CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-858

PHARMACOLOGY REVIEW

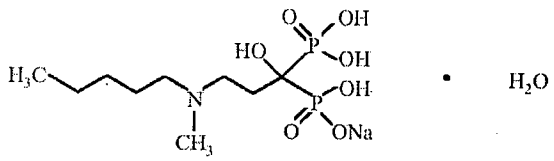
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number:	**21-858**
Compound:	**Ibandronate sodium**
Submission date:	December 6, 2004
Sequence number:	000
Type of submission:	N
Information to Sponsor:	Yes (x) (Labeling comments)
Sponsor:	Hoffman La Roche Inc., NJ, USA
Manufacturer for drug substance:	F.Hoffman-LaRoche Ltd., Basel, Switzerland
Reviewer name:	Gemma Kuijpers
Division name:	Division of Metabolic and Endocrine Drug Products
HFD #:	510
Review completion date:	August 16, 2005

Drug:

Trade name:	Boniva®
Generic name:	Ibandronate sodium
USAN name:	ibandronate sodium
Code name:	BM21.09955·Na·H_2O
Chemical name:	[1-hydroxy-3-(methylpentylamino) propylidene] bisphosphonic acid, monosodium salt, monohydrate
CAS registry number:	114084-78-5
Molecular formula:	$C_9H_{22}NO_7P_2Na.H_2O$
Molecular weight:	Mw 359.2 (sodium salt), 319.2 (free acid)
Conversions:	1 mg P= 5.14 mg free acid; 1 g free acid equivalent = 1.125 g ibandronate monosodium salt, monohydrate)

Structure:



Relevant INDs/NDAs/DMFs:	IND 46,266 (BM21.0955.Na, IV), IND 50,378 (BM21.0955.Na, oral), DMF 15429
Drug class:	Bisphosphonate (bone resorption inhibitor)
Indication:	Treatment of postmenopausal osteoporosis
Clinical formulation:	Film-coated tablet, containing [redacted] mg ibandronate monosodium monohydrate ([redacted] free acid), lactose monohydrate, povidone, cellulose, crospovidone, stearic acid, silicon dioxide, water
Route of administration:	Injection (IV)
Proposed use:	3 mg every three months

Pivotal clinical study:	Study BM16550; 1-year data from 2-year non-inferiority BMD study, with 2 mg every 2 months or 3 mg every 3 months
Disclaimer:	Tables and Figures from the electronic NDA submission have been copied for use in this review

Executive Summary

I. Recommendations

A. Recommendation on Approvability
Approval (AP)

Pharmacology/ Toxicology recommends approval of the NDA for ibandronate sodium (Boniva®), at a dose of 3 mg every 3 months by IV injection, for the treatment of postmenopausal osteoporosis.

Recommendations for Clinical Review
Based on non-clinical intermittent IV dose studies, safety margins for kidney toxicity were low. Pharmacology/Toxicology recommended that the Clinical Reviewer ensure that a sufficient number of patients were evaluated for a sufficient period of time for renal toxicity.

B. Recommendation for Nonclinical Studies
No additional nonclinical studies are required.

C. Recommendations on Labeling
Recommended labeling changes are appended to this Review.

II. Summary of Nonclinical Findings

Mechanism of action
In postmenopausal women bone loss is accelerated due to increased activation of basic multicellular units (BMU's) and a negative balance between bone formation and resorption in each remodeling cycle. Ibandronate prevents or reverses this bone loss because it reduces the size of the remodeling space, improves the bone balance in each newly formed BMU, and increases the degree of mineralization in formed bone. This results in an increase in bone volume and bone mass, reflected by an increase in bone mineral density (BMD, mg/cm2 or mg/cm3).

Primary pharmacology
Based on data from the intact growing rat and the aged ovariectomized rat, mineralization was not affected at doses 1000-5000x times higher than the doses optimally inhibiting bone resorption and turnover.

In a 12-month study in OVX rats, daily dosing (0.2, 1, 5, 25 ug/kg/day) or intermittent, once every 25 day dosing (25, 125 ug/kg) by the s.c. route prevented the loss of bone induced by ovariectomy (OVX) through inhibition of bone resorption and turnover. At optimal dose levels of 1-5 ug/kg/day vertebral bone strength was preserved in parallel with BMD, histologic bone volume and trabecular structure. Ibandronate also protected femoral cortical bone BMD, thickness and strength. Femoral neck strength was not affected by ovariectomy or treatment. Daily treatment with 1 or 5 ug/kg had similar effects as once every 25 day dosing with 25 or 125 ug/kg. There was no indication that the positive correlation between vertebral BMD and strength was different when dosing was carried out daily or intermittently. In a 5-month study in OVX rats, it was shown that optimal efficacy to prevent bone loss can be obtained with drug-free periods up to 6 weeks, equivalent to 12-18 weeks in humans.

In a 16-month study in OVX monkeys, once monthly dosing (0.01, 0.03, 0.15 mg/kg) by the IV route preserved vertebral BMD in parallel with histologic bone volume, trabecular structure and bone strength of whole vertebrae and vertebral cores. BMD at the ulna and femoral neck were also preserved, but bone strength at those sites was not significantly protected. The lack of effect on non-vertebral bone strength may have been due to methodological variability, or relative inefficacy of ibandronate to protect against structural effects of estrogen deficiency such as cortical thinning that are not reflected by BMD. There was a significant positive correlation

between BMD and strength (ultimate load) of the vertebrae and femoral neck. At the ulnar mid shaft, bone strength was much more strongly correlated to BMC than to BMD. BMC was the single most important predictor for bone strength, indicating an effect of both intrinsic (mineral density) and extrinsic (size) bone properties on overall bone strength.

Although the efficacy of ibandronate to preserve bone strength at cortical or mixed cortical/cancellous sites appears to be limited, the animal data do not predict an adverse effect on cortical/mixed bone strength. There were no deleterious effects on bone histology or mineralization in the rat or monkey at monthly doses up to 8 times (rat) and 4 times (monkey) the proposed human 3 mg IV dose, on the basis of mg/m2 (rat) or AUC (monkey) comparison.

The non-clinical bone pharmacology data indicate that within a given time and dose range, the main determinant of efficacy is total or cumulative dose administered. The data support bone safety of an intermittent 3-monthly clinical dosing regimen with optimally efficacious doses of ibandronate.

Safety pharmacology
Safety pharmacology studies in mice, rats and dogs showed that ibandronate did not affect CNS, gastrointestinal, or cardiovascular function at doses up to 1-10x the proposed human 3 mg IV dose, based on body surface area comparison (mg/m2). Inconsistent changes in urinary electrolyte (Na+ and K+) and volume excretion were observed in one oral dog study and a rat screening study at low human dose (mg/m2) multiples (0.3-0.6x). Ibandronate did not affect in vitro hERG K+ channel current at 17x the human C_{max} at the human 3 mg IV dose.

Pharmacokinetics
In rats and dogs, ibandronate is poorly absorbed after oral administration (1% of dose or less) and food markedly suppresses oral bioavailability. After oral administration, Tmax is 0.5-1 h and compound is rapidly cleared (within hours) from plasma by uptake in bone and renal excretion. $T_{1/2}$ (oral or i.v.) is approximately 56 hours in the dog, and 24 hours in the rat. Approximately 40-50% of an absorbed dose is taken up and stored by bone, and approximately 50% is eliminated unchanged via the kidney.

Uptake in bone is linear and related to total dose rather than treatment schedule. Bone levels attained after even a single dose remain high for several months, and $T_{1/2}$ for bone tissue in the rat is 400-500 days. Ibandronate is accumulated in kidney, liver and spleen tissue, but does not cross the blood-brain barrier. Binding to plasma proteins is similar for rat, dog and human (80-99%). In pregnant rats, ibandronate is distributed to the placenta and the fetus, and in lactating rats it is excreted in the milk.

There is no evidence for metabolism in rats or dogs, and no evidence for hepatic or renal drug-drug interaction.

General toxicology
Acute toxicity studies were performed in rats, mice and dogs. IV toxicity studies of up to 6-month duration with daily or (bi)weekly dosing were carried out in rats and dogs. Target organs identified were kidney, liver, lung, esophagus, stomach, thymus and testes. Renal tubular integrity was especially sensitive to ibandronate in rats and dogs.

A number of new IV toxicity studies were carried out for NDA 21-455/S-001 in the rat to address renal safety of an intermittent IV dose regimen. The studies showed that a variety of histologic renal lesions are induced by relatively low doses of ibandronate, and that most lesions were dependent on both AUC and Cmax (i.e. infusion rate).

In a 6-month intermittent IV study with 9 tri-weekly doses of 1 mg/kg, the most sensitive histologic parameters of renal damage were degeneration and necrosis in the proximal convoluted tubule (PCT) and hypertrophy and hyperplasia of the distal tubules. Impairment of renal function, believed to be related to the proximal tubule damage, was not seen in this study but occurred in

other studies at doses higher than those causing histologic renal damage. The intermittent dosing study showed that histologic renal damage is increased after repeated monthly administrations. Renal safety margins for the 3 mg IV clinical dose based on AUC comparison were approximately 1-2x for rat and dog single dose and intermittent IV studies.

Indicators of liver toxicity were observed in a 6-month weekly IV study in the dog but not in the rat. Safety margins based on AUC comparison were 20x (rat), and 6.2x (dog). Gastrointestinal effects were observed in a 6-month weekly IV study in the dog, with safety margins of 20x (rat) and 6.2x (dog). Lung, thymus and testicular toxicities observed in a 6-month weekly IV dog study were associated with safety margins of 6.2x (dog). The 16-month OVX monkey bone pharmacology study with monthly dosing provided a 4.3x safety margin for renal and liver toxicity.

Based on low renal safety margins, Reviewer recommended that the Clinical Reviewer ensure that a sufficient number of patients were evaluated for a sufficient period of time for renal toxicity.

Genetic toxicology and carcinogenicity
Ibandronate had no mutagenic or clastogenic potential, as demonstrated by negative results in *in vitro* and *in vivo* genotoxicity assays. In a carcinogenicity study mice dosed daily via the drinking water for 90 weeks, an increase in the incidence of adrenal subcapsular adenoma was observed in females at high human exposure multiples (cumulative 91-day exposure 32-51x human exposure at the 3 mg human IV, based on AUC). Carcinogenicity studies in rats and mice dosed daily via oral gavage for 18-24 months did not show an increased incidence of tumors. Highest cumulative exposure multiples achieved in the oral gavage studies were 2.5x and 1.4 for male and female rats, and 96x and 14x for male and female mice, respectively.

Reproductive toxicology
In reproductive toxicity studies in the rat, ibandronate decreased fertility in male and female rats at high human exposure multiples (≥40x). When given before or during delivery, ibandronate caused severe maternal dystocia and maternal and fetal periparturient mortality, at a low human exposure multiple (≥2x). This effect has been observed with other bisphosphonates and is believed to be result of hypocalcemia due to suppression of skeletal calcium mobilization required for delivery in the rat. When rats were dosed during gestation, RPU syndrome, a fetal kidney anomaly, was observed at relatively high human exposure multiples (47x), while fetal weight and pup growth were reduced at lower multiples (≥5x). In the rabbit, no teratogenic effects were identified at human dose (mg/m^2) multiples of 20x. In rats, ibandronate is transferred across the placenta and excreted in milk. Exposure multiples for the rat are based on cumulative AUC over the time of animal dosing relative to AUC at the quarterly 3 mg IV clinical dose.

In conclusion, the data from pharmacology and toxicology studies support the safety of the long term use of ibandronate for the treatment or prevention of osteoporosis in postmenopausal women at an IV dose of 3 mg every three months.

III. Administrative

A. Reviewer signature: Gemma Kuijpers

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

CC: list:
NDA Arch
HFD-510
HFD-510/Kuijpers/Davis-Bruno/Hedin

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PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION

This NDA #21-858 is for once every 3 months iv dosing with 3 mg ibandronate (BONIVA). The dose is to be administered over a period of 15 to 30 seconds (bolus injection). NDA #21-455 for BONIVA™ (ibandronate sodium), 2.5 mg orally once daily, or 20 mg orally every other day for 12 doses every 3 months, (tablets), was approved for the treatment and prevention of postmenopausal osteoporosis on May 16, 2003. NDA 21-455 supplement S-001 for BONIVA, 150 mg orally, once monthly, was approved for the osteoporosis indication on March 24, 2005. Sponsor is now submitting a new NDA for an IV formulation (3 mg/3mL) to be administered at 3-monthly intervals for the treatment of postmenopausal osteoporosis.

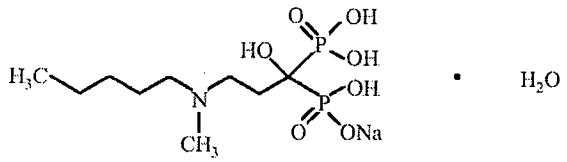
For the original NDA 21-455, a complete preclinical program including chronic toxicity studies with daily oral, s.c., or i.v. dosing in rats and dogs was carried out. The oral and IV toxicity studies were done with daily or (bi)weekly dosing regimens. The kidney was identified as a major target organ of systemic toxicity. Six additional toxicology studies were carried out later to further evaluate renal safety of the intermittent dosing regimen and these studies were submitted to NDA 21-455/S-001. The Sponsor did not submit additional preclinical studies to this NDA 21-858, but cross-referenced studies submitted to NDA 21-455 and NDA 21-455/S-001, as agreed at the pre-NDA meeting (telecon) of September 16, 2004 . The previous data are re-discussed in the context of the new IV dosing regimen.

Ibandronate (2.5 mg daily, orally) has been approved in the US, Europe and Canada for the treatment and prevention of postmenopausal osteoporosis in postmenopausal women. An IV formulation (BONDRONAT®) (1, 2, 4, 6 mg) is registered in Europe for the treatment of malignancy-associated hypercalcemia in patients with or without bone metastases. An IV (6 mg, every 3-4 weeks) and oral (50 mg daily) formulation is also registered in Europe for prevention of skeletal events in patients with breast cancer and bone metastases.

BACKGROUND

Ibandronate belongs to the class of bisphosphonates, which are compounds taken up in bone preferentially where they inhibit osteoclastic bone resorption. The pharmacologic action of ibandronate is based on binding of the compound to the bone matrix and bone cells (osteoblasts and osteoclasts). The effects on osteoclastic bone resorption are thought to be both direct and indirect through cellular actions of the bisphosphonate on the osteoblast. In turn, inhibition of osteoclastic bone resorption inhibits the coupled process of bone formation.

The compound has a core P-C-P bond with a nitrogen-containing side chain:



Bisphosphonates are known to act at the molecular, cellular and tissue level. N-substituted compounds such as ibandronate have also been reported to inhibit farnesyl pyrophosphate synthase, and enzyme in the mevalonate pathway in the osteoclast. This leads to reduced synthesis of isoprenoid geranylgeranyl pyrophosphate and reduced prenylation of small GTP-

binding proteins that are essential for the osteoclast cytoskeletal integrity and intracellular signaling. The result is inhibition of osteoclast activity and effects on osteoclast recruitment and differentiation.

In postmenopausal osteoporosis, bone turnover is generally accelerated due to estrogen deficiency. This leads to a decrease in bone mass because of the increase in skeletal remodeling space. Postmenopausal decreases in bone mass are also believed to result from a negative bone balance (resorption>formation) in each microscopic bone remodeling unit (BRU) leading to microarchitectural deterioration of bone tissue. These events cause a macroscopic decrease in bone mineral density (BMD), which is inversely related to an increase in fracture risk. This has been demonstrated most convincingly for the spine.

Maintenance or increase of bone mass (BMD) with a bisphosphonate is believed to result from both a reduction in remodeling space due to inhibition of the coupled processes of resorption and formation (bone turnover), and a reversal of the negative bone balance. The latter may occur as a consequence of increased bone formation and mineralization accompanying the bisphosphonate-induced decrease in activation frequency and increase in life time of the BRU, or as a result of other direct positive effects on the bone-forming osteoblast.

Bisphosphonates exert their effect by binding to the target tissue (bone and bone cells that adhere to bone) and plasma levels are not direct determinants of their pharmacologic action. This is reflected in the (pre)clinical finding that a single dose administration can be effective for several months. However, with regard to non-skeletal toxicities as well as for the purpose of species comparison, systemic exposure (C_{max}, AUC) is an adequate parameter to relate dose to effect.

Known adverse effects of bisphosphonates are GI and renal events. The renal toxicity is believed to be related to the renal excretion of these compounds. GI events (esophageal, gastric, intestinal irritation, ulceration, perforation) have been observed with several bisphosphonates. The mechanism of GI toxicity is unclear. IV dosing has also been associated with GI events.

CLINICAL STUDIES

This NDA is based on a single Phase 3 pivotal trial, BM16550 (DIVA Trial). This was a non-inferiority trial in postmenopausal women comparing changes in lumbar spine BMD after 1 year of treatment with IV ibandronate 3 mg every 3 months and 2 mg every 2 months, to 2.5 mg once daily orally. The trial is a bridging study with BMD changes at 12 months as the primary endpoint. The trial is continued for a second year. Biopsy data will be obtained in the second year. Other trial data on safety and efficacy of IV treatment were included in the NDA. Daily oral treatment with 2.5 mg has demonstrated anti-fracture efficacy in the 3-year pivotal study **MF4411** and served as the reference arm in Study BM 16550.

The rationale for 3-monthly IV dosing is based on the inconvenience of daily dosing with potential gastrointestinal intolerance, and the efficacy of intermittent dosing. A previous 3-year IV trial (**MF4380**) in women with osteoporosis has been completed with 0.5 mg and 1.0 mg IV every 3 months (2 mg and 4 mg annual doses). At 36 months, there was no significant effect on the incidence of new vertebral fractures (14% reduction) and a small increase in BMD (4.6% at Month 36). The doses selected for the 1-year pivotal study with quarterly treatment (**BM 16550**) were chosen based on the conclusion that the previous IV fracture trial had failed because the 0.5 and 1 mg doses were too low. This was supported by BMD data from other IV studies with higher doses (up to 2 mg every 3 months).

Bone biopsy histomorphometry evaluations were conducted in 3-year trial **MF4411** (2.5 mg oral daily, 20 mg intermittently) and trial MF4380 (0.5 mg or 1 mg IV every 3 months). Bone quality appeared normal in the treated women. ECG data were obtained in a sub-study of IV trial BM 16550.

Efficacy

Study BM 16550 showed that both IV regimens of 2 mg/q 2 mo and 3 mg/q 3mo increased BMD at the lumbar spine (L2-L4) by ca. 5% at Month 12 (compared to ca. 4% with 2.5 mg daily oral tablets). The difference of the BMD effect between the IV regimens and the oral regimen was statistically significant. Serum CTx was suppressed by ca. 60% with both IV regimens, similar to that with 2.5 mg daily oral. Efficacy of the two IV regimens (BMD, turnover) was not statistically significantly different. Clinical fractures occurred with similar incidence in the 3 groups. The improvement in BMD is anticipated to lead to anti-fracture efficacy.

Safety

IV ibandronate was well tolerated. Adverse events most likely associated with IV treatment were influenza-like symptoms, musculoskeletal events, fatigue and headache. There was no symptomatic hypocalcemia in the pivotal trial. Renal toxicity is an expected potential toxicity with bisphosphonates. Sponsor concluded that 2 mg/q 2mo and 3 mg/q 30 IV has no impact on renal function in osteoporotic women with creatinine clearance >30mL/min at baseline. Some women had deterioration of serum creatinine but this was ascribed to other underlying conditions (hypertension, diabetes). Use of the 3 mg IV dose is not recommended in patients with severe renal impairment (<30 mL/min). Clinical trials with IV inbandronate in patients with metastatic disease did not indicate effects on renal function, i.e., creatinine clearance or urinary excretion of tubular proteins (NAG, b-microglobulin). Ocular events occurred with similar frequency in treated and placebo groups in combined IV and oral studies. The 3-montn regimen was generally preferred by patients and doctors.

Biopharmaceutics

Several IV formulations have been used during clinical development. All have the same concentration (1 mg/mL), and similar excipients including NaCl, glacial acetic acid, sodium acetate, water. Bioanalytic methods for urine and serum measurements included GC-MS or ELISA (LLQ 1000 and 10 pg/mL).

The human clinical pharmacology program included 10 pharmacology studies in various study populations. Doses of 0.125 to 2 mg were given as injections over 30 seconds, while doses of 4-6 mg were given as IV infusions of 15-120 min duration. The pooled study results showed linear kinetics over the 0.5-6 mg range. The data predict an AUC of 808.5 ngxh/mL for the 3 mg bolus injection.

Clinical pharmacology data

		Cmax (ng/mL)	AUC (ngxh/mL
All doses (up to 6 mg)	-	16-400	17-908
3 mg IV dose	30 sec injection	582	**808.5**

Figure 1 **Mean Ibandronate Dose - AUC Relationships after IV Administration**

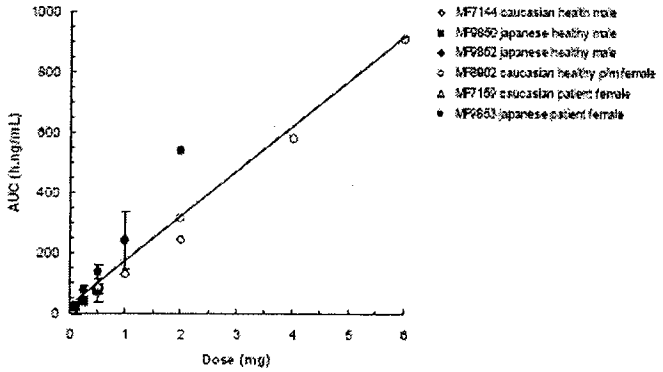
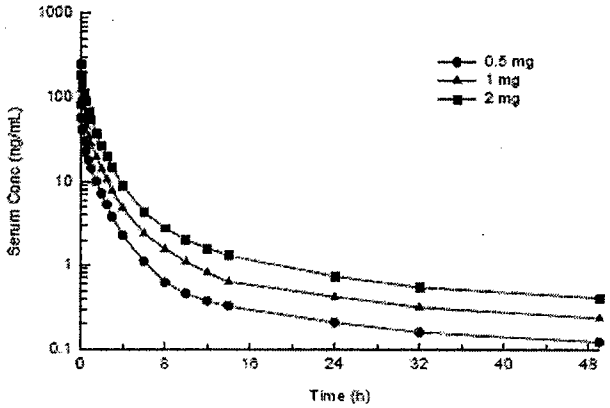


Figure 2 Mean Serum Ibandronate Concentrations after IV Administration of 0.5, 1, and 2 mg to Healthy Male Volunteers (Study MF7144)



Serum concentrations decline >90% within 4h after dosing and accumulation is not expected. Clearance is by bone uptake (40-50%) and renal excretion (50-60%) of unchanged drug. Ibandronate is not metabolized.

PK-PD modeling was performed to predict the time course of the decrease of urinary CTx excretion following oral or IV dosing. The model predicts an optimal annual IV dose of 12 mg, with regard to bone resorption inhibition.

Table 1 Model Predicted uCTX Percent Decrease from Baseline versus Time for Various Ibandronate Dosing Regimens

Time (month)	Ibandronate Regimen					
	po 2.5 mg od	iv 0.5 mg q3m	iv 1 mg q3m	iv 2 mg q3m	iv 3 mg q3m	iv 2 mg q2m
0	0	0	0	0	0	0
1	35	30	42	56	64	56
2	48	29	39	51	59	51
3	55	29	36	46	53	67
4	59	43	54	67	73	61
5	61	39	49	60	67	71
6	62	36	44	54	61	65

Human doses

Dose	Route	Regimen	Annual dose (mg)	Annual IV dose equivalent (mg) (assume 0.6% BA)
2.5 mg	oral	Daily	913	5.5
150 mg	oral	monthly	1800	10.8
20 mg	oral	Q 3mo, 12 doses	960	5.8
0.5	IV	Q 3mo, single	2	2
1	IV	Q 3mo, single	4	4
2	IV	Q 3mo, single	8	8
3	IV	Q 3mo, single	12	12
2	IV	Q 2mo, single	12	12

PHARMACOLOGY/TOXICOLOGY PROGRAM

A complete nonclinical pharmacology/toxicology program was conducted to support the original NDA (#21-455, #000). Drug was administered by i.v., p.o., s.c. and other routes. The program included toxicity and reprotoxicity studies with oral and iv dosing, genotoxicity studies, carcinogenicity studies, and local tolerance studies. All studies were in compliance with GLP regulations. No new studies were submitted to the current NDA.

For Pharmacology/Toxicology, the main issue for this NDA is the potential for renal toxicity associated with infrequent high doses. Renal disease has been associated with zoledronate treatment (ATN, acute tubular necrosis), and prolonged treatment with high doses of pamidronate (nephritic syndrome with glomerular sclerosis). Another issue is that at relatively high doses and/or long term treatment with bisphosphonates excessive suppression of bone turnover can occur which can lead to an impairment of (micro)fracture healing. Biopsy data have not yet been obtained in the pivotal trial BM16550, but are available from previous clinical studies (MF4411 oral, MF4380 IV) at lower total annual doses.

This NDA review focuses on the results of bone pharmacology and renal toxicity studies.
Bone pharmacology studies were performed in OVX rat and monkeys, and OHX dogs, with daily or intermittent treatment regimens. Toxicity studies performed in the original development program included daily oral, daily IV and (bi)weekly IV regimens in rat and dogs. In addition, renal safety was addressed in more detail in six rat toxicology studies with single or repeat IV doses, submitted and reviewed for NDA 21-455/S-001 for the 150 mg monthly oral dose regimen. The studies with single or intermittent (IV) doses are most relevant for the current NDA.

In toxicology studies, the doses of ibandronate are expressed as either weighed drug substance (WDS; ibandronate monosodium salt, monohydrate, dry substance) or free acid equivalents (FAE; ibandronic acid). Ibandronic acid is the active ingredient. The conversion factor in the studies ranges from 1.125 to 1.15 (1g free acid equivalent = 1.125-1.15g monosodium salt). In clinical studies, doses are expressed as free acid equivalents. The 150 mg clinical dose refers to the free acid.

I. PHARMACOLOGY

A central finding in the non clinical pharmacology studies is that ibandronate inhibits bone resorption and that within a given time frame the total cumulative dose is the main determining factor for its efficacy. Another important finding is that bone strength is positively correlated to BMD, even though the slope and the strength (scatter) of the correlation are different for different bone sites. At the doses tested in animals, bone mineralization was not affected in such a way that it led to features of osteomalacia (increased osteoid width combined with increased mineralization lag time and decreased mineral apposition rate). Cortical bone histology was normal lamellar.

Bone studies were reviewed in the original NDA review (#21-455). Relevant studies are discussed in this review in light of the proposed new dose regimen (3 mg//q 3mo, IV). For details see the original NDA review for NDA 21-455.

Studies in estrogen-deficient animals

Species	Report	Study description	Prevention/ Treatment
RAT	D14	20-week study in aged Wistar rats. Treatment started 1 day after OVX. Doses 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03 mg/kg/day s.c.	Prevention
	D15 D22	20-22 week treatment in aged Wistar rats, starting after OVX. Doses 0.0001, 0.001 mg/kg/day s.c. or higher intermittent doses resulting in similar cumulative doses	Prevention
	D25 D26 D27	12-month treatment of aged Wistar rats with 0.0002, 0.001, 0.005, 0.025 mg/kg/day, or 0.025 and 0.125 mg/kg every 25 days, starting 10 weeks after OVX	Treatment
MONKEY	D30	16 month study in OVX cynomolgus monkeys. Treatment started at day OVX, with i.v. doses of 10, 30, 150 ug/kg, once monthly.	Prevention
	D31	Micro-tomographical imaging and biomechanical testing of bones from cynomolgus monkeys treated for 16 months (Study D30)	
DOG	D9	4-week study in OHX dog, doses 0.0001, 0.0003, 0.001, 0.01, 0.1 mg/kg were given s.c. for 6 out of 7 days per week.	Prevention
	D13	16-month study with 12-month treatment starting 4 months after OHX in dogs, doses of 0.0008, 0.0012, 0.0041, 0.014 mg/kg (5 days/wk), or 0.065 mg/kg for 14 days followed by 11 wks off	Treatment

OVARIECTOMIZED (OVX) RAT

Summary and evaluation of 12-month rat study (D25, 26, 27)

- Rats were treated for 12 months by s.c. injection, starting 10 weeks after OVX, with 0, 0.2, 1, 5, 25 ug/kg/day or 25, 125 ug/kg/25 days.
- In cancellous bone, ibandronate suppressed the OVX-induced decrease in bone mass (BMD, BV/TV) and deterioration of trabecular structure.
- Ibandronate preserved bone mass (BMD) and strength at vertebra and femoral midshaft
- OVX or ibandronate had no effect on strength of femoral neck.
- Effects were optimal at 5 ug/kg/day or 125 ug/kg/25 days. Effects were preserved at the high dose of 25 ug/kg/day.
- At equivalent cumulative ("total") doses, daily treatment and intermittent treatment (once every 25 days) had similar effects on bone.
- There was no indication that the relation between BMD and bone strength is different for the intermittent dosing regimen as compared to the daily regimen.

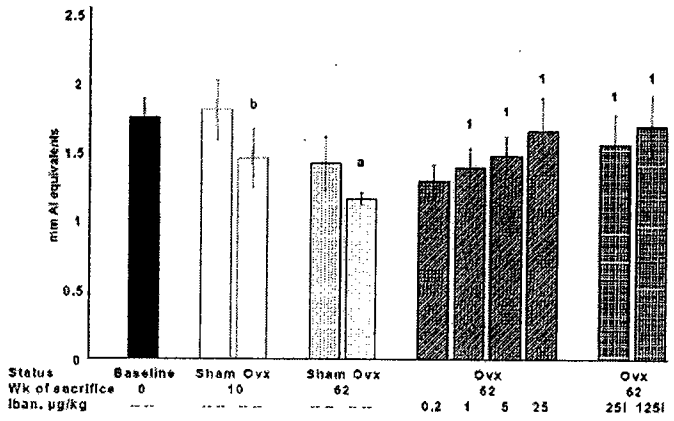
Multiple calculations

- Based on cumulative dose (mg/m2) per remodeling cycle, and assuming remodeling cycles in the rat and human are 25 days and 3 months, respectively, the 5 ug/kg/day s.c. dose is

equivalent to a human IV dose of 1.2 mg. The high dose of 25 ug/kg/day is equivalent to a human IV dose of 6 mg. This is **0.4x** and **2x** the proposed 3 mg IV dose. (*Assume: human body weight is 60 kg; and conversion factor for mg/kg→mg/m2 rat→human = 1/6*)

- Based on cumulative dose (mg/m2) per 91 days, the 5 ug/kg/day dose is equivalent to a 4.5 mg human IV dose, and the high dose of 25 ug/kg/day to a 22.5 mg human IV dose, Thus, the 5 ug/kg/day and 25 ug/kg/day are equivalent to **1.5x** and **7.5x** the 3 mg human IV dose.
- The latter (cumulative dose over 91 days, mg/m2) is the preferred multiple calculation, since the 90-day-AUC-multiple is similar to the bone-dose/remodeling-period multiple, as shown for the monkey study (D30, below), and Reviewer believes that bone dose is the most appropriate basis for comparison. Thus, Reviewer recommends to use the 7.5 multiple to describe the rat high dose of 25 ug/kg/day s.c. in the label.

Figure 1 **Intermittent and Daily Treatment : X-ray Density of the Distal Right Femora in Ovariectomized (OVX) and Sham-Operated (Sham) Aged Rats.**



OVARIECTOMIZED (OVX) MONKEY

A study to determine the effects of ibandronate on bone mass, strength and architecture after 16 months of treatment in the ovariectomized cynomolgus monkey (CTBR project nr. 87284) (D30)

Cynomolgus monkeys (15 or 12/group) were sham-operated (SHAM), or ovariectomized (OVX) and dosed with 0 (OVX vehicle control), **0.01, 0.03, 0.15 mg/kg**, every 30 days, by IV injection. Dosing was started immediately after surgery. Measured were BMD (DXA or pQCT), bone markers, histomorphometry of cancellous and cortical bone, and bone strength. Serum PK was measured at 1 and 16 months. Bone concentration (tibia, L6) was measured after 16 monts.

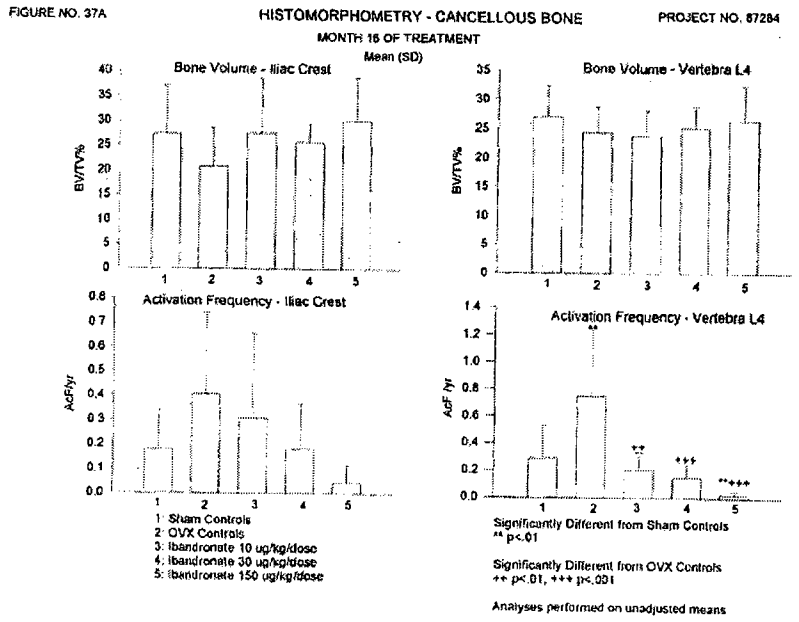
On a mg/m2 basis, the doses correspond to human IV doses of 0, 0.2, 0.6, 3 mg every remodeling cycle (1 mo in monkey, 3 mo in human), or on strict time base they correspond to human IV doses of 0, 0.6, 1.8, 9 mg every 3 months.

Biomarkers
Bone resorption and formation increased by OVX; ibandronate prevented increase dose-dependently

Bone mineral density

BMD (lumbar spine and femoral neck, proximal tibia) decreased by OVX, ibandronate prevented BMD loss at cancellous and cortical bone sites

Histomorphometry



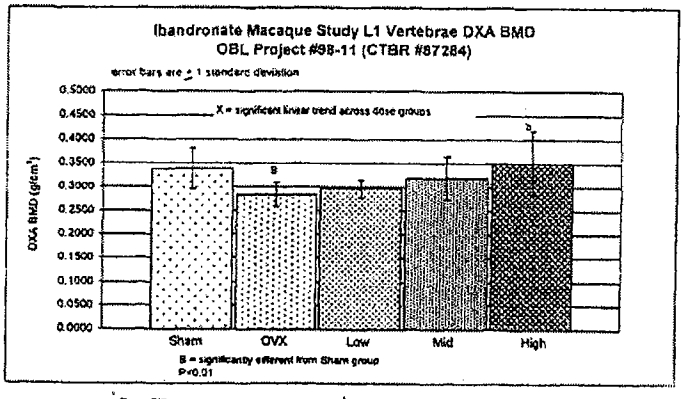
Histomorphometry at 6 (iliac crest) and 16 months (various sites):

- Ibandronate acts by suppression of OVX-induced bone turnover with restoration of trabecular separation
- OVX reduced bone volume, increased activation frequency and increased bone turnover parameters in cancellous bone (iliac crest, femur, lumbar vertebrae, radius). Ibandronate suppressed the OVX-induced increases (Ac.F, BFR, MS/BS), below sham levels at the HD of 150 ug/kg.
- The suppression of Ac.F was 20% (LD), 55% (MD) and 90% (HD) of OVX at iliac crest. The suppression was to below sham control levels at the HD at both iliac crest and vertebra L4.
- Osteod thickness was increased by OVX, and this was suppressed by ibandronate treatment, at some sites below sham control levels.
- Mineralization lag time (Mlt, a measure of mineralization and osteomalacia) was not significantly affected by ibandronate in iliac crest, femur and vertebra, but was increased at the 150 ug/kg dose in the radius. The latter increase was accompanied by a slight decrease in osteoid width.
- Mineral apposition rate (MAR) was either unaffected (femur, radius) or decreased by ibandronate (iliac crest, vertebra).
- Suppression of OVX-effects on cortical bone remodeling parameters (rib, femoral neck, radius) was less than in cancellous bone (i.e., different dose response).
- Histologically, there was no evidence of a mineralization defect, abnormal bone, or abnormal accumulation of unmineralized osteoid. Bone tissue was normal lamellar at all bone sites.

Data showed that there was a decreased bone turnover rate but no evidence of an osteomalacia-like mineralization defect (as defined by increased osteoid width and Mlt, and decreased MAR) in cancellous or cortical bone at any dose.

Biomechanics

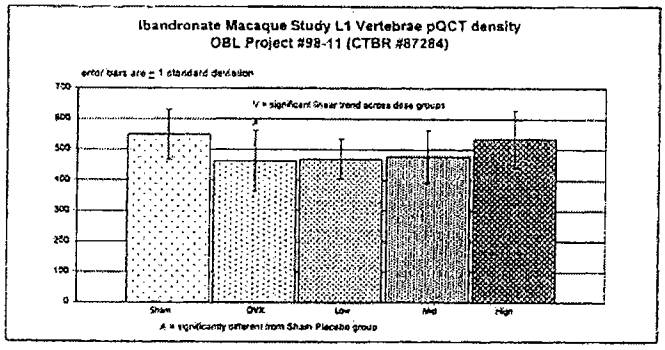
Bone strength decreased by OVX, effect prevented partially or completely by ibandronate



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	*+ 19%	13%
OVX	*- 18%	-	9%
Low Ibband	- 12%	+ 4%	6%
Mid Ibband	- 5%	* 12%	14%
High Ibband	+ 4%	*+ 23%	19%

* = significant difference

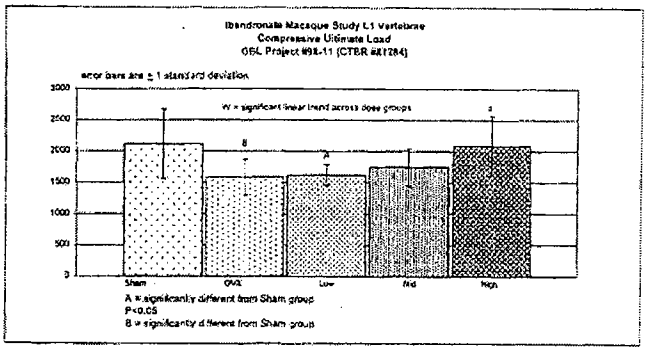
Figure 14: L1 Vertebrae BMD



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	*+ 19%	15%
OVX	*- 19%	-	21%
Low Ibband	- 15%	+ 1%	14%
Mid Ibband	- 13%	+ 3%	18%
High Ibband	+ 3%	* 19%	17%

* = significant difference

Figure 18: L1 Vertebrae pQCT density



Group Differences in percent	from Sham	from OVX	Group COV
Sham	-	*+ 33%	28%
OVX	*- 25%	-	18%
Low Ibband	*- 24%	+ 2%	19%
Mid Ibband	- 14%	* 10%	17%
High Ibband	- 1%	*+ 32%	23%

* = significant difference

Figure 19: L1 Vertebrae Compression Test - Ultimate Load

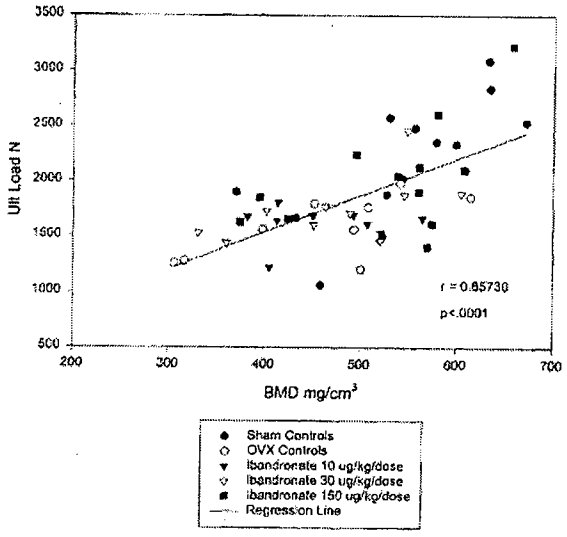
Effect of OVX and ibandronate on BMD, BMC, size, and strength after 16 monthly iv doses

		Sham	OVX			
			Vehicle	10 ug/kg	30 ug/kg	150 ug/kg
L5 vertebral cores	BMD pQCT (mg/cm3)	290	210 (a)	261	316 (b)	352 (b)
	BMC pQCT (mg/mm)	3.35	2.16	2.97	3.64	3.85
	Ultimate Load (N)	48	39	40	48	50
L1 whole vertebrae	BMD pQCT (mg/cm3)	549	462 (a)	469	477	534
	BMC pQCT (mg/mm)	51	43	43	44	51
	BMD DXA/APØ (g/cm3)	0.31	0.25 (a)	0.27 (a)	0.29 (b)	0.31 (b)
	BMC DXA (g)	0.70	0.59	0.57	0.67	0.77 (b)
	Ultimate Load (N)	2116	1586 (a)	1614 (a)	1745	2089 (b)
Humerus	Ultimate Load (N)	198	152	142	145	197
	Width (of specimen)	4.47	4.31	3.83 (a)	4.33	4.85 (b)
	Strength (N/mm2)	199	178	208	203	203
Ulna	BMD pQCT (mg/cm3)	1357	1297 (a)	1296 (a)	1324	1345 (b)
	BMC pQCT (mg/mm)	28.2	23.4 (a)	22.7 a)	24.0	24.2
	Area (mm2)	20.9	18.0	17.5	18.1	18.0
	AP diameter (mm)	4.96	4.60	4.56	4.73	4.56
	MI (mm4)	47	35	34	39	34
	Ultimate Load (N)	211	160 (a)	158 (a)	172	172
Femoral neck	BMD pDXA (g/cm2)	0.45	0.40	0.40	0.41	0.45
	BMC pDXA (gr)	0.18	0.16	0.13 (a)	0.17	0.20 (b)
	Ultimate Load (N)	1624	1229 (a)	1246 a)	1372	1362

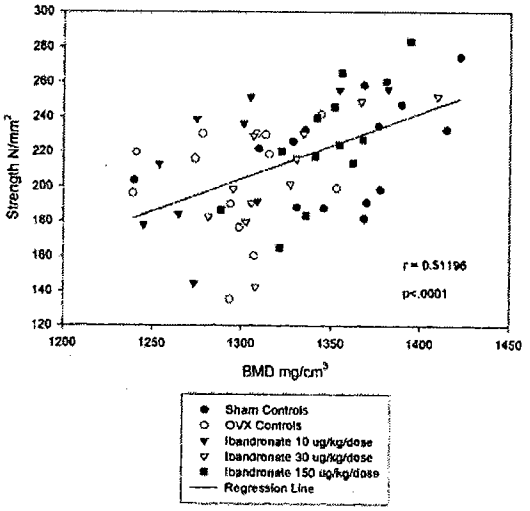
(a) significantly different from sham
(b) significantly different from OVX

Correlation between BMD and ultimate load for L5 cores: r=0.75794; for L1 vertebrae: r=0.6573. The correlations appeared similar for the different dose groups, and data points for sham and HD bone were similarly distributed. The data for vertebral core and whole vertebrae confirm the biomechanical test data that an increase in BMD is accompanied by an increase in strength. Best correlation was between BMC and strength in ulna, probably because BMC reflects both BMD and bone geometry/size.

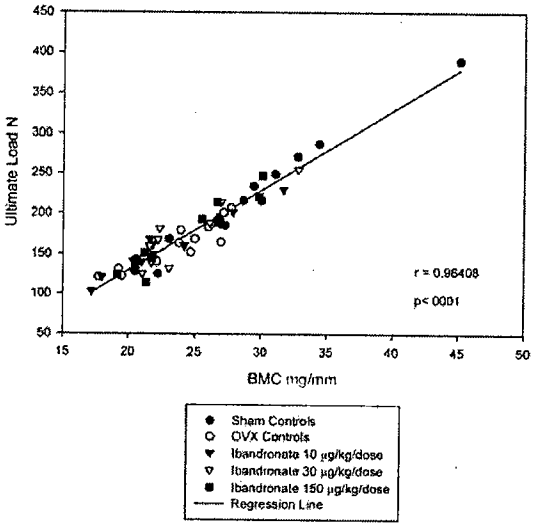
Correlation of Density and Ultimate Load - L1 Whole Vertebrae



Correlation of Density and Strength - Ulna



Correlation of Bone Mineral Content and Ultimate Load - Ulna



PK data

Determination of ibandronate in monkey serum (Month 16)
AUC was dose-proportional

Monkey PK data (serum, ELISA): (Appendix 31, pp.1549-1550, Report L16)

Group	Dose (ug/kg)	Cmax (ng/mL) median and range	AUC (ngxh/mL) median and range	Multiple of AUC at human 150 mg oral monthly dose (cumulative over 30 days)	Multiple of AUC at human 3 mg IV dose (cumulative over 91 days)	Multiple of AUC at human 3 mg IV dose (cumulative per remodeling cycle)	Multiple of bone dose (mg/m2 bone surface per remodeling period)
LD	10	55.7 (40-64)	62.2 (46-79)	0.3x	0.23x	0.08x	0.2x
MD	30	200.0 (145-367)	198 (131-479)	1x	0.8x	0.2x	0.6x
HD	150	877.3 (630-1436)	1115 (840-1956)	5.8x	4.3x	1.4x	3x

F (oral bioavalilability) 0.63% (based on human data with oral 20 mg dose)
AUC (human 150 mg oral dose, 2.5 mg/kg): 192 ngxh/mL (Study SB 743830/002).
AUC (human 3 mg IV dose, 50 ug/kg): 808.5 ngxh/mL

Multiple calculation

Remodeling cycle time in the monkey is ca. 1 month while in the human it is 3 months. AUC multiples can be calculated on basis of (1) strict time, or (2) remodeling cycle time. Based on cumulative AUC over time, the monkey dose of 150 ug/kg is a **4.3x** multiple of the human 3 mg IV dose. Based on AUC/remodeling cycle, the monkey dose of 150 ug/kg is a **1.4x** multiple of the human 3 mg IV dose.

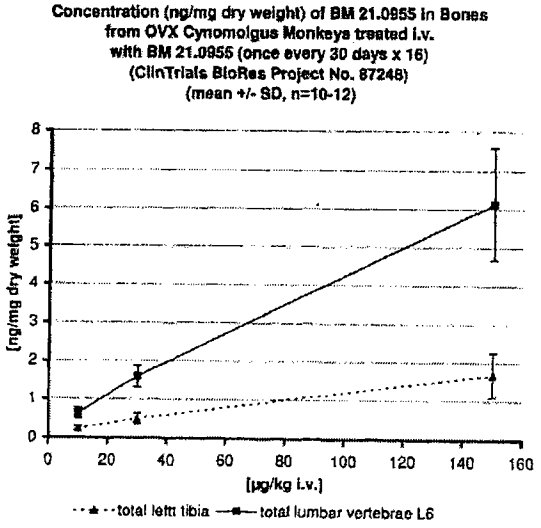
Another approach would be to base exposure multiples on (3) bone dose (mg/m2 bone surface) per remodeling period. This would be the most appropriate basis of comparison based on the compound's mechanism of action. Since ca. 40-50% of an administered dose is taken up in bone in both species, and bone surface is more likely related to body weight than to body surface area (since trabecular thickness is very similar in monkeys and humans), relative bone dose can be equated to dose/kg body weight. According to this approach, the high dose in monkeys (150 ug/kg) is **3x** the 3 mg human IV dose (50 ug/kg).

A last method of exposure comparison could be based on the suppression of activation frequency (Ac.F) in monkey and human studies using available biopsy data. In clinical trial # MF4380 with 1 mg i.v. every 3 months (4 mg yearly), Ac.F was ca. 70% suppressed in the iliac crest. Extrapolating from monkey data (dose vs. Ac.F) this translates to a suppression of ca. 90% with 3 mg every 3 months (12 mg yearly), which is similar to the suppression at the monkey HD of 150 ug/kg. This indicates the monkey high dose is equivalent to **1x** the 3 mg human IV dose.

Reviewer feels the multiple based on bone dose per remodeling period (similar to 91-day cumulative AUC multiple) is most appropriate.

Concentrations of ibandronate in bone

- Bone concentration was higher in L6 than in tibia. This was probably due to higher surface/volume ratio in trabecular bone.
- In tibia ibandronate was concentrated in proximal > distal metaphysis and diaphysis.
- Bone concentration was nearly dose-proportional.



In all doses, the concentration in vertebrae is significantly higher than those in tibiae ($p \leq 0.001$, Mann-Whitney rank sum test, one-tailed)

Other data

- BUN and creatinine were not affected at any dose

Summary monkey study

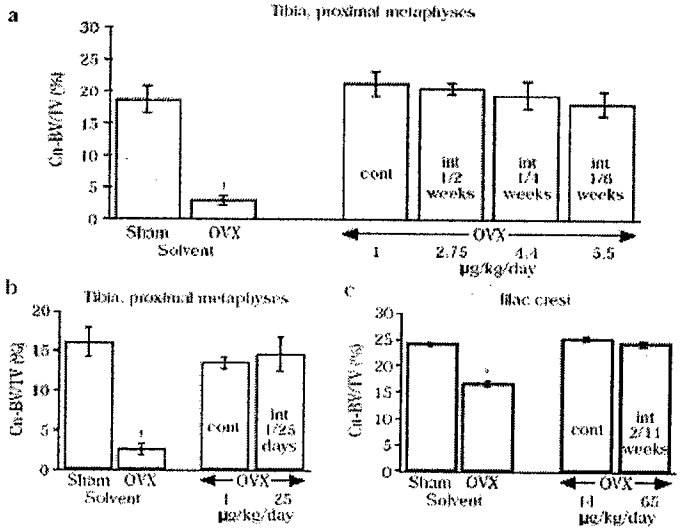
- Intermittent monthly IV doses of 10, 30, 150 ug/kg suppressed effects of OVX on BMD, histomorphometry and strength
- There was a positive correlation between bone mass and strength
- Ibandronate decreased bone turnover rate but there was no evidence of an osteomalacia-like mineralization defect in cancellous or cortical bone at any dose.

OVARIOHYSTERECTOMIZED (OHX) DOG

Ibandronate appeared to suppress bone turnover and increase histomorphometric bone volume and mineralization in the OHX dog. In Study D13, intermittent treatment with 65 ug/kg for 14 days every 11 weeks prevented the decrease in cancellous bone volume (Cn. BV/TV) similarly as continuous treatment with 14 ug/kg/day (5days/wk). However, the data showed that in the dog OHX

has no persistent effects on bone BMD, histomorphometry or strength. Thus, the OHX dog was not an adequate long term animal model for osteoporosis.

Figure 2 **Comparison of Continuous (cont) and Intermittent (int) Ibandronate Administration on Cancellous Bone Volume (Cn-BV/TV, %) in OVX Rats or OHX Dogs**



(a) preventive treatment in aged rats (n = 10–13) for 5 months (adapted from [1721] nonclinover.pdf - 26 ; (b) therapeutic treatment in aged rats (n = 10–12) for 1 year (adapted from [1723] nonclinover.pdf - 26 ; (c) therapeutic treatment in adult dogs (n = 9–10) for 1 year (adapted from [1727] nonclinover.pdf - 26 . The doses represent the optimal dose in the respective model, which is the lowest dose that fully prevented or restored bone mass when compared to age-matched sham controls.
Cycles as indicated within the bars are weeks or days on/off ibandronate treatment. Data are expressed as mean ± SEM. Difference between sham and OVX/OHX: *$p \leq 0.001$, †$p \leq 0.0001$. When compared to sham-controls, continuous and intermittent treatment revealed equivalent results, i.e. not statistically different.
Figure obtained from [2587] nonclinover.pdf - 26

CONCLUSIONS FROM OVX ANIMAL BONE STUDIES

- In OVX rats and monkeys bone mass, structure and strength are improved by daily and intermittent dosing with ibandronate, via suppression of bone resorption and formation. The positive relation between vertebral BMD and strength is maintained at all doses evaluated.
- The total cumulative dose appeared to determine the efficacy with regard to bone mass, structure and strength.
- Maximum studied dose-free interval was 6 weeks in OVX rats (Studies D15, D22), 11 weeks in OHX dog (D13), 30 days in OVX monkeys (D30). At these intervals efficacy was maintained within the dose range evaluated. Intervals in rat and monkey correspond to 2-4 months in human (remodeling period 2-3x rat, monkey).
- Data indicate a smaller effect of ibandronate on non-vertebral than on vertebral BMD and strength

- Data suggest that an increase in non-vertebral BMD in ibandronate-treated may occur in absence of a significant effect on strength
- High doses in most relevant OVX animal studies were equivalent to **7.5x** (rat) and **4.3x** (monkey) the human 3 mg IV dose, based on cumulative mg/m2 and cumulative AUC comparisons, in rats and monkeys respectively.

Clinical relevance

- Increase in lumbar spine BMD after 1 year treatment in pivotal clinical study BM16550 is likely to predict a reduction in vertebral fracture risk
- Value of BMD as surrogate marker for non-vertebral fracture risk is not clear
- BMC may be a better predictor than BMD for strength of non-vertebral (cortical) bone

Multiple calculations for label (Animal Pharmacology)

Sponsor based multiples for animal pharmacology studies on cumulative exposure over the time of dosing in the animals, based on mg/m2 (rat, monkey) or AUC (monkey). Sponsor provided the calculations (2.6 Non-Clinical Summary; Section 2.6.7 Toxicology Tabulated Summary, Appendix 1, pp.292-299). For the label ("Animal Pharmacology"), Sponsor used cumulative dose (mg/m2) over 91 days as basis for multiples. Reviewer calculated multiples based on cumulative AUC/remodeling cycle, cumulative AUC/3-months, or bone dose/remodeling cycle. Reviewer believes that the latter is the most appropriate basis for exposure multiple calculation. However, cumulative mg/m2 or AUC over 91 days can be used for rats and monkeys, respectively, since this parameter adequately reflect bone dose/remodeling cycle.

The data are adequate to support the current NDA. Labeling changes were proposed by sponsor and reviewed for this NDA (APPENDIX).

II. SAFETY PHARMACOLOGY

2.6.3.4 Safety Pharmacology Test Article: Ibandronate

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses[a] (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular System	hERG K^+ channel, expressed in CHO cells	*In vitro*	30 µM ibandronate (corresponding to approximately >10,000 ng/mL), and 10 µM of E-4031 (hERG K^+ channel blocker, used as a positive control)		Ibandronate, tested for its pro-arrhythmic potential in the hERG K^+ channel assay at a concentration of approximately 10 times the C_{max} in humans had no influence on in inward and outward K^+ currents.	NR	1006141
Central Nervous System	Mouse, NMRI	i.p.	1 and 3 mg/kg[a];	6 females	Ibandronate was negative in the Irwin behavioral test.	NR	E2
	Mouse, anesthetized (urethane) NMRI	i.p.	ibandronate: 3 and 10 mg/kg[a] diazepam: 2 mg/kg	10 females	Ibandronate did not potentiate the anesthetic effect of urethane, whereas diazepam resulted in 80% potentiation of the anesthetic effect.	NR	E10
	Mouse, NMRI	i.p.	ibandronate: 1 and 3 mg/kg[a] diazepam: 2 mg/kg (p.o.) pervitin: 2 mg/kg (p.o.)	6 females	Ibandronate had no effect on spontaneous locomotor activity at 1 mg/kg. At 3 mg/kg, ibandronate caused decreased motility comparable to that caused by 2 mg/kg of diazepam. Motility was potentiated by pervitin.	NR	E6
	Mouse, conscious NMRI	i.p.	ibandronate: 1 and 3 mg/kg[a] diazepam: 2 mg/kg Dopram®: 100 mg/kg:	10 females	Ibandronate had no effect on pentetrazole-induced cramps. In comparison, diazepam almost completely inhibited cramps and Dopram® almost doubled the intensity of cramps.	NR	E7
Gastrointestinal System	Mouse, NMRI	i.p.	ibandronate: 1 and 3 mg/kg[a] atropine: 2 mg/kg	10 – 17 females	Ibandronate had no effect on intestinal motility, whereas atropine decreased.	NR	E1

2.6.3.4 Safety Pharmacology (cont.) Test Article: Ibandronate

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses[a] (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
	Mouse, SWISS	s.c.	ibandronate: 0.01, 0.1, and 1 mg/kg[a] indomethacin: 3 mg/kg	10 males	Ibandronate had no effect in the phenyl-p-benzoqzuinone writhing test in the mouse while indomethacin had a marked analgesic effect characterized by a decrease in the number of writhing responses.	YES	E16
Gastrointestinal System	Rat, anesthetized Sprague-Dawley	i.v.	ibandronate: 1 mg/kg[a] cimetidine: 8 mg/kg carbachol: 9 µg/kg: single 15 min infusion	6 – 7 males	Ibandronate had no effect on gastric acid secretion. The production of gastric acid was promoted by carbachol and reduced by cimetidine.	NR	E4
Cardiovascular System	Rat, conscious, normotensive Wistar	i.v.	0.01 to 3 mg/kg[a] in increasing doses at 15 min intervals	6 –7 males	Ibandronate had no effect on blood pressure or heart rate when administered by i.v. or s.c. routes.	NR	E12
		s.c.	3 mg/kg[a]; daily for 3 consecutive days	4 – 9 males			
	Dog, conscious, normotensive mongrel	i.v.	Cumulative dose of 1 mg/kg (0.1, 0.2, and 0.7 mg/kg given at 10 min intervals)[a]	6, either sex	Ibandronate had no relevant effects on hemodynamic parameters (blood pressure, heart rate, cardiac output, stroke volume, total peripheral resistance, and ECG).	NR	E13

2.6.3.4 Safety Pharmacology (cont.) Test Article: Ibandronate

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses[a] (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
	Dog, conscious beagle	i.v.	Cumulative dose of 1 mg/kg (0.1, 0.2, and 0.7 mg/kg)[a] given at 15 min intervals)	6., either sex	Protracted administration of ibandronate had no effect on hemodynamic parameters (blood pressure, heart rate, and ECG) or blood chemistry parameters indicative of respiratory function (pH, pCO_2, pO_2, HCO_3, and BE).	NR	E5
Renal Function	Dog, conscious, episiotomized beagle	i.v.	0.1 and 1 mg/kg[a]	4 females	Ibandronate had no effect on urine volume, electrolyte excretion, or Na^+/K^+ ratio.	NR	E8
Renal Function	Dog, conscious, episiotomized beagle	p.o.	5 and 10 mg/kg[a]	4 females	The 5 mg/kg group had a significant ($p < 0.05$) increase in K+ excretion in the 2- to 6 h sampling period, whereas the 10 mg/kg group experienced no change. At 10 mg/kg, urine volume and Na^+/K^+ ratio were significantly decreased. No significant differences from controls were noted in the 0 to 2- or 0 to 6 h ibandronate samples at either dose.	NR	E3

Additional information:
Single dose unless specified otherwise.
ASA – Active systemic anaphylactic reaction. [a] – Doses expressed as the weighed drug substance. BE – Base excess. (CHO) cells. – Chinese hamster ovary cells. HCO_3 – Actual bicarbonate concentration. hERG K^+ – human ether-a-gogo related gene potassium channel. NR – Not required. NS – Not specified. PCA – Passive cutaneous anaphylaxis test. pCO_2 – Carbon dioxide partial pressure. PHA – Passive hemagglutination anaphylaxis test. pO_2 – Oxygen partial pressure.

Species/Sex/Model	Test Article (Batch No.)	Route	Dose/Administration/Duration	Results	GLP Status	Reference & Date (Laboratory)
Other Investigations						
Rabbit, conscious, crossbred	ibandronate (90017-88-8)	i.v.	1 mg/kg[a] via infusion pump (1 mg/mL; 0.5 mL/min)	Ibandronate had no effect on body temperature or blood glucose concentration.	NR	[1742] 10/88 [redacted]
Global screening	Ibandronate (NS)	Various	Various *in vivo* and *in vitro*	A minimal increase in urinary elimination of Na^+ and K^+ occurred in rats given a single p.o. dose of 20 mg/kg. No other effects were noted.	NR	[1743] 1/80 [redacted]
Guinea pig, Dunkin-Hartley	ibandronate (487 624-01)	Various	Various	Ibandronate was determined not to be antigenic in the following antigenicity assays: ASA, PCA, PHA.	NR	[1744] 6/96 [redacted]
Peripheral human leukocytes, enzyme immunoassay	ibandronate (45 480-02) Clodronate, NS Alendronate, NS Pamidronate, NS	*In vitro*	0.0001 to 0.01 mg/mL	None of the bisphosphonates at concentration 0.0001 to 0.01 mg/mL had an effect on the synthesis of TNFα, IL-1β, IL-1ra, or IL-6 by LPS-stimulated human peripheral mononuclear cells.	NR	[1745] 7/97 [redacted]

NR = Not reported; NS = Not specified; ASA = Active systemic anaphylactic reaction; PCA = Passive cutaneous anaphylaxis test; PHA = Passive hemagglutination anaphylaxis test; [redacted]

a: Doses expressed as the weighted drug substance

CNS effect

In a mouse study (E6), at 3 mg/kg i.p. (5x human 3 mg IV dose, mg/m2 basis), ibandronate decreased locomotor activity similarly to 2 mg/kg oral diazepam. However, 3 mg/kg (i.p.) was negative in the mouse Irwin test, and the significance of the result was unclear.

Renal effects

In a renal function study in the dog (E3) with oral doses of 5 and 10 mg/kg, K+ excretion was increased at 5 mg/kg in the 2-6-h sampling period. At 10 mg/kg, urine volume and Na+/K+ ratio were decreased. The doses of 5 and 10 mg/kg are equivalent to 0.3-0.6x the 3 mg IV dose, on mg/m2 basis.

In another dog study (E8), IV doses of 0.1 and 1 mg/kg had no effect on these parameters. The 1 mg/kg IV dog dose is equivalent to a 0.5 mg/kg human IV dose (30mg), or 10x the 3 mg IV dose.

In an oral global screening study in rats, 20 mg/kg p.o. minimally increased urinary elimination of Na and K (Study #E11). This dose is 0.3x the 3mg IV dose (mg/m2 basis).

The data indicate a potential effect on urine and electrolyte excretion.

Cardiovascular hERG channel study

The in vitro hERG channel study indicated that 10,000 ng/mL had no effect on channel K+ current. This concentration is 17x the Cmax (582 ng/mL) in humans dosed with 3 mg IV.

Summary

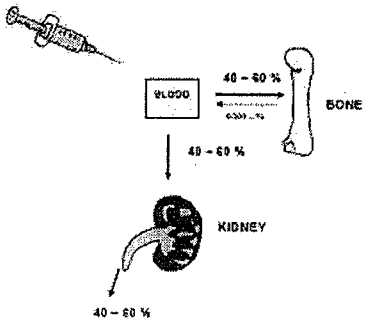
- Ibandronate was tested for effect on CNS, GI system, cardiovascular and renal function. Safety pharmacology studies in mice, rats and dogs showed that ibandronate did not affect CNS, gastrointestinal, or cardiovascular function at doses up to 1-10x the proposed human 3 mg IV dose, based on body surface area comparison (mg/m2). Multiples are calculated based on those determined for the 150 mg oral dose (NDA 21-455/S-001)
- There were inconsistent effects on renal function (electrolyte and volume excretion) in oral studies in dogs and rats, at 0.3-0.6x the human 3 mg IV dose (mg/m2 comparison).
- There was no signal for a QT effect (in vitro hERG channel study)

III. PHARMACOKINETICS/TOXICOKINETICS

Bioavailability is 100% with s.c. administration in rats and dogs.

Distribution is similar after single and repeated doses. Less than 2% of the dose distributes to soft tissues. The PD effect of the drug is related to the part of the dose bound to bone. Protein binding is relatively low (86%). Placental passage is marginal.

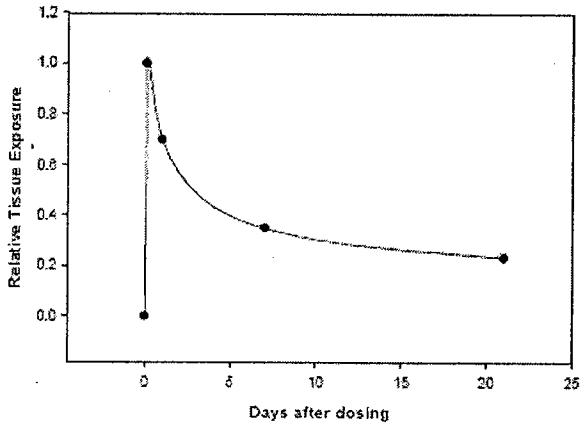
Figure 1 Distribution and Elimination of Ibandronate



Uptake in bone is linearly dose-dependent and related to total dose rather than dosing schedule within the dose range studied (0.2-25 ug/kg/day in rat). In other tissues, exposure is highest in spleen, kidney, liver. Terminal tissue half-lives were 22d (liver), 77d (spleen), 24d (kidney). Thus, with the quarterly dosing regimen, steady state tissue concentrations are achieved after 2 doses (liver, kidney) or 3 (spleen) doses.

Figure 4 Renal Tissue Kinetics

Relative renal tissue exposure (1 = after 2 hours) after a single i.v. dose of ibandronate in the rat)



Kidney elimination is biphasic with $T_{1/2, elim}$ of 6d, and $T_{1/2, term}$ of 24d. Renal tissue kinetics in the rat predict that 3 months after dosing the residual amount of drug in the tissue is 3%. After the initial

dose, maximal exposure (at 2h) is ca. 0.5% of administered dose. Thus, after the second dose the exposure is 1.03x exposure after the first dose and no further accumulation occurs.

Ibandronate is not metabolized. No induction of P450 activity was observed in the liver. Hepatic and renal drug-drug interactions are unlikely.

Excretion is predominantly renal, through the urine. Fecal excretion after IV dosing is low, and biliary excretion is negligible. Drug is secreted into milk in rats. Renal clearance was not affected by anionic/cationic inhibitors of renal transport systems, i.e., there was no competition for these systems. In rats, upon IV dosing with 0.1 mg/kg, total body clearance was 13.7 mL/min/kg, renal clearance was 3.25 mL/min/kg, terminal serum T1/2 was 7h, and distribution phase was <2h (Study I5).

Exposure data

RAT

Study #	Regimen	Dose (mg/kg/day)	Cmax (ng/mL)	AUC (ngxh/mL)
I 5 (PK)	Single dose (iv)	0.1	-	125
I 11 (PK)	Single dose (sc)	0.03	40	38
G6 (2-wk)	2-wk tox (oral)	5 10	3.6 15	17 35
H1	26-wk tox (oral, fasted)	10.1	19	85 (80-140)
H3	26-wk tox (fed)	3 10.1	0.5 2.2	6.0 13
H4	12-mo tox (fed)	10	5.9	42
H7	26-wk (iv)	0.15 weekly 0.3 biweekly	487 1113	464 1069
H9	26-wk (iv)	0.3 weekly 0.9 weekly 1.8 weekly 2.7 weekly	1617 3105 - -	1178 (400-1900) 3406 (1700-5100) 8251 15879

DOG

Study #	Regimen	Dose (mg/kg/day)	Cmax (ng/mL)	AUC (ngxh/mL)
I 4 (PK)	Single dose (i.v.)	0.1	-	746
I 4 (PK)	Single dose (oral)	1	33	323
I 12 (PK)	Single dose (s.c.)	0.01	20	79
H2	26-wk tox (oral)	2 5 13	24 160 1505	51 350 3000
H5	12-mo tox (oral)	2 5	18 118	70 320
H7	26-wk (iv)	0.075 weekly 0.15 weekly 0.3 biweekly	205 563 1187	263 633 1352
H9	26-wk (iv)	0.3 weekly 0.9 weekly 1.8 weekly 2.7 weekly	1291 6444 - 15890	1214 4994 - 14431

MONKEY (OVX)

Study #	Regimen	Dose (ug/kg/month)	Cmax (ng/mL)	AUC (ngxh/mL)
D30	16-month bone study	0.01	55.7 (40-64)	62.2 (46-79)
		0.03	200.0 (145-367)	198 (131-479)
		0.15	877.3 (630-1436)	1115 (840-1956)

IV. GENERAL TOXICOLOGY

Introduction

Target organ toxicities identified in the earlier oral and IV studies in rats and dogs were kidney, GI tract, liver, lung, testis, thymus. The kidney was one of the main target organs, and renal toxicity in the rat was associated with the lowest safety margins.

Since the proposed dose is 3 mg/q 3mo by IV injection, the most relevant toxicity studies are acute and (bi)weekly IV studies in rats and dogs. To support intermittent clinical dose regimens, renal toxicity was further investigated in single and intermittent (triweekly) IV rat studies.

Single dose studies

Original studies (NDA#21-455).

LOAEL/NOAEL for toxicities in acute toxicity studies (RAT*, MOUSE*, DOG)

Study		Doses (mg/kg)	Organ	Findings	LOAEL/NOAEL (mg/kg)	Multiple of human 3mg IV dose (mg/m2 basis)
Mouse	IV	10, 20, 40, 50, 64, 80	CNS Intestine Liver Lung Stomach	Sedation Intestine dilation Congestion Edema Hemorrhage	40/20 80/64	72x/36x 144x/116x
Mouse	Oral	100, 200, 800, 1200, 1600	CNS Lung Stomach Intestine Liver Kidney	Sedation Edema Hemorrhage Dilatation/hemorrhage Discoloration Discoloration	1200/800 1600/1200	260x/174x 346x/260x
Rat	IV	10, 20, 25, 28, 32, 40, 64, 100	CNS Lung Intestine	Sedation, rough pelt Edema, hydrothorax Dilatation Hemorrhage	25/20 40/32 100/64	90x/72x 144x/116x 361x/231x
Rat	Oral	100, 200, 400, 640, 1000	Stomach Intestine Lung	Gastric hemorrhage Intestinal dilation Edema, hemorrhage	640/400	20x/12x
Dog	IV	5	Kidney	Tubule damage (basophilia, cellular infiltation, epithelial vacuolation) BUN increase (females) Urine specific gravity decrease	5/<5	54x/<54x
		5	Bone	Decreased ALP, decreased or increased serum calcium		

*Only animals that died were necropsied
NOTE: Doses and multiples are for weighed drug substance, WDS (i.e. 1.13x free acid equivalents), thus slightly overestimated (1.1x)

Target organs identified were CNS, GI tract, liver, lung, kidney. All safety margins were very high. The lowest NOAEL-associated margin was for GI toxicity in the oral rat study. At IV doses up to 32 mg/kg, 6x higher than the 640 mg/kg LOAEL rat oral dose, there was no GI toxicity.

New IV studies in rat (NDA 21-455/S-001)

		Ref/CTD location	Study Nr.	Report Nr. (pdf file)	Administration	Treatment	Doses (mg/kg)	
1A	Rat	[1079]	191P00	1003699	IV bolus injection	Acute	1, 3, 10, 20	
1B	Rat	[1079]	245P00	1003699	IV bolus injection or 2.5- or 15-min infusion	Acute	3	

1C	Rat	[1079]	421P00	1003699	IV bolus injection or 60-min infusion	Acute	1	
2	Rat	[1080]	904P01	1004737	IV bolus injection or 15-min infusion	Acute	1	UNX model
3	Rat	[1077]	959P00	1003696	IV bolus injection	Acute	1, 3 (ibandronate) 1, 3, 6, 10 (zoledronate)	Zoledronate vs. ibandronate
4	Rat	[1078]	924P00	1003698	IV bolus injection, or 15 min- or 1h-infusion	2 weeks (14 doses)	1, 3	
5	Rat	[1081]	045P01	1008284	Bolus injection or 15-min or 30-min infusion, 3-weekly, or injection once only	26 weeks (9 doses)	1	

1. Report 1003699
Ref [1079]
3 studies
Conclusions

- IV doses (1 to 20 mg/kg) caused renal histologic toxicity, consisting mainly of proximal tubule degeneration/necrosis, without changes in serum urea or creatinine levels
- Most sensitive renal lesion was degeneration and necrosis in P1/P2 segments of proximal convoluted tubules (PCT). Lesion had dose-related incidence and severity, was seen at all doses from 1 mg/kg up to 20 mg/kg i.v., and occurred with delayed onset (4-days).
- Tubule dilation in collecting ducts was observed at 20 mg/kg
- PCT lesion occurred with slightly higher incidence in bolus groups than in long infusion (60-min) group, but was similar in bolus and 15-min infusion groups.
- Cmax was 3-20x higher in bolus group vs. 60-min group
- Based on minimal PCT damage at 1 mg/kg, this dose is at borderline of renal tolerability (LOAEL= 1mg/kg). NOAEL for this toxicity (PCT degeneration/necrosis) is 0.3 mg/kg, based on data from repeat dose IV studies (4-week rat IV daily dose toxicity study G2; 6-month IV rat biweekly dose toxicity study H7).
- In the 6-month IV toxicity study with weekly dosing (H9), LOAEL for renal toxicity was 0.3 mg/kg (serum creatinine increase, renal tubule hyperplasia and hypertrophy)

2. Report No. 1004737
Ref [1080]
Study No. 904 P01

- A single dose of 1 mg/kg (IV) was given to control (sham) rats (bolus), unilaterally nephrectomized (UNX) rats (bolus), or UNX rats (15-min infusion). In UNX rats, renal function and number of intact nephrons is 30-40% reduced.
- The 1 mg/kg dose had no effect on renal function. Renal histologic lesions were similar in all groups, i.e., focal PCT changes, minimal acute tubular necrosis in one sham and 1 UNX (15-min infusion) animal.
- Acute renal safety of ibandronate is not affected by impaired kidney structure and function.
- Reviewer agrees with conclusion for the dose of 1 mg/kg (IV).

Data from the UNX rat model suggested that impairment of renal function does not increase the sensitivity of the remaining kidney tissue to ibandronate at 1 mg/kg (4x human AUC at the human 3 mg IV dose) in an acute setting.

3. Report No. 1003696
Ref [1077]
Study No. 959P00 (incl. 150P01)

- Nephrotoxicity in the rat was studied in a comparative acute IV dose range-finding study with ibandronate (1, 3 mg/kg) or zoledronate (1, 3, 6, 10 mg/kg).
- Ibandronate and zoledronate caused renal histologic toxicity at doses ≥3 mg/kg, at 4 days but not 1 day postdose. The absence of a finding at 1 mg/kg was ascribed to examination of only half of the kidneys.

- Toxicity consisted mainly of degeneration and single cell necrosis of proximal tubules (PCT). In a previous study, 1 mg/kg ibandronate also caused PCT damage,
- At 10 mg/kg, zoledronate caused proteinaceous casts and deposits in distal tubule lumen (not formed by compound, since no P in it).
- Serum Ca and P were slightly decreased in 1 and 3 mg/kg groups after 1 day.
- Sponsor concluded that 1 mg/kg ibandronate is probably similarly nephrotoxic as 1-3 mg/kg zoledronate.

4. Report No. 1003698
Ref [1078]
Study 924P00 (2-week IV study)
Conclusions

- Rats were dosed by daily IV injections, for 14 days, with 0, 1, 3 mg/kg/day.
- At 1 and 3 mg/kg, kidney toxicity (biochemical and histologic manifestations) was main toxicity and was positively correlated to dose.
- Incidence of kidney toxicity (histopathology lesions) similar in bolus and 15-minute groups (re: incidence), and less in 60-min groups. Severity of acute tubular necrosis and other kidney lesions higher in bolus> 15-min> 60-min groups.
- Biochemical evidence of renal toxicity (serum creatinine) positively related to Cmax (bolus > 15min > 60min)
- Dose-dependent liver toxicity (AST, ALT increase, congestion, necrosis) at 1 and 3 mg/kg, not clearly related to Cmax (i.e. infusion time)
- Spleen and testis toxicity at 1 and 3 mg/kg
- Decrease in serum calcium (and P), and effects on red blood cells were probably due to pharmacological inhibition of bone resorption and hematopoiesis. There was no clear relation to rate of infusion (i.e., Cmax)

- NOAEL for kidney toxicity (based on serum creatinine) was ≤1 mg/kg/day for 1-h infusion, and <1 mg/kg for bolus or 15-min infusion
- NOAEL for kidney toxicity (based on histopathology) was ≤1 mg/kg using 1-h infusion, and <1 mg/kg for bolus or 15-min infusion

Sponsor concluded that 1 mg/kg dose was at "limit of renal tolerability". Reviewer agrees. NOAEL was <1mg/kg, and 3 mg/kg was clearly above NOAEL, at any infusion rate.

5. Report No. 1008284
Ref [1081]
Study No. 045P01
Conclusions

- Rats were dosed with a single IV injection, or once 3-weekly IV injections of 1 mg/kg.
- Degeneration/necrosis in proximal tubule is more pronounced (incidence and severity) after once 3-weekly repeated IV injections of ibandronate (1 mg/kg) than after one single dose only (data available only for bolus injection).
- Degeneration/necrosis in proximal tubule after once 3-weekly IV injections of ibandronate (1 mg/kg) is similar (incidence and severity) when given as boluses as when given as 30-min infusions. Thus, finding does not depend on Cmax.
- Hypertrophy/plasia of medullary collecting ducts and distal tubules was observed after repeat doses but not single dose. Incidence and severity were not dependent on infusion rate.
- Histologic kidney lesions were seen in absence of effects on indicators of kidney function, including creatinine clearance
- Acute lung congestion was seen in 1/6 animals in a repeat dose group
- There were no clearly drug-related liver findings
- Data suggest upon once monthly clinical dosing, there is potential exacerbation of histologic renal damage to PCT, and a potential risk of histologic damage to distal tubules and collecting ducts.

- Sponsor concluded single 1 mg/kg dose had similar effect as multiple (9) 1 mg/kg doses on PCT structure. Reviewer does not agree with conclusion. Repeating the 1 mg/kg dose 9x increased average incidence (3-fold) and severity (slightly) of the PCT lesions in the 3 repeat-dose groups, and also of most other renal lesions. Reviewer believes this suggests a potential for exacerbation of (histologic) renal damage over time.

Based on studies 1077, 1079, 1081 the dose-dependence of the renal PCT finding was as follows:

Table 3 Incidence and Severity of PCT Findings in Single i.v. Dose Experiments with Bolus Injection in the Rat

	Ibandronate i.v.			
Severity of PCT changes	1 mg/kg	3 mg/kg	10 mg/kg	20 mg/kg
Minimal	5/16	3/9		
Mild	2/16	3/9		
Moderate		2/9	2/2	1/2
Severe		1/9		1/2

Data from studies Ref. 1077, 1079, 1081
n/n = number of affected animals / total number of animals examined by light microscopy

For the rat, the NOAEL for renal toxicity based on single and repeat dose studies was 0.3 mg/kg dose (see below) and the LOAEL was 1 mg/kg (borderline tolerated dose in new single dose IV studies). Sponsor calculated exposure margins for these doses:

Table 1 Comparison of Plasma Exposure in Rats and Humans

	3 mg i.v. Human [1]	0.3 mg/kg i.v. Rat	1 mg/kg i.v Rat
AUC (ng h/mL)	808.5	1142	3406
Multiple of human AUC	-	1.4	4.2
C_{max} (ng/mL)	582	1059	4159
Multiple of human C_{max}	-	1.8	7.1

[1] Reference to 2.7.2 Summary of Clinical Pharmacology, Section 3.2.2.4

Data for 1 mg/kg are from single dose experiments
Data for 0.3 mg/kg are derived from multiple dose experiments (H7, H9)

Repeat dose rat and dog studies

Oral Studies

Repeat dose oral studies were reviewed in detail in the original NDA review (NDA #21-455; Review April 16, 2003).

Rat studies with daily oral dosing (4-week, 6 months,12 months)

- CNS effects
- red blood cells (decrease) and white blood cell changes
- liver enzymes (AST, ALT increases)
- serum ALP decrease
- serum mineral perturbations (Ca, P, K, Na, Cl) indicative of bone and kidney effects
- urine specific gravity increase
- increased kidney, spleen, liver weights
- gross and histo-pathologic findings (kidney tubule changes and hypertrophy, increased bone tissue), stomach irritation

Dog studies with daily oral dosing (4 weeks, 6 months, 12 months)

- mortality (10-13 mg/kg/day)
- vomiting
- decreased body weight/food consumption
- liver enzyme increase (AST, GGT)
- serum ALP decrease
- increase in serum lipids
- anemia, WBC change
- decreases in serum Ca and P,
- serum urea and creatinine increase
- increased weight of kidney and spleen
- histologic renal tubule changes, liver cell vacuolation, esophagitis, pneumonia, stomach irritation, testis atrophy, bone increase and bone marrow fibrosis, bone-cartilage necrosis, spleen extramedullary hematopoiesis.

Conclusion

In rat and dog oral repeat dose studies, target organs were, in order of sensitivity: Kidney, GI tract, liver, stomach, esophagus.

IV Studies

Repeat dose IV studies were reviewed in the original NDA review (NDA #21-455; Review April 16, 2003).

Repeat dose IV toxicity studies

Species	Study (REF #]	Study Nr.	Route	Dosing frequency	Study Duration	Doses (mg/kg/day)
Rat	1015	G2	IV	daily	4 weeks	0.09, 0.28, 0.9
	1016	H7	IV	weekly* or biweekly**	6 months	0.075*, 0.15*, 0.3**
	1017	H9	IV	weekly	6 months	0.3, 0.9, 1.8, 2.7
Dog	1018	G4	IV	daily	4 weeks	0.09, 0.28, 0.9
	1019	H6	IV	weekly* or biweekly**	6 months	0.075*, 0.15*, 0.3**
	1020	H8	IV	weekly	6 months	0.3, 0.9, 2.7

Rat

- Data from the 4-week rat study (G2; doses 0.1, 0.3, 1 mg/kg/day, WDS) showed poor local tolerance of IV administration route, at MD and HD. Main target organ was kidney, and moderate tubular nephrosis was observed at the HD.
- In study H7 (0.075, 0.15, weekly or 0.3 mg/kg, biweekly) there were minimal effects on hematology parameters in all treated male groups, concomitant with new trabecular bone formation and spleen extramedullary hematopoiesis. Biweekly doses of 0.3 mg/kg had no histologic renal effects.
- In Study H9 (0.3, 0.9, 1.8, 2.7 mg/kg/week) local tolerance was poor at MD and HD, and dosing was changed to s.c. dosing. Renal toxicity was observed reflected by changes in serum parameters (BUN, creatinine), increased kidney weight, and dose-related histologic effects. These included epithelial hypertrophy/hyperplasia, focal basophilic tubules (NOAEL <0.3 mg/kg), tubule dilation, tubule protein casts (NOAEL 0.9 mg/kg). There were no liver effects.
- Bone changes (endochondral ossification, new trabecular bone formation), increased extramedullary hematopoiesis and anemia and serum Ca and P decreases were observed in all dose groups.
- The rat IV NOAEL for kidney changes is 0.15 mg/kg/wk. The LOAEL is 0.3 mg/kg/wk IV.

Dog

- Data from the 4-week IV dog study (G4; doses 0.1, 0.3, 1 mkd; WDS) showed toxicity in kidney, liver and GI tract at 0.3 and 1 mg/kg/day. Kidney damage included tubulonephrosis, necrosis, and papillary and pelvis pathology. Liver damage included fatty liver, icterus, necrosis. GI toxicity included diarrhea (all groups) and intestinal bleeding at 1 mg/kg/day. NOAEL <0.1 mkd.
- In Study H6 (doses 0.075, 0.15, weekly, or 0.3 mg/kg biweekly) there were no treatment related histopathologic findings. Biweekly dosing with 0.3 mg/kg had no histologic renal effects.
- In Study H8 (0.3, 0.9, 2.7 mg/kg/wk, WDS) there was renal toxicity at all doses reflected by a variety of histologic changes that were severe at 2.7 mg/kg/wk. Severe lung lesions were observed at 2.7 mg/kg/wk. GI irritation, testicular atrophy and thymus involution and liver toxicity judging from serum chemistry changes occurred at 2.7 mg/kg/wk. Histologic kidney changes included epithelial hypertrophy/hyperplasia, congestion, and tubule dilation (NOAEL <0.3 mg/kg), interstitial nephritis, papilla necrosis (NOAEL 0.3 mg/kg), focal necrosis (NOAEL 0.9 mg/kg).
- Bone effects included enlarged zone of endochondral ossification in all treated groups in all IV dog studies.
- The dog IV NOAEL for kidney changes is 0.15 mg/kg/wk. The LOAEL is 0.3 mg/kg/wk IV.

NOTE: The presence of GI toxicity (irritation, bleeding) in the IV toxicity studies in rats, mice and dogs indicates that systemic exposure can lead to GI events that are usually ascribed to local GI irritation. GI effects have been observed in animals dosed by the i.v. route with other bisphosphonates and in humans treated with intermittent i.v. doses of ibandronate. These GI effects may be due to exsorption (transport from interstitium to epithelial lumen). This process might involve active transcellular transport mechanisms and may be related to the low oral bioavailability of bisphosphonates.

Table 4 **Dose and Exposure Values at Renal NOAEL in Intravenous Repeat Dose Toxicity Studies in the Rat and Dog**

Study	Dosing regimen	Renal NOAEL		
		[mg/kg]	AUC [ng*h/mL]	Cmax [ng/mL]
4-week i.v. rat	daily	0.3	n.d.	n.d.
4-week i.v. dog	daily	0.1	682 ± 68.3[1]	621 ± 18.6[1]
6-month i.v. rat	Twice monthly	0.3	1069 ± 44	1113 ± 197
6-month i.v. dog	Twice monthly	0.3	1352 ± 232	1187 ± 258

[1] values from a single dose kinetic study [2002 nonclinsum.pdf - 301].

Conclusion
In rat and dog repeat dose IV studies, target organs were, in order of sensitivity:
Kidney, GI tract, liver, lung, testis, thymus.

Evaluation of toxicity data

Renal failure has been observed clinically in cancer patients treated with IV bisphosphonate (zoledronate, 4 mg IV infusion over 15 minutes, monthly for 3-9 months). The renal failure was histologically apparent as ATN (acute tubular necrosis) with tubule cell degeneration, loss of brush border and apoptosis. Thus, renal toxicity is considered a potential safety problem with bisphosphonates at relatively high doses.

Sponsor's new toxicology studies with single or intermittent IV doses were carried out to characterize renal toxicity. AUC levels were not measured in these studies, and were extrapolated based on data obtained in previous 4-week repeat dose IV studies (0.1, 0.15, 0.3, 0.9 mg/kg).

Two different types of renal lesions were observed: (1) degeneration and necrosis of proximal convoluted tubules, PCT, and (2) hypertrophy and hyperplasia at distal tubules and collecting ducts. The proximal convoluted tubule (PCT) is the most sensitive part of the kidney. PCT are localized to the renal (subcapsular) cortex and are extensively involved in active tubular transport processes and their damage when severe can result in acute renal failure. The PCT lesion is associated with acute tubular necrosis (ATN), which has been observed in patients treated with IV zoledronate. PCT damage was not noted in earlier IV rat or dog studies. The other renal lesion, tubule hypertrophy/plasia was observed in distal nephron parts, in the new studies with IV doses of 1 and 3 mg/kg. The lesion is believed to be less relevant to kidney function, since the distal nephron is the site of urinary concentration.

Functional effects (creatinine increase) were not seen in single dose studies, but were seen in the 2-week repeat doses study. This indicates that histologic damage can occur without systemic manifestation. It was also shown that kidney toxicity is related to both Cmax and AUC. In the most relevant study (rat, 6-month study, 9 repeated doses, 1 mg/kg, IV), the incidence and severity of the PCT damage was increased after 9 doses, possibly due to low half life (24 days) and renal tissue accumulation. Acute liver congestion was observed in one animal after 9 doses, but there were no liver enzyme effects.

Safety margin calculation

1. Sponsor

Sponsor considered renal toxicity only, and calculated the following AUC and Cmax multiples at the renal NOAEL's ("safety margins"). They are based on single dose studies in rats, and repeat dose studies in both species. Safety margins are very low (1.4x in rat, 1.7x in dog).

Table 2 from Non-Clinical Overview (p.21)

Table 2 Comparison of Plasma Exposure in Animals and Humans

	3 mg i.v. Human [1]	0.3 mg/kg i.v. Rat	0.3 mg/kg i.v. Dog
AUC (ng h/mL)	808.5	1142	1352
Multiple of human AUC	-	1.4	1.7
C_{max} (ng/mL)	582	1059	1187
Multiple of human C_{max}	-	1.8	2.0

[1] Reference to 2.7.2 Summary of Clinical Pharmacology, Section 3.2.2.4

Animal: human dose multiples (human IV dose of 3 mg = 0.05 mg/kg)

	0.3 mg/kg i.v. Rat	0.3 mg/kg i.v. Dog
Dose comparison based on:		
mg/kg	6x	6x
mg/m2	1x	3x

This comparison shows that AUC multiples are close to mg/m2 multiples, generally confirming the value of dose expressed as mg/m2 as basis for plasma or tissue exposure.

Sponsor also provided the following renal safety assessment (Non-Clinical Overview, pp.23-24)

5.2 Renal Safety Assessment

As noted above, the kidney is the primary target of systemic toxicity with ibandronate.
We have considered above the risk of kidney toxicity based on relative plasma exposures

(see Table 1, Table 2). However, an alternative method to assess the renal safety of ibandronate can be used by estimating and comparing the relative exposure of the primary target, the kidney, instead of plasma exposures in animals and humans. For bisphosphonates as a class, it has become common practice to determine bisphosphonate bioavailability in preclinical studies according to the amount of drug retained in bone, and in clinical experiments according to the amount of drug in urine **[Ref. 2540]**. Accordingly, comparing the amount of bisphosphonate in urine in both, animals and human, is an accurate method to compare systemic exposures. Moreover, taking into account the kidney weights, the exposures of renal tissue can be calculated and directly compared between animals and human. The data used for this approach are:

Rat (BW 200-300 g):
– Rel. Kidney weight = 0.7% of BW
– Renal LOEL – single dose: 1 mg/kg
– 40% of dose eliminated by renal excretion **[2000]**
– Rat (250 g): 1.75 g kidney tissue exposed to 0.1 mg ibandronate: **0.06 mg/g**

Rat (BW 200-300 g):
– Rel. Kidney weight = 0.7% of BW
– Renal NOEL – repeat-dose: 0.3 mg/kg
– 40% of dose eliminated by renal excretion **[2000]**
– Rat (250 g): 1.75 g kidney tissue exposed to 0.03 mg ibandronate: **0.017 mg/g**

Dog (BW 8-9 kg):
– Rel. Kidney weight = 0.5% of BW
– Renal NOEL – repeat-dose: 0.3 mg/kg
– 67.5 % of dose eliminated by renal excretion = 1.7 mg **[2002]**
– Dog (8500g): 42,5 g kidney tissue exposed to 1.7 mg ibandronate: **0.04 mg/g**

Human (60-70 kg):
– Kidneys weight 300 g
– Therapeutic dose: 3 mg
– 50% of dose eliminated by renal excretion **[5003]**
– 300 g kidney tissue exposed to 1.5 mg: **0.005 mg/g**

These values translate into the safety margins based on renal exposure shown in Table 3.

Table 3 Safety Margins Based on Relative Tissue Exposure in Animals and Humans

		Renal LOAEL	Renal NOAEL	Renal NOAEL
	3 mg i.v. Human	1 mg/kg i.v. Rat	0.3 mg/kg i.v. Rat	0.3 mg/kg i.v. Dog
Renal Exposure (mg/g)	0.005	0.06	0.017	0.04
Multiple of human renal	-	12	3.4	8

Sponsor argued that renal exposure is a more appropriate basis of comparison. Reviewer does not agree with this evaluation. "Renal exposure" is the total amount of ibandronate to which the kidney is exposed (in mg/g) over time, i.e. the tissue equivalent of the whole body dose in mg/kg. This is not the optimal measure for exposure since it does not take into account the time period over which the tissue is exposed to drug. It is an over-estimate of tissue Cmax and AUC. Therefore, Reviewer feels exposure multiples based on plasma AUC (or Cmax) are more relevant.

Sponsor summarized animal exposure multiples in Table 8 (below). The LAOEL is 1 mg/kg, derived from tri-weekly IV study #045P01 (rat), and the NOAEL's are 0.3 mg/kg, derived from 6-month biweekly IV studies H7 (rat) and H6 (dog).

Table 8 from Toxicology Written Summary (p.142)

Table 8 Animal Exposures (AUC, C_{max}, Renal Exposure) Expressed as Multiples of Human Exposure

Study	Renal Effects	Based on Comparison of		
		AUC	C_{max}	Renal exposure
Single i.v. dose in the rat	LOAEL	4.2	7.1	12.0
6-month i.v. in the rat	NOAEL	1.4	1.8	3.4
6-month i.v. in the dog	NOAEL	1.7	2.0	8.0

2. Reviewer

Renal toxicity

Based on lowest observed NOAEL values in IV studies, Reviewer calculated the following safety margins for renal toxicities:

Rat (IV studies)

	Study #	NOAEL (mg/kg)	AUC at NOAEL (ngxh/mL)	Multiple of human AUC at IV dose of 3 mg/q3mo (NOAEL)
PCT degeneration/necrosis, hypertrophy/hyperplasia (distal) tubules and collecting ducts, serum creatinine increase	191P00, 045P01	<1 mg/kg single or triwkly	<3406	<4.2x
	H7	0.3 biwkly	1069	1.3x
	H9	<0.3 wkly	<1069	<1.3x

Human AUC: 808.5 ngxh/mL

Dog (IV studies)

	Study #	NOAEL (mg/kg/day)	AUC at NOAEL	Multiple of human AUC at IV dose of 3 mg/q3mo (NOAEL)
Epithelial hypertrophy/hyperplasia, congestion, tubule dilation, serum creatinine increase	H6	0.3 biwkly	1352	1.7x
	H8	<0.3 wkly	<1214	<1.7x

In the 16-month monkey OVX monkey study there was no indication of renal or liver toxicity, at monthly IV doses up to 150 ug/kg once monthly (AUC =1115 ngxh/mL). This provides a 4.3x safety margin for renal or liver toxicity, based on cumulative AUC comparison..

GI toxicity

There were no single dose studies to determine the minimal toxic dose of IV doses of ibandronate with regard to other than kidney organ toxicities. However, the following safety margins based on repeat dose IV studies can be calculated for GI, liver and other toxicities:

Rat (IV study)

	Study #	NOAEL (mg/kg/day)	AUC at NOAEL	Multiple of human AUC at IV dose of 3 mg/q3mo (NOAEL)
Stomach irritation, hemorrhage	H9	2.7 wkly	15,879	20x

Dog (IV study)

	Study #	NOAEL (mg/kg/day)	AUC at NOAEL	Multiple of human AUC at IV dose of 3 mg/q3mo (NOAEL)
Esophagitis, stomach and/or GI irritation, cachexia	H8	0.9 wkly	4994	6.2x

Liver toxicity

Rat

	Study #	NOAEL (mg/kg/day)	AUC at NOAEL	Multiple of human AUC at IV dose of 3 mg/q3mo (NOAEL)
Liver histopathology, enzyme increases	H9	2.7 wkly	15879	20x

Dog

	Study #	NOAEL (mg/kg/day)	AUC at NOAEL	Multiple of human AUC at IV dose of 3 mg/q3mo (NOAEL)
Liver histopathology, enzyme increases	H8	0.9 wkly	4994	6.2x

Other toxicity

Dog

	Study Code	NOAEL (mg/kg/day)	AUC at NOAEL	Multiple of human AUC at IV dose of 3 mg/q3mo (NOAEL)
Lung lesions (severe), testicular atrophy, thymus involution	H8	0.9 wkly	4994	6.2x

Conclusions

Safety margins (AUC at animal NOAEL/AUC at human 3 mg IV dose*)

		NOAEL (mg/kg)		AUC multiples	
	Dose frequency in animal study	Rat	Dog	Rat	Dog
Kidney	biweekly	0.3	0.3	**1.3x**	**1.7x**
GI	weekly	2.7	0.9	**20x**	**6.2x**
Liver	weekly	2.7	0.9	**20x**	**6.2x**
Lung, testis, thymus	weekly	-	0.9	-	**6.2x**

*AUC = 808.5 ngxh/mL

Safety margins based on Cmax are slightly higher than those based on AUC and are not shown.

The lowest safety margin for renal toxicity at the 3 mg human IV dose is 1.3x, based on a biweekly rat study (NOAEL 0.3 mg/kg). At the LOAEL of 1 mg/kg in the 6-month triweekly study there was minimal-to-slight histologic toxicity at the PCT tubules but no functional renal impairment. Safety margins for renal toxicity, although very low, are acceptable since they are based on NOAEL values from weekly (dog) or biweekly (rat) studies and merely histologic toxicity was noted at the LAOEL. Nevertheless, since renal toxicity appeared to increase over time, it is recommended that in clinical studies sufficient data were obtained over a sufficiently long period of time to support the renal safety of a long term 3 mg quarterly IV dose regimen. Safety margins for other toxicities (mainly GI tract and liver) are acceptable.

V. GENETIC TOXICOLOGY

There was no evidence for a mutagenic or clastogenic potential of ibandronate in two in vitro bacterial mutagenesis tests in Salmonella typhimurium and Escherichia coli (Ames test), the mammalian cell mutagenesis assay in Chinese hamster V79 cells, and the chromosomal aberration test in human peripheral lymphocytes, each with and without metabolic activation. Ibandronate was not genotoxic in two (oral and IV) in vivo mouse micronucleus tests for chromosomal damage.
No new studies were performed to support the change of treatment regimen to a quarterly 3 mg IV dose. The data are adequate to support the current NDA. No labeling changes were proposed.

VI. CARCINOGENICITY

Three carcinogenicity studies were conducted with ibandronate. A two year carcinogenicity study was conducted in rats by oral intubation and two carcinogenicity studies were conducted in mice. Dosing was performed daily by the oral route. The first mouse carcinogenicity study was conducted by oral gavage. In this study an increased mortality rate was observed at the mid-and high dose as a result of respiratory distress due to the irritant nature of the dosing solutions. Therefore, a second study was conducted with ibandronate administered orally in the drinking water to avoid the respiratory problems encountered with oral gavage. In all carcinogenicity studies, ibandronate was administered at the maximum tolerated dose (MTD) based on daily administration.

Sponsor calculated multiples in oral carcinogenicity studies based on cumulative (91 days) mg/m2 comparison, assuming 1% bioavailability to compare with the three-monthly 3 mg IV human dose.

Table 5 **Cumulative Dose Multiples in the Rat Carcinogenicity Study as Compared to the Single Human 3 mg Dose**

Daily oral dose [mg/kg/day]	Cumulative dose[1] [mg/m^2]	Multiple of human dose (1.75 mg/m^2)
3	16.4	9.4
7	38.2	21.8
15	81.9	46.8

[1] corrected for 1% oral bioavailability

Table 6 **Cumulative Dose Multiples in the Mouse Carcinogenicity (Gavage) Study as Compared to the Single Human 3 mg Dose**

Daily oral dose [mg/kg/day]	Cumulative dose[1] [mg/m^2]	Multiple of human dose (1.75 mg/m^2)
5	13.7	7.8
20	54.6	31.2
40	109.2	62.4

[1] corrected for 1% oral bioavailability

Table 7 **Cumulative Dose Multiples in the Mouse Carcinogenicity (Drinking Water) Study as Compared to the Single Human 3 mg Dose**

Daily oral dose [mg/kg/day]	Cumulative dose[1] [mg/m²]	Multiple of human dose (1.75 mg/m²)
5	13.7	7.8
20	54.6	31.2
80	218.4	124.8

[1] corrected for 1% oral bioavailability

However, for the label Sponsor based multiples for carcinogenicity studies on cumulative AUC comparison. Sponsor provided calculations based on cumulative 91-day exposure (AUC) over the time of dosing in the animals (2.6 Non-Clinical Summary; Section 2.6.7 Toxicology Tabulated Summary, Appendix 1, pp.292-299). Reviewer agrees with this approach and the calculations are acceptable. AUC data were obtained in the rat and mouse oral gavage studies (NDA #21-455 P/T Review). The AUC at the 80 mg/kg/day dose in the mouse drinking water study was assumed to be similar to the AUC measured in a 3-month study at 50 mg/kg/day (NDA #21-455 P/T Review, Carcinogenicity, Mouse drinking water study, p33, and Mouse oral gavage study, p.22). This is acceptable.

The data are adequate to support the current NDA. Labeling changes were proposed by sponsor and reviewed for this NDA (APPENDIX).

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Oral and IV reprotoxicity studies in rats and rabbits were submitted to the original NDA (#21-455). Detailed study reviews from the Review of NDA#21-455 are included here.

Study of Fertility and Early Embryonic Development to Implantation with BM 21.0955-Na in the Rat (IV Injection)
Report No. K19
RCC Project Nr. 379438
Study period 1994/1995
Batch Nr. 447-624-01
Segment I (Fertility): C-section (on GD14)

Rat (Segment I, IV dosing)

Species, strain	RAT, Wist Hanlbm: WIST (SPF)					
Route	IV injection					
N	24/sex/grp					
Dosing	Males: 28 days before pairing, during pairing (max 14 days), and up to 7 days p.c.					
	Females: 14 days before pairing, during mating, and until GD7					
Dose selection	Based on MTD (dose range finding Study K10)					
Procedure	C-section on GD14					
Group			Control	LD	MD	HD
Dosing	Doses (mg/kg/day)	Males	0	0.1	0.3	1.0
		Females	0	0.1	0.4	1.2

Doses are free-acid equivalents

Results

Group			Control	LD	MD	HD
Doses M/F (mg/kg/day)			0/0	0.1/0.1	0.3/0.4	1.0/1.2
Reproduction Findings	Females with plug	N	24	24	24	23
	Pregnant females	N	24	24	23	21
	Evaluated pregnant females*	N	23	23	22	20
	Precoital time	# days	3	2	3	4
	Mating index	%	100	100	100	96
	Fertility index	%	100	100	96	88
	Conception rate	%	100	100	96	91
	Corpora lutea	N (mean)	14.2	13.8	14.2	11.3*
	Preimplantation loss	% of CL	2.1	4.1	1.9	8.0*
	Implantation sites (IS)	N (mean)	13.9	13.2	13.9	10.4*
	Postimplantation loss	% of IS	5.3	5.6	6.5	4.8
	Embryos (all alive)	Total N	302	287	286	197
		N (mean)	13.1	12.5	13.0	9.9
		% of IS	95	94	94	95

* Females not evaluated in which mating was not detected
*p<0.05, ** p<0.01

Group			Control	LD	MD	HD	
Doses M/F (mg/kg/day)			0/0	0.1/0.1	0.3/0.4	1.0/1.2	
Mortality						1M (D28)	
Body weight	Males	D1	272	268	270	271	
		D28	315	305	309	288**	*decrease
		D53 (D10 after pairing)	337	332	333	292*	*decrease
	Females	D1	201	200	205	203	
		GD14	264	263	268	255	
Body weight gain	Males	D1-D28 prepairing	43	37	39	17	No stat analysis
		D1-D14 pairing	23	22	16	6	
		D1-D10 after pairing	3	8	9	3	
		Total D1-D53	65	64	63	21	
	Females	D1-D14 prepairing	11	11	12	4	
		GD1-GD8	25	25	23	20	
Food consumption (g/day)	Males	D1-D28 prepairing	22	22	22	21	* decrease
		D22-28 prepairing	22	21	22	19**	
		D1-D7 after pairing	23	22	21**	18**	* decrease
	Females	D1-D14 prepairing	No sign effects				
Sperm analysis		Motility	No effects				
		Conc ($x10^6$/mL), left vas deferens	8.4	7.5	4.7**	3.2**	* decrease
		Conc ($x10^6$/mL), right vas deferens	8.1	6.8	4.8**	3.0**	* decrease
		Morphology	See below				

*p<0.05, ** p<0.01

Other findings:
One male died at D28. From D20, this animal had body weight loss, sedation, ruffled fur, stiff gait, poor condition. Sponsor considered the death incidental. Reviewer feels it may have been drug-related.

Clinical signs:
Males: Increased incidence of reddened tails, encrusted injection sites in HD.

Sperm morphology:
Significant effect: Increased % of sperm with abnormal hook (D) or reversed head (E) in MD and HD males

Morphology		Ctrl	LD	MD	HD	T-test
	A (sperm) (%)	97	97	97	97	
	D (%)	0.85	0.75	1.05	1.45	* increase
	E (%)	0	0.01	0.02	0.09	* increase

Hematology (prior to necropsy) - significant effects:
Males: slight RBC decrease in HD; slight decrease in Hb and Hct in MD, HD; slight increase in platelet count in HD; slight increase in WBC in HD; slightly altered differential WBC count in MD, HD. All were drug-dose-related effects.
Females: slight RBC increase in HD; slight decrease in platelet count in HD.

Necropsy (macroscopic findings):
No obvious treatment effects

Summary

Wistar rats were dosed by IV injection, once daily, with (males/females) 0/0, 0.1/0.1, 0.3/0.4, 1.0/1.2 mg/kg/day. Males were treated from 28 days before pairing, females from 14 days before pairing. C-section was performed on GD14.

- Mortality (1/24) in HD males.
- Decreased fertility in HD group. Decrease in number of pregnant females, increase in precoital time and decreases in mating index, fertility index and conception rate in HD.
- Decreased food consumption and decreased body weight gain during prepairing and pairing periods in HD males. Slight but not statistically significant decrease in body weight gain during prepairing and gestation in HD females.
- Females: decrease in corpora lutea, increase in preimplantation loss, and decrease in # implantation sites and #embryos in HD females.
- Males: significant decrease in sperm count (concentration, $x10^6$/mL) in MD (40%) and HD (60%), and significant change in sperm morphology (increased % of sperm with abnormal hook or reversed head) in HD.
- Effect on hematology parameters in MD and HD males, indicating anemia and inflammation

BM 21.0955.NA: Embryo-Fetal Toxicity Study in The Rat (Intravenous Injection) Segment II– Caesarean Section

Report No. K12
Study period 1992
Batch Nr. 780-462-59A (formulation for injection)
Segment II- Caesarean Section

Rat (Segment II, IV dosing)

Species, strain	RAT, [redacted] CD BR Sprague Dawley				
Route	IV injection (2x1ml/day)				
N	25 females/grp				
Dosing	GD6-GD15 (females)				
Dose selection	Based on MTD determined in study with 1, 2, 5, 10 mkd. Dystocia occurred at 1mkd, lethality at 2 mkd (K11).				
Procedure	F0 females C-sectioned and sacrificed on GD21				
Group		Control	LD	MD	HD
Dosing	Doses (mg/kg/day)*	0	0.1	0.4	1.5

*Doses are free-acid equivalents

Definitions

Postimplantation loss		# living pups at 1st check/#implantation sites

Results

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.1	0.4	1.5
F0 dams	Pregnant females	24	25	21	24
	Premature decedents		1(#115, GD21)	1(#202, GD9)	
	F0 females evaluated	24	24	20	24
Signs	Apathy on GD21 (related	0	0	2	2

	to low serum calcium)				
	Vaginal hemorrhage	1 (GD14)	0	0	0

Autopsy F0
1LD (#115) that died on GD21 had hemorrhagic pulmonary edema, 1MD (#202) had acute cardiovascular failure. Death of LD animal on GD21 may be related to low serum Ca and dystocia, or drug-related systemic toxicity.

F0 findings
Body weight in HD females slightly (<5%) but statistically significantly (pairwise, and trend test in some cases) decreased compared to controls on GD 10, 12,13, 15, 16.

Litter data

Group F0 dams		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.4	1.5	
Dams evaluated		24	24	20	24	
	Weight of male fetuses (gr)	5.4	5.4	5.3	5.2*	*
	Weight of female fetuses (gr)	5.1	5.0	5.0	4.9	ns

No effects on litter averages of : #implantation sites IS, % postimplantation loss/IS, % live fetuses/IS, % dead fetuses/IS, % sex ratio, weight of female fetuses, placental weight, uterine weight (full/empty).

F1 fetuses: Externally visible anomalies (GD21; all fetuses):
No treatment effects

F1 fetuses: Visceral anomalies (GD21; ca. 50% of fetuses):

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.4	1.5	
N litters		24	24	20	23	
Variations	% (litter average)	64	62	68	67	ns
Malformation	% (litter average)	0.5	0	0	1.5	ns
N fetuses		191	205	169	192	
Variation	Urinary organs: RPU syndrome	67	81	74	84	Not tested

Even though no effect on average % variation was apparent, there appeared to be a slight increase in all treated groups of RPU (as seen in other ibandronate reprotox studies). This finding confirms the biological significance of the other study findings.

F1 fetuses: Skeletal anomalies (GD21; ca. 50% of fetuses):

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.4	1.5	
N litters		24	24	20	24	
Retardations	% (litter average)	79	84	84	88	ns
Variations	% (litter average)	17	14	10	6	ns
Malformation	% (litter average)	3.8	3.1	1.9	1.1	ns
N fetuses		193	205	167	191	
Retardations	Sternum, 6th sternal center, asymmetry	3	9	12	11	
	Hindfoot, phalanges of toes,	1	4	4	7	

	poorly ossified					
	Hindfoot, phalanges of toes, unossified	11	5	6	3	
	Fore foot, phalanges of fingers, poorly ossified	16	20	10	13	
	Fore foot, phalanges of fingers, unossified	8	2	7	16	
	Head, interparietal bone, poorly ossified	8 (4.2%)	28 (13.7%)	12 (7.2%)	29 (15.2%)	
	Head, interparietal bone, unossified	0	1	0	0	
Variation	Rudimentary rib	17 (8.6%)	17 (8.3%)	6 (3.6%)	9 (4.7%)	
Deformation (transitory)	Thoracic vertebrae, ribs, wavy ribs at thoracic vertebrae	2 (1.0%)	3 (1.5%)	1 (0.6%)	10 (5.2%)	

- Even though no effect on average % retaradations or other anomalies was apparent, there appeared to be a dose-related increase in the fetal incidence of sternum asymmetry at the 6th center (LD, MD, HD), poorly ossified hindfoot phalanges (LD, MD, HD), unossified fore foot phalanges (HD), and an increase in wavy ribs at thoracic vertebra (HD).
- The retardation finding of sternal asymmetry at the 6th center was not seen at other centers (1st through 5th) and appears not biologically significant. Also there was no increase in the incidence of sternal 6th center asymmetry, classified as variation.
- The hindfoot poorly ossified phalanges finding was accompanied by a decrease in hindfoot unossified phalanges and is of questionable significance.
- The forefoot unossified phalanges finding in HD was not accompanied by an increase in fore foot poorly ossified phalanges. This finding may have some significance.
- Taken together, the significance of the impaired ossification findings at the foot is unclear.
- The head interparietal bone finding of poor ossification and the wavy rib finding may have been drug-related. In the oral Segment 2 (C-section) study (0, 10, 30 , 60, 100 mkd) the control incidence of the interparietal bone poor ossification was 15.7% and the incidence was reduced in treated groups. Thus, this finding in the IV study was probably not significant. Also, in this oral study the control wavy rib incidence was 5%, suggesting that the finding in the IV study was not significant.
- There was no effect on the fetal incidence of rudimentary rib.

Serum chemistry F0 dams:

Group F0 dams		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.4	1.5	
Dams evaluated		24	24	20	24	
GD21	Serum calcium (mmol/L)	2.6 ±0.2	2.4 ±0.3	2.3 ±0.6	2.2 ±0.7*	*
	Serum P (mmol/L)	1.9	1.9	1.9	1.9	ns
	BUN (mmol/L)	7.1	6.7	7.1	7.3	ns
	Creatinine (umol/L)	53	48	56	56	*

Clear dose-related reduction in serum Ca (sign in HD) and dose-related increase in creatinine on GD21.

Summary

Pregnant SD rats were dosed by IV injection, daily from GD6-GD15, with 0, 0.1, 0.4, 1.5 mg/kg/day. C-section was performed on GD21.

- Clinical signs and possibly mortality (1/25 in LD) related to drug-induced decrease in serum Ca observed on GD21

- Slight but significant decrease in maternal gestational body weight in HD.
- Decrease in fetal body weight in males (significant) and females (non-significant) in HD
- Slight increase in RPU (renal pelvis ureter) syndrome in all treatment groups (no trend test)
- Significant, dose-related reduction in serum calcium on GD21 in all dose groups.

BM 21.0955.Na: Embryo-Fetal Toxicity Study in The Rat (Intravenous Injection) Segment II-Spontaneous Delivery

Report No. K14
Study period 1992
Batch Nr. 780-462-59A (formulation for injection)
Segment II- Spontaneous Delivery

Rat (Segment II, IV dosing)

Species, strain	RAT, [redacted] CD BR Sprague Dawley				
Route	IV injection (2x1ml/day)				
N	15 females/grp				
Dosing	GD6-GD15 (females)				
Dose selection	Based on MTD, ie dose at which dystocia was minimized with Ca substitution (Drf Study K13)				
Procedure	F0 females allowed to deliver				
	All F0 received s.c. Ca substitution from GD 18-PPD0 (32 mg/kg/day)				
	F0 dams sacrificed on PPD21, F1 pups sacrificed on PPD42 All F1 pups were evaluated (no culling)				
	F1 pups (1/s/litter) reared, mated and allowed to deliver, sacrificed with F2 pups on PPD7				
Group		Control	LD	MD	HD
Dosing	Doses (mg/kg/day)*	0	0.1	0.3	1.0
	Ca (mg/kg/day) Administered on GD18-PPD0, twice daily, sc injection	32	32	32	32

*Doses are free-acid equivalents

COMMENT: Calcium supplementation was based on results from drf studies, K11, K13, K12. In those studies, levels of 1.0 mg/kg/day that were not toxic in other ways lead to disturbances of birth causing maternal deaths. A dose-dependent decrease in serum calcium concentration was observed at the end of pregnancy, presumably due to inhibition of bone. resorption. This is thought to affect parturition (dependent on extracellular calcium). The lack of maternal calcium could also affect fetal development at end of pregnancy or during parturition or lactation. Ca substitution of dams was found to overcome the dystocia at 1.0 mg/kg, but not at 1.5 mg/kg/day. Thus, 1.0 mg/kg was selected as HD. Calcium (approximately 13mg/day) was given as a 4- to 6-day daily sc supplement from GD18-PPD0.

Definitions

Postimplantation loss		# living pups at 1st check/#implantation sites
Postnatal loss (PPD0-PPD4)		#pups that died from PPD0-4/#living pups at PPD0
Breeding loss (PPD5-PPD21)		# pups that died from PPD5-21/# of living pups at PPD4
Live birth index (%)		(Number of pups born alive/Number of implantations)x100
Viability index 1 (%)		(Number of pups alive on PPD4/Number of pups born alive)x100
Weaning index (%)		(Number of pups alive on PPD21/Number of pups alive on PPD4)x100

Results

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.1	0.3	1.0
F0 dams	Pregnant females	15	15	15	15
	Unscheduled kill	0	0	0	2 (#312 GD22, #313

					PPD1)
	Females with signs of sedation	0	0	0	2 (#312,313)
	Females with delivery	15	15	15	14
	Females with live pups at parturition	15	15	15	13
	Females with total postnatal loss				1 (#313: all pups stillborn)
	Females with live pups on PPD21	15	15	15	13

Two HD dams were killed, one on GD22, the other on PPD1 after all pups were stillborn. Both dams had signs of sedation (no tremor or other signs of dystocia).

Autopsy findings in F0 dams;
Vaginal bleeding in 0-0-0-1(#312).

F0 findings
No effect on body weight in F0 dams during gestation or lactation

Litter data

Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.1	0.3	1.0
Litters evaluated		15	15	15	13-15
	Gestation duration	21.9	22.1	22.0	21.9
	No. implantations (# IMP) (mean)	16.1	16.3	16.1	16.8
	Postimplantation loss (% of IMP)	5.3	6.3	4.2	5.2

F0 dams (exclusive)	Live birth index (%)	92	97	98	92
	Viability index 1(%)	96	99	98	98
	Weaning index (%)	96	98	97	98

No effects on # live or dead pups, sex ratio in F0 litters

F1 pup findings

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.3	1.0	
Litters evaluated		15	15	15	13	
Body weight (males)	BW PPD14 (gr)	32	29*	30*	29*	*
	BW PPD21 (gr)	53	49	49	47*	*
	BW PPD42 (gr)	224	214	216	214	
	AUC PPD7-42 (sum of diff's to initial BW)	2989	2810	2842	2780	
Body weight (females)	BW PPD14 (gr)	31	28	29*	27*	*
	BW PPD21 (gr)	51	47	48	45*	*
	BW PPD42 (gr)	177	171	170*	170	ns
	AUC PPD7-42 (sum of diff's to initial BW)	2615	2484	2495*	2437	ns
Neuromuscular coordination/refl	Air righting	0.97	0.83**	0.95	0.92*	ns

exes						

Nd=no data; ns = not significant

No effect on body weight at PPD0 (m,f) (7gr-6gr)
No effects on physical development parameters (pinna detachment, incisor eruption, full coat, eye opening, vaginal opening, testicular descent) in F1 pups
No effects on neuromuscular behavior (negative geotaxis, air righting, grip strength, pupillary reflexes, auditory startle) in F1 pups
Cliff avoidance test not performed
No effects on water maze behaviour tests in F1 pups.

Anomalies
Premature deaths: Visceral/skeletal anomalies in F1 pups that died prematurely

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.1	0.3	1.0
N examined		26	9	6	3
Visceral					
Variation	Urinary organs (RPU syndrome)	5 (19%)	5 (56%)	1 (17%)	2 (67%)

No skeletal treatment effects in premature deaths

Surviving pups: Visceral anomalies in F1 pups that survived (autopsy findings)

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.3	1.0	
N litters		15	15	15	13	
N examined		169	188	187	173	
Individual Variation	Urinary organs (RPU syndrome)	5	9	5	23	
	% of fetuses with RPU	3%	5%	3%	13%	
Variations	% (litter average)	13.5	18.0	19.8	22.7**	*

*p<0.05

No visceral malformations, pathological findings
NOTE: Skeletons were not examined in surviving pups

The effect on variations was due to a high incidence of renal pelvis ureter (RPU) syndrome in HD animals. This finding is relatively common according to Sponsor (concurrent control incidence =3%; however, control incidence in oral Segment II C-section study No. K17; same SD rat strain: 29/116=25%, and in oral Segment II delivery Study K9: 9%).
The RPU syndrome includes various states of dilatation of the ureter and the renal pelvis without evidence of functional defects. According to Sponsor, it is a consequence of the nephrotoxicity of the class of compounds (bisphosphonates), and also seen with others in the class (???).

F1 generation mating and breeding performance
Female F1 performance

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.1	0.3	1.0
F1 dams	Mated females	15	15	15	13
	Pregnant females	15	13	14	13
	Females with live pups at parturition	15	13	13	13

No treatment related signs, no mortalities

F1 dams body weight

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.1	0.3	1.0	
N		15	13	14	13	
Body weight (females)						
Gestation	BW GD1 (gr)	285	262	268*	270	
	BW GD21 (gr)	502	471*	450***	**466	**
	AUC GD7-GD21 (sum of diff's to initial BW)	1495	1472	1288**	1375	*
Lactation	BW PPD0 (gr)	367	344	334**	339*	**
	BW PPD7 (gr)	397	375*	372**	365**	**

*p<0.05; **p<0.01;***p<0.001

No treatment effects on litter data (#corpora lutea, #implantation sites, % preimplantation loss, % postimplantation loss, % live pups, % dead pups, % sex ratio, % live birth index, % viability index 1 and 2)

No effect at necropsy (autopsy) of F1 parents (m or f)

F2 pups
No effects in F2 pups on:

- Clinical findings or external anomalies
- BW development (BW in PPD0 and BW gain on PPD0-7)
- Visceral and skeletal anomalies in F2 pups that died prematurely. However, N was too small (10-4-4-14) to conclude anything regarding F2 anomalies.

Summary
Pregnant SD rats were dosed by IV injection, daily from GD6-GD15, with 0, 0.1, 0.3, 1 mg/kg/day. Calcium (32 mg/kg/day) was administered s.c. from GD18-PPD0. Dams were allowed to deliver and raise the pups until weaning. F1 was evaluated for development and reproductive performance.

- Two HD dams with signs of dystocia killed on GD22 or PPD1. The second one had all stillborn pups.
- Treatment-related dystocia and perinatal mortality reduced by perinatal calcium administration (result from dose range finding study with 1 and 1.5 mg/kg and with/without 16-32 mg/kg/day sc Ca administration)
- Slight decrease in body weight in male and female F1 pups in first 2-3 weeks after delivery. Effect was significant in HD, and partially resolved after 6 weeks.
- Increased incidence of fetuses with RPU (renal pelvis ureter) syndrome, significant in HD.
- Decreased body weight in mated F1 females during gestation and lactation (through PPD7), significant in all dose groups. No clear dose relationship of this finding.
- No effect on F1 reproductive performance.

BM 21.0955.Na: Embryo-Fetal Toxicity Study (Segment II) in The Rabbit (Intravenous Administration)
Report No. K7
Study period 1992
Batch Nr. 780-462-59A (formulation for injection)
Segment II- Caesarean Section GD29

Rabbit (Segment II, IV dosing)

Species, strain	RABBIT/CHbb:HM				
Route	IV injection ear vein				
N	17 females/grp				
Dosing	GD6-GD18				
Dose selection	Based on MTD determined in drf Studies K5, K6				

Procedure	F0 females C-sectioned and sacrificed on GD29				
Group		Control	LD	MD	HD
Dosing	Doses (mg/kg/day)*	0	0.03	0.07	0.2

*Doses are free-acid equivalents

Definitions

Postimplantation loss		# living pups at 1st check/#implantation sites

Results

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.03	0.07	0.2
F0 dams	Inseminated	17	17	17	17
	Pregnant	17	17	15	16
	Prematurely killed			1 (broken paw) (GD6)	2 (#308,312; GD25)
	Dams with abortions				2 (#308, 312; GD23)
	Dams with delivery				1 (#302; GD29)
	Dams with total resorptions				1 (#311)
	Vaginal hemorrhage	0	1 (#104; GD22-26)	0	0
	Pregnant females evaluated	17	17	14-15	13-16

Autopsy F0

Dam with all resorptions (#311; 6 fetuses) had kidney hypertrophy

Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.03	0.07	0.2
Dams evaluated		17	17	17	17
Hypertrophy of gallbladder		0	2	3	1
Lung edema		0	0	1	0
Hindlimb fracture		0	0	1	0
Gallbladder missing		0	0	0	1
Hypertrophy of kidney		0	0	0	1 (#311)

F0 findings

Body weight

Group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0.2	
N litters		17	17	14-15	13-16	
F0 dams	BW GD0	2573	2704*	2658	2662	ns
	BW GD6	2619	2742	2656	2707	
	BW GD23	2701	2801	2744	2548	*
	BW GD29	2850	2940	2876	2783	
	AUC1	2078	1597	714	-559	

AUC1 = area-under-time curve for GD6-GD29 differences to initial body weight

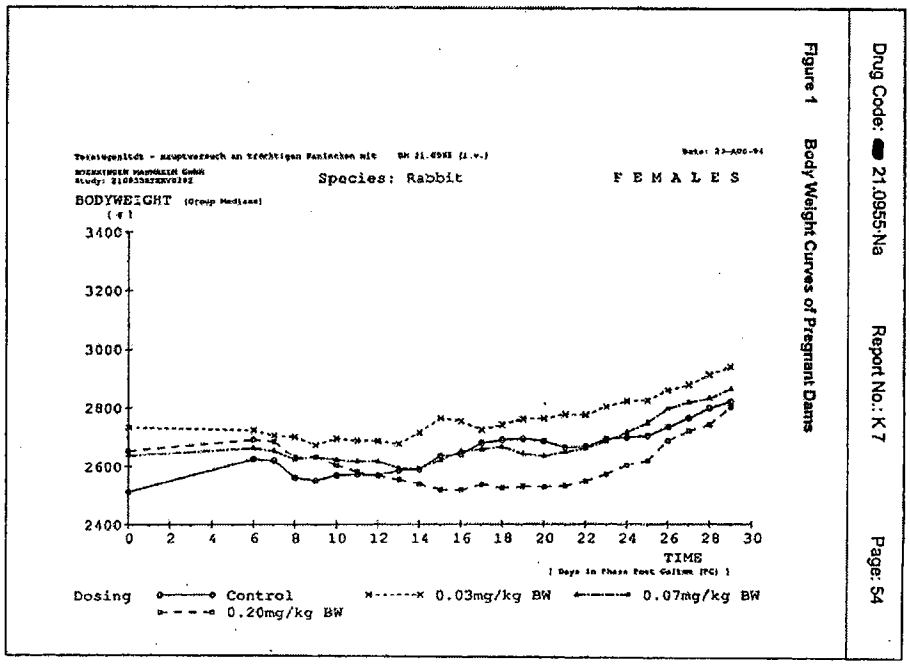


Figure 1 Body Weight Curves of Pregnant Dams

Litter data:

No effects on litter averages of: #implantation sites IS, % sex ratio, uterine weight (full/empty).

Group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0.2	
Dams evaluated		17	17	14	12-16	
	Postimplantation loss/IS (%)	8.3	8.5	10.7	16.6	Ns increase in HD
	Live fetuses/IS (%)	92	92	89	83	Ns decrease in HD
	Weight of male fetuses (gr)	39	40	39	36	Ns decrease in HD
	Weight of female fetuses (gr)	39	39	39	37	Ns decrease in HD
	Placenta weight of all fetuses (gr)	5.1	5.3	4.9	4.5*	*
	Body weight gain since GD0 (minus uterine content) (gr)	-35	-86	-106*	-145*	**

*p<0.05; **p<0.01; ns= non-significant

- Increased PI loss was due to an increased rate of resorptions (no dead fetuses)
- Resorption range: Ctrl 0-3, LD 0-3, MD 0-2, HD 0-6. Number of litters with late resorptions: ctrl 1/17- LD 3/17- MD 4/14- HD 5/12. LD dam with vaginal hemorrhage (#104) had 2/7 late resorptions. These events may have been related.
- #Live fetuses was reduced probably as a result of postimplantation loss
- The fetal weight reduction in HD m and f was not statistically significant. The effect was due to the fact that in some HD litters there were pups with lower body weights than the lowest observed in ctrl, LD, MD. Thus, there was a larger range of avg. body weights in the HD litters.
- Body weight gain: (Body weight – uterine weight: < 0) was significantly reduced in dose-related manner during gestation (all does lost body weight during gestation)

F1 fetuses:
No external anomalies

F1 fetuses: Visceral anomalies (GD29):

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0.2	
N litters		17	17	14	12	
Variations	% (litter average)	42	46	53	56*	*
N fetuses		111	112	88	77	
Variation	Gall bladder enlargement or underdevelopment	14	10	18	20	

The cause of the increase in average % variations was gallbladder anomalies

F1 fetuses: Skeletal anomalies (GD29):
No effect on average % retardations, variations or malformations. No clear effects on individual fetal findings either.

F1 group		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0.2	
N litters						
Malformation	% (litter average)	3.5	3.5	2.7	5.7	Ns

Serum chemistry
(GD 0,20,29: Ca, P, BUN, creatinine) in F0 dams:

Group F0 dams		Ctrl	LD	MD	HD	Trend test
Dose (mg/kg/day)		0	0.03	0.07	0.2	
Dams evaluated		17	17	14	14	
GD29	Serum Ca (mmol/L)	3.1	3.1	3.2	3.0	
	Range of Ca	2.3-3.5	2.6-3.6	2.6-3.7	1.8-3.7	
	BUN (mmol/L)	6.6	6.2**	6.8	7.7	*
GD20	Creatinine (umol/L)	90	85	99	132***	***
GD29	Creatinine (umol/L)	88	91	93	96	
	Range of creat.	63-105	73-124	77-118	77-158	

- Serum Ca was significantly decreased in GD29 (not on GD20) in some HD dams, although no statistical overall effect was seen.
- No effect on serum P at any day
- Serum BUN increased on GD29 in HD
- Serum increased in most HD on GD20 (stat sign group effect), and increased in some HD dams on GD29 (ns group effect). This effect on kidney function markers (BUN, creatinine) was biologically significant.

Summary
Pregnant Himalayan rabbits were dosed by IV injection, daily from GD6-GD18, with 0, 0.03, 0.07, 0.2 mg/kg/day. C-section was performed on GD29.

- Two (2/16) animals with abortions and one (1/16) with premature delivery in HD
- Dose-related reduction in gestational body weight gain, significant in MD and HD.
- One (1/16) HD animals with all resorptions (dam with kidney hypertrophy). Related non-significant increase in postimplantation loss in HD.

- Slight non-significant decrease in fetal weight and significant decrease in placental weight in HD.
- Increased incidence of fetuses with abnormal gallbladder (variation) in HD
- Serum Ca decrease on GD29 in HD
- Serum creatinine increase (significant) and BUN increase (not significant) in period GD20-GD29 in HD
- Conclusion: Maternal, embryo- and fetal toxicity observed in presence of maternal toxicity.

Peri- and Postnatal Study with BM 21.0955-Na in The Rat (Intravenous Injection)
Report No. K18
RCC Project 381262
Study period 1994/1995
Batch Nr. 447-624-00
Segment III- Spontaneous Delivery

RAT STUDY (Segment III, IV dosing)

Species, strain	RAT, WIST Hanlbm: Wist (SPF)				
Route	IV injection				
N	25 females/grp				
Dosing	GD17-PPD20 (females)				
Dose selection	Based on MTD (4-wk IV tox study with 0.1, 0.3, 1 mg/kg) (Study G2)				
Procedure	F0 dams delivered				
	F1 litters culled on PPD4 to N=8/group where possible				
	F1 pups (1/sex/litter) reared to maturity and mated				
	F1 dams delivered and F2 sacrificed at PPD4				
	Litters culled on PPD4 to N=8/litter when possible				
Group		Control	LD	MD	HD
Dosing	Doses (mg/kg/day) (target doses)*	0	0.05	0.15	0.50
	Real doses used (mg/kg/day)	0	0.0475	0.143	0.475

*Doses are free-acid equivalents, and expressed as target doses. Since batch used was determined to have lower free acid content than initially assumed, the real free acid doses used are 0.95x intended (target) doses

Definitions

Postimplanatation loss		# living pups at 1st check/#implantation sites
Postnatal loss (PPD0-PPD4)		#pups that died from PPD0-4/#living pups at PPD0
Breeding loss (PPD5-PPD21)		# pups that died from PPD5-21/# of living pups at PPD4
Birth index (%)		(Number of pups born alive/Number of implantations)x100
Viability index (%)		(Number of pups alive on PPD4/Number of pups born alive)x100
Weaning index (%)		Number of pups alive on PPD21/Number of pups alive on PPD4)x100

RESULTS

Female F0 performance (numbers)

Group		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.05	0.15	0.50
F0 dams	Mated females	25	25	25	25
	Pregnant females	23	25	23	24
	Mortalities (total)	0	3	1	4
	Prenatal	0	GD22: 1 GD23: 2	GD 23: 1	GD21:1 GD23:1
	Postnatal				PPD2: 2 (#80, #100)
	Deaths with	0	3	1	3

	dystocia finding				
	Deaths with no dystocia	0	0	0	1
	Females with dystocia that did not die (#, days with signs)		1 (#42, GD22-PPD7)	1 (#66, GD22)	
	Females with signs of dystocia	0	4	2	3
	Females with live pups at parturition	23	22	22	22
	Gestation duration (days)	21.4	21.5	21.5	21.4
	Females with total postnatal loss	1 (#10)	2 (#38, 42)	4 (#52, 56,65,66)	0
	Females with live pups on PPD21	22	20	18	20

Female #42 lost all pups (all dead at first litter check) on PP Day0/1, and female #62 also lost all pups (pups dead or missing on PPD1/2).

Signs of dystocia: tremor, ruffled fur, felt cold, dyspnea, bleeding from vagina, prostrate and lateral recumbency.
Dystocia was attributed to hypocalcemia resulting from inhibition of bone resorption and calcium mobilization. This has also been seen with other bisphosphonates. Sponsor mentions that lack of calcium is critical at the perinatal time when fetal demand and maternal demand are at its peak.

There were no remarkable treatment-related autopsy findings in F0 dams with dystocia

F0 findings

		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.05	0.15	0.50
F0 dams	Food consumption (PPD1-PPD4)	28.9	25.5 (ns)	27.7	25.8 (ns)
	Body weight gain (PPD7-PPD14)	21	18	22	17 (ns)

No effects on maternal body weight or food consumption during gestation.

Litter/breeding data
Data EXCLUDING dams with total litter loss (1-2-4-0) and excluding dams which died postpartum (0-0-0-2). This analysis did not include any animals with signs of dystocia.

Data for dams excluding those with total postnatal loss

Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.05	0.15	0.50
	Litters evaluated	22	20	18	20
	Gestation duration	21.4	21.6	21.4	21.4
	No. implantations (# IMP) (mean)	12.8	12.0	13.1	12.4
	Postimplantation loss (% of IMP)	10.7	15.8	17.4*	10.5
	Dead pups at first litter check (N/litter)	0	0.4*	1.1*	0.5*
	Living pups at first litter check (N/litter)	11.4	10.1	10.8	11.1
	Postnatal loss (PPD0-4) (%)	0.8	7.9**	4.1*	6.8**

	# Litters affected with postnatal loss (PPD0-4)	2	5	4	5
	Breeding loss (PPD5-21) (%)	0	1.3	0	0
F0 dams (exclusive)	Birth index (%)	89	84	83*	90
	Viability index (%)	99	92**	96*	93**
	Weaning index (%)	100	99	100	100

Data INCLUDING dams with total litter loss (1-2-4-0) and excluding dams which died postpartum (0-0-0-2) showed larger significant effects in LD and MD groups on PostImplantation (PI) loss and PostNatal (PN) loss.

Data for dams including those with total postnatal loss

Group F0 dams		Ctrl	LD	MD	HD
Dose (mg/kg/day)		0	0.05	0.15	0.50
	Litters evaluated	23	22	22	20
	Gestation duration	21.4	21.5	21.5	21.4
	No. implantations (# IMP) (mean)	12.7	12.0	12.9	12.4
	Postimplantation loss (% of IMP)	10.6	19.2*	24.6*	10.5
	Dead pups at first litter check (N/litter)	0	0.9*	1.5**	0.5*
	Postnatal loss (PPD0-4) (%)	4.6	13.1**	12.1**	6.8
	# Litters affected with postnatal loss (PPD0-4)	3	7	8	5

Indices for dams including those with total postnatal loss

F0 dams (inclusive)	Birth index (%)	89	81**	75**	90
	Viability index (%)	95	87**	88**	93
	Weaning index (%)	100	99	99	100

Early postnatal loss (in litters with total or partial loss) occurred mostly on PPD1, 2, and 3

Females with PPD0-4 loss (excluding dams that died pre- or postpartum)

	ctrl	LD	MD	HD
Dams (#)	#6,14,10	#31,32,37,43,50,38,42	#53,54,62,75,52,56,65,66	#79,84,87,88,99
Dams with total loss	#10	#38,42	#52,56,65,66	none
Dams with dystocia	none	#42	#66	none
Dams with no signs of dystocia, but with partial postnatal loss	#6,14	#31,32,37,43,50	#53,54,62,75	#79,84,87,88,99
Dams with no signs of dystocia, but with total postnatal loss	#10	#38	#52,56,65	none

Serum chemistry/hematology
PPD21: No effect on albumin, serum calcium or differential blood counts in plasma of dams. Calcium was measured only in dams that did not die perinatally or postnatally.

Necropsy (macroscopic findings)
None treatment-related in F0 females

F1 findings

F1 group		Ctrl	LD	MD	HD	
Dose (mg/kg/day)		0	0.05	0.15	0.50	
Body weight	BW PPD1 (gr)	5.8	5.8	5.2*	5.3ns	
	BW PPD21 (gr)	42.1	43.4	41.4	37.9*	
	BW PPD35 (gr)	110	112	105 ns	-	
Behavior tests	Negative geotaxis, PPD21(%)	93	86	85*	87	Sponsor concludes this was incidental
	Water maze 3. learning (out of 6 trials), ppd35-43 (%)	68	63	52*	nd	Sponsor concludes this was incidental
Findings during lactation and rearing	Lost or malpositioned lower incisors			25pups/8 litters	All pups/all litters	Significant finding

nd=no data

After PPD21 (weaning) HD and some MD pups revealed abnormal odontogenesis: when beginning eating food pellets, lower incisors were lost or missing or malpositioned, and pups could not eat, starved and died. Thus, all pups in Grp 4 HD were sacrificed on PPD24-28. Some pups in MD (23 pups of 8 litters) had same abnormality causing perforation of the palate. These pups were also sacrificed on PPD25-29. Abnormality caused reduced group body weight mean in MD on PPD35. Effect was not seen in oral Segment 3 study, or other studies with delivery and F1 evaluation.

No effects on physical developmental indices and behavioral test parameters in F1 pups, including cliff avoidance on PPD21.

F1 generation mating and breeding performance
Female F1 performance

		Ctrl	LD	MD
Dose (mg/kg/day)		0	0.05	0.15
F1 dams	Mated females	25	25	25
	Pregnant females	24	25	23
	Females with live pups at parturition	24	25	23
	Females with total postnatal loss	0	0	0

No treatment related signs, no mortalities in F1 dams
No effects on FC and BW of F1 female parents during gestation and lactation.
No effects on parental reproduction parameters (% mating, fertility index, conception rate, gestation index, PI loss, PN loss, birth, viability, weaning indices)
No effect on necropsy of F1 parents

F2 pups: external examination

Group		Ctrl	LD	MD
Dose (mg/kg/day)		0	0.05	0.15
	No. litters	24	25	23
F2 pups	No. live pups	279	306	259
	No. dead pups	0	0	0
	Head multiple malformed	0	0	1

No effect on BW in PPD0 and BW gain on PPD0-4 in F2 pups

Summary

Pregnant Wistar rats were dosed by IV injection, daily from GD17-PPD20, with target doses of 0, 0.05, 0.15, 0.5 mg/kg/day (actual doses 0, 0.048, 0.14, 0.48 mg/kg/day). Dams were allowed to deliver and raise the pups until weaning. F1 was evaluated for development and reproductive performance.

- Dystocia and perinatal mortality in F0 dams in all treatment groups (not dose-related)
- Increase in postimplantation loss due to increase in dead pups at first litter check, significant in LD and/or MD (not dose-related)
- Significant increase in early postnatal loss (PPD0-PPD4) and in #litters affected with postnatal loss in all dose groups. Postnatal loss (partial or total) also observed in dams with no signs of dystocia.
- Reduced F1 pup body weight at birth in MD and HD
- Reduced F1 pup body weight gain during lactation in HD
- Abnormal odontogenesis in MD and HD apparent after weaning (PPD 21). Upon start of eating food pellets, lower incisors were lost, missing or malpositioned. Affected pups could not eat, starved and died.
- Mortality and reduced body weight gain due to dental abnormality with palate perforation in MD and HD. HD (all) and MD (some) pups with abnormality were sacrificed on PPD24-28.
- No effect on reproductive performance of F1

Postimplantation and postnatal loss due to periparturient toxicity

In this Segment 3 IV study, It appears that there was a treatment effect, although not always dose-related, on birth and viability index due to increased postimplantation (PI) loss and increased postnatal (PN) loss. This was an effect seen in all dams/litters including those who survived and had no signs of dystocia. A similar effect was seen in a Segment 3 oral study (K15) in rats. The Sponsor speculated in that study that the increased postimplantation loss was really due to undetected peri-postnatal loss. Sponsor also concluded that in the oral study that PI and PN loss only occurred in dams with dystocia, and that there was no toxicity to pups unrelated to the maternal effect. Reviewer does not agree with that since in both this IV study and the oral Segment 3 study there was increased PI or PN loss in animals without signs of dystocia. Low serum calcium (in dams and/or fetuses) appears to be responsible for this postnatal fetal loss since it occurred in the Segment 3 studies in which Ca is not supplemented, but was not seen in IV and oral Segment 2 delivery studies with Ca-supplementation on GD18-PPD0 (K9, K14).

In a published study in SD rats with alendronate (oral dosing from 4 days prior to mating through GD20) (15 mg/kg/day), IV Ca supplementation (one 9.3 mg dose on GD21) prevented maternal dystocia/deaths, but some postnatal fetal deaths (on PPD1-2) were still observed and 1/155 pups was lost on PPD0 in alendronate-treated (Reference 1). The PPD1-2 postnatal loss incidence in the treated group was 4%, and the authors argued that this was not drug-related since it was within historical control values for postnatal loss PPD1-2 (range 0-7%). The historical range for postnatal loss from PPD0-PPD4 in Wistar rats from [redacted] is 0-2.6% (studies from 1984-1992) (Report K18, p.212). Thus, the increased postnatal loss in the ibandronate studies in dams without signs of dystocia was both statistically significant and exceeded the historical controls. Therefore, Reviewer concludes it is a drug-related effect that is related to low serum calcium in dams and/or fetuses.

Reference 1: Minsker DH, Manson, JM, and Peter CP (1993) Effects of the bisphosphonate, alendronate, on parturition in the rat. Toxicol. Appl. Pharmacol. 121, 217-223

ADME data in pregnant or lactating rats

In pregnant rats (single i.v. dose, 0.1 mg/kg) at 2-24h postdosing, dams retained 49%-35% of dose in carcass, and 2%-1% in kidney and liver. Spleen and sexual organ levels retained 0.5%-0.1% of dose. Fetuses retained 0.02% of dose after 2h, and 0.008% of dose after 24h. Placenta contained 0.07% of dose at 2h, 0.03% at 24h. Amniotic fluid contained 0.003% of dose at 2h only.

A single dose of radiolabeled ibandronate (0.08 mg/kg/day) administered by the i.v. route to lactating rats 12 days after delivery resulted in the appearance of compound in milk at 2, 6, 12 and 24h after dosing. The highest concentration in milk was seen at 2h after dosing (8.1 ng/mL). At 24h after dosing the milk concentration was 0.4 ng/mL. Higher concentrations of radioactivity in milk than in plasma (ca. 1.5-fold) may have been due to the higher calcium levels in milk.

TK data rat reprotoxicity studies

TK data summary (female or sex-pooled data)

Species, strain	Study #	Duration	Study	Route	Dose (mg/kg)	Cpl right after injection for iv studies	AUC_{0-24} (ngxh/mL)	Time of TK
Rat, SD	H7	26-week	Tox	i.v.	0.15 weekly	487	464	26 wks
					0.3 biweekly	1113	1069	26 wks
Rat, SD	H9	26-week	Tox	i.v. or s.c.	0.3 weekly i.v.	2573	1604	17-18wks
					0.9 weekly i.v.	3105	3553	17-18wks
					1.8 weekly s.c.			17-18 wks
					2.7 weekly s.c.			17-18 wks
					0.3 weekly i.v.	661	752	26-27 wks
					0.9 weekly s.c.	-	3259	26-27 wks
Rat, SD	I5	Single ^{14}C dose	PK	iv	0.1 i.v.	No data	125	NA
Rat, Wistar	I11	Single ^{14}C dose	PK	sc	0.03 s.c.	39.6	38.1	NA

Cmax and AUC (extrapolated values from repeat dose IV toxicity studies)

Species, strain	Study #	Study Ref #	Duration of dosing	Study	Doses (mg/kg/day)	Route	Mg/kg/d	AUC_{0-24} (ngxh/mL)	TK values extrapolated from
RAT, Wistar	K19	[1042]	21 days (f)	Seg 1	0.1, 0.4, 1.2 (f)	i.v.	0.1	345	Pooled (M,F) data from iv tox studies H7 and H9
							0.4	1380	,,
							1.2	4140	,,
			28 days (m)		0.1, 0.3, 1.0 (m)	i.v.	0.1	345	,,
							0.3	1035	,,
							1.0	3450	,,
RAT, SD	K12	[1045]	10 days	Seg 2	0.1, 0.4, 1.5	i.v.	0.1	345	,,
							0.4	1380	,,
							1.5	5175	,,
RAT, SD	K14	[1046]	10 days	Seg 2	0.1, 0.3, 1.0	i.v.	0.1	345	,,
							0.3	1035	,,
							1.0	3450	,,
RAT, Wistar	K18	[1047]	25 days	Seg 3	0.05, 0.15, 0.5	i.v.	0.05	166	,,
							0.15	483	,,
							0.5	1656	,,

Exposure multiples in rat reprotoxicity studies
(based on extrapolated TK data from repeat dose studies)

Species	Study #	Duration of dosing	Route	Mg/kg/d	AUC_{0-24} (ngxh/mL)	Cumulative AUC in reprotoxicity study (ngxh/mL)	Exposure multiple vs. humans at 3 mg IV dose (based on cumulative AUC comparison)
RAT	K19	21 days (females)	i.v.	0.1	345	7,245	9x
	Seg1			0.4	1380	28,980	36x
				1.2	4140	86,940	108x
		28 days (males)	i.v.	0.1	345	9,660	12x
				0.3	1035	28,980	36x
				1.0	3450	96,600	119x
RAT	K12	10 days	i.v.	0.1	345	3,450	4.3x
	Seg2			0.4	1380	13,800	17x
				1.5	5175	51,750	64x
RAT	K14	10 days	i.v.	0.1	345	3,450	4.3x
	Seg2			0.3	1035	10,350	12.8x
				1.0	3450	34,500	43x
RAT	K18	25 days	i.v.	0.05	166	4,150	5.1x
	Seg3			0.15	483	12,075	15x
				0.5	1656	41,400	51x

Human PK data: AUC (3 mg, IV) = 808.5 ngxh/mL

Rabbit (Segment II, Study K7)
13 days of dosing (GD6-GD18), doses 0, 0.03, 0.07, 0.2 mkd.
Dose multiples (based on cumulative dose in mg/m2): 2.8x, 6.6x, 19x

Sponsor based multiples for reprotoxicity studies on cumulative exposure over the time of dosing in the animals, based on AUC (rat) or mg/m2 (rabbit). Sponsor provided the calculations that were used for the labeling (2.6 Non-Clinical Summary; Section 2.6.7 Toxicology Tabulated Summary, Appendix 1, pp.292-299). Sponsor's multiples are slightly different from Reviewers multiples calculated.

Reviewer generally agrees with this approach and Sponsor's multiples are acceptable. However, for the periparturient findings in the Segment 3 rat study described in the "Pregnancy" section (dystocia, maternal mortality and early postnatal pup loss through PPD4), Reviewer calculated the cumulative AUC to be 10x the daily AUC rather than 25x the daily AUC, since dosing was for only (up to) 6 days during gestation plus 4 days during lactation (ie 10 days maximal) when the observations were made.

The data are adequate to support the current NDA. Labeling changes were proposed by sponsor and reviewed for this NDA (APPENDIX).

VII. SPECIAL TOXICOLOGY

Local tolerance
No new local tolerance studies were performed. There was sufficient information from previous studies to support the safety of an i.v. formulation for human use.
Summary of findings:

- The i.v. route is considered suitable for parenteral administration and the test compound should not be administered paravenously.
- The formulations used in clinical studies and intended for marketing were tested in i.v. tolerance studies and were found to be well tolerated.
- In the skin irritation test in rabbits, ibandronate was classified as causing burns.
- There was no evidence of a sensitizing potential in the guinea pig model.

The data are adequate to support the current NDA

VIII. OVERALL SUMMARY AND EVALUATION

See Executive Summary.

IX. APPENDIX/ATTACHMENTS

1. Dose and exposure comparisons for proposed package insert (2.6 Non-Clinical Summary; Section 2.6.7 Toxicology Tabulated Summary, Appendix 1, pp.292-299).
2. Sponsor's proposed label
3. Reviewer's label

Appendix 1 Supportive Information for Dose and Exposure Comparisons of Ibandronate Sodium in Animals and Humans: Proposed Package Insert for BONIVA Injection (3 mg every 3 months)

1. INTRODUCTION

The following data were used to compare human doses and exposure levels in women with postmenopausal osteoporosis treated with intravenous ibandronate 3 mg every 3 months with doses and exposure levels of ibandronate in intravenous or oral animal studies. These dose/exposure comparisons are presented in the Animal Pharmacology, Carcinogenesis, Impairment of Fertility, and Pregnancy sections of the draft prescriber information in this NDA.

2. METHODS

2.1 Human Exposure

AUC values used to estimate clinical exposure with intravenous ibandronate administered as 3 mg every 3 months were extrapolated from plasma concentrations measured in clinical study MF9853 [9056 ... biosum.pdf - 57]. In this study, postmenopausal females with osteopenia received two doses of 0.25 mg, 0.5 mg, 1 mg, or 2 mg intravenous ibandronate, delivered as a bolus injection over 30 seconds, separated by a 13 week interval. The results of this study are described in the Summary of Clinical Pharmacology (See 2.7.2. Section 3.2.2.4) in this NDA [9056 ... biosum.pdf - 57]. Based on these data, AUC exposure with 3 mg every 3 months was estimated to be:

$$AUC_{human} = 808.5 \text{ ng·h/mL}$$

2.2 Animal Exposure

AUC values in animals were derived from the respective animal studies listed below. Where no measured values are available, the exposures in rats were extrapolated according to a method described in the Nonclinical Written Summary submitted in NDA 21-455/S-001 (See 2.6.6, Section 1.8.1.2) [9035 nonclinsum.pdf - 299].

2.3 Human and Animal Doses

For comparison of doses, the human and animal doses were normalized to body surface according to a method recommended by the FDA / CDER [0010 nonclinsum.pdf - 300 [3].

In humans with a mean body weight of 65 kg and using a conversion factor of *38* , a clinical dose of 3 mg intravenous ibandronate, expressed in terms of body surface area, corresponds to:

$$1.75 \text{ mg/m}^2$$

In animals, the respective calculations are based on body weight (and correspondi conversion factor), 250 g (7) for rats, 2.0 kg (12.6) for rabbits, and 3.5 kg (12.9) f Cynomolgus monkeys.

2.4 Comparison of Doses and Exposures

Since the designs of animal studies do not match with the intended clinical treatme regimen, i.e. animals were dosed more frequently than the quarterly interval in human the doses that animals received during a 3-month treatment period or the correspondi exposures were added together and these cumulative values were compared with a sing quarterly dose or exposure in humans.

3. RESULTS

3.1 Animal Pharmacology

12-month rat study [1723 . nonclinsum.pdf - 299 1724 nonclinsum.pdf - 299]:

Dose: 25 µg / kg s.c.

Treatment regimen: daily

Cumulative dose (µg/kg) for 3 months (91 days): 2275 µg/kg

Cumulative dose (mg/m^2) for 3 months (91 days): **15.9 mg/m^2**

Animal to human dose ratio: 15.9 / 1.75 = 9

16-month monkey study [1728 nonclinsum.pdf - 299]:

Dose: 150 µg /kg i.v.

Treatment regimen: every 30 days

Cumulative dose (µg/kg) for 3 months: 450 µg/kg

Cumulative dose (mg/m^2) for 3 months: **5.8 mg/m^2**

Animal to human dose ratio: 5.8 / 1.75 = 3

Exposure (AUC): 1157.4 ng·h/mL

Cumulative exposure (AUC) for 3 months: **3472 ng·h/mL**

Animal to human exposure ratio: 3472 / 808.5 = 4

3.2 Carcinogenesis

104-week rat study [1023 ... nonclinsum.pdf - 298 1:

Males

Exposure (AUC) high dose (15 mg/kg) = 22 ng·h/mL

Treatment regimen: daily

Cumulative exposure for 3 months (91 days): **2002 ng·h/mL**

Animal (male) to human exposure ratio: 2002 / 808.5 = 2.5

Females

Exposure (AUC) high dose (15 mg/kg) = 12 ng·h/mL

Treatment regimen: daily

Cumulative exposure for 3 months (91 days): **1092 ng·h/mL**

Animal (female) to human exposure ratio: 1092 / 808.5 = 1.4

78-week (gavage) mouse study [1026 nonclinsum.pdf - 298 1:

Males

Exposure (AUC) high dose (40 mg/kg) = 856 ng·h/mL

Treatment regimen: daily

Cumulative exposure for 3 months (91 days): **77896 ng·h/mL**

Animal (male) to human exposure ratio: 77896 / 808.5 = 96

Females

Exposure (AUC) high dose (40 mg/kg) = 127 ng·h/mL

Treatment regimen: daily

Cumulative exposure for 3 months (91 days): **11557 ng·h/mL**

Animal (female) to human exposure ratio: 11557 / 808.5 = 14

90-week (drinking water) mouse study [1031 nonclinsum.pdf - 298]:

Males

Exposure (AUC) high dose (80 mg/kg) = 281 ng·h/mL

Treatment regimen: daily

Cumulative exposure for 3 months (91 days): **25571 ng·h/mL**

Animal (male) to human exposure ratio: 25571 / 808.5 = 32

Females

Exposure (AUC) high dose (80 mg/kg) = 449 ng·h/mL

Treatment regimen: daily

Cumulative exposure for 3 months (91 days): **40859 ng·h/mL**

Animal (female) to human exposure ratio: 40859 / 808.5 = 51

3.3 Pregnancy

Peri- and postnatal (Segment III) rat study [1047 nonclinsum.pdf - 298]:

Exposure (AUC $_{\text{rat, extrapolated}}$) low dose (0.05 mg/kg) = 199 ng·h/mL

Treatment regimen: daily

Total number of doses: 25

Cumulative exposure for 25 days: **4975 ng·h/mL**

Animal to human exposure ratio: 4975 / 808.5 = 6.1

Exposure (AUC $_{\text{rat, extrapolated}}$) mid dose (0.15 mg/kg) = 584.5 ng·h/mL

Treatment regimen: daily

Total number of doses: 25

Cumulative exposure for 25 days: 14613 ng·h/mL

Animal to human exposure ratio: 14613 / 808.5 = 18

Exposure (AUC $_{\text{rat, extrapolated}}$) high dose (0.5 mg/kg) = 1907 ng·h/mL

Treatment regimen: daily

Total number of doses: 25

Cumulative exposure for 25 days: 47675 ng·h/mL

Animal to human exposure ratio: 47675 / 808.5 = 59

Embryo-fetal toxicity (Segment II) rat study 11046 nonclinsum.pdf - 298 1:

Exposure (AUC $_{\text{rat, extrapolated}}$) high dose (1 mg/kg) = 3766 ng·h/mL

Treatment regimen: daily

Total number of doses: 10

Cumulative exposure for 10 days: 37660 ng·h/mL

Animal to human exposure ratio: 37660 / 808.5 = 47

Embryo-fetal toxicity (Segment II) rat study 11045 ... nonclinsum.pdf - 298 1:

Exposure (AUC $_{\text{rat, extrapolated}}$) high dose (1.5 mg/kg) = 5608 ng·h/mL

Treatment regimen: daily

Total number of doses: 10

Cumulative exposure for 10 days: 56080 ng·h/mL

Animal to human exposure ratio: 56080 / 808.5 = 69

Embryo-fetal toxicity (Segment II) rabbit study 11050 nonclinsum.pdf - 298 1:

Dose: 0.5 µg / kg i.v.

Treatment regimen: daily

Total number of doses: 13

Cumulative dose (µg/kg) for 13 days: 2.6 µg/kg

Cumulative dose (mg/m^2) for 13 days: 32.8 mg/m^2

Animal to human dose ratio: 32.8 / 1.75 = 19

Fertility (Segment I) rat f1042 nonclinsum.pdf - 298 1:

Males

Exposure (AUC) mid dose (0.3 mg/kg) = 1154.6 ng·h/mL

Treatment regimen: daily

Number of doses: 28

Cumulative exposure for 28 days: **32329 ng·h/mL**

Animal (male) to human exposure ratio: 32329 / 808.5 = 40

Females

Exposure (AUC rat, female, extrapolated) high dose (1.2) = 4504.5 ng·h/mL

Treatment regimen: daily

Total number of doses: 21

Cumulative exposure for 21 days: **94595 ng·h/mL**

Animal (female) to human exposure ratio: 94595 / 808.5 = 117

32 Page(s) Withheld

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