationship between the ratio of [% change in L1-L4 BMC in rats] vs [% change in lumbar spine BMC in humans] and osteosarcoma incidence; and FIG.2 shows the relationship between the ratio of [% change in proximal femur BMC in rats] vs. [% change in total hip BMC in humans] and osteosarcoma incidence. The human effect was the baseline corrected % BMC change in treated vs placebo at Month 18 (Phase 3 Study BA058-05-003 in postmenopausal women).

FIG. 1 (Vertebrae)

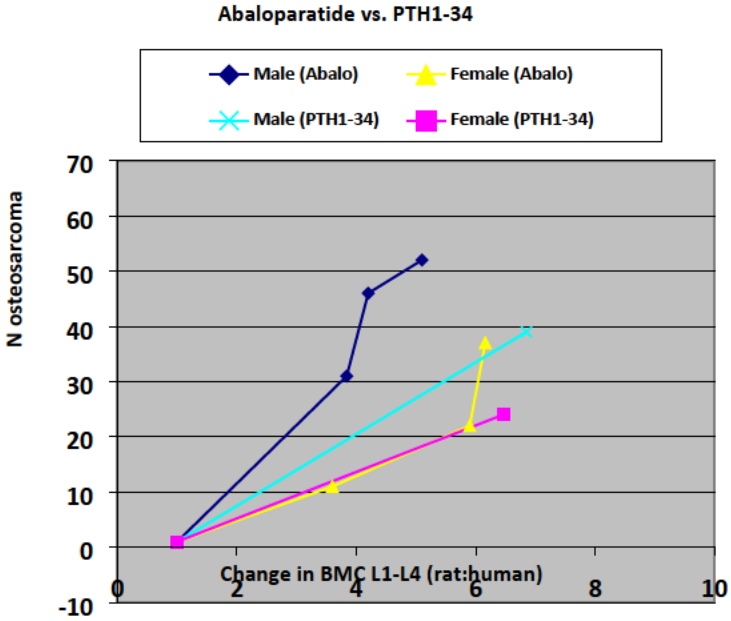
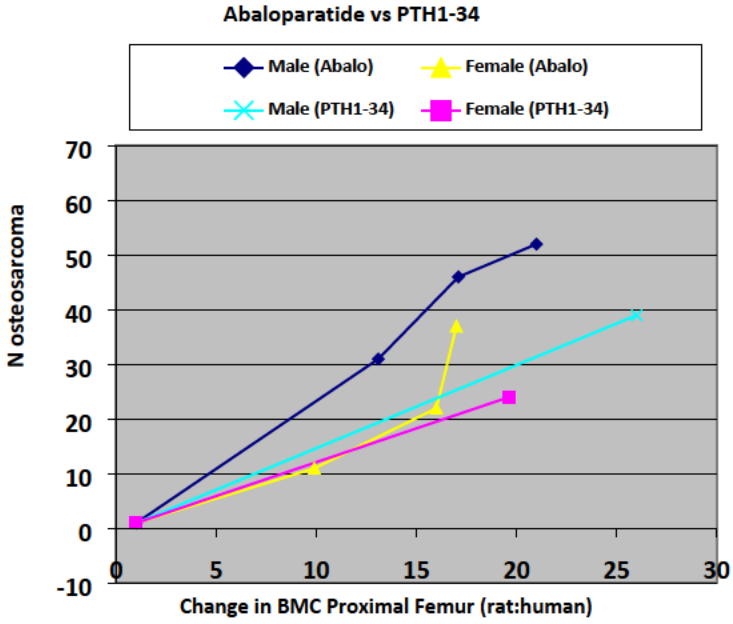


FIG. 2 (Proximal Femur)



FIGS. 1 and 2 show that osteosarcoma incidence was larger in ABL- than in PTH1-34-treated males when normalized by BMC-effect. However, the difference did not appear significant for females.

It should be noted that the rat BMC data were from survivors only and the actual bone effects were probably smaller for animals that died prematurely due to osteosarcoma. This may have caused a leftward shift in the data points, especially for the higher dose groups. However, since this would have happened for both ABL and PTH groups, the comparisons in these figures are probably still reasonably valid.

A change in BMC is the net result of changes in osteoblastic bone formation and osteoclastic bone resorption. Hence, osteoblastic bone formation (e.g. BFR) would be an alternative parameter to correlate to tumor incidence. However, comparative data on bone formation (e.g. BFR/BS) for ABL and PTH1-34 in rats were not available and these correlations could not be established.

CONCLUSIONS

- Abaloparatide causes osteosarcoma, osteoblastoma and osteoblast hyperplasia in rats
- The clinical relevance of the rat bone tumors is unclear
- Although the osrteosarcoma incidence appeared larger in male ABL vs PTH1-34 groups upon normalization by estimated multiples of BMC effect, the data do not provide conclusive information about the relative risk of tumorigenicity of ABL (80 ug/day) vs. PTH1-34 (20 ug/day) in humans.
- The Sponsor is proposing a black box warning for their product label. It includes the rat osteosarcoma finding and recommendations for clinical use.

WARNING: POTENTIAL RISK OF OSTEOSARCOMA

Reviewer comments on labeling:

- Reviewer agrees with Sponsor that the rat tumor findings warrant this warning.
- Exposure (AUC) multiples for the rat osteosarcoma finding need to be corrected.
- Osteosarcoma incidences (%) at **10, 25, 50 ug/kg/day** ABL were **52%, 78%, 87%** in males, and **18%, 36%, 62%** in females. Percentages at the high doses should be mentioned in the label.
- should not be mentioned in the label. the rats in these studies were sacrificed after 6 months of treatment, which may not be sufficient time to develop detectable tumors.
- Postmarketing surveillance studies with Forteo have not shown an increase in osteosarcoma when treatment duration was limited to 2 years. For approvability of abaloparatide (80 ug/day), a recommendation of a specific, e.g., maximum treatment duration, , may need to be included in the labeling. The proposed ABL label (Section Section 2.5 of the FORTEO label states: "*The safety and efficacy of FORTEO have not been evaluated beyond 2 years of treatment. Consequently, use of the drug for more than 2 years during a patient's lifetime is not recommended*". This Reviewer recommends that the label include a recommendation for a time limit to clinical treatment duration.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study for Effects of BA058 on Fertility and Early Embryonic Development to Implantation by Subcutaneous Administration in Male Rats.
(SBL062-288) (GLP)

Summary:
Abaloparatide had no effect on copulation rate, fertility rate, copulatory interval, necropsy findings, organ weight (testes or epididymides), sperm activity, motile sperm rate, sperm count, or abnormal sperm rate in rats, at doses up to 70 µg/kg/day. There were also no treatment related changes in female rats mated with treated males on number of corpora lutea, number of implantations, implantation rate, pre-implantation loss rate, number of live embryos, embryonic viability rate, number of post-implantation losses, or post-implantation loss rate.

Methods
Male SD rats (age 11 weeks) (N=20/grp) were dosed before mating (Day 0-13), through mating (Day 14-27), and until the day before necropsy (Days 42-44), for a total of 6 weeks, with **0, 10, 25, 70 ug/kg/day**, by SC injection in 0.9% NaCl (1 mL/kg). Females (N=20/grp) were not dosed and were 12 weeks old at start of mating. High dose selection (70 ug/kg/day) was based on data from a 4-week toxicity study with doses of 15, 70 and 300 ug/kg/day (Study No. 17348 TSR). Study objective was to evaluate functional male reproductive effects and early embryonic development in dams impregnated by dosed males.

7.9 **Study Design**

1 control group and 3 test article groups

Group	Test and Control Articles	Dose Level (µg/kg/day)	Dose Volume (mL/kg/day)	Concentration (µg/mL)	Number of Animals (Animal No.)	
					Male	Female (Non-treated)
1	Control Article	-	1	-	20 (10001 to 10020)	20 (10021 to 10040)
2	BA058	10	1	10	20 (10041 to 10060)	20 (10061 to 10080)
3	BA058	25	1	25	20 (10081 to 10100)	20 (10101 to 10120)
4	BA058	70	1	70	20 (10121 to 10140)	20 (10141 to 10160)

Observations/measurements:
- Mortality and clinical signs: Males 2x/day; Females 1x/day
- Body weight: Males twice weekly; Females 6 times before GD15
- Food consumption: Males every 3 days through 2 weeks of dosing
- Mating (Days 14-27): Pairing on 1:1 basis
- Copulation rate: # of copulated animals/# of paired animals

- Fertility rate: # of pregnant animals/# of copulated animals
- Necropsy:
  - Males: (Days 43-45) Gross pathology, testis and epididymes weight, no histopathology
  - Females: (Gestation Day 15) Gross pathology, # corpora lutea, # implantations
- Sperm exam: Activity, motile sperm rate, sperm count, abnormal sperm rate (in N=10M/grp)
- Embryo exam: # of live embryos, postimplantation loss (# and condition)
- Calculations:
  - Preimplantation loss rate = (number of corpora lutea – number of implantations) /number of corpora lutea × 100
  - Implantation rate = number of implantations / number of corpora lutea × 100
  - Embryonic viability rate = number of live embryos/number of implantations × 100
  - Postimplantation loss rate = number of postimplantation losses/number of implantations × 100

Results

Males (treated)

Mortality
No effects

Clinical observations
Reddening of the skin at all doses (probably due to vasodilation)

Body weight
No significant effects

Food consumption
No significant effects

Mating
No effects on copulation parameters (rate or interval) or fertility rate

Necropsy
No effects

Organ weight
No effect on testes or epididymis

Sperm Exam
No effects on sperm activity, motile sperm rate, sperm count, or abnormal sperm rate

Early embryonic development
No significant differences in number of corpora lutea, number of implantations, implantation rate, preimplantation loss rate, number of live embryos, embryonic viability rate, number of postimplantation losses, or postimplantation loss rate in any dose group compared with the control group.

Females (non-treated)
Clinical Signs
No deaths and no abnormalities

Body Weight
No abnormal weight effects

Necropsy
No abnormalities

Conclusions
In this fertility and early embryonic development study with doses of 10, 25 and 70 ug/kg/day (males dosed only), there were no treatment effects on body weight, body weight gain, food consumption, copulation rate, fertility rate, copulatory interval, necropsy findings, organ weight (testes or epididymides), sperm activity, motile sperm rate, sperm count, or abnormal sperm rate in any group. With respect to early embryonic development, no test article-related changes were noted in the number of corpora lutea, number of implantations, implantation rate, prereimplantation loss rate, number of live embryos, embryonic viability rate, number of postimplantation losses, or postimplantation loss rate in any dose group. The NOAEL was 70 ug/kg/day.

TK data were not collected in this study. However, TK data were available from the 4-week study that served as these basis for the dose selection in the current study. Serum levels were quantifiable up to 6h (at 70 ug/kg) or 12h (at 300 ug/kg) post dosing.

Table 3. Mean BIM44058 toxicokinetic parameters observed on days 1 and 28 following subcutane administration of 15, 70, 300 µg/kg/day for 4 weeks to male rats.

Dose (µg/kg/day)	Parameter	Units	Day 1	Day 28
15	T_{max}	*hr*	0.50	0.50
	C_{max}	*ng/ml*	7.073	7.624
	C_{max}/D	-	0.472	0.508
	AUC_t	*(ng/ml)·hr*	5.660	6.933
	AUC*	*(ng/ml)·hr*	5.815	6.995
	AUC/D	-	0.388	0.466
	AUC_{extr}	%	2.66	-
70	T_{max}	*hr*	0.50	0.50
	C_{max}	*ng/ml*	20.864	51.337
	C_{max}/D	-	0.298	0.733
	AUC_t	*(ng/ml)·hr*	20.246	42.416
	AUC*	*(ng/ml)·hr*	20.752	43.172
	AUC/D	-	0.296	0.617
	AUC_{extr}	%	2.43	-
300	T_{max}	*hr*	0.50	0.50
	C_{max}	*ng/ml*	62.386	296.053
	C_{max}/D	-	0.208	0.987
	AUC_t	*(ng/ml)·hr*	58.863	328.703
	AUC*	*(ng/ml)·hr*	58.944	328.820
	AUC/D	-	0.196	1.096
	AUC_{extr}	%	0.14	-

* AUC values corresponding to day 28 are AUCτ values

(-) not estimated

Reviewers Note: AUC (0-tau) = AUC from beginning to end of the dosing period (i.e. 24hrs)

Table 5. Comparative dose level ratios and exposure parameter ratios of BIM44058 on days 1 and 28 after subcutaneous administration of BIM44058 at doses of 15, 70 and 300µg/kg/day for 4 weeks to male rats.

Day 1					
Dose	Dose ratio	C_{max}	C_{max} ratio	AUC	AUC ratio
µg/kg/day	-	*ng/ml*	-	*(ng/ml)·hr*	-
15	1.0	7.073	1.0	5.815	1.0
70	4.7	20.864	2.9	20.752	3.6
300	20.0	62.386	8.8	58.944	10.1

Day 28					
Dose	Dose ratio	C_{max}	C_{max} ratio	AUC*	AUC ratio
µg/kg/day	-	*ng/ml*	-	*(ng/ml)·hr*	-
15	1.0	7.624	1.0	6.995	1.0
70	4.7	51.337	6.7	43.172	6.2
300	20.0	296.053	38.8	328.820	47.0

* AUC values corresponding to day 28 are AUC_τ values

Multiples of human exposure:

Dose (ug/kg/day)	AUC (Day 28) (pgxh/mL)	Human Exposure Multiple*
15	6995	4.5x
70	43172	28x
300	329820	213x

*Human AUC at 80 ug/day in postmenopausal women was 1546 pgxh/mL (Day 7; Study BA058-5-001B).

10 Special Toxicology Studies

Phototoxicity

The absorbance spectrum of a representative lot of abaloparatide shows that abaloparatide does not absorb the visible wavelengths (290 to 700 nm). Thus, abaloparatide is not expected to be phototoxic. No phototoxicity studies were conducted. No method with an absorbance at a wavelength range within the visible light spectrum was submitted.

APPEARS THIS WAY ON ORIGINAL

11 Integrated Summary and Safety Evaluation

The sponsor of NDA 208734, Radius Health Inc, is seeking regulatory approval of their marketing application for abaloparatide-SC (80µg/day) for the treatment of postmenopausal osteoporosis.

Abaloparatide is a synthetic PTHrP1-34 analog with 76% homology to PTHrP. It selectively activates the PTH1 receptor which plays in important role in bone and calcium metabolism. PTH1R agonists administered intermittently stimulate bone formation by the osteoblast. This outweighs a concomitant stimulation of osteoclastic bone resorption and causes a net anabolic effect on bone.

The applicant completed an adequate nonclinical development program, comprised of in vitro and in vivo pharmacology studies, PK/ADME studies, and toxicology studies. The latter included general toxicity studies in two species, genotoxicity studies, a 2-year carcinogenicity study, a male fertility study, local tolerance studies, and studies to qualify the degradant, (b) (4) . Pivotal animal studies were conducted by SC injection.

Pharmacology

In vitro pharmacology assays showed that abaloparatide selectively binds to the PTH1 receptor (PTH1R) and can signal via the AC/PKA pathway. In HEK-293 derived cells, abaloparatide bound with high affinity to the RG receptor conformation of the receptor, similar to other ligands (PTH1-34, PTHrP1-36, and LA-PTH), and this correlated with intracellular cAMP response. The affinity for the R^0 conformation, however, was 10 to 400 fold less for abaloparatide than for the other ligands. Thus, abaloparatide was considered an "RG-selective" ligand. The in vitro residual intracellular cAMP response after ligand removal appeared to be inversely related to the ligands' differential RG vs. R^0 selectivity and was least with Abl, at the selected concentrations. Sponsor hypothesized that this was causally related to preferential stimulation of bone formation with little effect on resorption by abaloparatide. However, both nonclinical and clinical data showing stimulation of bone resorption by abaloparatide contradicted the hypothesis. Support from other data was equivocal. The relationship between in vitro intracellular cAMP events and in vivo bone tissue responses is currently unclear and it is unknown how this relationship evolves upon prolonged treatment duration.

Short term in vivo studies demonstrated similar calcemic activity of abaloparatide and PTH1-34 in the parathyroidectomized rat. In 4 and 6 week studies in ovariectomized (OVX) rats, abaloparatide increased vertebral and femoral BMD, improved trabecular bone structure, and increased vertebral and femoral strength.

Bone quality studies

Rat

Long term primary pharmacology ("bone quality") studies were conducted in OVX rat and monkey models of osteoporosis. In both rat and monkey studies, treatment with abaloparatide was started after 3-month (rat) or 9-month (monkey) bone depletion periods. The studies did not include post-treatment recovery periods or teriparatide comparator groups.

In the 12-month OVX rat study, at doses of 0.3x, 1.2x and 12x human exposure (AUC) at the daily dose of 80 ug, abaloparatide increased bone formation markers, while resorption markers increased transiently and to a lesser extent. Abl increased DXA BMD, BMC and Bone Area (BA) in whole body, spine, femur and tibia. All effects, especially the Bone Area increases, continued throughout 12 months.

Cancellous bone effects, e.g. in lumbar spine, were due to increases in trabecular thickness and number (probably by 'tunneling'). Long bone changes consisted of increased periosteal bone apposition and slight bone widening, continuing through 12 months. In long bone diaphyses, marked endosteal bone apposition resulted in increased cortical thickness especially in the first 6 months of dosing. However, some endocortical resorption in the metaphyses also appeared to take place.

Histomophometry at end of study showed increases in BV/TV and % Obl surface, and increases in BFR (bone formation rate) and AcF in trabecular bone. In long bone diaphysis there was a marked increase in periosteal BFR (up to 5-fold), suggesting continued increase in bone formation. At the endosteal surface BFR was increased to a smaller extent. The increases in BFR at the peri- and endosteal surfaces were conceivably accompanied by increases in resorption. The finding that net endosteal bone apposition no longer occurred at this time and % endocortical eroded perimeter was also slightly increased was consistent with this conclusion. Since a large part of the treatment effect occurred in the first months, relevant information would have been obtained by earlier histomorphometric analysis. However, the 12-month data were still informative.

Biomechanical testing showed increases in compressive, bending and shear strength parameters in vertebrae, femur shaft and femur neck, respectively. Correlations between strength measures and BMC were unaffected, indicating maintenance of bone quality with abaloparatide treatment.

Most or all abaloparatide-induced changes were dose related, and most densitometry, structural and mechanical parameters in higher dose groups markedly exceeded sham control levels. The rat study was adequately conducted.

Monkey

In the 16-month OVX monkey study, at doses of 0.1x, 0.13x and 0.7x human AUC exposure at the daily dose of 80 µg, abaloparatide increased skeletal bone formation markers while resorption markers were less affected. VitD(OH)2 increases were observed in all treated groups. DXA BMD, Area and BMC were increased at spine, whole body, tibia, and femur, but distal radius Area and BMC were decreased. In long bone diaphysis metaphyses, cortical thickness wasincreased at some sites due to endosteal bone apposition. Slight bone widening without a change in cortical thickness was noted at other sites. Most treatment effects occurred in the first 8 months. The data suggested that abaloparatide had bone site, region and surface specific effects. Abaloparatide did not completely restore the OVX-induced bone changes to sham control levels, probably due to the low doses used in the study.

MicroCT and histomorphometry showed increases in trabecular thickness and number in cancellous bone. Vertebral wall thickness (microscopic bone balance) was increased. Histomorphometry at end of study failed to show increases in BFR in trabecular bone of vertebrae or femur neck. In the femur diaphysis there were increases in endosteal and periosteal BFR, even though bone and cortical thickness were not significantly affected by abaloparatide. This again suggested concomitant stimulation of bone resorption and continued enhancement of bone turnover. Unlike teriparatide (published data), abaloparatide did not significantly increase cortical porosity. As in the rat study, the timing of the histomorphometry evaluation was not optimal.

Bone strength was increased in vertebrae and femur neck, but abaloparatide had no effect on the strength of femur shaft or the intrinsic strength of humeral cortical bone. In vertebral bodies, OVX had a small negative effect on bone quality, when defined as the slope of the regression line for BMC vs peak load. Abaloparatide restored bone quality to sham levels at doses of 0.15-0.7x human AUC. However, the statistical and biological relevance of these small changes was questionable and the effect was not evident in vertebral cores. Regardless, the data indicated preservation of bone quality and did not raise bone safety concerns.

The monkey study was conducted at very low doses (<1x human exposure), and changes were sometimes difficult to resolve.

Safety pharmacology

Abaloparatide had no adverse effects on central nervous system, respiratory, renal/urinary, gastrointestinal systems and hemostasis. Marked hemodynamic effects were observed in anethesized dogs given IV doses of 0.1, 0.3, 1, 3 ug/kg (0.1x, 0.3x, 1x, 3x human AUC, based on mg/m^2 comparison) They included dose-related decreases in mean aortic blood pressure (up to 45%), increases in heart rate (up to 48%), increases in cardiac contractility and output, and decreases in peripheral resistance. Most effects were probably due to peripheral vasodilation known to occur with PTH1 receptor agonists. There are published data suggesting that hPTHrP1-34, and thus abaloparatide, may be more potent and effective in causing direct positive inotropic and chronotropic effects on the heart than rPTH(1-34). This may explain the higher incidence of palpitations, dizziness, nausea, and tachycardia in Phase 3 Study 003, in subjects treated with 80 ug/day abaloparatide vs 20 ug/day teriparatide, doses with equivalent bone efficacy. QTc was not affected in a dog safety pharmacology study or in the repeat dose monkey toxicity studies.

PK/ADME

Abaloparatide is absorbed quickly, reaching C_{max} within 30-40 minutes in rats and monkeys. The plasma $T_{1/2}$ is approximately 30-60 min (rat, monkey). In the rat, it is rapidly distributed to tissues and mainly excreted in the urine (95% within 2 days). Protein binding was similar in dogs and humans (25-30%), but slightly higher in monkeys (ca. 50%). Rat protein binding data were not available. Studies with radioactively labeled abaloparatide suggested rapid degradation by non-specific proteolytic mechanisms in rat and human liver and kidney preparations. Degradation resulted in the formation of relatively inactive peptide fragments. Proteolytic degradation appeared faster in kidney than in liver. Abaloparatide did not inhibit or induce CYP enzymes in human liver preparations. Antibody formation was observed at

relatively high doses in monkeys, and was associated with higher abaloparatide exposures. ADA formation did not appear to affect toxicity. TK data were available for all pivotal toxicity studies, allowing calculation of human exposure multiples. Repeat vs single dose TK data showed increases in exposure concomitant with decreases in clearance over time in rats but not in monkeys.

Toxicity

Single dose studies in mice and rats showed hypoactivity at high IV doses (42 mg/kg) but no mortality. Repeat dose studies in rats and monkeys revealed effects that were predominantly related to the pharmacologic actions of this PTH receptor agonist. Findings in pivotal studies are summarized below. Exposure multiples are based on the human AUC at steady state of 1546 pg·h/mL] (Study BA058-05-001b).

Rat studies

NOAEL and exposure multiples in rat repeat-dose toxicity studies

Study Nr.	**Species Study duration Doses**	**NOAEL (µg/kg/day)**	**LOAEL Findings @ LOAEL**	**AUC at NOAEL* (Females) (pg·h/mL)**	**NOAEL Exposure Multiple****
BA058-114	Rat 4 weeks 15, 70, 300 ug/kg/d	<15 µg/kg/day	15 µg/kg/day Skin reddening (vasodilation) RBC, thrombocyte, eosinophil decreases PT/APTT decrease Alb/Glob decrease Urine Ca and Ca/creatinine increases Bone cell proliferation, trabecular increases, marrow decrease Liver, spleen increased hematopoiesis	<4516	<2.9x
BA058-115	Rat 13 weeks 10, 25, 70 ug/kg/d	<10 ug/kg/day	10 ug/kg/day Skin reddening (vasodilation) RBC, eosinophil decreases APTT decrease, fibrinogen increase, Alb/Glob decrease Urine Ca and Ca/creatinine increases Bone cell proliferation, trabecular increase, marrow space decrease, woven bone formation Spleen increased hematopoiesis and megakaryocytosis	3979	2.6x
7801-124	Rat 26 weeks 10, 25, 70 ug/kg/d	<10 µg/kg/day	10 µg/kg/day Skin reddening RBC, thrombocyte, eosinophil, monocyte decreases Lung weight increase Adrenal cortex vacuolation (Zona Gran) Renal pelvis mineral	2783	1.8x

			No serum Ca effect (pre-dose) (?) Bone and marrow hyperostosis Spleen extramedullary hematopoiesis		
10RAD032	Rat 104 weeks 10, 25, 50 ug/kg/d	<10 µg/kg/day	10 µg/kg/day Osteosarcoma, osteoblastoma	5483 (M,F avg)***	<3.5x

* AUC values are from last sampling time in the toxicology studies
** Multiple = [Animal AUC @ NOAEL] / [Human AUC_{ss} of 1546 pg·h/mL] (Study BA058-05-001b)
*** AUC average for Wks 26 and 52

Monkey studies

NOAEL and exposure multiples in monkey repeat-dose toxicity studies

Study Nr.	**Species Study duration Doses**	**NOAEL (µg/kg/day)**	**LOAEL Findings @ LOAEL**	**AUC at NOAEL* (Males and Females) (pg·h/mL)**	**NOAEL Exposure Multiple****
BA058-118	Monkey 4 weeks 100, 200, 450 ug/kg/d	100 µg/kg/day	200 µg/kg/day BW decrease RBC decrease PT increase Kidney weight increase Bone cell proliferation, woven bone formation, trabecular and cortical bone increases	66985	43x
BA058-119	Monkey 13 weeks 10, 50, 200 ug/kg/d	50 µg/kg/day	200 µg/kg/day Mortality in 1/6 M and 1/6 F due to cardiac degeneration/ necrosis/ mineralization and nephropathy Bone subendosteal cell proliferation, woven bone formation, woven bone formation, no effect on sternum marrow volume Heart, thickened pericardium Kidney weight increase, enlarged Kidney: Tubulo-interstitial nephropathy (basophilia, tubular degen/regen, peritubular fibrosis, mineralization)	15630	10x

7801-125	Monkey 39 weeks 10, 25, 70/50 ug/kg/d	<10 µg/kg/day	10 µg/kg/day Mortality (Animal with Ca 15 mg/dL and kidney mineralization) BW decrease RBC, Hct decreases No serum Ca effect (pre-dose) (?) Serum P decrease BUN increase Kidney weight increase Mineralization in kidney tubules and papilla, adrenal cortex, lung	<4745	< 3.1

* AUC values are from last sampling time in the toxicology studies
** Multiple = [Monkey AUC @ NOAEL] / [Human AUC of 1546 pg·h/mL] (Study BA058-05-001b); Correction for species difference in protein binding was not performed, thus multiples are conservative estimates

Most effects occurred in both species and both genders. The majority of the findings was related to the pharmacology of the product and constituted potential clinical safety issues. While additional toxicities occurred upon longer treatment duration, several effects were already observed in the 4-week studies. In monkeys, upon increase of treatment duration, more organs were affected by mineralizations and kidney pathology was exacerbated.

Target organs/systems

Treatment-related mortality in the 13 and 39 week monkey studies was associated with cardiac and/or kidney pathology. Mortality in the 39-week study occurred in one low dose animal at 3x human exposure. The mortality was probably related to kidney mineralization and preceded by high plasma Ca levels up to 15 mg/dL.

Body weight loss was observed at high doses and multiples in the 4-week monkey study, but at a lower multiple (3x) in the 39-week study. The effect was probably secondary to other toxicities.

Anemia and other blood cell decreases were seen at low exposure multiples (2-3x) in the pivotal rat studies and a dose related progressive RBC decrease was observed in the 39-week monkeys study at ≥3x exposure multiples. Unlike rats, the hematology changes in monkeys were not correlated to decreases in marrow space and increased extramedullary hematopoiesis. Thus, a direct effect on blood forming elements may have occurred. Clinical safety data also identified anemia at a higher incidence in treated vs placebo patients.

Thrombocytopenia and eosinophil or monocyte decreases in rats at 3x human exposure multiples were of unclear significance.

Increases in serum and urine calcium were seen in most of the rat and monkey studies and reflected changes in Ca fluxes due to the effects of abaloparatide on kidney, bone and possibly gut through an effect on renal VitD hydroxylation. The latter was shown in the 16-month monkey bone study. The serum Ca changes were maximal at 3h post dosing. In both species, they were characterized in detail in the 13-week animal studies where they occurred at higher doses in rats (44x human exposure) than monkeys (3x human exposure). Being a recognized potential safety issue, changes in urine and serum calcium were monitored in the Phase 3 clinical study. Serum P was also routinely evaluated.

Bone responses to treatment were seen at the lowest doses in the rat studies and were evidenced by bone increases, subendosteal bone cell proliferation and woven bone formation (2-3x exposure multiples). In monkeys, bone increases were observed at higher exposure multiples (10x) in 4- and 13 week studies. They were not identified in the 9-month study conducted in another test facility.

Mineralization of soft tissues (kidney, heart, lung, urine bladder, mandibular gland) was one of the main toxicologically relevant findings in the monkey studies. It was probably associated with transient hypercalcemia or hypercalciuria, or with local effects on Ca metabolism. The finding was associated with mortality and evident at the high dose of 50x human exposure in the 13-week study, but it was also apparent in the 9-month study at all doses ≥3x human exposure. Soft tissue mineralization in monkeys did not clearly recover in the 4 week treatment-free period after 9 months of dosing. In rats, mineralization of the renal pelvis was seen at 3x human exposure after 26 weeks. Kidney and heart mineralization were also observed at 25-50 µg/kg abaloparatide (13-25x human exposure), and 30 ug/kg PTH(1-34) in the 2-year rat carcinogenicity study.

Kidney pathology especially in monkeys was one of the major toxicological concerns. The potential for kidney calcifications was specifically addressed in a subset of the Phase 3 study population by performing renal CT scans. Clinical monitoring of serum and urine calcium and phosphorus and AE reporting was carried out to detect safety issues and/or mitigate risks related to altered calcium metabolism.

Apart from the hematology changes of unclear origin, obvious off-target effects were not identified.

Local tolerance

There were no adverse findings in rabbits injected SC with 51-100 ug/mL, and in rats or monkeys given up to 300 or 400 ug/mL (0.5-1 mL/kg) by SC dosing. Data suggest that local tolerance is not a significant safety issue. Clinical injection site reactions occurred in <1% of Phase 3 patients.

Genotoxicity

There was no evidence of genotoxicity with abaloparatide in a standard battery of genotoxicity tests. The genotoxicity study findings are included in the product label (Section 13.1), conforming to regulations.

Carcinogenicity

The most significant nonclinical result was the finding of bone neoplasms in the 2-year rat carcinogenicity study. The study was conducted with abaloparatide doses of 10, 25 and 50 ug/kg/day, yielding 4x, 13x and 25x exposure multiples of the recommended human dose of 80µg/day. Exposure multiples are AUC multiples averages for male and female rats. Osteosarcoma and osteoblastoma incidences were statistically significantly increased in a dose-related manner, in all male and female dose groups. Osteosarcoma incidences in low, mid and high dose groups were 35%, 57% and 74% (male and female averages). Tumor incidence in the

30 ug/kg/day hPTH1-34 positive control group, were similar to those in the 25 ug/kg/day abaloparatide group.

The rat tumors induced by abaloparatide or PTH1-34 occurred in parallel with relatively large bone increases. For example, at the low dose of 10ug/kg abaloparatide (3-4x human exposure), the vertebral BMC increase in rats after 24 months was approx. 4 times the 14% BMC increase at the human lumbar spine after 18 months of treatment with 80ug/day in Phase 3 Study 003. The the proximal femur BMC increase in rats was ca. 10-fold the 4.4% BMC increase in human total hip in Study 003.

Bone tumors have previously been observed with rhPTH1-34 and hPTH1-84 (Forteo, Lilly; Natpara, NPS Pharmaceuticals, respectively). They are probably due to the stimulation of osteoblast activity and recruitment and/or the inhibition of osteoblast apoptosis. In the 2-year rhPTH1-34 carcinogencity study, conducted for NDA 21318, osteosarcoma incidences were 6%, 28% and 45% at doses of 5, 30 and 75 ug/kg/day, equivalent to 3x, 21x and 58x human exposure. These incidences were lower and observed at higher exposure multiple than those in the abaloparatide study. However, caution is necessary when comparing these study outcomes because there were differences between the study protocols, particularly the use of radiographical evaluation to increase tumor detection sensitivity in the abaloparatide study (discussed further below).

When osteosarcoma incidence in the abaloparatide study was correlated to BMC-change, the incidence was larger with abaloparatide than with PTH1-34 for males, but not clearly for females. The usefulness of this comparison to predict relative human risk is unclear, because rats and humans may have different bone and tumor responses to different PTH1R agonists.

The earliest fatal osteosarcomas were noted in Months 11 and 12 in the male abaloparatide groups (4-24x human exposure) and the male PTH1-34 group (at ca. 40% of the rat's lifespan). In females, the earliest fatal cases were in Month 14 in the 25 and 50 ug/kg groups (12-25x human exposure) and the PTH1-34 group. Thus, onset of osteosarcoma was similar in 10-25 ug/kg abaloparatide vs 30 ug/kg PTH1-34 groups. Obviously, the times of tumor-related deaths are later than the times of tumor initiation and/or promotion.

The applicant argued that the larger bone tumor incidences in the 30 ug/kg PTH1-34 groups in their carcinogenicity study (39/60 in males, 24/60 in females) as compared to the previous rhPTH1-34 study (21/60 males, 12/60 females) (Vahle et al, 2002), were potentially due to the use of radiography to detect additional bone tumors. However, the larger Cmax, and likely AUC, at 30 ug/kg/day hPTH1-34 in the current vs the previous study may be a more plausible explanation for the difference. The fact that in parallel with the larger tumor incidences the earliest fatal osteosarcoma in the 30 ug/kg hPTH1-34 groups in the current study, were noted at an earlier time point than in the previous study, supports this conclusion.

It has been argued that the relatively long treatment duration of 2 years in these rat studies, i.e. approx. 80% of the rat's average lifespan, diminishes the human relevance of the tumor finding. It has also been noted that the tumors develop in rats in conjunction with an exacerbated anabolic bone response including occlusion of the marrow space. Furthermore, differences in bone

biology between rats and humans have been emphasized to devalue the clinical relevance of the tumors. In an updated Expert Panel Report (July 23, 2015) submitted with the NDA these arguments were put forward to conclude that there is negligible increased risk of osteosarcoma formation in subjects receiving 18-24 months of abaloparatide therapy.

This Reviewer believes that the larger bone response in the 2-year rat studies does not necessarily invalidate the relevance of the tumor findings occurring before end of study. In addition, it is not clear whether differences between rat and human bone physiology negate the tumor findings. Hence, the clinical relevance of the rat osteosarcoma findings with abaloparatide is uncertain and the animal findings should not be dismissed. Based on unknown potential human risk, the animal tumor findings, similar to Forteo, merit inclusion in a black box warning.

In the abaloparatide clinical trial population (Phase 3, N= ca. 800/grp, pbo or Abl) no bone neoplasms were observed. Considering the low human osteosarcoma prevalence (4 per 10^6) a signal is unlikely to be seen in this population of this size, even if the risk were considerably increased. The absence of bone tumors in the 16-month OVX monkey bone quality study is not reassuring, since that study was conducted at low human exposure multiples (<1x) and had relatively small group sizes (N=16F/grp). Bone tumors were also not observed in the 12-month bone quality study in Sprague Dawley OVX rats, at doses up to 12x human exposure and a group size of 18F/grp. In this study, bone mass was markedly increased beyond sham control levels, probably to similar extent as in the intact F344 rats at the 1-year time point in the 2-year carcinogenicity study. Hence, this study did provide partial reassurance.

The rat osteosarcoma findings with rhPTH1-34 (Forteo®) and hPTH1-84 (Natpara®) were included in black box warnings in the labels of these products. Forteo® is approved for the treatment of osteoporosis or to increase bone mass, and Natpara® is approved for the control of hypocalcemia in patients with hypoparathyroidism. Forteo has been on the market since 2002 and use of the drug for more than 2 years is not recommended. Based on available postmarketing and epidemiological surveillance data no clear human osteosarcoma signal has been identified in the Forteo treatment population. However, the low background prevalence and a potentially long latency period are a hindrance to definitive detection of a signal. Hence, the potential human risk increase remains unknown.

As appropriate, the applicant included the rat carcinogenicity finding in a black box warning and in all other relevant sections of the product label. The applicant did not propose [redacted] the Division is recommending limiting cumulative treatment duration with abaloparatide and other parathyroid hormone analogs to 2 years. This Reviewer strongly concurs with this recommendation.

While inclusion of the rat tumor findings in the label is an essential for risk communication, additional approaches for risk evaluation and mitigation may be warranted. REMS considerations and recommendations are discussed in clinical and other discipline reviews.

Fertility

A fertility and early embryofetal development study in male rats showed no effects on male fertility or adverse effects on embryofetal toxicity in females mated with treated males. In a 4-

week toxicity study no effects on epididymal sperm properties were observed. The findings do not suggest that male fertility would be a significant clinical safety issue. Reproductive toxicity study data are included in the label, conforming to regulations. Since the product is intended for use in postmenopausal women, the lack of animal EFD and PPND studies is acceptable for the indication.

Impurity

(b) (4) is the main impurity (degradant) present in drug product and substance. Based on specification limits (b) (4) ICH thresholds for DP and DS (b) (4)%, Q3B(R2), (b) (4)%, Q3A), the degradant was qualified in rat toxicity studies using Abl spiked with degradant, and in genotoxicity assays with degradant only. The results of these studies were not concerning and the compound does not pose a safety or potency risk. The toxicological qualification was adequate.

Taken together, the submitted nonclinical information is adequate. Additional nonclinical PMR studies are not needed.

Label

Pharmacology/Toxicology Labeling Recommendations are in the Executive Summary.

- Black Box warning and Section 5 (Warning and Precautions): Edit language to accurately represent rat carcinogenicity study findings
- Section 12.1: Mechanism of Action: Edit language based on above discussion
- Section 13.1 Carcinogenicity: Edit language to accurately represent rat carcinogenicity findings
- Section 13.2: Animal Toxicology: Include nonclinical toxicities with potential clinical relevance, and include animal bone pharmacology study findings

---

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

GEMMA KUIJPERS
12/22/2016

MUKESH SUMMAN
12/22/2016
Nonclinical supports AP