

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
125320

PHARMACOLOGY REVIEW(S)

**Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research**

Date: May 7, 2010

Reviewer: Lynnda Reid, Ph.D.
Supervisory Pharmacologist

BLA #/SS#/date: 125-320, CR, January 25, 2010

Sponsor: Amgen, INC.

Drug Product: Denosumab (Prolia®)

Indication: Treatment of postmenopausal osteoporosis at high risk for fracture

Recommended Action: Nonclinical data support approval for the treatment of osteoporosis in postmenopausal women.

Background: This submission is in response to a Complete Response issued by FDA on October 16, 2009. The data submitted in the original Biologics License Application (BLA) supported approval for the treatment of osteoporosis in postmenopausal women, and provided supportive data for a prevention indication (see MEMO dated 9/1/10). There were no outstanding Pharmacology/Toxicology items in the Complete Response for the proposed indication.

The resubmission on January 25, 2010, contained two additional nonclinical studies in transgenic and surrogate models which supported sections of labeling dealing with potential effects on growth and development.

Pharmacology: Denosumab is a fully human monoclonal IgG2 antibody that binds to and inactivates RANKL (receptor activator of nuclear factor kappa B ligand). The mechanism of action for this antibody involves blocking the binding site of RANKL with its receptor RANK, thereby preventing receptor activation and clustering, and downstream signaling. RANKL-induced RANK signaling is essential for the formation, function and survival of mature osteoclasts, which are responsible for bone resorption. RANKL is produced by osteoblastic lineage cells and activated T cells, and its expression is regulated by various cytokines, glucocorticoids, and PTH.

Surrogate models used to evaluate the pharmacodynamic role of RANKL in rodents included a mouse knock-in (KI) model expressing human RANKL, a rat transgenic model overexpressing OPG and a surrogate fusion protein construct of osteoprotegerin bound to antibody Fc region (OPG-Fc). OPG (osteoprotegerin) is the natural endogenous regulator of RANKL activity in both rodents and humans, inhibiting RANKL activity in a manner comparable to denosumab.

Toxicology: Denosumab is pharmacologically active only in humans and nonhuman primates. Pharmacology and chronic toxicology studies were performed in cynomolgus monkeys. Although nonclinical studies could only be performed in a single species, and many of the studies were not considered optimal, the overall nonclinical program is considered adequate to support treatment in women who are not at risk for pregnancy. A causative role for denosumab in the deaths and oral abscesses observed in high-dose animals cannot be ruled out and are potentially secondary to denosumab-induced immunosuppression and an inability to mount an adequate immunologic response.

Carcinogenicity: Carcinogenicity risk has not been evaluated due to the lack of an appropriate model. However, there may be some increased risk associated with denosumab based denosumab-induced immunotoxicity: disrupting RANK/RANKL signaling has the potential to cause immune suppression. There was some evidence of increased infection rates in animals and humans.

Reproductive toxicology: Reproductive and developmental studies were performed in cynomolgus monkeys. There was no evidence of impairment of fertility following once weekly administration of denosumab through 2 menstrual cycles, mating and gestational day 20 of presumed pregnancy. When administered to pregnant monkeys once weekly during the time of major organogenesis (gestational days 20-50) there were no observed adverse effects on the mother or fetus. Although there were no gross teratogenic effects observed, fetuses had only limited exposure and limited fetal tissues were examined histologically.

Inhibition of the RANK/RANKL in knock-out mice resulted in lymph node agenesis, and postnatal impairment of bone growth, dentition and tooth eruption. These mice also showed altered maturation of the maternal mammary gland during pregnancy, leading to impaired lactation postpartum.

Young mice (2 weeks of age at initiation of treatment) treated with OPG-Fc for 6 weeks had significant decreases in body weight gain and axial skeletal length. Decreased upper and lower incisor length, and delayed molar eruption proportional to the magnitude of bone resorption suppression were also

observed. Transgenic rats overexpressing OPG had narrow femur midshafts and reduced peak load and energy to failure levels.

Summary and Discussion:

Clinical Safety: Ongoing clinical concerns associated with denosumab treatment include hypocalcaemia, serious infections, new malignancies, dermatologic adverse events, pancreatitis, adverse events related to suppression of bone turnover, and cardiovascular safety.

Nonclinical issues:

- Reproductive and Developmental Toxicity: Submitted data inadequate to support dosing in fertile women. Additional studies would be necessary to assess potential adverse effects on skeletal, immune and nervous system development, and would be required to support indications which would include women of child bearing potential in the patient population.
- Data suggest that denosumab should not be used in pediatrics where the epiphyseal plates are not fully closed. In young mice, where the epiphyseal plates had not fully closed, treatment with OPG-Fc fusion protein resulted in growth plates that were markedly enlarged with reduced chondroclasis and expanded growth plates associated with cartilage calcification (zone 4) and cartilage erosion and calcification (zone 5). Overexpression of OPG in young rats results in narrow femur midshafts and reduced peak load and energy to failure levels.

Data in mice and rats treated with OPG-Fc also indicate that denosumab may interfere with tooth development.

A study in 2-week old rats treated with OPG-Fc demonstrated partial reversibility of effects on bones and teeth when dosing of OPG-Fc during the recovery phase of the study.

- Carcinogenicity: Carcinogenicity studies were not performed due to the lack of an appropriate animal model. In the two long term studies (12-month toxicity and 16-month pharmacology), there were no incidences of tumor formation indicated with the exception of squamous metaplasia (benign) in two females, one each dosed at 1 and 50 mg/kg/month for 12 months. Standard organ histopathology was not conducted in the 16-month pharmacology study. Immunosuppressive agents, in general, increase the risk of cancer.
- Immunotoxicity: The RANK/RANKL signaling pathway interacts with the immune system in several ways including lymphocyte development and lymph node organogenesis, monocyte / dendritic cell maturation,

activation and longevity, antigen presentation and CD40 ligand-independent T helper cell activation. The absence of RANKL or RANK genes in knock-out mice leads to the complete failure of lymph node development and an absence of lactation by inhibiting mammary gland maturation.

- There was no evidence of ocular toxicity or cardiovascular toxicity in the non-human primate studies.
- Delayed fracture healing was not assessed in animals.

Conclusions:

- I concur with the primary nonclinical reviewer, Dr. Kimberly Hatfield, that the submitted nonclinical data support approval of denosumab for the treatment of postmenopausal osteoporosis.
- From a Pharmacology/ Toxicology perspective, labeling submitted on April 26, 2010, is acceptable.

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 125320
Supporting document/s: EDR 0047
Applicant's letter date: January 25, 2010
CDER stamp date: January 25, 2010
Product: Prolia (denosumab)
Indication: Treatment of postmenopausal women with
osteoporosis at high risk of fracture
Applicant: Amgen, Inc.
Review Division: Division of Reproductive and Urologic Products
Reviewer: Kimberly Hatfield, PhD
Supervisor/Team Leader: Lynnda Reid, PhD
Division Director: Scott Monroe, MD
Project Manager: Nenita Crisostomo, RN

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

Nonclinical data support approval of denosumab (AMG 162), 60 mg subcutaneous injection once every 6 months, for the treatment of postmenopausal women with osteoporosis at high risk of fracture.

1.1.2 Additional Nonclinical Recommendations

No additional nonclinical studies are required.

1.1.3 Labeling

The following are recommended changes in labeling made in Section 13.2 of the March 19, 2010 submission to address reversibility of adverse effects observed in bone and tooth development. All other labeling submitted by the Sponsor is acceptable with only minor changes made regarding the use of the terms "denosumab" versus "[TRADENAME]".

(b) (4)

(b) (4)

1.2 Brief Discussion of Nonclinical Findings

The main pharmacology/toxicology review of denosumab concluded that the nonclinical data did not support the use of denosumab in pediatric populations, pregnant women, or in patients with open epiphyses. This was due, in part, to findings implicating denosumab-associated deleterious changes in epiphyseal

growth plates that were not closed prior to treatment, and findings that osteoprotegerin (OPG), a RANKL inhibitor that is active in the rodent, caused treatment-related decreased axial skeleton and femur length, flared and club-like morphometry of the femur, delayed molar eruption, and decreased upper/lower incisor length.

The nonclinical study supplements included in this BLA resubmission further address the potential use of denosumab in younger populations (i.e. pediatric) where bone growth is still in progress. Since denosumab itself is not active in rodents as it does not recognize mouse or rat RANKL, these studies utilized either OPG transgenic (OPG-Tg) rats (overexpressing OPG), or rats dosed with OPG-Fc, to simulate the potential effects of denosumab on a young population.

Previous studies in OPG-overexpressing rats showed deleterious changes in long bone geometry at 12 months of age (narrow femur midshafts and decreased bending strength). Study R20090069 examined long bone geometry in OPG-overexpressing rats at 1 and 2 months of age to determine when these changes could first be seen. Two-month old rats showed no significant changes in femur geometry, density or strength, therefore long bone geometry appears to be most affected between 2 and 12 months, a time period when bone development is still taking place.

Study R20090070 concluded that after 10 weeks of recovery following 6 weeks of OPG-Fc or alendronate treatment in 2-week old rats, negative effects on bone resorption/growth/strength and tooth eruption that occurred during the exposure period showed partial recovery. Outcomes were less severe than directly after active treatment, but still included low body weight, decreased bone size and strength, and altered morphology of growth plates. Molar eruption occurred but was still fairly delayed with roots of late erupting 2nd and 3rd molars having impaired growth and orientation within the jaw.

The results of these two studies further confirmed our recommendation that denosumab should not be used to treat pediatric populations, populations that are still undergoing bone development, or pregnant women due to concerns over the developing fetus.

2 Drug Information

2.1 Drug: Prolia

2.1.2 Generic Name: Denosumab**2.1.3 Code Name:** AMG 162

Other names: (b) (4) anti-RANKL mAb

2.1.5 Molecular Formula/Molecular Weight:

(b) (4)
147,352 Daltons (b) (4)

2.1.6 Structure

Monoclonal antibody; denosumab is a fully human full-length immunoglobulin. The molecule is a (b) (4) consisting of 2 heavy chains (HC) of the IgG₂ subclass and 2 light chains (LC) of the kappa subclass, which are covalently linked through disulfide bonds. Each HC contains an N-linked glycan at the canonical glycosylation site. Each LC contains 215 amino acids, with 2 intramolecular disulfides. Each HC, with its 4 intramolecular disulfides, contains 447 amino acids, (b) (4). (b) (4) Each LC is covalently linked to a separate HC through a single disulfide bond; the 2 HC are covalently linked through 4 intermolecular disulfide bonds.

The following figure was provided by the Sponsor; N000 12/19/08 Section 2.4

Figure 1: Schematic of denosumab structure



2.1.7 Pharmacologic class:

1) RANK Ligand (RANKL) Inhibitor; 2) Anti-osteoporetic, osteoclast activation inhibitor

2.2 Relevant IND/s, NDA/s, and DMF/s

BLA 125331 – prevention of postmenopausal osteoporosis

BLA 125332 – treatment and prevention of bone loss in patients undergoing hormone ablation for breast cancer

BLA 125333 – treatment and prevention of bone loss in patients undergoing hormone ablation for prostate cancer

BB-IND 9837 – initial IND – treatment of postmenopausal osteoporosis (DRUP)

(b) (4)

(b) (4)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Table 1: Qualitative and quantitative composition of denosumab drug product

Component	Grade	Function	Quantity / mL
Denosumab	In-house ^a	Active Ingredient	60 mg
Sorbitol	NF, PhEur, JP	(b) (4)	47 mg
Acetate	USP, PhEur, JP	(b) (4)	1 mg
Polysorbate 20 ^b	NF, PhEur	(b) (4)	0.1 mg
Sodium hydroxide	NF, PhEur, JP ^c	(b) (4)	(b) (4)
Water for Injection	USP, PhEur	(b) (4)	(b) (4)

(b) (4)

^a Tested to internal specifications (3.2.S.4.1, Specifications)

^b Prefilled syringe presentation only.

^c Supplier tests sodium hydroxide pellets to NF, PhEur, and JP standards.

(b) (4)

2.4 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is postmenopausal women with osteoporosis at high risk of fracture. The dosing regimen for denosumab in clinical populations is a 60 mg subcutaneous injection every 6 months, administered by a healthcare provider.

2.5 Regulatory Background

In December 2008, the Sponsor (Amgen) submitted a BLA for denosumab for 4 indications, each of which was represented by separate BLA numbers:

- BLA 125320: Treatment of postmenopausal osteoporosis
- BLA 125331: Prevention of postmenopausal osteoporosis
- BLA 125332: Treatment and prevention of bone loss in patients undergoing hormone ablation for breast cancer
- BLA 125333: Treatment and prevention of bone loss in patients undergoing hormone ablation for prostate cancer

BLAs 125320 and 125331 were in the Division of Reproductive and Urologic Drug Products (DRUP), and BLAs 125332 and 125333 were in the Division of Biologic Oncology Products (DBOP). Treatment of postmenopausal osteoporosis was considered to be the primary indication.

In October 2009, Amgen was issued a Complete Response letter for BLAs 125332 and 125333 from DBOP and for BLAs 125320 and 125331 from DRUP. As a result, Amgen has now submitted a Complete Response to the action letter from DRUP for BLA 125320 (treatment of osteoporosis). The nonclinical studies reviewed here are a part of the complete response submission dated 1-25-2010.

2.6 Drug Information

Denosumab (AMG 162) is a fully-human monoclonal IgG₂ antibody (mAb) that binds to RANKL (receptor activator of nuclear factor-kappa B ligand). RANKL is a tumor necrosis factor superfamily cytokine member (b) (4), and stimulates its specific receptor, RANK, initiating intracellular signaling cascades which promote osteoclast formation, fusion, differentiation, activation and survival, leading to enhanced bone resorption and bone loss. Another important function of RANKL is in the immune system where RANKL is involved in B-cell and T-cell differentiation as well as dendritic cell maturation. RANKL is produced by osteoblastic lineage cells and activated T cells and its expression is modulated by various cytokines, glucocorticoids and PTH.

Osteoporosis and osteopetrosis are examples of abnormal bone architecture, and are the result of an imbalance between the activities of osteoclasts and osteoblasts, which regulate bone remodeling. The change in sex steroid levels that accompanies menopause in women leads to just such an imbalance between the activities of osteoblasts and osteoclasts, with osteoclastic activity predominating. The result is osteoporosis and an increased risk of bone fracture risk, which is an important source of morbidity and mortality in older women. The mechanism of action for denosumab involves a blocking mechanism, where the antibody binds to RANKL and inhibits the interaction of RANKL with its receptor RANK on the cell surface of osteoclasts, resulting in decreased bone resorption, and increased bone mass. As such, denosumab functions to decrease osteoclast activity and restore the balance between osteoblast and osteoclast activity to achieve normal bone remodeling and architecture.

3 Studies Submitted

3.1 Studies Reviewed

R20090069: Long bone geometry in 1- and 2-month old transgenic Sprague-Dawley rats overexpressing the soluble RANKL inhibitor OPG during growth and development.

R20090070: The effects of OPG-Fc or alendronate treatment on tooth eruption and on bone density, geometry, and strength in neonatal rats: A recovery study.

3.2 Studies Not Reviewed: None

3.3 Previous Reviews Referenced

BLA review for 125320, 125331, 125332 and 125333

Authors: Kimberly Hatfield, PhD (DRUP) and Michael Orr, PhD (DBOP)

Date submitted: August 28, 2009

4 Pharmacology

4.2 Secondary Pharmacology

4.2.1 Study title: Long bone geometry in 1- and 2-month-old transgenic Sprague-Dawley rats overexpressing the soluble RANKL inhibitor OPG during growth and development

Key study findings:

- Overexpression of OPG results in neutral or favorable effects on bone properties (bone mass, geometry, strength).
- Narrow femur midshafts and reduced peak load and energy to failure levels due to overexpression of OPG may occur between the ages of 2 and 12 months.

Study no.: R20090069

Volume #, and page #: EDR 0047 1/25/10 Section 4.2.1.2

Conducting laboratory and location: Amgen, Inc., Thousand Oaks, CA

Date of study initiation: November 2007

GLP compliance: No

QA report: yes () no (X)

Methods

The main objective of this study was to examine the long bone geometry and strength in young (1- and 2-month old) OPG-Tg (osteoprotegerin overexpressing) rats to determine the age at which the previously observed suboptimal long bone

phenotype becomes apparent (refer to studies in 12-month old rats¹). OPG has a similar mechanism of action to denosumab. Since denosumab does not recognize mouse or rat RANKL, OPG-overexpressing rats were used to simulate the potential effects of denosumab on developing bones. As such, this study provided information on potential use of denosumab in pediatric populations.

Doses: There were no treatments during this study. The study is a comparison between transgenic rats that overexpress osteoprotegerin (OPG-Tg) and their wild-type (WT) counterparts.

Age	Breeding Group	No. males	No. females
1 month	WT	7	7
1 month	OPG-Tg	7	3
2 months	WT	8	9
2 months	OPG-Tg	5	3

Species/strain: OPG-Transgenic (OPG-Tg) Sprague-Dawley rats
Wild-type (WT) Sprague-Dawley rats

Number/sex/group or time point (main study): 27 males, 22 females

Route, formulation, volume, and infusion rate: no treatment

Age: 1- and 2-months old

Weight: unknown

Observations and times:

OPG-Tg and WT rats were compared at 1 and 2 months of age. WT controls and OPG-Tg rats were matched for age, sex and background strain. Blood samples were collected at necropsy by cardiac puncture. Serum OPG and the osteoblast marker osteocalcin were measured using microbead kits. The osteoclast marker serum TRACP-5b was evaluated by ELISA. Right femurs were analyzed by micro-CT for density and geometry parameters. Femur midshafts were tested to failure in 3-point bending. All of the analyses were limited by the small numbers of OPG-Tg rats that were available from breeding efforts.

Results

Serum OPG and biochemical markers of bone turnover

Overexpression of OPG (and hence RANKL inhibition) was apparent in OPG-Tg males and females at 1 and 2 months of age (130 to 300-fold over WT). In both 1- and 2-month old OPG-Tg males, osteoclast marker TRACP-5b

¹ Ominsky MS, Stolina M, Li X, Corbin TJ, Asuncion FJ, Barrero M, Niu Q-T, Dwyer D, Adamu S, Warmington KS, Grisanti M, Tan HL, Ke HZ, Simonet WS, and Kostenuik PJ (2009) One Year of Transgenic Overexpression of Osteoprotegerin in Rats Suppressed Bone Resorption and Increased Vertebral Bone Volume, Density, and Strength. *J Bone Miner Res*, 24:1234–1246.

(remodeling-based bone formation) was decreased 2- and 7-fold respectively from WT counterparts, and osteoblast marker osteocalcin (growth-based bone formation) was increased 1.4-fold at both timepoints over WT counterparts. In OPG-Tg females, TRACP-5b was only significantly decreased 2-fold from WT counterparts at 2-months of age, and there was no change at either age for osteocalcin levels.

Femur bone mass and geometry

There was no difference in femur length between WT and OPG-Tg male or female rats at 1- and 2-months. At 1- and 2-months, mid-femur and distal femur vBMC (volumetric bone mineral content) and BVF (bone volume fraction) were increased in OPG-Tg males and females (1.5 to 3-fold). Distal femur measurements had higher fold change than mid-femur. Increases in BMC were associated with retention of trabecular bone. Slight increases in mid-femur CSMI (cross-sectional moment of inertia) (1.2 to 1.5-fold) were found in 1- and 2-month old males and females indicating increased bone strength.

Bone strength parameters

In 1- and 2-month OPG-Tg males and females, peak load, and stiffness levels in the femur midshaft were increased over WT counterparts by 1.3 to 1.8 fold. Energy to failure was only increased significantly in males at 2-months (1.4-fold). In 1-month OPG-Tg males and females, ultimate strength and elastic modulus levels were only slightly increased 1.1 to 1.3-fold. No significant changes were observed for these parameters at 2-months in either males or females. Energy to failure was increased at 2-months in OPG-Tg males and females (1.4-fold), but not at 1-month. Femur toughness was decreased in OPG-Tg males and females at 2-months, but was not changed at 1-month.

Comparison to previous studies (12-month OPG-Tg rats)

- 12-month OPG-Tg female rats had narrower midshafts and reductions in peak load and energy to failure – these results were not observed at 1 or 2-months of age indicating potential adverse effects may be linked with prolonged inhibition of clastogenic activity.

4.2.2 Study title: The effects of OPG-Fc or alendronate treatment on tooth eruption and on bone density, geometry, and strength in neonatal rats: A recovery study.

Key study findings:

- Partial reversal of OPG-Fc related increases in bone mass and strength (femur and vertebrae), and reductions in bone resorption, bone growth, and weight gain
- Lessening of flared morphometry and trabecular bone in femur

- Partial restoration of decreased incisor length and tooth eruption: 3rd molar eruption was still delayed, with roots of late erupting 2nd and 3rd molars having impaired growth and orientation within the jaw

Study no.: R20090070

Volume #, and page #: EDR 0047 1/25/10 Section 4.2.1.2

Conducting laboratory and location: Amgen, Inc., Thousand Oaks, CA

Date of study initiation: November 2008

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Alendronate (ALN) Lot# D00041255

Rat OPG-Fc (OPG) Lot# 14032406

Methods

The main objective of this study was to determine the extent to which changes induced by 6 weeks of treatment with RANKL inhibitor OPG-Fc or alendronate (ALN) on tooth eruption, tooth root development, bone density/geometry/histology/strength in neonatal (2-week old) rats were reversible within 10 weeks of discontinuing treatments. The preceding study examining the effects of treatment over 6 weeks without recovery (R20080340) was reviewed in the main BLA pharmacology/toxicology review (8-28-2009). OPG-Fc has a similar mechanism of action to denosumab, and since denosumab does not recognize mouse or rat RANKL, OPG-Fc was used as a surrogate. ALN was used as an active comparator. This study provided information on potential use in pediatric populations.

Doses: Select concentrations of sterile saline (vehicle), OPG-Fc, or alendronate (ALN) once per week for 6 weeks per the experimental design below. Dosing ceased after 6 weeks, followed by 10 weeks recovery.

Treatment	No. males	No. females	Route	Dose level (mg/kg)	Conc (mg/mL)	Volume (mL/kg)	Dose Schedule
Vehicle	10	10	s.c.	0	0	5.0	1x/week
OPG-Fc	10	10	s.c.	1	0.2	5.0	1x/week
OPG-Fc	10	10	s.c.	3	0.6	5.0	1x/week
OPG-Fc	10	9	s.c.	10	2	5.0	1x/week
ALN	11	10	s.c.	1	0.2	5.0	1x/week

Species/strain: Sprague Dawley rats

Number/sex/group or time point (main study): 51 males, 49 females

Route, formulation, volume, and infusion rate: subcutaneous (s.c.) injection; volume = 5 mL/kg

Age: 2 weeks

Weight: At start of study: Males = 29 g; Females = 29 g

Observations and times:

2-week old male and female rats were treated once per week for 6 weeks. They were weaned at 4 weeks of age and caged according to sex. After weaning, females received standard pelleted rodent chow diet, while males had access to both pelleted and pulverized rodent chow for the remainder of the study. Body weights were measured prior to each of the first 5 weekly treatments, then weekly from weeks 9-16 of recovery. Blood samples were collected (tail vein) at weeks 6, 11 and 16 to measure serum TRACP-5b and OPG-Fc. Right femur and 5th lumbar vertebra were harvested at necropsy for micro-CT and biomechanical analyses. The mandible and maxilla were dissected to assess eruption of molars. Incisor length was measured from gingiva to tip of the tooth with electronic calipers. Incisor overgrowth was evident in some animals in the OPG-Fc and ALN recovery groups, and was managed by regular trimming with 3.5" toe nail scissors. The left hemi-mandible was decalcified and sectioned for histopathology, and the right hemi-mandible for male rats only was scanned by micro-CT. Length of the axial skeleton was assessed at week 13 and at necropsy (tip of the nose to the anus).

Results**Previous study in neonatal rats (without recovery)**

In the previous study that was conducted to examine the effects of OPG-Fc, RANK-Fc and alendronate treatment in neonatal rats (R20080340), rats were treated for 6 weeks and sacrificed (no recovery). The review of this study can be found in the main BLA pharmacology/toxicology review (8-28-2009). For ease of comparison to the current study, the key study findings from this non-recovery study included:

- Decreased upper and lower incisor length and delayed molar eruption of 2nd and 3rd molars with OPG-Fc and ALN in both sexes.
- Decreased bone resorption (TRACP-5b reduction), body weights, axial skeleton length and femur length were observed with all treatments to varying degrees, however femoral and vertebral bone volume, density and strength were increased (no evidence of skeletal fragility).
- By micro-CT examination, the femur and L5 vertebrae showed marked accumulation of trabecular bone, as well as 'flared' morphometry in the femoral metaphysis (all treatments, males and females), and generally smaller vertebral cross sectional area (CSA).

Serum OPG-Fc and Serum TRACP-5b

Serum OPG-Fc concentrations rapidly declined in male and female rats once treatment stopped after 6 weeks, returning to control levels by week 16 (10 weeks post recovery) (Table 2). Serum TRACP-5b levels showed a dose dependent decrease at week 6 with lowest levels in response to OPG-Fc 10 mg/kg (Table 3). At week 11, TRACP-5b levels were greater than WT controls in males and females at all doses of OPG-Fc, with maximum

increases for OPG-Fc in 3 mg/kg females and OPG 10 mg/kg males. By week 16, OPG-Fc 3 and 10 mg/kg females still had higher levels of TRACP-5b than WT controls, while only OPG-Fc 10 mg/kg males were increased over controls (+64%). ALN decreased TRACP-5b in both males and females at week 6, returned to control levels by week 11 in males and females, but were increased in males at week 16.

Table 2: Serum OPG levels in male and female rats

Dose of OPG-Fc	Week 6		Week 11		Week 16	
	Males	Females	Males	Females	Males	Females
Vehicle	0.61±0.09	0.46±0.03	0.24±0.03	0.26±0.02	0.26±0.02	0.24±0.02
1 mg/kg	2898.35±111.37*	1701.67±121.45*	2.81±0.18*	1.98±0.17*	0.53±0.06	0.36±0.04
3 mg/kg	4845.56±191.33*	3666.95±201.74*	3.02±0.27*	2.37±0.22*	0.46±0.04	0.55±0.06*
10 mg/kg	8118.85±141.20*	6266.16±271.44*	4.92±0.29*	3.14±0.31*	1.33±0.20*	0.43±0.05*

* Significantly different from vehicle control (p<0.05).

Table 3: Serum TRACP-5b levels in male and female rats

Values represented as % difference from WT control

Dose	Week 6		Week 11		Week 16	
	Males	Females	Males	Females	Males	Females
OPG-Fc 1 mg/kg	-78% *	-54% *	+28%	+66%	+1%	+18%
OPG-Fc 3 mg/kg	-88% *	-79% *	+53% *	+170% *	+12%	+106% *
OPG-Fc 10 mg/kg	-94% *	-91% *	+176% *	+131% *	+64% *	+88% *
ALN 1 mg/kg	-57% *	-51% *	+20%	+11%	+70% *	+19%

* Significantly different from vehicle control at the corresponding timepoint (p<0.05).

Clinical chemistry

In female OPG-Fc and ALN rats, small decreases in red cell mass were apparent (decreased RBC, HGB and/or HCT; 3-9%). OPG-Fc 10 mg/kg and ALN groups also showed increased % neutrophils (88-100%) and globulins (12-15%), and slight decreases in % lymphocytes (10-12%). These changes were considered by the pathologist to be secondary to inflammation (the source of which was unidentified), and males were not affected. Small dose dependent decreases in AST (26-32%), creatinine (15-31%, males only), TBIL (16-37%), triglycerides (28-47%, females only), albumin (6-12%, females only), and dose-dependent increases in phosphorus (14-24%, females only) were evident in OPG-Fc 3 and 10 mg/kg and ALN animals. These were considered by the Sponsor to be minimal to mild, and could be partly due to effects on body weight/muscle mass or secondary to treatment-related effects on bone and teeth.

Body weight and skeletal growth

At the time of last dose (week 5), male body weights were slightly decreased from controls for OPG-Fc 1 and 10 mg/kg and ALN (-13% to -10%). Body weights somewhat recovered for OPG-Fc 1 mg/kg males, but remained statistically significantly lower at a -7% decrease. OPG-Fc 10 mg/kg and ALN males did not recover, and remained lower than controls (16-19%, OPG-Fc; 9-11%, ALN) from weeks 9-16. Female body weights were more sensitive to OPG-Fc treatment than males, and were lower than controls at the time of last dose for OPG-Fc 10 mg/kg and ALN (29% and 11%). Again, body weights did not recover, and remained lower than controls for OPG-Fc 10 mg/kg (25-35%) and ALN (10-12%) from weeks 9-16.

REVIEW NOTE: The Sponsor notes that the impairment in tooth eruption that occurs with OPG-Fc and ALN treatment may have contributed to lower weight gain. As such, males given access to a supplemental powdered diet after weaning did not have as much of a deficit in weight gain as females given only standard hard food. In the previous study (20080340), males were only fed hard food, and weights were decreased by up to 43%, while a powdered diet in the current study only decreased weights by 13%. Females were not offered a powdered diet in the current study.

Axial skeleton length was statistically significantly lower in OPG-Fc 10 mg/kg treated males and females (6-11%) and ALN treated males and females (3-4%) at weeks 13 and 16 (7 and 10 weeks post dose). Skeleton lengths did not appear to recover from week 6 values documented in the previous study (male and female OPG-Fc 10 mg/kg, 15-16%; male and female ALN, 3%).

Long bone geometry, density and strength

In the previous study, 6 weeks of OPG-Fc and ALN treatment resulted in visible changes on femur geometry and density by micro-CT, namely:

- marked accumulation and retention of trabecular bone in the distal half of the femur marrow cavity – males and females – all treatments,
- “flared” morphometry in the distal femoral metaphysis – males and females – all treatments, and
- presence of trabecular bone in the femur midshaft.

After 10 weeks recovery, the trabecular bone in the femur marrow cavity partially resorbed in the OPG-Fc groups, but resorption was less pronounced in the ALN groups. In male and female femurs following recovery, OPG-Fc treated animals had dose dependently decreased length and distal cortical thickness, with length showing partial recovery compared to values after 6 weeks of treatment. Midshaft CSA increases following 6 weeks of OPG-Fc treatment actually showed higher increases following 10 weeks recovery. Midshaft CSMI and vBMC, and distal CSA increases following 6 weeks of OPG-Fc treatment partially resolved following 10 weeks recovery. Increases

in distal vBMC were almost completely resolved in OPG-Fc males and females following recovery. ALN treatment generally showed similar results to OPG-Fc 10 mg/kg though not always to the same extent. With ALN treatment, recovery of femur length was not apparent following the 10-week recovery period. The only parameter that showed recovery from ALN treatment was distal vBMC. While increased differences from controls were apparent following recovery for midshaft CSA, CSMI, and vBMC, there was no change following recovery from ALN treatment for distal CSA. The Sponsor notes that bone in the femur midshaft was created during active treatment, while distal femur bone was produced during recovery, hence the small differences between vehicle versus treated after recovery in the distal femur, and lack of recovery in the midshaft.

Table 4: Micro-CT results for male and female femurs

Values represented as % difference from vehicle control

	Length				Midshaft CSA			
	Males		Females		Males		Females	
	6wks	16wks	6wks	16wks	6wks	16wks	6wks	16wks
OPG-Fc 1 mg/kg	-3%*	-4%*	-3%*	-1%	13%*	5%	10%*	8%
OPG-Fc 3 mg/kg	--	-7%*	--	-5%*	--	18%*	--	22%*
OPG-Fc 10 mg/kg	-21%*	-13%*	-18%*	-12%*	6%	20%*	11%*	19%*
ALN 1 mg/kg	-6%*	-9%*	-7%*	-6%*	15%*	24%*	14%*	29%*

* Significantly different from vehicle control at the corresponding timepoint (p<0.05).

	Midshaft CSMI				Midshaft vBMC			
	Males		Females		Males		Females	
	6wks	16wks	6wks	16wks	6wks	16wks	6wks	16wks
OPG-Fc 1 mg/kg	59%*	9%	47%*	25%*	67%*	6%	59%*	24%*
OPG-Fc 3 mg/kg	--	24%*	--	58%*	--	12%*	--	44%*
OPG-Fc 10 mg/kg	36%*	18%	43%*	28%*	48%*	14%*	49%*	17%*
ALN 1 mg/kg	47%*	76%*	34%*	75%*	41%*	63%*	33%*	53%*

* Significantly different from vehicle control at the corresponding timepoint (p<0.05).

	Distal CSA				Distal vBMC			
	Males		Females		Males		Females	
	6wks	16wks	6wks	16wks	6wks	16wks	6wks	16wks
OPG-Fc 1 mg/kg	29%*	10%	23%*	7%	143%*	0%	111%*	11%*
OPG-Fc 3 mg/kg	--	28%*	--	16%*	--	8%	--	20%*
OPG-Fc 10 mg/kg	41%*	34%*	66%*	19%*	148%*	9%	136%*	-2%
ALN 1 mg/kg	27%*	22%*	21%*	18%*	128%*	41%*	92%*	55%*

* Significantly different from vehicle control at the corresponding timepoint ($p < 0.05$).

	Distal cortical thickness [#]			
	Males		Females	
	6wks	16wks	6wks	16wks
OPG-Fc 1 mg/kg	--	-14%*	--	-17%*
OPG-Fc 3 mg/kg	--	-27%*	--	-24%*
OPG-Fc 10 mg/kg	--	-36%*	--	-40%*
ALN 1 mg/kg	--	-22%*	--	-10%*

* Significantly different from vehicle control at the corresponding timepoint ($p < 0.05$).

[#] Distal cortical thickness not examined at 6 weeks.

The flared morphometry seen at 6 weeks in males and females was not as prominent following the recovery period.

In regards to bone strength, sex-related differences were apparent with ALN treatment and low dose OPG-Fc. Males recovering from ALN treatment showed increases in energy to failure and in toughness compared to controls, while females had decreases in both parameters compared to controls. OPG-Fc 1 and 3 mg/kg recovery males had decreased stiffness, while females had increased stiffness, all compared to controls. However, all strength parameters (peak load, stiffness, energy, ultimate strength, elastic modulus and toughness) were decreased in OPG-Fc 10 mg/kg recovery males and females compared to controls.

Bone geometry was associated with differences in fracture patterns in OPG-Fc 10 mg/kg recovery males and females. OPG-Fc 10 mg/kg rat femurs did not consistently fracture by the typical transverse pattern (across the midshaft where force is applied). Because they had greater distal femur CSA and thinner distal femur cortex, they fractured from the posterior edge of the distal

femur cortex and propagated across to the anterior midshaft. The thin cortex of these higher dosed animals could also have contributed to reduced peak load and stiffness, since lower doses of OPG-Fc showed increases in these parameters. The biomechanical testing had limitations though such that the young rats did not have optimal ratios of span length to femur width as adult rats do, especially OPG-Fc 10 mg/kg treated animals which had shorter femurs than controls. Therefore true bending strength was hard to assess.

Overall, OPG-Fc discontinuation resulted in reduced femur strength proportional to reduced length and reduced distal femur cortical thickness. In comparison to the previous study, 6 weeks of OPG-Fc treatment generally increased all of the strength parameters in males and females. It appears that after cessation of treatment, the phenotype returned to normal.

Tibial Histopathology

Increased cancellous bone in the epiphysis and diaphysis was present in tibias of OPG-Fc and ALN animals following recovery, but was more severe with ALN treatment. OPG-Fc animals also had increased thickness and disorganization of the growth plate, but it was not predominant with ALN treatment. Decreased cancellous bone of the metaphysis was observed with OPG-Fc treatment, but the opposite was observed with ALN treatment.

Vertebral geometry, density and strength

OPG-Fc recovery rats had L5 phenotypes that were similar to controls as observed by micro-CT, while ALN recovery rats still retained increased bone volume and density of L5. Vertebral height remained lower than controls for OPG-Fc 10 mg/kg males and females, but was slightly less severe following the recovery period. ALN treated males and females had no difference in vertebral height following 6 weeks of treatment (previous study), but after recovery, were slightly shortened in both males and females. Central vBMC and central vBMD were increased in males and females after 6 weeks of treatment, with recovery from OPG-Fc 1, 3 and 10 mg/kg and ALN treatment showing significant decreases toward control levels. Central CSA showed return to control levels in males or females from either OPG-Fc or ALN recovery. OPG-Fc 3 mg/kg recovery males and females showed small but significant increases in central CSA over controls.

Table 5: Micro-CT results for male and female L5 vertebra

Values represented as % difference from vehicle control

	Vertebral height				Central CSA			
	Males		Females		Males		Females	
	6wks	16wks	6wks	16wks	6wks	16wks	6wks	16wks
OPG-Fc 1 mg/kg	6%*	-4%*	1%	-1%	-2%	5%	-4%	2%
OPG-Fc 3 mg/kg	--	-4%*	--	-5%*	--	11%*	--	13%*
OPG-Fc 10 mg/kg	-16%*	-12%*	-19%*	-14%*	-10%*	6%	-12%*	0%
ALN 1 mg/kg	3%	-4%*	0%	-5%*	-7%*	2%	-7%*	1%

* Significantly different from vehicle control at the corresponding timepoint (p<0.05).

	Central vBMC				Central vBMD			
	Males		Females		Males		Females	
	6wks	16wks	6wks	16wks	6wks	16wks	6wks	16wks
OPG-Fc 1 mg/kg	95%*	22%	74%*	20%*	56%*	16%*	49%*	17%*
OPG-Fc 3 mg/kg	--	27%*	--	28%*	--	16%*	--	13%*
OPG-Fc 10 mg/kg	77%*	20%*	50%*	-5%	55%*	13%*	42%*	-5%
ALN 1 mg/kg	85%*	52%*	62%*	48%*	57%	49%*	45%*	44%*

* Significantly different from vehicle control at the corresponding timepoint (p<0.05).

In regards to vertebral strength, sex-related differences were apparent in recovery animals following OPG-Fc treatment. Recovery males treated with any dose of OPG-Fc showed no difference from controls for any of the strength parameters (peak load, stiffness, energy, ultimate strength, elastic modulus and toughness). This indicates recovery of the initial phenotype, as increases in peak load and stiffness were observed following 6 weeks treatment. Recovery OPG-Fc 1 mg/kg females however had significant increases in peak load, stiffness and energy, which was also observed at this dose following 6 weeks of treatment. However, recovery OPG-Fc 10 mg/kg females showed decreases in each of these parameters from control levels, along with dose-dependent decreases in ultimate strength, elastic modulus and toughness. This is a reversal of levels observed directly following 6 weeks treatment since at that time, peak load and stiffness were increased, and there was no difference from controls for ultimate strength, elastic modulus and toughness. Recovery ALN-treated males and females also had increased peak load, stiffness and energy compared to controls, that was also

observed after 6 weeks of treatment. The Sponsor noted that discontinuing treatment with OPG-Fc did not affect vertebral ultimate strength in males (peak load/bone area), but reduced vertebral ultimate strength in OPG-Fc 10 mg/kg discontinued females. A potential cause for this was the tendency for OPG-Fc 10 mg/kg recovery females to have thinner cortices, inducing failure at the posterior cortex between the neural fossae when subjected to destructive strength testing.

Tooth eruption and root development

In the previous study, upper incisor length was significantly decreased in OPG-Fc 10 mg/kg and ALN males and females after 6 weeks of treatment, while lower incisor length was decreased as well, but not as dramatically. Decreased incisor length had led to reduced growth in these animals due to inability to eat well. After 10 weeks of recovery, incisor length in OPG-Fc 10 mg/kg males and females was visibly restored (-72 to -76% versus -28 to -35% for upper incisors; -22 to -24% versus -11 to +2.5% for lower incisors). The Sponsor notes that malocclusion was prevalent, likely due to the later eruption of upper incisors. Malocclusion of incisors can result in their overgrowth², and this was apparent in this study as some animals in the OPG-Fc and ALN recovery groups had incisor overgrowth that needed to be regularly managed by trimming. This also led to the unreliability of measured incisor length as an indicator of growth. ALN recovery males and females had positive changes in incisor length following recovery, but since the initial effects of ALN on decreased incisor length were not dramatic, the positive changes were not large.

In regards to molar eruption, 6 weeks of OPG-Fc treatment in both males and females resulted in decreased eruption of left and right 2nd molars by 80 to 100% and by 100% in left and right 3rd molars (maxillary and mandibular molars). High dose ALN also caused a 100% decrease in tooth eruption for left and right 3rd maxillary and mandibular molars in both males and females, with no effect on 2nd molars. In this study, the Sponsor notes that the previous findings likely relate to the age of animals when treatment was initiated since 2nd molars erupt between 14-20 days of age, and 3rd molars between 30-40 days of age. Following recovery, all 2nd molars of all treatment groups were erupted, while all 3rd molars remained unerupted in ALN-recovery groups, and only 33% erupted in OPG-Fc 10 mg/kg recovery groups. Of note, the 3rd molars that did erupt were only located in the mandible.

Micro-CT analysis and histopathology were performed on mandibles. Micro-CT showed absence of root development for all impacted 3rd molars in the ALN-recovery groups. Histopathology of these molars determined that lack of

² Marks SC (1981) Tooth eruption depends on bone resorption: Experimental evidence from osteopetrotic (la) rats. *Metab Bone Dis Rel Res*,3:107-115.

eruption was due to the deep location of the tooth in the mandible, major deficit in root development, ankylosis between bone and dentin, and minimal or absent cementum development. Erupted 3rd molars from OPG-Fc 10 mg/kg recovery groups were small, poorly developed, and not consistently embedded, while erupted 2nd molars from OPG-Fc 10 mg/kg recovery groups also had impaired root development. Histopathology of OPG-Fc 10 mg/kg 3rd molars indicated that eruption delay was associated with a sub-gingival location of the tooth in the mandible, convoluted roots at right angle with crown, normal development of cementum, and no ankylosis between bone and dentin. First molar root length in all treatment groups was not affected.

11 Integrated Summary and Safety Evaluation

The nonclinical study supplements for this application dealt with potential use of denosumab in younger populations (i.e. pediatric) where bone growth is still in progress. Since denosumab itself is not active in rodents as it does not recognize mouse or rat RANKL, these studies utilized either OPG transgenic (OPG-Tg) rats (overexpressing OPG), or rats dosed with OPG-Fc, to simulate the potential effects of denosumab on a young population. OPG is a RANKL inhibitor and is active in rodents.

Adult OPG-Tg rats (OPG overexpressing) were previously observed to have stronger vertebrae than their wild-type littermates, but their femurs had narrower midshafts and decreased bending strength (Ominsky et al., 2009). It was unknown at what stage of development (prenatal, neonatal, or later) these deficiencies began, since many studies only investigated the effects of OPG-overexpression after significant bone growth had occurred (6 months or 12 months). As a result, the first study reviewed here investigated the effects of OPG overexpression on 1 and 2 month old rats to determine when the femur deficiencies might occur. It concluded that effects on long bone geometry and strength in OPG overexpressing rats likely occur between 2 and 12 months of age, as there appeared to be no significant changes in femur geometry, density or strength at 2 months. However, since changes in the long bones eventually occur prior to 12 months, it appears that denosumab administration has a negative effect on bone growth, geometry or strength in pediatric populations that are still undergoing significant bone development.

The Sponsor did note that there were two limitations to this study. The first issue concerned use of WT littermates versus sex/strain matched WT controls. The referenced study by Ominsky (2009) with adult OPG-Tg and WT rats used littermates that were either WT or OPG overexpressing from a cross mating of heterozygous transgenic animals. However, the younger rats used in the currently reviewed study (R20090069) were not littermates, but from separate inbred colonies that were sex and strain matched. The Sponsor noted that results may not be completely comparable since drifts in skeletal phenotypes could have developed that are unrelated to OPG overexpression. The second issue concerned differences in

circulating OPG levels. In OPG-Tg rats, circulating OPG levels are more than 100-fold lower than in normal rats receiving a subcutaneous injection of recombinant OPG-Fc. This could explain the significant suppression of bone growth seen in recombinant OPG-Fc treated neonatal rats (Study R20090070) which was not apparent in young or old OPG-Tg rats (Study R20090069). It has been shown that circulating OPG can only regulate bone resorption at supraphysiological or pharmacological levels, which are not seen in the transgenic mice. As such, normal rats exposed from birth to clinical RANKL inhibitors would show a more negative long bone phenotype than young OPG-Tg rats.

The second study (R20090070) was a follow up recovery study to investigate the potential reversal of effects on bone growth/strength and tooth eruption that were apparent following neonatal treatment with OPG-Fc or alendronate. The original study concluded that treatment of 2 week old rats for 6 weeks with OPG-Fc or alendronate suppressed bone resorption, inhibited bone growth, increased whole bone femur and vertebral strength, and impaired molar eruption and incisor growth. After 10 weeks of recovery, following 6 weeks of active treatment, partial reversal of changes in bone growth, resorption and molar eruption were observed. At the end of recovery, body weight remained low, decreased bone size and strength were still evident, growth plates had altered morphology, and 3rd molar eruption was still delayed, with roots of late erupting 2nd and 3rd molars having impaired growth and orientation within the jaw. These results further confirmed our recommendation that denosumab should not be used to treat pediatric populations or populations that are still undergoing bone development.

Suggested labeling: The following are recommended changes in labeling in the 3/19/2010 submission (additions are underlined, deletions are crossed through).

(b) (4)





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

PHARMACOLOGY/TOXICOLOGY REVIEW

BLA number: 125320 and 125331

Review number: 2

Sequence number/date/type of submission: 0000, December 19, 2008

Information to sponsor: Yes (X) No ()

Sponsor: Amgen Inc.

Drug supplier/manufacturer:

Site 1: Boehringer Ingelheim Pharma, GmbH & Co. Kg, Birkendorfer Strasse 65
88397 Biberach an der Riss, Germany

Site 2: Amgen Inc., 5550 Airport Boulevard, Boulder, CO 80301

Site 3: Amgen Inc., 4000 Nelson Rd, Longmont, CO 80503

Site 4: Amgen Manufacturing Limited, State Road 31, Kilometer 24.6,
Juncos, Puerto Rico 00777

Reviewer name: Kimberly Hatfield, Ph.D.

Division name: Division of Reproductive and Urologic Products

Review completion date: October 30, 2009

Drug:

Trade name: Prolia

Generic name: Denosumab

Code name: AMG 162

Other names: (b) (4) anti-RANKL mAb

Molecular formula/molecular weight: (b) (4)
147,352 Daltons (b) (4)

Structure: monoclonal antibody; denosumab is a fully human full-length immunoglobulin.

The molecule is a (b) (4) consisting of 2 heavy chains (HC) of the IgG₂ subclass and 2 light chains (LC) of the kappa subclass, which are covalently linked through disulfide bonds. Each HC contains an N-linked glycan at the canonical glycosylation site. Each LC contains 215 amino acids, with 2 intramolecular disulfides. Each HC, with its 4 intramolecular disulfides, contains 447 amino acids, (b) (4)

(b) (4). Each LC is covalently linked to a separate HC through a single disulfide bond; the 2 HC are covalently linked through 4 intermolecular disulfide bonds.

Drug class: anti-osteoporetic, osteoclast activation inhibitor

Intended clinical population: postmenopausal women with osteoporosis (treatment) or at risk for osteoporosis (prevention)

Route of administration: subcutaneous injection

Relevant INDs/NDAs:

The Sponsor (Amgen) has submitted this BLA for denosumab for 4 indications, each of which is represented by separate BLA numbers:

- BLA 125320: Treatment of postmenopausal osteoporosis
- BLA 125331: Prevention of postmenopausal osteoporosis
- BLA 125332: Treatment and prevention of bone loss in patients undergoing hormone ablation for breast cancer
- BLA 125333: Treatment and prevention of bone loss in patients undergoing hormone ablation for prostate cancer

Additional relevant INDs/NDAs are:

BB-IND 9837 – initial IND – treatment of postmenopausal osteoporosis (DRUP)

(b) (4)

(b) (4)

Background:

An initial nonclinical review of studies submitted to the BLA by the Sponsor has been completed, signed, and submitted to the file as of August 28, 2009. While reviewing the labeling, it was noted that the Sponsor referenced nonclinical studies from the literature in the “Nonclinical Overview” section of their BLA in support of the safety of denosumab. These literature articles were not formally reviewed in the initial BLA nonclinical review. The Sponsor intends to use the results from these studies to support safety claims in the labeling for denosumab, therefore a formal review of those studies is documented here.

On the BLA PDUFA date (10-19-09), a complete response letter was mailed to the Sponsor. This labeling review supports anticipated future submissions by the Sponsor in order to gain Agency approval of Prolia for BLAs 125320, 125331, 125332 or 125333.

Literature articles reviewed in this submission:

Kong Y-Y, Yoshida H, Sarosi I, Tan H-L, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ and Penninger JM (1999). OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397:315-323.

Fata JE, Kong Y-Y, Li J, Sasaki T, Irie-Sasaki J, Moorehead RA, Elliott R, Scully S, Voura EB, Lacey DL, Boyle WJ, Khokha R and Penninger JM (2000). The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. *Cell* 103:41-50.

Nonclinical review of literature

1. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis.

(Kong Y-Y, Yoshida H, Sarosi I, Tan H-L, Timms E, Capparelli C, Morony S, Oliveirados-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ and Penninger JM (1999) *Nature* 397:315-323.)

Methods: This study investigated mice (C57BL/6 background) with the *opgl* gene ablated (*opgl*^{-/-}, *opgl*^{+/-}, *opgl*^{+/+}). For in vivo studies, groups of *opgl*^{-/-}, *opgl*^{+/-} and *opgl*^{+/+} mice were sacrificed at days 21 and 28 after birth.

Results: This study first investigated whether disruption of *opgl* altered bone physiology in vivo in 3-4 week old *opgl*^{+/+} and *opgl*^{-/-} mouse littermates. In *opgl*^{-/-} mice, osteopetrosis was evident via whole body autoradiograph as early as postnatal day 2, and characterized by radio-opaque long bones, vertebral bodies and ribs. Long bones were shortened and club-shaped (bone remodeling defect), and none of the *opgl*^{-/-} mice had incisor or molar teeth that had erupted into the oral cavity. The investigators noted that "failure of tooth eruption is a typical finding in osteopetrosis as bone resorption is required to open an avenue through the bone of the jaw for eruption of teeth." Of note was that bone formation in the skull was radiographically normal, suggesting that bone formed by intramembranous bone formation is not affected by OPGL. Trabecular and total bone density were also increased in 4-week old *opgl*^{-/-} mice versus *opgl*^{+/-} and *opgl*^{+/+} mice. Histological analysis of long bones in *opgl*^{-/-} mice also showed osteopetrotic long bones, with accumulation of cartilage and bone and the epiphysis, metaphysis and diaphysis, and vertebral body osteopetrosis characterized by thin calvaria and marrow spaces. There was also no evidence of periosteal bone modeling adjacent to growth plates, and there was disorganized columnar structure of chondrocytes at the epiphyseal growth plates, consistent with the visible finding of shortened femurs. TRAP (tartrate-resistant acid phosphatase) staining in *opgl*^{-/-} and *opgl*^{+/+} mice showed that ^{-/-} mice completely lacked TRAP-positive immature and mature multinucleated osteoclasts, while TRAP-positive cells were observed in ^{+/+} littermates.

Further investigation showed that thymic cellularity and thymus size were decreased in 4-week old *opgl*^{-/-} mice. The reduced thymic cellularity is likely due to blocked differentiation of CD4⁺CD8⁻ double-negative CD44⁺CD25⁺ precursors to CD44⁺CD25⁻ thymocytes. Scattered expression of the OPGL receptor (RANK) in the thymus was also observed by in situ hybridization, and OPGL is expressed in CD4⁺CD8⁻ thymocyte precursors. In chimeric *opgl*^{-/-}*ragl*^{-/-} mice, a block in the progression of CD25⁺CD44⁺ to CD25⁻CD44⁺ thymocytes and reduced thymic cellularity was observed. This was also observed in osteoprotegerin (OPG) transgenic mice and normal mice injected with

recombinant OPG, which confirmed the critical role of OPGL in early thymic development.

Also, spleens of *opgl*^{-/-} mice were 2-3 times larger than spleens from control littermates, due to reduced body weight and splenic hematopoiesis in *opgl*^{-/-} mice. Increased percentages of CD4⁺ and CD8⁺ T cells were also observed in *opgl*^{-/-} spleens. OPGL was also shown to be important in the development of B-cell precursors.

Anatomical analysis showed that *opgl*^{-/-} mice had a defect in lymph node organogenesis, and completely lacked mesenteric, cervical, mandibular, inguinal, axillary, para-aortic and popliteal lymph nodes. Serial sectioning also confirmed the complete absence of any tissues resembling early lymph-node anlagen. The lack of lymph nodes was shown not to be the result of a defect in cellular homing, and transfer of normal bone marrow cells in to newborn *opgl*^{-/-} mice did not restore lymph node formation. Despite the lack of lymph node formation, Peyer's patches were observed in *opgl*^{-/-} mice, though they were reduced in size, and contained defined T-cell and B-cell areas.

Conclusions: These results show that the absence of OPGL causes osteopetrosis in newborn mice, characterized by shortened and club shaped long bones, absence of tooth eruption (incisors and molars), increase in trabecular and total bone density, and lack of bone modeling at bone growth plates. As a result, there is a dependence on the expression of OPGL for osteoclast differentiation and development. Knocking out *opgl* also decreases thymus size, and reduces thymic cellularity, and results in enlarged spleens (though with intact architecture). Finally, though the absence of OPGL appears to have no effect on splenic architecture or formation of Peyer's patches, OPGL was found to be essential for lymph node formation due to the complete absence of lymph nodes in *opgl*^{-/-} mice.

Regulatory decision: This study was well-designed and adequately showed that in newborn mice, lack of OPGL results in osteopetrosis, impaired bone growth, absence of tooth eruption, decreased thymus size, reduced thymic cellularity, along with complete absence of lymph node formation. Including these findings in labeling for denosumab is appropriate.

2. The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development.

(Fata JE, Kong Y-Y, Li J, Sasaki T, Irie-Sasaki J, Moorehead RA, Elliott R, Scully S, Voura EB, Lacey DL, Boyle WJ, Khokha R and Penninger JM (2000) *Cell* 103:41-50.)

Methods: This study investigated mice (C57BL/6 background) genetically deficient in *opgl* and *rank* (*opgl*^{-/-}, *opgl*^{+/-} and *opgl*^{+/+}, *rank*^{-/-}, *rank*^{+/-} and *rank*^{+/+}). For timed pregnancies, male and female mice were mated overnight and female mice were scored for vaginal plugs the next morning (presence of vaginal plug indicated pregnancy day 0.5). It is noted that *opgl*^{+/-} and *rank*^{+/-} mice had a mammary gland phenotype that was indistinguishable from that of wild-type mice.

Results: This study first investigated whether OPGL function was required during pregnancy and lactation. *Opgl*^{-/-} mothers were fertile, and gave birth to normal pups, with litters comparable to *opgl*^{+/-} and *opgl*^{+/+} mothers. While normal nursing and mothering was evident and normal for all genotypes, all pups from the *opgl*^{-/-} mothers died within 48 hours. Lethality of pups was independent of their genotype, and further investigation showed a lack of milk in their stomachs. Fostering with *opgl*^{+/-} or *opgl*^{+/+} mothers rescued the pups born to *opgl*^{-/-} females, indicating a potential defect in the production of breast milk in *opgl*^{-/-} mothers.

To investigate this further, mammary gland morphogenesis was compared between *opgl*^{-/-}, *opgl*^{+/-} and *opgl*^{+/+} females. Age-matched nulliparous *opgl*^{-/-}, *opgl*^{+/-} and *opgl*^{+/+} females had comparable development of the parenchymal breast tissue during postnatal life, puberty, and as nulliparous adult females (normal development of mammary gland anlage, elongation and extension of the ductal tree, ductal side branching; normal epithelial ductal structures with normal stromal matrix and fibroblasts, and normal numbers of myoepithelial cells). However, further investigation of mammary gland development during pregnancy showed that while pregnant *opgl*^{-/-} females had increased ductal side branching and formation of the initial alveolar buds, as did wild-type mice, "differentiation and expansion of the alveolar buds into mature lobulo-alveolar mammary structures was arrested in *opgl*^{-/-} females", with earliest defects in development being observed at pregnancy day 14.5. The defects remained until lactation, even though usual dilation of primary ducts occurred. Expression of β -casein mRNA (major milk protein) was also impaired in *opgl*^{-/-} mice at pregnancy day 14.5 and continuing until lactation.

In an attempt to rescue the phenotype, slow-release pellets of soluble recombinant OPGL were implanted into the mammary glands of pregnant *opgl*^{-/-} females. Lobulo-alveolar development was rescued along with restored expression of β -casein mRNA (recovered milk production). Control pellets did not induce these changes.

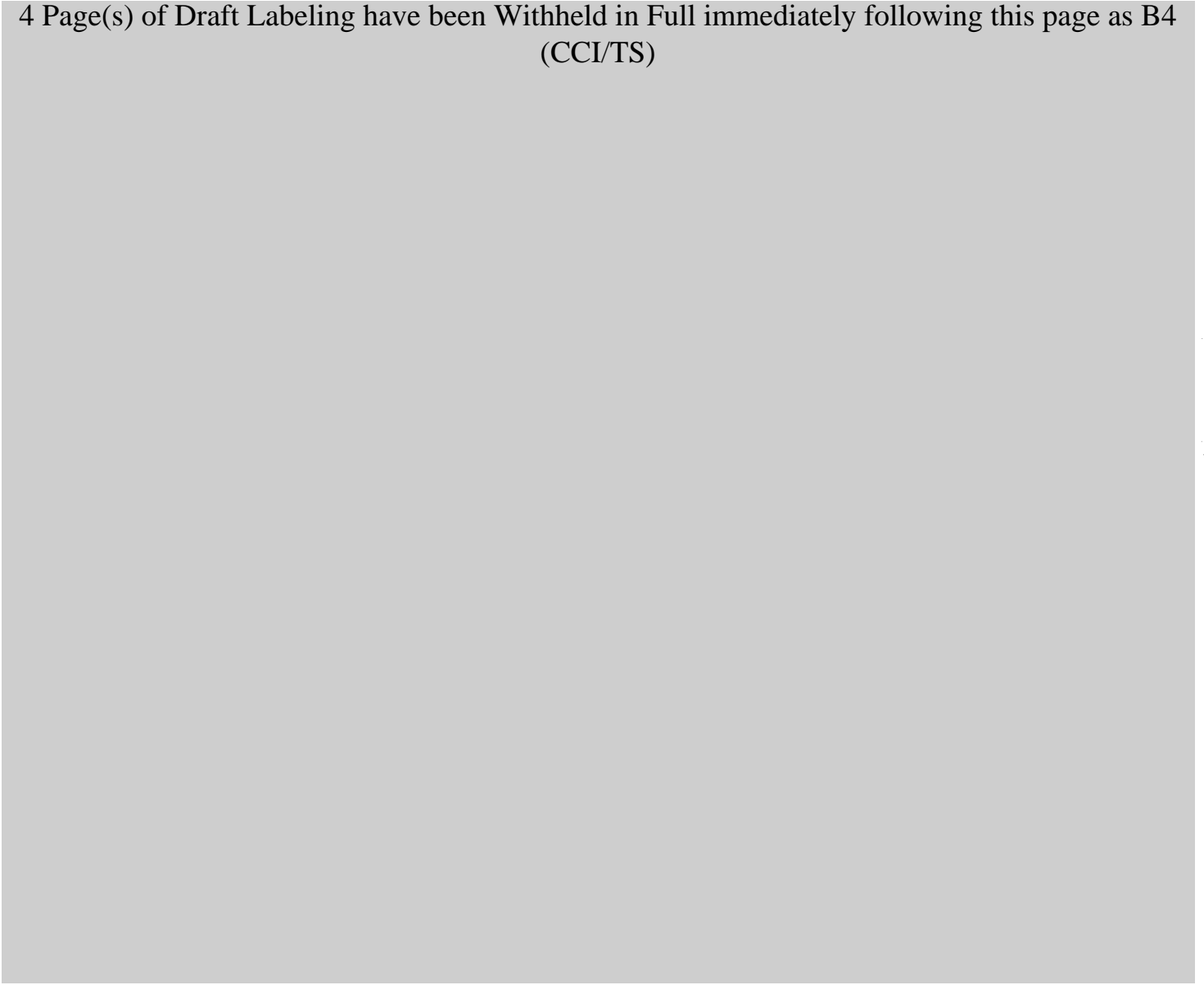
Comparable observational findings to *opgl*^{-/-} female mice were observed when *rank*^{-/-} female mice were investigated (failed lactation, and lethality of litters). Further investigation into mammary gland development during pregnancy in *rank*^{-/-} females showed the same lack of differentiation of alveolar buds into mature lobulo-alveolar mammary structures as in *opgl*^{-/-} females. An attempt to rescue development with implantation of recombinant OPGL pellets into the mammary tissue did not have any effect, and defects were not rescued.

Conclusions: These results show that OPGL and RANK expression are necessary for the development of alveolar buds into mature lobulo-alveolar epithelial structures and the formation of a functioning mammary gland during pregnancy. Developmental arrest of *opgl*^{-/-} mammary glands is directly due to a lack of OPGL, and OPGL functions through RANK alone to mediate these developmental changes. The mechanism of action is that loss of OPGL expression results in apoptosis of mammary alveolar epithelial cells during pregnancy.

Regulatory decision: This study was well-designed and adequately showed that 1) OPGL and RANK are necessary for mammary gland development during pregnancy, and 2) knockout mice deficient in either of these two genes have a lack of lobulo-alveolar development of the mammary gland, and impaired postpartum lactation. Including these findings in labeling for denosumab is appropriate.

Post-review labeling:

4 Page(s) of Draft Labeling have been Withheld in Full immediately following this page as B4 (CCI/TS)



**Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research**

Date: September 1, 2009

Reviewer: Lynnda Reid, Ph.D.
Supervisory Pharmacologist

BLA #/SS#/date: 125-320 (S000) & 125-331 (S000), December 19, 2008

Sponsor: Amgen, INC.

Drug Product: Denosumab (Prolia®)

Indication: Treatment (BLA 125-320) and prevention (BLA 125-331) of postmenopausal osteoporosis

Recommended Action: Nonclinical data support approval for the treatment of osteoporosis in postmenopausal women. Nonclinical data is supportive for a prevention claim provided there is a positive risk:benefit profile for treatment of osteopenic postmenopausal women.

Background: The Sponsor (Amgen) has submitted marketing applications for denosumab for 4 indications:

- Division of Reproductive and Urologic Products
 - BLA 125-320: Treatment of postmenopausal osteoporosis
 - BLA 125-331: Prevention of postmenopausal osteoporosis
- Division of Biologic Oncology Products
 - BLA 125-332: Treatment and prevention of bone loss in patients undergoing hormone ablation for breast cancer
 - BLA 125-333: Treatment and prevention of bone loss in patients undergoing hormone ablation for prostate cancer

Treatment of postmenopausal osteoporosis is considered to be the primary indication. If approved, denosumab would be the first biologic agent available in the U.S. for the treatment or prevention of postmenopausal osteoporosis. In addition, there are currently no products approved for bone loss associated with hormone ablation for prostate cancer or breast cancer, although the current

standard of care includes treatment with bisphosphonates and calcium and Vitamin D supplementation.

The clinical dosing regimen for denosumab for all indications is a 60 mg subcutaneous injection every 6 months, administered by a healthcare provider.

Pharmacology: Denosumab is a fully human monoclonal IgG2 antibody that binds to and inactivates RANKL (receptor activator of nuclear factor kappa B ligand). The mechanism of action for this antibody involves blocking the binding site of RANKL with its receptor RANK, thereby preventing receptor activation and clustering, and downstream signaling. RANKL-induced RANK signaling is essential for the formation, function and survival of mature osteoclasts, which are responsible for bone resorption. RANKL is produced by osteoblastic lineage cells and activated T cells, and its expression is regulated by various cytokines, glucocorticoids and PTH.

A 16-month pharmacology study was conducted in ovariectomized (OVX) cynomolgus monkeys, a model which mimics postmenopausal bone loss. Once monthly treatment with denosumab for 12 months decreased biomarkers of bone formation (osteocalcin, sALP) and resorption (CTx, TRAP-5b, NTx), and increased bone mineral density (BMD), prevented OVX-induced BMD changes in both cortical and cancellous bone, and increased bone strength in most examined bones. Following cessation of treatment, BMD and bone parameters returned to original baseline levels.

Surrogate models used to evaluate the pharmacodynamic role of RANKL in rodents include a mouse knock-in (KI) model expressing human RANKL, and a surrogate fusion protein construct of osteoprotegerin bound to antibody Fc region (OPG-Fc). Osteoprotegerin is the natural endogenous regulator of RANKL activity in both rodents and humans, inhibiting RANKL activity in a manner comparable to denosumab.

Secondary Pharmacodynamics: High levels of protein expression have been observed in skeletal and lymphoid tissues. In addition, RANKL mRNA expression has been detected in keratinocytes of skin, mammary epithelial cells, heart, skeletal muscle, lung, stomach, placenta, thyroid gland and brain.

The RANK/RANKL signaling pathway interacts with the immune system in several ways including lymphocyte development and lymph node organogenesis, monocyte / dendritic cell maturation, activation and longevity, antigen presentation and CD40 ligand-independent T helper cell activation. The absence of RANKL or RANK genes in knock-out mice leads to the complete failure of lymph node development and an absence of lactation by inhibiting mammary gland maturation.

Toxicology: Denosumab is pharmacologically active only in humans and nonhuman primates. Although nonclinical studies could only be performed in a single species, and many of the studies were not considered optimal, the overall nonclinical program is considered adequate to support treatment in women who are not at risk for pregnancy.

One-month and twelve-month toxicology studies were conducted in cynomolgus monkeys. In the 1-month study, adult male and female monkeys were dosed weekly with 0, 0.1, 1 or 10 mg/kg s.c. or 10 mg/kg i.v. There were no significant non-pharmacodynamically related effects observed in this study. In the 12-month study young adult male and female monkeys were dosed monthly with 0, 1, 10 or 50 mg/kg s.c. The majority of the animals in this study (47/64), control and treated, were infected with protozoa (*giardia lamblia* and/or *cryptosporidium*) infections. Two males treated at study 50 mg/kg died due to acute renal failure (most likely secondary to diarrhea and dehydration caused by the protozoan infections). One female treated at 10 mg/kg and one female treated at 50 mg/kg developed abscess of the teeth and/or jaw (not observed in control females, females treated at 1 mg/kg or in any of the males). In animals where the epiphyseal plates had not fully closed prior to treatment, growth plates were markedly enlarged with reduced chondroclasis and expanded growth plates which were associated with cartilage calcification and cartilage erosion and calcification. A causative role for denosumab in the deaths and oral abscesses observed at the high-dose cannot be ruled out and are potentially secondary to denosumab-induced adverse effects on the immune system resulting in an inability to mount an adequate immune response.

Genotoxicity: Genotoxicity potential was not been studied. The standard genotoxicity studies routinely conducted for pharmaceuticals are not applicable to monoclonal antibodies and therefore are not recommended. Denosumab is not expected to interact directly with DNA or other chromosomal material.

Carcinogenicity: The potential carcinogenicity potential of denosumab has not been assessed. However, there may be some increased risk associated with denosumab since there is some evidence, e.g., increased infection rates in animals and humans, that it may be immunosuppressive.

Denosumab does not bind to rodent RANKL and therefore an appropriate model was not available. Although the Sponsor generated a knock-in human RANKL transgenic mouse and a surrogate rodent antibody (OPG-Fc fusion protein), these models were not considered relevant for carcinogenicity testing. It should also be noted that huRANKL KI mice have lower osteoprotegerin levels than wild type mice.

Reproductive toxicology: Reproductive and developmental studies were performed in cynomolgus monkeys. There was no evidence of impairment of fertility following once weekly administration of denosumab through 2 menstrual

cycles, mating and gestational day 20 of presumed pregnancy. When administered to pregnant monkeys once weekly during the time of major organogenesis (gestational days 20-50) there were no observed adverse effects on the mother or fetus. However, IgG antibodies do not readily cross the placenta during this period and therefore, this study only assessed potential secondary or indirect effects on the fetus due to maternal exposure. Although there were no gross teratogenic effects observed, only limited fetal tissues were examined histologically. Of particular note, the lymph nodes, where RANK signaling plays a major role in the developing immune system, were not examined. **Additional studies would be necessary to assess potential adverse effects on skeletal, immune and nervous system development, and would be required to support indications which would include women of child bearing potential in the patient population.**

Inhibition of the RANK/RANKL in knock-out mice resulted in lymph node agenesis, and postnatal impairment of bone growth, dentition and tooth eruption. These mice also showed altered maturation of the maternal mammary gland during pregnancy, leading to impaired lactation postpartum.

Young mice (2 weeks of age at initiation of treatment) treated with OPG-Fc for 6 weeks had significant decreases in body weight gain and axial skeletal length. Decreased upper and lower incisor length, and delayed molar eruption proportional to the magnitude of bone resorption suppression were also observed.

Summary and Discussion:

Clinical Issues:

Immunotoxicity: Current clinical findings indicate that the overall incidence of serious adverse events of infection is slightly higher in denosumab versus placebo-treated subjects. These events include pneumonia, endocarditis, serious skin infections, gastrointestinal infections, urinary tract infections, infective arthritis, and ear infections, along with serious bacterial infections and serious infections due to an unspecified pathogen.

Data from animal models indicate that RANK/RANKL signaling is necessary for development of lymph nodes and plays a major role in immune regulation and response. High levels of RANKL have been detected in lymphoid tissues; therefore it is not surprising that in the tissue distribution studies, high concentrations of ¹²⁵I-denosumab were detected in lymph nodes.

An immunology assessment was performed as part of the 16-month pharmacology study in relatively healthy adult male and female monkeys. There were no remarkable findings in regards to T-cell dependent antibody response. However, blood immunophenotyping did show the total lymphocyte count was slightly and statistically significantly decreased at pre-necropsy for

animals treated at 50 mg/kg/month. Absolute counts of CD3+/CD8+ cytotoxic T lymphocytes were also slightly and statistically significantly decreased at the high dose compared to ovariectomized controls at pre-necropsy. In the 12-month study in adult male and female animals, two males treated at 50 mg/kg died due to acute renal failure secondary to protozoan infections, and one female treated at 10 mg/kg and one female treated at 50 mg/kg developed abscess of the teeth and/or jaw. Immunoglobulin levels and immunophenotyping of lymphocytes did not show evidence of immunosuppression. However, potential effects on the immune function are difficult to assess and the assays performed do not cover all aspects of immune competence. Available data do not provide information on the level of suppression of RANK/RANKL signaling that is necessary to impact immune function. **Disrupting RANK/RANKL signaling has the potential to cause immune suppression.**

- 2) **Delayed fracture healing:** In the 16-month pharmacology study in cynomolgus monkeys, denosumab treatment delayed remodeling of bone biopsy sites and resorption of woven bone and bone fragments compared to untreated controls. A delay in fracture healing was also observed in the huRANKL KI mouse model characterized by delays in the removal of cartilage and induced changes in morphology and time course of tissue remodeling of the fracture site. However, following healing, overall bone strength in denosumab treated animals was increased.

In clinical studies, information on fracture healing complications was collected for all nonvertebral fractures. In the primary clinical study (20030216), there was a reduction in the number of nonvertebral fractures, and fracture healing complications were actually few and well-balanced between placebo and treated groups.

- 3) **Ocular Toxicity:** In clinical trial 20040138, a signal for cataract (9 vs. 34 cases in placebo vs. denosumab group) was noted. In tissue distribution studies in monkeys, ¹²⁵I-denosumab appeared to accumulate in the cornea of the eye in cynomolgus monkeys. However, there were no indications of ocular toxicity noted in the 12 month toxicology study in monkeys. In addition, tissue cross reactivity studies with human, monkey, rat and rabbit tissues did not show binding of denosumab to ocular tissues. However, it should be noted that in these same studies, denosumab had very limited binding to normal human tissues and only lymphocytes in the paracortex of the lymph node had a positive signal in monkeys.
- 4) **Cardiovascular Toxicity:** An association between osteoprotegerin (OPG) levels, RANKL and coronary artery disease, i.e., arterial calcification following OPG/RANKL induced osteolysis in bone, has been reported in the literature. In the 12-month toxicity study in monkeys treated at 50 mg/kg/month, minimal to slight focal myocarditis/pericarditis was observed in one male and slight

myocardial degeneration/necrosis was observed in two males. Minimal to slight inflammatory cell foci were observed in male and female treated animals at all sacrifices but was also observed in 3 female control animals. No calcification was noted by the investigator and there were no apparent test item-related effects on blood pressure or heart rate. The incidence rate and types of cardiac findings reported in denosumab treated monkeys were similar to the reported spontaneous lesions; therefore the lesions could not be definitively classified as drug-related.

Outstanding nonclinical issues:

- **Reproductive and Developmental Toxicity:** Only secondary maternal effects on fetal organogenesis were assessed in primates, however, given the primary indication of treatment of osteoporosis in postmenopausal women, DRUP did not consider that additional reproductive and developmental studies were necessary for approval. If denosumab were ever to be evaluated for treatment in a population that included fertile women, further evaluation of the risks on reproduction and development would be necessary.
- Preliminary data suggest that denosumab should not be used in patients where the epiphyseal plates are not fully closed. In animals where the epiphyseal plates had not fully closed prior to treatment, growth plates were markedly enlarged with reduced chondroclasis and expanded growth plates associated with cartilage calcification (zone 4) and cartilage erosion and calcification (zone 5).
- **Carcinogenicity:** As for cancer risk, no carcinogenicity studies were performed due to the lack of an appropriate animal model. In the two long term studies (12-month toxicity and 16-month pharmacology), there were no incidences of tumor formation indicated with the exception of squamous metaplasia (benign) in two females, one each dosed at 1 and 50 mg/kg/month for 12 months. Standard organ histopathology was not conducted in the 16-month pharmacology study. Immunosuppressive agents, in general, increase the risk of cancer.
- **Immunotoxicity:** A causative role for denosumab in the deaths and oral abscesses observed at the high-dose cannot be ruled out and are potentially secondary to denosumab-induced immunosuppression and an inability to mount an adequate immunologic response.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Kimberly Hatfield, that the submitted nonclinical data support approval of denosumab for the treatment of postmenopausal osteoporosis. Nonclinical data is supportive for

a prevention claim provided there is a positive risk:benefit profile for treatment of osteopenic postmenopausal women.



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER(S): 125320 & 125331 (Division of Reproductive and Urologic Products)
125332 & 125333 (Division of Biologic Oncology Products)

SERIAL NUMBER: 0000

DATE RECEIVED BY CENTER: 12/19/08

PRODUCT: Prolia (denosumab)

INTENDED CLINICAL POPULATION: Treatment (BLA 125320) and prevention (BLA 125331) of
postmenopausal osteoporosis

SPONSOR: Amgen, Inc.

DOCUMENTS REVIEWED: Module 4: Nonclinical Study Reports

REVIEW DIVISION: Division of Reproductive and Urologic Products (HFD-580)

PHARM/TOX REVIEWER: Kimberly Hatfield, Ph.D.

PHARM/TOX SUPERVISOR: Lynnda Reid, Ph.D.

DIVISION DIRECTOR: Scott Monroe, M.D.

PROJECT MANAGER: Celia Peacock

Date of review submission: August 28, 2009

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: Nonclinical data support approval of denosumab (AMG 162), 60 mg subcutaneous injection once every 6 months, for treatment of postmenopausal osteoporosis, and for prevention of postmenopausal osteoporosis if a positive clinical risk:benefit profile has been demonstrated.
- B. Recommendation for nonclinical studies: No additional nonclinical studies are required.
- C. Recommendations on labeling: The Sponsor's submitted labeling for Sections 8.1, 8.3, 8.4, 13.1 and 13.2 are acceptable with minor changes. Recommended labeling is shown below. Annotated labeling can be found on page 211 of this review.

(b) (4)



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacology: Denosumab is a fully human IgG₂ monoclonal antibody that binds to the receptor activator of nuclear factor- κ B (RANK) ligand (RANKL). RANKL binds to RANK on osteoclast precursors and mature osteoclasts, stimulates osteoclasts to resorb bone, and promotes differentiation of the precursor cells into osteoblasts. The binding of denosumab to RANKL inhibits binding of the ligand to target receptors such as RANK, thereby neutralizing the effects of RANKL. Since RANKL binding to RANK is involved with the formation, function, and survival of cells that resorb bone such as osteoclasts, the inhibition of RANKL binding to RANK by denosumab leads to the suppression in osteoclast-mediated bone turnover.

Denosumab is specific to human and non-human primate RANKL, and is not active in the rodent. In ovariectomized (OVX) monkeys used as a model of postmenopausal osteoporosis, denosumab was efficacious in treating and preventing bone loss. Denosumab decreased biomarkers of bone formation and resorption, and increased bone mineral density (BMD) predominantly of the lumbar and thoracic spine, whole body, and proximal femur, and to a lesser extent, in the central tibia and distal radius, prevented OVX-induced BMD changes in both cortical and cancellous bone, and increased bone strength in femur and vertebrae. BMD and bone parameters returned to original baseline levels once treatment was stopped. In a comparison pharmacology study in monkeys, treatment with denosumab was comparable to treatment with alendronate in reducing biomarkers of bone turnover, and inducing similar changes in bone histomorphometry and bone strength. Denosumab was also found to be comparable to alendronate in regards to fracture healing, in huRANKL knock-in (KI) mice. Both alendronate and denosumab treatment delayed fracture healing in KI mice compared to control mice with fractured femurs, but did not compromise mechanical parameters such as strength and stiffness.

Pharmacokinetics / Toxicokinetics: Weekly (up to 4 doses) and monthly (up to 16 doses) s.c. administration (0.1-50 mg/kg) of denosumab in monkeys resulted in dose-proportional increases in AUC, with no accumulation. Antibody formation was prevalent and inversely proportional to dose, with neutralizing antibodies forming in 15-34% of animals. Denosumab is primarily distributed to thyroid/parathyroid, serum, lymph nodes, blood, spleen, and ovaries. Unexpectedly, ¹²⁵I-denosumab was found to concentrate in the cornea of the eye in monkeys.

When comparing animal doses to human doses, dose extrapolations have been made based on body weight (mg/kg) as the most appropriate measure due to the absence of complete AUC data in monkeys, and since systemic exposures in the monkey were very similar following subcutaneous and intravascular injections.

General Toxicology: Overall, the nonclinical program is considered adequate. According to ICH guidance, safety evaluations of biologics should include two relevant species, however, since denosumab is only pharmacologically active in the monkey and does not bind to rodent RANKL, the use of one relevant species for toxicity testing is justified. In addition, carcinogenicity studies were not conducted with denosumab due to lack of an appropriate test

species. While the Sponsor does have a surrogate huRANKL knock-in mouse model that was used primarily for pharmacology studies, it is not an appropriate model for carcinogenicity studies. Also, based on Agency guidance, genetic toxicology studies are not applicable or necessary for monoclonal antibodies.

Two repeat dose toxicology studies in cynomolgus monkeys (1-month and 6/12-month) were conducted to support the general safety of denosumab. Toxicity was evaluated as well as effects on bone parameters and potential immunogenicity. In the 1-month study (4 doses, once per week), there did not appear to be any overt treatment-related toxicological findings that were not pharmacodynamic (PD)-related. Bone parameters were evaluated and denosumab was found to dose-dependently reduce serum osteocalcin and N-telopeptide in males and females, but only increase total and cortical BMD in males only. No treatment-related changes in BMD were noted in female monkeys in any dose group. Immunogenicity was high as 28/30 animals were positive for anti-denosumab antibodies, thereby compromising exposure. In the chronic toxicology study, monkeys were treated once monthly for either 6 or 12 months. As in the 1-month study, there did not appear to be any overt treatment-related toxicological findings that were not PD-related. Findings of note included a high incidence of infection in the majority (73%) of control and treated animals (protozoa: giardia lamblia and/or cryptosporidium), which led to diarrhea and poor health. In addition, abscesses of the teeth/jaws in females were observed, which were likely PD-related. Antibody formation occurred following treatment and was inversely related to dose (100% low dose, 50% mid-dose, 13% high dose), with neutralizing antibodies also forming (81% low dose, 43% mid-dose, 13% high dose). Two high dose males died while on treatment (50 times higher than the proposed clinical dose), which was determined to likely be the result of infection. Effects of denosumab on bone were evidenced by a reduced rate of bone remodeling, reduced serum levels of bone turnover biomarkers, and increased BMD in both cortical and trabecular bone of the radius, tibia and femur.

The unexplained male deaths (possible impairment of the ability to control infection), abscesses of the teeth/jaw, and additional supportive data found in the literature, indicated that denosumab may be immunosuppressive. However, further literature-based examination indicated that a clear association could not be established between denosumab treatment and definitive immunosuppression, and further nonclinical studies were not recommended. The NOAEL for this study was 50 mg/kg since the primary effects of denosumab were PD-related, and correlates with a dose multiple of 50X compared to the proposed clinical dose. However, PD-related abscesses of the teeth/jaws occurred at doses ≥ 10 mg/kg, which is 10 times higher than the proposed clinical dose.

Reproductive toxicology: The fertility study in female monkeys showed no effect of denosumab on cycle length, mating performance, hormone analysis, or confirmed pregnancies. The embryofetal study showed no effect on prenatal loss or maternal clinical signs or weight, and there were no teratogenic effects of denosumab. Nonsignificant trends in delayed ossification and increased incidence of shortened, isolated, rudimentary and/or vestigial ribs were observed, along with slight decreases in adrenal, heart and fetal body weight, and a slight increase in ovary weight. A limited tissue panel was examined by histopathology, so gross findings could not be confirmed or correlated with histopathology. While both studies were adequate, and dosing during the embryofetal study covered the period of primate organogenesis, antibodies do not

typically cross the placenta until later in fetal primate development. Therefore, the study likely only assessed potential secondary effects of denosumab on the fetus resulting from maternal exposure. In addition, only limited organs were evaluated by histopathology in the embryofetal study, and fetal lymph nodes were not examined. This would have been beneficial since signaling via RANK has been shown to be required for lymph node development in mice.

B. Pharmacologic activity

Denosumab is a fully-human monoclonal antibody (mAb) that binds to RANKL (receptor activator of nuclear factor-kappa B ligand). RANKL is a tumor necrosis factor superfamily cytokine member (b) (4), and stimulates its specific receptor, RANK, initiating intracellular signaling cascades which promote osteoclast formation, fusion, differentiation, activation, and survival, leading to enhanced bone resorption and bone loss. Another important function of RANKL is in the immune system where RANKL is involved in B-cell and T-cell differentiation as well as dendritic cell maturation. RANKL expression is modulated by various cytokines, glucocorticoids, and PTH, and it is produced by osteoblastic lineage cells and activated T cells.

Osteoporosis and osteopetrosis are examples of conditions characterized by abnormal bone architecture, and are the result of an imbalance between the activities of osteoclasts and osteoblasts, which regulate bone remodeling. The change in sex steroid levels that accompanies menopause leads to just such an imbalance between the activities of osteoblasts and osteoclasts, with osteoclastic activity (bone loss) predominating. The result is osteoporosis and an increased risk of bone fracture risk, which is an important source of morbidity and mortality in older women. The mechanism of action for denosumab involves a blocking mechanism, where the antibody binds to RANKL and inhibits the interaction of RANKL with its receptor RANK on the cell surface of osteoclasts, resulting in decreased bone resorption, and increased bone mass. As such, denosumab would serve to decrease osteoclast activity and gain more of a balance between osteoblast and osteoclast activity to decrease abnormal bone remodeling and architecture.

C. Nonclinical safety issues relevant to clinical use

The following items are safety concerns identified in nonclinical studies submitted with this application, and from information obtained from the literature:

- The 6/12-month toxicity study raised concerns about the potential of denosumab to suppress immune system function: i.e., unexplained deaths at doses 50 times the proposed clinical dose, potential impairment of the ability to control infection, and teeth/jaw abscesses at doses 10 times the proposed clinical dose. Published literature also indicates: 1) RANK mediates activation of T cells by dendritic cells; 2) RANK mediates activation of CD4⁺ T helper cells in response to viral infection in the CD40^{-/-} mouse; and 3) disruption of RANK signaling during pancreatitis potentiates the development of diabetes through CD4⁺/CD25⁺ regulatory T cell disruption in the pancreas.
 - This is a relevant clinical concern. However, based on the available data, disruption of RANKL/RANK signaling in immunologically intact patients would be of negligible clinical significance, but it is unclear whether disruption in signaling would be of clinical significance in immune-compromised patients as a result of concurrent therapy or age-related immunosenescence.

- Fracture healing and callus remodeling were delayed in both denosumab and bisphosphonate treated huRANKL KI and WT mice. Denosumab treatment was 10 times higher than the proposed clinical dose. Denosumab treated bones also showed decreased woven bone formation.
 - This is a relevant clinical concern. However, mechanical strength was not affected, and strength and stiffness were increased compared to controls.
- Following 12-month exposure to denosumab, treatment-related deleterious changes in epiphyseal growth plates that were not closed prior to treatment were observed at doses 10 times the proposed clinical dose (≥ 10 mg/kg).
 - This is of low concern for this patient population. Denosumab is only indicated for use in postmenopausal women. These results indicate that denosumab should not be indicated for use in patients with open epiphyses, i.e. pediatric populations, or in pregnant women.
- Osteoprotegerin (OPG) treatment in neonatal mice caused treatment-related decreased axial skeleton and femur length, flared and club-like morphometry of the femur, delayed molar eruption, and decreased upper/lower incisor length.
 - This is of low concern for this patient population. Denosumab is only indicated for use in postmenopausal women. Since denosumab acts similarly to OPG, the results indicate that denosumab should not be indicated for use in pediatric populations or in pregnant women.
- The embryo/fetal reproductive toxicity study may not have afforded optimal exposure to the fetus since denosumab was administered earlier than the time of development when it could cross the placenta, and only limited organs were evaluated by histopathology. Lymph node evaluation was also not included, even though RANK/RANKL knockout mice have impaired lymph node formation and absence of lactation due to inhibition of mammary gland maturation.
 - This is of low concern for this patient population. Denosumab is only indicated for use in postmenopausal women. Denosumab should not be indicated for use in pregnant women or women of childbearing potential unless additional studies are completed that accurately assess exposure.
- The published literature indicates that RANK/RANKL knockout mice, or disruption of RANK signaling in mice is associated with developmental defects such as: 1) absence of mature osteoclasts; 2) severe osteopetrosis with total occlusion of bone marrow space; 3) defective tooth eruption; 4) absence of peripheral lymph nodes; 5) absence of lactation due to inhibition of mammary gland maturation; and 6) defective B cell development with a 60% increase in splenic B220+ B cells.
 - This is of low concern for this patient population. Denosumab is only indicated for use in postmenopausal women. Denosumab should not be indicated for use in pregnant women or women of childbearing potential unless additional studies are completed that accurately assess exposure.
- Carcinogenicity studies were not conducted with this application due to absence of a relevant animal model. However, clinical studies in postmenopausal women have shown an imbalance in the number of denosumab-treated subjects developing a new malignancy (female reproductive, gastrointestinal, breast, endocrine and haematopoietic neoplasms). In addition, agents which suppress immune function potentially increase carcinogenic risk by decreasing immune surveillance. The 6/12- and 16-month nonclinical studies did not show

evidence of tumor formation with the exception of benign squamous metaplasia in the uterus in one low dose (1 mg/kg) and one high dose (50 mg/kg) female in the 6/12-month toxicology study.

- Since denosumab is not active in a standard rodent model, only surrogate models could be used (the huRANKL KI mouse, or use of osteoprotegerin as a surrogate for denosumab treatment in a standard rat model). Both of these models have challenges of their own in terms of clinical relevance. At this time, the use of these models is not recommended for carcinogenicity testing due to their unknown relevance to humans.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA numbers: 125320 & 125331

Review number: 1

Sequence number/date/type of submission: N000, December 19, 2008

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Amgen, Inc.

One Amgen Center Drive, Thousand Oaks, CA 91320-1799

Manufacturer for drug substance:

Site 1: Boehringer Ingelheim Pharma, GmbH & Co. Kg, Birkendorfer Strasse 65
88397 Biberach an der Riss, Germany

Site 2: Amgen Inc., 5550 Airport Boulevard, Boulder, CO 80301

Site 3: Amgen Inc., 4000 Nelson Rd, Longmont, CO 80503

Site 4: Amgen Manufacturing Limited, State Road 31, Kilometer 24.6
Juncos, Puerto Rico 00777

Reviewer name: Kimberly Hatfield, Ph.D.

Division name: Division of Reproductive and Urologic Products (DRUP)

HFD #: 580

Review completion date: August 21, 2009

Drug:

Trade name: Prolia

Generic name: Denosumab

Code name: AMG 162

Other names: (b) (4) anti-RANKL mAb

Chemical name: n/a

CAS registry number: n/a

Molecular formula/molecular weight: (b) (4)
147,352 Daltons (b) (4)

Structure: monoclonal antibody; denosumab is a fully human full-length immunoglobulin. The molecule is a (b) (4) consisting of 2 heavy chains (HC) of the IgG₂ subclass and 2 light chains (LC) of the kappa subclass, which are covalently linked through disulfide bonds. Each HC contains an N-linked glycan at the canonical glycosylation site. Each LC contains 215 amino acids, with 2 intramolecular disulfides. Each HC, with its 4 intramolecular disulfides, contains 447 amino acids, (b) (4)

(b) (4) Each LC is covalently linked to a separate HC through a single disulfide bond; the 2 HC are covalently linked through 4 intermolecular disulfide bonds.

(b) (4)

Relevant INDs/NDAs/DMFs:

BB-IND 9837 – initial IND – treatment of postmenopausal osteoporosis (DRUP)

(b) (4)

Drug class: anti-osteoporetic, osteoclast activation inhibitor**Intended clinical population:** postmenopausal women with osteoporosis (treatment) or at risk for osteoporosis (prevention)**Clinical formulation:** provided by the Sponsor; EDR 0000 12/19/08 Section 2.3.P**Table 1. Qualitative and Quantitative Composition of Denosumab Drug Product**

Component	Grade	Function	Quantity / mL
Denosumab	In-house ^a	Active Ingredient	60 mg
Sorbitol	NF, PhEur, JP	(b) (4)	47 mg
Acetate	USP, PhEur, JP	(b) (4)	1 mg
Polysorbate 20 ^b	NF, PhEur	(b) (4)	0.1 mg
Sodium hydroxide	NF, PhEur, JP ^c	(b) (4)	(b) (4)
Water for Injection	USP, PhEur	(b) (4)	(b) (4)

(b) (4)

^a Tested to internal specifications (3.2.S.4.1, Specifications)^b Prefilled syringe presentation only.^c Supplier tests sodium hydroxide pellets to NF, PhEur, and JP standards.^d

(b) (4)

Route of administration: subcutaneous injection

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Background: Denosumab (AMG 162) is a fully-human monoclonal antibody (mAb) that binds to RANKL (receptor activator of nuclear factor-kappa B ligand). RANKL is a tumor necrosis factor superfamily cytokine member (b) (4), and stimulates its specific receptor, RANK, initiating intracellular signaling cascades which promote osteoclast formation, fusion, differentiation, activation, and survival, leading to enhanced bone resorption and bone loss. Another important function of RANKL is in the immune system where RANKL is involved in B-cell and T-cell differentiation as well as dendritic cell maturation. RANKL expression is modulated by various cytokines, glucocorticoids, and PTH and it is produced by osteoblastic lineage cells and activated T cells.

Osteoporosis and osteopetrosis are examples of abnormal bone architecture, and are the result of an imbalance between the activities of osteoclasts and osteoblasts, which regulate bone remodeling. The change in sex steroid levels that accompanies menopause in women leads to just such an imbalance between the activities of osteoblasts and osteoclasts, with osteoclastic activity predominating. The result is osteoporosis and an increased risk of bone fracture risk, which is an important source of morbidity and mortality in older women. The mechanism of action for denosumab involves a blocking mechanism, where the antibody binds to RANKL and inhibits the interaction of RANKL with its receptor RANK on the cell surface of osteoclasts, resulting in decreased bone resorption, and increased bone mass. As such, denosumab would serve to decrease osteoclast activity and gain more of a balance between osteoblast and osteoclast activity to decrease abnormal bone remodeling and architecture.

The Sponsor (Amgen) has submitted this BLA for denosumab for 4 indications, each of which is represented by separate BLA numbers:

- BLA 125320: Treatment of postmenopausal osteoporosis
- BLA 125331: Prevention of postmenopausal osteoporosis
- BLA 125332: Treatment and prevention of bone loss in patients undergoing hormone ablation for breast cancer
- BLA 125333: Treatment and prevention of bone loss in patients undergoing hormone ablation for prostate cancer

BLAs 125320 and 125331 are in the Division of Reproductive and Urologic Drug Products, and BLAs 125332 and 125333 are in the Division of Biologic Oncology Products. Treatment of postmenopausal osteoporosis is considered to be the primary indication.

The dosing regimen for denosumab in clinical populations is a 60 mg subcutaneous injection every 6 months, administered by a healthcare provider. If approved, denosumab would be the first biologic agent available in the U.S. for the prevention and treatment of postmenopausal

osteoporosis. In addition, there are currently no products approved for bone loss associated with hormone ablation for prostate cancer or breast cancer.

Nonclinical study justifications: Overall, the nonclinical program is adequate.

Denosumab is a specific mAb for human RANKL, and does not cross react with other members of the tumor necrosis factor family (e.g. TNF α ; TNF β ; TNF-related apoptosis-inducing ligand [TRAIL]; or CD40 ligand [CD40L]). Denosumab also does not bind to rodent RANKL. As a result, the use of mice and rats to assess the pharmacologic and toxicologic profile of denosumab is not useful, and would be inappropriate. Denosumab is specific to non-human primate RANKL, therefore toxicity studies were carried out only in cynomolgus monkeys as non-human primates are the only relevant species. The Sponsor has also developed a “knock-in” mouse that expresses human RANKL (huRANKL) at the murine RANKL (muRANKL) locus, and this model has also been used to assess efficacy and toxicity. For evaluation of nonclinical pharmacology and efficacy in rodents, studies have been carried out using osteoprotegerin (OPG) fused to an antibody Fc region, since OPG is an endogenous regulator of RANKL activity, binds to both human and rodent RANKL, and the binding of OPG to RANKL inhibits RANKL activity in a manner comparable to that of denosumab.

According to Agency guidance, safety evaluations should normally include two relevant species, however, since the biologic activity of denosumab is specific to non-human primates, and denosumab has no activity in rodent models, the use of one relevant species for toxicity testing is justified. In addition, carcinogenicity studies were not conducted with denosumab due to lack of an appropriate test species. While the Applicant does have a surrogate knock-in mouse model with huRANKL that was used primarily for pharmacology studies, it is not an appropriate model for carcinogenicity studies. Also, based on Agency guidance, since denosumab is a biologic product, genetic toxicology studies were not applicable or necessary.

Studies reviewed within this submission:

Studies reviewed by Kimberly Hatfield, Ph.D.

Pharmacology:

- R2004430 – Effects of denosumab (AMG 162) on bone mass and bone resorption in human RANKL knock-in mice
- R2004321 – Effects of denosumab (AMG 162) on bone mass and bone resorption in aged human RANK ligand knock-in mice
- R2006351 – Denosumab, a fully human monoclonal antibody, has selective effects on human RANK ligand and human osteoclasts
- 103981 – AMG 162 - A monthly subcutaneous injection osteoporosis prevention study for 16 months in the cynomolgus monkey
- R20080340 – The effects of OPG-Fc, RANK-Fc, or alendronate on tooth eruption and on bone density, geometry, and strength in neonatal rats

Pharmacokinetics:

- 106892 – Pharmacokinetics report for “A single dose pharmacokinetics study of denosumab (AMG 162) following intravenous administration to male or female huRANKL knock-in and wild-type mice
- 101398 – A single-dose intravenous and subcutaneous pharmacokinetic and pharmacodynamic study of AMG 162 in cynomolgus monkeys
- 103948 – Pharmacokinetic and pharmacodynamic comparability study for two manufacturing processes of AMG 162 in female cynomolgus monkeys

Toxicology:

- 102843 – Subcutaneous fertility evaluation of AMG 162 in the female cynomolgus monkey

Studies reviewed by Michael Orr, Ph.D.Pharmacology:

- 106564 – A 12-month osteoporosis prevention study of denosumab with and without 6-month alendronate pretreatment in the cynomolgus monkey
- R2006458 – Comparison of two anti-resorptive therapies (alendronate versus AMG 162 monoclonal anti-RANKL antibody) on murine fracture healing
- 101606 – A single-dose subcutaneous administration of AMG 162 for cardiovascular and respiratory evaluation in cynomolgus monkeys

Pharmacokinetics:

- 101494 – Pharmacokinetic study of denosumab (AMG 162) in male mice following intravenous or subcutaneous administration
- 106893 – A single dose pharmacokinetics study of denosumab (AMG 162) following intravenous administration to male or female FcRn knockout and wild type mice
- 104105 – Quantitative whole body autoradiography of cynomolgus monkeys following a single subcutaneous administration of ¹²⁵I-AMG 162.
- 104192 – Absorption, distribution, and excretion in cynomolgus monkeys following a single subcutaneous administration of ¹²⁵I-AMG 162

Toxicology:

- 101447 – A 1-month study evaluating the effect on bone of AMG 162 administered subcutaneously or intravenously in cynomolgus monkeys with a 3-month recovery period
- 101758 – Cross-reactivity of AMG 162 with normal cynomolgus monkey and human tissues
- 101348 – Cross-reactivity of AMG 162 with normal human tissues
- 102700 – Cross-reactivity of AMG 162 with cynomolgus monkey, rat, and rabbit tissue ex vivo

Studies reviewed by Ronald Wange, Ph.D.Toxicology:

- 102090 – A 6/12-month subcutaneous toxicity study of AMG 162 in the cynomolgus monkey with an interim kill after 6 months and a 3-month recovery period
- 102842 – Subcutaneous embryo-fetal development study of AMG 162 in the cynomolgus monkey

Studies not reviewed within this submission:

(b) (4)

Abbreviations used for bone turnover markers, and histomorphometry parameters:**Bone turnover markers:**

Abbreviation	Term	Formation or resorption?	Description
Osteocalcin	Osteocalcin	Formation	Produced by osteoblasts and inserted into bone matrix
sALP	Bone specific alkaline phosphatase	Formation	Early enzyme marker of osteoclastogenesis
CTx	Serum C-terminal crosslinked telopeptide of type I collagen	Resorption	Released during osteoclastic bone resorption
NTx	Urine N-terminal telopeptide of type I collagen	Resorption	Released during osteoclastic bone resorption
TRAP-5b	Tartrate-resistant acid phosphatase-5b	Resorption	Enzyme produced by osteoclasts

Bone histomorphometry parameters:

Abbreviation	Term	Definition	Formation or resorption?	Structural or dynamic?
DXA or DEXA	Dual energy x-ray absorptiometry			
BMD	Bone mineral density			
UD	Ultra distal			
ENDO	Endosteal circumference			
ENDO-C	Endosteal circumference			
PERI	Periosteal circumference			
THICK	Cortical thickness			
A/P	Anterior/posterior			
pQCT	Peripheral quantitated computed tomography			
BMC	Bone mineral content			
CSMI	Cross sectional moment of inertia			
TV	Tissue volume			
BS	Bone surface			
T.Ar	Tissue area			structural
BV/TV	Bone volume			structural
OV/BV	Osteoid volume		formation	structural
Tb.Th	Trabecular thickness			structural
Tb.Sp	Trabecular separation			structural
b.N	Trabecular number			structural
.Th	Osteoid thickness		formation	structural

OS/BS	Osteoid surface	Osteoid surface as a fraction of bone surface	formation	structural
Os.S/BS	Osteoblast surface	Osteoblast surface as a fraction of bone surface		structural
Oc.S/BS	Osteoclast surface	Osteoclast surface as a fraction of bone surface	resorption	structural
ES/BS	Erosion surface	Erosion surface as a fraction of bone surface	resorption	structural
W.Th	Wall thickness		formation	structural
MS/BS	Mineralizing surface	Mineralizing surface as a fraction of bone surface	formation	dynamic
sLS/BS	Single label surface	Labeled surface as a fraction of bone surface		dynamic
dLS/BS	Double label surface	Labeled surface as a fraction of bone surface		dynamic
MAR	Mineral apposition rate	Distance between labels divided by the labeling period	formation	dynamic
Aj.AR	Adjusted apposition rate	$MAR \times (LS/OS)$		dynamic
Omt	Osteoid maturation time			dynamic
Mlt	Mineralization lag time	$O.Th/Aj.AR$	formation	dynamic
BFR	Bone formation rate		formation	dynamic
BFR/BS	Bone formation rate based on surface			dynamic
BFR/BV	Bone formation rate based on volume	$MS \times MAR \times (BS/BV)$	formation	dynamic
Ac.f	Activation frequency	Rate of formation of bone remodeling units = $(FP \times [BS/OS])^{-1}$	formation	dynamic
P	Formation period	Lifespan of osteoid surface = $W.Th/Aj.AR$		dynamic
Rs.P	Resorption period			dynamic
Tt.T.Ar	Tissue area (total)			structural
Ct.Ar	Cortical area			structural
Me.Ar	Medullary area			structural
%Ct.Ar	Cortical area (relative)			structural
%Me.Ar	Medullary area (relative)			structural
Ct.Wi	Cortical width			structural
Ps.Pm	Periosteal perimeter			structural
Ec.Pm	Endocortical perimeter			structural
%Po.Ar	Percent porosity area			structural
H.W.Th	Haversian wall thickness			structural
Ps.sL.Pm/Ps.Pm	Periosteal single label surface	Periosteal surface = important in appositional growth and fracture repair		dynamic
Ps.dL.Pm/Ps.Pm	Periosteal double label surface	Periosteal surface = important in appositional growth and fracture repair		dynamic
Ps.L.Pm/Ps.Pm	Periosteal labeled surface	Periosteal surface = important in appositional growth and fracture repair		dynamic
Ps.MAR	Periosteal mineral apposition rate			dynamic
Ps.BFR/BS	Periosteal bone formation rate surface referent			dynamic
Ec.sL.Pm/Ec.Pm	Endocortical single label surface			dynamic

Ec.dL.Pm/Ec.Pm	Endocortical double label surface			dynamic
Ec.L.Pm/Ec.Pm	Endocortical labeled surface			dynamic
Ec.MAR	Endocortical mineral apposition rate			dynamic
Ec.BFR/BS	Endocortical bone formation rate surface referent			dynamic
H.sL.Pm/H.Pm	Haversian single labeled surface			dynamic
H.dL.Pm/H.Pm	Haversian double labeled surface			dynamic
H.L.Pm/H.Pm	Haversian labeled surface			dynamic
H.MAR	Haversian mineral apposition rate			dynamic
H.BFR/BS	Haversian bone formation rate surface referent			dynamic
H.BFR/BV	Haversian bone formation rate volume referent			dynamic

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacology studies with denosumab examined the relative efficacy of the drug in monkeys, in human RANKL knock-in (huRANKL KI) mice, and with a surrogate drug product in WT mice. In a pivotal 16-month pharmacology study in ovariectomized (OVX) monkeys, denosumab was found to be effective in decreasing biomarkers of bone formation including osteocalcin and sALP (bone specific alkaline phosphatase), and of bone resorption including CTx (C-terminal telopeptides), TRAP-5b (tartrate resistant acid phosphatase 5b), and NTx (N-telopeptides). Denosumab was also effective at increasing bone mineral density (BMD) predominantly of the lumbar and thoracic spine, whole body, and proximal femur, and to a lesser extent, in the central tibia and distal radius. In OVX animals, denosumab also prevented OVX-induced BMD changes in both cortical and cancellous bone. Bone strength in femur and vertebrae was also increased with denosumab treatment, as measured by stiffness, peak load and toughness. However, upon stopping treatment with denosumab, BMD and bone parameters returned to original baseline levels.

In a 12-month pharmacology study in OVX monkeys, denosumab and alendronate treatment regimens were compared. Treatment with denosumab alone, alendronate alone, or denosumab in combination with alendronate pretreatment all induced significant reductions in biochemical markers of bone formation (serum osteocalcin; sALP) and resorption (CTx). Bone biomarkers remained elevated in the vehicle treated OVX monkeys, and these animals developed mild osteopenia with increased bone turnover and loss of bone mass, based on bone densitometry measurements. Transient reductions in serum calcium were observed after treatment with denosumab alone. Treatment with denosumab alone, alendronate alone, and the combination increased BMD in the whole body, lumbar spine, tibial diaphysis, and radial cortical diaphysis, and also prevented OVX-induced BMD changes in both cortical and cancellous bone, and increased bone strength. Overall, denosumab treatment was able to induce robust reductions in bone biomarkers of bone turnover as compared to dosing with alendronate, to induce similar modulations in histomorphometry parameters as alendronate treatment did, and bone strength was similar across the different treatment groups. For the denosumab alone group, similar changes in bone turnover markers, bone strength, and BMD

changes in cortical and cancellous bone were observed in the 16-month study, thereby providing data from two independent OVX cynomolgus monkey studies showing that denosumab was able to prevent OVX induced BMD changes in both cortical and cancellous bone. Overall, this nonclinical data provides evidence that denosumab is able to prevent bone loss due to reductions of estrogen in the OVX monkeys

A safety pharmacology study in male cynomolgus monkeys was performed to evaluate cardiovascular and respiratory parameters following a single subcutaneous administration of denosumab. In the mid-dose group (3 mg/kg), 1/3 monkeys had a run of four ventricular premature complexes, which returned to normal for rest of the 7 days following the single dose administration. The toxicological significance of this finding is unknown at this time, as it only occurred in 1/12 monkeys in the study and there were no dose dependent effects observed. Furthermore, this cardiovascular effect was not observed in the 1-month (study # 101447) or 6/12-month (study #102090) repeat dose toxicology studies, in which denosumab plasma levels were able to reach steady state and potentially allow deep tissue exposure to the test article.

In vitro binding and activity studies with denosumab were performed, and indicated that denosumab does not bind to murine RANKL and is not pharmacologically active in rodents. Denosumab was also noted to have 10-fold less binding affinity to huRANKL than the natural endogenous inhibitor for RANKL, osteoprotegerin, but still remained effective in suppressing osteoclastogenesis, more so even than bisphosphonate comparators.

HuRANKL KI mice were used in studies to compare the effects of alendronate and denosumab on murine fracture healing. The results showed that both alendronate and denosumab treatment delayed the removal of cartilage and remodeling of the fracture callus compared to control mice with fractured femurs. However, treatment with either denosumab or alendronate actually induced increases in strength and stiffness relative to the nonfractured control or vehicle control group. Overall, fractures took longer to repair when the huRANKL mice were treated with denosumab or alendronate, as compared to the vehicle control. Patients with new and/or healing fractures should consider starting denosumab treatment once the fracture has healed, as denosumab could delay fracture repair.

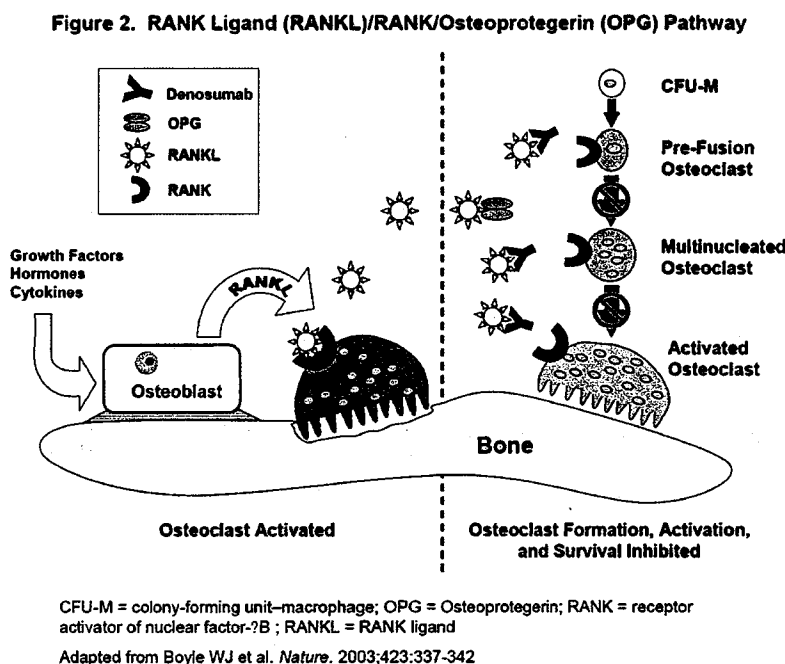
Finally, an additional study showed that the natural endogenous inhibitor for RANKL, osteoprotegerin (OPG), was effective as a surrogate for the pharmacologic effects of denosumab in a rodent model, since denosumab itself is not pharmacologically active in the rodent. Using a huRANKL KI mouse model comparing the effects of OPG and denosumab, both drugs elicited similar effects on inhibiting bone resorption and formation, and increasing BMD in both adult and aged rodents. As such, OPG was used in a WT neonate mouse to examine the potential effects of pediatric exposure to denosumab. This study indicated that OPG, and potentially denosumab, decreased axial skeleton and femur length, caused a flared and club-like morphometry of the femur, decreased incisor length, and delayed molar eruption. These deleterious effects to the neonate skeleton indicate that denosumab should not be used in young patients.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Denosumab is a fully human IgG₂ monoclonal antibody that binds to the receptor activator of nuclear factor- κ B (RANK) ligand (RANKL). RANKL binds to RANK on osteoclast precursors and mature osteoclasts, stimulates osteoclasts to resorb bone, and promotes differentiation of the precursor cells into osteoblasts. The binding of denosumab to RANKL inhibits binding of the ligand to target receptors such as RANK, thereby neutralizing the effects of RANKL. Since RANKL binding to RANK is involved with the formation, function, and survival of cells that resorb bone such as osteoclasts, the inhibition of RANKL binding to RANK by denosumab leads to the suppression of osteoclast-mediated bone turnover.

The following figure was provided by the Sponsor; N000 12/19/08 Section 2.4



Drug activity related to proposed indication:

Denosumab is proposed to act in the same manner as the soluble endogenous inhibitor of RANKL, OPG. As stated previously, RANKL is produced by osteoblasts, and plays a role in terminal differentiation, activation and survival of osteoclasts, leading to an increase in the ability of osteoclasts to resorb bone and cause osteopetrosis or osteoporosis. OPG inhibits RANKL, and administration of recombinant OPG to animals reduces bone resorption by inhibiting osteoclasts (this is the same mechanism of action of denosumab). As such, denosumab is expected to act similarly to OPG by inhibiting the terminal differentiation of osteoclasts, and inhibiting the activity of mature osteoclasts.

2.6.2.2.1 Study title: AMG 162: A monthly subcutaneous injection osteoporosis prevention study for 16 months in the cynomolgus monkey.

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings:

- AUC and Cmax increased linearly with dose. No drug accumulation was evident.
- Anti-denosumab antibodies were found in 35% of all denosumab-treated animals, and neutralizing antibodies in 57% of antibody-positive animals.
- Denosumab increased BMD in lumbar and thoracic spine, whole body and right proximal femur (neck/global/trochanter), central tibia and distal radius.
- Denosumab decreased bone formation and resorption parameters from OVX controls in cancellous and cortical bone. No dose or time response was evident.
- Bone strength was significantly increased in the femur and vertebrae.

Study no.: 103981

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.1.1

Conducting laboratory and location: [REDACTED] (b) (4)

Date of study initiation: September 10, 2004

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Denosumab (AMG 162); Lot# A0309190001/X000145;
Purity conforms to standard; Potency = 102% of specific activity.

Methods

Doses: Group 1: Sham vehicle control Group 3: Low dose 25 mg/kg (OVX)
Group 2: OVX vehicle control Group 4: High dose 50 mg/kg (OVX)

Species/strain: Female cynomolgus monkey (*Macaca fascicularis*)

Number/sex/group or time point (main study): 20 females/dose group

Route, formulation, volume, and infusion rate: Subcutaneous (s.c.) injection; formulation (70 mg/mL in 10 mM Na acetate, 5% sorbitol, pH 5.2); volume = 0.71 mL/kg.

Satellite groups used for toxicokinetics or recovery: none

Age: 9 years

Weight: 3.1-7.1 kg

Sampling times: see below for observations and times

Unique study design or methodology (if any): Doses were administered s.c. in the dorsal region, once every 28 days for 16 doses starting at 28 days following ovariectomy. While animals were randomized to treatment, groups were verified to be homogeneous based on age, weight, whole body bone mineral content (BMC) and lumbar spine bone mineral density (BMD). All animals assigned to Groups 2-4 were ovariectomized (OVX) prior to treatment start. Group 1 animals were sham operated. In addition, bone biopsies were performed on all animals following doses 6 and 12.

For statistical analysis and analysis of numerical data, a subset of animals was separately analyzed as “denosumab-negative”. The denosumab-negative group consisted of animals that:

1. Tested positive for anti-denosumab antibodies via immuno- and bioassays.
2. Had no detectable denosumab in their serum when measured at trough.
3. Had reversal of serum CTx suppression.

Observations and times:

Mortality: twice daily (except for once on day of arrival and last day of necropsy)

Clinical signs: twice daily (except for once on day of arrival and last day of necropsy)

Body weights: weekly (acclimation, through baseline, through treatment)

Food consumption: once daily

Ophthalmoscopy: not examined

EKG: not examined

Hematology: once during acclimation, prior to OVX, prior to dosing, and prior to dose 3, 6, 9, 12, 16

Clinical chemistry: once during acclimation, prior to OVX, prior to dosing, and prior to dose 3, 6, 9, 12, 16

Urinalysis: once during acclimation, prior to OVX, prior to dosing, and prior to dose 3, 6, 9, 12, 16. Urine collected daily by catheterization 1-4 days following blood collection.

Hormone analysis: with blood collection. Parameters included PTH(1-84), 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D, estradiol.

Bone biopsy: following doses 6 and 12 (samples taken from the 7th rib and right side ilium at dose 6, and 7th rib and left side of ilium at dose 12).

Biochemical markers of bone turnover: Serum bone formation markers [osteocalcin and bone specific alkaline phosphatase (sALP)], serum bone resorption markers [C-terminal telopeptides (CTx) and TRAP-5b], and urine bone resorption marker [N-telopeptides (NTx)] were analyzed at acclimation, baseline pre-OVX, baseline post-OVX, and postdoses 3, 6, 9, 12 and 16.

Bone densitometry measurements and radiographs: DXA (dual energy x-ray absorptiometry) and pQCT (peripheral quantitative computed tomography) were performed once during acclimation (pre-OVX), and once following doses 3, 6, 12, 16 of treatment. Radiographs were taken once during acclimation (pre-OVX), and at end of treatment.

Histomorphometry: Sections of rib and ilium biopsy samples collected after Doses 6 and 12, as well as sections of lumbar vertebra (L2), right central tibia, right proximal femur (femoral neck) and right rib (9th) from each euthanized animal at end of treatment, were prepared for all groups, without decalcification, for histomorphometric evaluation. Evaluation of cancellous bone was done on the femoral neck, ilium and lumbar vertebra, and evaluation of cortical bone was done on the right mid tibia and ribs.

Fluorescent bone labeling: At 15 days prior, and again at 5 days prior to bone biopsy (after doses 6, 12 and 16), each animal was given an injection of fluorochrome label. Tetracycline and alizarine complexone were injected i.v. prior to biopsy at months 6

and 12, respectively. Bicarbonate buffered calcein solution was injected s.c. prior to necropsy at study termination.

Biomechanical testing: Bone samples included vertebrae (L3, L4, L5 and L6), left whole femur, right humerus – cortical beams, vertebra T11, T12 (back-up), left tibia (back-up), left radius (back-up). At necropsy, after collection, all bone specimens were individually wrapped in gauze soaked saline, covered with plastic film, placed in a plastic bag and stored at -20°C until testing. Left femur was scanned using pQCT and DXA at the expected fracture site and bone parameters analyzed. Left femur was tested to failure in 3-point bending, and the neck tested to failure in simulated single-legged stance. L3 and L4 vertebral bodies and L5 and L6 vertebral cores were scanned using pQCT and DXA for various bone parameters. L3 and L4 were tested to failure in compression. A core of trabecular bone was removed from L5 and L6 and compressed to failure. Cortical beams were milled from the right humerus-shaft and subjected to 3-point bending. Machined bone from the mid-shaft of the humerus was also tested with 3-point bending.

Gross pathology: at necropsy

Organ weights: Only uterus (with cervix) and lymph nodes (mandibular and mesenteric) were dissected free of fat and weighed. Other soft tissues were collected and fixed/preserved in formalin, but not weighed.

Histopathology: Adequate Battery: yes (), no (X)
Peer review: yes (X), no ()

Only selected tissues (injection sites and bone defect sites) were prepared for histopathological examination. A variety of tissues (most of the adequate battery) were collected at necropsy for soft tissue preservation, but not analyzed microscopically (only grossly).

Toxicokinetics: pre-OVX and 24, 48, 96, 168, 336 and 504 hrs after the 1st, 12th and 15th doses. A single sample was collected prior to the 2nd, 4th, 6th, 8th, 10th, 13th, and 16th doses.

Antibody assessments: pre-OVX and following doses 1, 3, 6, 12 and 16: sampled 1 week prior to next dose.

Immunology assessments: Blood immunophenotyping was performed prior to dosing, during month 8 and prior to necropsy. Results reported as relative proportions and absolute numbers of CD4+, CD8+, CD3+, CD20+ and CD16+ lymphocyte subsets. T-cell dependent antibody response using KLH was conducted 21 days prior to last day of dosing. Anti-KLH IgM and anti-KLH IgG were determined 5, 6, 7, 10, 14 and 21 days after KLH injection. Measurement of total immunoglobulins (IgG, IgM and IgA) was conducted following dose 15.

Results:

Mortality: Three animals were euthanized (two sham vehicle and one low dose) and one high dose animal was found dead during treatment.

Group #	Animal #	Cause of death
1	166	Euthanized–Day 43–severe regenerative anemia–histology confirmed animal developed an autoimmune hemolytic anemia
1	156	Euthanized–Day 292–intra-abdominal mass originating from right adrenal gland, invading pancreas and contralateral adrenal noted at necropsy
3	353	Euthanized–Day 189–cause of death undetermined due to limited histopathological evaluation
4	473	Found dead–Day 453–cause of death considered due to dilatation of stomach with gas and foodstuff

Clinical signs: There were no clinical signs that were significantly different from controls or appeared to be treatment-related. However, animals in all treatment groups did exhibit a reduced appetite (Grp1: 20/20; Grp2: 18/20; Grp3: 18/20; Grp4: 19/20).

Body weights: No mean body weight or body weight gain changes were noted between groups from pre-dose week -10 through treatment week 64 (all animals included). This was consistent even after the exclusion of animals from analysis that were considered denosumab-negative.

Food consumption: As mentioned above, reduced appetite was occasionally noted in both control and treated animals. This did not have an effect on body weight, and was not considered treatment-related.

Hematology: See table below for a listing of parameters that had observed changes during treatment. There were minimal changes in hematology parameters throughout the study in denosumab positive animals. Neutrophils (% and absolute) were increased at postdose 9 compared to OVX controls at the low and high dose, while eosinophils were consistently decreased compared to OVX controls at the low and high dose at all time points, with the most significant changes at postdose 3, 6 and 9. All other parameters listed showed some change from OVX controls at various timepoints, but changes were not large, do not appear to be biologically significant, and are more likely due to variation even though some statistical significance was noted.

Group mean hematology – based on exclusion of animals considered denosumab-negative

Time	Dose (mg/kg)	Parameter								
		NEUT %	NEUT (abs)	LYMP %	LYMP (abs)	MONO %	MONO (abs)	EOS %	EOS (abs)	LUC (abs)
Baseline	OVX con	24.64	1.884	59.01	4.563	6.54	0.494	8.06	0.618	0.105
	25	32.44 (+32%)	2.459 (+30%)	53.13 (-10%)	3.527 (-23%)	6.08 (-7%)	0.399 (-19%)	6.85 (-15%)	0.451 (-27%)	0.075 (-29%)
	50	34.11 (+38%)	2.610 (+39%)	52.47 (-11%)	3.479 (-24%)	5.68 (-13%)	0.388 (-21%)	5.95 (-26%)	0.405 (-34%)	0.092 (-12%)
Postdose 3	OVX con	37.25				5.10		5.77	0.470	0.107
	25	44.38				5.10		3.26 (-44%)	0.279 (-41%)	0.075 (-30%)
	50	52.32 (+40%) *				3.76 (-26%)		1.95 (-66%) *	0.151 (-97%) *	0.074 (-31%) *
Postdose 6	OVX con							5.15	0.432	
	25							2.51 (-51%)	0.195 (-55%)	
	50							1.82 (-65%)	0.142 (-67%) *	
Postdose 9	OVX con	25.37	2.033	61.47	4.948			6.15	0.490	0.135
	25	40.95 (+61%) *	3.749 (+84%)	49.30 (-20%)	3.675 (-26%) *			3.15 (-49%) *	0.249 (-49%) *	0.133
	50	51.34 (+102%) *	4.132 (+103%) *	40.80 (-33%) *	3.223 (-35%) *			2.32 (-62%) *	0.184 (-62%) *	0.086 (-36%)
Postdose 12	OVX con							4.78	0.431	
	25							2.31 (-52%)	0.188 (-56%)	
	50							2.59 (-46%)	0.235 (-45%)	
Postdose 16	OVX con							5.51	0.504	
	25							3.01 (-45%)	0.254 (-50%)	
	50							2.74 (-50%)	0.221 (-56%)	

NEUT=neutrophils; LYMP=lymphocytes; MONO=monocytes; EOS= eosinophils; LUC=large unstained cells

Empty boxes indicate that no significant changes were observed for this parameter at the particular postdose measurement.

* p≤0.05

Clinical chemistry: In denosumab positive animals, ALP was consistently decreased compared to OVX control throughout treatment at both low dose (-57-68%) and high dose (-59-67%). This correlates with decreased bone specific alkaline phosphatase, and is considered indicative of a reduction in bone formation. Ca and Phos levels were also decreased throughout treatment in both low and high dose animals, but while statistically significant, changes were only between 5-8% (Ca) and 14-32% (Phos) compared to OVX controls. No changes were observed in serum protein electrophoresis levels (albumin, α 1, α 2, β and γ).

Urinalysis: No remarkable changes were observed. Electrolyte levels and urine creatinine were highly variable, making comparisons difficult.

Radiographs: There were no radiographic findings or progression of skeletal disease that would impact interpretation of bone densitometry data. Incidence of findings was unrelated to group assignment or treatment.

Gross pathology: No treatment-related changes were observed.

Upon necropsy, it was noted that one of the sham vehicle controls had no ovaries, which the Sponsor says is compatible with ovarian aplasia or severe hypoplasia. The absence of ovaries for this animal was not noted following the sham surgery where ovaries were extruded but not ligated. It is unclear why the absence of ovaries was not noted, and it would appear rare that the ovaries would have resorbed during the 16 months of treatment. No estradiol surges were noted during the study for this animal, and a small uterus weight was noted. Bone-related data for this animal was excluded from statistical analyses. No remarkable observations were noted.

Organ weights: Only the uterus (with cervix) and lymph nodes were weighed at necropsy. As expected with ovariectomy, decreased uterus weights were observed in Groups 2-4 versus sham vehicle controls (-56-62%).

Histopathology: As mentioned under methods, only injection sites and bone defect sites (created during bilateral sampling of biopsies from the rib and ilium, right and left side, months 6 and 12) were analyzed by histopathology. There were no treatment-related histopathological changes at the injections sites of any animal. In the bone defect sites, the pathologist noted treatment-related effects. Remodeling of the bone defect sites in the rib was retarded at 2 and 9 months post-biopsy and prior to necropsy, and persistence of the rib osteotomy line was also observed in treated animals at the same timepoints. In both control and treated groups, the rib biopsies resulted in a fibrous tissue gap between ends of severed bone. Woven bone and/or periosteal new bone deposition at the defect margin/gap was present in most animals 6 months following biopsy, however denosumab prevented the resorption of woven bone 9 months after biopsy. Overall, the pathologist notes that at this rib cortical site, the observations suggest that the initial repair process following osteotomy (woven bone deposition) is unaffected by denosumab, and treatment actually detracted from attempts to remodel defect edges and woven bone deposits, and to resorb bone

fragments. Denosumab was also found to reduce the amount of osteoclast resorption in the iliac defect sites compared to OVX controls, indicating its anti-resorptive effects that were most noted 9 months post-biopsy versus 2 months post-biopsy. Persistence of bone fragments in the iliac defect site compared to their absence in controls also supported this finding, as well as the increased severity grading for woven bone at the defect compared to controls at 9-month post-biopsy.

Toxicokinetics: After multiple dosing, anti-denosumab antibodies developed in 5 low dose animals, and 3 high dose animals, which confounded exposure. Animals that were antibody positive had serum concentrations and exposures that were 100 to 1000-fold lower than antibody negative animals. The following tables were submitted in the Toxicokinetic Report, and represent TK values in antibody negative (denosumab positive) animals.

Mean TK parameters after the 1st, 12th and 15th dose of 25 mg/kg denosumab (antibody negative animals)

Parameter (units)	First Dose		12th Dose		15th Dose	
	Mean	SD	Mean	SD	Mean	SD
T_{max}^b (hr)	96	24-168	96	24-168	96	48-168
C_{max} (µg/mL)	143	58.7	234	34.1	222	49.9
C_{last} (µg/mL)	40.7	21.5	96.7	29.0	80.8	29.9
$AUC_{(0-t)}$ (hr•mg/mL)	59.6	22.9	113	21.0	101	26.1
$AUC_{(0-t)/D}$ [(hr•mg/mL)/(mg/kg)]	2.38	0.914	4.51	0.841	4.04	1.05
Accumulation Ratio	N/A	N/A	1.83	0.554	1.65	0.503

^aExcept for T_{max} . Values have been rounded to 3 significant figures

^b T_{max} shown as Median and range

Mean TK parameters after the 1st, 12th and 15th dose of 50 mg/kg denosumab (antibody negative animals)

Parameter (units)	First Dose		12th Dose		15th Dose	
	Mean	SD	Mean	SD	Mean	SD
T_{max}^b (hr)	48	48-96	48	24-168	48	24-96
C_{max} (µg/mL)	336	67.6	511	132	413	160
C_{last} (µg/mL)	109	51.6	177	95.4	142	81.4
$AUC_{(0-t)}$ (hr•mg/mL)	139	34.6	212	71.9	171	72.4
$AUC_{(0-t)/D}$ [(hr•mg/mL)/(mg/kg)]	2.78	0.692	4.24	1.44	3.41	1.45
Accumulation Ratio	N/A	N/A	1.52	0.311	1.20	1.293

^aExcept for T_{max} . Values have been rounded to 3 significant figures

^b T_{max} shown as Median and range

At both doses, T_{max} remained consistent throughout the 16-month dosing (96 hrs for low dose and 48 hrs for high dose). AUC and C_{max} were also consistent over time. Dose normalized AUC was consistent between doses indicating that AUC increased linearly with dose. C_{max} also appeared to increase linearly with dose. No accumulation was noted at either dose.

Antibody assessment: Three vehicle control animals tested positive for anti-denosumab antibodies: one sham at doses 12 and 16, one sham at dose 1, and one OVX control at

dose 1. These antibodies were not neutralizing. The Sponsor notes that it appears that these samples have pre-existing antibodies that cross-react with denosumab, but their nature is not clear. Seven (out of 20) animals treated at low dose tested positive for antibodies, 5 of which had neutralizing antibodies. Antibodies were detected as early as dose 1, while neutralizing antibodies were evident after dose 3. The two animals that did not have neutralizing antibodies appeared to have a transient immune response as they only tested positive at one postdose time point. Finally, seven (out of 20) animals treated at high dose tested positive for antibodies, 3 of which had neutralizing antibodies. Antibodies were detected as early as dose 1, while neutralizing antibodies were evident after dose 3. A transient immune response was also detected in the 4 animals without neutralizing antibodies as they tested positive at only 1 or 2 postdose time points.

Immunology assessment: There were no remarkable findings related to test article in the assay assessing T-cell dependent antibody response using KLH. With blood immunophenotyping, total lymphocyte (CD3+) count was slightly and statistically significantly decreased at pre-necropsy for the high dose. Absolute counts of CD3+/CD8+ cytotoxic T lymphocytes were also slightly and statistically significantly decreased at the high dose compared to OVX controls at pre-necropsy.

Efficacy Endpoints:

Hormones: In denosumab positive animals, PTH (1-84) levels increased with both low and high dose versus OVX controls. At postdose 3, 6, 9, 12 and 16, levels were increased over OVX controls by 131, 68, 48, 26, and 73% (low dose), and 148, 67, 51, 34 and 89% (high dose). Levels of 1,25-dihydroxyvitamin D also increased with both low and high dose, but only at postdoses 3 and 6 (61% and 32% for low dose and 54% and 19% for high dose); levels returned to OVX control levels at postdoses 9-16. There were no changes in estradiol levels or 25-hydroxyvitamin D levels with treatment at any timepoint.

Biochemical markers: Both ovariectomy and treatment with denosumab had an effect on bone markers. In the ovariectomized controls, osteocalcin, sALP, CTx, and NTx were increased about 1.5-2-fold compared to sham controls throughout the 16-mo treatment phase while increased levels of TRAP-5b were only observed at postdoses 3-9. With denosumab treatment, all markers of bone formation and resorption were decreased at both the low and high doses compared to OVX controls at all dosing timepoints for denosumab positive animals. There was no dose or time response. Osteocalcin was decreased 85-88% at low dose, and 84-92% at high dose. sALP was decreased 69-80% at low dose, and 70-79% at high dose. CTx was decreased 72-90% at low dose, and 83-92% at high dose. TRAP-5b was decreased 75-83% at low dose, and 72-80% at high dose. NTx was decreased 83-91% at low dose, and 87-92% at high dose.

All bone markers were analyzed in antibody positive animals as well, and according to the Sponsor, revealed the time course for recovery from treatment. Markers that were initially decreased at postdose (pd) 3 and 6, but returned to OVX control levels

by postdose 9, maintaining through postdose 16 were osteocalcin (87% at pd 3 and 56% at pd 6), CTx (70% at pd 3 and 52% at pd 6), TRAP-5b (69% at pd 3 and 52% at pd 6). NTx was only decreased at postdose 3 (67%) and returned to OVX control levels at postdose 6. There was no difference between antibody positive animals and OVX controls with sALP.

In vivo bone densitometry (DXA): The table below shows the percent change from baseline of BMD as measured by DXA. Variability in standard deviations was high for all percent calculations (not shown), but despite this, statistical significance was often achieved.

According to the medical officer for this application, a clinically significant increase in BMD is 1.25% from baseline. Many of the increases in BMD shown in the table were greater than 1.25% at postdose 3, so a clinical response was observed. In OVX controls, BMD was consistently below baseline (as expected), and often significantly different from sham controls. Both doses of denosumab had an effect on BMD, and there was not often a dose response. Time response increases in BMD % from baseline were observed for anterior/posterior (A/P) lumbar spine, thoracic spine, whole body, and right proximal femur neck. Right proximal femur (global and trochanter) increased up to postdose 12 then leveled off. Right distal femur appears to remain static as it is statistically significant increased from OVX controls, but does not have a time or dose response increase or decrease in BMD over treatment. Right central tibia initially shows a loss of BMD, but steadily increases over treatment. Right distal radius (1/3) does not have an increase from baseline until postdose 16, and right distal radius (ultra distal) has a slow increase in BMD until postdose 16. The responses in the tibia and radius bones appear to be slower than for the spine and/or femur. Animals that were determined to be denosumab-negative by the end of the study were also analyzed. It appears that these animals had some time response to treatment in that BMD was increased over OVX controls (but lower than baseline) up through postdose 6, but by postdose 12 these effects tapered off (as anti-denosumab antibody production decreased the effect), and BMD in these animals were similar to OVX controls. As a result, the return to baseline after effectively 'stopping' treatment (as evidenced by denosumab-negative animals) does occur, and denosumab does not provide a long term increase in BMD if treatment is stopped. Overall, treatment with both doses of denosumab prevented the OVX induced bone loss in these animals.

BMD by DXA – Percent change from baseline

Parameter	Postdose 3				Postdose 6				Postdose 12				Postdose 16			
	OVX n=20	25 mg/kg N=14	50 mg/kg n=17	AMG neg n=8	OVX n=20	25 mg/kg n=14	50 mg/kg N=17	AMG neg n=8	OVX N=20	25 mg/kg n=14	50 mg/kg n=17	AMG neg n=8	OVX n=20	25 mg/kg n=14	50 mg/kg n=17	AMG neg n=8
A/P lumbar spine Total (L1-L4)	-2.46 ^b	6.13 ^f	3.35 ^f	3.31	-5.02 ^c	7.28 ^f	8.39 ^f	1.93	-5.31 ^b	9.21 ^f	10.84 ^f	-3.24	-4.95 ^b	10.58 ^f	12.01 ^f	4.09
Thoracic spine Total (T9-T12)	-0.44	3.56 ^d	3.28 ^d	3.75	-4.32	5.43 ^f	4.77 ^f	-0.17	-3.31	10.73 ^f	7.85 ^f	-3.46	-1.24	11.57 ^f	11.00 ^f	-0.35
Whole body	-0.84 ^a	5.15 ^f	4.11 ^f	1.43	-1.20 ^c	7.30 ^f	6.95 ^f	0.78	-1.11 ^a	7.86 ^f	7.37 ^f	-2.80	3.52	12.83 ^f	12.60 ^f	4.09
Right proximal femur (global)	-4.84 ^b	3.16 ^f	2.33 ^f	1.89	-7.72 ^c	5.54 ^f	3.35 ^f	-1.97	-4.23 ^b	10.82 ^f	7.88 ^f	-3.45	-7.40 ^c	10.33 ^f	7.35 ^f	-8.55
Right proximal femur (neck)	-4.35	6.24 ^f	3.26 ^e	2.90	-5.54	5.85 ^f	3.55 ^e	-0.64	-3.81	8.73 ^e	6.57 ^e	-2.26	-5.60	11.29 ^f	8.64 ^f	-1.03
Right proximal femur (trochanter)	-5.48 ^a	4.95 ^f	3.14 ^e	3.37	-8.05 ^c	7.98	3.32 ^f	-0.39	-4.27	12.13 ^f	6.98 ^f	-2.25	-5.39 ^a	11.55 ^f	9.73 ^f	-7.57
Right distal femur	-4.67	-0.62 ^d	-0.52 ^e	-2.47	-7.13 ^b	-1.55 ^f	-0.68 ^f	-3.06	-8.28 ^c	0.63 ^f	-1.00 ^f	-7.76	-9.14 ^b	0.00 ^f	1.16 ^f	-8.55
Right central tibia (1/3 distal)	-6.09	-2.57 ^d	-2.07 ^e	-2.86	-7.71 ^a	-0.67 ^f	0.02 ^f	-4.10	-10.71 ^c	-0.00 ^f	0.17 ^f	-8.08	-10.86 ^c	0.59 ^f	1.39 ^f	-9.60
Right distal radius (1/3)	-2.11	0.45 ^d	-0.36	-0.42	-3.26	1.09 ^e	0.78 ^e	-0.01	-7.04 ^a	0.08 ^e	0.23 ^f	-5.45	-4.33	2.75 ^e	3.12 ^e	-3.90
Right distal radius (ultra distal)	-2.55	0.08	1.27 ^e	0.67	-3.85 ^c	1.63 ^f	2.12 ^f	-0.56	-7.15 ^b	1.37 ^f	1.46 ^f	-3.95	-5.36 ^a	2.56 ^f	2.99 ^f	-3.65

AMG neg = denosumab-negative

Values rounded to two decimal points

Sham controls not included since primary comparison was to OVX controls.

No statistical analysis appears to have been done for AMG neg groups.

a= p≤0.05 from sham controls; b= p≤0.01 from sham controls; c= p≤0.001 from sham controls

d= p≤0.05 from OVX controls; e= p≤0.01 from OVX controls; f= p≤0.001 from OVX controls

In vivo bone densitometry (pQCT): The table below shows the percent change from baseline of BMD as measured by pQCT. Variability in standard deviations was high for all percent calculations (not shown), but statistical significance was often achieved despite this.

Sham controls (not shown in the table below) were comparable to baseline throughout treatment. However, at the end of treatment, slight to minimal decreases from baseline were observed for BMC (total, cortical/subcortical, trabecular), BMD (total and trabecular (radius only)), and area (trabecular and cortical/subcortical). In the diaphysis of both tibia and radius, decreases were noted in sham controls for cortical area, thickness and BMC, and slight increases were noted in endosteal circumference for both.

Ovariectomy had the expected negative effect on bone densitometry for many of the bone areas examined except for total area and trabecular area and BMC, which were not statistically significant compared to sham controls. The decreases in BMD were consistent with the findings using DXA analysis. The large increase in trabecular area of OVX animals was noted by the Sponsor to be consistent with endosteal bone resorption.

At the metaphysis of the right distal radius, denosumab prevented the OVX-induced change in bone densitometry as early as postdose 3 and continuing to postdose 16 for all parameters except total area, trabecular area, and trabecular BMC. The trabecular area increase with OVX was decreased with denosumab treatment over time. The same was observed at the metaphysis of the right proximal tibia. However, cortical BMD in the tibia showed no improvement compared to OVX controls at postdose 16 as it had in the radius.

At the diaphysis of the right distal radius, denosumab prevented the OVX-induced change in bone densitometry as early as postdose 3 and continuing to postdose 16 for cortical area, cortical BMC, cortical BMD and CSMI (cross sectional moment of inertia). Cortical thickness and periosteal circumference only appeared to improve at postdose 16. Total area and endosteal circumference did not appear to have any changes compared to OVX controls with denosumab over time.

pQCT in denosumab-negative animals was similar to that observed with DXA, returning to sham levels after postdose 3 or 6.

Bone Densitometry Values by pQCT**Percent change from baseline – Metaphysis and Diaphysis**

Parameter			Postdose 3				Postdose 16			
			OVX	25	50	AMG	OVX	25	50	AMG
			n=20	mg/kg n=14	mg/kg n=17	neg n=8	n=20	mg/kg n=14	mg/kg n=17	neg N=8
Metaphysis Right Distal Radius	Total	Area	0.88	0.18	2.09	2.81	-1.53	-1.51	-1.04	2.84
		BMC	-5.96	-0.94 ^e	-0.04 ^f	-0.73	-9.45	0.42 ^f	0.81 ^f	-11.46
		BMD	-6.49	-0.99 ^e	-1.95 ^d	-3.39	-7.95	2.04 ^f	2.00 ^f	-13.76
	Cortical/ subcortical	Area	-3.18	-1.36	0.65	0.72	-8.07	-1.53 ^e	-1.56 ^e	-7.59
		BMC	-6.60	-1.07 ^e	-0.38 ^f	-1.27	-9.92	0.82 ^f	0.94 ^f	-13.27
		BMD	-3.51	0.44 ^d	-1.01	-1.95	-2.07	2.53 ^e	2.59 ^f	-5.76
	Trabecular	Area	15.05	6.30	8.12	12.61	21.80	-0.51 ^f	1.85 ^e	43.96
		BMC	7.81	3.25	8.54	15.58	0.36	-5.47	-0.82	35.91
		BMD	-5.57	-2.88	0.14	2.37	-17.17 ^c	-4.92 ^e	-2.96 ^g	-4.54
Metaphysis Right Proximal Tibia	Total	Area	0.15	1.18	-0.11	1.81	-0.58	1.68	1.09	2.63
		BMC	-8.83	0.06 ^f	1.67 ^f	-1.60	-11.80 ^c	4.84 ^f	1.76 ^f	-8.90
		BMD	-8.96 ^a	-1.00 ^f	-1.52 ^f	-3.28	-11.29 ^b	3.14 ^f	0.73 ^f	-11.06
	Cortical/ subcortical	Area	-5.53 ^c	2.34 ^e	-1.33 ^f	1.32	-12.08 ^c	6.53 ^f	3.09 ^f	-3.55
		BMC	-9.81 ^a	0.29 ^f	-1.91 ^f	-1.83	-12.48 ^b	6.37 ^f	2.35 ^f	-9.22
		BMD	-4.42	-1.92	-0.45 ^e	-3.01	-0.21	-0.04 ^f	-0.59	-5.87
	Trabecular	Area	13.74	1.64	4.69	16.32	34.35	-4.70 ^f	-1.37 ^e	90.00
		BMC	6.00	1.52	3.42	14.34	3.12	-5.27	-1.64	64.40
		BMD	-5.31	-0.12	-0.98	-1.15	-24.08 ^c	-0.55 ^f	0.10 ^f	-15.83
Diaphysis Right Distal Radius	Total	Area	-1.06	0.20	0.17	-0.44	-2.12	0.58 ^d	-0.06	-1.17
	Cortical	Area	-1.43	0.00	-0.41	-0.16	-2.58	0.58	0.59 ^d	-2.12
		BMC	-2.32	0.20 ^d	-0.46 ^d	0.07	-5.60	1.52 ^f	1.27 ^f	-4.66
		BMD	-0.89	0.21 ^e	-0.05	0.23	-3.11 ^c	0.92 ^f	0.68 ^f	-2.67
		THICK	-1.15	0.23	-0.34	0.12	-1.75	0.27	1.00	-2.21
		PERI	-0.54	0.11	0.09	-0.22	-1.08	0.29 ^d	0.03	-0.59
		ENDO	0.40	-0.45	-0.01	-0.22	-0.07	0.46	-1.08	2.06
		CSMI	-2.47	-1.33	0.22	-0.35	-4.25	0.06	1.08	-1.96
Diaphysis Right Proximal Tibia	Total	Area	-0.26	0.85	0.06	1.37	-2.15 ^a	1.33 ^d	0.83 ^d	1.21
	Cortical	Area	-2.87	-1.03	-1.05	-2.29	-7.82 ^b	1.37 ^f	0.72 ^f	-6.13
		BMC	-6.06 ^b	-1.55 ^f	-0.71 ^f	-1.82	-11.32 ^c	3.07 ^f	2.36 ^f	-10.02
		BMD	-3.27 ^c	-0.54 ^f	0.35 ^f	0.48	-3.86 ^c	1.66 ^f	1.66 ^f	-4.19
		THICK	-3.60	-1.93	-1.39	-4.12	-8.55 ^b	0.71 ^f	0.28 ^f	-8.91
		PERI	-0.13	0.42	0.03	0.68	-1.10 ^a	0.66 ^d	0.41 ^d	-0.60
		ENDO	2.47	1.80	1.07	4.05	4.41	0.78	0.76	-7.40
		CSMI	-1.92	-0.42	-2.16	1.88	-6.69	1.25 ^d	0.49 ^d	-5.231

AMG neg = denosumab-negative

THICK=cortical thickness; PERI=periosteal circumf.; ENDO=endosteal circumf.; CSMI=cross sectional moment of inertia

Values rounded to two decimal points

Sham controls not included since primary comparison was to OVX controls.

No statistical analysis appears to have been done for AMG neg groups.

a= p≤0.05 from sham controls; b= p≤0.01 from sham controls; c= p≤0.001 from sham controls

d= p≤0.05 from OVX controls; e= p≤0.01 from OVX controls; f= p≤0.001 from OVX controls

Histomorphometry:

Cancellous bone – Iliac biopsy: (Text Table 7 from the Sponsor) OVX caused reduced BV/TV (bone volume), Tb.N (trabecular number), and increased bone formation, bone turnover and activation frequency. No changes in resorption were noted. Denosumab prevented OVX-induced bone loss in the ilia as early as 6 months, continuing (at 25 mg/kg) through 12 months as measured by BV/TV. The Sponsor notes that the increase in bone volume in denosumab treated animals versus OVX controls was primarily due to preservation of Tb.N. Bone formation was significantly decreased (at both doses and time points) in denosumab treated animals compared to OVX controls to levels similar to or less than sham controls. This is shown through the following parameters: OV/BV (osteoid volume), O.Th (osteoid thickness), OS/BS (osteoid surface), Ob.S/BS (osteoblast surface), sLS/BS (single label surface), dLS/BS (double label surface), MS/BS (mineralizing surface), MAR (mineral apposition rate), Aj.Ar (adjusted apposition rate), BFR/BS (bone formation rate based on surface) and BFR/BV (bone formation rate based on volume). Bone resorption was also decreased at both doses compared to OVX controls as evidenced by Oc.S/BS (osteoclast surface) and ES/BS (erosion surface). As a result, it appears that in the ilia, denosumab is preventing and treating the normal bone remodeling that occurs in shams and the OVX-induced bone loss.

Interlabel widths were reportedly difficult to measure in treated animals, therefore minimal MAR and O.Th values were relied on. Also, MS/BS was not fully measurable in all animals; therefore variables derived from Aj.Ar could not be fully calculated. As a result, values for Mlt (mineralization lag time), Ac.f (activation frequency), FP (formation period) and Rs.P (resorption period) cannot be relied on based on absent information for calculation.

Denosumab-negative animals demonstrated the reversal of treatment effects, with values for many of the parameters having smaller changes compared to denosumab-positive animals at 6 months, and progressing to control levels by 12 months.

Cortical bone – rib biopsy: (Text Table 8 from the Sponsor) OVX caused an increase in bone turnover and formation at both the endocortical and haversian sites of the rib, but overall size and cortical width remained constant. Increases in % porosity, and BFR were also noted. Treatment with denosumab decreased the OVX-induced increases in %Po.Ar (percent porosity area), Ec.BFR/BS (endocortical bone formation rate surface referent), H.BFR/BS (haversian bone formation rate surface referent) and H.BFR/BV (haversian bone formation rate volume referent), and labeled surface in the endocortical and haversian sites to levels lower than sham controls at both doses and time points. Ec.MAR (endocortical mineral apposition rate) and H.MAR (haversian mineral apposition rate) were both decreased as well which contributed to the decreased bone formation rates. No significant changes were observed at the periosteal site. Total tissue area, cortical area, medullary area, and cortical width were unchanged with treatment.

As with cancellous bone, denosumab-negative animals demonstrated reversal of treatment effects with values for many of the parameters having smaller changes compared to denosumab-positive animals at 6 months, and comparable/increased levels similar to OVX controls by 12 months.

Cancellous bone– femoral neck and lumbar vertebra (L2) – study termination: (Text Table 9 from the Sponsor) OVX alone increased variables involved in bone formation and turnover in both the femoral neck and L2: OV/BV, OS/BS, MS/BS, labeled surface, BFR and Ac.f. OVX did not increase bone loss (BV/TV) or trabecular changes in either the femoral neck or L2, and only decreased Oc.S/BS in the femoral neck. Denosumab treatment at both doses reversed the increased bone formation and turnover caused by OVX in both the femoral neck and L2, and suppressed the levels to lower than those in sham controls (as observed with OV/BV, O.Th, OS/BS, Ob.S/BS, labeled surface, MS/BS, MAR, Aj.Ar, BFR and Ac.f). Denosumab also increased BV/TV, Tb.N and Tb.Th (trabecular thickness), and decreased bone resorption markers (Oc.S/BS and ES/BS) at both doses and in both bones.

As with the iliac biopsies, interlabel widths were reportedly difficult to measure in treated animals, therefore minimal MAR and O.Th values were relied on. Also, MS/BS was not fully measurable in all animals; therefore variables derived from Aj.Ar could not be fully calculated. As a result, values for Mlt, Ac.f, FP and Rs.P cannot be relied on based on absent information for calculation.

Denosumab-negative animals showed a reversal of treatment effects with values for bone formation, turnover and resorption being comparable to or increased over OVX controls in both the femoral neck and L2.

Cortical bone – 9th rib and tibia – study termination: (Text Table 10 from the Sponsor) OVX had similar effects on tibia and 9th rib as it had on the initial rib biopsy samples. Increased porosity was observed in both, but increased remodeling at haversian and endocortical sites was primarily observed with the tibia. Increased remodeling at periosteal sites was more pronounced in the rib than tibia. Despite the remodeling effects, no significant changes in size or cortical width were observed in either rib or tibia. Denosumab treatment reversed the OVX-induced increase in porosity in both rib and tibia at both doses, but the high dose increased cortical width and area in the tibia alone. Both doses of denosumab also completely reversed and decreased the OVX-induced increases in labeled surface, MAR and BFR/BS in both the endocortical and haversian sites, to levels lower than sham controls. This was also observed in periosteal sites but the decreases were not as dramatic or significant compared to OVX. As a result, both doses of denosumab reduced bone formation in both the tibia and rib, and reversed the effects of ovariectomy.

Denosumab-negative animals showed a reversal of treatment effects with values for many of the parameters having comparable/increased levels similar to OVX controls for tibia and rib by study termination.

Text Table 7

Differences in Selected Cancellous Bone Site Variables from Iliac Biopsies[^]

Time Point Treatment	Post Month 6				Post Month 12			
	Sham	OVX + AMG 162	Neg.	Sham	OVX + AMG 162	Neg.	Sham	OVX + AMG 162
Dose (mg/kg)	0	25	50	0	25	50	0	25
BV/TV	21	28	34	42	38	59 *	18	37
Tb.Th	8	11	9	5	2	4	-10	5
Tb.N	9	13	19	33	35 *	54 *	32	28
OV/BV	-51 *	-85 *	-90 *	-44	-36 *	-99 *	-98 *	-17
O.Th	-19 *	-52 *	-53 *	-18	-9	-47 *	-58 *	-7
OS/BS	-40 *	-59 *	-69 *	-37	-30 *	-97 *	-97 *	-13
Ob.S/BS	-34 *	-93 *	-96 *	-14	-29	-100 *	-100 *	-13
Oc.S/BS	-11	-78 *	-88 *	-17	-34	-100 *	-97 *	-25
ES/BS	-14	-45 *	-60 *	17	-2	-62 *	-69 *	-14
dLS/BS	-25	-97 *	-99 *	-32	21	-100 *	-99 *	4
dIS/BS	-55 *	-100 *	-100 *	-45	-48 *	-100 *	-100 *	2
MS/BS	-46 *	-99 *	-100 *	-41	-26 *	-100 *	-100 *	2
MAR@	-5	-73 *	-73 *	-31	-8 *	-77 *	-77 *	-1
Aj.Ar	-8	-100 *	-100 *	-30	-7	-96 *	-89 *	1
BFR/BS	-47 *	-100 *	-100 *	-44	-33 *	-100 *	-100 *	0
BFR/BV	-49 *	-100 *	-100 *	-41	-35 *	-100 *	-100 *	-3
Ac.f	-54 *	-97 *	-100 *	-20	-39 *	&	-100 *	-22

[^] Compared to the OVX vehicle control group expressed as percent difference from group mean.

Based upon statistical analysis of group means, asterisk (*) and pound (#) flagged differences are significantly different from OVX and Sham vehicle control groups, respectively; - P ≤ 0.05; refer to data tables for actual tests performed and significance levels. No statistical comparisons were conducted against control groups for the AMG 162 negative (Neg.) subset.

@ MAR assumed to be 0.24 µm/day in animals with no double labels.

& = Could not be computed from any animal in group.

Text Table 8

Differences in Selected Variables of Cortical Bone Sites from Rib Biopsies[^]

Time point Treatment	Post month 6				Post month 12			
	Sham	OVX + AMG 162	Neg.	Sham	OVX + AMG 162	Neg.	Sham	OVX + AMG 162
Dose (mg/kg)	0	25	50	0	25	50	0	25
Tb.T.Ar	5	10	8	23	7	11	6	18
Ct.Ar	0	-3	-5	5	4	5	2	2
Me.Ar	16	36	37	61	13	22	12	44
Ct.Wi	-5	-13	-8	-7	3	-3	1	-11
%Po.Ar	-49 *	-68 *	-72 *	-35	-23 *	-58 *	-62 *	6
Ps.L.Pm/Ps.Pm	-38	-78	-69	-20	-56	-31	-64	-20
Ps.MAR@	-14	-35	-25	-24	-8	-4	-17	33
Ps.BFR/BS	-22	-83	-70	-30	-52	-19	-66	20
Ec.L.Pm/Ec.Pm	-46 *	-94 *	-88 *	-47	-11	-84 *	-95 *	0
Ec.MAR@	-19 *	-67 *	-59 *	-23	-8	-64 *	-68 *	7
Ec.BFR/BS	-53 *	-96 *	-92 *	-53	-15	-88 *	-97 *	6
HL.Pm/H.Pm	-44 *	-96 *	-98 *	-51	-28 *	-95 *	-97 *	7
H.MAR@	-18 *	-72 *	-76 *	-15	-7	-74 *	-71 *	15
H.BFR/BS	-53 *	-99 *	-99 *	-51	-33 *	-99 *	-99 *	20
H.BFR/BV	-61 *	-99 *	-100 *	-52	-39 *	-99 *	-99 *	24

[^] Compared to the OVX vehicle control group expressed as percent difference from group mean.

Based upon statistical analysis of group means, asterisk (*) and pound (#) flagged differences are significantly different from OVX and Sham vehicle control groups, respectively; - P ≤ 0.05; refer to data tables actual tests performed and significance levels. No statistical comparisons were conducted against control groups for the AMG 162 negative (Neg.) subset.

@ MAR assumed to be 0.30 µm/day in animals with no double labels.

Text Table 9 Differences in Selected Variables of Cancellous Bone Sites at Study Termination[^]

Tissue Treatment	Lumbar Vertebral Body (L2)			Femoral Neck		
	Sham	OVX	AMG 162	Sham	OVX	AMG 162
Dose (mg/kg)	0	25	50	0	25	50
			Neg.			Neg.
BV/TV	4	31 *	26	8	15	30
Tb.Th	-3	14	13	1	-6	8
Tb.N	6	16 *	13	9	23	22
OV/BV	-32 *	-97 *	-97 *	-44 *	-91 *	-89 *
O.Th	-7	-35 *	-41 *	-9	-36 *	-37 *
OS/BS	-16 *	-94 *	-90 *	-46 *	-88 *	-88 *
Ob.S/BS	0	-92 *	-82 *	-39	-93 *	-91 *
Oc.S/BS	-3	-93 *	-92 *	-48 *	-97 *	-100 *
ES/BS	20	-66 *	-74 *	-17	-86 *	-93 *
sLS/BS	-2	-97 *	-94 *	-36 *	-88 *	-87 *
dLS/BS	-37 *	-100 *	-99 *	-52 *	-91 *	-96 *
MS/BS	-21 *	-99 *	-97 *	-43 *	-90 *	-91 *
MAR [@]	-8	-78 *	-67 *	-7	-67 *	-57 *
Aj.Ar	-11	-93 *	-84 *	-16	-75 *	-75 *
BFR/BS	-27 *	-100 *	-99 *	-37	-91 *	-95 *
BFR/BV	-25 *	-100 *	-99 *	-40 *	-93 *	-97 *
Ac.f	-23 *	-99 *	-98 *	-48 *	-81 *	-92 *

[^] Compared to the OVX vehicle control group expressed as percent difference from group mean.

Based upon statistical analysis of group means, asterisk (*) and pound (#)-flagged differences are significantly different from OVX and Sham vehicle control groups, respectively; - P ≤ 0.05; refer to data tables for actual tests performed and significance levels. No statistical comparisons were conducted against control groups for the AMG 162 negative (Neg.) subset.

[@] MAR assumed to be 0.24 µm/day in animals with no double labels.

Text Table 10 Differences in Selected Variables of Cortical Bone Sites at Study Termination[^]

Tissue Treatment	Rib (9 th Right)			Tibia (Right Central)		
	Sham	OVX	AMG 162	Sham	OVX	AMG 162
Dose (mg/kg)	0	25	50	0	25	50
			Neg.			Neg.
Ti.T.Ar	-9	6	-8	-1	9	6
Cl.Ar	-16	1	-14	3	10	13 *
Me.Ar	4	17	4	-8	7	-12
Ct.Wi	-12	-2	-10	6	5	14 *
%Po.Ar	-20	-38 *	-50 *	-15 *	-28 *	-28 *
H.W.Th	10 *	8	13 *	2	3	4
Ps.L.Pm/Ps.Pm	-46	-84 *	-63	-24	-30	-43
Ps.MAR [@]	-45 *	-53 *	-48	-32	-47 *	-47 *
Ps.BFR/BS	-66	-94	-77	-57	-73	-78 *
Ec.L.Pm/Ec.Pm	-24	-85 *	-91 *	-20	-97 *	-97 *
Ec.MAR [@]	-6	-70 *	-77 *	9	-73 *	-73 *
Ec.BFR/BS	-19	-93 *	-96 *	36	-99 *	-99 *
H.L.Pm/H.Pm	-28 *	-99 *	-99 *	2	-59 *	-97 *
H.MAR [@]	3	-76 *	-81 *	14	-17 *	-76 *
H.BFR/BS	-29 *	-100 *	-100 *	12	-58 *	-99 *
H.BFR/BV	-40	-100 *	-100 *	17	-62 *	-99 *

[^] Compared to the OVX vehicle control group expressed as percent difference from group mean.

Based upon statistical analysis of group means, asterisk (*) and pound (#)-flagged differences are significantly different from OVX and Sham vehicle control groups, respectively; - P ≤ 0.05; refer to data tables actual tests performed and significance levels. No statistical comparisons were conducted against control groups for the AMG 162 negative (Neg.) subset.

[@] MAR assumed to be 0.30 µm/day in animals with no double labels.

Bone strength:

Femur 3-point bending: (Text Table 11 from the Sponsor) OVX resulted in significant decreases in bone mass and cortical thickness as measured by DXA and pQCT (BMD and BMC). This was correlated with bone strength measurements of OVX femurs which showed decreased peak load, ultimate stress, stiffness, modulus and CSMI, and increased work to failure (area under the load displacement curve) and toughness. Denosumab treatment at both doses increased bone mass and stiffness and decreased toughness compared to OVX controls. By DXA and pQCT, denosumab significantly increased both BMC and BMD in the mid femur and cortical section compared to OVX controls, as well as CSMI. Levels were generally equal to or better than that of sham controls. No changes were noted with denosumab treatment for work to failure, ultimate strength, or elastic modulus.

Correlation analyses indicated that femur bone mass was strongly correlated with bone strength (peak load and ultimate stress). There was also a significant positive association for all groups combined for peak load vs. pQCT-derived cortical BMC and ultimate stress vs. cortical BMD.

Humerus cortical beams 3-point bending: (Text Table 12 from the Sponsor) OVX had no significant effects on cortical beam strength. Increases in work to failure and toughness, and decreases in modulus may be related to decreased cortical BMC as observed in the femur. Denosumab treatment at the high dose increased peak load, ultimate stress, stiffness, and modulus, but the changes were not statistically significant.

Femoral neck shear: (Text Table 13 from the Sponsor) OVX decreased bone mass (BMC and BMD) and strength in the femoral neck, as well as peak load, stiffness, and work to failure, but the changes were not statistically significant. Treatment with denosumab showed a dose response and significant increase in peak load and stiffness compared to OVX controls. By DXA, increases were also observed in proximal area, BMC and BMD compared to OVX controls. These changes were all increased over that of sham controls as well.

Correlation analyses indicated that by individual group associations, proximal femur bone mass (BMD and BMC) was strongly correlated with bone strength (peak load). Significant positive associations were noted for each group for peak load vs. DXA BMC, and controls and low dose denosumab for peak load vs. BMD. In addition, for equivalent BMC, increases in peak load were greater for the denosumab 50 mg/kg group compared to other groups. The Sponsor notes that this suggests that denosumab at 50 mg/kg improves bone quality, and that some other parameter other than BMC accounted for a portion of the increased bone strength in this group.

Vertebral compression L3/L4: (Text Table 14 from the Sponsor) OVX decreased bone mass BMD and BMC in the lumbar spine, and also decreased all bone strength parameters to some degree compared to sham controls. Treatment with both doses of denosumab increased all bone strength parameters significantly compared to OVX

controls, except for work to failure and toughness which were increased dose responsively, but not with statistical significance. All values were increased over sham controls as well. Both DXA and pQCT showed significant increases in BMC and BMD compared to OVX controls, including increased trabecular BMC and BMD. Total area however was not significantly increased with treatment.

Correlation analyses indicate significant positive associations for OVX, sham and denosumab groups for yield load vs. lumbar spine total slice BMC, and yield stress vs. total slice BMD. Denosumab (50 mg/kg) was also significantly different from OVX controls for yield load and yield stress vs. BMD. For equivalent bone mass (BMC and/or BMD), increases in yield stress and load were greater in denosumab treated groups compared to both controls, and as with the femoral neck, it suggests that something other than bone mass accounts for a portion of the increased bone strength, and that denosumab also improves bone quality at lumbar spine.

Vertebral core compression L5/L6: (Text Table 15 from the Sponsor) OVX decreased vertebral core BMD, BMC and bone strength parameters, but not statistically significantly compared to sham controls. The Sponsor notes that the slight increase in trabecular area for L5 was considered incidental. OVX did not alter bone or trabecular area. Treatment with both doses of denosumab increased all bone strength parameters over both OVX and sham controls, and to a greater extent than that observed with vertebral compression of L3/L4. Work to failure and toughness were particularly increased, more so at the low dose than the high dose, compared to controls. Both doses of denosumab also increased BMD and BMC, and area by DXA scan, but not trabecular area by pQCT.

Correlation analyses indicated that significant positive associations were found for all groups for yield load vs. lumbar spine trabecular BMC and trabecular BMD, and yield stress vs. trabecular BMD and BMC. For equivalent bone mass (BMC and BMD), increases in yield stress and load were greater in denosumab treated groups compared to both controls, and as with the femoral neck and whole vertebrae, it suggests that something other than bone mass accounts for a portion of the increased bone strength, and that denosumab also improves trabecular bone quality at lumbar spine.

Text Table 11

Femur 3-Point Bending: Effect of OVX and Treatment on Bone Densitometry and Strength Parameters

	Units	Group	OVX + Vehicle		Sham + Vehicle		OVX - 25 mg/kg AMG 162		OVX - 50 mg/kg AMG 162		AMG 162 Negative	
			Raw	%	Raw	%	Raw	%	Raw	%	Raw	%
Peak Load	Mean	Mean	1289	14.2	1481	10.2	1444	14.2	1479	13.9	1384	5.0
	SD	SD	213.1	19.9	213.1	19.9	213.1	19.9	213.1	19.9	213.1	19.9
Stiffness	Mean	Mean	1085	11.9	1196	10.2	1153	13.9	1252	15.4	1191	9.3
	SD	SD	184.4	17.6	176.6	17.6	187.3	17.6	194.2	17.6	184.4	17.6
AUC	Mean	Mean	3247	10.2	3042	4.3	3102	11.7	3075	3.3	3190	1.7
	SD	SD	621.5	807.7	621.5	807.7	621.5	807.7	621.5	807.7	621.5	807.7
Ult. Strength	Mean	Mean	233.6	14.9	233.6	4.4	233.6	0.9	233.6	3.8	233.6	4.4
	SD	SD	25.84	27.64	25.84	27.64	25.84	27.64	25.84	27.64	25.84	27.64
Elastic Modulus	Mean	Mean	9434	9.82	9434	5.8	9434	2.0	9434	4.3	9434	0.8
	SD	SD	1203	12.9	1203	12.9	1203	12.9	1203	12.9	1203	12.9
Toughness	Mean	Mean	12.15	10.59	12.15	10.59	12.15	10.59	12.15	10.59	12.15	10.59
	SD	SD	2.192	1.629	2.192	1.629	2.192	1.629	2.192	1.629	2.192	1.629
DXA	Mean	Mean	2.078	2.352	2.078	8.4	2.078	13.98	2.078	12.1	2.078	6.7
	SD	SD	0.255	0.320	0.255	0.320	0.255	0.320	0.255	0.320	0.255	0.320
pQCT	Mean	Mean	0.563	0.610	0.563	8.5	0.563	10.19	0.563	9.8	0.563	2.3
	SD	SD	0.051	0.065	0.051	0.065	0.051	0.065	0.051	0.065	0.051	0.065
Central	Mean	Mean	46.18	48.65	46.18	5.4	46.18	8.3	46.18	8.6	46.18	5.2
	SD	SD	4.989	5.613	4.989	5.613	4.989	5.613	4.989	5.613	4.989	5.613
Central	Mean	Mean	58.91	63.31	58.91	8.2	58.91	10.4	58.91	10.4	58.91	4.2
	SD	SD	6.232	7.063	6.232	7.063	6.232	7.063	6.232	7.063	6.232	7.063
BMC	Mean	Mean	1371.8	13.62	1371.8	2.7	1371.8	1.7	1371.8	1.9	1371.8	0.9
	SD	SD	313.3	31.44	313.3	31.44	313.3	31.44	313.3	31.44	313.3	31.44
BMD	Mean	Mean	302.8	32.19	302.8	6.6	302.8	22.4	302.8	12.7	302.8	11.9
	SD	SD	55.41	52.19	55.41	52.19	55.41	52.19	55.41	52.19	55.41	52.19

% = percent difference from OVX Vehicle Control
Values in bold are significantly different from OVX Vehicle Group

Text Table 12

Humerus Cortical Beam 3-Point Bending: Effect of OVX and Treatment on Strength Parameters

	Units	Group	OVX + Vehicle		Sham + Vehicle		OVX - 25 mg/kg AMG 162		OVX - 50 mg/kg AMG 162		AMG 162 Negative	
			Raw	%	Raw	%	Raw	%	Raw	%	Raw	%
Peak Load	Mean	Mean	21.27	20.43	21.27	20.43	21.27	2.3	21.27	9.2	21.27	1.4
	SD	SD	5.27	4.162	5.27	4.162	5.27	4.162	5.27	4.162	5.27	4.162
Ultimate Stress	Mean	Mean	255.6	258.5	255.6	258.5	255.6	3.0	255.6	7.1	255.6	1.2
	SD	SD	46.19	47.79	46.19	47.79	46.19	47.79	46.19	47.79	46.19	47.79
Stiffness	Mean	Mean	15.26	15.87	15.26	15.87	15.26	8.6	15.26	18.5	15.26	2.0
	SD	SD	5.045	4.631	5.045	4.631	5.045	4.631	5.045	4.631	5.045	4.631
Modulus	Mean	Mean	18718	20.683	18718	20.683	18718	10.1	18718	16.7	18718	1.3
	SD	SD	4392	48.98	4392	48.98	4392	48.98	4392	48.98	4392	48.98
AUC	Mean	Mean	33.32	25.37	33.32	25.37	33.32	27.2	33.32	6.0	33.32	1.8
	SD	SD	14.35	11.80	14.35	11.80	14.35	11.80	14.35	11.80	14.35	11.80
Toughness	Mean	Mean	3.895	3.092	3.895	3.092	3.895	26.8	3.895	6.6	3.895	1.9
	SD	SD	1.578	1.416	1.578	1.416	1.578	1.416	1.578	1.416	1.578	1.416

% = percent difference from OVX Vehicle Control

Text Table 13

Femoral Neck Shear: Effect of OVX and Treatment on Bone Densitometry and Strength Parameters

	Units	Group	OVX + Vehicle		Sham + Vehicle		OVX - 25 mg/kg AMG 162		OVX - 50 mg/kg AMG 162		AMG 162 Negative	
			Raw	%	Raw	%	Raw	%	Raw	%	Raw	%
Peak Load	Mean	Mean	1480	16.40	1480	10.8	1480	18.9	1480	31.6	1480	1.0
	SD	SD	207.3	295.7	207.3	295.7	207.3	295.7	207.3	295.7	207.3	295.7
Stiffness	Mean	Mean	1376	13.3	1376	12.3	1376	21.5	1376	26.2	1376	1.1
	SD	SD	296.1	283.6	296.1	283.6	296.1	283.6	296.1	283.6	296.1	283.6
AUC	Mean	Mean	1990	22.12	1990	11.2	1990	4.9	1990	9.5	1990	1.8
	SD	SD	634.5	693.4	634.5	693.4	634.5	693.4	634.5	693.4	634.5	693.4
Prox Femur	Mean	Mean	5.961	6.066	5.961	6.066	5.961	10.1	5.961	4.6	5.961	1.1
	SD	SD	0.475	0.531	0.475	0.531	0.475	0.531	0.475	0.531	0.475	0.531
Prox Femur	Mean	Mean	3.227	3.532	3.227	3.532	3.227	22.2	3.227	18.8	3.227	3.3
	SD	SD	0.472	0.611	0.472	0.611	0.472	0.611	0.472	0.611	0.472	0.611
BMC	Mean	Mean	0.541	0.581	0.541	0.581	0.541	10.4	0.541	13.6	0.541	1.1
	SD	SD	0.059	0.072	0.059	0.072	0.059	0.072	0.059	0.072	0.059	0.072

% = percent difference from OVX Vehicle Control
Values in bold are significantly different from OVX Vehicle Group

AUC indicates work to failure (area under the load displacement curve)

Text Table 14

Vertebral Compression L3/L4: Effect of OVX and Treatment on Bone Densitometry and Strength Parameters

Unit	Group	OVX - Vehicle		OVX - 25 mg/kg		OVX - 50 mg/kg		ANG 162	
		Raw	%	Raw	%	Raw	%	Raw	%
Peak Load	N	2383	18.5	3176	54.0	3549	55.6	2331	-2.5
	SD	602.0		680.8		817.5		896.1	
Ultimate Stress	MPa	2131	21.2	3126	48.7	3277	53.8	2144	0.6
	SD	4694		5103		5673		7268	
Yield Load	N	2204	16.6	3417	55.0	3414	54.9	2118	-3.9
	SD	570.6		667.1		768.9		912.6	
Yield Stress	MPa	20.62	19.4	30.36	47.3	31.33	52.9	20.01	-3.0
	SD	4.578		4.938		8.048		6.338	
Stiffness	N/mm	11897	18.0	17417	46.4	16590	39.4	12373	4.0
	SD	3202		3376		2064		3402	
Modulus	MPa	870.9	24.2	1219	40.0	1215	39.6	947.3	8.3
	SD	198.0		201.2		261.8		193.8	
AUC	N-mm	820.4	17.9	1140	39.0	1473	79.5	184.3	7.8
	SD	383.7		409.8		1010		739.1	
Toughness	MPa	0.981	20.7	1.382	33.5	1.702	77.3	1.053	9.6
	SD	0.385		0.403		1.131		0.727	
Area	cm ²	1.409	-1.4	1.511	7.2	1.458	3.5	1.393	-1.2
	SD	0.153		0.147		0.160		0.249	
BMC	g	0.363	10.1	0.487	34.0	0.474	30.6	0.379	-4.5
	SD	0.088		0.066		0.083		0.119	
BMD	g/cm ³	0.256	12.2	0.322	24.0	0.337	27.8	0.246	-5.0
	SD	0.044		0.033		0.055		0.046	
Total Area	mm ²	106.9	-1.6	113.2	5.9	110.3	3.2	102.8	-3.8
	SD	12.91		14.77		14.47		14.70	
Total BMC	mg/mm	45.41	7.7	54.06	24.8	55.78	22.8	45.55	2.5
	SD	9.171		7.983		9.109		14.08	
Total BMD	mg/cm ³	423.9	10.2	503.0	18.7	500.3	20.2	447.8	5.1
	SD	58.30		51.56		79.43		90.02	
Trabecular BMC	mg/mm	16.18	11.9	22.05	36.3	21.06	30.2	17.59	8.1
	SD	4.641		3.908		6.165		7.381	
Trabecular BMD	mg/cm ³	230.7	14.3	327.5	30.6	320.3	27.8	230.0	11.7
	SD	56.68		57.03		90.16		90.17	

*1 = percent difference from OVX Vehicle Control

Values in bold are significantly different from OVX Vehicle Group

Text Table 15

Vertebral Core Compression L5/L6: Effect of OVX and Treatment on Bone Densitometry and Strength Parameters

Unit	Group	OVX - Vehicle		OVX - 25 mg/kg		OVX - 50 mg/kg		ANG 162	
		Raw	%	Raw	%	Raw	%	Raw	%
Peak Load	N	150.8	25.6	275.0	81.4	255.1	69.2	151.0	0
	SD	61.65		61.93		73.16		34.93	
Ultimate Stress	MPa	6165	28.1	12460	84.5	11433	69.4	6931	0
	SD	3653		2466		3151		1577	
Yield Load	N	133.3	16.5	247.4	85.6	233.3	75.4	133.5	0
	SD	49.15		44.18		63.40		34.57	
Yield Stress	MPa	6183	19.0	11577	87.2	10344	74.3	6267	1
	SD	2378		2645		2719		1651	
Stiffness	N/mm	1381	9.4	2225	61.1	2215	60.4	1363	-1
	SD	565.2		452.0		550.9		376.9	
Modulus	MPa	127.8	13.0	243.9	64.5	243.9	62.9	208.3	0
	SD	127.8		103.0		114.3		87.1	
AUC	N-mm	31.65	36.1	73.19	117.6	53.75	59.7	27.07	-19
	SD	22.68		37.17		41.33		11.47	
Toughness	MPa	0.332	35.3	0.768	114.0	0.513	55.6	0.202	-20
	SD	0.249		0.473		0.258		0.139	
Area	cm ²	0.207	2.0	0.228	9.0	0.234	7.9	0.211	1
	SD	0.011		0.011		0.010		0.015	
BMC	g	0.022	13.9	0.042	40.0	0.042	46.3	0.030	-4
	SD	0.009		0.007		0.011		0.005	
BMD	g/cm ³	0.135	11.8	0.187	38.3	0.184	36.1	0.139	3
	SD	0.031		0.027		0.041		0.018	
Trabecular Area	mm ²	21.73	-2.6	21.17	-1.5	21.45	-1.3	21.36	-1
	SD	0.779		0.549		0.831		0.524	
Trabecular BMC	mg/mm	7.615	8.7	9.037	30.5	9.794	28.6	7.615	0
	SD	1.258		1.178		1.412		0.863	
Trabecular BMD	mg/cm ³	331.2	11.4	391.9	31.7	425.6	29.5	325.2	0
	SD	64.53		59.58		92.15		45.21	

*1 = percent difference from OVX Vehicle Control

Values in bold are significantly different from OVX Vehicle Group

AUC indicates work to failure (area under the load displacement curve)

2.6.2.2.2 Study title: A 12-Month Osteoporosis Prevention Study of Denosumab With and Without 6-Month Alendronate Pretreatment in the Cynomolgus Monkey

Reviewed by: Michael Orr, Ph.D.

Key findings:

- Denosumab exposure based on C_{max} and AUC was maintained during the duration of the study, but 25/32 (~78%) monkeys developed anti-drug antibodies (ADA). In the monkeys positive for ADA, 7/32 tested positive for neutralizing antibodies. The development of ADA corresponded with approximately 50% reduction in denosumab exposure based on AUC as compared to animals that were antibody negative.
- The estrogen depletion in monkeys following ovariectomy (OVX) resulted in mild osteopenia, based on loss of bone mass as measured by bone densitometry with DXA or pQCT. In addition, increases were observed in the biochemical markers of bone turnover during the 12 month duration of the study.
- Denosumab treatment increased bone mineral density (BMD) in the whole body by 4% following 6 months of dosing and 7% after 12 months of dosing relative to the OVX vehicle controls. Pretreatment with alendronate and subsequent treatment with denosumab resulted in whole body BMD increases of 3.6% at 6 months, and 1.5% at 12 months as compared to baseline.
- Denosumab treatment increased BMD and significantly increased bone strength at the lumbar spine, based on bone densitometry and biomechanical tests.
- Denosumab treatment increased BMD in trabecular and cortical bone mass at the lumbar spine (5% at 6 months and 8.5% at 12 months), femur (1.6% and 8.7%), proximal tibia and distal radius (1% and 4%), respectively, based on bone densitometry measurements by either DXA or pQCT evaluation.
- Alendronate (Fosamax, ALN) treatment, which is currently approved for osteoporosis treatment increased the BMD in the lumbar spine (5.8% at 6 months and 5.5% at 12 months), proximal femur (6.5% and 4.2%), and central tibia (1.6% and 1.3%).
- Pretreatment with alendronate followed by denosumab treatment induced similar increases in BMD as was observed in the denosumab-only treated group. Furthermore, the reductions in biochemical biomarkers of bone turnover in the combination of alendronate and denosumab treatment group were greater than alendronate treatment group alone.
- There were significant reductions in biochemical markers of bone turnover such as C-telopeptide, TRAP-5b, sALP, and osteocalcin (OC) relative to both baseline and the ovariectomized vehicle control monkeys, providing evidence to support the sponsor's hypothesis that denosumab treatment is inhibiting bone resorption. Alendronate induced a less robust reduction in the bone biomarkers as compared to the denosumab and denosumab in combination with alendronate pretreatment dose groups.
- Histomorphometry evaluation provided evidence that both alendronate and denosumab treatment were associated with significant decreases in tissue-level bone

resorptions/formation parameters in cortical and trabecular sites. Specifically, denosumab prevented bone resorption, formation and turnover parameters in the ilia, L2, rib (6 and 12 months), tibial diaphysis and proximal tibia as compared to the OVX vehicle controls.

- Significant increases in PTH (parathyroid hormone) were noted following the first dose of denosumab in treatment-naïve monkeys, and PTH remained elevated during the first 6 months of treatment. The PTH levels were reduced to control or baseline levels during the last 6-months of the 12-month treatment period.
- Treatment with denosumab induced a statistically significant decrease (4.5-15% reduction) in serum calcium for up to 14-28 days postdose relative to baseline or vehicle control levels.

Study # (b) (4) Amgen Study # 106564

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.1.1

Conducting Laboratory and Location (b) (4)

Date of Study Initiation: not specified (final report dated July 9, 2008)

GLP Compliance: yes

QAU statement: yes (X) no ()

Drug Lot #: 049A053686 (denosumab), 124K4712 (alendronate), 049A022940 (denosumab vehicle)

Methods: The objectives of this study were to investigate the effects of a pretreatment of ovariectomized (OVX) cynomolgus monkeys (*Macaca fascicularis*; weight range 3.3 to 5.9 kg) with bi-weekly dosing with Alendronate for 6 months, followed by once monthly subcutaneous injections of denosumab on the bone mineral density (BMD), serum calcium, phosphorous levels, bone markers, pharmacokinetics, and immunogenicity of the test articles.

Dosing Procedure:

The vehicle and denosumab were administered by subcutaneous injection once every 28 days. For the group 1 animals (denosumab vehicle), a total of 12 doses were subcutaneously administered once every 28 days and the animals also received 24 doses of PBS administered intravenously once every 14 days.

For group 2 (vehicle + denosumab), for the first 6 months of the study, this group of animals were subcutaneously administered a total of 6 doses of vehicle (vehicle for denosumab) once every 28 days. A single dose level of denosumab at 25 mg/kg was used in this study, and the monkeys were dosed s/c every 28 days for an additional 6 months. The animals in group two also received 24 doses of PBS (vehicle for alendronate) administered intravenously once every 14 days during the study.

Group 3 animals received a total of 24 doses of alendronate, administered intravenously once every 14 days. The dose level of 50 µg/kg alendronate was the effective dose used in previous primate studies.¹

For group 4, the animals received a total of 12 doses of alendronate administered intravenously once every 14 days. Following 6 months of dosing with alendronate, group 4 monkeys then received 6 doses of denosumab administered subcutaneously once every 28 days, and received 12 doses of phosphate buffered saline intravenously once every 14 days, starting with the first dose of denosumab on Day 169.

The group 5 animals were subcutaneously administered 12 doses of denosumab once every 28 days for 1 year. The monkeys in group 5 also received 24 doses of phosphate buffered saline once every 14 days starting on Day 1. Animals were euthanized 14 days following last dose.

Comment: The dose level of denosumab tested in this study was 25 mg/kg, administered every 28 days for 6 months, while a dose of approximately 1 mg/kg denosumab administered every 6 months was used in the clinical study. The bi-weekly dose of alendronate provided an approximately 3.5-fold exposure margin compared to the weekly clinical dose (70 mg/week, PO; assuming 0.64% bioavailability as per Fosamax label, and a 60 kg patient).

The following parameters were evaluated in the study: clinical signs, menstrual regularity, body weight, appetite, hematology, clinical biochemistry, urinalysis, hormones, biochemical markers of bone turnover, bone densitometry (DXA, pQCT), radiographs, pharmacokinetics, immunogenicity, macroscopic observations at necropsy, organ weights, histopathology, histomorphometry, micro CT evaluation, and biomechanical testing.

Serum Bone Formation Markers: Osteocalcin, Bone specific alkaline phosphatase (sALP)

Serum Bone Resorption Marker: C-telopeptide (CTx), TRAP-5b

Dose Group Number Reference:

Group Number	Phase I: (Dose 1 to 6)	Phase II: (Dose 7 to 12)
1	Vehicle	Vehicle
2	Vehicle	Denosumab
3	ALN	ALN
4	ALN	Denosumab
5	Denosumab	Denosumab

¹ Balena R, Toolan BC, and Shea M (1993) The effects of 2-year treatment with the aminobisphosphonate alendronate on bone metabolism, bone histomorphometry, and bone strength in ovariectomized nonhuman primates. J.Clin. Invest. 92:2577-2586.

Blood Collection Time points:
Text Table 5 - Blood Collection Time Points

	Base-line 1	Base-Line 2	Dose 1					Dose 2	Dose 3	Dose 4	Dose 7					Dose 8	Dose 10	Term
			Pre	24	72	168	336				Pre	24	72	168	336			
Urine	X																	
Hematology	X	X																
Clinical Chemistry	X	X																
Selected Clinical Chemistry			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hormones																		
PTH	X	X	X	X	X	X		X		X	X	X	X	X			X	X
1,25-VIT D	X	X	X							X								
25-OH VIT D	X	X	X							X								
Estradiol	X	X	X							X	X						X	X
Biomarkers																		
Osteocalcin	X	X	X							X	X						X	X
sALP	X	X	X							X	X						X	X
CTX	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
TRACP-5b	X	X	X							X	X						X	X

Pre - predose

1,25-VIT D - 1,25-dihydroxyvitamin D

25-OH VIT D - 25-hydroxyvitamin D

Dual Energy X-Ray Absorptiometry (DXA): DXA is used to measure bone mineral density (BMD), bone mineral content (BMC) and area using the Hologic Discovery A bone densitometer.

Scan site	Scan Type	Scan Mode	Analysis Method	Reporting
Whole Body	Infant Whole Body	Array	Infant Whole Body	Global BMD
AP Lumbar Spine	AP Spine	Array	Lumbar Spine (L1-L4)	Total BMD
Right Prox. Tibia	Left Forearm	Array	Left Forearm	Distal (1/3) BMD
Right Prox. Femur	Right Hip	Array	Subregion Array Hip	Global BMD, Neck (R1), BMD, Trochanter (R2) BMD
Right Dist. Radius	Right Forearm	Array	Right forearm	Total, Distal (1/3) BMD, ultra distal (UD) BMD

* Whole body area, BMC and BMD reported for acclimation/baseline occasion only.

Peripheral Quantitative Computed Tomography (pQCT): Peripheral QCT was performed on all animals. Scans were acquired once during the acclimation/baseline period and once following doses 3, 6, and 12 during treatment.

Radiographs: Radiographs of both radii (caudo-cranial views), both femora (caudo-cranial views), both tibia (medio-lateral views) and the lumbar and thoracic spine (dorso-ventral and lateral views), were taken once during the acclimation/baseline period, and at the end of the treatment period.

Bone Tissues at Necropsy:

On completion of the necropsy of each animal examined during or at the end of the treatment period, the following bone samples were retained:

Bone	Histomorphometry	Biomechanics
Femur	-	Left whole
Tibia	Right proximal and middle	-
Lumbar vertebrae	L2	L3, L4, L5, L6
Ilium	Left	-
Rib	Left 7 th	-
Back-up bones:		
Femur	Right, proximal and middle	-
Tibia	Left, proximal and middle	-
Thoracic vertebrae	T10	L1, T11, T12
Mandible (bisected)	Right half	Left half *
Humerus	Right	Left*
Radius	Right	Left*

* - Ultimate use to be documented and maintained in study file.

Histomorphometry: Sections of right ilium and rib biopsy samples collected after Dose 6, as well as sections of the left ilium, left 7th rib, lumbar vertebra (L2), and right tibia from each euthanized animal at the end of the treatment period were prepared for all groups, without decalcification, for histomorphometric evaluation. The thoracic vertebra T10, left proximal and middle tibia, right proximal and middle femur, right half mandible, right humerus and right radius were retained and stored in fixative for possible future analyses. Shortly after bone biopsy or necropsy, the bones were trimmed using a diamond cutting saw to expose the bone marrow and placed immediately into 10% neutral buffered formalin, then transferred to 70% alcohol. Specimens processed included medially and frontally cut tissue blocks through the L2 vertebral body and proximal tibia, respectively. For the ilium specimens, two parallel slices were cut at the vicinity of the cortical dorsal spine, starting approximately 1.0 cm caudal to the spine. For the rib specimens, transverse sections were taken from the middle of each specimen. At least one block from each bone was dehydrated then infiltrated and embedded in methyl-methacrylate (MMA). Unstained sections were cut and ground for evaluation of cortical bone. Sections stained with toluidine blue and Goldner's trichrome stain, as well as unstained sections were prepared to evaluate the cancellous bone.

Reference Terms Key for results section:

Evaluation of the cancellous bone region was done on the proximal tibia (1 section level), ilium (biopsies and terminally sampled specimens, 2 section levels) and lumbar vertebra (1 section level). The following static and dynamic parameters of bone were reported using a BIOQUANT/TCW image analyzer:

STRUCTURAL

Tissue area (T.Ar)
 Bone volume (BV/TV)
 Mineralized volume (Md.V/TV)
 Osteoid volume (OV/BV)
 Osteoid thickness (O.Th)
 Trabecular thickness (Tb.Th)
 Trabecular number (Tb.N)
 Trabecular separation (Tb.Sp)
 Osteoblast surface (Ob.S/BS)
 Osteoclast surface (Oc.S/BS)
 Osteoclast number (N.Oc/BS)
 Eroded surface (ES/BS)
 Osteoid surface (OS/BS)
 Wall thickness (W.Th)

*BFR: Bone Formation Rate

DYNAMIC

Mineralizing surface (MS/BS)
 Single label surface (sLS/BS)
 Double label surface (dLS/BS)
 Mineral apposition rate (MAR)
 Adjusted apposition rate (Aj.AR)
 Osteoid maturation rate (Omt)
 Mineralization lag time (Mlt)
 BFR*, surface referent (BFR/BS)
 BFR*, volume referent (BFR/BV)
 Activation frequency (Ac.F)
 Formation period (FP)
 Resorption period (Rs.P)

Evaluation of cortical bone was done on the right mid tibia and ribs (biopsies and terminally sampled specimens), using two section levels per bone (one section level for Haversian system of right mid tibia). The following static and dynamic parameters of bone were reported using a BIOQUANT/TCW image analyzer:

STRUCTURAL

Total tissue area (Tt.T.Ar)
 Cortical area (Ct.Ar)
 Medullary area (Me. Ar)
 Cortical area, relative (%Ct.Ar)
 Medullary area, relative (%Me.Ar)
 Cortical width (Ct.Wi)
 Periosteal perimeter (Ps.Pm)
 Endocortical perimeter (Ec.Pm)
 Percent porosity area (%Po.Ar)
 Haversian wall thickness (H.W.Th)

DYNAMIC

Periosteal single label surface (Ps.sL.Pm/Ps.Pm)
 Periosteal double label surface (Ps.dL.Pm/Ps.Pm)
 Periosteal labelled surface (Ps.L.Pm/Ps.Pm)
 Periosteal MAR (Ps.MAR)
 Periosteal BFR, surface referent (Ps.BFR/BS)
 Endocortical single label surface (Ec.sL.Pm/Ec.Pm)
 Endocortical double label surface (Ec.dL.Pm/Ec.Pm)
 Endocortical labelled surface (Ec.L.Pm/Ec.Pm)
 Endocortical mineral apposition rate (Ec.MAR)
 Endocortical BFR, surface referent (Ec.BFR/BS)
 Haversian single label surface (H.sL.Pm/H.Pm)
 Haversian double label surface (H.dL.Pm/H.Pm)
 Haversian labelled surface (H.L.Pm/H.Pm)
 Haversian mineral apposition rate (H.MAR)
 Haversian BFR, surface referent (H.BFR/BS)
 Haversian BFR, volume referent (H.BFR/BV)

MicroCT Scanning and Evaluation: The right femur from all animals was scanned using a Micro-CT system, and analyzed using the 3-D morphometry evaluation program by the Sponsor. The scans were done in the fixative (70% denatured alcohol).

Biochemical Testing: Testing was performed for each animal euthanized at the end of the treatment period, using an MTS Servohydraulic test system, model 242.03, using TestWorks™ version 3.8A for TestStar® software version 4.0C. The following bone samples were cleaned of excess tissue and muscle and retained frozen (ca -20°C) at

necropsy for each animal euthanized as scheduled or prior to the end of the treatment period:

vertebrae (L3, L4, L5 and L6)
left whole femur
vertebra L1, T11, T12 - back-up

The bones that were evaluated and tests that were performed are listed below:

Bone Specimen	Test Type	Test Rate	Results Reported
Left femur (shaft)	3-point bending	1 mm/sec	Peak load, Ultimate Stress, Stiffness, Modulus, Energy to Break (area under the curve), Toughness
	pQCT		Cross Sectional Moment of Inertia (IX-CRT-A) Area Periosteal circumference (PERI) BMC (CRT-CNT), BMD (CRT-DEN) Cortical Area (CRT_A), Cortical Thickness (CRT_THICK), Endosteal circumference (ENDO_C)
	DXA		Area, BMC, BMD
Left proximal femur	Femoral neck shear	1 mm/sec	Peak load, Stiffness Energy to Break (area under the curve)
	DXA		Area, BMC, BMD
L3, L4 vertebrae	Compression	20 mm/min	Peak Load, Apparent strength, Yield Load, Yield Stress, Stiffness, Modulus, Energy to Break (area under the curve), Toughness
	Measurement by caliper		Height
	DXA		Area, BMC, BMD
	pQCT		Area (TOT_A), BMC (TOT_CNT), BMD (TOT_DEN), Area (TRAB_A), BMC (TRAB_CNT), BMD (TRAB_DEN)
L5, L6 vertebral core	Trabecular Core Compression	20 mm/min	Peak Load, Ultimate Stress, Yield Load, Yield Stress, Stiffness, Modulus, Energy to Break (area under the curve), Toughness
	Measurement by Caliper		Height
	DXA		Area, BMC, BMD
	pQCT		Area (TOT_A), BMC (TOT_CNT), BMD (TOT_DEN)

Results for Study 106564:

Mortality: There were no unscheduled deaths during this study.

Clinical Observations: There were no clinical signs associated with the treatments during the course of this study. No compound related histological changes were identified at the injection sites of the monkeys.

Food consumption: Nothing noteworthy

Ophthalmoscopy: Not investigated

EKG: Not investigated

Body weights: There were no treatment related changes in the body weights.

Hematology: Not investigated

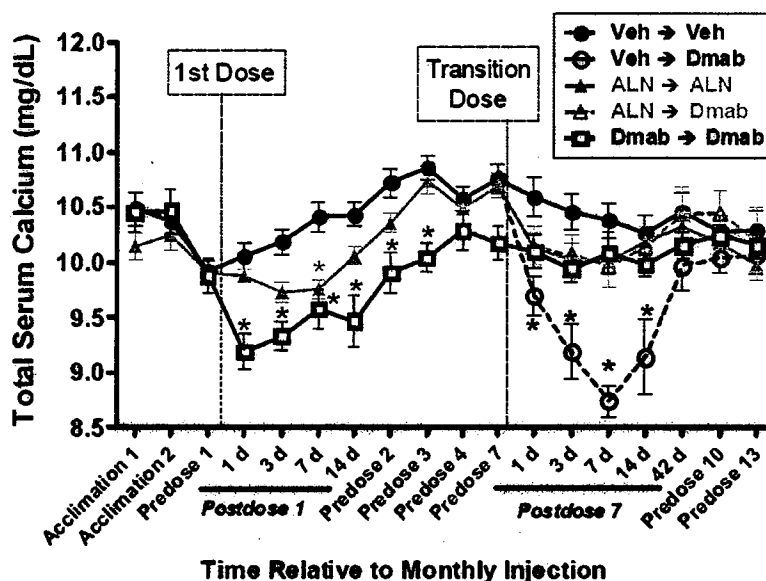
Dose Group Numbers for Reference:

Group Number	Phase I: (Dose 1 to 6)	Phase II: (Dose 7 to 12)
1	Vehicle	Vehicle
2	Vehicle	Denosumab
3	ALN	ALN
4	ALN	Denosumab
5	Denosumab	Denosumab

Efficacy End-point Results:

Results from Study 106564- Serum Calcium Levels: There was a statistically significant, transient reduction in serum calcium relative to the vehicle controls observed following the first and second dose of denosumab (see Figure 6 below). The transient reduction in serum calcium was even more pronounced in the cohort previously treated with vehicle as the first dose. Alendronate displayed a trend for reductions in serum calcium levels; however, the reductions observed in the alendronate (dose 1) and alendronate (dose 2) cohorts were not statistically significant, except for the 7 day time point. The reduction in serum calcium by denosumab was diminished in animals previously exposed to denosumab, (i.e. first versus second dose of denosumab). The serum calcium levels returned to baseline levels approximately three to five weeks following the initial dose and the second (transition) dose of denosumab (see Figure 6 below, from the sponsor's final study report).

Figure 6. Total Serum Calcium in OVX Cynomolgus Monkeys Treated With Denosumab With and Without 6 Months of Alendronate Pretreatment

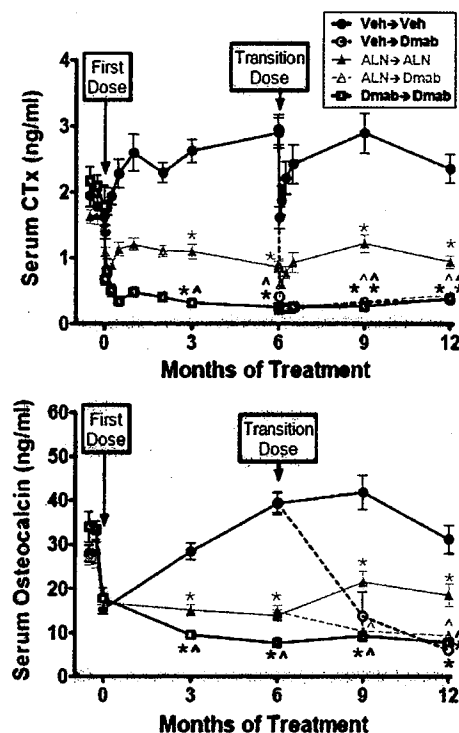


Serum calcium was assessed before and after therapeutic transition from vehicle (Veh) or alendronate (ALN) to denosumab (Dmab) at month 6. Time is expressed relative to the monthly injections such that Predose 1, 2, 3, 4, 7, 10, and 13 refer to samples drawn at baseline and after 1, 2, 3, 6, 9, and 12 months of treatment, respectively. Data are presented as the mean \pm SE, $n = 7$ to 10/group. * $p \leq 0.05$ vs Veh \rightarrow Veh. Prior to the transition dose, group differences in baseline-adjusted serum calcium between the 3 treated groups (vehicle, alendronate, and denosumab) were assessed using an ANOVA followed by contrast pairwise post-tests. From the transition dose to termination, group differences in pre-transition-adjusted serum calcium were assessed across all 5 treatment groups and Tukey's pairwise comparisons were performed. No significant differences were found between continued alendronate treatment (ALN \rightarrow ALN) and transition from ALN to denosumab (ALN \rightarrow Dmab), significant differences between Veh \rightarrow Dmab and ALN \rightarrow Dmab or Dmab \rightarrow Dmab were found but are not shown. Equal variance across groups was assessed using Levene's tests; rank-transformation was used to equalize variance if $p \leq 0.05$. If variance remained unequal, an ANOVA model accounting for unequal variance was utilized.

Source: Study Report No. 106564

Results from Study 106564- Biomarkers of Bone Turnover: After ovariectomy of female cynomolgus monkeys, the serum biomarkers of bone formation and resorption were elevated from baseline for animals in the vehicle control group. Both alendronate and denosumab treatments significantly reduced the levels of these biomarkers (i.e. bone resorption and formation) during this 12-month pharmacology study, as shown in Figure 7. As shown in Table 6, the percent change was calculated relative to predose levels of the bone biomarkers; there was an 87% reduction in CTx, a 73% reduction in TRAP-5b, a 60% reduction in sALP, and a 57% reduction in OC at the end of phase I treatment with denosumab relative to the vehicle controls. Alendronate alone reduced the levels of CTx (39%), TRAP-5b (23%), sALP (37%), and OC (18%) relative to the vehicle controls, at the end of phase I treatment. Similar reductions in the bone biomarkers were observed at the end of phase II of the study in both the denosumab and alendronate treated monkeys (see Text Table 6).

Figure 7. Biomarkers of Bone Turnover in OVX Cynomolgus Monkeys Treated With Denosumab With and Without 6 Months of Alendronate Pretreatment



Serum CTx (resorption marker, upper panel) and serum osteocalcin (formation marker, lower panel). Therapeutic transition occurred at month 6. Data are presented as the mean \pm SE, $n = 7$ to 10/group. * $p \leq 0.05$ vs Veh \rightarrow Veh and ^ $p \leq 0.05$ vs ALN \rightarrow ALN; for CTx, statistics are shown only for the month 3, 6, 9, and 12 timepoints. One way ANOVA was performed across all groups, with Tukey's post-tests for the changes from baseline in the Veh, ALN, and Dmab 12-month treatment groups. For the transition groups after 6 months, additional Tukey's post-test comparisons (Veh \rightarrow Veh vs. Veh \rightarrow Dmab and ALN \rightarrow ALN vs ALN \rightarrow Dmab) were performed to examine biomarker changes from the pre-transition timepoint. Equal variance across groups was assessed using Levene's tests; rank-transformation was used to equalize variance if $p \leq 0.05$. If variance remained unequal, an ANOVA model accounting for unequal variance was utilized.

Source: Study Report No. 106564

Text Table 6

Percent (%) Change in Biochemical Markers of Bone Turnover

Rx	% Change Relative to Predose 1						% Change Relative to End Phase I				
	End Phase I			End Phase II			End Phase II				
Phase II				Veh	ALN	Denosumab	Veh	Denosumab	ALN	Denosumab	Denosumab
Rx	Veh	ALN	Denosumab	(1)	(3)	(5)	(1)	(2)	(3)	(4)	(5)
Phase I	(1)	(3)	(5)	(1)	(3)	(5)	(1)	(2)	(3)	(4)	(5)
CTx	+54	-39	-87	+47	-35	-78	-4	-90	+7	-51	+64*
TRACP-5b	+45	-23	-73	+12	-33	-60	-23	-77	-12	-54	+47*
sALP	+31	-37	-60	+39	-25	-63	+6	-75	+19	-26	-7
OC	+131	-18	-57	+74	+1.4	-56	-24	-83	+24	-35	+1.8

Rx (treatment) for Phase I and II, group number in parentheses.

% change calculated using group mean values.

* Note negligible change in very low absolute values becomes a large percent change.

Veh: Vehicle

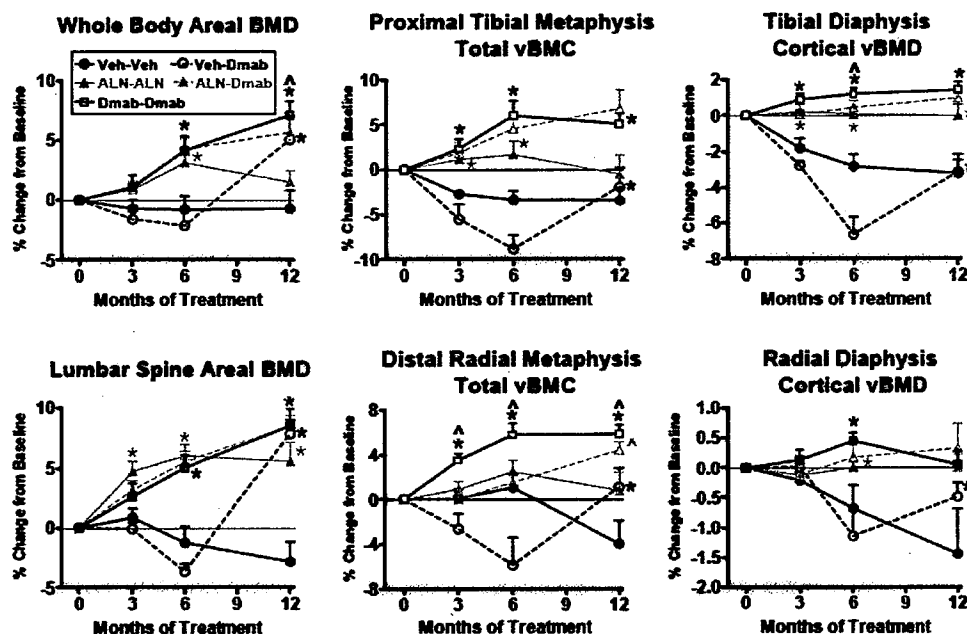
Results from Study 106564- In Vivo Densitometry: Denosumab treatment of OVX cynomolgus monkeys with and without 6 months of alendronate pretreatment induced statistically significant increases in the whole body BMD, lumbar spine BMD, and

radial diaphysis cortical BMD, proximal tibial metaphysis BMC, and radial diaphysis cortical BMD. Denosumab alone was unable to increase the tibial diaphysis cortical BMD following 6 months of weekly subcutaneous injections. Overall, the female OVX cynomolgus monkeys provide data showing that denosumab treatment prevents the reduction of BMD in the whole body, and in specific bone areas such as the lumbar spine, tibial diaphysis cortical, and radial diaphysis cortical bone, based on *in vivo* densitometry of female OVX cynomolgus monkeys treated with and without 6 months of alendronate pretreatment.

Specifically, statistically significant increases in cortical BMD of 1 and 4% at the distal radius and proximal tibia were observed, relative to the vehicle controls at the end of phase I of the study

Comment: According to the medical officer reviewing this application, a clinically significant increase in BMD is $\geq 1.25\%$ from baseline. Many of the increases in BMD shown above were greater than 1.25% following 12-months of treatment, so a clinical response was observed in the OVX cynomolgus monkeys treated with denosumab, with or without 6 months of alendronate pretreatment. Furthermore, alendronate (positive control comparator, currently marketed for the treatment of osteoporosis by inhibiting osteoclast-mediated bone-resorption) increased the BMD as well as the combination of alendronate and denosumab (see Figure 9 below, from the sponsor).

Figure 9. In Vivo Densitometry in OVX Cynomolgus Monkeys Treated With Denosumab With and Without 6 Months of Alendronate Pretreatment



Data are presented as mean + SE, $n = 7$ to 10 /group. * $p \leq 0.05$ vs. OVX + vehicle. # $p \leq 0.05$ vs. ALN. Statistics for months 3 and 6 are presented after combining the groups similarly treated before the transition to denosumab, although these groups are illustrated separately for clarity. Group differences in the percent change from baseline for the Veh-Veh, ALN-ALN, and Dmab-Dmab groups at 3, 6, and 12 months were assessed by one way ANOVA followed by Tukey's pairwise comparisons. For the transition groups at month 12, additional Tukey's post-test comparisons (Veh→Veh vs. Veh→Dmab and ALN→ALN vs. ALN→Dmab) were performed to examine changes from the pre-transition timepoint.

Source: Study Report No. 106564

Whole Body (Text from Study No. 106564)

Denosumab vs. Vehicle: Vehicle control groups showed only minor decreases in whole body BMD in response to OVX relative to baseline. Treatment of OVX monkeys with denosumab resulted in increases in whole body BMD of approximately 4% following 6 monthly doses (Group 5), continuing to increase to 7% after 12 months of treatment relative to baseline. The increases in BMD in the denosumab treated animals were statistically significant compared to changes observed in vehicle controls. Treatment with denosumab for 6 months starting 6 months post-OVX (Group 2) resulted in a significant increase in whole body BMD of approximately 7% (relative to end of phase I), and an increase of approximately 5% relative to baseline (pre-OVX). Thus denosumab appeared to reverse the effects of OVX-induced bone loss following a 6 month treatment-free period. The overall effect of 6 months of treatment relative to baseline was similar (4 to 5%) for these two groups.

Alendronate vs. Vehicle: Compared to changes in vehicle controls, treatment of OVX monkeys with bimonthly doses of ALN for 6 months (Group 3) resulted in a significant increase in whole body BMD of approximately 3.6% relative to baseline, an effect which was not sustained after 12 months of treatment. Whole body BMD decreased slightly (-1.6%, relative to end of phase I) during the last 6 months of

treatment, although values did remain slightly increased relative to baseline, compared to changes observed in vehicle controls.

Denosumab vs. Alendronate: Although treatment with denosumab and ALN for 6 months resulted in similar increases in whole body BMD (4.1% and 3.6%, Group 5 and 3, respectively), the increase following treatment with denosumab for 12 months was significantly greater than that observed after 12 months of ALN treatment (7.1% and 1.5%, respectively).

Pretreatment with Alendronate: OVX monkeys pretreated with ALN for 6 months, then dosed with denosumab for 6 months showed increases in whole body BMD of 1.4% compared to 2.9% (relative to end of phase I) for animals continuing on denosumab treatment for an additional 6 months. This schedule resulted in increases in BMD for ALN-denosumab-treated animals which were only slightly below animals treated continuously with denosumab for 12 months (5.7% and 7.1%, Groups 4 and 5, respectively, relative to baseline). Increases were greater following 6 months of denosumab treatment compared to continued ALN treatment (Group 4 vs. Group 3). Denosumab administered to treatment-naïve, OVX animals (Group 2) and OVX animals pretreated with ALN for 6 months (Group 4) increased whole body BMD to similar levels. The magnitude of the changes relative to predose levels were therefore significantly different for these two groups since BMD was markedly increased following administration of denosumab to treatment-naïve animals compared to the relatively smaller additional increases seen in animals pretreated with ALN (Group 2 vs. Group 4).

Lumbar Spine (Text from Study No. 106564)

Denosumab vs. Vehicle: Vehicle control groups showed slight decreases in lumbar spine BMD ($\leq 3\%$) in response to OVX relative to baseline. Treatment of OVX monkeys with denosumab resulted in increases in lumbar spine BMD of approximately 5% following 6 monthly doses (Group 5), continuing to increase to 8.5% after 12 months of treatment relative to baseline. These findings were statistically significant compared to changes observed in vehicle controls. Treatment with denosumab for 6 months starting 6 months post-OVX (Group 2) resulted in a significant increase in lumbar spine BMD of approximately 12% (relative to end of phase I), and an increase of approximately 7.8% relative to baseline (pre-OVX). Thus denosumab reversed the effects of OVX-induced bone loss which occurred in this group during the 6 month treatment-free period. The overall effect of 6 months of denosumab treatment relative to baseline was slightly greater starting 6 months post-OVX in high turnover, osteopenic animals (i.e. BMD increased 7.8%) compared to the effects of treatment given immediately post-OVX in animals with normal rates of bone turnover (i.e. BMD increased 5%).

Alendronate vs. Vehicle: Compared to changes in vehicle controls, treatment of OVX monkeys with bimonthly doses of ALN for 6 months (Group 3) resulted in a significant increase in lumbar spine BMD of approximately 5.8% relative to baseline, an effect which was sustained during the last 6 months of treatment (5.5%).

Denosumab vs. Alendronate: Treatment with denosumab or ALN for 6 months resulted in similar increases in lumbar spine BMD (5.0% and 5.8%, Groups 5 and 3, respectively), with a slight although not significantly greater increase following

treatment with denosumab compared to ALN for 12 months (8.5% and 5.5%, respectively).

Pretreatment with Alendronate: OVX monkeys pretreated with ALN for 6 months then dosed with denosumab for 6 months showed increases in lumbar spine BMD similar to animals treated with denosumab for a total of 12 months, resulting in comparable increases in BMD for ALN-denosumab-treated animals and animals on denosumab for 12 months (8.5% for both Group 4 and 5, relative to baseline). Increases were greater in monkeys treated with ALN followed by 6 months of denosumab treatment compared to the group receiving continued ALN treatment (Group 4 vs. Group 3). Denosumab administered for 6 months to treatment-naïve animals (Group 2) and animals pretreated with ALN for 6 months (Group 4) increased lumbar spine BMD to similar levels. The magnitude of the changes relative to predose levels were therefore significantly different for these two groups since BMD was markedly increased following administration of denosumab to treatment-naïve animals compared to the relatively smaller additional increases seen in animals pretreated with ALN (Group 2 vs. Group 4).

Proximal Femur

The effects on global proximal femur data will be discussed, with reference to the femoral neck and trochanteric subregions of interest made as appropriate. At the femoral neck and trochanteric region, vehicle control Group 1 showed slight increases in BMD while Group 2 showed decreases relative to baseline during the first 6 months of the study, confounding interpretation of the effects of OVX and treatment at these subregions of interest.

Denosumab vs. Vehicle: Vehicle control groups showed no consistent decreases in proximal femur BMD ($\leq 2\%$) in response to OVX relative to baseline, with a slight increase noted during the last 6 months of the study (1.6%, relative to end of phase I). Relative to baseline, denosumab treatment of OVX monkeys resulted in a significant increase in proximal femur BMD of approximately 10% following 6 monthly doses (Group 5), with no further increases observed following dosing for an additional 6 months. Proximal femur BMD was increased 8.7% after 12 months of treatment relative to baseline. Treatment with denosumab for 6 months starting 6 months post-OVX (Group 2) resulted in a significant increase in proximal femur BMD of 8.4% (relative to end of phase I), representing an increase of approximately 6.2% relative to baseline (pre-OVX). At this site, the overall effect of 6 months of denosumab treatment starting 6 months post-OVX (i.e. BMD increased 8.4%) was essentially comparable to the effects seen when given immediately post-OVX (BMD increased 10.1%).

Alendronate vs. Vehicle: Compared to changes in vehicle controls, treatment of OVX monkeys with bimonthly doses of ALN for 6 months (Group 3) resulted in a significant increase in proximal femur BMD of approximately 6.5% relative to baseline. This effect was reduced slightly during the last 6 months of treatment (i.e. 4.2% increase overall).

Denosumab vs. Alendronate: Treatment with denosumab for up to 12 months resulted in slightly greater increases in proximal femur BMD compared to ALN, although differences relative to baseline did not attain statistical significance (6.5% and 10.1%,

Group 5 and 3, respectively at 6 months and 4.2% and 8.7%, respectively, at 12 months).

Pretreatment with alendronate: OVX monkeys pretreated with ALN for 6 months then dosed with denosumab for 6 months showed minimal increases in proximal femur BMD of approximately 1% (relative to end of phase I), compared to no change observed in animals continuing on denosumab a further 6 months. Relative to baseline, increases in BMD for ALN-denosumab-treated animals were only slightly lower than for animals dosed with denosumab for 12 months (7.7% for Group 4 and 8.7% for Group 5). Increases were slightly greater following 6 months of denosumab treatment compared to continued ALN treatment (Group 4 vs. Group 3).

Distal Radius (Text from Study No. 106564)

No effects of either denosumab or alendronate on the distal radius.

Central Tibia (Text from Study No. 106564)

Denosumab vs. Vehicle: Vehicle control groups showed consistent decreases in tibial BMD ($\leq 6\%$) in response to OVX during the study relative to baseline. Relative to baseline, treatment of OVX monkeys with denosumab resulted in a significant increase in tibial BMD of approximately 1.6% following 6 monthly doses (Group 5) compared to vehicle controls, with no further increases observed following dosing for an additional 6 months. Tibial BMD was significantly increased 1.3% after 12 months of treatment relative to baseline compared to vehicle controls. Treatment with denosumab for 6 months starting 6 months post-OVX (Group 2) resulted in a significant increase in tibial BMD of 3.2% (relative to end of phase I), although values were approximately -2.6% below baseline values (pre-OVX). At this site, the overall effect of 6 months of denosumab treatment starting 6 months post-OVX (i.e. BMD increased 3.2%) was slightly greater than the effects seen when given immediately post-OVX (i.e. BMD increased 1.6%).

Alendronate vs. Vehicle: Similar to the distal radius, the effects of treatment of OVX monkeys with bimonthly doses of ALN during the first 6 months of the study differed between Groups 3 and 4. The effect of combining data for these two groups for analysis (Group 3/4) resulted in a significant increase in tibial BMD of approximately 1.0% relative to baseline, compared to changes in vehicle controls. Continued treatment of animals in Group 3 with ALN resulted in a slight loss in BMD relative to baseline (i.e. 3.7% decreases) with values comparable to controls.

Denosumab vs. Alendronate: Treatment with denosumab for up to 12 months resulted in slight increases in tibial BMD relative to baseline compared to slight losses for the groups dosed with ALN, although differences between these two groups did not attain statistical significance.

Pretreatment with Alendronate: OVX monkeys pretreated with ALN for 6 months then dosed with denosumab for 6 months showed slight decreases in tibial BMD of approximately -2.2% (relative to end of phase I), compared to no change observed in animals continuing on denosumab a further 6 months. BMD for ALN-denosumab-treated animals was similar to animals dosed with denosumab for 12 months and both groups showed no meaningful changes relative to baseline.

There were no meaningful differences between ALN-treated groups treated for 6 months with denosumab compared to continued ALN treatment (Group 4 vs. Group 3). Denosumab administered for 6 months to treatment-naïve animals (Group 2) significantly increased tibial BMD (3.2% relative to end of phase I) compared to changes in vehicle controls, while animals pretreated with ALN for 6 months (Group 4) showed slight decreases in tibial BMD (-2.2%, similar to losses seen in vehicle controls). The magnitude of the changes relative to predose levels was therefore significantly different for these two groups, since BMD was increased following administration of denosumab to treatment-naïve animals compared to slight losses seen in animals pretreated with ALN (Group 2 vs. Group 4).

Histomorphometry (Text from Study No. 106564)

Cancellous Bone Evaluation at the End of Phase I

No qualitative histological changes in the iliac biopsies, other than those that were quantifiable with histomorphometry, were noted in any animals.

Effects of Denosumab upon Cancellous Bone at Postdose 6

At postdose 6, treatment with denosumab markedly suppressed bone resorption, formation and turnover variables when compared to the OVX controls.

All measured resorption variables (eroded surface [ES/BS], osteoclast surface [Oc.S/BS] and osteoclast number [N.Oc/BS] were significantly decreased in the denosumab-treated group. The effect of the compound was striking upon osteoclasts, as these cells were not found at all in the iliac biopsy region of interest of any of the treated monkeys. All bone formation variables (osteoid volume [OV/BV], osteoid thickness [O.Th], osteoid surface [OS/BS], osteoblast surface [Ob.S/BS], mineralizing surface [MS/BS], mineral apposition rate [MAR], adjusted apposition rate [Aj.Ar] and surface referent bone formation rate [BFR/BS]) were significantly decreased after six doses of denosumab. Even though a sham-operated group was not available to confirm prevention of the OVX-induced increases in bone turnover, levels reached for these bone resorption and formation variables in the denosumab-treated group were consistent with complete prevention of these effects in the model. Bone turnover was significantly decreased, as measured with the volume referent bone formation rate (BFR/BV) and activation frequency (Ac.f.) Other significant changes associated with denosumab treatment included an increase in formation period (FP) along with a decrease in resorption period (Rs.P.). There was no significant effect upon the cancellous bone volume (BV/TV) in the denosumab-treated group when compared to the controls. Nevertheless, a slight increase (13%) was noted after Phase I in the denosumab-treated group.

Effects of Alendronate upon Cancellous Bone at the End of Phase I

At the end of phase I, significant reductions in all bone resorption variables (ES/BS, Oc.S/BS and N.Oc/BS) were found in the ALN-treated group when compared to the controls. Treatment with ALN markedly suppressed bone formation and turnover variables as shown by significant reductions of OV/BV, O.Th, OS/BS, Ob.S/BS,

MS/BS, MAR, Aj.Ar, BFR/BS, BFR/BV and Ac.f. Significant increases in FP completed the response associated with this treatment. Despite these effects upon bone dynamics, there were no effects upon BV/TV in the ALN-treated group.

Comparisons Between Denosumab and Alendronate upon Cancellous Bone at the End of Phase I

At the end of phase I, significant differences between denosumab and ALN treatments were limited to decreased osteoclast-derived variables Oc.S/BS, N.Oc/BS and Rs.P in the denosumab-treated group.

Cortical Bone Evaluation at the End of Phase I

No qualitative histological changes in the rib biopsies, other than those that were quantifiable with histomorphometry, were noted in any animals examined.

Effects of Denosumab upon Cortical Bone at Postdose 6

Compared to controls, denosumab treatment caused marked effects at the haversian systems, which included significant decreases in percent porosity (%Po.Ar), harversian labeled surface (H.L.Pm/H.Pm), harversian mineral apposition rate (H.MAR), surface referent haversian bone formation rate (H.BFR/BS) and volume referent haversian bone formation rate (H.BFR/BV). The magnitude of these changes was regarded as indicating a complete prevention of the expected OVX-induced increases in bone turnover. The overall size of the bone compartments and cortical width (Ct.Wi) of the rib biopsies was unaffected by denosumab at postdose 6. In addition when compared to the vehicle controls, no significant changes were noted at the periosteal and endocortical envelopes.

Effects of Alendronate upon Cortical Bone at the End of Phase I

Compared to controls, ALN treatment caused significant decreases in %Po.Ar, H.L.Pm/H.Pm, H.MAR, H.BFR/BS and H.BFR/BV at the haversian systems. The overall size of the bone compartments including Ct.Wi in the rib biopsies was unaffected by ALN at postdose 6. In addition, when compared to the vehicle controls, no significant changes were noted at the periosteal and endocortical envelopes.

Comparisons Between Denosumab and Alendronate Upon Cortical Bone at the End of Phase I

At postdose 6, significant differences between denosumab and ALN treatments were limited to several haversian-derived variables. Decreases in %Po.Ar, H.L.Pm/H.Pm, H.MAR, H.BFR/BS and H.BFR/BV were found in the denosumab-treated group.

Cancellous Bone Evaluation at Study End

No qualitative histological changes in the ilia collected at necropsy, other than those that were quantifiable with histomorphometry, were noted in any animals examined at the end of Phase II. Treatments with denosumab, ALN or their combination were not associated with histological defects of bone mineralization or collagen arrangement at L2 and proximal tibia in any of the monkeys. In these sites, several other histological changes were noted. Based upon their nature and low incidence, either sporadic or

scattered in most groups including the vehicle controls, they were regarded as incidental in origin and unrelated to the compounds tested. The most common findings were subchondral osteosclerosis in the proximal tibia and degeneration/loss of end plate(s) and/or focal/multifocal osteosclerosis in L2. Histopathological findings noted during qualitative evaluation did not justify exclusion from histomorphometric analyses with the exception of the right proximal tibia of one animal (Animal #557). This monkey had marked focal bone atrophy associated with medullary fibrosis in the region of interest, which correlated with severe degenerative joint disease detected radiologically at baseline. This finding was the justification to use the animal's contralateral proximal tibia for conducting histomorphometric measurements.

Effects of Denosumab Upon Cancellous Bone at Study End

At postdose 12, treatment with denosumab markedly suppressed bone resorption, formation and turnover variables in the ilia, L2 and proximal tibia when compared to the OVX controls. Most resorption variables measured (Oc.S/BS and N.Oc/BS) were significantly decreased in the denosumab-treated group at L2 and proximal tibia. This compound-related effect upon osteoclasts was so marked that these cells were absent in the measured region of interest at both cancellous bone sites in most treated monkeys. All bone formation variables measured in L2 and tibia (OV/BV, O.Th [L2 only], OS/BS, Ob.S/BS [L2 only], MS/BS, MAR, Aj.Ar and BFR/BS) were significantly decreased after 12 doses of denosumab. In the absence of a reliable determination of Ac.f in these bones, significant decreases in BFR/BV were regarded as indirect evidence that the bone turnover was depressed. Other significant changes associated with denosumab treatment were limited to the proximal tibia and included an increase in FP, osteoid maturation time (Omt) and mineralization lag time (Mlt) along with a decrease in resorption period (Rs.P). The increased Omt and Mlt were not regarded as indicative of a defective mineralization because the O.Th was reduced. Compared to controls, there were no significant differences in the ilium for the denosumab-treated group when using results adjusted to the end of Phase I. This reflects that the response to the compound had reached a plateau during the first half of the study. Even though no significant effects upon BV/TV were noted in the denosumab-treated group when compared to controls, all three cancellous bone sites showed mild increases in BV/TV, ranging from 13% to 20%, at postdose 12.

Effects of Alendronate Upon Cancellous Bone at Study End

Compared to Vehicle controls at study end, treatment with ALN resulted in paradoxical effects upon bone resorption variables. While the osteoclast-derived variables (Oc.S/BS and N.Oc/BS) were reduced (attaining significance only for N.Oc/BS in proximal tibia), the ES/BS were markedly increased (attaining significance in ilium and L2) in all three cancellous bone sites for the ALN-treated group, attaining significance for the ileum and L2 sites. Comparison with the biopsy data showed that this effect upon ES/BS became apparent in the second half of the study, with significant increase in the ilium for the ALN-treated group when using results adjusted to the end of Phase I. Marked and significant decreases in all bone formation markers (OV/BV, O.Th, OS/BS, Ob.S/BS, MS/BS, MAR, Aj.Ar and

BFR/BS) were noted in L2 after ALN treatment. The reductions in bone formation and turnover variables were similar in the ilium compared to L2 but there were no significant differences in the latter bone for the ALN-treated group when using results adjusted to the end of Phase I. This finding reflects that the response to ALN had reached a plateau during the first half of the study. The effects of ALN upon bone formation were less conclusive in the proximal tibia, for which only the Aj.Ar was significantly decreased. Bone turnover variables (BFR/BV and Ac.f) measured in L2 and proximal tibia were significantly decreased in the ALN-treated group. Other significant effects included increases in Mlt and FP at the proximal tibia and increased Rs.P at L2. The increased Mlt was not indicative of defective mineralization because O.Th was concurrently reduced. No significant or consistent effects upon BV/TV were noted in various cancellous bone sites evaluated the ALN-treated group when compared to the controls.

Effects of a Treatment Switch to Denosumab in Phase II Upon Cancellous Bone at Study End

Compared to ALN treatment, cancellous bone changes in the group switched from ALN to denosumab (ALN + denosumab) were characterized by decreases of resorption variables, reaching significance for N.Oc/BS and ES/BS in L2 and N.Oc/BS and Oc.S/BS in tibia. Most bone formation and turnover variables were significantly decreased in the proximal tibia of the ALN + denosumab-treated group. In L2 and ilium, differences for bone formation and turnover variables were generally small for the compared groups. No significant or consistent effects upon BV/TV were noted in the ALN + denosumab-treated group when compared to the ALN group-treated group.

The cancellous bone response in the group switched from vehicle to denosumab (Vehicle + denosumab) included marked and significant decreases of all calculated bone resorption, formation and turnover variables in L2 and proximal tibia. Compared to the controls, affected variables included Oc.S/BS, N.Oc/BS, ES/BS, OV/BV, O.Th, OS/BS, Ob.S/BS, MS/BS, MAR, Aj.Ar, BFR/BS, BFR/BV and Ac.f, with the exception of tibial Ob.S/BS. The ilium responded similarly based upon marked decreases in variables of bone resorption, formation and turnover but these differences were less consistently significant. Even though no significant effects upon BV/TV were noted in the Vehicle + denosumab-treated group, all three cancellous bone sites showed mild increases in BV/TV, ranging from 13% to 18% increases, when compared to vehicle controls.

Effects of the Phase I Treatment Upon Denosumab Response in Phase II Upon Cancellous Bone at Study End

Compared to the vehicle + denosumab-treated group, there were no significant differences noted in any histomorphometric variables measured at L2, ilium and proximal tibia in the group treated continuously with denosumab for 12 months. In the ilium, significant differences were noted for all bone resorption and most bone formation and turnover variables when using adjusted results to the end of Phase I. These observations reflect differences present in Phase I because of the small

intergroup variations noted for these variables at study end. No meaningful effects upon BV/TV were noted at any of the cancellous bone sites between the denosumab and Vehicle + denosumab-treated groups. Compared to the Vehicle + denosumab-treated group, there were no significant differences noted in any variables measured at L2 and proximal tibia in the ALN + denosumab treated group. In the ilium, significant differences were noted for most bone formation and turnover variables when using adjusted results. These observations reflect differences present in Phase I because of the small intergroup variations noted for these variables at study end. No significant or consistent effects upon BV/TV were noted at any of the cancellous bone sites between the Vehicle + denosumab and Alendronate + denosumab-treated groups. Compared to the ALN + denosumab-treated group, there were no significant differences in bone structure or dynamics for the denosumab-treated group at any of the three measured cancellous bone sites.

Cortical Bone Evaluation at Study End

No qualitative histological changes in the rib and mid tibia, other than those that were quantifiable with histomorphometry, were noted in any animals examined at study completion.

Effects of Denosumab Upon Cortical Bone at Study End

At study end compared to controls, the denosumab treatment caused marked effects at the haversian systems marked effects which included decreases in %Po.Ar, H.L.Pm/H.Pm, H.MAR, H.BFR/BS and H.BFR/BV. These responses were very similar at the rib and tibia and were comparable to those measured in the rib biopsies at postdose 6. At least for the rib, the effects of denosumab upon the haversian systems had attained by postdose 6 had attained a plateau that was maintained through postdose 12. Compared to controls, the denosumab treatment was associated with marked decreases in the dynamic variables measured at the periosteal and endocortical envelopes. At the tibia, significant decreases in Ps.L.Pm/Ps.Pm, Ec.L.Pm/Ec.Pm, Ps.MAR, Ec.MAR and, consequently, bone formation rates (Ps.BFR/BS and Ec.BFR/BS) were noted. The rib at study end responded similarly for these variables when compared to the tibia. Unlike the haversian systems, the decreases in periosteal and endocortical turnover were less rapid and decrescent over the study course based on the rib data. Despite these effects upon bone dynamics, the overall size of the bone compartments including Ct.Wi in the rib and mid tibia remained unaffected by the treatment when compared to the controls.

Effects of Alendronate Upon Cortical Bone at Study End

Compared to controls at study end, the ALN treatment caused effects at the tibial haversian systems which included decreases of several dynamic variables including H.L.Pm/H.Pm, H.MAR, H.BFR/BS and H.BFR/BV. These variables were similarly decreased at postdose 12 in the ribs but based on comparison with the biopsy data, it appeared that these effects had attained a plateau by postdose 6 had attained that was maintained through postdose 12. No significant decrease in %Po.Ar was evident at the tibia after 12 months of ALN treatment. A consistent response was noted at the rib cortex so comparison between both time points suggests that the %Po.Ar decrease

noted at postdose 6 was a transient effect of ALN. Compared to controls at postdose 12, ALN treatment was associated with marked and significant decreases of Ec.L.Pm/Ec.Pm, Ec.MAR and Ec.BFR/BS at the mid tibia. At the rib, the response for these variables in the ALN-treated group headed in the same direction but was less marked and non-significant. The overall size of the bone compartments including Ct.Wi in the rib and tibia remained unaffected by ALN at postdose 12. In addition when compared to the controls, no consistent changes were noted at the periosteal envelope.

Effects of a Treatment Switch to Denosumab in Phase II Upon Cortical Bone at Study End

Compared to ALN treatment, cortical bone changes in the group switched from ALN to denosumab (ALN + denosumab) were mainly evident at the haversian systems with significant decreases of H.MAR at both tested sites and H.L.Pm/H.Pm, H.BFR/BS and H.BFR/BV at the tibia. A significant decrease of tibial Ps.BFR/BS was another significant difference noted between these two groups. Compared to the Vehicle controls, the cortical bone response in the group switched from vehicle to denosumab (Vehicle + denosumab) was characterized at the haversian systems by significant decreases of %Po.Ar (tibia only), H.L.Pm/H.Pm, H.MAR, H.BFR/BS and H.BFR/BV at both cortical bone sites. In addition, significant decreases in formation variables at the endocortical surface were limited to Ec.MAR in both cortical bone sites. Significant decreases of periosteal variables (Ec.L.Pm/Ec.Pm, Ec.MAR and Ps.BFR/BS) following the switch to denosumab appeared site-dependent, being limited to the tibia. The overall size of the bone compartments including Ct.Wi in the rib and tibia remained unaffected when comparing these data sets for differences during Phase II when treatments were changed to denosumab.

Effects of the Phase I Treatment upon Denosumab Response in Phase II Upon Cortical Bone at Study End

Compared to the Vehicle + denosumab-treated group, most tibial haversian-derived variables were significantly decreased in the denosumab-treated group, including H.L.Pm/H.Pm, H.BFR/BS and H.BFR/BV. At the rib, only the H.L.Pm/H.Pm was significantly reduced in the denosumab-treated group. No other significant differences were noted at other costal and mid-tibial bone envelopes for this comparison.

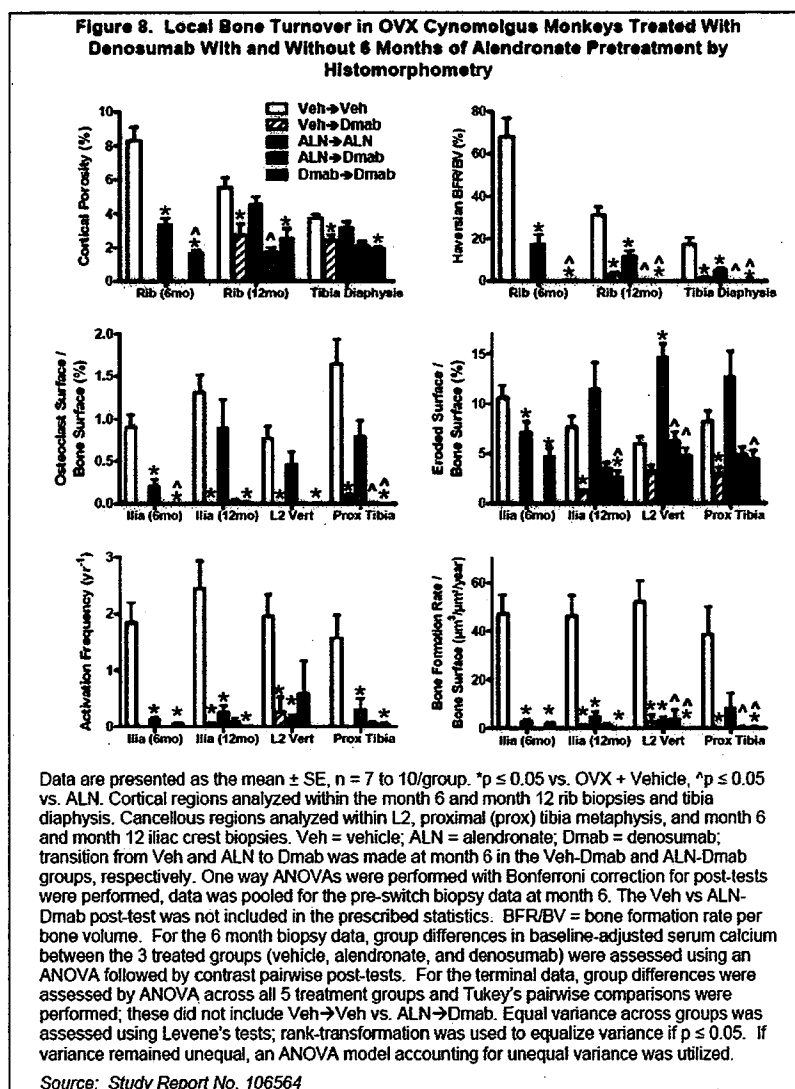
Compared to the Vehicle + denosumab-treated group, several of the haversian-derived variables at the tibia were significantly decreased in the alendronate + denosumab-treated group, including H.L.Pm/H.Pm, H.BFR/BS and H.BFR/BV. No other significant differences were noted at other costal and mid-tibial bone envelopes for this comparison.

Compared to the ALN + denosumab-treated group, there were no significant differences for the denosumab-treated group at the mid-tibial and costal site. At the rib, a few haversian variables (H.MAR and H.BFR/BS) were significantly decreased in the denosumab-treated group. These differences reflected the smaller relative

contribution of the ALN + denosumab treatment in Phase I despite that at study end the magnitude of these decreases were very similar. No other significant differences were noted at other costal and mid tibial bone envelopes for this comparison.

As shown in Figure 8 provided by the sponsor, the histomorphometry evaluation provides evidence that denosumab prevented bone resorption, formation and turnover parameters in the ilia, L2, rib and proximal tibia as compared to the OVX vehicle controls. A similar response was observed in the alendronate alone group and in the combination of alendronate and denosumab cohort except for the ES/BV (%) parameter the alendronate alone group was initially reduced in the ilia at 6 months but was present at greater levels as compared to the vehicle control in the ilia, L2, and proximal tibia at 12 months.

Summary of Histomorphometry (Figure 8 below, as provided by the sponsor):



Bone Strength (Text from Study No. 106564)**Femur 3-point Bending**

Denosumab vs. Vehicle: Femur BMD (DXA and pQCT derived) was increased relative to vehicle controls. Peak load, ultimate stress and stiffness and modulus were increased up to 11% compared to vehicle controls for monkeys treated with denosumab for 12 months, although the work to failure and toughness were similar to vehicle controls. Slight increases in mean ultimate stress (2%) and modulus (1%) were noted compared to vehicle controls. Peak load and stiffness were increased by 9% and 11%, respectively, compared to the vehicle controls. Work to failure and toughness increased slightly (7% and 4%, respectively) relative to controls.

Alendronate vs. Vehicle: Treatment with ALN for 12 months showed slight increases in all biomechanics parameters relative to vehicle controls (up to 13%) with the greatest increases in work to failure and toughness of all treated groups.

Denosumab vs. Alendronate: Compared to treatment with ALN, treatment with denosumab for 12 months resulted in slightly greater increases in ultimate stress (2%) and modulus (6%) Peak load and stiffness were similar between the two dose groups while work to failure and toughness were slightly lower (13%).

Pretreatment with alendronate: Overall, ultimate stress and modulus were increased slightly (2% and 9%, respectively) for OVX monkeys in the group pretreated with ALN for 6 months followed by dosing with denosumab for 6 months (Group 4) compared to OVX monkeys that did not receive prior treatment (i.e. received vehicle only before denosumab treatment for 6 months, Group 2). Peak load, stiffness, work to failure and toughness were similar for these two groups. Samples from animals pretreated with ALN followed by denosumab compared to continuous denosumab treatment for 12 months showed a tendency for marginally greater biomechanics strength parameters with the exception of ultimate stress which was slightly lower (2%).

Overall, denosumab-treated bones were similar to controls with respect to the work required for failure and toughness. Femurs also tended to be stiffer and less ductile from animals dosed with denosumab for 12 months relative to monkeys treated with ALN treatment for 12 months. The biomechanics strength parameters ultimate stress and modulus were increased for animals pretreated with ALN followed by denosumab relative to denosumab treatment for 6 months post-OVX. Pretreatment with ALN followed by denosumab showed slight increases in work to failure and toughness relative to denosumab alone.

Correlation Analyses:

A statistically significant linear and positive association was found for all groups for peak load versus pQCT-derived cortical BMC ($r=0.89$). Femur bone mass (BMC) was correlated with bone strength.

Femoral Neck Shear:

There were no statistically significant differences between Groups 1 through 5 for any biochemical strength parameters measured at the femoral neck.

Vertebral Compression L3/L4:

Data were analyzed for L3 and L4 separately and averaged. The results discussed below are for the averaged data.

Denosumab vs. Vehicle: Lumbar spine (L3/L4) BMC and BMD (DXA and pQCT-derived) were slightly increased relative to vehicle controls following 12 months of denosumab treatment and were the highest among the denosumab-treated groups (Group 2, 4 and 5). For animals treated with denosumab for 12 months, peak load, apparent strength, yield load, yield stress, stiffness and modulus were statistically significantly increased 31 to 47% compared to vehicle controls.

Alendronate vs. Vehicle: Treatment with ALN for 12 months showed slight increases in all biomechanics parameters compared to vehicle controls of 8 to 19%.

Denosumab vs. Alendronate: Compared to treatment with ALN, treatment with denosumab for 12 months resulted in greater increases in all biomechanical strength parameters (peak load, apparent strength, yield load, yield stress, stiffness, modulus, work to failure and toughness) of 14 to 30%, and the findings were statistically significant for stiffness when compared to the ALN treated group.

Pretreatment with alendronate: Biomechanical parameters with the exception of stiffness and modulus were similar for OVX monkeys pretreated with ALN for 6 months followed by dosing with denosumab for 6 months (Group 4) compared to OVX monkeys that did not receive prior treatment (i.e. received vehicle only before denosumab treatment for 6 months, Group 2). Stiffness and modulus were slightly lower (13% and 15%, respectively) for Group 2 compared to Group 4. Biomechanical parameters were similar to or slightly lower (up to 8%) for the group pretreated with ALN followed by denosumab (Group 4) compared to the group given 12 months of denosumab treatment (Group 5). Compared to continued treatment with ALN for 12 months (Group 3), animals treated with ALN for 6 months followed by denosumab for 6 months (Group 4) showed increases in all biomechanical strength parameters (peak load, apparent strength, yield load, yield stress, stiffness, modulus, AUC and toughness) of 8 to 27%. At the lumbar spine, biomechanical strength parameters were increased relative to vehicle controls for all groups treated with denosumab, with the greatest effects noted following denosumab treatment for 12 months. Prior treatment with ALN did not meaningfully modify the response to denosumab. The effect of denosumab treatment for 12 months on lumbar spine strength was superior to 12 months ALN treatment.

Correlation Analyses:

For pQCT and biomechanics parameters (peak load vs. total BMC and apparent strength vs. total BMD) the slopes of the regression lines were significantly different between groups precluding an analysis of all groups combined. Statistically significant linear and positive associations were found for individual groups for peak load vs. lumbar spine total slice BMC ($r = 0.76$ to 0.96). Positive and significant associations were found for all groups combined for peak load vs. DXA BMC ($r = 0.88$). Significant but generally weaker associations were found for other associations for some but not all dose groups.

Vertebral Core Compression L5/L6:

Data were analyzed for L5 and L6 separately and averaged. The results discussed below are for the averaged data.

Denosumab vs. Vehicle: Lumbar spine vertebral core (L5/L6) BMC and BMD (DXA and pQCT-derived) were slightly increased relative to vehicle controls for all treated groups. DXA BMC and BMD were statistically significantly increased for animals in Group 5 that were treated for 12 months with denosumab. Specifically, peak load, apparent strength, yield load, yield stress, stiffness and modulus were statistically significantly increased 37 to 46% compared to vehicle controls.

Alendronate vs. Vehicle: Treatment with ALN for 12 months showed non-significant increases in all biomechanics parameters of 23 to 43%, which were the least changes of all treated groups relative to vehicle.

Denosumab vs. Alendronate: Compared to treatment with ALN, treatment with denosumab for 12 months resulted in slightly greater increases in all biomechanical strength parameters (peak load, apparent strength, yield load, yield stress, stiffness, modulus, work to failure and toughness) of 8 to 18%.

Pretreatment with Alendronate: Biomechanical parameters with the exception of work to failure and toughness were generally similar (within 5%) for OVX monkeys pretreated with ALN for 6 months then dosed with denosumab for 6 months (Group 4) compared to OVX monkeys that did not receive prior treatment (received vehicle only before denosumab treatment for 6 months, Group 2). Work to failure and toughness were slightly lower (16% and 10%, respectively) for Group 2 compared to Group 4. Biomechanical parameters were similar (within 3%) for the group pretreated with ALN followed by denosumab treatment (Group 4) compared to the group given 12 months of denosumab treatment alone (Group 5). Compared to treatment with ALN for 12 months (Group 3), animals treated with ALN for 6 months followed by denosumab treatment for 6 months (Group 4) showed increases in all biomechanical strength parameters (peak load, apparent strength, yield load, yield stress, stiffness, modulus, work to failure and toughness) of 9 to 18%. Biomechanical strength of lumbar spine vertebral cores was increased relative to vehicle controls for all groups treated with denosumab, with the greatest effects noted following denosumab treatment for 12 months. Prior treatment with ALN did not meaningfully modify the response to denosumab. The effect of denosumab treatment for 12 months on lumbar spine vertebral core strength was superior to 12 months ALN treatment alone.

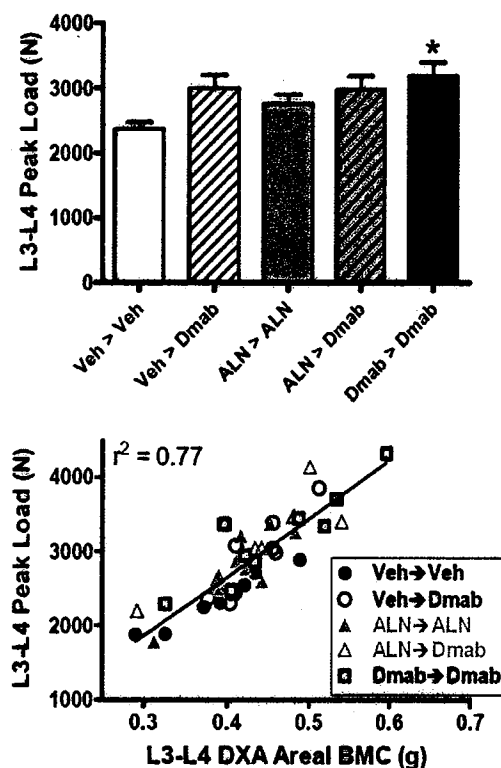
Correlation Analyses:

Lumbar vertebral core BMC and BMD were positively and significantly correlated with bone strength. Statistically significant linear and positive associations were found for peak load vs. DXA BMD/BMC ($r = 0.90$ to 0.91). For pQCT and biomechanics parameters (peak load/apparent strength vs. total BMC) the slopes of the regression lines were significantly different between groups precluding an analysis of all groups combined.

Based on the L3-L4 Peak Load parameters displayed in Figure 10 below, bone strength in the OVX cynomolgus monkeys were similar whether the OVX cynomolgus monkeys were treated with the vehicle for 12 months, vehicle followed

by denosumab, alendronate alone for 12 months, pretreatment with alendronate (6 months) followed by denosumab (6 months), or denosumab treatment for 12 months.

Figure 10. Bone Strength in OVX Cynomolgus Monkeys Treated With Denosumab With and Without 6 Months of Alendronate Pretreatment



Peak load data are presented as mean \pm SE, $n = 7$ to 10/group. * $p \leq 0.05$ vs OVX + vehicle. Group differences were assessed by ANOVA across all 5 treatment groups and Tukey's pairwise comparisons were performed, not including Veh \rightarrow Veh vs. ALN \rightarrow Dmab. Linear regression analysis of peak load vs. ex vivo areal BMC demonstrated similar and strong correlations for each group. Data for all treatment groups were therefore pooled; the line shown illustrates the regression across groups with an $r^2 = 0.77$.

Source: Study Report No. 106564

Toxicokinetics:

^a Immunoassay negative animals Mean (SD) Denosumab PK Parameter Estimates Following Monthly SC Administration of 25 mg/kg to Ovariectomized Cynomolgus Monkeys						
Group (Treatment)	Month	t_{max}^c (day)	C_{max} (ug/mL)	AUC_{0-336} (mg*hr/mL)	AR	N
2 (Vehicle + denosumab)	7	7 (3-14)	145 (31)	41.6 (10.2)	N/A N/A	10
4 (ALN + denosumab)	7	3 (3-14)	172 (65)	43.7 (10.8)	N/A N/A	11
5 (denosumab)	1	7 (1-7)	152 (45)	40.3 (10.2)	N/A N/A	11
5 (denosumab) ^a	7	3 (1-7)	372 (132)	90.4 (25.8)	2.25 (0.46)	5
5 (denosumab) ^b	7	3 (3-14)	220 (142)	45.6 (24.9)	1.14 (0.53)	6

^aValues calculated from immunoassay negative animal data.
^bValues calculated from immunoassay positive animal data.
^c t_{max} reported as a median (min-max) value
 All values were rounded to 3 significant figures after calculations were performed, except t_{max} which is presented as a whole number. SD is reported to the same precision as the mean value.
 C_{max} : maximum observed concentration.
 t_{max} : time at observed maximum concentration
 AUC_{0-336} : area under the concentration-time curve from 0 to 336 hours postdose.
 AR: $AUC_{0-336, month7} / AUC_{0-336, month1}$.
 N/A: not applicable

Comment: The development of antibodies to denosumab in treated monkeys corresponded with an approximately 50% reduction in exposure, based on the AUC values in monkeys with ($AUC_{0-t} \sim 45$ mg*hr/mL) or monkeys without ($\sim AUC_{0-t}$ 90 mg*hr/mL) anti-denosumab antibodies. Twenty-five of 32 (78%) monkeys in the s.c. denosumab treatment groups tested positive for the development of binding antibodies to denosumab. Seven of 32 (21%) monkeys in the s.c. treatment group tested positive for the neutralizing antibodies.

On the basis of body weight (mg/kg) for determining the dose multiple between animals and humans, denosumab (25 mg/kg) exposure was 25 fold greater in the monkeys relative to the human exposure of approximately 1 mg/kg of denosumab.

Study conclusion: This study was designed to evaluate the effects of biweekly intravenous dosing of alendronate for 6 months, followed by once monthly subcutaneous injection of denosumab for 6 months in ovariectomized monkeys. Parameters evaluated included BMD, serum calcium and phosphorous levels, and bone biochemical turnover of markers. Statistically significant decreases in calcium levels relative to the control values in the study were observed following the first dose and second dose of denosumab but they were transient, and the levels of calcium returned close to control or baseline levels 3-5 weeks following dosing. Denosumab treatment induced statistically significant increases in PTH compared to vehicle controls, which remained elevated for approximately 28 days following the 3rd and 6th

monthly doses. The PTH levels declined gradually and returned to vehicle controls and baseline levels during the remaining 6 months of the monthly administration of denosumab.

Treatment of the ovariectomized monkeys with denosumab suppressed the biochemical markers of bone turnover below baseline levels. In addition, after 6 months of treatment with 25 mg/kg/dose of denosumab, animals displayed slightly increased BMD and increased lumbar bone strength. Both alendronate and denosumab reversed the increases in biochemical markers of bone turnover such as C-telopeptide, TRAP-5b, sALP and OC relative to baseline and OVX vehicle controls, providing evidence that both alendronate and denosumab treatment were able to induce reductions in bone turnover. In conjunction with the biochemical bone turnover markers, there were statistically significant increases in BMD, and increased bone strength at the lumbar spine based on bone densitometry and biomechanical tests. Denosumab increased BMD in trabecular and cortical bone mass at the lumbar spine (5% at 6 months and 8.5% at 12 months), femur (1.6% and 8.7%), proximal tibia and distal radius (1% and 4%) respectively based on bone densitometry measurements by DXA and pQCT evaluation. Alendronate (Fosamax) treatment, which is currently approved for osteoporosis treatment, increased the BMD in the lumbar spine (5.8% at 6 months and 5.5% at 12 months), proximal femur (6.5% and 4.2% respectively), and central tibia (1.6% and 1.3% respectively). Histomorphometry evaluation provided evidence that denosumab prevented bone resorption, formation and turnover parameters in the ilia, L2, rib and proximal tibia, as compared to the OVX vehicle controls. A similar response was observed in the alendronate alone group and in the combination of alendronate and denosumab cohort except for the ES/BS (%) parameter, which in the alendronate alone treated group was initially reduced in the ilia at 6 months, but was at greater levels as compared to the vehicle control in the ilia, L2, and proximal tibia at 12 months.

Overall, denosumab treatment was able to induce robust reductions in bone biomarkers of bone turnover as compared to dosing with alendronate, to induce similar modulations in histomorphometry parameters as alendronate treatment did, and bone strength was similar across the different treatment groups. Denosumab induced immunogenicity in 78% of the cynomolgus monkeys evaluated and reduced denosumab exposure by 50% in ADA positive animals; however, reasonable pharmacological activity was maintained for the duration of the study based on efficacy endpoints such as BMD, biochemical markers of bone turnover, histomorphometry parameters, and biomechanic endpoints. These data provide substantial evidence that denosumab treatment of OVX cynomolgus monkeys at 25 mg/kg/dose was able to prevent the OVX-induced increases in markers of bone turnover, and based on histomorphometry parameters, maintain bone mass and bone strength. Pretreatment with alendronate did not change the activity of denosumab treatment, and dosing with alendronate alone at 50 µg/kg was able to induce similar modulations in the bone biomarkers, bone mass and bone strength. However, the response at this dose was not as robust as what was observed for denosumab in some of the parameters, such as in the biochemical markers of bone turnover. This may be

just a reflection that alendronate was used at a dose approximately 3-fold higher than the clinical dose, while denosumab treatment was at a dose approximately 25-fold higher than the clinical dose based on body weight (mg/kg)..

2.6.2.2.3 Study Title: Comparison of Two Anti-Resorptive Therapies (Alendronate Versus AMG-162 Monoclonal Anti-RANKL Antibody) on Murine Fracture Healing

Reviewed by: Michael Orr, Ph.D.

Key Findings:

- Alendronate and denosumab (AMG 162) treatment delayed the removal of cartilage, remodeling of the fracture callus, and induced changes in the morphology and time course of tissue remodeling of the fractured femur, as compared to the control mice with fractured femur.
- In addition, the fractured calluses for the denosumab treated knock-in mice 42 days post fracture had increased bone volume (BV), BV (%), bone mineral content (BMC), and BV/TV (%) relative to alendronate and vehicle control mice.
- The mechanical strength of healing/healed bone was not negatively affected in this study. At the 42 day time point, the denosumab and alendronate treated mice had increases in fractured bone strength and stiffness either relative to the contralateral (nonfractured) control, or to the vehicle control group.

Study #: R2006458

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.1.1

Conducting Laboratory and Location (b) (4)

Date of Study Initiation: November 15, 2007 (final report dated March 14, 2008)

GLP Compliance: No

QAU statement: yes () no (X)

Drug Lot #: This reviewer could not find the lot # for either denosumab or alendronate in the report.

Methods:

The purpose of this study was to compare the effects of denosumab and alendronate on fracture healing to determine if denosumab was capable of interfering with normal fracture healing process.

Denosumab neutralizes human RANKL, but based on the scientific data provided to date it does not appear to bind or neutralize mouse or rat RANKL. Thus, transgenic, knock-in (KI) mice were created by Amgen Inc. that were genetically engineered to express a chimeric form of RANKL by exchanging the 5th exon of the murine RANKL gene for the human 5th exon, which is thought to include the critical denosumab binding or neutralizing epitope. The chimeric RANKL expressed in the KI mice is bound and neutralized by denosumab. This model was used to test the effects of denosumab on bone mass and bone resorption in study R2004430, which provides data to support that

the genetically engineered KI mouse was a useful model to study the pharmacology of denosumab *in vivo*. Furthermore, the genetically engineered mice were used to evaluate the bone mass and bone resorption in aged human RANKL KI mice, in study R2004321.

In this study, the C57/B6/ human RANKL knock in mice were shipped directly from a breeding colony at (b) (4) to (b) (4)

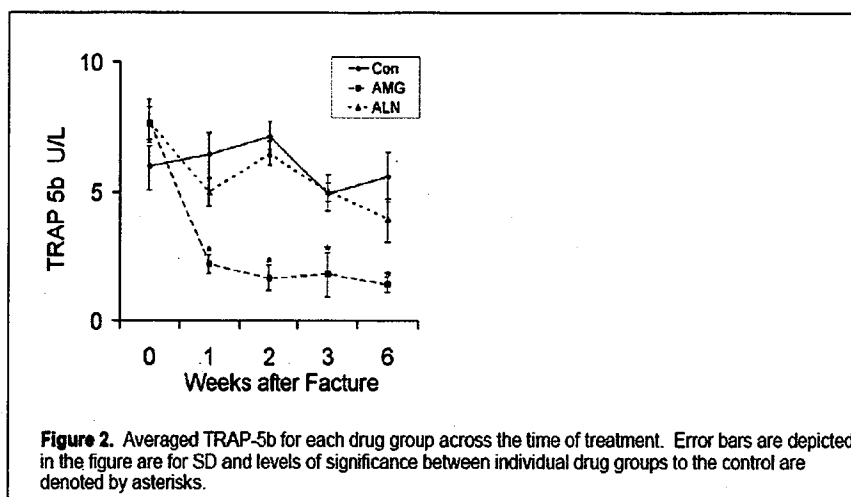
(b) (4) Ninety male C57/B6/RANK Human RANKL KI mice received unilateral transverse femur fractures on the right femora. Mice (8 to 17 weeks of age) then received biweekly subcutaneous injections of alendronate at 0.1 mg/kg, denosumab at 10 mg/kg, or the vehicle (PBS, 0.1 mL) until sacrifice at days 21 or 42 post-fracture. Each treatment group evaluated 15 mice per time point at terminal necropsy (day 21 or day 42). The right fractured and the normal contralateral femurs were then evaluated by microCT analysis and torsional testing. Subsets of fractured femora from day 21 were also examined by histological evaluation, which included special staining techniques for the marker TRAP-5b staining, to demonstrate the presence of osteoclasts.

Micro-computed Tomography (mCT)-generated images were used to evaluate the three dimensional structure of the bone. The following were quantified: total volume, bone volume, bone volume fraction (BV%), total callus bone mineral density (BMD), bone mineral content (BMC), and average cross-sectional area (Cs.Ar).

After the microCT scans were performed, the fractured and non fractured femora were subjected to torsion testing using an Instron 55MT1 MicroTorsion testing system. The torsional testing evaluated the maximum torque at failure, torsional stiffness, and toughness for each femur. Mechanical testing parameters were calculated using MATLAB.

The figures and tables below were copied from sponsor's submission.

Results: For the alendronate (0.1 mg/kg) treated mice, a statistically significant reduction in TRAP-5b occurred as early as 1 week after initiation of the biweekly subcutaneous (s.c.) injections of denosumab at 10 mg/kg. However, biweekly s.c. injections of alendronate did not induce significant reductions in TRAP-5b within 6 weeks of treatment.



Overall, a total of 157 bones (84 fracture bones and 73 contralateral bones) were included in the data collected from torsion testing. Ten fracture bones were excluded due to physical damage to the bone during handling prior to testing, and ten contralateral bones were excluded due to technical reasons. Based on the summarized mechanical assessment data for the fractured bones in Table 1 and Figure 3, fractured bones from mice in all treatment groups (i.e. alendronate, denosumab and vehicle) required higher maximum torque to failure as compared to the contralateral bones, but the differences were not significantly different except on day 42 for both the alendronate and denosumab treated groups (see Figure 3A). Alendronate treatment significantly increased the maximum torque required to failure on day 42 relative to day 21 post-fracture, while results from the denosumab-treated animals were similar to control values (see Figure 3B). Comparison of the contralateral bone (Figure 3C) indicated that the maximum torque to fracture was similar for the contralateral bone on day 21 and day 42 across all treatment groups. As shown in Table 1, denosumab treatment induced statistically significant increases in torsional rigidity on day 42 relative to the vehicle control fractured bones, while alendronate increased the maximum torque on day 42 relative to the vehicle control. Alendronate induced a transient but statistically significant reduction in maximum torque on day 21 that changed to a statistically significant increase in maximum torque on day 42 relative to the vehicle controls. Other than the changes described above, maximum torque and torsional rigidity values in samples from alendronate and denosumab treated mice were similar to those obtained for the vehicle control group.

TABLE 1. MECHANICAL TESTING OF FRACTURED BONES

	ALN	AMG	Control
<i>Post-fracture - Day 21</i>			
Max Torque (N·m)	0.0461 ± 0.0188*	0.0544 ± 0.0166	0.0502 ± 0.0121
Torsional Rigidity (N·mm²/rad)	4515.34 ± 1918.15	6184.77 ± 1332.02 ^C	5133.41 ± 1892.51 ^C
<i>Post-fracture - Day 42</i>			
Max Torque (N·m)	0.0752 ± 0.0343 ^{*C}	0.0661 ± 0.0227 ^C	0.0513 ± 0.0138
Torsional Rigidity (N·mm²/rad)	8578.15 ± 3227.85 ^C	9249.96 ± 2957.62 ^{*C}	6590.09 ± 2089.07 ^C

Data shown are mean values ± SD. Significant differences between ALN or AMG, to control within the same time point are shown by* C indicates between that treatment group and contralateral value. All values indicated as significant are at $p < 0.05$.

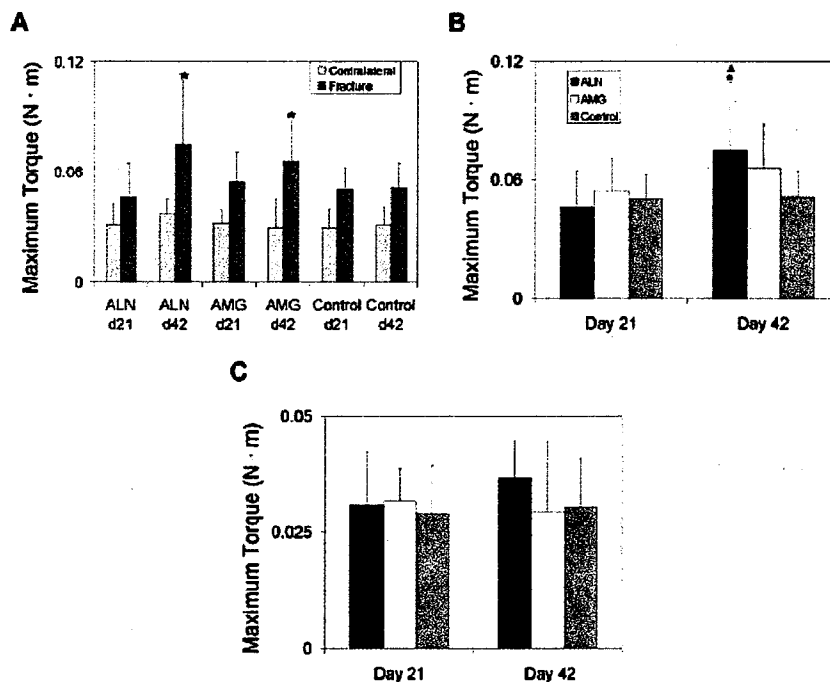


Figure 3. Graphical analysis of maximum torque to failure. (A) Comparison between fracture and intact bones. Stars indicate significance between fracture and contralateral bones, $p < 0.05$ (B) Comparison of fracture bones at both time points after fracture. Triangles indicate significance relative to the same treatment group at day 21. Asterisks indicate significance relative to the control at the same time point, $p < 0.05$. (C) Comparison of contralateral bone at both time points after fracture. For all graphs, error bars are ± SD.

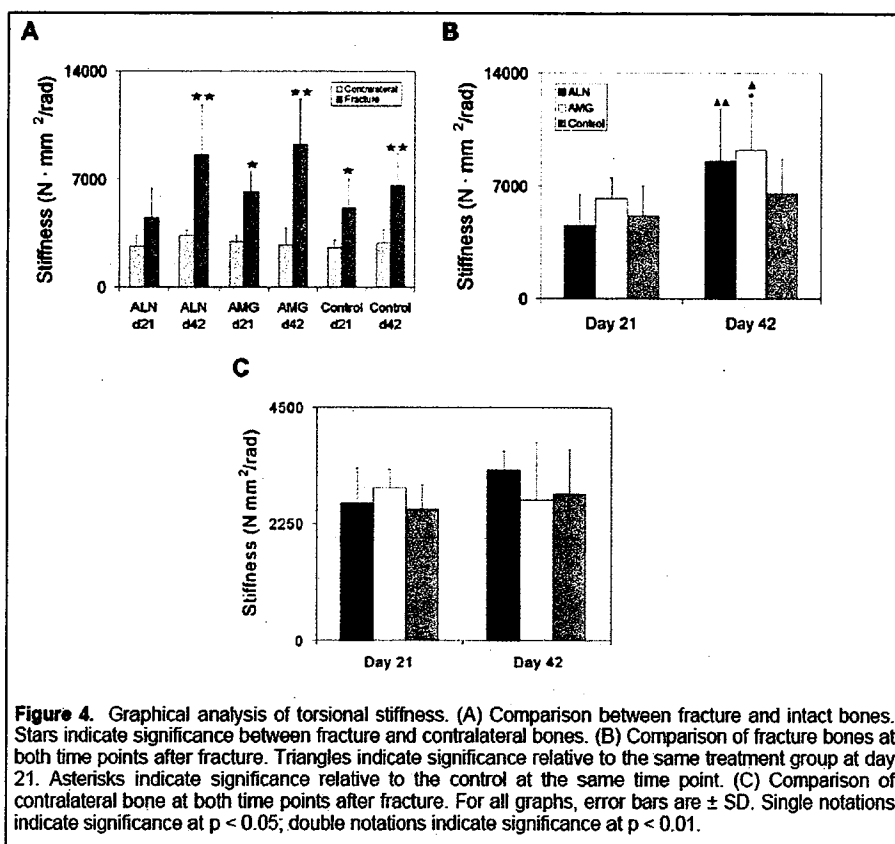
As shown in Table 3, there were statistically significant differences in BV, BV/TV (%), callus Cs.AR (mm²) and BMC for the denosumab treated mice, as compared to the alendronate treated mice at 42 days post fracture. Alendronate treatment increased BV, BV/TV (%), and BMC significantly relative to the vehicle control 42 days post-fracture. In addition, denosumab had an increase in cross sectional area as compared to the vehicle control mice, based on MicroCT analysis of the fractured bones.

TABLE 3. MICROCT ANALYSIS OF FRACTURED BONES

	ALN	AMG	Control
<i>Post-fracture - Day 21</i>			
TV (mm ³)	49.79 ± 21.49	50.23 ± 15.88	41.12 ± 13.70
BV (mm ³)	23.74 ± 7.98	33.99 ± 8.44 ^{*D}	17.31 ± 5.83
BV/TV (%)	50.28 ± 9.15	70.72 ± 16.17 ^{*D}	42.38 ± 3.85
BMC (mg HA)	23.152 ± 7.83	30.47 ± 6.64 [*]	18.26 ± 5.92
Callus Cs.Ar (mm ²)	7.53 ± 2.39	7.46 ± 1.59	6.35 ± 1.63
<i>Post-fracture - Day 42</i>			
TV (mm ³)	42.33 ± 16.60	43.13 ± 11.35	28.41 ± 15.11
BV (mm ³)	23.12 ± 9.08 [*]	39.20 ± 11.83 ^{*D}	11.91 ± 4.65
BV/TV (%)	56.36 ± 10.69 [*]	90.15 ± 10.5 ^{*D}	46.94 ± 11.91
BMC (mg HA)	26.70 ± 9.23 [*]	41.07 ± 11.44 ^{*D}	14.00 ± 5.29
Callus Cs.Ar (mm ²)	6.34 ± 1.96	6.77 ± 1.24 [*]	4.51 ± 1.92

Data shown are mean values ± SD. Significant differences between ALN, AMG, and the control within the same time point are denoted with Significant differences between ALN or AMG, to control within the same time point are shown by*. D indicates significant difference between AMG and ALN. All values indicated as significant are at $p < 0.05$.

In Figure 4B below, the stiffness of the fractured bone between vehicle, denosumab, and alendronate treated, knock-in mice were similar on day 21, while both alendronate and denosumab treatment induced statistically significant increases in stiffness in the mice femurs. As shown in Figure 5, the twist to failure parameter was similar between all treatment groups, and at both the time points. Furthermore, in Figure 6, the toughness parameter was similar between all treatments and at both the time points.



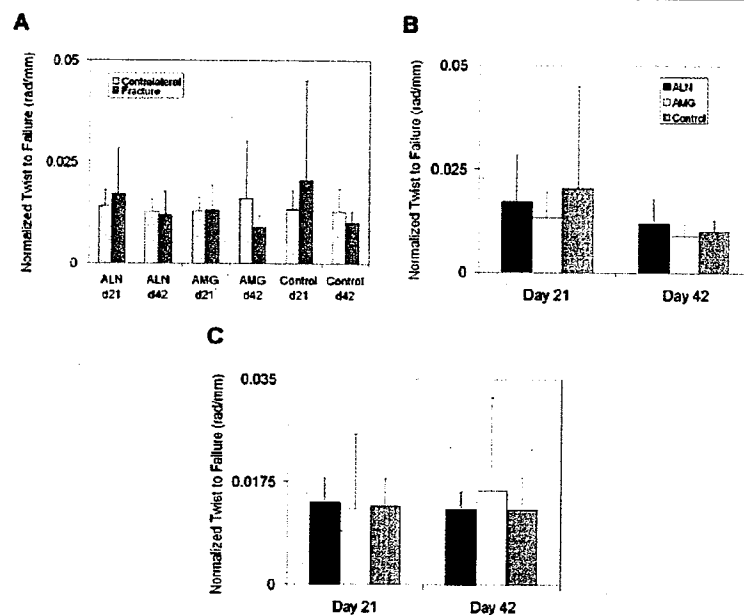


Figure 5. Graphical analysis of twist to failure normalized by gauge length. (A) Comparison between fracture and intact bones. (B) Comparison of fracture bones at both time points after fracture. (C) Comparison of contralateral bone at both time points after fracture. For all graphs, error bars are \pm SD. No significant differences were observed.

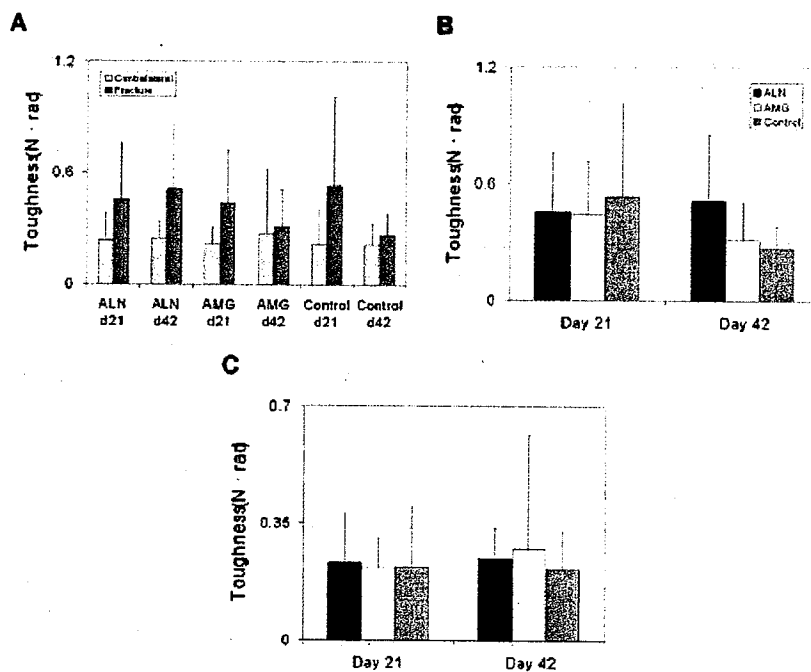


Figure 6. Graphical analysis of toughness (work to failure). (A) Comparison between fracture and intact bones. (B) Comparison of fracture bones at both time points after fracture. (C) Comparison of contralateral bone at both time points after fracture. For all graphs, error bars are \pm SD. No significant differences were observed.

As displayed in Figure 8 below showing the representative microCT single slices of the fracture calluses 42 days after fracture, the morphometry of bone healing, i.e. fracture calluses from the denosumab-treated mice were morphologically distinct as compared to the alendronate and vehicle-treated mice. In addition, the fracture calluses for the denosumab treated knock-in mice 42 days post fracture had increased bone volume (BV), BV (%), bone mineral content (BMC), trabecular thickness, and BV/TV (%) relative to alendronate and vehicle control mice (see Figure 9, below).

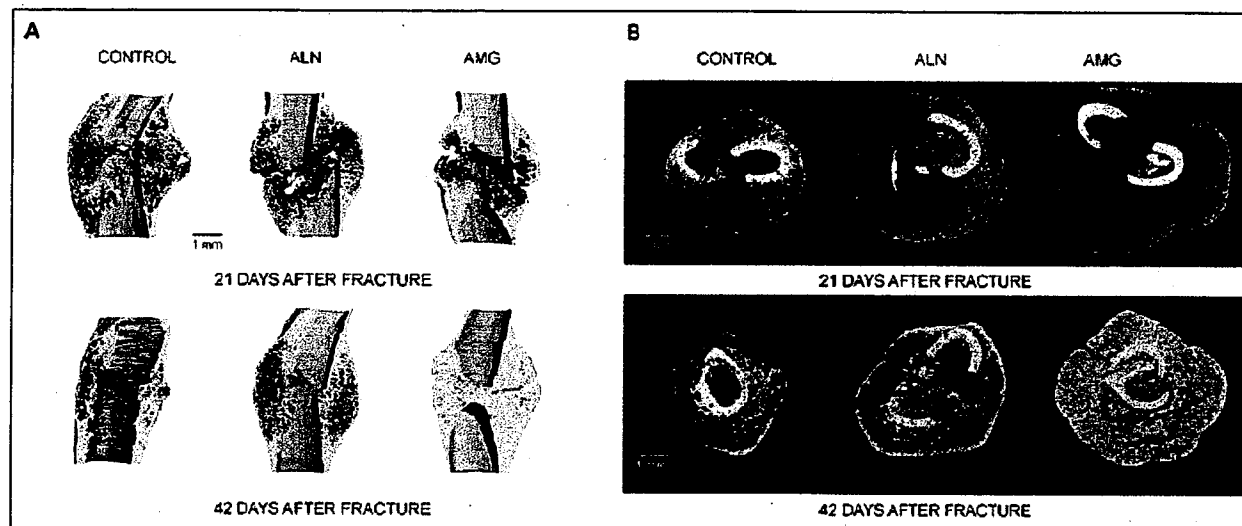
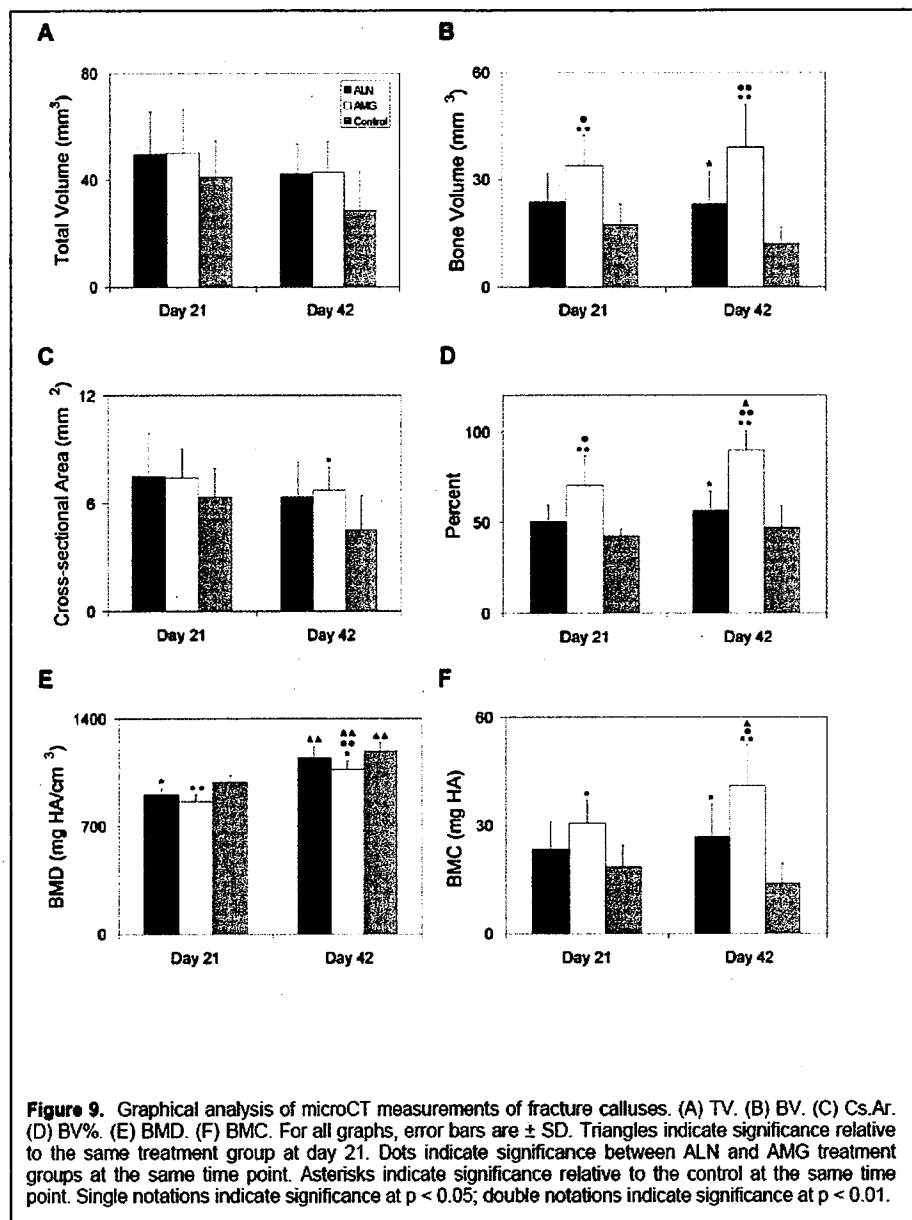


Figure 8. MicroCT images of fracture healing in control, ALN, and AMG mice showing delay in remodeling. (A) Representative 3-D reconstructions of fracture calluses at 21 and 42 days after fracture (coronal plane cutaway). (B) Representative microCT single slices of fracture calluses at 21 and 42 days after fracture (transverse section).



Study Conclusions:

The results from this study provide evidence that both alendronate and denosumab induced morphological delays in fracture healing, based on the results from the 21 and 42 post-fracture time points. Furthermore, both alendronate and denosumab treatment delayed the removal of cartilage, remodeling of the fracture callus, and resulted in altered bone morphologies as compared to the control mice. Both alendronate and denosumab treated mice had greater areas of tissues with reduced amounts of mineral content in the 21 day post-fracture calluses, as compared to the control calluses. The areas of low mineral content correlated with areas of unresorbed cartilage based on the qualitative histological analysis at day 21. The data indicate that the time course of cartilage

resorption and tissue remodeling was delayed in mice treated with either denosumab or alendronate fracture healing, as compared to fracture healing in the control mice.

The fracture calluses in the denosumab-treated mice were morphologically distinct as compared to the vehicle control or alendronate-treated mice. In addition, the fracture calluses for the denosumab treated knock-in mice 42 days post fracture had increased bone volume (BV), BV (%), bone mineral content (BMC), trabecular thickness, and BV/TV (%), relative to alendronate and vehicle control mice (see Figure 9).

Even with the morphological differences observed on Day 42, and the increased BV, BV (%), BV/TV (%), BMC, and trabecular thickness, the mechanical strength of the bone was not negatively affected. Instead, at the 42 day time point both denosumab and alendronate treatment induced increases in the strength and stiffness (see Table 1; Figures 4 and 5 above for the supporting data). Alendronate and denosumab increased the torsional stiffness of the bone as compared to the contralateral (nonfractured) femur on days 21 and 42. In addition, alendronate and denosumab treatment increased the stiffness of the healing, fractured femur relative to the vehicle control mice at day 42 post-fracture.

There was a small (~ 8%) but significant reduction in maximum torque in the alendronate treated group femurs as compared to the control, and an increase in maximum torque relative to vehicle control on days 21 and 42 post-fracture. Denosumab increased the torsional rigidity at day 42 post-fracture relative to the control. Overall, the maximum torque and torsional rigidity were approximately similar between the treatment groups (alendronate, denosumab, and vehicle control) on day 21 and day 42 post-fracture.

2.6.2.2.4 Study title: Effects of denosumab (AMG 162) on bone mass and bone resorption in human RANK ligand knock-in mice.

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings:

The main objective of this study was to determine whether human OPG-Fc (recombinant human osteoprotegerin) is a reasonable surrogate for the pharmacologic effects of denosumab (in a human RANKL knock-in mouse), and confirm that the 5th exon of human RANKL possesses a crucial binding or neutralizing epitope for denosumab. The results of this study concluded:

- OPG-Fc significantly inhibited bone resorption and increased BMD in both WT and KI mice.
 - Since OPG is the natural endogenous inhibitor of RANKL, it follows that it should be effective in both WT and KI
- Denosumab significantly inhibited bone resorption and increased BMD in huRANKL KI mice, but not in WT mice.
 - Since denosumab neutralizes human RANKL, but does not bind or neutralize mouse or rat RANKL, it follows that it should only be effective in KI mice.

- Since the change in RANKL gene between human and mouse was created at the 5th exon (murine RANKL was knocked in with the 5th exon of human RANKL), this signifies that this site has a specific denosumab binding/neutralizing site.
- Denosumab and OPG-Fc act similarly in KI mice
 - OPG-Fc represents a reasonable surrogate for pharmacologic effects of denosumab.

Study no.: R2004430

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.1.1

Conducting laboratory and location: Amgen, Inc., Thousand Oaks, CA

Date of study initiation: June 2003

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: AMG 162 (denosumab), Lot# 49A013190

huOPG-Fc, Lot# 43001B (for 03-20)

Lot# 0115643001 (for 04-15)

Methods

Doses: Studies used a fixed dose of 5 mg/kg denosumab. Study designs are shown below.

Study 03-20:

Group-Treatment	Animal Nos.	n	Sex	Route	Dose Level (mg/kg)	Conc. (mg/mL)	Volume (mL/kg)	Dosing Schedule
WT-Vehicle	1, 2, 3	3	2 male 1 female	SC	N/A	N/A	5	2x/week
WT-Denosumab	4, 5, 6	3	1 male 2 female	SC	5	1	5	2x/week
HET-Denosumab	8, 9	2	2 male	SC	5	1	5	2x/week
KI-Vehicle	10, 11 12, 13	4	4 male	SC	N/A	N/A	5	2x/week
KI-Denosumab	14, 15 16, 17	4	4 female	SC	5	1	5	2x/week
KI-OPG-Fc	18, 19 20	3	3 male	SC	5	1	5	2x/week

HET = heterozygous, KI = knock-in, N/A = not applicable, OPG-Fc = huOPG-Fc, WT = wild type

Study 04-15:

Group-Treatment	Animal Nos.	n	Sex	Route	Dose Level (mg/kg)	Conc. (mg/mL)	Volume (mL/kg)	Dosing Schedule
WT-Vehicle	17, 18, 20, 28, 31, 38, 40, 45	8	Male	SC	N/A	N/A	5	2x/week
WT-Denosumab	19, 21, 30, 35, 36, 37, 41, 46	8	Male	SC	5	1	5	2x/week
KI-Vehicle	1, 3, 7, 9, 10, 11, 25, 33	8	Male	SC	N/A	N/A	5	2x/week
KI-Denosumab	2, 5, 6, 8, 12, 26, 32, 34	8	Male	SC	5	1	5	2x/week
KI-OPG-Fc	13, 14, 15, 27, 42, 44, 48, 49	8	Male	SC	5	1	5	2x/week
WT-Vehicle	50, 53, 56, 57, 59, 62, 65, 68	8	Female	SC	N/A	N/A	5	2x/week
WT-Denosumab	22, 51, 54, 60, 61, 63, 66, 69	8	Female	SC	5	1	5	2x/week
WT-OPG-Fc	23, 24, 52, 55, 58, 64, 67, 70	8	Female	SC	5	1	5	2x/week

KI = huRANKL KI, N/A = not applicable, OPG-Fc = huOPG-Fc, SC = subcutaneous, WT = wild type

Species/strain: Mice were of the Black Swiss strain. Transgenic animals were created by injecting 129 ES cells into a B6 blast. The line was expanded by breeding the chimeras with a BLACK SWISS mouse to create the transgenic colony. Wild-type (WT) and human RANKL knock-in (KI) mice were used for these studies.

Number/sex/group or time point (main study):

Study 03-20: 12 males, 7 females; 3-4/group

Study 04-15: 40 males, 24 females; 8/group

Route, formulation, volume, and infusion rate: s.c. injection; volume = 5 mL/kg

Denosumab = 70 mg/mL in 10 mM Na acetate, 5% sorbitol, pH 5.2

huOPG-Fc = 10 mg/mL liquid in phosphate buffered saline (both lots);

Age: 6-8 weeks (Study 03-20) and 8-12 weeks (Study 04-15)

Weight: M=21-45 g; F=19-29 g

Observations and times:

Study 03-20: WT mice were treated s.c. with vehicle (PBS) or denosumab at 5 mg/kg, twice weekly for 3 weeks. huRANKL KI mice were treated s.c. with vehicle (PBS), denosumab or huOPG-Fc at 5 mg/kg twice weekly for 3 weeks. Heterozygous mice were treated with denosumab at 5 mg/kg twice weekly for 3 weeks (results not discussed b/c n=2 and no control). Blood samples were collected at necropsy for measurement of serum TRAP-5b (specific marker for osteoclasts). At the end of the study, tibiae were collected for static histomorphometry and pQCT analysis.

Study 04-15: WT male mice were treated with vehicle (PBS) or denosumab at 5 mg/kg, s.c., twice weekly for 3 weeks. WT female mice were treated with vehicle, denosumab or huOPG-Fc at 5 mg/kg, s.c., twice weekly for 3 weeks. huRANKL KI mice were treated with vehicle, denosumab or huOPG-Fc at 5 mg/kg, s.c., twice weekly for 3 weeks. Spine (lumbar vertebrae 1-4) BMD was measured at baseline by

DXA, and at week 1, 2 and 3 after treatment for males, and only at baseline and week 3 for females. Body weights were measured weekly. Blood samples were collected at 24 hrs and week 3 for serum TRAP-5b analysis. At necropsy, study tibiae were collected for static histomorphometry, and lumbar vertebrae and femur were collected for micro-CT analysis.

Results

Effects on bone densitometry – pQCT in huRANKL KI mice (Study 03-20)

In the proximal tibia metaphysis of male and female KI mice, denosumab significantly increased total and trabecular BMD and total and trabecular BMC over vehicle control, and to the same extent as OPG-Fc. Neither denosumab nor OPG-Fc had any effect on total or trabecular area in either KI or WT mice. Denosumab did not have a similar effect on total or trabecular BMD or BMC in WT mice.

Effects on spine BMD in male and female mice – DXA (Study 04-15)

Denosumab significantly increased spine BMD in male KI mice over vehicle controls during weeks 1-3. While OPG-Fc also increased BMD to some extent, the increase was not significant. There was no change in BMD with denosumab treatment in WT male mice. In female mice, only WT were examined in response to denosumab or huOPG-Fc treatment. OPG-Fc, and not denosumab, caused a significant increase in BMD over vehicle controls after 3 weeks of treatment. Since the actions of denosumab are unique to the KI mice, it indicates that the change in the 5th exon of the human RANKL gene to make the KI has a key binding or neutralizing epitope for denosumab.

Effects on serum TRAP-5b in male and female mice (Studies 03-20 and 04-15)

Serum TRAP-5b is a marker for osteoclast number and a measure of bone resorption. In Study 03-20, in KI mice, both denosumab and OPG-Fc significantly decreased TRAP-5b from vehicle control levels. Denosumab had no effect on WT mice. In Study 04-15, denosumab significantly decreased serum TRAP-5b in KI mice as early as 24 hrs after treatment, and continuing to 3 weeks after treatment. Denosumab did not have a significant effect on TRAP-5b in WT mice, but OPG-Fc significantly decreased TRAP-5b in female WT mice from vehicle controls after 24 hrs, but was not significant after 3 weeks. As a result, denosumab only decreases bone resorption in KI mice, and not WT mice.

Effects on bone histomorphometry parameters (Studies 03-20 and 04-15)

BV/TV (bone volume/tissue volume), Oc.S/BS (osteoclast surface/bone surface) and Ob.S/BS (osteoblast surface/bone surface) parameters in the proximal tibia were measured. In Study 03-20, denosumab and OPG-Fc increased BV/TV (2.5-fold) and decreased Oc.S/BS (to near zero levels) compared to vehicle controls in KI mice. Denosumab showed a slight but not significant increase in Ob.S/BS compared to vehicle control in KI mice. There was no effect of denosumab on any parameter in WT mice. In Study 04-15, neither denosumab nor OPG-Fc had any significant effect on BV/TV, Oc.S/BS or Ob.S/BS in KI male mice. OPG-Fc significantly increased BV/TV in female WT mice, and had a slight but non-significant decrease effect on

Oc.S/BS and Ob.S/BS. Denosumab did not have any effect on any parameter in WT male or female mice.

Micro-CT analysis of trabecular and cortical bone (Study 04-15)

Trabecular bone – In L6 vertebrae, denosumab non-significantly increased BMD, BVF, Tb.Th and Tb.N in male KI mice. OPG-Fc only appeared to non-significantly increase BMD. There were slight but non-significant increases by denosumab in BMD, BVF, Tb.Th and Tb.N in WT mice as well. In the distal femur, denosumab non-significantly increased BVF in male KI mice, while OPG-Fc had no effect. In the L6 vertebrae and distal femur of female WT mice, OPG-Fc significantly increased BMD, BVF, Tb.Th, Tb.N and Tb.Cn. Denosumab had slight but non-significant increases in BMD and Tb.N of L6.

Cortical bone – Denosumab had no effect on cortical area, periosteal perimeter or endocortical perimeter in WT male and female or KI male mice. OPG-Fc slightly but non-significantly increased cortical area in female WT mice.

2.6.2.2.5 Study title: Effects of denosumab (AMG 162) on bone mass and bone resorption in aged human RANK ligand knock-in mice.

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings:

The main objective of this study was to observe the effects of denosumab on bone mass and bone resorption in aged (10 months) huRANKL KI mice, since a previous study has demonstrated reduced bone resorption and increased BMD in 6-8 week old huRANKL KI mice treated with denosumab. The results of this study concluded:

- Denosumab decreased TRAP-5b and osteocalcin levels in KI mice, indicating decreased bone resorption and formation.
- Decreased biomarker levels correlated with decreased osteoclast and osteoblast surface measurements in relation to total bone surface.
- Denosumab increased trabecular BMD, BVF, thickness and number in lumbar vertebrae, femur and proximal tibia.
- Control KI animals inherently had lower osteocalcin levels and increased QCT endpoints such as BMD compared to WT mice, which could indicate that KI mice have less efficient bone resorption, and a slower rate of bone turnover.
- There did not appear to be substantial differences in bone mass or resorption in aged KI mice compared to younger KI mice evaluated previously.

Study no.: R2004321

Volume #, and page #: EDR 0012 3/11/09 Section 4.2.1.1

Conducting laboratory and location: Amgen, Inc., Thousand Oaks, CA

Date of study initiation: March 2004

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Denosumab, Lot# 49A013190

Methods

Doses:

Group-Treatment	Animal Nos.	n	Route	Dose Level (mg/kg)	Conc. (mg/mL)	Volume (mL/kg)	Dosing Schedule
WT-Vehicle	37-42	6	SC	N/A	N/A	0.021	1x/week
KI-Vehicle	1-6	6	SC	N/A	N/A	0.021	1x/week
KI-Denosumab	13-18	6	SC	2	0.095	0.021	1x/week
KI-Denosumab	19-24	6	SC	10	0.476	0.021	1x/week

KI = knock-in, N/A = not applicable, SC = subcutaneous, WT = wild type

Species/strain: wild-type (WT) and human RANKL knock-in (KI) mice of the Black Swiss strain.

Number/sex/group or time point (main study): 24 females; 6/group

Route, formulation, volume, and infusion rate: s.c. injection; volume = 21 mL/kg (this volume was provided in an amended report submitted March 2009 (SDN#12) which is why it is different from the table above).

Denosumab = 70 mg/mL in 10 mM Na acetate, 5% sorbitol, pH 5.2

Age: 10 months

Weight: 21-40 g

Observations and times:

huRANKL KI mice were s.c. treated with vehicle (PBS) or denosumab (2 or 10 mg/kg) once weekly for 3 weeks. WT mice were treated s.c. with vehicle only, once weekly for 3 weeks. Blood samples were collected before treatment and weekly after treatment for TRAP-5b and osteocalcin measurements. BMD (by DXA) was measured before treatment (baseline) and weekly after treatment. At the end of the study, right tibiae were collected for static histomorphometry. Lumbar vertebrae, left femur and tibia were collected for micro-CT analysis.

Results

Effects on serum TRAP-5b and osteocalcin

TRAP-5b is a marker of bone resorption and osteocalcin is a marker of bone formation. In KI mice, denosumab (2 and 10 mg/kg) significantly decreased serum TRAP-5b from vehicle controls after 1 week, but the decrease was only maintained through weeks 2 and 3 by 10 mg/kg denosumab. The Sponsor notes that the low response of 2 mg/kg denosumab may be due to an immune response by the immunocompetent KI mice to denosumab, though there was insufficient serum to confirm an anti-denosumab response. There was no difference in TRAP-5b levels between WT and KI mice when treated with vehicle. Osteocalcin levels in KI mice were significantly decreased from vehicle controls with both doses of denosumab, starting at week 2, and continuing to week 3. Interestingly, KI mice treated with vehicle had significantly lower osteocalcin levels than WT mice treated with vehicle.

This indicates that KI mice inherently have lower amounts of bone formation than their WT counterparts. It is unknown if this is inherent in aged mice versus young mice as osteocalcin was not measured in the previous study in younger mice.

Effects on areal BMD measurements

In the lumbar vertebrae and whole leg, there were no significant differences in areal BMD during treatment with either dose of denosumab from KI control values. The denosumab groups did have a higher baseline BMD than the KI controls in the lumbar vertebrae, but when looking at change from baseline, there were no differences. WT controls had a significant decrease in BMD in the lumbar vertebrae at Week 2 that seemed to recover closer to KI control values by week 3. This could be a study artifact. A similar decrease was not observed in the whole leg.

Effects on bone histomorphometry parameters

Denosumab showed a dose response increase in BV/TV (statistically significant at 10 mg/kg), a dose response decrease in Oc.S/BS (statistically significant at 10 mg/kg, and at zero), and a statistically significant decrease in Ob.S/BS with both doses, all compared to KI vehicle controls.

Effects on trabecular and cortical BMD by micro-CT

Lumbar vertebrae – Denosumab (2 and 10 mg/kg) significantly increased trabecular BMD (37-45%), BVF (71-80%) and thickness (Tb.Th) (41-52%) in KI mice over vehicle controls. There was no effect of denosumab on trabecular number (Tb.N). In comparison between vehicle treated WT and KI mice, KI mice had significantly higher levels for BMD (+62%), BVF (+103%) and Tb.N (+70%) than WT mice, but not for Tb.Th.

Femur – Denosumab (2 and 10 mg/kg) increased trabecular BMD (49-82%), BVF (116-140%), thickness (35-46%) and number (51-66%) in KI mice over vehicle controls. The increases had varying significance with the low dose having a significant increase in BMD and thickness, while the high dose was significantly increased for all parameters except BMD. As in the lumbar vertebrae, vehicle treated KI mice had higher (but not statistically significant) levels than vehicle treated WT mice for BMD (+46%), BVF (+190%), Tb.Th (+50%) and Tb.N (+117%).

Proximal tibia cancellous region – Denosumab (2 and 10 mg/kg) increased trabecular BMD (82-127%), BVF (165-267%), and number (81-157%) in KI mice over vehicle controls, but not thickness. The increases had varying significance with the low dose having a significant increase in BMD, BVF and number, while the high dose was significantly increased for only BMD. As in the femur, non-significant increases in BVF (+64%) and number (+102%) were observed in vehicle treated KI mice compared to WT mice, but were not observed for BMD or thickness.

Femur diaphysis – Denosumab (2 mg/kg) significantly increased cortical area (42%), cortical vBMD (3.5%) and cortical thickness (22%), while the higher 10 mg/kg dose only significantly increased cortical vBMD (2.5%). No changes in periosteal or

endocortical perimeter were observed after denosumab treatment. Of note, cortical thickness was significantly increased (+29%) in vehicle treated KI mice over WT mice, but no other parameters showed this difference.

Comments: Overall, there were no dramatic differences in the response of aged mice to denosumab treatment as compared to young mice. Small changes included an increase in trabecular BMD, BVF, thickness and number in the femurs of aged KI mice treated with denosumab, while young mice only showed a non-statistically significant increase in trabecular BVF of femur. In addition, cortical area was increased in aged KI mice treated with denosumab, while young mice showed no change from controls. These changes however, do not appear to be substantial.

This study labeled these mice as 'aged', however, 10-month old mice may not necessarily be considered 'aged', since a mouse's lifespan is approximately 2 years. Also, if the intent was to relate an 'aged' mouse to a postmenopausal woman, 10-month old mice are still reproductively capable, and a better way to evaluate that would be to include OVX mice, which was not done.

2.6.2.2.6 Study title: Denosumab, a fully human monoclonal antibody, has selective effects on human RANK ligand and human osteoclasts.

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings:

The objectives of the various in vitro assays and in vivo studies were to 1) determine the binding potency and selectivity of denosumab for human RANKL; 2) describe the proliferation and survival of murine osteoblast and bone marrow stromal cell cultures exposed to various concentrations of denosumab, huOPG-Fc, or various bisphosphonates; and 3) determine if denosumab exerts any obvious effects on the skeletons of normal mice or rats.

- Denosumab has 10-fold less binding affinity than huOPG-Fc to huRANKL, and does not bind murine RANKL.
- Denosumab only suppressed osteoclastogenesis in murine cells in vitro when they were stimulated with huRANKL, and had no effect on cancellous bone volume in mice or rats in vivo, indicating its inactivity towards murine RANKL.
- Denosumab was more effective than bisphosphonates in decreasing osteoclastogenesis in vitro.
- Denosumab decreases huRANKL-induced hypercalcemia.

Study no.: R2006351

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.1.1

Conducting laboratory and location: Amgen, Inc., Thousand Oaks, CA

Date of study initiation: September 2000

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity:

Drug	Lot #	Formulation
denosumab	11102300, A0010170015, 1110239, 49A013190	α -MEM + 10% FBS
huOPG-Fc	1811148M8, 1808029	α -MEM + 10% FBS
alendronate	not recorded	α -MEM + 10% FBS
zoledronic acid	not recorded	α -MEM + 10% FBS
Raloxifene	not recorded	α -MEM + 10% FBS
human RANKL	042499, 0078H159	10mM MES pH 6, 0.5M NaCl + PBS; α -MEM + 10% FBS
murine RANKL	061798	10mM MES pH 6, 0.5M NaCl + PBS; α -MEM + 10% FBS
human flag-tagged RANKL	5.12.99, pool 29-37	10mM MES pH 6, 0.5M NaCl + PBS; α -MEM + 10% FBS
chimeric RANKL (murine w/ human DE loop)	not available	10mM MES pH 6, 0.5M NaCl + PBS; α -MEM + 10% FBS
murine flag-tagged RANKL	011698	10mM MES pH 6, 0.5M NaCl + PBS; α -MEM + 10% FBS
murine M-CSF	not recorded	10mM MES pH 6, 0.5M NaCl + PBS; α -MEM + 10% FBS
TNF- α	03308	FACS buffer (Ca ²⁺ and Mg ²⁺ free PBS + 0.1% BSA + 0.01% Na azide)
TNF-b	not recorded	FACS buffer (Ca ²⁺ and Mg ²⁺ free PBS + 0.1% BSA + 0.01% Na azide)
TRAIL	hTrail 95-281-Flag L04248	FACS buffer (Ca ²⁺ and Mg ²⁺ free PBS + 0.1% BSA + 0.01% Na azide)
CD40L	hCD40L 9-29-00	FACS buffer (Ca ²⁺ and Mg ²⁺ free PBS + 0.1% BSA + 0.01% Na azide)

Methods**Osteoclastogenesis assay**

Cell line: murine RAW macrophage cells

Concentration: 5×10^3 cells/mL

Brief method: Macrophages were cultured with murine M-CSF (30 ng/mL) and huRANKL (30 ng/mL) for 5 days. Cultures were treated every 3 days with fresh media w/ various concentrations of huOPG-Fc or denosumab. Osteoclast formation was measured by quantifying TRAP using a solution assay.

Osteoclastogenesis co-culture assay

Species/strain: mouse/C3H/HEN

Number/sex: 3-10 male mice per sample (10^6 cells/mouse)

Age: 4-6 weeks old Weight: 20-30 g

Tissue type: bone marrow

Species/strain: mouse

Number: 1×10^5 cells/mL

Tissue type: ST-2 stromal cells

Brief method: Bone marrow cells were harvested from mouse femurs and tibiae.

Non-adherent murine bone marrow cells were co-cultured with murine ST-2 stromal cells (source of murine RANKL and M-CSF), and incubated in presence of 1,25-(OH)₂D₃, dexamethasone and PGE₂. Cultures were treated w/ various

concentrations of human Fc, huOPG-Fc or denosumab. Osteoclast formation was measured by quantifying TRAP using a solution assay.

Osteoclastogenesis assay with murine bone marrow cells + human RANKL + megakaryocyte colony-stimulating factor (M-CSF)

Species/strain: mouse/C3H/HEN

Number/sex: 3-10 male mice per sample (10^6 cells/mouse)

Age: 4-6 weeks old Weight: 20-30g

Tissue type: bone marrow

Brief method: Non-adherent murine bone marrow cells were incubated in 96-well dishes in presence of murine M-CSF (30 ng/mL) and huRANKL (30 ng/mL) for 5-6 days. Cultures were treated once with various concentrations of denosumab, alendronate, zoledronic acid, or raloxifene. Osteoclast formation was measured by quantifying TRAP using a solution assay.

Competitive assay for denosumab binding to RANKL-expressing cells

Cell line: Chinese hamster ovary (CHO) (b) (4)

Brief method: Denosumab (100 mg/mL) was preincubated with various concentrations of huRANKL or other ligands (TNF- α , TNF- β , TRAIL, or CD40L). These mixtures were added to cells incubated with FITC-labeled F(ab')₂ goat anti-human IgG. Cell surface fluorescence was measured by flow cytometry.

Osteoblast proliferation and survival assay

Cell line: mouse MC3T3-E1 osteoblasts (20,000 cells/well)

Cell line: ST-2 bone marrow stromal cells

Brief method: Murine osteoblasts and murine ST-2 bone marrow stromal cells were cultured in 96-well dishes and drugs were added to cultures for 72 hrs. Total live cell number was evaluated with a non-radioactive cell proliferation assay.

Radiographic bioassay in normal mice

Species/strain: mouse/BDF1

Number/sex: 4 males/group Age: 3-4 weeks Weight: 18-22g

Brief method: Male mice were injected once daily for 4 days with varying doses of denosumab or huOPG-Fc, and then sacrificed. Vehicle and treated mice were included in each radiograph. Cancellous bone volume was scored by comparing the left and right proximal tibial metaphysis of control and treated mice. A positive score was recorded if tibia from treated animals had obviously greater radiographic opacity compared with vehicle control bone.

Radiographic bioassay in normal rats

Species/strain: Sprague-Dawley rat

Number/sex: 4 males/group Age: 4 weeks Weight: 75-125g

Brief method: The same method was used as in mice (see above). Differences included use of rats, and daily injections of denosumab or huOPG-Fc for 11 days.

Hypercalcemia assay

Species/strain: mouse/BDF1

Number/sex: 35; 5 males/group Age: 4 weeks Weight: 16-21g

Brief method: Intact male mice were injected with huRANKL (0.5 mg/kg) twice daily, or with PBS. Mice treated with RANKL were immediately treated with a single s.c. injection of either huOPG-Fc (3 mg/kg) or denosumab (0.3-10 mg/kg). Blood ionized calcium was measured daily.

Results

Osteoclastogenesis assay with murine RAW macrophage cell line

Denosumab and huOPG-Fc both decreased TRAP activity (suppressed osteoclastogenesis) in RAW macrophages stimulated with huRANKL. The IC_{50} for denosumab was 1.64 ng/mL, and for huOPG-Fc was slightly less at 1.15 ng/mL. According to the Sponsor, since the weight of denosumab is roughly twice that of huOPG-Fc, the potency of denosumab was similar to or slight greater than that of huOPG-Fc.

Osteoclastogenesis coculture assay

In murine bone marrow cells co-cultured with murine ST-2 stromal cells (as a source of murine M-CSF and murine RANKL), huOPG-Fc significantly decreased TRAP activity from control (human Fc) (more than 50% decrease) at a concentration as low as 10^{-11} M, up to 10^{-5} M. Denosumab did not alter TRAP activity compared to control at any concentration. This indicates the inability of denosumab to affect murine RANKL.

Osteoclastogenesis assay with murine bone marrow cells + huRANKL + M-CSF

In murine bone marrow cells cultured with murine M-CSF and huRANKL, denosumab was most effective at decreasing TRAP activity (suppressing osteoclastogenesis) with an IC_{50} of 10^{-14} M, compared to alendronate ($IC_{50} = 10^{-7}$ M), zoledronic acid ($IC_{50} = 10^{-7}$ M) or raloxifene ($IC_{50} = 10^{-5}$ M).

Enzyme immunoassay to measure binding of huOPG-Fc or denosumab to murine or human RANKL

The binding of denosumab or huOPG-Fc to various constructs of murine and human RANKL were investigated. These constructs included a fragment of native human RANKL (amino acids 143-317), human flag-tagged RANKL (143-317), full-length murine RANKL, murine flag-tagged RANKL, and a chimeric RANKL protein comprised of full-length murine RANKL with the DE loop substituted for the human DE loop (DE loop thought to represent a critical binding domain for denosumab). Denosumab did not bind to either murine RANKL or murine flag-tagged RANKL, as expected, but showed strong binding to human RANKL, human flag-tagged RANKL and chimeric RANKL. These results show that substituting the human DE loop into murine RANKL affords binding by denosumab, and that this is a critical binding domain. Binding of huOPG-Fc to RANKL appeared to be fairly equivalent among the multiple constructs, showing no real preference for human or murine RANKL.

Binding affinities of denosumab and huOPG-Fc to human RANKL

BIAcore and KinExA assays were used to assess binding affinity of denosumab and huOPG-Fc to huRANKL. The Sponsor notes that the data reached the BIAcore instrument limitations for kinetic analysis, so the KinExA data will be evaluated here. The data show that huOPG-Fc has a 10-fold higher binding affinity for huRANKL than denosumab, with a K_D of 2.6×10^{-13} M (denosumab = 3×10^{-12} M). As such, less huOPG-Fc is necessary to occupy half of the available huRANKL receptors at equilibrium.

Competitive assay for denosumab binding to RANKL-expressing cells

Denosumab was able to bind to the cell surface of CHO cells transfected with huRANKL. Upon titration of increasing concentrations of soluble huRANKL, there was decreased binding of denosumab to CHO cells due to competition. The additional ligands added to the incubation, TNF- α , TNF- β , TRAIL, CD40L, did not inhibit denosumab binding to CHO cells.

Competitive assay for denosumab binding to immobilized RANKL

Denosumab was able to bind to immobilized huRANKL coated on EIA plates. As in the CHO cell assay, increasing concentrations of soluble huRANKL were able to decrease denosumab binding as a sign of competition. Addition of ligands TNF- α , TNF- β , TRAIL, or CD40L did not inhibit denosumab binding to immobilized huRANKL.

Osteoblast proliferation and survival assay

Both denosumab and huOPG-Fc did not have any deleterious effect on live cell numbers compared to controls when incubated at various concentrations with either stromal cells or osteoblasts. The bisphosphonates pamidronate and zoledronic acid both decreased live cell number at concentrations ≥ 1 μ M in stromal cells and 100 μ M in osteoblasts.

Radiographic bioassay in normal mice and rats

Denosumab did not have any effect on the radiographic density of any of the eight mouse or rat proximal tibial metaphysis samples examined, while huOPGFc induced significant increases in cancellous bone volume in all eight tibial metaphysis samples in both mice and rats. The Sponsor notes that suppression of bone resorption is expected to increase cancellous bone volume in this region due to preservation of primary spongiosa normally resorbed during longitudinal bone growth. Denosumab likely has no effect since it does not bind to murine RANKL, while huOPG-Fc does.

Effects of denosumab or huOPG-Fc on hypercalcemia induced by huRANKL

Mice were treated with either huRANKL alone, or in combination with huOPG-Fc or concentrations of denosumab to examine the effects on blood ionized calcium levels in the mice. huRANKL alone significantly increased calcium levels over vehicle controls (hypercalcemia) (+35%) from days 2-4 after treatment. This indicates that huRANKL was able to activate murine RANK, leading to increased mobilization of calcium due to increased osteoclast activity. The addition of increasing concentrations

of denosumab (0.3-10 mg/kg) decreased this effect, and at 10 mg/kg, brought calcium levels to control levels by day 4. huOPG-Fc plus huRANKL decreased calcium levels from RANKL alone (~7%), but not to the same extent as denosumab.

2.6.2.3 Secondary pharmacodynamics

In addition to the RANK/RANKL pathway being a key regulator of osteoclast formation, survival, and function, RANKL is expressed on activated T and B cells, and RANK is expressed in mature dendritic cells. The RANKL-RANK pathway has been shown to modulate Aire⁺ (autoimmune regulator) thymic medullary epithelial cells and RANKL mRNA is expressed in the adult thymus in mice^{2,3}. It should be noted that Aire-expressing medullary thymic epithelial cells play a role in preventing autoimmunity. Of importance, dendritic cells are specialized cells designed to capture and process antigens. Contact with an antigen induces the maturation of the dendritic cells in response to inflammatory stimuli. The mature dendritic cells then migrate to secondary lymphoid organs and interact with T and B cells that are involved with the adaptive immune responses. There is evidence in the literature indicating that RANKL expression is induced in the skin following inflammation of the skin by ultraviolet radiation exposure or infection⁴. Furthermore, mice engineered to overexpress RANKL, albeit high levels via the keratin-14 promoter, displayed decreases in cutaneous contact hypersensitivity responses⁴. There are data in the literature using animal models which indicate that the inhibition of the RANK/RANKL signal transduction pathway could potentially compromise immune functions necessary to avoid the development of infections, result in increases in contact hypersensitivity responses (contact dermatitis), or increase autoimmunity responses. It is currently unclear what threshold of suppression of the RANK/RANKL pathway is necessary to modulate the immune functions discussed above, as many of the studies were performed in animals completely devoid of RANKL or RANK expression, or in animal models that express exceedingly high levels of RANKL or RANK. Studies investigating osteoprotegerin (OPG) that inhibits RANKL in mice indicated that OPG did not affect cell-mediated reactions such as contact hypersensitivity to the hapten oxazolone, or liver damage, granuloma formation, and infectious load in response to mycobacterial infection. However, OPG increased humoral reactions such as production of IgM, IgG, and IgE against the T cell dependent keyhole limpet hemocyanin, and the production of IgM against the T cell independent antigen Pneumovax⁵.

² White AJ, Withers DR, Parnell SM, Scott HS, Finke D, Lane PJ, Jenkinson EJ and Anderson G (2008) Sequential phases in the development of Aire-expressing medullary thymic epithelial cells involve distinct cellular input. *Eur. J. Immunol.* 38:942-947.

³ Rossi SW, Kim MY, Leibbrandt A, Parnell SM, Jenkinson WE, Glanville SH, McConnell FM, Scott HS, Penninger JM, Jenkinson EJ, Lane PJ and Anderson G (2007) RANK signals from CD4(+)3(-) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. *J. Exp. Med.* 204:1267-72.

⁴ Loser K, Mehling A, Loeser S, Apelt J, Juhn A, Grabbe S, Schwarz T, Penninger JM and Beissert S (2006) Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. *Nat. Med.* 12:1372-1379.

⁵ Stolina M, Guo J, Faggioni R, Brown H and Senaldi G (2003) Regulatory effects of osteoprotegerin on cellular and humoral immune responses. *Clin Immunol.* 109:347-54.

The absence of RANKL or RANK genes in knock-out mice leads to the complete failure of lymph node development⁶ and an absence of lactation via the inhibition of mammary gland maturation.⁷ High levels of protein expression have been observed in skeletal and lymphoid tissues. In addition, RANKL mRNA expression has been detected in keratinocytes of skin, mammary epithelial cells, heart, skeletal muscle, lung, stomach, placenta, thyroid gland and brain.⁸

Inhibition of the RANK/RANKL in knock-out mice and in rats expressing osteoprotegerin (OPG) induced a failure of incisor tooth eruption. In addition, the 6/12-month toxicology study in cynomolgus monkeys (study #102090) provides evidence that inhibition of the RANKL/RANK pathway with denosumab induced deleterious changes in the epiphyseal growth plates that were not closed prior to treatment. Based on this study and additional data provided in this submission, the sponsor has indicated that denosumab should not be used in pediatric patients in the proposed product label.

2.6.2.4 Safety pharmacology

2.6.2.4.1 Study title: A Single-Dose Subcutaneous Administration of AMG 162 for Cardiovascular and Respiratory Evaluation in Cynomolgus Monkeys

Reviewed by: Michael Orr, Ph.D.

Key findings:

- A single subcutaneous injection of denosumab (AMG 162) at 0.3, 3, 30 mg/kg did not induce changes in clinical signs, body weight, blood pressure, heart rate, body temperature or respiration rate, relative to the vehicle control dose group.
- The ECG recordings were normal for animals in all dose groups evaluated throughout the seven day monitoring period, except for one male monkey in the 3 mg/kg dose group that had a run of four ventricular premature complexes approximately 45 minutes following administration of denosumab. Following this run of four ventricular premature complexes, the ECG readings for this animal returned to normal for the rest of the 7 day duration of this study.

Comment: Female monkeys were not evaluated in this safety pharmacology study. As the indicated patient populations include women with breast cancer undergoing hormone ablation therapy and women with postmenopausal osteoporosis, it is unclear why female monkeys were not evaluated in this safety pharmacology study

⁶ Kong Y-Y, Yoshida H, Sarosi I, Tan H-L, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ and Penninger JM (1999) OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 397:315-323.

⁷ Martin TJ and Gillespie MT (2001) Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL): Another Link Between Breast and Bone. *Trends Endocrinol. Metab.* 12:2-4.

⁸ Leibbrandt A and Penninger J (2008) RANK/RANKL: Regulators of Immune Responses and Bone Physiology. *Ann. N.Y.Acad.Sci.* 1143:123-150.

Study #: (b) (4), Amgen study number 101606

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.1.3

Conducting Laboratory and Location (b) (4)

Date of Study Initiation: not specified (final report dated July 27, 2001)

GLP Compliance: yes

QAU statement: yes (X) no ()

Drug Lot #: 049A053686 (denosumab), 124K4712 (alendronate), 049A022940 (denosumab vehicle)

Methods: (abstracted from the final study report)

The test article, AMG 162, and the vehicle control article, AMG 162 Placebo, were supplied by the Sponsor as preformulated aqueous solutions in 1- and 5-mL vials, respectively, and were maintained at -70° to -76° and 3° to 5°C, respectively, when not in use. The concentration of the active ingredient in the test article solution was 30 mg/mL. This solution was used as received for administration of the highest dose, and was diluted with AMG 162 Placebo for preparation of the middle- and low-dose solutions.

A total of 12 male cynomolgus monkeys were used in this study. The animals were experimentally naive, and ranged from 2.3 to 7.3 years of age and 2.4 to 5.2 kg in weight at the outset of the study. At least 10 days prior to administration of the test or control article, a radiotelemetry transmitter was surgically implanted into each animal for monitoring and recording cardiovascular parameters.

The animals were assigned to treatment groups as shown in the table below.

Group No.	Day of Dosing	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Concentration (mg/mL)	Route of Administration	Number of Animals (Male)
1	Day 1	0 (control)	1.0	0	Subcutaneous	3
2	Day 1	0.3	1.0	0.3	Subcutaneous	3
3	Day 1	3	1.0	3	Subcutaneous	3
4	Day 1	30	1.0	30	Subcutaneous	3

Each animal received a single dose of test or vehicle-control article by subcutaneous injection on Day 1. Cardiovascular data (i.e., blood pressure, heart rate and electrocardiographic activity) and body temperature data were collected via telemetry prior to and following dose administration, beginning approximately one hour prior to dose administration and continuing until approximately 168 hours (7 days) postdose. In addition, the study animals were evaluated for changes in clinical signs by way of routine cageside observations, and respiration rate was measured by visual observation predose and 72 hours postdose. Blood samples were collected for test article concentration analysis prior to dosing, at approximately 48 and 96 hours (T_{max}) postdose, and on Day 8 (final day of the study). At the end of the study, the animals were returned to the Sierra Biomedical animal colony.

Results: A single subcutaneous injection of denosumab at 0.3, 3, 30 mg/kg did not induce changes in clinical signs, body weight, blood pressure, heart rate, body temperature or respiration rate based on the evaluation of three male monkeys per dose group relative to the vehicle control cohort. The ECG recordings were normal for animals in all dose groups evaluated throughout the seven day monitoring period, except for one monkey (F7481CQM) in the 3 mg/kg dose group, who had a run of four ventricular premature complexes (VPC) approximately 45 minutes following administration of denosumab. This particular monkey did not display changes in blood pressure or heart rate near the time of the multiple VPC occurrences (see Table 4a and 5a below provided by the Sponsor). Furthermore, the ECG readings returned to normal at the subsequent time points following this isolated event.

Table 4a: Individual Animal and Group Mean Blood Pressure
Study Number: 1129-01

		Mean Arterial Pressure													
		mmHg													
Animal Number	Sex	Approximate Recording Intervals (minutes)													
		-60	-50	-40	-30	-20	-10	10	20	30	40	50	60	70	
Group 1: Control (0 mg/kg)															
F20-122M	M	124	126	128	129	139	142	141	142	141	130	120	120	122	
FN1493QM	M	123	123	119	119	120	130	131	120	120	116	109	108	103	
FN16038M	M	134	133	123	110	111	129	130	113	105	98	97	97	96	
Mean		127	127	123	119	128	134	134	126	122	116	108	108	107	
S.D.		6	5	5	9	14	7	6	15	18	16	12	12	13	
Group 2: AMG 162 (0.3 mg/kg)															
FN14001M	M	119	130	124	121	119	111	115	114	111	110	104	98	98	
FN1496QM	M	138	123	125	134	139	134	129	118	114	109	105	109	108	
F15659M	M	94	90	87	89	87	91	88	79	75	78	82	78	70	
Mean		117	114	112	118	116	112	111	104	100	98	97	96	92	
S.D.		22	21	22	23	26	22	21	21	21	18	13	16	20	
Group 3: AMG 162 (3 mg/kg)															
F15544M	M	104	101	92	89	85	105	89	82	84	83	79	86	95	
F15676M	M	95	101	102	99	95	94	98	94	89	87	84	96	86	
F7481CQM	M	123	122	118	117	114	115	112	102	103	109	105	103	99	
Mean		108	108	104	101	98	106	109	83	92	93	91	92	88	
S.D.		14	12	13	15	13	11	12	10	10	14	13	10	7	
Group 4: AMG 162 (30 mg/kg)															
F7959CQM	M	105	102	99	105	108	106	101	100	97	91	90	90	90	
FN14028M	M	150	146	142	145	157	159	154	145	133	131	126	127	127	
FN15411M	M	131	131	126	129	134	138	132	127	112	110	110	108	106	
Mean		129	128	122	128	133	134	129	124	114	111	108	108	108	
S.D.		23	22	22	20	25	27	27	23	18	20	18	19	19	

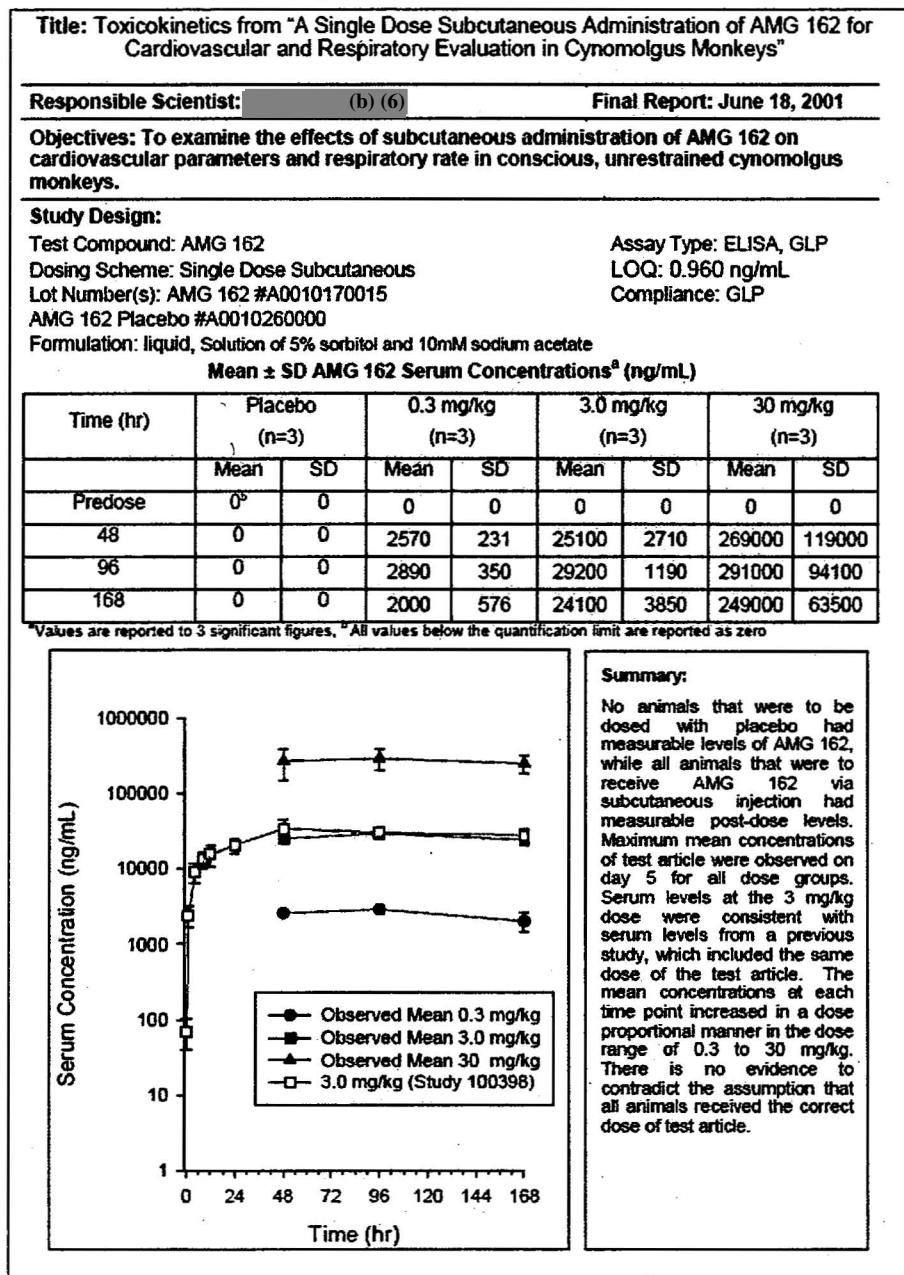
Table 5a: Individual Animal and Group Mean Heart Rate
Study Number: 1129-01

Animal Number	Sex	Heart Rate beats per minute													
		Approximate Recording Intervals (minutes)													
		-60	-50	-40	-30	-20	-10	10	20	30	40	50	60	70	
Group 1: Control (0 mg/kg)															
F20-122M	M	243	234	236	233	238	241	242	246	240	219	196	201	204	
FN1493QM	M	230	230	229	227	219	230	239	235	229	218	205	193	180	
FN16038M	M	239	236	228	204	219	245	246	222	202	183	172	170	166	
Mean		237	238	232	221	226	240	242	234	224	207	191	188	188	
S.D.		7	3	6	15	11	10	4	12	20	21	17	16	19	
Group 2: AMG 162 (0.3 mg/kg)															
FN14001M	M	134	228	218	210	215	223	206	184	180	171	149	139	134	
FN1496QM	M	250	226	221	231	240	250	238	216	204	183	167	184	177	
F15659M	M	232	231	200	192	187	203	155	169	150	142	144	131	114	
Mean		226	228	219	211	214	226	213	189	178	166	163	161	142	
S.D.		29	3	11	20	27	24	22	24	27	21	12	29	32	
Group 3: AMG 162 (3 mg/kg)															
F15544M	M	214	219	204	186	176	204	192	183	170	140	130	146	158	
F15676M	M	179	204	213	202	189	187	156	206	197	169	164	148	144	
F7481CQM	M	255	238	226	231	231	236	216	187	170	162	160	158	154	
Mean		216	220	214	208	188	208	201	182	178	167	161	161	162	
S.D.		38	17	11	23	29	25	13	12	16	15	19	6	7	
Group 4: AMG 162 (30 mg/kg)															
F7959CQM	M	242	242	230	233	241	246	242	232	226	202	199	199	196	
FN14028M	M	220	208	190	190	202	209	215	201	169	167	154	152	148	
FN15411M	M	258	261	250	247	251	258	254	244	223	206	201	202	193	
Mean		240	237	229	233	281	288	287	228	208	182	186	184	179	
S.D.		19	27	31	30	26	26	20	22	32	21	27	28	27	

Comment: The toxicological significance of the ECG findings for the one monkey (F7481CQM) in the 3 mg/kg dose cohort is unknown at this time. The isolated ventricular premature complexes were not evident at later time points in this particular monkey. No other abnormalities in the electrocardiograms were observed in animals in the vehicle control, 0.3 or 30 mg/kg dose groups, which comprised the evaluation of 11 other monkeys. This reviewer does not believe that the ECG

abnormality documented for this one monkey was due to the administration of denosumab, based on the collective information provided in this study.

Toxicokinetics: (as provided in the final study report)



Comments: Denosumab exposure levels were maintained for the duration of this study. A dose-dependent increase in the concentrations of denosumab from 0.3, 3, and 30 mg/kg were observed in the monkeys following subcutaneous administration.

Study conclusions: A single subcutaneous dose of denosumab from 0.3, 3, and 30 mg/kg did not induce blood pressure, heart rate, body temperature, or respiration rate

changes over a seven day follow-up evaluation period. This reviewer believes that the lack of time and dose dependence for the development of the ventricular premature complexes in one out of the 12 male monkeys suggest that this ECG abnormality was not due to a treatment effect of the 3 mg/kg dose of denosumab. Currently, there is no clear scientific explanation for why the four ventricular premature complexes were observed in the single male monkey from this dose cohort.

Comment: The ability to identify cardiac or pulmonary safety pharmacology issues based on single dose of denosumab is limited. Furthermore, this study utilized a single dose of denosumab, which would not enable concentrations of denosumab to reach steady state and subsequent penetration of deep tissue compartments in the animals.

2.6.2.4.2 Study title: The effects of OPG-Fc, RANK-Fc, or alendronate on tooth eruption and on bone density, geometry, and strength in neonatal rats

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings:

The main objective of this study was to observe the effects of RANKL inhibitors OPG-Fc or RANK-Fc, versus the bisphosphonate alendronate, on tooth eruption and on bone density geometry, and strength in neonatal rats. RANK-Fc is a RANKL inhibitor with similar mechanism of action to denosumab, and since denosumab does not recognize mouse or rat RANKL, OPG-Fc and RANK-Fc were used as surrogates. This study provided information on potential use in pediatric populations. The results of this study concluded:

- Decreased upper and lower incisor length and delayed molar eruption of 2nd and 3rd molars with OPG-Fc and ALN in both sexes.
- Decreased bone resorption (TRAP-5b reduction), body weights, axial skeleton length and femur length were observed with all treatments to varying degrees, however femoral and vertebral bone volume, density and strength were increased (no evidence of skeletal fragility).
- By micro-CT examination, the femur and L5 vertebrae showed marked accumulation of trabecular bone, as well as 'flared' morphometry in the femoral metaphysis (all treatments, males and females), and generally smaller vertebral cross sectional area (CSA).

Study no.: R20080340

Volume #, and page #: EDR 0012 3/11/09 Section 4.2.1.3

Conducting laboratory and location: Amgen, Inc., Thousand Oaks, CA

Date of study initiation: July 2008

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Alendronate (ALN) Lot# D00041255
 Murine RANK-Fc Lot# 80108-16
 Rat OPG-Fc Lot# 14032406

Methods

Doses: Select concentrations of sterile saline (vehicle), rat OPG-Fc, murine RANK-Fc or alendronate (ALN) once per week for 6 weeks per the experimental design below

Treatment	No. males	No. females	Route	Dose level (mg/kg)	Conc (mg/mL)	Volume (mL/kg)	Dose schedule
Vehicle	13	16	s.c.	0	10	5.0	1x/week
Rat OPG-Fc	12	12	s.c.	1.0	0.2	5.0	1x/week
Rat OPG-Fc	10	9	s.c.	10	2	5.0	1x/week
Murine RANK-Fc	12	10	s.c.	10	2	5.0	1x/week
ALN	12	8	s.c.	0.1	0.002	5.0	1x/week
ALN	12	12	s.c.	1.0	0.2	5.0	1x/week

Species/strain: Sprague Dawley rats

Number/sex/group or time point (main study): 71 males, 67 females

Route, formulation, volume, and infusion rate: s.c. injection; volume = 5 mL/kg

Age: 2 weeks

Weight: Males = 106 g; Females = 86 g

Observations and times:

2-week old male and female rats were treated once per week for 6 weeks. They were kept with their mothers until 4 weeks of age, at which point their sex was determined and they were weaned. They were caged according to sex and fed standard rodent chow diet for the remaining 4 weeks. Blood samples were collected at weeks 2, 4 and 6 (7 days after previous treatment) to measure serum TRAP-5b. Right femur and 5th lumbar vertebra were harvested at necropsy for micro-CT and biomechanical analyses. Heads were collected at necropsy and mandible and maxilla were dissected to assess eruption of molars. Incisor length was measured from gingiva to tip of the tooth. Length of the axial skeleton was assessed at necropsy (tip of the nose to the anus).

Results

Effects on incisor length

Upper incisor length was dramatically decreased with high dose OPG-Fc treatment in both males and females (72% and 77%). Smaller but significant decreases were observed with high dose ALN in males and females. Slight increases in upper incisor length were observed with low dose ALN in males and females (statistically significant in females). As for lower incisors, decreases in size were not as dramatic, but were observed following treatment in males with high dose OPG-Fc and low and high dose ALN, and in females with only high doses of OPG-Fc and ALN.

Effects on molar eruption

In both maxillary and mandibular molars, high dose OPG-Fc in males decreased eruption of the left and right 2nd molars by 80% (2/10 eruptions occurring) and by 100% in left and right 3rd molars. Similar findings were observed in females with 100% of both left and right 2nd and 3rd maxillary molars and left and right 3rd mandibular molars not erupting, and 80% of left and right 2nd mandibular molars not erupting. High dose ALN also caused significant changes in tooth eruption with a 100% decrease in eruption for left and right 3rd maxillary and mandibular molars in both males and females. Low dose ALN caused more slight decreases in eruption of left and right 3rd maxillary and mandibular molars in males only at 25%.

Effects on serum TRAP-5b

TRAP-5b is a specific marker of osteoclasts and identifies bone resorption. In both male and female rats, OPG-Fc, RANK-Fc and ALN caused a decrease in TRAP-5b as early as 2 weeks after dosing, and continuing to week 6. The decrease with OPG-Fc was dose response-related, while with ALN the lower dose was more effective at 2 weeks, but did not maintain through week 6, while the higher dose was consistent throughout treatment. Overall, high dose OPG-Fc was most effective (-92% reduction at week 6), followed by RANK-Fc \approx low dose OPG-Fc, high dose ALN and low dose ALN.

Effects on body weights

In males, high dose OPG-Fc caused a significant decrease in body weight, beginning at week 2 and continuing to week 6 (6-43%). RANK-Fc caused significantly decreased body weights from week 2 to week 4 (4-9%) but weights were not different from controls at week 5 or 6. A slight decrease in weight was also observed with high dose ALN treatment after week 6 (9%). All other compounds did not show any significant decreases in body weight.

In females, significantly decreased body weights were observed at all weeks for each compound (except low dose OPG-Fc and week 2 low dose ALN). Decreases between weeks 2 and 6 were as follows: high dose OPG-Fc (10-39%), RANK-Fc (10-11%), low dose ALN (6-9%) and high dose ALN (8-13%). Clearly, high dose OPG-Fc caused the largest effects in both male and female rats.

Effects on axial skeleton lengths

In males, high dose OPG-Fc and high dose ALN both significantly decreased skeleton lengths at 16% and 3%, respectively, compared to controls. In females, all compounds decreased skeletal length except low dose OPG-Fc. The decreases were led by high dose OPG-Fc (15%), followed by high dose ALN (3%), low dose ALN (3%) and RANK-Fc (2%). Again, high dose OPG-Fc had the greatest effect on decreasing axial skeleton length.

Visible changes on femur geometry and density (micro-CT)

The following visible changes with treatment (micro-CT) were noted by the Sponsor:

- Marked accumulation and retention of trabecular bone in the distal half of the femur marrow cavity – males and females – all treatments.
- “Flared” morphometry in the distal femoral metaphysis – males and females – all treatments.
- Presence of trabecular bone in the femur midshaft

Effects on femur geometry and density

All of the compounds had significant effects on femur geometry and density patterns. All treatments resulted in reduced femur lengths in males and females, with high dose OPG-Fc causing the greatest reductions (21% and 18%) and more of the appearance of a club-shape. Increased midshaft vBMC was noted in males and females (33-67%) with all treatments except low dose ALN. The sponsor notes that this correlates with trabecular bone accumulation in the femur marrow. Increased distal femur cross-sectional area (CSA) was also noted in both sexes (20-65%) with all treatments except low dose ALN in females. The sponsor notes that this correlated with the ‘flared’ morphometry in the distal femur. Increased femur midshaft CSA was also increased in most groups (10-15%), which correlated with the presence of trabecular bone in this area, and resulted in the increased midshaft cross-sectional moment of inertia (CSMI) with all treatments except low dose ALN. Distal vBMC was also increased significantly (108-149%) in all treatment groups

Effects on femur bone strength

All treatment groups showed increased femur bending strength in males and females as measured by femur midshaft peak load. This correlates with the increased midshaft CSMI, as it is an important biomechanical parameter of bending strength. Stiffness was also increased in all treatment groups in males, but was only increased in females with low dose OPG-Fc and high dose ALN. Toughness was decreased with all treatment groups (except for low dose ALN in females). Ultimate strength was only increased after treatment with high dose ALN in males, and elastic modulus was only decreased after treatment with RANK-Fc. No changes in energy were noted with treatment.

In a separate cohort of rats, males and females treated with high dose OPG-Fc showed increased peak load, decreased stiffness, and decreased elastic modulus compared to controls. No significant changes in energy to failure, ultimate strength or toughness were observed. This cohort was analyzed separately (with separate controls and modified 3-point bending test) due to the shortness of their femurs after treatment with high dose OPG-Fc. The Sponsor notes that the modified test parameters actually caused changes in the control animals, which is why the high dose OPG-Fc animals had opposing outcomes from other treatments in regards to stiffness, elastic modulus and possibly toughness.

Visible changes on lumbar vertebral body (L5) geometry and density (micro-CT)

The following visible changes with treatment (micro-CT) were noted by the Sponsor:

- Increased trabecular bone volume – males and females – all treatments.

- Smaller L5 in males and females treated with high dose OPG-Fc vs. controls, as shown by reduced L5 height and central width (cross sectional area).

Effects on L5 vertebra geometry and density

All treatment groups showed increased central vBMC and vBMD over vehicle controls in males and females. Vertebral height and central CSA were decreased mostly with high dose OPG-Fc in males and females, and also with high dose ALN in males and females, and low dose ALN in females. This correlates with the overall visible smallness of L5 vertebra with high dose OPG-Fc.

Effects on vertebral strength

Peak load and stiffness were increased with all treatments in males and females. Elastic modulus was increased only with RANK-Fc treatment in females and toughness was decreased only with RANK-Fc treatment in females. Energy to failure was increased with all treatments in males and females, but was not always statistically significant. No changes in ultimate strength were observed. The Sponsor notes that vertebral peak load values were strongly correlated with central vertebral bone area in all treatments in both sexes.

Overall correlations in findings

Correlations in findings were found between:

- Trabecular bone accumulation correlated with increased midshaft vBMC and CSA.
- Flared morphometry correlated with increased distal femur CSA.
- Increased midshaft CSA correlated with increased midshaft CSMI.
- Decreased body weight correlated with decreased axial skeleton length.
- Increased peak load correlated with increased femur midshaft CSMI and central vertebral bone area.
- Treatment-related increases in bone mass and density correlated with increased L5 bone strength parameters.
- Inhibition of tooth eruption and growth was proportional to the magnitude of suppression of bone resorption.

All agents had significant effects on bone growth, strength and development, though the majority resulted from high dose OPG-Fc. Since the RANK inhibitors and bisphosphonates both had effects, the results are likely related to osteoclast inhibition rather than drug-specific effects.

REVIEW NOTE: Since OPG-Fc and RANK-Fc inhibit osteoclasts similarly to denosumab, it could be hypothesized that denosumab may act with similar results in neonatal/pediatric populations.

Neurological effects: None available

Renal effects: None available

Gastrointestinal effects: None available

Abuse liability: Not applicable

2.6.2.5 Pharmacodynamic drug interactions

No studies have been conducted. The Sponsor notes that hepatic microsomal metabolism plays a negligible role in the elimination of denosumab, and the specificity of denosumab for RANKL indicates that pharmacodynamic interaction with other drugs is unlikely. No effects on the pharmacodynamic activity of alendronate or denosumab were noted in a therapeutic switch study in monkeys.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

The following tables were submitted by the Sponsor (found in Modules 2.6.2 and 2.6.3)

Table 2. Effects of Denosumab on Serum TRAP-5b in Human RANKL Knock-in Mice

Group-Treatment	Dose (mg/kg)	Serum TRAP-5b (U/L)			
		Baseline	Day 7	Day 14	Day 21
WT-Vehicle	N/A	10.663 ± 0.736	7.926 ± 1.686	13.038 ± 2.093	14.720 ± 2.354
KI-Vehicle	N/A	11.331 ± 2.649	8.888 ± 1.725	9.113 ± 1.822	16.648 ± 4.382
KI-Denosumab	2	15.663 ± 3.827	0.845 ± 0.165 ^c	4.182 ± 2.548	16.535 ± 4.323
KI-Denosumab	10	17.655 ± 1.396	0.330 ± 0.023 ^c	0.322 ± 0.057 ^a	0.3700 ± 0.047 ^b

KI = huRANKL knock-in, WT = wild type, U = unit

Ten months old female WT and huRANKL KI mice were subcutaneously administered vehicle control (PBS) or denosumab at 2 or 10 mg/kg, 1x/week for 3 weeks. Serum TRAP-5b was measured as described in Research Report No. R2004321. Results are expressed as the mean ± SEM for n = 6 mice per group.

^ap < 0.05, ^bp < 0.01, and ^cp < 0.001 compared with vehicle-treated KI mice. One way ANOVA followed by Dunnett's comparison was used to determine the effect of treatment by comparing with vehicle-treated group in KI mice.

Source: Research Report No. R2004321

Table 6. Percent Change from Baseline in Densitometric Parameters After 16 Months of Denosumab Treatment in OVX Cynomolgus Monkeys

DXA aBMD Site	Sham + Vehicle	OVX + Vehicle	OVX + Dmab 25 mg/kg	OVX + Dmab 50 mg/kg
Lumbar Spine	0.84* (1.49)	-4.95 (1.16)	10.58** (1.64)	12.01** (1.25)
Total Hip	-0.60* (1.61)	-7.40 (1.41)	10.33** (1.00)	7.35** (1.52)
Femur Neck	-1.98 (2.78)	-5.60 (1.95)	11.29** (2.68)	8.64** (2.75)
Central Tibia	-4.98* (0.99)	-10.88 (1.40)	0.59** (0.92)	1.39** (0.75)
1/3 Distal Radius	-0.25 (1.45)	-4.33 (1.64)	2.75* (0.87)	3.12* (0.77)
Ultra-Distal Radius	-0.97* (1.17)	-5.38 (1.38)	2.56* (0.74)	2.83* (0.64)

pQCT Variable	Sham + Vehicle	OVX + Vehicle	OVX + Dmab 25 mg/kg	OVX + Dmab 50 mg/kg
Radial Meta. Total vBMD	-4.99* (1.67)	-7.95 (1.58)	2.04** (0.97)	2.00** (0.91)
Tibial Meta. Total vBMD	-4.97* (1.73)	-11.28 (1.24)	3.14** (1.23)	0.73** (1.06)
Radial Dia. Cortical Area	-2.07 (0.81)	-2.58 (1.05)	0.58 (0.67)	0.59** (0.33)
Tibial Dia. Cortical Area	-2.85* (1.17)	-7.81 (1.18)	1.37** (0.71)	0.71* (0.70)
Radial Dia. Cortical vBMD	-0.09 (0.31)	-3.11 (0.66)	0.92** (0.17)	0.68* (0.23)
Tibial Dia. Cortical vBMD	-0.76* (0.50)	-3.88 (0.65)	1.66** (0.32)	1.66** (0.34)

OVX = ovariectomy; Meta = Metaphysis, Dia = Diaphysis. Dmab = Denosumab

Data are presented as the mean \pm (SE), n = 14 to 20/group. *p \leq 0.05 vs. OVX-Veh, **p \leq 0.05 vs sham-vehicle. An ANOVA was performed, and if p < 0.05, then 4 pairwise comparisons (with Bonferroni's correction) were performed to compare each denosumab group to both the Sham + Vehicle and OVX + Vehicle controls. An additional t-test was used to compare OVX + Vehicle and Sham + Vehicle controls. Equal variance across groups was assessed using Levene's tests; rank-transformation was used to equalize variance if p \leq 0.05. If variance remained unequal, an ANOVA model accounting for unequal variance was utilized.

Source: Study Report No. 103981

Table 7. Bone Strength Parameters After 16-months of Denosumab Treatment in OVX Cynomolgus Monkeys

Peak Load (N)	Sham-Veh	OVX + Vehicle	OVX + Dmab 25 mg/kg	OVX + Dmab 50 mg/kg
L3-L4 Vert. Bodies	2704 (197)	2293 (135)	3516 ^{*,A} (182)	3549 ^{*,A} (206)
L5-L6 Vert. Cores	189 (15)	161 (15)	275 ^{*,A} (17)	255 ^{*,A} (18)
Femur Neck	1640 (74)	1490 (48)	1759 [*] (72)	1978 ^{*,A} (76)
Femur Diaphysis	1431 (53)	1299 (47)	1494 (64)	1479 (53)

Stiffness (N/mm)	Sham + Vehicle	OVX + Vehicle	OVX + Dmab 25 mg/kg	OVX + Dmab 50 mg/kg
L3-L4 Vert. Bodies	14040 [*] (907)	11897 (716)	17417 ^{*,A} (902)	16590 [*] (500)
L5-L6 Vert. Cores	1511 (110)	1381 (126)	2225 ^{*,A} (145)	2215 ^{*,A} (135)
Femur Neck	1433 (71)	1278 (86)	1550 (73)	1611 [*] (88)
Femur Diaphysis	1196 (43)	1085 (42)	1256 [*] (52)	1252 [*] (47)

OVX = ovariectomy; Vert = Vertebral.

Data are presented as the mean \pm (SD), n = 14 to 20/group. *p \leq 0.05 vs. OVX-vehicle. *p \leq 0.05 vs. OVX-vehicle and sham-vehicle. An ANOVA was performed, and if p < 0.05, then 4 pairwise comparisons (with Bonferroni's correction) were performed to compare each denosumab group to both the Sham + Vehicle and OVX + Vehicle controls. An additional t-test was used to compare OVX + Vehicle and Sham + Vehicle controls. Equal variance across groups was assessed using Levene's tests; rank-transformation was used to equalize variance if p \leq 0.05. If variance remained unequal, an ANOVA model accounting for unequal variance was utilized.

Source: Study Report No. 103981

Table 8. Biomechanical Testing of Fractured Bones

	Alendronate	Denosumab	Control
Post-fracture - Day 21			
Max Torque (N•m)	0.0461 \pm 0.0168 *	0.0544 \pm 0.0166	0.0502 \pm 0.0121
Torsional Stiffness (N•mm ² /rad)	4515.34 \pm 1918.15	6194.77 \pm 1332.02 ^C	5133.41 \pm 1892.51 ^C
Post-fracture - Day 42			
Max Torque (N•m)	0.0752 \pm 0.0343 * ^C	0.0661 \pm 0.0227 ^C	0.0513 \pm 0.0138
Torsional Stiffness (N•mm ² /rad)	8578.15 \pm 3227.85 ^C	9249.98 \pm 2957.82 * ^C	6690.09 \pm 2099.07 ^C

N = newtons.

Data shown are mean values \pm SD. Significant differences between alendronate, denosumab to control within the same time point are shown by *. The C denotes significant difference between the treatment group and contralateral value. Significant group differences were based on a two-way ANOVA followed by Tukey HSD post-hoc test with significance as p < 0.05.

Source: Research Report No. R2006458

Table 1. Pharmacology Overview – Safety Pharmacology								
Test Article: AMG 162								
Type of Study	Species/Strain	Method of Administration	Doses (mg/kg)	Sex and No. per Group	Evaluation	Noteworthy Findings	GLP Compliance	Study Number
	Cynomolgus Monkeys	SC (single dose)	0 (vehicle), 0.3, 3.0, 30	3M	Cardiovascular	No treatment-related effects on evaluated cardiovascular parameters (heart rate, systolic pressure, diastolic pressure, mean arterial pressure, body temperature, or ECG recordings). NOAEL: 30 mg/kg	Yes	101606 (1129-01)
					Respiratory	No treatment-related effects on evaluated respiratory parameter (respiratory rate) NOAEL: 30 mg/kg		
					Toxicokinetics	Concentration (ng/mL) ^a		
						Dose (mg/kg)	Mean	SD
						0 (Vehicle)	0	(0)
						0.3	2890	(350)
						3.0	29200	(1190)
						30	29100	(94100)
							0	

^a Serum concentration measured at 96 hours postdose; n = 3/group. ECG = electrocardiogram; GLP = Good Laboratory Practice; SC = subcutaneous; M = male; NOAEL = no observed adverse effect level; SD = standard deviation.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

- Elimination of denosumab is more rapid in WT mice than in huRANKL KI mice, due to absence of available ligand for denosumab to bind in WT mice.
- Serum concentration levels were similar in s.c. and i.v. treated mice.
- The F value (bioavailability) in s.c. treated mice was 86% compared to i.v. treated mice.
- In monkeys, single dose i.v. or s.c. administration of denosumab demonstrates triphasic, exposure, with nonlinear and greater than dose proportional AUC observed for doses <1 mg/kg, but was linear and dose proportional from 1-3 mg/kg.
- During weekly s.c. dosing in monkeys, AUC increased dose proportionally following both the first dose at Week 1 and the 4th dose at Week 4.
- Radiolabeled denosumab primarily distributes to the dose site skin, dose site subcutaneous tissue, thyroid/parathyroid, serum, lymph nodes, blood, spleen, and ovaries in monkeys.
- Unexpected concentration of radiolabeled denosumab was observed in the cornea of the eye in monkeys.
- Major route of elimination for denosumab is via the urine.
- Tissue cross reactivity studies in a variety of species tissue samples noted that denosumab bound to cells in the periphery of the cortex of lymph nodes and lymphoid nodules of the spleen, the periphery of lymphoid nodules in the gut-associated lymphoid tissue of the small and large intestines, cells in the lymph node of 1/3 human donors, lymphocytes lining the periphery of the paracortex in the lymph node, and to chondrocytes and the margins of their surrounding lacunae in the articular cartilage.
- In repeat dose toxicology studies, the AUC for denosumab was linear from 1-50 mg/kg, no accumulation was observed, and antibody formation was prevalent and inversely proportional to dose.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

2.6.4.3.1 Study title: Pharmacokinetics report for “A single dose pharmacokinetics study of denosumab (AMG 162) following intravenous administration to male or female huRANKL knock-in and wild-type mice”

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings: The results showed that in the presence of huRANKL in KI mice, plasma clearance of denosumab was increased 6-fold over that in WT mice where there was not a RANKL target to bind. In WT mice the AUC was 881 $\mu\text{g}\cdot\text{hr}/\text{mL}$ with a $t_{1/2}$ of 426 hrs, while in KI mice, the AUC was 150 $\mu\text{g}\cdot\text{hr}/\text{mL}$ with a $t_{1/2}$ of 78 hrs.

Study no.: 106892

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.2.2

Conducting laboratory and location: Amgen, Inc., Thousand Oaks, CA

Date of study initiation: July 2008

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Denosumab (AMG 162) (60 mg/mL) Lot# 049A041380

Methods

Doses: 0.1 mg/kg single bolus i.v. dose of denosumab

Species/strain: Wild-type, Group 1: Black Swiss mice

huRANKL knock-in, Group 2: OPG Ligand #86 CK P3 mice

Number/sex/group or time point (main study): 45 males or females in each Group (numbered 1-45 in Group 1, and numbered 46-90 in Group 2). Males and females were not separated out.

Route, formulation, volume, and infusion rate: i.v. injection; formulation = 10 mM sodium acetate, 5% sorbitol, pH 5.2; volume = 0.1 mg/kg single bolus

Age: 9-12 weeks

Weight: 19-35 g

Observation and times: Whole blood was collected via cardiac puncture from 3 test systems per time point per group (using a composite sampling approach) at pre-dose, 1, 8, 24, 48, 96, 168, 240, 336, 504, 672, 840, 1008, and 1344 hrs postdose for characterization of serum PK. Concentrations were determined using ELISA (LLOQ = 10.000 ng/mL).

All serum samples were also measured for levels of anti-denosumab antibodies. Concentrations less than 1 $\mu\text{g}/\text{mL}$ were considered to be at the level of background. Serum denosumab concentrations were excluded from PK analysis if the result from

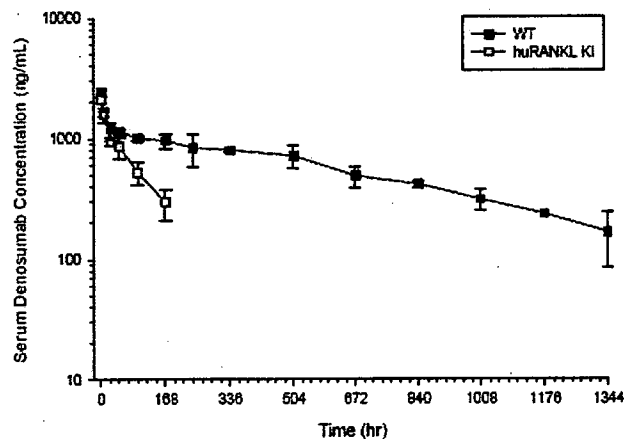
the antibody assay was greater than 2-fold over background ($>2 \mu\text{g/mL}$ = positive antibody result).

Results:

Pharmacology studies have shown that denosumab does not bind murine RANKL, but will bind to monkey RANKL. To investigate PK in mice, a huRANKL knock-in (KI) mouse model was used to compare to that seen in a wild-type (WT) mouse, since denosumab should bind to the huRANKL in the knock-in mouse, but not the murine RANKL in the wild type.

In WT mice, measurable concentrations of denosumab were observed throughout the study up to 1344 hrs. In KI mice, denosumab was only measured up to 168 hrs. In the anti-denosumab antibody analysis, only 1 WT mouse was anti-denosumab antibody positive (and excluded from analysis), while 25 KI mice were anti-denosumab antibody positive (and excluded from analysis). The initial serum concentration of denosumab was comparable in both WT and KI mice, however AUC was almost 6 times higher in WT mice versus KI, and $t_{1/2}$ was 5.4 times longer in WT than in KI mice. This correlates with the almost 6-fold increase in clearance of denosumab in KI mice. The initial volume of distribution and volume at steady state were comparable between WT and KI.

Overall, these results suggest that when denosumab is able to bind its target (e.g. huRANKL in KI mice), clearance is increased, as opposed to when there is not a specific target to bind to, and denosumab remains in the system (e.g. murine RANKL in WT mice).



Composite means for Ab negative animals are shown.

Denosumab PK parameter estimates following a single i.v. administration of 0.1 mg/kg denosumab to wild-type or huRANKL knock-in mice

Parameter	Unit	Group 1 WT	Group 2 KI
C_0	$\mu\text{g/mL}$	2.60	2.19
$\text{AUC}_{0-\infty}$	$\text{hr} \cdot \mu\text{g/mL}$	881	150
$t_{1/2,z}$	hr	426	78.6
CL	mL/hr/kg	0.114	0.667
V_0	mL/kg	38.5	45.7
V_{ss}	mL/kg	74.0	72.7

C_0 = initial serum concentration

$t_{1/2,z}$ = terminal half-life

CL = clearance

V_0 = initial volume of distribution

V_{ss} = volume of distribution at steady state

2.6.4.3.2 Study title: Pharmacokinetic Study of Denosumab (AMG-162) in Male Mice Following Intravenous or Subcutaneous Administration

Reviewed by: Michael Orr, Ph.D.

Key Findings:

- Following subcutaneous (s.c.) administration of 1 mg/kg, the C_{max} was reached 72 hours postdose and the half-life was 18.5 hours, which was similar to the half-life observed following i.v. administration. The clearance was similar between the 1 mg/kg s.c. and 1 mg/kg i.v. dose groups.
- Systemic bioavailability was 86.1% following s.c. administration

Study Number: Amgen Study No. 101494

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.2.2

Conducting Laboratory and Location: Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799

Date of Study Initiation: Not specified when study was initiated (final report dated February 1, 2007)

GLP Compliance: No

QAU statement: yes () no (X)

Drug Lot #: Denosumab # A0010170015; Denosumab formulation buffer A0010260000

Methods: Two hundred male C57BL/6J mice between 7 and 11 weeks old and weighing between 15 and 30 grams were dosed with a single s.c. or i.v. injection of denosumab (Lot Number A0010170015) or vehicle (denosumab formulation buffer, lot number A0010260000), as shown in Table 6-1. Approximately 0.5 mL of whole blood was collected via cardiac puncture from 2 animals from each group at sampling times (pre-dose, 15 and 30 minutes postdose, 1, 4, 8, 12, 24, 48, 72, 120, 168, 216, 336, 408, 504, 576, 672, 744, 912, 1080, 1248, 1416, 1584, and 1752 hours postdose). Whole blood samples were collected into Microtainer Brand Serum Separator Tubes, and kept at room temperature for approximately 20 minutes to clot. The samples were then centrifuged at approximately 11,500 rpm for 10 minutes at 26°C and the collected serum stored at approximately -70°C. Concentrations of denosumab in serum were determined using a sandwich ELISA assay with a limit of detection (LOD) of 0.781 ng/mL. The ELISA used utilized osteoprotegerin (OPG) for capture and detection of the analyte (denosumab). Noncompartmental analysis was performed using WinNonlin Professional (version 4.1e, Pharsight Corporation, Mountain View, CA). This was a non-GLP study.

Study Design (from the sponsor's study report):**Table 6-1. Experimental Design**

Group	Animal Numbers	Route	Dose (mg/kg)	Dose Rate (mL/kg)	Approximate Dose Volume ^a (mL)
1	1-50	SC	1.0	1	0.025
2	51-100	IV	0.1	1	0.025
3	101-150	IV	1.0	1	0.025
4	151-200	IV	10.0	1	0.025

^aApproximate dose volumes assumes a body weight of approximately 0.025 kg per mouse.

Results: The V_{ss} was approximately 40.1 mL/kg following i.v. administration of 1 mg/kg denosumab in mice, which indicates that denosumab is staying in the vascular space and is not widely distributed in the body. Following s.c. administration of denosumab at 1 mg/kg, the half-life was similar as compared to 1 mg/kg administered via i.v., the C_{max} was reached 72 hours postdose, and systemic bioavailability was approximately 86% in mice. Furthermore, as displayed in Figure 8-1 (provided by the Sponsor), the serum concentrations of denosumab in mice over time were similar regardless whether the route of administration was 1 mg/kg i.v. or 1 mg/kg s.c.

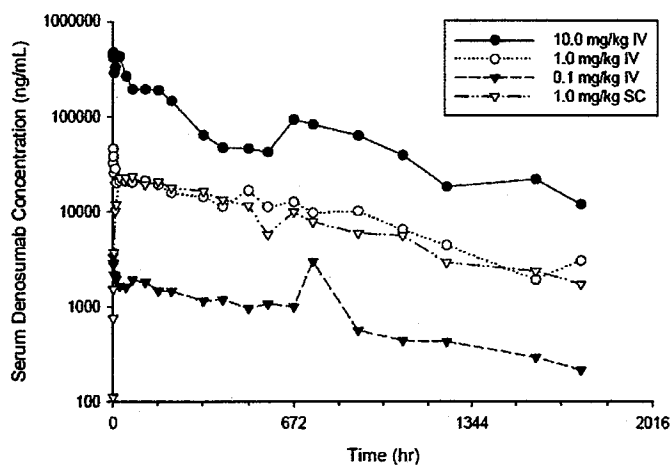
Figure 8-1. Composite Mean Serum Denosumab Concentration-Time Profiles Following SC or IV Administration to Male Mice (N=2/timepoint/group)

Table 8-1. Denosumab Pharmacokinetic Parameters Following SC or IV Administration of Denosumab to Male Mice
(based on composite sampling, N=2 mice/timepoint/group)

Parameter	Units	Route (Dose, mg/kg)			
		SC (1.0)	IV (0.1)	IV (1.0)	IV (10.0)
T_{max}	hr	72	NA	NA	NA
C_0^a	$\mu\text{g/mL}$	23.1	3.91	31.9	511
$t_{1/2,z}$	days	18.5	17.5	19.3	19.2
$AUC_{0-\infty}$	$\mu\text{g}\cdot\text{hr/mL}$	15600	1680	18100	128000
CL^b	mL/hr/kg	0.0642	0.0594	0.0553	0.0778
V_{ss}	mL/kg	NA	43.5	40.2	48.6
F	%	86.1	NA	NA	NA

^a C_{max} for SC dose group

^b CL/F for SC dose group

NA = Not applicable

C_0 = initial serum concentration

C_{max} = maximum observed concentration

T_{max} = time of C_{max}

$t_{1/2,z}$ = half-life

$AUC_{0-\infty}$ = Area under the concentration time curve from time 0 to infinity

CL = Clearance

CL/F = Apparent clearance

V_0 = Initial volume of distribution

V_{ss} = Volume of distribution at steady state

F = Bioavailability

Values are reported to 3 significant figures, except for T_{max} which is reported to 2 significant figures.

Study Conclusion: The serum concentrations of denosumab in mice over time were similar regardless of whether the route of administration was 1 mg/kg subcutaneous or 1 mg/kg intravenous. Furthermore, systemic bioavailability was approximately 86% in mice administered denosumab through the subcutaneous route of administration.

2.6.4.3.3 Study title: A Single Dose Pharmacokinetics Study of Denosumab (AMG-162) Following Intravenous Administration to Male or Female FcRn Knockout and Wild Type Mice

Reviewed by: Michael Orr, Ph.D.

Key Findings:

- The presence of the FcRn decreases the clearance and tissue distribution of denosumab in mice.
- The half-life in the WT mice was ~ 26-fold longer as compared to the FcRn KO mice, and there was ~ 15-fold increase in the clearance rate for the FcRn KO mice as compared to the WT mice administered denosumab.
- There was a reduction in the V_{ss} in the FcRn KO animals (V_{ss} = 52.3 mL/Kg at 0.1 mg/kg and 58.6 mL/kg at 1 mg/kg) as compared to the WT animals (V_{ss} = 97.6 at 0.1 mg/kg and 100 at 1 mg/kg). The WT mice displayed ~ 15-fold greater exposure based on the $AUC_{0-\infty}$.

Study Number: Amgen Study No. 106893

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.2.2

Conducting Laboratory and Location: Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320-1799

Date of Study Initiation: Not specified when study was initiated (final report dated February 1, 2007)

GLP Compliance: No

QAU statement: yes () no (X)

Drug Lot #: Denosumab # 049A041380; Denosumab formulation buffer 049A037350

Methods: The purpose of the study was to characterize the single dose pharmacokinetics of denosumab following intravenous administration to male or female FcRn knockout (KO) and wild type (WT) mice, with a weight range of 15-27 grams. One hundred and eighty male and female FcRn KO or WT mice were administered 0.1 mg/kg or 1 mg/kg denosumab via a single bolus i.v. injection. Whole blood was collected via cardiac puncture from three mice per time point specified below, under section 6.3 (from the sponsor's final study report). The concentration of denosumab in serum was determined using an ELISA assay with a Lower Limit of Quantitation (LLOQ) of 10 ng/mL. Noncompartmental analysis was performed using WinNonlin Professional (version 4.1e, Pharsight Corporation, Mountain View, CA).

Study Design:

6.2 Control and Test Articles

Test Article: Denosumab (AMG 162) (60 mg/mL)
 Lot Number: 049A041380
 Dose Vehicle: Denosumab (AMG 162) Placebo
 Lot Number: 049A037350
 Formulation: 10mM Sodium Acetate, 5% Sorbitol, pH 5.2

6.3 Study Design

One hundred eighty male or female B6.129P2-B2m^{mtUnc/J} (FcRn Knockout) and C57BL/6J (Wild Type) mice between 5 and 15 weeks of age and weighing between 15 and 27 grams, were administered a 0.1 or 1.0 mg/kg single bolus IV dose of denosumab as described in Table 6-1. Whole blood was collected via cardiac puncture from three mice per time point per group at pre-dose, 1, 8, 24, 48, 72, 96, 120, 168, 240, 336, 504, 672, 1008, and 1344 hours post-dose. Whole blood samples were collected in Microtainer Brand Serum Separator Tubes and stored at room temperature for approximately 20 minutes or until fully clotted. Serum was extracted by centrifugation at approximately 11,500 rpm for 10 minutes.

Table 6-1. Study Design

Group	Test System Number	Strain	Dose (mg/kg)
1	1-45	Wild Type	0.1
2	46-90	FcRn KO	0.1
3	91-135	Wild Type	1.0
4	136-180	FcRn KO	1.0

Results: Following a single intravenous dose of 0.1 or 1 mg/kg denosumab, pharmacokinetic parameters were similar for the FcRn KO and WT mice (see Table 8-1 below, provided by the Sponsor). There was a dose-proportional increase based on the serum concentration and $AUC_{0-\infty}$ from 0.1 and 1 mg/kg denosumab in both the FcRn KO and WT mice, and the initial volume of distribution was similar as well.

However, the half-life in the WT mice was ~ 26-fold longer as compared to the FcRn KO mice, and there was ~ 15-fold increase in the clearance rate for the FcRn KO mice as compared to the WT mice administered denosumab. There was a reduction in the V_{ss} in the FcRn KO animals (V_{ss} = 52.3 mL/Kg at 0.1 mg/kg and 58.6 mL/kg at 1 mg/kg) as compared to the WT animals (V_{ss} = 97.6 at 0.1 mg/kg and 100 at 1 mg/kg). The WT mice displayed ~ 15-fold greater exposure based on the $AUC_{0-\infty}$.

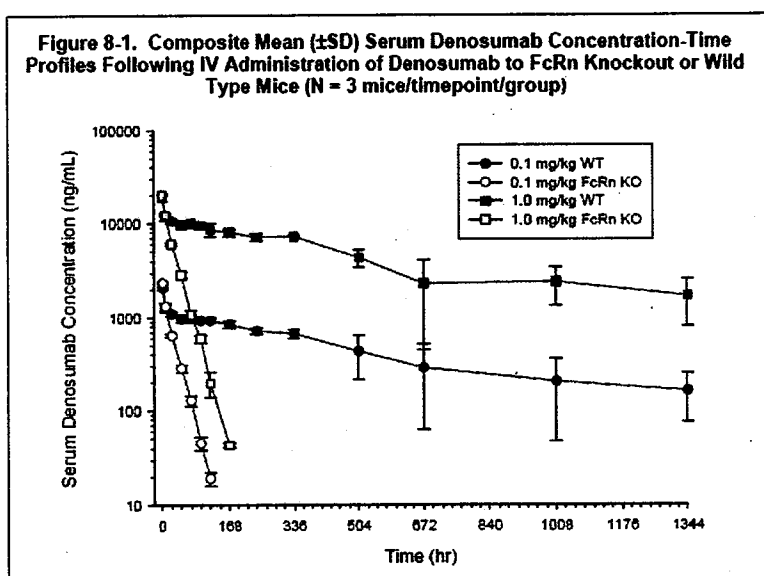


Table 8-1. Pharmacokinetic Parameter Estimates Following a Single IV Administration of 0.1 mg/kg or 1.0 mg/kg Denosumab to FcRn Knockout or Wild Type Mice

Parameter	0.1 mg/kg IV		1.0 mg/kg IV	
	Group 1 (WT)	Group 2 (FcRn KO)	Group 3 (WT)	Group 4 (FcRn KO)
C_0 (μ g/mL)	2.21	2.55	20.8	21.7
$AUC_{0-\infty}$ (μ g·hr/mL)	685	48.5	6910	455
$t_{1/2z}$ (hr)	489	18.1	506	20.2
CL (mL/hr/kg)	0.146	2.06	0.145	2.20
V_0 (mL/kg)	45.2	39.3	48.0	46.0
V_{ss} (mL/kg)	97.6	52.3	100	58.6

Values are reported to 3 significant figures

Study Conclusion: Overall, the presence of the FcRn in mice leads to an increase in the clearance of denosumab, and a reduction in the steady-state tissue distribution, based on the calculated V_{ss} values.

2.6.4.3.4 Study title: A single-dose intravenous and subcutaneous pharmacokinetic and pharmacodynamic study of AMG 162 in cynomolgus monkeys.

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings: The purpose of this study was to evaluate the pharmacokinetics and pharmacodynamics of denosumab (AMG 162) when administered as a single i.v. or s.c. injection to cynomolgus monkeys. The results showed that denosumab exposure was tri-phasic with an initial distribution phase (i.v.) or absorption phase (s.c.), followed by a secondary phase with longer half-life, and followed lastly by a rapid terminal phase. Greater than dose-proportional increases in C_{max} and AUC were observed up to 1 mg/kg, MRT increased with dose, and CL/F decreased with dose. Antibody formation was observed in both i.v. and s.c. dosing in all animals except for one treated with >0.01 mg/kg. Finally, measurements of sNTx were indicative of an anti-resorptive effect of denosumab on bone that was observed within 24 hrs of s.c. or i.v. dosing with ≥ 0.1 mg/kg that had a dose dependent duration of up to 8 weeks.

Study no.: 101398

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.2.2

Conducting laboratory and location:

In-life: (b) (4)

PK and antibody analysis: Amgen Inc., Thousand Oaks, CA

Bone turnover marker analysis: (b) (4)

Date of study initiation: unknown; final report dated February 2002.

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Denosumab (AMG 162) (30 mg/mL) Lot# A0010170015

Methods

Doses: 0, 0.003, 0.01, 0.1, 1, and 3 mg/kg

Species/strain: Cynomolgus monkey

Number/sex/group or time point (main study): 33 females (animals chosen had the lowest coefficient of variation in pre-dose serum N-telopeptide (sNTx) levels; 6/dose/group for treatment, and 3/control.

Group	# of animals		Dose level (mg/kg)	Actual dose (mg/kg) ^a	Concentration (mg/mL)
	s.c.	i.v.			
1 (placebo)	3	--	0	0	0
2	3	3	0.003	0.0016	0.03
3	3	3	0.01	0.00532	0.03
4	3	3	0.1	0.0848	0.1
5	3	3	1.0	1.0	1.0
6	3	3	3.0	3.0	3.0

a: Dose solution analyses based on total protein concentrations showed that animals scheduled to receive 0.003, 0.01 and 0.1 mg/kg actually received the doses listed. Animals scheduled to receive 1 and 3 mg/kg doses received doses that deviated less than 10% from nominal.

Route, formulation, volume, and infusion rate: i.v. or s.c. injection; formulation = 10 mM sodium acetate, 5% sorbitol, pH 5.2; i.v. injections administered in either the cephalic or saphenous vein, and s.c. injections administered into the intracapsular area of the back. No injection volumes were provided.

Age: 3-5 years

Weight: 2.2-3.5kg

Observation and times:

Blood samples were collected at pre-dose, 0.0883, 1, 4, 8, 12 and 24 hrs postdose, and 3, 5, 11, 15, 18, 22, 29, 36, 43, 50, 57, 64 and 71 days postdose for serum test article concentration. Animals in groups 1, 5, and 6 had further samples drawn on study days 43, 50, 57, 64 and 71. Concentrations were determined using ELISA (LLOQ = 0.96-5.0 ng/mL; ULOQ = 40 ng/mL or 16 ng/mL).

Serum for antibody analysis was collected from all animals at pre-dose, days 15 and 29, with further samples drawn from animals in groups 1, 5, and 6 on study days 43, 57, and 71 days postdose. Serum was also collected to analyze for bone turnover marker N-telopeptide, total serum calcium and total serum alkaline phosphatase (days -21, -14, -7, immediately pre-dose, 0.0833, 1, 4, 8, 12 and 24 hrs postdose and on days 3, 5, 8, 11, 15, 18, 22, 29 and 36). Animals in groups 1, 5, and 6 had further samples drawn on study days 43, 50, 57, 64 and 71.

Results:

Serum concentration:

The serum concentration-time profiles for single dose i.v. administration of denosumab were dose dependant. Exposure went through 3 phases, which the Sponsor characterized as an initial distribution phase (first 2 days), a secondary phase with a longer half life (first 10 days after administration) and a rapid terminal phase once serum levels dropped below 1000 ng/mL. The serum concentration-time profiles for single dose s.c. administration of denosumab were also dose dependant. Exposure went through 3 phases as with i.v. dosing, but the Sponsor notes the absence of the initial distribution phase as with i.v. The 3 phases consisted of absorption phase (first 4 days), a secondary phase with a longer half life (first 7-42 days after administration depending on dose), and a

rapid terminal phase once serum levels dropped below 1000 ng/mL (see Figures 8.1 and 8.2 below, provided by the Sponsor).

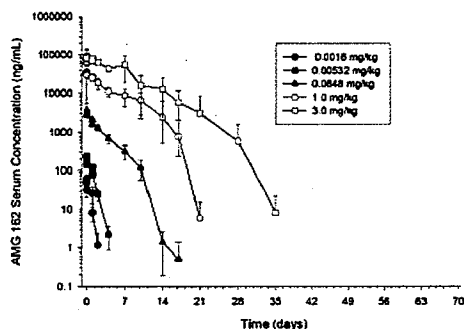


Figure 8-2. Mean (SD) Serum Concentration-Time Profiles of AMG 162 in Cynomolgus Monkeys Following Single Dose Intravenous Administration

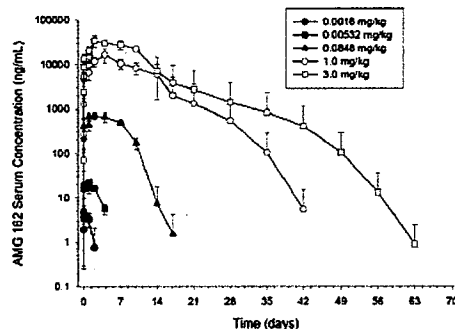


Figure 8-1. Mean (SD) Serum Concentration-Time Profiles of AMG 162 in Cynomolgus Monkeys Following Single Dose Subcutaneous Administration

Antibody analysis

All animals receiving doses greater than 0.01 mg/kg (0.00532 mg/kg) tested positive for denosumab antibodies, but at varying time points. Only one animal receiving a s.c. dose of 0.1 mg/kg did not test positive. Development of antibodies was not dose route-specific as both animals treated s.c. or i.v. were antibody positive. Early development of antibodies correlated with increased clearance of denosumab.

Noncompartmental PK analysis

After s.c. administration, C_{max} and AUC increased greater than dose proportionally up to 1 mg/kg, and were relatively dose proportionally increased between 1 and 3 mg/kg (AUC ratio of 2, and dose ratio of 3). After i.v. administration, C_{max} increased dose proportionally, but AUC increased greater than dose proportionally up to 1 mg/kg, and was dose proportional between 1 and 3 mg/kg. For both s.c. and i.v., MRT increased with dose, and CL/F decreased with dose. T_{max} increased relatively dose-dependently from 10-96 hrs. The Sponsor notes that both s.c. and i.v. dosing resulted in a rapid terminal phase half-life at all dose levels, averaging 21.3 hrs for i.v. and 31.0 hrs for s.c. (see Tables 7-3 and 7-4 below, provided by the Sponsor).

Table 7-3. Summary of Pharmacokinetic Parameter Estimates* Following Single Dose Subcutaneous Administration of AMG 162 to Cynomolgus Monkeys

Group		2		3		4		5		6	
Dose (mg/kg)		0.0016		0.00532		0.0848		1.0		3.0	
Parameters	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T _{max}	hr	10.7	11.5	18.7	9.24	56	36.7	96	0	64	27.7
C _{max}	ng/mL	4.33	1.66	22.9	0.500	728	91.1	16500	5830	35800	9020
t _{1/2,γ}	hr	41.9	0.134	35.8	6.30	24.1	7.00	28.9	18.8	29.5	16.8
AUC _(0-∞)	μg*hr/mL	0.143	0.0339	1.54	0.119	126	21.2	3940	1820	8790	2080
CL/F	mL/hr/kg	10.6	3.56	6.15	0.720	0.808	0.125	0.299	0.147	0.353	0.0737
MRT _(0-∞)	hr	17.0	27.4	40.4	3.17	112	4.54	182	55.6	192	90.3

*Values are reported to 3 significant figures

Table 7-4. Summary of Pharmacokinetic Parameter Estimates* Following Single Dose Intravenous Administration of AMG 162 to Cynomolgus Monkeys

Group		2		3		4		5		6	
Dose (mg/kg)		0.0016		0.00532		0.0848		1.0		3.0	
Parameters	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_{max}	ng/mL	62.5	12.0	243	19.9	3720	245	35900	5110	105000	40500
$t_{1/2\gamma}$	hr	8.37	1.15	14.3	1.80	36.9	17.5	19.3	4.18	27.5	11.7
AUC ₍₀₋₁₎	$\mu\text{g}\cdot\text{hr}/\text{mL}$	0.779	0.310	4.80	0.320	187	34.1	3590	1540	12400	4850
CL	$\text{mL}/\text{hr}/\text{kg}$	4.40	1.80	2.10	0.133	0.542	0.106	0.310	0.110	0.277	0.134
MRT ₍₀₋₁₎	hr	12.1	1.50	21.5	2.31	72.5	13.6	106	33.9	131	63.6
V_{ss}	mL/kg	51.4	15.3	45.1	1.88	38.4	1.38	30.7	5.21	31.2	7.98

*Values are reported to 3 significant figures

Exponential PK analysis

After i.v. administration, denosumab had an initial rapid distribution into a volume similar to serum or plasma volume, characterized as V_{init} , and averaging 25.3 mL/kg. As mentioned above, denosumab had a triphasic disposition, but a secondary phase was not observed in the two lowest doses. As a result, the 3 higher doses had a mean distribution half life ($t_{1/2,\alpha}$) of 3.91 hrs, and a mean secondary phase half-life ($t_{1/2,\beta}$) of 96.1 hrs (see Table 7-9 below, provided by the Sponsor).

Table 7-9. Exponential Pharmacokinetic Parameter Estimates* For Cynomolgus Monkeys Administered Intravenous Doses of AMG 162 from 0.0016 to 3.0 mg/kg

Parameter	Units	Dose (mg/kg)					Mean	SD
		0.0016	0.00532	0.0848	1.0	3.0		
C_0	ng/mL	61.9	224	3450	34700	128000	33300	54900
$V_{initial}$	mL/kg	25.9	23.7	24.6	28.8	23.4	25.3	2.19
$t_{1/2,\alpha}$	hr	N/A*	N/A	5.06	5.73	0.931	3.91	2.60
$t_{1/2,\beta}$	hr	N/A	N/A	94.9	94.3	99.1	96.1	2.60

*Values are reported to 3 significant figures

*Not Applicable

 V_{init} = initial volume of distribution; $t_{1/2,\alpha}$ = distribution half-life; $t_{1/2,\beta}$ = secondary half-life

Pharmacodynamics

The tables and figures below (Tables 7-5, 7-6 and Figures 8-5, 8-6, provided by the Sponsor) represent the non-compartmental PD parameter estimates using placebo-corrected values of sNTx expressed as inverse percent change from baseline following i.v. or s.c. administration of denosumab. After s.c. and i.v. dosing, the two lowest doses produced sNTx levels above and below baseline, therefore there was no consistent effect. After s.c. dosing with the 3 higher doses, the maximum effect of sNTx decrease from baseline was dose dependent with 1 and 3 mg/kg having the greatest effect at 67-74% decrease at a timepoint of 132-240 hrs postdose (5.5-10 days). After i.v. dosing with the 3 higher doses, the maximum effect of sNTx decrease from baseline was dose dependent with 1 and 3 mg/kg having the greatest effect at 64-70% decrease at a timepoint of 76-188 hrs postdose (3-8 days). In addition, the anti-resorptive doses (the 3 highest doses) caused a rapid decrease in sNTx levels within 24 hrs, and the duration of effect increased with dose (0-8 weeks). While the maximum effect levels appear to be the same for s.c. and i.v. dosing at 1 and 3 mg/kg, the ETmax is longer for s.c. dosing at 1 mg/kg, but returns to similar levels between i.v. and s.c. at 3 mg/kg. Overall, the results indicate that denosumab is anti-resorptive and inhibits bone resorption at doses greater than 0.00532 mg/kg (0.01 mg/kg).

Table 7-5. Summary of Non-Compartmental Pharmacodynamic Parameters Estimated Following Single Dose Subcutaneous Administration of AMG 162 to Cynomolgus Monkeys

Group		2		3		4		5		6	
Dose (mg/kg)		0.0016		0.00532		0.0848		1.0		3.0	
Parameters	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ET _{max}	hr	20	25.0	36	20.8	88	69.3	240	166	132	178
ME	%	7.7	7.18	25	17.7	48.4	10.2	67.7	9.03	74.5	3.44
AUEC	%*hr	-1870	1370	-89.3	2410	12900	3640	35800	21400	47300	25400

Table 7-6. Summary of Non-Compartmental Pharmacodynamic Parameters Estimated Following Single Dose Intravenous Administration of AMG 162 to Cynomolgus Monkeys

Group		2		3		4		5		6	
Dose (mg/kg)		0.0016		0.00532		0.0848		1.0		3.0	
Parameters	Units	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ET _{max}	hr	25.3	22.0	88	69.3	76	81.7	76	81.7	188	274
ME	%	14.1	9.80	9.37	6.49	53.8	15.6	64.9	11.2	70.6	4.40
AUEC	%*hr	-1030	1140	-3770	1510	12600	11000	28100	12500	39100	18000

ET_{max} = time of maximum effect

ME = maximum effect

AUEC = area under the inverted effect-time curve from time zero to the first BQL timepoint

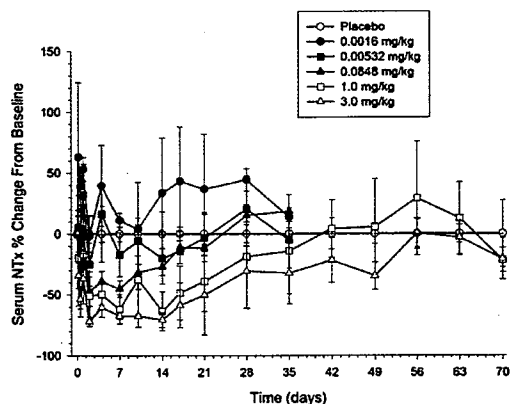


Figure 8-5. Mean (SD) Placebo-Corrected Percent Change From Baseline in Serum N-Telopeptide Following Single Dose Subcutaneous Administration of Placebo, AMG 162 to Cynomolgus Monkeys

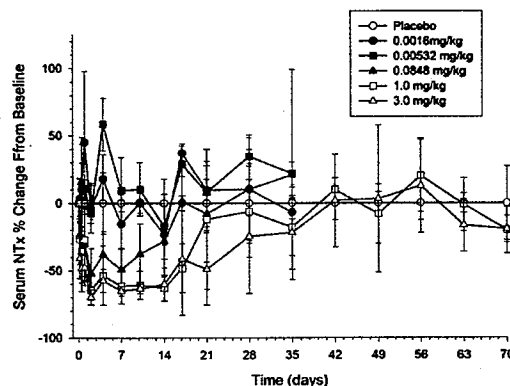


Figure 8-6. Mean (SD) Placebo-Corrected Percent Change From Baseline in N-Telopeptide Following Single Dose Intravenous Administration of Placebo, AMG 162 to Cynomolgus Monkeys

2.6.4.4 Distribution

2.6.4.4.1 Study title: Absorption, Distribution, and Excretion in Cynomolgus Monkeys Following a Single Subcutaneous Administration of ¹²⁵I-AMG 162

Reviewed by: Michael Orr, Ph.D.

Key findings:

- The highest concentrations of radioactivity were observed in the dose site skin, dose site subcutaneous tissue, thyroid/parathyroid, axillary lymph nodes, inguinal lymph nodes, serum blood, lungs, spleen and ovaries following administration of 0.1 or 1 mg/kg of ¹²⁵I-denosumab.
- The major route of elimination of ¹²⁵I-denosumab and or radioactively labeled protein fragments was in the urine, as 76-95% of the administered activity was

present in the urine 672 hours postdose. Fecal elimination represented 1.1 to 3% of the administered radioactivity.

Study Number: Amgen Study No. 104192 and (b) (4)

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.2.2

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: September 2, 2004 (final report dated December 20, 2007)

GLP Compliance: Yes

QAU statement: yes () no (X)

Drug Lot #: ¹²⁵I-Denosumab # 52318-18; Denosumab # A0206120000, Vehicle # A0108030000

Methods: The goal of this study was to determine the extent of absorption, distribution, and excretion of radioactivity following a single subcutaneous administration of ¹²⁵I-denosumab given to female monkeys. The study took serum samples to determine if anti-denosumab antibodies were present or absent, determined C-Telopeptide concentrations, and denosumab concentrations. Fourteen female drug naïve Cynomolgus monkeys from (b) (4) were used, but these animals were originally of Chinese origin. The animals were fasted overnight prior to the subcutaneous dose administered via syringe and needle in the dorsal scapular region. The animals were observed twice daily and weighed on the day of dose administration, and every two weeks during the test period. Blood collections were taken predose 1 (3 days prior to dose administration), predose 2 (on the day of dose administration), and at 0.5, 4, 12, 24, 120, 168, 336, 408, 504, 672, 1008, 1176, and 1344 hours postdose.

Study Design below (taken from the study report):

Study Design

Monkeys were assigned to two groups for this study. At designated times following dosing, blood, urine, feces, and selected tissues were collected. The group designations, number of animals, target dose levels, and target dose volumes were as follows:

Group	Number of Female Animals	Target Dose Level (mg/kg)	Dose Route	Target Dose Volume (mL/kg)	Samples Collected
1	6	0.1	Subcutaneous	1	Blood, Urine, Feces, and Tissues
2	8	1	Subcutaneous	1	Blood, Urine, Feces, and Tissues

Note: Animals in Group 1 received approximately 64 μ Ci/kg. Animals in Group 2 received approximately 77 μ Ci/kg.

Tissue distribution was evaluated in the tissues listed below for 2 monkeys per/time point at 12, 120 and 672 hours, following blood collections and necropsy:

Adrenal glands	Lungs
Bladder (urinary)	Lymph nodes (axillary)
Blood	Lymph nodes (inguinal)
Bone (femur)	Muscle (thigh)
Bone (lumbar vertebrae L3 and L4), see protocol deviations	Ovaries
Bone (thoracic vertebrae T3 and T4), see protocol deviations	Salivary glands
Bone marrow (from femur)	Serum
Brain	Skin (abdominal)
Dose site (skin)	Small intestine
Dose site (subcutaneous tissue)	Spleen
Eyes (both)	Stomach
Fat (reproductive)	Synovial fluid (knee) ^a
Heart	Thymus
Kidneys	Thyroid/parathyroid
Large intestine	Uterus
Liver	

^a Collection was attempted. In some instances, no synovial fluid was obtained.

Radioanalysis: The radioactivity in the serum samples was analyzed for radioactivity in a Packard COBRA II 5003 solid scintillation counter for at least 5 minutes or until 1,000,000 counts. The results were calculated as ¹²⁵I dpm/g sample. All samples were analyzed in duplicate as long as the sample size was of sufficient size to process in duplicate.

Results:

The ¹²⁵I-denosumab was widely distributed in animals administered the 0.1 and 1 mg/kg doses. In the 0.1 mg/kg dose group, radioactivity was identified in all tissues except bone marrow, brain and muscle at 672 hours postdose. The highest concentrations were observed at the dose site skin, dose site subcutaneous tissue, thyroid/parathyroid, serum, axillary lymph nodes, inguinal lymph nodes, blood, spleen, and ovaries.

In the 1 mg/kg dose group, radioactivity was quantified in all tissue types examined at 672 hours and in approximately half of the tissues at the 1344 hours postdose. The highest concentrations of radioactivity were observed at the dose site skin, dose site subcutaneous tissue, thyroid/parathyroid, axillary lymph nodes, serum, blood, ovaries, and lungs.

Anti-denosumab antibodies were present in all animals that were on-study through 672 and 1344 hours postdose.

The percent of radioactive dose in urine following a single subcutaneous dose of ¹²⁵I-denosumab at 0.1 mg/kg was approximately 80 to 95% of the administered radioactivity and fecal elimination represented approximately 1.8 to 3.1% of the administered amount

at 672 hours postdose. Overall recoveries of radioactivity at the 672 hour postdose time were approximately 92-106% of the dosed radioactivity.

For the animals administered 1 mg/kg of ^{125}I -denosumab, approximately 76-79% of the radioactive dose was recovered in the urine, and 1.1 to 2.8% administered radioactivity was recovered in the feces at the 1344 hours postdose. At the 1344 hour time-point, approximately 81 to 88% of the dosed radioactivity was recovered in the study. Approximately 12-19% of the ^{125}I -denosumab and or radioactively labeled fragments of ^{125}I -denosumab were not accounted for at the 1344 hour time-point.

Study conclusion:

The major route of elimination for denosumab is in the urine with approximately 80-95% of the administered radiolabel detected in urine by 1344 hours postdose. The minor route of elimination was in the feces, with approximately 1-3% being recovered through this route of elimination.

The highest concentrations of radioactivity were observed at the dose site skin, dose site subcutaneous tissue, thyroid/parathyroid, axillary lymph nodes, serum, blood, and ovaries for both the 0.1 mg/kg and 1 mg/kg dose groups of ^{125}I -denosumab. For monkeys treated with the 0.1 mg/kg dose of ^{125}I -denosumab, there were higher concentrations of radioactivity in the inguinal lymph nodes and spleen that were not observed in the 1 mg/kg dose group. The 1 mg/kg dose group had higher concentrations of radioactivity in the lungs; this finding was not observed in the lower dose group.

Comment:

The data provides evidence that denosumab concentrates in the lung. However, based on the 1 month and 6/12 month toxicology studies that utilized doses that were 10 and 50-fold higher than concentrations that were administered here (10 mg/kg and 50 mg/kg respectively), no lung toxicity was observed in these repeat dose toxicology studies.

2.6.4.4.2 Study title: Cross-Reactivity of AMG-162 with Normal Human Tissues

Reviewed by: Michael Orr, Ph.D.

Key findings:

- Denosumab (AMG 162) cross-reacted to the lymph node of one human donor. Denosumab displayed weak to moderate (1-2⁺) membrane staining of lymphocytes lining the periphery of the paracortex in the lymph node.

Study Number: Amgen Study No. 101348 and (b) (4)

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.3.7.7

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: ~ initiation on January 8, 2001 (final report dated May 15, 2001)

GLP Compliance: Yes

QAU statement: yes (X) no ()

Methods: Fresh unfixed tissue samples were stored in OCT embedding medium and maintained at -70°C until sectioning. Tissue sections were cut to approximately 5 µm thickness and fixed in acetone for 10 minutes, desiccated and stored below -70°C. Sections were then fixed in 10% neutral buffered formalin (NBF) for 10 seconds just prior to staining. Denosumab conjugated with FITC was applied to human tissues (three donors per tissue) at 1 and 10 µg/mL. The negative control antibody was a Human IgG₂ conjugated with FITC. The (b) (4) cell line capable of overexpressing osteoprotegerin ligand was used as the positive control and the cell line (b) (4) that did not express osteoprotegerin was used as the negative control.

Results: Denosumab cross-reacted to the lymph node tissue sample from one human donor, with weak to moderate (1-2⁺) membrane staining of lymphocytes lining the periphery of the paracortex in the lymph node at 1 and 10 µg/mL of denosumab.

Study conclusion: Denosumab had very limited binding to normal human tissues based on this cross-reactivity study. Only lymphocytes in the paracortex in the lymph node provided a positive signal in this study.

2.6.4.4.3 Study title: Cross-Reactivity of AMG-162 with Normal Cynomolgus Monkey and Human Tissues

Reviewed by: Michael Orr, Ph.D.

Key findings:

- Denosumab cross-reacted in the lymph node in 3/3 cynomolgus monkey donors.
- Denosumab cross-reacted to the lymph node tissue from one human donor.

Study Number: Amgen Study No. 101758 and (b) (4)

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.3.7.7

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: Initiation date was not identified in the report (final report dated July 2, 2001)

GLP Compliance: No

QAU statement: yes () no (X)

Methods: Fresh unfixed tissue samples were stored in OCT embedding medium and maintained at -70°C until sectioning. Tissue sections were cut to approximately 5 µm thickness and fixed in acetone for 10 minutes, desiccated and stored below -70°C. Sections were then fixed in 10% neutral buffered formalin (NBF) for 10 seconds just prior to staining. Denosumab conjugated with FITC was applied to human tissues

(three donors per tissue) at 1 and 10 µg/mL. The negative control antibody was a Human IgG₂ conjugated with FITC. The (b) (4) cell line capable of overexpressing osteoprotegerin ligand was used as the positive control, and the cell line (b) (4) that did not express osteoprotegerin was used as the negative control.

Results: Denosumab cross-reacted to the lymph node tissue from one human donor. Denosumab displayed weak to moderate (1-2⁺) membrane staining of lymphocytes lining the periphery of the paracortex of lymph nodes in normal cynomolgus monkey tissues. Nonspecific staining based on binding of both denosumab and negative control antibodies to multiple tissue structures was observed for bone marrow cells, lymphoid organs, gastrointestinal lamina propria and whole blood neutrophils and eosinophils.

Study conclusion: Denosumab had very limited binding to normal human tissues based on this cross-reactivity study. Only lymphocytes in the paracortex of the lymph node provided a positive signal in the monkey.

2.6.4.4.4 Study title: Cross-Reactivity of AMG 162 with Cynomolgus Monkey, Rat and Rabbit Tissue *Ex Vivo*.

Reviewed by: Michael Orr, Ph.D.

Key findings:

- In the rabbit and monkey, denosumab (AMG 162) bound to the periphery of the cortex of lymph nodes and lymphoid nodules of the spleen, and at the periphery of lymphoid nodules in the gut-associated lymphoid tissue of the small and large intestines.
- In the rat, denosumab bound to chondrocytes and the margins of their surrounding lacunae in the articular cartilage.

Study Number: Amgen Study No. 102700 and (b) (4)

(b) (4)

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.3.7.7

Conducting Laboratory and Location: (b) (4)

(b) (4)

Date of Study Initiation: August 30, 2002 (final report May 8, 2003)

GLP Compliance: Yes

QAU statement: yes (X) no ()

Methods: Cynomolgus monkey, rat and rabbit tissues were from the (b) (4) tissues bank collected by (b) (4). Tissues used in this study were from naive animals. Fresh unfixed cynomolgus monkey, rat and rabbit tissue samples were collected as necropsy specimens, frozen in Tissue-Tek OCT compound and maintained at

approximately -65 to -80°C until use. The positive control was (b) (4) cells that expressed human OPG ligand, and the negative control was (b) (4) cells that lacked expression of human OPG. Tissue sectioning and fixation process consisted of placing fresh unfixed tissue samples into molds filled with Tissue-Tek OCT compound and frozen on dry ice. Sections of approximately 7 µm in thickness were cut and mounted onto slides. The slides with the tissues were fixed in acetone for 10 minutes at room temperature and then air dried overnight. Slides were used immediately or stored at approximately 65 to -86°C until they were stained. The staining procedure consisted of incubating dilutions of denosumab or human IgG₂ kappa with biotinylated goat anti-human IgG and adding this mixture to the slides. Avidin/biotin incubations were performed and the labeling reagent utilized was (b) (4). After immunohistochemical staining with denosumab, sections were visualized under light microscopy for determination of denosumab binding.

Slide Evaluation Procedure (taken from the study report):

Test and Measurements

All slides were evaluated by the Pathologist and/or Study Director to ensure that the quality of stain was sufficient for interpretation. Each slide was examined for the presence and strength of labeling, as well as the distribution and relative density of positive cells for each antibody.

The relative density of positive cells was graded on the following scale:

0 or – or Blank	No labeled cells
1+ or +	Light stain and/or occasional cells
2+ or ++	Light-medium stain and/or small numbers of cells/types of cells
3+ or +++	Moderate stain and/or medium numbers of cells/types of cells
4+ or ++++	Dark stain and/or large numbers of cells/types of cells
N/A	Not applicable or Not available

The distribution and intensity of each of the markers studied was summarized in a Microsoft Excel (Version Excel 2000) spreadsheet detailing the finding for individual slides. There were no statistical analyses performed on these data.

The following tissues from three separate individuals per species were evaluated in this study (table taken from the study report):

Cynomolgus Monkey Tissue from 3 Separate Individuals		
• Adrenal	• Heart	• Spinal Cord
• Bladder	• Kidney (glomerulus)	• Spleen
• Blood	• Kidney (tubule)	• Striated Muscle
• Bone	• Liver	• Testes
• Bone Marrow	• Lung	• Thymus
• Breast	• Lymph Node	• Thyroid
• Cerebellum	• Ovary	• Tonsils
• Cerebral Cortex	• Pancreas	• Ureter
• Colon	• Parathyroid	• Uterus (cervix)
• Endothelium*	• Pituitary	• Uterus (endometrium)
• Eye	• Prostate	
• Fallopian Tube	• Skin	
• Gastrointestinal Tract		
*Cynomolgus monkey endothelium was evaluated from multiple tissue types.		
Rat Tissue from 3 Separate Individuals		
• Adrenal	• Heart	• Spinal Cord
• Bladder	• Kidney (glomerulus)	• Spleen
• Blood	• Kidney (tubule)	• Striated Muscle
• Bone	• Liver	• Testes
• Bone Marrow	• Lung	• Thymus
• Breast	• Lymph Node	• Thyroid
• Cerebellum	• Ovary	• Uterus (cervix)
• Cerebral Cortex	• Pancreas	• Uterus (endometrium)
• Colon	• Parathyroid	
• Endothelium (Aorta)	• Pituitary	
• Eye	• Prostate	
• Fallopian Tube	• Skin	
• Gastrointestinal Tract		
Rabbit Tissue from 3 Separate Individuals		
• Adrenal	• Heart	• Spinal Cord
• Bladder	• Kidney (glomerulus)	• Spleen
• Blood	• Kidney (tubule)	• Striated Muscle
• Bone	• Liver	• Testes
• Bone Marrow	• Lung	• Thymus
• Breast	• Lymph Node	• Thyroid
• Cerebellum	• Ovary	• Uterus (cervix)
• Cerebral Cortex	• Pancreas	• Uterus (endometrium)
• Colon	• Parathyroid	
• Endothelium	• Pituitary	
• Eye	• Prostate	
• Fallopian Tube	• Skin	
• Gastrointestinal Tract		

Results: In the rabbit and monkey, denosumab bound to the periphery of the cortex of lymph nodes and lymphoid nodules of the spleen, and at the periphery of lymphoid nodules in the gut-associated lymphoid tissue of the small and large intestines. In the rat, denosumab bound to chondrocytes and the margins of their surrounding lacunae in the articular cartilage based on evaluating tissue from 3 separate rats.

Study conclusion: In both cynomolgus monkey and rabbit, denosumab bound to lymph nodes, lymphoid nodules in the spleen, and gut-associated lymphoid tissue (GALT) of the small and large intestine.

2.6.4.4.5 Study Title: Quantitative Whole Body Autoradiography of Cynomolgus Monkeys Following a Single Subcutaneous Administration of ^{125}I -AMG-162.

Reviewed by: Michael Orr, Ph.D.

Key Findings:

- As expected, high concentrations of ^{125}I -denosumab were present in the various lymph nodes.
- An unexpected finding was that ^{125}I -denosumab accumulated in the cornea of the eye in both the male and female cynomolgus monkeys.

Comments: Based on the toxicology studies, there was no evidence that the eye was a target organ for toxicity following monthly subcutaneous administration of 1, 10, or 50 mg/kg doses of denosumab to cynomolgus monkeys for 12 months. In addition, there was no evidence of an increased incidence of cataracts in the monkey toxicology studies. It should be noted that a higher incidence of cataract formation was only observed in the clinical study of prostate cancer patients treated with denosumab.

Study Number: Amgen Study No. 104105

Volume # and Page #: EDR 0000 12/19/08 Section 4.2.2.3

Conducting Laboratory and Location (b) (4)

Date of Study Initiation: August 30, 2002 (final report May 8, 2003)

GLP Compliance: No

QAU statement: yes () no (X)

Drug Lot #: ^{125}I -Denosumab # 52318-35; Denosumab # A0206120000, Vehicle # A0108030000

Methods: ^{125}I -denosumab was administered subcutaneously in the back to non-fasted cynomolgus monkeys at 0.1 (3 males and 3 females) and 1 mg/kg doses (4 males and 4 females). The animals were observed twice daily for any signs of pain or distress. Once daily observations of general health and appearance were performed, and the monkeys were weighed every 2 weeks during the study. Blood was collected at two predose time points and at time intervals from 0.5, 4, 12, 24, 120, 336, 408, 504, 672, 840, 1008, 1176, and 1344 hours postdose. Furthermore, whole-body autoradiography was performed at 12, 120 and 672 hours following the 12, 120 and 672 hour blood collections (1 animal/sex/time) in 0.1 mg/kg dose group. In the 1 mg/kg dose group, blood collections and whole body autoradiography were performed on 1 animal/sex/time at 12, 120, 672, and 1344 hour time points.

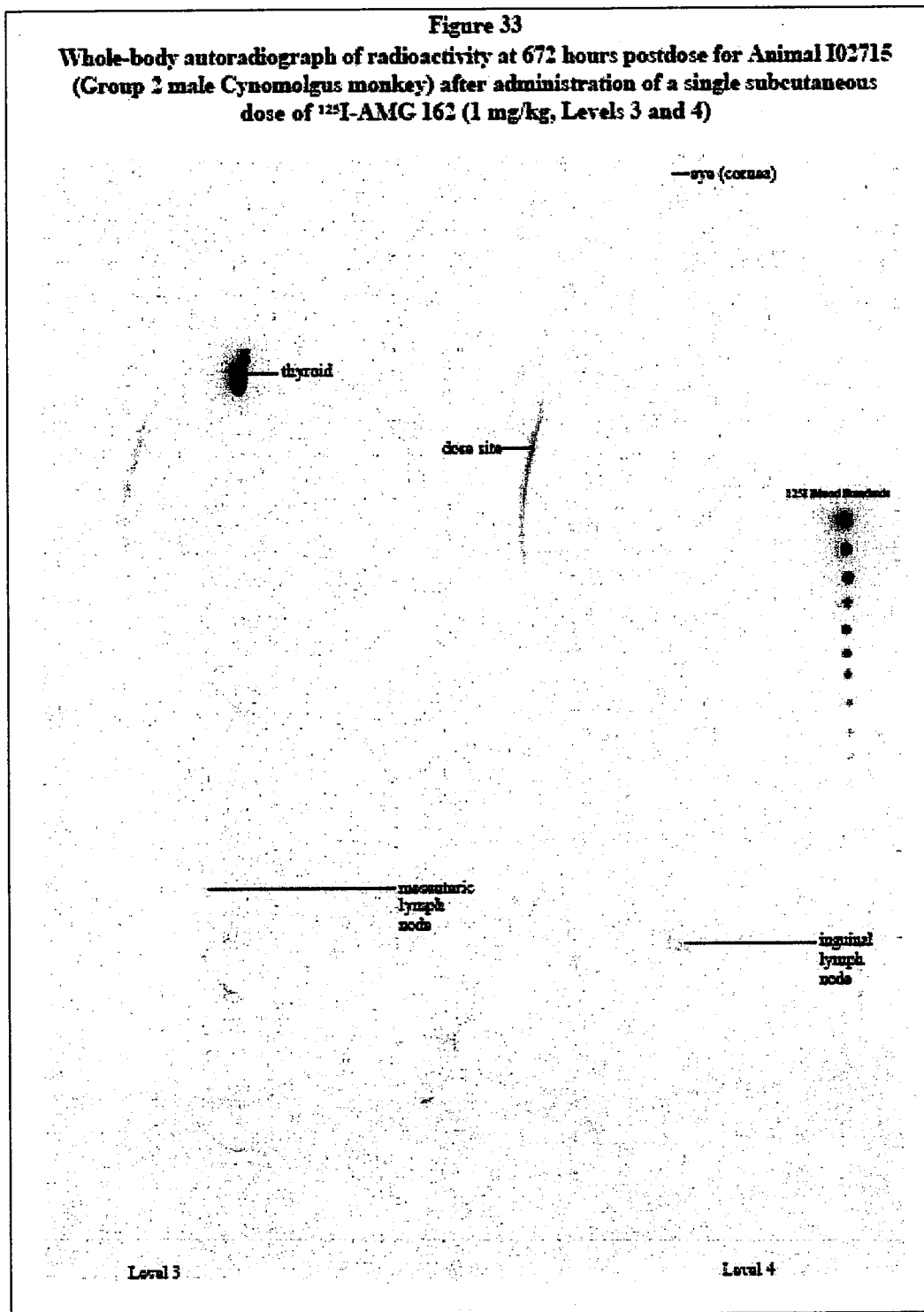
Group	Number of Animals		Target Dose Level (mg/kg)	Dose Route	Target Dose Volume (mL/kg)	Samples Collected
	Males	Females				
1	3	3	0.1	Subcutaneous	1	Blood and Carcasses for WBA
2	4	4	1	Subcutaneous	1	Blood and Carcasses for WBA
WBA	Whole-body autoradiography.					

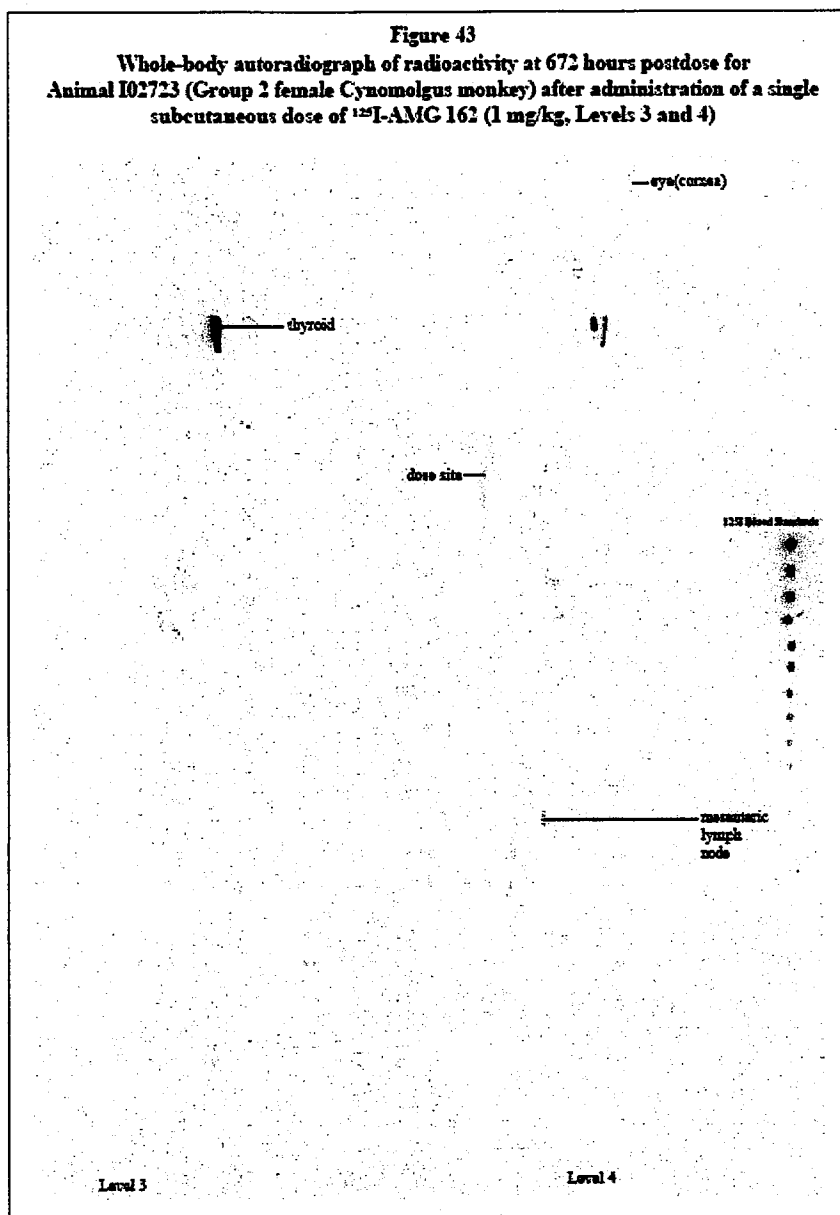
Results: Highest concentrations of radioactivity were observed following administration of 0.1 mg/kg dose of ^{125}I -denosumab at the dose site, thyroid, axillary lymph nodes, inguinal lymph nodes, cervical lymph nodes, gastric mucosa, esophageal contents, stomach contents, and areas in the spleen for both males and females. For the 1 mg/kg ^{125}I -denosumab, the highest concentrations of ^{125}I -denosumab was observed in the dose site, thyroid, gastric mucosa, blood, lung, liver and cervical lymph nodes in both males and females. Males had high levels of ^{125}I -denosumab in the stomach contents, mesenteric lymph nodes, prostate, and stomach. Females had high levels of radiolabel present in the axillary lymph nodes, ovary, and nasal turbinates.

Comment: No basis identified for the differences in the distribution patterns for either gender or different doses of denosumab.

All animals in the 0.1 and 1 mg/kg groups of ^{125}I -denosumab displayed anti-denosumab antibodies. Male monkeys in the 0.1 mg/kg dose group gave positive ADA results at the 672 hour time point, and female monkeys in this same dose group were positive for ADA at the 336 and 672 hour time points. For the 1 mg/kg ^{125}I -denosumab group, both male and female monkeys tested positive for anti-denosumab antibodies at the 336 hour, 672 hour, and Day 56 time points.

Figures 33 and 43 below were copied from the submission. The figures indicated that ^{125}I -denosumab is accumulating in the cornea in both male and female monkeys 672 hours postdose.





Except for the 0.5 hours postdose at which time TCA-precipitable radioactivity was 51.5% for males and 42.9% for females, the mean serum TCA-precipitable radioactivity values in males and females were greater than 83% for all collection time points.

Comments: It is unknown what the affects of anti-denosumab antibodies have on the distribution of 125 I-denosumab in the cynomolgus monkey at this time. It is clear that denosumab induces a robust immunogenicity response in monkeys.

Based on the previous experience in the monkey, naked denosumab has maintained pharmacological activity between monthly dosing intervals. During the study, C-telopeptide levels were monitored, and there were reductions in C-telopeptide (serum bone resorption biomarker) from 38-70% depending on the time point and dose group

evaluated. This provides supporting data that the ^{125}I -denosumab was pharmacologically active during the duration of the study. It is unclear, however, how much intact ^{125}I -denosumab versus ^{125}I -denosumab antibody fragments were present in the tissues that the ^{125}I -denosumab is accumulating in, such as the cornea. Based on the tissue-cross reactivity study, denosumab did not bind to the monkey or human eye tissue (see studies 101348 [human] and 101750 [monkey]). However, ^{125}I -denosumab accumulated in the lymph nodes in both female and male monkeys (see Figures 33 and 43), which was an expected finding, i.e. based on the tissue cross-reactivity studies (Studies #101348 and #101750, above), denosumab bound to the lymph nodes in both monkey and human tissues.

It is currently unclear what the physiological significance of potentially intact ^{125}I -denosumab and/or ^{125}I -denosumab antibody fragment(s) accumulation in the cornea of the monkey is at this time. Based on the toxicology studies provided in this submission, ocular toxicity was not detected in the cynomolgus monkeys at the doses and time points examined in the 1- and 6/12-month repeat dose toxicology studies (Study #101447 and Study #102090).

2.6.4.5 Metabolism

No metabolism studies were conducted as this is a monoclonal antibody product.

2.6.4.6 Excretion

After a single s.c. administration of ^{125}I -denosumab, approximately 76-95% of the administered activity was recovered in the urine, while 1-3% of the administered radioactivity was recovered in feces. The overall recovery of administered radioactivity was 83-100% (See section 2.6.4.4.1). No specific studies were conducted to evaluate excretion of denosumab in milk.

2.6.4.7 Pharmacokinetic drug interactions

No studies have been conducted. The Sponsor notes that hepatic microsomal metabolism plays a negligible role in the elimination of denosumab, and the specificity of denosumab for RANKL indicates that pharmacodynamic interaction with other drugs is unlikely.

2.6.4.8 Other Pharmacokinetic Studies

2.6.4.8.1 Study title: Pharmacokinetic and pharmacodynamic comparability study for two manufacturing processes of AMG 162 in female cynomolgus monkeys

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings: Following subcutaneous administration to female cynomolgus monkeys of CP1 and CP2, PK and PD parameters were sufficiently similar to determined comparability.

Study no.: 103948

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.2.7

Conducting laboratory and location (b) (4)

Date of study initiation: unknown; final report on June 2, 2004

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: Denosumab (AMG 162) Lot # 049A014925 (CP1)
Denosumab (AMG 162) Lot # A0310220001 (CP2)

Background

The purpose of this study was to compare the PK profiles of denosumab manufactured by two different processes (CP1 and CP2) that were administered subcutaneously to female cynomolgus monkeys. Phase 1 and 2 clinical studies used the CP1 drug product, while CP2 will be used for Phase 3 clinical studies. CP2 development used the same cell line, but improves on the media seed train and feed strategy for expression of denosumab. There are also changes in recovery and purification.

Methods

Doses: 0.1 mg/kg single s.c. dose of denosumab

Species/strain: Cynomolgus monkey

Number/sex/group or time point (main study): 8 females/group

Route, formulation, volume, and infusion rate: s.c. injection; formulation = 30 mg/mL, 10 mM sodium acetate, 5% sorbitol, pH 5.2; volume = 0.1 mL/kg

Age: 3.5-8.7 years

Weight: 2.9-5.2 kg

Observation and times: Blood was collected for PK/PD analysis by venipuncture (cephalic vein) once weekly for 3 weeks prior to dosing (Days -21, -14, -7), pre-dose (day of dosing), and 4, 24, 48, 72, 168, 336, 504, and 672 hrs postdose. Blood was collected for anti-denosumab antibody analysis at Day -4 pre-dose, and at 336, 504 and 672 hrs postdose. Urine for PD analyses were collected once weekly for the 3 weeks prior to dosing (Days -21, -14 and -7), pre-dose, and 24, 48, 72, 168, 336, 504, 672 hrs postdose. Concentrations were determined using ELISA (LLOQ = 0.960 ng/mL).

TRAP-5b was analyzed in serum samples using the BoneTRAP assay kit (IDS, Inc.). Osteocalcin samples were analyzed at Amgen using the Osteocalcin assay (b) (4). Bone specific alkaline phosphatase (BAP) was analyzed at Amgen Inc. using a Metra BAP quantitative immunoassay (b) (4). Serum CTx and urine NTx were analyzed by ELISA. Anti-denosumab antibodies were analyzed by ECL immunoassay. A sample to negative control ratio <1.4 was reported as antibody negative. Samples were reported positive if the sample to negative control ratio was ≥ 1.4 and the post to pre-dose ratio was ≥ 2.0 .

Results:

Antibody analysis: Thirteen of the 16 animals treated with a single dose of denosumab developed antibodies, starting at day 14 postdose (table below provided by the Sponsor).

AMG 162 Formulation	# of Animals Antibody Positive			
	Day 7	Day 14	Day 21	Day 28
CP1	0/0	3/8	4/8	5/8
CP2	0/0	5/8	8/8	8/8

Since both CP1 and CP2 treated animals developed antibodies, there was a similar antigenic response in both formulations, though the incidence leaned toward CP2, since the 3 animals that did not develop antibodies were all treated with CP1.

Serum concentrations: When including both antibody positive and negative animals, CP1 treated animals had overall higher serum concentrations and higher standard deviations, though the difference was not statistically significant up to 336 hrs as the concentration-time profiles correlated up to this point. At 336 hrs (Day 14), antibody formation was detected, and an increased rate of elimination of denosumab was noted in the concentration-time profile which is likely attributed to shortened exposure in antibody positive animals.

PK analysis: There is a small difference in exposure upon PK analysis of all animals (regardless of antibody formation). CP2 animals have lower C_{max}, AUC and T_{max} values compared to CP1 animals, and the standard deviations are high. However, when analysis was performed with only antibody negative animals (on Day 14 postdose), the standard deviations remained high, C_{max} and T_{max} were lower in CP2 animals, but AUC was comparable. (Tables below provided by the Sponsor)

PK parameters (analysis of all animals)

Process	T _{max} (hr)		C _{max} (ng/mL)		AUC ₍₀₋₄₎ (µg·hr/mL)		
	Mean	SD	Mean	SD	Mean	SD	n
CP1	102	57.0	1010	376	226	48.7	8
CP2	75	39.4	749	135	161	25.1	8

PK parameters (Day 14 antibody negative animals)

Process	T _{max} (hr)		C _{max} (ng/mL)		AUC ₍₀₋₃₃₆₎ (µg·hr/mL)		
	Mean	SD	Mean	SD	Mean	SD	n
CP1 Ab _{-ve} Day 14	101	64	958	479	188	46.4	5
CP2 Ab _{-ve} Day 14	56	14	785	163	163	40.4	3

By eliminating antibody positive animals, only a small number of animals were analyzed which reduced the power to detect differences in the two groups. The adjusted means using antibody negative animals had p-values >0.44, so the C_{max} and AUC were not statistically significant, but the power was very low.

Pharmacodynamic analysis: Mean uNTx/CRN values were comparable between CP1 and CP2, and were not statistically different up to 336 hrs for either all animals, or only antibody negative animals. Mean sCTX values were also comparable between CP1 and CP2 animals, though the standard deviation was very high at 336 hrs (possible due to improper sample handling). There was no analysis for sCTX in antibody negative animals, so the only analysis was for all treated animals. In addition, values for Ca^{2+} , BAP, osteocalcin and TRAP5b were comparable between CP1 and CP2. As with sCTX, there was no analysis for antibody negative animals, only all treated animals.

Conclusion: On Day 14 postdose, a number of animals developed antibodies, so analysis of only antibody negative animals for PK and PD caused the study to be underpowered. However, upon analysis, it was determined that the data above show that CP1 and CP2 are sufficiently similar. While the antigenicity appears slightly different, this should not be an issue in the clinic. As a result, it was acceptable for safety and efficacy data generated using CP1 to also support use of CP2.

Upon discussion, the DBOP pharm/tox reviewer noted that the PK study misses the mark with the 90% confidence interval, so it can't be said statistically that these are comparable. However, due to the formation of anti-product antibodies, it is an underpowered study, so the likelihood of showing equivalence is already low. The PD does correlate between CP1 and CP2, and the PK is fairly similar, so overall, this should be acceptable.

2.6.4.9 Discussion and Conclusions

Single dose and multiple dose pharmacokinetic studies were conducted using both s.c. and i.v. doses of denosumab in mice (WT and huRANKL KI) and cynomolgus monkeys. Studies in mice showed that after single dose i.v. administration of denosumab, elimination was much faster in KI mice versus WT mice, since in WT mice, there was no ligand for denosumab to bind. A second study comparing s.c. and i.v. administration of a 1 mg/kg dose in male WT mice indicated that serum concentration was similar regardless of the route of administration. The F value (systemic bioavailability) in s.c. treated mice was 86.1% compared to i.v. treated mice. Following i.v. administration, the V_{ss} values observed indicated that denosumab was residing in the vascular space, and was not distributing to other extravascular compartments.

Studies were also conducted in male and female FcRn knockout (KO) and WT mice administered an i.v. dose of denosumab. WT mice had a 26-fold longer half-life for denosumab and a 15-fold greater exposure (based on $\text{AUC}_{0-\infty}$) than FcRn KO mice. There was also a 15-fold increase in the clearance rate for denosumab, and reduced steady state volume of distribution in FcRn KO mice compared to WT mice.

Studies in monkeys characterized the PK further since denosumab was pharmacologically active in this species. After single dose i.v. or s.c. administration of denosumab, exposure was determined to be triphasic, with an initial distribution or absorption phase, secondary phase with long half-life, and rapid terminal phase. Nonlinear and greater than dose proportional AUC was observed for doses <1 mg/kg, but was linear and dose proportional from 1-3 mg/kg. Mean residence time also increased with dose, while clearance decreased. Antibody formation in the monkeys was highly prevalent with both i.v. and s.c. dosing, with all but one animal developing antibodies. Antibody formation is expected however, and is noted in the repeat dose toxicology studies as well. During weekly s.c. dosing in monkeys, AUC increased dose proportionally following the first dose at Week 1 for doses between 0.1-10 mg/kg, and also following the 4th dose at Week 4.

Radiolabeled ¹²⁵I-denosumab was widely distributed in monkeys administered 0.1 and 1 mg/kg s.c. doses. At 0.1 mg/kg, radioactivity was identified in all tissues except bone marrow, brain and muscle at 672 hours postdose. The highest concentrations were observed at the dose site skin, dose site subcutaneous tissue, thyroid/parathyroid, serum, axillary lymph nodes, inguinal lymph nodes, blood, spleen, and ovaries. At 1 mg/kg, radioactivity was quantified in all tissues at 672 hours and in approximately half of the tissues at 1344 hours postdose. The highest concentrations of radioactivity were observed at the dose site skin, dose site subcutaneous tissue, thyroid/parathyroid, axillary lymph nodes, serum, blood, ovaries, and lungs. As expected, high concentrations of ¹²⁵I-denosumab were present in the various lymph nodes. An unexpected finding was that ¹²⁵I-denosumab accumulated in the cornea of the eye in both the male and female cynomolgus monkeys.

The major route of elimination of ¹²⁵I-denosumab and or radioactively labeled protein fragments in monkeys was in the urine, with 76-95% of the administered activity present in the urine 672 hours postdose. Fecal elimination represented 1.1 to 3% of the administered radioactivity.

The distribution of denosumab was also evaluated *ex vivo* in a series of tissue cross-reactivity studies using adult, fetal human, cynomolgus monkey, rabbit, and rat tissue sample sections. In the rabbit and monkey, denosumab bound to cells in the periphery of the cortex of lymph nodes and lymphoid nodules of the spleen, and the periphery of lymphoid nodules in the gut-associated lymphoid tissue of the small and large intestines. In human tissues, denosumab cross reacted to cells in the lymph node of 1/3 human donors, and displayed weak to moderate membrane staining of lymphocytes lining the periphery of the paracortex in the lymph node. In the rat, denosumab bound to chondrocytes and the margins of their surrounding lacunae in the articular cartilage.

Additional toxicokinetics are discussed in the primary review of toxicology study 102090 in section 2.6.6.3.2, and in the primary review of pharmacology study 103981 in section 2.6.2.2.1. Briefly, in the 6/12-month toxicology study, AUC was relatively linear from 1-50 mg/kg, no accumulation was observed between the 1st and 13th dose, and antibody formation was prevalent and inversely proportional to dose (100% LD, 50% MD, 13% HD). In the 16-month pharmacology study, C_{max} and AUC increases were linear over the dose range, and no accumulation of drug was observed over the duration of the study.

In human subjects, s.c. denosumab administration has dose-dependent, nonlinear PK over a wide dose range, but exhibits dose-proportional increases at doses ≥ 60 mg. Absorption is rapid, with measurable levels evident at 10 minutes postdose. C_{max} is typically observed 1-4 weeks postdose, after which serum levels decline over 4-5 months ($t_{1/2} \approx 25-30$ days). Repeat doses of denosumab at 6 month intervals (clinical regimen) do not lead to accumulation, and over a 4 year administration period, PK does not appear to change with time. The amount of denosumab from a s.c. dose that is pharmacologically available has been estimated to be 61% based on degradation of monoclonal antibodies in the plasma.

Evaluation of human dose multiples:

NOAELs for the pivotal toxicology and pharmacology studies (102090, 103981 and 102842) were derived and are presented in the table below. To determine the human dose multiples of the nonclinical doses, it was decided that they would be based on body weight (mg/kg) comparison. The basis for this decision was three-fold: 1) nonclinical exposure to denosumab was either monthly or weekly, compared to the Q6M clinical regimen of treatment, 2) systemic exposure from nonclinical subcutaneous dosing correlated with exposure following nonclinical intravenous dosing, and 3) only AUC_{0-τ} data were provided for nonclinical and clinical PK, and not AUC_{0-∞}. The proposed clinical dose of denosumab is a single s.c. injection of 60 mg every 6 months, which for a 60 kg female, equates to a 1 mg/kg dose every 6 months.

Study	Dose (NOAEL in animals)	Dose Multiple (based on mg/kg)
12-mo monkey (102090)	50 mg/kg (monthly)	50
16-mo monkey (103981)	50 mg/kg (monthly)	50
Embryo/fetal in monkey (102842)	12.5 mg/kg (weekly)	13
PK in human (20010223)	1 mg/kg (Q6M)	

The calculated dose multiples based on mg/kg comparison provide an appropriate safety margin for clinical use of the recommended 60 mg subcutaneous dose.

2.6.4.10 Tables and figures to include comparative TK summary

The following tables were submitted by the Sponsor (found in Module 2.6.5)

Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies

Single Dose Pharmacokinetics

Study	Species	Sex	Route	Dose (mg/kg)	n/ timepoint	T _{max} (hr)	C _{max} or C ₀ (µg/mL)	t _{1/2} (hr)	AUC ₀₋₁ (µg*hr/mL)	AUC _{0-∞} (µg*hr/mL)	CL or CL/F (mL/hr/kg)	V _{ss} (mL/kg)	MRT _{0-∞} (hr)
101494	Mouse	M	SC	1	2	72	23.1	444	NA	15600	0.0642	NA	NA
		M	IV	0.1	2	NA	3.91	420	NA	1680	0.0594	43.5	NA
		M	IV	1	2	NA	31.9	463	NA	18100	0.0553	40.2	NA
		M	IV	10	2	NA	511	461	NA	128000	0.0778	48.6	NA
101002	Rat	F	SC	1	1	72	6.87	106	1780	1970	0.507	NA	NA
		M/F	IV	0.0628	2	NA	1.97	240	148	201	0.318	98.6	NA
		M/F	IV	1	2	NA	22.9	270	3090	3580	0.287	107	NA
		M/F	IV	10	2	NA	318	290	34700	41800	0.242	97.2	NA
101398	Cynomolgus Monkey	F	SC	0.0016	3	10.7 (11.5)	0.00433 (0.00166)	41.9 (0.134)	0.143 (0.0339)	0.301 (NA)	10.6 (3.56)	NA	17.0 (9.00)
		F	SC	0.0053	3	18.7 (9.24)	0.0229 (0.000500)	35.8 (6.30)	1.54 (0.119)	1.64 (0.203)	6.15 (0.720)	NA	40.4 (3.17)
		F	SC	0.0848	3	56.0 (36.7)	0.728 (0.0911)	24.1 (7.00)	126 (21.2)	126 (21.2)	0.808 (0.125)	NA	111 (4.29)
		F	SC	1.0	3	96.0 (0.00)	16.5 (5.83)	28.9 (18.8)	3940 (1820)	3940 (1820)	0.298 (0.147)	NA	182 (55.5)
		F	SC	3.0	3	64.0 (27.7)	35.8 (9.02)	29.5 (16.8)	8790 (2080)	8790 (2080)	0.353 (0.0737)	NA	192 (90.3)
		F	IV	0.0016	3	NA	0.0625 (0.0120)	8.37 (1.15)	0.779 (0.310)	0.763 (0.302)	4.40 (1.80)	51.4 (15.3)	12.1 (1.50)
		F	IV	0.0053	3	NA	0.243 (0.0199)	14.3 (1.80)	4.80 (0.320)	4.77 (0.310)	2.10 (0.133)	45.1 (1.88)	21.5 (2.31)
		F	IV	0.0848	3	NA	3.72 (0.245)	36.9 (17.5)	187 (34.1)	189 (37.0)	0.542 (0.106)	38.4 (1.38)	72.5 (13.6)
		F	IV	1.0	3	NA	35.9 (5.11)	19.3 (4.18)	3590 (1540)	3590 (1540)	0.310 (0.110)	30.7 (5.21)	106 (33.9)
		F	IV	3.0	3	NA	105 (40.5)	27.5 (11.7)	12400 (4850)	12400 (4850)	0.277 (0.134)	31.2 (7.96)	131 (63.6)

Median (range) for T_{max} not applicable for Studies 101494 and 101002 and not reported for Study 101398.

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Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies

Single Dose Toxicokinetics

Study	Species	Sex	Route	Dose (mg/kg)	n/timepoint	T _{max} (hr)	C _{max} or C ₀ (µg/mL)	t _{1/2} (hr)	AUC ₀₋₁ (µg*hr/mL)	AUC _{0-∞} (µg*hr/mL)	CL or CL/F (mL/hr/kg)	V _{ss} (mL/kg)	MRT _{0-∞} (hr)
101606	Cynomolgus Monkey	M/F	SC	0.3	3	96	2.89 (0.350)	NA	NA	NA	NA	NA	NA
		M/F	SC	3	3	96	29.2 (1.19)	NA	NA	NA	NA	NA	NA
		M/F	SC	30	3	96	291 (94.1)	NA	NA	NA	NA	NA	NA

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Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies

Multiple Dose Toxicokinetics

Study	Species	Dosing Schedule	Study Week	Sex	Route	Dose (mg/kg)	n/timepoint	T _{max} (hr)	C _{max} (ug/mL)	AUC ₀₋₁ (ug*hr/mL)
101447	Cynomolgus Monkey	Weekly	1	M/F	SC	0.1	12	72.0 (20.5)	1.51 (0.486)	195 (65.7)
				M/F	SC	1	12	92.0 (32.1)	14.0 (3.14)	1800 (430)
				M/F	SC	10	12	108 (36.2)	155 (27.6)	20200 (3640)
				M/F	IV	10	12	6.83 (8.56)	615 (183)	48700 (14400)
			4	M/F	SC	0.1	12	26.9 (47.7)	11.3 (31.2)	349 (869)
				M/F	SC	1	12	30.7 (33.1)	27.5 (14.0)	3410 (2080)
				M/F	SC	10	12	35.0 (38.6)	302 (151)	42000 (22700)
				M/F	IV	10	12	4.00 (6.66)	663 (133)	68600 (23000)
102090	Cynomolgus Monkey	Monthly	1 ^a	M/F	SC	1	16	96 (48-96)	15.9 (1.81)	4100 (1150)
				M/F	SC	10	16	96 (48-336)	162 (25.4)	61500 (16000)
				M/F	SC	50	16	96 (48-96)	853 (79.3)	343000 (52200)
			13 ^a	M/F	SC	10	4	24 (12-96)	115 (37.1)	48200 (21100)
				M/F	SC	50	7	48 (24-96)	666 (156)	268000 (90300)

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a Dose number

Median (range) for T_{max} not reported for Study 101447**Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies**

Multiple Dose Toxicokinetics

Study	Species	Dosing Schedule	Study Week	Sex	Route	Dose (mg/kg)	n/timepoint	T _{max} (hr)	C _{max} (ug/mL)	AUC ₀₋₁ (ug*hr/mL)
103981	Cynomolgus Monkey	Monthly	First Dose	F	SC	25	15	96 (24-168)	143 (58.7)	59600 (22900)
			12th Dose	F	SC	25	14	96 (24-168)	234 (34.1)	113000 (21000)
			15th Dose	F	SC	25	14	96 (48-168)	222 (49.9)	101000 (26100)
			First Dose	F	SC	50	17	48 (48-96)	336 (67.6)	139000 (34600)
			12th Dose	F	SC	50	17	48 (24-168)	511 (132)	212000 (71900)
			15th Dose	F	SC	50	17	48 (24-96)	413 (160)	171000 (72400)

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a Dose number

Median (range) for T_{max} not reported for Study 101447**Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies**

Drug-Drug Interactions

Study	Species	Dosing Schedule	Study Week	Sex	Route	Dose (mg/kg)	n/timepoint	T _{max} (hr)	C _{max} (ug/mL)	AUC ₀₋₁ (ug*hr/mL)
106564	Cynomolgus Monkey	Monthly	Month 7	F	SC	Vehicle + 25	10	168 (72-336)	145 (31)	41600 (10200)
			Month 7	F	SC	ALN + 25	11	72 (72-336)	172 (65)	43700 (10800)
			Month 1	F	SC	25	11	168 (24-168)	152 (45)	40300 (10200)
			Month 7	F	SC	25	5	72 (24-168)	372 (132)	90400 (25800)
			Month 7	F	SC	25	6	72 (72-336)	220 (142)	45600 (24900)

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Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies

Toxicokinetics in Pregnant or Nursing Animals

Study	Species	Dosing Schedule	Dose #	Sex	Route	Dose (mg/kg)	n	T _{max} (hr)	C _{max} (ug/mL)	AUC ₀₋₂₄ (mg*hr/mL)	AR
102842	Cynomolgus Monkey	Weekly doses	5	F	SC	2.5	7	24 (8-120)	58.8 (19.8)	8.80 (3.05)	2.46 (0.428)
			5	F	SC	5	10	16 (8-72)	114 (52.3)	15.5 (6.35)	2.59 (0.926)
			5	F	SC	12.5	14 ^a	24 (8-72)	282 (89.6)	41.0 (10.6)	2.79 (0.656)
102843	Cynomolgus Monkey	Weekly doses	NA	F	SC	2.5	6	72 (24-120)	48.8 (11.9)	6.77 (1.50)	NA
			NA	F	SC	5	6	72 (72-120)	79.0 (16.4)	11.7 (2.53)	NA
			NA	F	SC	12.5	6	72 (72-168)	186 (24.4)	26.9 (2.70)	NA
			11	F	SC	2.5	4	24 (8-72)	26.5 (36.7)	4.22 (5.94)	0.787 (1.09)
			9	F	SC	5	4	24 (8-24)	115 (81.7)	16.4 (11.4)	1.54 (1.06)
			10	F	SC	12.5	5	24 (8-72)	476 (279)	67.8 (39.6)	2.59 (1.57)
			18	F	SC	2.5	2	64 (8-120)	121 (84.0)	17.6 (14.8)	3.27 (2.64)
			17	F	SC	5	3	24 (8-72)	163 (27.2)	16.9 (2.32)	1.61 (0.320)
			20	F	SC	12.5	4	3 (8-24)	727 (148)	85.5 (20.7)	3.31 (1.25)

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^a n = 13 for AR**Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies**

In Vivo Metabolism Studies

Study	Species	Route	Dose (mg/kg)	Sex	n/timepoint	C ₀ (ug/mL)	AUC ₀₋₂₄ (ug*hr/mL)	t _{1/2z} (hr)	CL (mL/hr/kg)	V _d (mL/kg)	V ₁₆ (mL/kg)
106892	WT mouse	IV	0.1	M/F	3	2.60	881	426	0.114	38.5	74.0
	KI mouse	IV	0.1	M/F	3	2.19	150	78.6	0.667	45.7	72.7
106893	WT mouse	IV	0.1	M/F	3	2.21	685	489	0.146	45.2	97.6
	FcRn KO mouse	IV	0.1	M/F	3	2.55	48.5	18.1	2.06	39.3	52.3
	WT mouse	IV	1	M/F	3	20.8	6910	506	0.145	48.0	100
	FcRn KO mouse	IV	1	M/F	3	21.7	455	20.2	2.2	46.0	58.6

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Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies

Other Pharmacokinetic Studies

Study	Species	Route	Dose (mg/kg)	Sex	N ^a	Process	T _{max} (hr)	C _{max} (ng/mL)	AUC _{0-336 hr} (ug*hr/mL)
103948	Cynomolgus Monkey	SC	0.1	F	5	CP1	72 (24-169)	958 (479)	188 (46.4)
		SC	0.1	F	3	CP2	78 (48-72)	765 (163)	163 (40.4)

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^a Anti-denosumab antibody-negative on Day 14**Table 14. Summary of Denosumab PK or TK Parameters from Preclinical Pharmacokinetics Studies**

Tissue Distribution / Excretion Studies

Study	Species	Route	Dose (mg/kg)	Sex	n/timepoint	T _{max} (hr)	C _{max} (ng/mL)	t _{1/2z} (hr)	AUC ₀₋₂₄ (ug*hr/mL)	AUC ₀₋₂₄ (ug*hr/mL)	CL/F (mL/hr/kg)	MRT (hr)
104105	Cynomolgus Monkey	SC	0.1	M/F	7	120	564	ND	107	ND	ND	ND
		SC	1	M/F	7	120	9750	36.6	2760	2770	0.361	204
104192	Cynomolgus Monkey	SC	0.1	M/F	7	120	612	34.6	NR	126	0.795	139
		SC	1	M/F	7	120	8870	37.9	NR	2930	0.341	274

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2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Table 1. Pharmacokinetics Tabular Summary: Absorption After a Single Dose Test Article: Denosumab

Type of Study / Title	Status	Test System	Objective	Methods	Summary of Results
101494- Pharmacokinetic Study of Denosumab (AMG 162) in Male Mice Following Intravenous or Subcutaneous Administration	Single Dose/ Non- GLP	Mouse	To determine the pharmacokinetics of denosumab in mice following intravenous or subcutaneous administration.	IV, SC bolus dosing 0.1, 1.0, 10.0 mg/kg IV, 1.0 mg/kg SC; Serum samples were collected to determine denosumab concentrations; serum denosumab concentration-time data were analyzed by non- compartmental methods.	Following IV dosing of 0.1 to 10.0 mg/kg, denosumab PK was approximately linear with dose. Exposure based on C_0 and $AUC_{0-\infty}$ values increased approximately dose-proportionally (76- to 130-fold for the 100-fold increase in dose). Across the IV dose range, CL values were similar (<41% different, mean value 0.0642 mL/hr/kg), as were V_{ss} (<21% different, mean value 44.1 mL/kg) and $t_{1/2\alpha}$ (<11% different, mean value 18.7 days) values. Following SC administration of 1.0 mg/kg, C_{max} was reached 72 hours post-dose, $t_{1/2\alpha}$ was similar to those observed following IV dosing, and bioavailability was approximately 88%. Denosumab displayed dose-linear IV pharmacokinetics in the dose range of 0.0628 to 10 mg/kg, with a steady state volume of distribution of approximately 101 mL/kg. The mean terminal-phase half-life was 270 ± 19.4 hrs. Following SC administration of 1.0 mg/kg, peak levels of 8,870 ng/mL occurred 72 hr post-dose and mean bioavailability was 58%.
101002 – Pilot Pharmacokinetic Study of AMG 162 Administered Subcutaneously or Intravenously in Male and Female Sprague Dawley Rats	Single Dose/ Non- GLP	Rat	To determine the pharmacokinetic profiles of a human IgG ₂ monoclonal antibody (Hu-Mab) to osteoprotegerin ligand (OPGL), AMG 162 in rats following intravenous or subcutaneous administration.	IV, SC bolus dosing 0.0628, 1.0, 10 mg/kg IV, 1.0 mg/kg SC; Serum samples were collected to determine denosumab concentrations and the presence of anti-denosumab antibodies; serum denosumab concentration-time data were analyzed by non- compartmental methods.	

Table 1. Pharmacokinetics Tabular Summary: Absorption After a Single Dose Test Article: Denosumab

Type of Study / Title	Status	Test System	Objective	Methods	Summary of Results
101398 – A Single Dose Intravenous and Subcutaneous Pharmacokinetic and Pharmacodynamic Study of AMG 162 in Cynomolgus Monkeys	Single Dose/ GLP	Cynomolgus Monkey	To evaluate the pharmacokinetics and pharmacodynamics of AMG 162 when administered, as a single subcutaneous or intravenous injection to cynomolgus monkeys.	IV, SC bolus 0.0016, 0.00532, 0.0848, 1.0, 3.0 mg/kg (IV or SC); Serum samples were collected to determine denosumab concentrations and the presence of anti- denosumab antibodies; serum denosumab concentration-time data were analyzed by non- compartmental methods.	Denosumab displayed non-linear pharmacokinetics in cynomolgus monkeys following IV and SC administration. CL increased 16-fold (0.277 to 4.40 mL/hr/kg) following IV administration and CL/F increased 30-fold (0.353 to 10.6 mL/hr/kg) following SC administration as dose decreased from 3.0 mg/kg to 0.0016 mg/kg. However, denosumab PK was approximately dose-linear from 1 to 3 mg/kg for both IV and SC administration. Mean V_{ss} was similar to that of plasma volume (~40 mL/kg). Denosumab IV PK was well described by a two-compartmental model with parallel linear and nonlinear Michaelis-Menten elimination. Following SC administration, bioavailability showed a trend of increasing from 28% to 100% with increasing dose (based on compartmental analysis). SC dosing of denosumab caused a rapid (within 24 hours) reduction in bone resorption based on N-telopeptide in serum (sNTx), at doses above 0.00532 mg/kg. The duration of sNTx reduction increased with dose up to 8 weeks over the dose range investigated. A serum denosumab concentration corresponding to half- maximal inhibition of bone resorption (EC_{50}) of 464 ng/mL was estimated based on PK/PD modeling using an indirect effect model. The development of antibodies to denosumab was not route dependent and was associated with more rapid elimination of denosumab for doses above 0.0848 mg/kg.

Table 1. Pharmacokinetics Tabular Summary: Absorption After a Single Dose Test Article: Denosumab

Type of Study / Title	Status	Test System	Objective	Methods	Summary of Results
101606 - A Single Dose Subcutaneous Administration of AMG 162 for Cardiovascular and Respiratory Evaluation in Cynomolgus Monkeys	Single Dose/ GLP	Cynomolgus Monkey	To examine the effects of subcutaneous administration of AMG 162 on cardiovascular parameters and respiratory rate in conscious, unrestrained cynomolgus monkeys.	SC bolus 0.3, 3.0, 30 mg/kg; A limited number of serum samples were collected to determine denosumab concentrations; due to limited data, summary statistics were generated by timepoint, but no PK analysis was performed.	No animals that were to be dosed with placebo displayed quantifiable serum denosumab concentrations, while all animals that were to receive denosumab displayed quantifiable levels. Based on limited TK sampling, maximum mean serum denosumab concentrations were observed on day 5 for all dose groups and increased in an approximately dose-proportional manner.

Table 2. Pharmacokinetics Tabular Summary: Absorption After Repeated Doses Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
101447 – Toxicokinetics From A 1-Month Study Evaluating the Effect on Bone of AMG 162 Administered Subcutaneously or Intravenously in Cynomolgus Monkeys with a 3-Month Recovery Period	Multiple Dose/ GLP	Cynomolgus Monkey	To assess the toxicity of AMG 162 when administered by intravenous or subcutaneous injection to monkeys once weekly for 1 month, and to evaluate recovery from any effects of the test article after a 13-week treatment-free period following 4-weeks of treatment.	IV, SC bolus 0.1, 1.0, 10 mg/kg SC, 10 mg/kg IV once-weekly; Serum samples were collected to determine denosumab concentrations and the presence of anti-denosumab antibodies; serum denosumab concentration-time data were analyzed by non-compartmental methods.	There was no evidence of serum denosumab exposure in control animals, and sex had no impact on denosumab toxicokinetics. Exposures based on AUC over the (1 week) dosing interval (AUC_{0-1}) increased approximately dose-proportionally over the SC dose range of 0.1 to 10 mg/kg after the first dose. For the SC dose regimens, there was approximately 2-fold accumulation after the fourth vs. first weekly dose (based on AUC_{0-1}), while there was no appreciable accumulation following 4 weekly IV doses of 10 mg/kg. Antibodies to denosumab were detected in 28 of 30 treated animals that were evaluated during the recovery period and were associated with decreased exposure in those animals relative to antibody-negative animals.

Table 2. Pharmacokinetics Tabular Summary: Absorption After Repeated Doses Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
102090 – Toxicokinetics Report for a ~8/12-Month Subcutaneous Toxicity Study of AMG 162 in the Cynomolgus Monkey with an Interim Kill after 6 Months and a 3-Month Recovery Period	Multiple Dose/ GLP	Cynomolgus Monkey	To evaluate the toxicity of the test article, AMG 162, following subcutaneous administration to the Cynomolgus monkey for 6 or 12 months and to assess the reversibility of effects observed – if any – during a 3-month treatment free-period.	SC bolus 1.0, 10, 50 mg/kg SC once-monthly for 6 or 12 months; Serum samples were collected to determine denosumab concentrations and the presence of anti-denosumab antibodies; serum denosumab concentration-time data were analyzed by non-compartmental methods.	There was no evidence of serum denosumab exposure in control animals. Sex had no apparent impact on denosumab toxicokinetics. Following the first dose, exposure based on mean C_{max} and AUC_{0-1} values increased approximately dose-proportionally from 1 to 50 mg/kg. Following the last dose, exposure increased approximately dose-proportionally from 10 to 50 mg/kg and no accumulation was observed. Anti-denosumab antibodies were detected in 55.3 % of denosumab-treated animals, which was associated with decreased serum denosumab exposure. However, importantly, only 2 of 16 animals treated with 50 mg/kg monthly (the No-Observed-Adverse-Effect-Level or NOAEL) for 12 months were antibody positive. In the remaining (antibody-negative) animals, the mean AUC_{0-1} value after the last dose was 268 mg*hr/mL, corresponding to an estimated cumulative AUC over a 6-month period of 1808 mg*hr/mL.

Table 2. Pharmacokinetics Tabular Summary: Absorption After Repeated Doses Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
103981 - A Monthly Subcutaneous Injection Osteoporosis Prevention Study of AMG 162 for 16 Months in the Cynomolgus Monkey	Multiple Dose/ GLP	Cynomolgus Monkey	The purposes of this study were 1) to determine the efficacy of two dose levels of monthly subcutaneous injections of AMG 162 after 16 months of treatment on the preservation of cortical and cancellous bone mass as determined by bone mineral density (BMD) and on strength as determined by biomechanical testing. 2) to evaluate mechanisms by which AMG 162 affects bone by evaluation of biomarkers of bone function and histomorphometric indices of bone function in surgically postmenopausal (ovariectomized or OVX) monkeys.	SC bolus 25, 50 mg/kg once-monthly for 16 months; Serum samples were collected to determine denosumab concentrations and the presence of anti-denosumab antibodies; serum denosumab concentration-time data were analyzed by non-compartmental methods.	There was no evidence of serum denosumab exposure in sham(-operated) or OVX control animals treated with vehicle. In denosumab-treated animals, exposure based on mean C_{max} and AUC_{0-16} values increased approximately dose-proportionally from 25 to 50 mg/kg following the first and last doses, and no accumulation was observed. Anti-denosumab antibodies developed in 25 and 15% of animals receiving 25 and 50 mg/kg, respectively, and were associated with decreased serum denosumab exposure.

Table 3. Pharmacokinetics Tabular Summary: Organ Distribution Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
104105 – Quantitative Whole Body Autoradiography of Cynomolgus Monkeys Following a Single Subcutaneous Administration of 125 I-AMG 162 [see also Study 104192, Table 10]	Single Dose/ Non-GLP	Cynomolgus Monkey	To assess the extent of absorption and distribution of radioactivity following a single subcutaneous administration of 125 I-AMG 162 given to male and female monkeys.	Single SC bolus dose 0.1 or 1.0 mg/kg At 12, 120, 672, and 1344 (1 mg/kg only) hours post-dose, 1 animal/sex/time-point was sacrificed to examine the tissue distribution by QWBA. Serum samples collected up to 672 or 1344 hours were analyzed for total radioactivity, trichloroacetic acid (TCA)-precipitable radioactivity, and the development of antibodies to denosumab. In addition, serum denosumab and C-telopeptide (CTx) concentrations were determined. Serum denosumab concentration-time data were analyzed by non-compartmental methods.	For both the 0.1 and 1 mg/kg doses, radioactivity was widely distributed in both males and females, with radioactivity quantifiable in nearly all analyzed tissues at 12 and 120 hours post-dose, but at levels generally markedly less than those in serum. In the 0.1 mg/kg group, concentrations of radioactivity declined in all tissues to non-quantifiable levels by 672 hours post-dose except the injection site, eye (cornea), large intestinal contents (males), lymph nodes, spleen, stomach content (males), and thyroid. For the 1 mg/kg dose, concentrations of radioactivity declined in all tissues to non-quantifiable levels by 1344 hours post-dose, with the exception of the injection site, lymph nodes, ovary, spleen, and thyroid. Thus, radioactivity was generally measurable in the lymph nodes and spleen at 672 or 1344 hours post-dose for the 0.1 and 1 mg/kg doses, respectively. Overall, the results for male and female animals were similar, indicating a lack of sex difference in the distribution of 125 I-denosumab-derived radioactivity. Serum denosumab exposure based on AUC_{0-16} values increased greater than dose-proportionally (approximately 26-fold, respectively, for the 10-fold increase in dose) from 0.1 to 1 mg/kg. The percent change from baseline serum CTx ranged from approximately -38 to -54% from 12 to 336 hours post-dose for the 0.1 mg/kg dose group, while it ranged from approximately -49 to -70% over that time period for the 1 mg/kg dose group.

Table 5. Pharmacokinetics Tabular Summary: Study in Pregnant or Nursing Animals Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
102843 – Subcutaneous Fertility Evaluation of AMG 162 in the Female Cynomolgus Monkey	Multiple Dose/ GLP	Cynomolgus Monkey	To evaluate the potential effect on fertility of the test article AMG 162, when administered subcutaneously to the female cynomolgus monkey over two consecutive menstrual cycles until Day 20 post-mating.	Multiple SC bolus doses; 2.5, 5, 12.5 mg/kg weekly; Serum samples were collected to determine denosumab concentrations; serum denosumab concentration-time data were analyzed by non-compartmental methods.	Because the development of antibodies to denosumab was not assessed in this study, results for exposure dose-proportionality or accumulation may be confounded by the effects of anti-drug antibodies. Mean C_{max} and AUC_{0-24} values in all animals at the highest dose level (12.5 mg/kg weekly) were 476 µg/mL and 67,800 µg*hr/mL, respectively, prior to the first mating.
102842 – Subcutaneous Embryo-Fetal Development Study of AMG 162 in the Cynomolgus Monkey	Multiple Dose/ GLP	Cynomolgus Monkey	To Investigate the embryonic and teratogenic effects of the test article AMG 162, when administered subcutaneously to the pregnant cynomolgus monkey during the period of organogenesis.	Multiple SC bolus doses; 2.5, 5, 12.5 mg/kg weekly; Serum samples were collected to determine denosumab concentrations and the presence of anti-denosumab antibodies; serum denosumab concentration-time data were analyzed by non-compartmental methods.	One animal in the control (vehicle-treated) group displayed quantifiable serum denosumab concentrations. Neutralizing antibodies were detected in 34% of denosumab-treated animals and were associated with decreased serum denosumab exposure. In antibody-negative animals, exposure based on mean C_{max} and AUC_{0-24} values increased approximately dose-proportionally from 2.5 to 12.5 mg/kg and moderate accumulation (>2 fold) was observed by the 5 th weekly dose. Mean C_{max} and AUC_{0-24} values in antibody-negative animals at the highest dose level (12.5 mg/kg weekly) were 282 µg/mL and 41,000 µg*hr/mL, respectively, following the 5 th dose. Quantifiable denosumab concentrations were observed in 70% of fetal serum samples, indicating that denosumab crosses the placental barrier.

Table 7. Pharmacokinetics Tabular Summary: Metabolism In Vivo Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
106892 – A Single Dose Pharmacokinetics Study of Denosumab (AMG 162) Following Intravenous Administration to Male or Female huRANKL Knock-In and Wild-Type Mice	Single Dose/ Non-GLP	Mouse	To characterize the single dose pharmacokinetics (PK) of denosumab (AMG 162) following intravenous administration to male or female huRANKL knock-in and wild-type mice.	Single IV bolus dose of 0.1 mg/kg; Serum denosumab concentrations were determined up to 1344 hr post-dose and composite mean serum denosumab concentration-time data were analyzed by non-compartmental methods.	Exposure based on AUC_{0-24} was 6.6-fold greater and the terminal half-life was 11-fold longer in wild-type relative to huRANKL knock-in mice. The difference in exposure reflects 6.6-fold higher clearance in the huRANKL knock-in animals. These results suggest that, in mice expressing a form of RANKL to which denosumab binds (huRANKL), binding of denosumab to its target antigen leads to an accelerated rate of elimination (relative to elimination in wild-type animals). Thus, binding of denosumab to huRANKL appears to play a significant role in the elimination of the antibody.

Table 7. Pharmacokinetics Tabular Summary: Metabolism In Vivo Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
106893 - A Single Dose Pharmacokinetics Study of Denosumab (AMG 162) Following Intravenous Administration to Male or Female FcRn Knockout and Wild Type Mice	Single Dose/ Non-GLP	Mouse	To characterize the single dose pharmacokinetics of denosumab (AMG 162) following intravenous administration to male or female FcRn knockout and wild type mice.	Single IV bolus dose of 0.1 or 1.0 mg/kg; Serum denosumab concentrations were determined up to 1344 hr post-dose and composite mean serum denosumab concentration-time data were analyzed by non-compartmental methods.	Following a single bolus IV dose of 0.1 or 1.0 mg/kg denosumab, C_0 and V_0 values were similar in FcRn KO and WT mice. The WT mice displayed 14- to 15-fold greater exposure based on AUC_{0-24} values, reflecting 14- to 15-fold higher CL values in the FcRn KO animals. V_{ss} values were approximately 2-fold higher than V_0 values in WT animals, indicating modest tissue distribution of denosumab. In contrast, V_{ss} values were ≤33% different than V_0 values in FcRn KO animals, indicating more limited tissue distribution of denosumab in the absence of FcRn. The higher CL and smaller V_{ss} in FcRn KO animals resulted in markedly (25- to 27-fold) shorter $t_{1/2}$ values relative to WT mice.

Table 10. Pharmacokinetics Tabular Summary: Excretion Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
104192 – Absorption, Distribution and Excretion in Cynomolgus Monkeys Following a Single Subcutaneous Administration of ¹²⁵ I-AMG 162	Single Dose/ Non-GLP	Cynomolgus Monkey	To assess the extent of absorption, distribution, and excretion of radioactivity following a single subcutaneous administration of ¹²⁵ I-AMG 162 given to female cynomolgus monkeys.	Single SC bolus dose of 0.1 or 1.0 mg/kg Up to 672 or 1344 hours post-dose, 2 animals per time point were sacrificed and tissues processed prior to radioactivity analysis by liquid scintillation counting. Serum samples collected up to 672 or 1344 hours were analyzed for total radioactivity, trichloroacetic acid (TCA)-precipitable radioactivity, and the development of antibodies to denosumab.	In both dose groups, ¹²⁵ I-denosumab-derived radioactivity was widely distributed and quantifiable in all tissues examined, but generally at levels markedly lower than those in blood or serum. The highest concentrations were observed in the injection site (skin and subcutaneous tissue), thyroid/parathyroid, serum, axillary and inguinal lymph nodes, blood, spleen, ovaries, and lungs. The concentration of radioactivity declined to levels less than those in serum by 672 or 1344 hours post-dose in all tissues except at the injection site (skin and subcutaneous tissue), axillary and inguinal lymph nodes, and spleen. The radioactivity in lymph nodes and spleen continually decreased, with higher concentrations than serum at end-of-study reflecting slightly longer persistence in those tissues. It was not determined whether the radioactivity in these tissues was acid-precipitable. Thus, it is unknown if the radioactivity corresponds to intact antibody, a catabolic product, or free ¹²⁵ I.

Table 10. Pharmacokinetics Tabular Summary: Excretion Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
				In addition, serum denosumab and C-telopeptide (CTX) concentrations were determined. Urine and fecal samples were also collected up to 672 and 1344 hours post-dose in the 0.1 and 1.0 mg/kg dose groups, respectively. Serum denosumab concentration-time data were analyzed by non-compartmental methods	All animals that remained on-study to 672 or 1344 hours developed antibodies to denosumab, the effects of which on the distribution of the radiolabel are unknown. Radioactivity in bone tissues was low relative to serum and declined in parallel, indicating a lack of specific uptake or sequestration in osseous tissues. Except for the 0.5 hr time-point, the majority of circulating radioactivity (86 to 99% at each time point) in serum was acid-precipitable in both dose groups, indicating that most of the circulating species was intact antibody. Urine was the major route of elimination of ¹²⁵ I-denosumab-derived radioactivity, accounting for approximately 80-95% and 76-79% of the administered radioactivity in the 0.1 and 1.0 mg/kg dose groups, respectively. The corresponding fecal recovery values were 1.8-3.1% and 1.1-2.8%. The percentage of urinary radioactivity that was acid-precipitable generally ranged from 3.4-25% and represented on average $\pm 0.75\%$ of the administered radioactivity at each collection interval.

Table 10. Pharmacokinetics Tabular Summary: Excretion Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
					<p>The corresponding values for feces were 20-60% of sample radioactivity and $\leq 0.15\%$ of administered radioactivity. Therefore, ^{125}I-denosumab-derived radioactivity was primarily excreted as free iodide or small iodinated peptide fragments. Overall recovery of radioactivity ranged from 92-106% and 83-89% in the 0.1 and 1.0 mg/kg dose groups at 672 and 1344 hours post-dose, respectively.</p> <p>Denosumab serum PK was nonlinear with dose, with a 23-fold increase in $\text{AUC}_{0-\text{inf}}$ for the 10-fold increase in dose. The percent change from baseline serum CTx ranged from approximately -41 to -59% from 12 to 336 hours post-dose for the 0.1 mg/kg dose group, while it ranged from approximately -56 to -70% from 12 to 672 hours post-dose for the 1 mg/kg dose group.</p>

Table 12. Pharmacokinetics Tabular Summary: Other Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
103948 – Pharmacokinetic and Pharmacodynamic Comparability Study for Two Manufacturing Processes of AMG 162 in Female Cynomolgus Monkeys	Single Dose/ Non-GLP	Cynomolgus Monkey	To compare the pharmacokinetic profiles of AMG 162 manufactured by two different processes (CP1 and CP2) following subcutaneous administration of 0.1 mg/kg to naive female cynomolgus monkeys. The secondary objective of this study was to compare the pharmacodynamic effects of AMG 162 manufactured by two processes (CP1 and CP2) following SC administration of 0.1 mg/kg to naive female cynomolgus monkeys by assessing the serum biomarker Type 1 C-telopeptide and urine N-telopeptide.	<p>SC bolus</p> <p>0.1 mg/kg of CP1 or CP2;</p> <p>Serum samples were collected to determine denosumab concentrations and the presence of anti-denosumab antibodies; serum denosumab concentration-time data were analyzed by non-compartmental methods.</p>	<p>A majority of animals (13 of 16) developed antibodies to denosumab that, as observed in other studies, appeared to lead to more rapid elimination of denosumab. However, in a subset of animals (N=5, CP1; N=3, CP2) that did not develop antibodies to denosumab at 336 hours, mean C_{max} and $\text{AUC}_{0-336 \text{ hr}}$ values were less than 23 and 16% different, respectively. In addition, overall changes in bone turnover markers were similar in animals administered CP1 and CP2. Therefore, the PK and PD of denosumab CP1 and CP2 were determined to be comparable in the cynomolgus monkey.</p>

Table 13. Pharmacokinetics Tabular Summary: Drug-Drug Interactions Test Article: Denosumab

Study Title	Type of Study / Status	Species	Objective	Methods	Summary of Results
106564 - A 12-Month Osteoporosis Prevention Study of Denosumab with and without 6 Month Alendronate Pretreatment in the Cynomolgus Monkey	Multiple Dose/ GLP	Cynomolgus Monkey	To investigate the effects of biweekly intravenous injection doses of Alendronate (ALN) for 6 months followed by once monthly subcutaneous injection doses of denosumab for 6 months on bone mineral density (BMD), serum calcium and phosphorous levels and bone markers. This study also analyzed the pharmacokinetic and antibody data from the test article (denosumab) and vehicle treated ovariectomized (OVX) monkeys.	<p>SC bolus</p> <p>25 mg/kg SC;</p> <p>Serum samples were collected to determine denosumab concentrations and the presence of anti-denosumab antibodies; serum denosumab concentration-time data were analyzed by non-compartmental methods.</p>	<p>No detectable serum concentrations of denosumab were found in the vehicle treated group. Mean C_{max} and $\text{AUC}_{0-336 \text{ hr}}$ values at month 7 in monkeys that received 6-months pretreatment with alendronate prior to switching to SC administration of denosumab were less than 19 and 9% different, respectively, when compared to animals that received 6 months pretreatment with a control test article or to data at month 1 for animals that received denosumab throughout the study. These data suggest that previous treatment with alendronate does not markedly alter the PK of denosumab.</p> <p>Twenty-five of 32 (78%) monkeys in the SC denosumab treatment groups tested positive for the development of binding antibodies to denosumab during the study. Seven of 32 (22%) monkeys in the SC denosumab treatment groups tested positive for the development of neutralizing antibodies towards the end of the study. The development of antibodies to denosumab corresponded with lower denosumab serum concentrations.</p>

Table 17A. Other Toxicity Studies
Test Article: AMG 162

Species/ Test Model	Method of Administration	Doses	Sex and No. Per Group	Noteworthy Findings	Study Number
Tissue Cross-reactivity: Cynomolgus Monkey, Rat, Rabbit	In vitro	5 or 25 µg/mL	NA	<u>Cynomolgus monkey and rabbit</u> : Binding consisted of cytoplasmic staining of cells in a reticular pattern at the periphery of the cortex of lymph nodes, outlining the periarteriolar lymphoid sheaths and lymphoid nodules of the spleen, and at the periphery of lymphoid nodules in the gut-associated lymphoid tissue of the small and large intestines. Staining was more intense in rabbits, suggesting that AMG 162-positive cells in the rabbit contain more of the AMG 162 target antigen than similar cells in the cynomolgus monkey. <u>Rat</u> : Binding was observed in chondrocytes and the margins of their surrounding lacunae in the articular cartilage. There was no evidence of specific AMG 162 binding in other rat tissues; however, high levels of background staining seen with both AMG 162 and the human IgG2 isotype control may have obscured specific staining. The significance of the differential pattern of AMG 162 binding among the 3 species is unclear.	102700

NA = not applicable

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Table 17A. Other Toxicity Studies
Test Article: AMG 162

Species/ Test Model	Method of Administration	Doses	Sex and No. Per Group	Noteworthy Findings	Study Number
Tissue Cross-reactivity: Cynomolgus Monkey, Human	In vitro	1 or 10 µg/mL	NA	<u>Cynomolgus monkey</u> : Reactivity was observed in the lymph node with weak to strong membrane staining of lymphocytes lining the periphery of the paracortex in all donors. <u>Human</u> : No reactivity.	101758
Tissue Cross-reactivity: Human	In vitro	1 or 10 µg/mL	NA	Immunoreactivity was observed in the lymph node from 1 human donor with weak to moderate membrane staining in few to moderate numbers of lymphocytes lining the periphery of the paracortex.	101348

NA = not applicable

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2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Two repeat dose toxicology studies in cynomolgus monkeys were conducted to support the general toxicity of denosumab – a 1-month and a 6/12-month study. Toxicity was evaluated as well as effects on bone parameters and potential immunogenicity.

1-month toxicology study:

- There were no overt treatment-related toxicological findings that were not pharmacodynamic-related.
- Denosumab dose-dependently reduced bone turnover marker levels, and increased total and cortical BMD in males only (no treatment-induced changes in BMD in females at any dose).
- High incidence of immunogenicity likely compromised exposure.

6/12-month toxicology study:

- High incidence of infection in the majority of control and treated animals (73%).
- Two high dose males died while on treatment, which was determined to be the likely result of infection.
- Development of abscesses of the teeth/jaw in 2 females at as early as 37 weeks (doses 10 times higher than the proposed clinical dose based on body weight (mg/kg)).
- Immunogenicity and neutralizing antibody formation was observed, and inversely proportional to dose.
- Denosumab reduced the rate of bone remodeling, reduced bone turnover marker levels, and increased BMD and BMC in cortical and trabecular bone.
- Some indications were present that denosumab may be immunosuppressive (unexplained male deaths (possible impairment of the ability to control infection), abscesses of teeth/jaw, additional information on RANKL signaling found in the literature). However, a clear association between denosumab treatment and immunosuppression could not be established.
- NOAEL determined to be 50 mg/kg (primary effects were PD-related) which represents a dose 50 times higher than the proposed clinical dose based on body weight (mg/kg).

Genetic toxicology: Genetic toxicology studies were not conducted for this application and are not required or recommended for monoclonal antibody therapies.

Carcinogenicity: Carcinogenicity studies were not conducted for this application since an appropriate animal model was not available. However, immunosuppressive agents are generally considered to increase the risk for carcinogenicity.

Reproductive toxicology:

Two studies were conducted in female cynomolgus monkeys to assess the reproductive toxicity of denosumab – a fertility study, and an embryo/fetal developmental study.

- Fertility study in female monkeys showed no effects of denosumab on cycle length, mating performance, hormonal levels, or confirmed pregnancies.
- Embryo/fetal study in female monkeys showed no effect on prenatal loss or adverse maternal clinical parameters.
- Denosumab was not teratogenic.
- The embryo/fetal study likely only assessed potential secondary effects of denosumab on the fetus resulting from maternal exposure since the dosing interval preceded the gestational age when antibodies typically cross the placenta in fetal primate development.
- Only limited organs were evaluated by histopathology in the embryo/fetal study, and fetal lymph nodes were not examined. This would have been beneficial since signaling via RANK has been shown to be required for lymph node development in mice.
- The NOAEL for both reproductive studies was 12.5 mg/kg, which represents a dose 13 times higher than the proposed clinical dose based on body weight (mg/kg).

2.6.6.2 Single-dose toxicity

Single dose toxicity studies were not conducted for this application. See single dose pharmacokinetic studies with denosumab in section 2.6.4.

2.6.6.3 Repeat-dose toxicity**2.6.6.3.1 Study title: A 1-Month Study Evaluating the Effect on Bone of AMG 162 Administered Subcutaneously or Intravenously in Cynomolgus Monkeys, with a 3-Month Recovery Period**

Reviewed by: Michael Orr, Ph.D.

Key study findings:

- No denosumab (AMG 162) induced changes were observed in the clinical signs, food consumption, body weight change, and ophthalmology.
- Ca levels were significantly lower in males of the 1.0 (s.c.) and 10 (s.c. and i.v.) mg/kg groups relative to the control. The reductions in blood Ca levels were not observed in the female monkeys. In the recovery group at week 13, there were no significant differences in Ca levels for the denosumab treated groups relative to the control group.
- There is evidence to support that the pharmacological activity of denosumab was maintained during the duration of the study, as reductions in serum osteocalcin, N-telopeptide, and alkaline phosphatase were observed in all treatment groups in both males of the 10 mg/kg i.v. and s.c. groups and in all denosumab treated groups for females at week 4 of dosing.
- There were statistically significant increases in total and cortical bone mineral density in males in the 1 mg/kg denosumab s.c. group at week 4 of dosing. Denosumab did not induce changes in bone mineral density in the female monkeys in any dose group following 4 weeks of dosing. Also, there were increases in the bone mineral density of the proximal tibia in male monkeys of the 1 mg/kg s.c. and 10 mg/kg (i.v. and s.c.) dose groups during the recovery period. Similar to the 4 week time-point, denosumab did not change the bone mineral density following recovery in the female monkeys in this study.
- Moderate occult blood in urine samples was observed at week 4 of dosing in one male in the 10 mg/kg i.v. group, and severe urinary occult blood was noted at week 4 of dosing in one female each in the 0.1 mg/kg s.c. group and the 10 mg/kg i.v. group.
- There was a high incidence of immunogenicity in this study with 28/30 animals testing positive for anti-denosumab antibodies. In the animals testing positive for anti-denosumab antibodies, there was approximately 30% reduction in exposure in these animals as compared to anti-denosumab antibody negative monkeys.
- There was an approximate 2-fold increase in accumulation observed between the first and last dose of denosumab administered subcutaneously.
- There is approximately 4-fold greater exposure in the cynomolgus monkeys for the subcutaneous route of administration for the 10 mg/kg dose group (AUC_{0-t} 42,000

$\mu\text{g}\cdot\text{hr}/\text{mL}$) and 6-fold greater exposure via the intravenous route of administration in the 10 mg/kg dose group (AUC_{0-t} 68,600 $\mu\text{g}\cdot\text{hr}/\text{mL}$) as compared to the human clinical exposure based on the AUC_{0-t} (human clinical study # 20010223; AUC_{0-t} 10,752 $\mu\text{g}\cdot\text{hr}/\text{mL}$). [Based on body weight (mg/kg), the 10 mg/kg dose in monkeys is 10-fold higher than the recommended human dose of 60 mg administered once every 6 months.]

Study no.: (b) (4) Amgen Study Number 101447

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.3.2

Conducting laboratory and location: (b) (4)

Date of study initiation: December 22, 2000

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Denosumab (AMG 162), Lot #A0010170015, 99.4% purity

Methods:

Doses: 0.1, 1, 10 mg/kg (s.c); 10 mg/kg (i.v.); 4 doses on Days 0, 7, 14 and 21

Species/strain: cynomolgus monkeys (*Macaca fascicularis*)

Number/sex/group or time point (main study): 3 males/group; 3 females/group; males and females necropsied on Day 28

Route, formulation, volume, and infusion rate:

Test Article: Denosumab

Lot Number S01344

Formulation: 30 mg/mL denosumab, 10 mM Na Acetate, 5% Sorbitol, pH 5.2

Route of Administration:

s.c., Dose Volume 0.1 to 1 mL/kg/day

i.v., Dose Volume 1 mL/kg/day with an infusion rate of 5 mL/min

Control Article:

Lot Number A0010260000, s.c.

Formulation: 0 mg/mL denosumab, 10 mM Na Acetate, 5% Sorbitol, pH 5.2

Route of Administration:

s.c., Dose Volume 1 mL/kg/day

Satellite groups used for toxicokinetics or recovery: 3 males/group; 3 females/group; necropsied on week 13 of recovery

Age: 3-5 years of age

Dose Groups and Observations Made (from the sponsor's final study report):

(b) (4)

Amgen study number : 101447
Final Report**SUMMARY**

The purpose of this study was to evaluate the effect of AMG 162 on bone when administered subcutaneously or intravenously to cynomolgus monkeys once weekly for 4 weeks, and to assess the reversibility of any effects following a 13-week recovery period. The study design was as follows:

Group	Test Article	Dose Level (mg/kg/day)	Dose Route	Number of Animals (Animal No.)*	
				Male	Female
1	Placebo	0	SC	3+3*	3+3*
2	AMG 162	0.1	SC	3+3*	3+3*
3	AMG 162	1.0	SC	3+3*	3+3*
4	AMG 162	10.0	SC	3+3*	3+3*
5	AMG 162	10.0	IV	3+3*	3+3*

*: 3 animals/group were necropsied on Day 28 and 3 animals/group following a 13-week recovery period.

The test article was administered on Days 0, 7, 14 and 21 of dosing (total 4 times). All animals were observed for clinical signs of toxicity and mortality once daily during the acclimation period and twice daily during the dosing and recovery periods (except on the day of necropsy when animals were observed once). Food consumption was measured once daily from 7 days before dosing until the end of the experiment. The animals were weighed twice during the acclimation period and weekly during the dosing and recovery period. Ophthalmological examinations, urinalysis, hematological, serum biochemistry, and blood Ca⁺⁺ examinations, and bone mineral density examinations, by peripheral quantitative computed tomography (pQCT), were performed once during the acclimation period and at Week 4 of dosing and at Week 4 or 13 of recovery. Urine and serum for bone metabolic marker analysis and serum for antibody measurement and toxicokinetics were collected periodically throughout the experimental period. At the scheduled necropsies on Day 28 of dosing and at the end of the recovery period, a gross pathological examination was conducted, organ weights were measured and a full light microscopy examination was also conducted.

Weight: Males 3.6 to 4.58 kg; Females 2.87 to 4.04 kg on day of initiation of dosing
Sampling times: Scheduled necropsies were performed on week 4 following dosing and week 13 of the recovery phase of the study.

Results

Mortality: No remarkable findings

Clinical signs: No remarkable findings

Body weights: No remarkable findings

Food consumption: No remarkable findings

Ophthalmoscopy: No remarkable findings

EKG: Not evaluated in this study

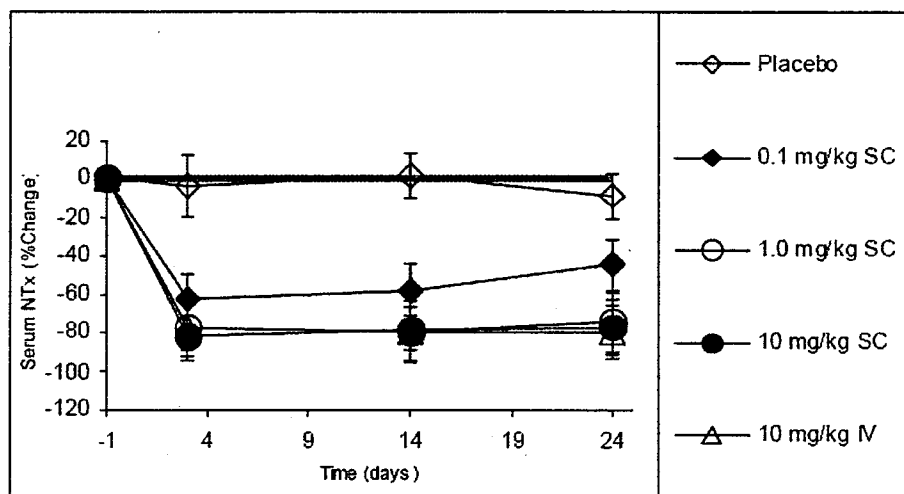
Hematology: A statistically significant decrease in mean platelet volume was observed in females of the 10 mg/kg i.v. group. This was not observed in the male group.

Comment: It is not clear what the toxicological significance of the decrease in mean platelet volume in females as this finding was not observed in the male monkeys.

Clinical chemistry: As expected based on the pharmacological action of denosumab, statistically significant reductions in ALP were observed in the male monkeys following i.v. administration of denosumab at 10 mg/kg. In females, denosumab induced an approximately 47% reduction in ALP at the 1 mg/kg (s.c.) dose, 42% reduction in ALP at the 10 mg/kg dose (s.c.), and 49% reduction in ALP at the 10 mg/kg dose (i.v.). A decrease in Ca^{+2} levels was observed in males of the 1 mg/kg (s.c.) and 10 mg/kg (s.c. and i.v.) groups.

Bone Biomarkers: Serum osteocalcin and N-Telopeptide were reduced in a dose-dependent manner in all treatment groups. The 0.1 mg/kg s.c. cohort had initial reductions of serum osteocalcin of 23% at the 24 hr time point but the levels increased to approximately 10% below baseline at later points. More robust reductions were observed in the 1 mg/kg and 10 mg/kg dose groups with approximately 40% reductions in serum osteocalcin being observed (data not shown).

Figure 2. Serum NTx in Cynomolgus Monkeys Treated Weekly With Denosumab
(% Change From Baseline +/- SD [n = 12])



NTx = cross-linked N-telopeptides; SC = subcutaneous, IV = intravenous.
Source: Data from Study 101447, Appendix 2.

As shown in Figure 2 above, the serum cross-linked N-telopeptide (NTx; biomarker of bone resorption) was decreased by 60 to 80 % at all dose levels.

Urinalysis: Moderate occult blood in urine samples was observed at week 4 of dosing in one male in the 10 mg/kg i.v. group, and severe urinary occult blood was noted at week 4 of dosing in one female each in the 0.1 mg/kg s.c. group and the 10 mg/kg i.v. group.

Bone Mineral Density: There were statistically significant increases in total and cortical bone mineral density (BMD) in males in the 1 mg/kg denosumab s.c. group at week 4 of dosing. Denosumab did not induce changes in BMD in the female monkeys in any dose group following 4 weeks of dosing.

Also, there were increases in the BMD of the proximal tibia in male monkeys of the 1 mg/kg s.c. and 10 mg/kg (i.v. and s.c.) dose groups during the recovery period. Similar to the 4 week time-point, denosumab did not change the BMD following recovery in the female monkeys in this study.

Gross pathology: Cysts in the ovaries (bilateral) were observed in 1/3 females. No changes were observed at the end of the recovery period.

Organ weights (specify organs weighed if not in the histopathology table): There was an elevation in thyroid weights in females in the 10 mg/kg i.v. group following denosumab dosing. There was no evidence of increased thyroid weights following the recovery period in either male or female monkeys. At the end of the recovery phase of the study, there were statistically significant increases in liver weights relative to the control group observed in female monkeys in the 0.1 mg/kg and 1 mg/kg denosumab groups. It is not clear what the toxicological significance of this finding is, as the 10 mg/kg i.v. and s.c. dose groups lacked the increase in liver response observed in the lower dose cohorts.

Histopathology: Adequate Battery: yes (x), no ()—explain
Peer review: yes (), no (x)

No test article related changes were identified.

Comment: The Sponsor's SOP requires a pathology peer review for this study. However **no peer review** was performed, as no findings were identified following the initial histopathological examination.

Toxicokinetics: As shown in Figure 9-1 and Tables 7-1 to 7-4 from the submission, dose-dependent increases in exposure levels were evident over the duration of the study even with a high incidence of immunogenicity (28/30 treated monkeys were anti-denosumab antibody positive).

Comments: There were decreases in the pharmacodynamic markers alkaline phosphatase, serum osteocalcin, and N-telopeptide at all doses evaluated in this study, which provides evidence that denosumab was pharmacologically active in this study (data not shown). Furthermore, there were increases in BMD of the proximal tibia in

male monkeys of the 1 s.c. and 10 (i.v. and s.c.) mg/kg groups. It is not clear why no significant changes in BMD were observed in the female monkeys in any of the dosing groups or at any time-points evaluated (i.e. end of 4 week dosing phase or end of 13 week recovery phase).

There was approximately a 2-fold increase in accumulation observed between the first and last dose of denosumab administered s.c. (Tables 7-1 to 7-4, below).

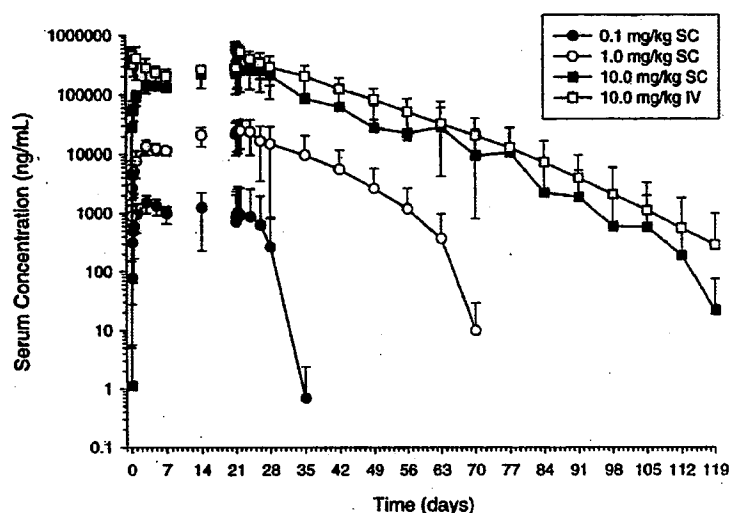


Figure 9-1. Mean (SD) Serum Concentration-Time Profiles Following Multiple Dose IV/SC Administration of AMG 162 to Cynomolgus Monkeys

Table 7-1. Combined Comparison of Mean (\pm SD) PK Parameters Following First and Last SC Dose of 0.1 mg/kg AMG 162 to Cynomolgus Monkeys

Parameter	Units	First		Last		AR
		Mean	SD	Mean	SD	
T_{max}	hr	72	20.5	26.9	47.7	N/A
C_{max}	$\mu\text{g/mL}$	1.51	0.486	11.3	31.2	7.48
$AUC_{(0-168)}$	$\text{hr}\cdot\mu\text{g/mL}$	195	65.7	349	869	1.79
$AUC_{(0-168)/Dose}$	$(\mu\text{g}\cdot\text{hr/mL})/(\mu\text{g/kg})$	1950	657	3490	8690	

Table 7-2. Combined Comparison of Mean (\pm SD) PK Parameters Following First and Last SC Dose of 1.0 mg/kg AMG 162 to Cynomolgus Monkeys

Parameter	Units	First		Last		AR
		Mean	SD	Mean	SD	
T_{max}	hr	82	32.1	30.7	33.1	N/A
C_{max}	$\mu\text{g/mL}$	14.0	3.14	27.5	14.0	1.96
$AUC_{(0-168)}$	$\text{hr}\cdot\mu\text{g/mL}$	1800	430	3410	2080	1.89
$AUC_{(0-168)/Dose}$	$(\mu\text{g}\cdot\text{hr/mL})/(\mu\text{g/kg})$	1800	430	3410	2080	

Table 7-3. Combined Comparison of Mean (\pm SD) PK Parameters Following First and Last SC Dose of 10.0 mg/kg AMG 162 to Cynomolgus Monkeys

Parameter	Units	First		Last		AR
		Mean	SD	Mean	SD	
T_{max}	hr	108	38.2	35	38.6	N/A
C_{max}	μ g/mL	155	27.6	302	151	1.95
$AUC_{(0-168)}$	$hr \cdot \mu$ g/mL	20200	3640	42000	22700	2.08
$AUC_{(0-168)/Dose}$	$(\mu g \cdot hr/mL)/(\mu g/kg)$	2020	364	4200	2270	

Table 7-4. Combined Comparison of Mean (\pm SD) PK Parameters Following First and Last IV Dose of 10 mg/kg AMG 162 to Cynomolgus Monkeys

Parameter	Units	First		Last		AR
		Mean	SD	Mean	SD	
T_{max}	hr	5.83	8.56	4	6.66	N/A
C_{max}	μ g/mL	615	183	663	133	1.08
$AUC_{(0-168)}$	$hr \cdot \mu$ g/mL	48700	14400	68600	23000	1.41
$AUC_{(0-168)/Dose}$	$(\mu g \cdot hr/mL)/(\mu g/kg)$	4870	1440	6860	2300	

Immunogenicity: Overall, denosumab administered to cynomolgus monkeys was highly immunogenic in both male and female monkeys. The incidence of anti-denosumab antibody increased during the 13-week recovery group relative to the study day 28 (for references see Tables 2A, 2B, and 2C below).

**Table 2A
Anti-AMG 162 Positive Incidence - Through Day 28 for Male Monkeys per Dosing Group**

Group	Total No. of Monkeys	No. of Positive Monkeys			% Incidence of Positives			Total No. of Positive Monkeys	% Incidence of Positives
		Pre Dose (day -9)	Post dose (Day 14)	Post dose (Day 28)	Pre Dose (day -9)	Post dose (Day 14)	Post dose (Day 28)		
2 (0.1mg/kg SC)	6	0	6	6	0	100	100	6	100
3 (1.0mg/kg SC)	6	0	1	4	0	17	67	4	67
4 (10.0mg/kg SC)	6	0	0	1	0	0	17	1	17
5 (10.0mg/kg IV)	6	0	0	3	0	0	50	3	50

**Table 2B
Anti-AMG 162 Positive Incidence Through Day 28 for Female Monkeys per Dosing Group**

Group	Total No. of Monkeys	No. of Positive Monkeys			% Incidence of Positives			Total No. of Positive Monkeys	% Incidence of Positives
		Pre Dose (day -2)	Post dose (Day 14)	Post dose (Day 28)	Pre Dose (day -2)	Post dose (Day 14)	Post dose (Day 28)		
2 (0.1mg/kg SC)	6	0	1	6	0	17	100	6	100
3 (1.0mg/kg SC)	6	1	0	4	17	0	67	4	67
4 (10.0mg/kg SC)	6	0	1	3	0	17	50	3	50
5 (10.0mg/kg IV)	6	0	0	0	0	0	0	0	0

Table 2C
Anti-AMG 162 Positive Incidence in the Recovery Period for Male Monkeys per
Dosing Group

Group	Total No. of Recovery Monkeys	No. of Positive Monkeys			% Incidence of Positives			Total No. of Positive Monkeys	% Incidence of Positives
		Through Day 28 of Dosing	WEEK 4 of Recovery	WEEK 8 of Recovery	WEEK 13 of Recovery	WEEK 4 of Recovery	WEEK 8 of Recovery	WEEK 13 of Recovery	
2 (0.1mg/kg SC)	3	3	3	3	3	100	100	100	3
3 (1.0mg/kg SC)	3	1	1	3	3	33	100	100	3
4 (10.0mg/kg SC)	3	1	1	1	3	33	33	100	3
5 (10.0mg/kg IV)	3	1	1	2	2	33	67	67	2

Comments: A high incidence of anti-denosumab antibodies (ADA) was detected in this study (28 of 30 treated monkeys). In the animals testing positive for ADA, there was approximately 30% reduction in exposure as compared to ADA negative monkeys.

Histopathology inventory (optional)

Study	SBL 39-50			
Species	Cyno			
Adrenals	X			
Aorta	X			
Bone Marrow smear	X			
Bone (femur)	X			
Brain	X			
Cecum	X			
Cervix	X			
Colon	X			
Duodenum	X			
Epididymis	X			
Esophagus	X			
Eye	X			
Fallopian tube				
Gall bladder	X			
Gross lesions				
Harderian gland				
Heart	X			
Ileum	X			
Injection site	X			
Jejunum	X			
Kidneys	X			
Lachrymal gland				
Larynx				
Liver	X			
Lungs	X			

Lymph nodes, cervical				
Lymph nodes mandibular	X			
Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity				
Optic nerves	X			
Ovaries	X			
Pancreas	X			
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary	X			
Prostate	X			
Rectum	X			
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	X			
Skin	X			
Spinal cord	X			
Spleen	X			
Sternum	X			
Stomach	X			
Testes	X			
Thymus	X			
Thyroid	X			
Tongue	X			
Trachea	X			
Urinary bladder	X			
Uterus	X			
Vagina	X			
Zymbal gland				

X, histopathology performed

*, organ weight obtained

2.6.6.3.2 Study title: A 6/12-month subcutaneous toxicity study of AMG 162 in the cynomolgus monkey with an interim kill after 6 months and a 3-month recovery period

Reviewed by: Ronald Wange, Ph.D. August 31, 2006

Key study findings:

- Once monthly s.c. injection of denosumab at doses ≥ 10 mg/kg in the cynomolgus monkey for a period of one year reduced the rate of bone remodeling, as indicated by reductions in serum levels of osteocalcin and C-telopeptide and urine levels of

N-telopeptide, and increased BMD and BMC in cortical and trabecular bone of the radius, tibia and femur.

- Denosumab was immunogenic in monkeys. 100% of LD, 50% of MD and 13% of HD animals developed antibodies to denosumab. The majority of anti-denosumab antisera were neutralizing in a bioassay.
- Two MD and 2 HD females developed abscesses of the teeth and/or jaws. This was not seen in any of the C or LD females, and suggests that doses of denosumab ≥ 10 mg/kg may have been immunosuppressive.
- The majority of the animals used in this study (controls and treated alike) were found to be infected with protozoa (giardia lamblia and/or cryptosporidium) around study day 79. Accordingly, most animals had periods of diarrhea and poor health. However, the only two animals to die in the study, presumably as a consequence of infection (probable cause of death was acute renal failure due to dehydration) were 2 HD monkeys. This suggests that denosumab may have contributed to the morbidity in these animals, hypothetically via a denosumab-induced immunosuppressive effect on these animals (this is discussed more fully below).

Study no.: Sponsor: 102090 (b) (4)

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.3.2

Conducting laboratory and location: (b) (4)

Date of study initiation: 04 January 2002

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, and % purity: Denosumab (AMG 162), lots A0106290000, purity = 99.6 & A0111300000, purity = 99.3

Methods

Doses: 0, 1, 10 or 50 mg/kg delivered once per month by s.c. injection for 6 or 12 months

Species/strain: Cynomolgus monkey

Number/sex/group or time point (main study): 3/s/g for 6 month and 12 month sacrifice

Route, formulation and volume:

s.c. injection into the back (between scapulae)

Soln. for injection in 5% sorbitol + 10 mM sodium acetate, pH 5.2; 1 mL/kg (LD & MD), 1.66 mL/kg (C & HD)

Satellite groups used for toxicokinetics or recovery: 2/s/g for 3 month recovery

Age: 2.5 to 4 years old (adolescent to young adult)

Weight: 3.5 to 5.0 kg (males) and 2.5 to 4.0 kg (females)

Source: Naïve and purpose-bred, Herpes B retrovirus-free Cynomolgus monkeys (*Macaca fascicularis*) were obtained from (b) (4)

Unique study design or methodology (if any):

Assay for anti-test item antibodies

Assay for neutralizing activity of anti-test item antibodies

Assay for serum and urinary bone markers

pQCT assay of BMD and BMC
Immunoglobulin levels (IgA, IgG & IgM)
Flow cytometric analysis of lymphocyte immunophenotype
Sperm motility and morphology

Observation and Times:

Clinical signs: Detailed clinical observations weekly. Morbidity and mortality checks twice daily.

Body weights: Pre-dose. Weekly thereafter.

Food consumption: Daily. Recorded as either normal or reduced food consumption.

Ophthalmoscopy: Indirect ophthalmoscopy and slit-lamp examination was performed on all animals once pretreatment and then in weeks 13 and 25. Also performed in all surviving animals week 53 and at end of 3 month recovery period.

EKG: All animals once pretreatment and then in weeks 13 and 25. Also performed in all surviving animals week 53 and at end of 3 month recovery period.

Hematology & Clinical Chemistry: Blood samples were withdrawn from all animals once pre-treatment and in weeks 12 and 24, and from all surviving animals in weeks 36 and 52 of treatment and in the last week of the recovery period. Samples were collected from the brachial or femoral vein before dosing and after an overnight period without food.

Urinalysis: Urine samples were collected overnight (16 hours) from all animals once pre-treatment and in weeks 12 and 24, as well as from all surviving animals in weeks 36 and 52 of treatment and in the last week of the recovery period. Animals were deprived of water throughout the collection period.

Immunoglobulins: Control and HD animals only, weeks 25 and 52.

Immunophenotyping: From blood samples collected in weeks 25 and 52. Antibodies against CD3, CD4, CD8, CD16, CD20 and CD45 were used in combination with flow cytometry to identify T-cell, B-cell, NK-cell and NKT-cell fractions in the total lymphocyte pool (defined as CD45 positive cells).

α -denosumab antibodies: Titers of anti-denosumab antibodies in study animal sera was measured by the Sponsor. Sera samples were collected during weeks 12, 24, 52 and 66 in all surviving animals.

Sperm quality: At necropsy (terminal kill after Week 53 of study), the epididymis from all surviving male animals was prepared for CASA analysis using the IVOS Sperm Analyzer and microscopic evaluation. Sperm motility of cauda epididymides was examined. Sperm morphology was not assessed from Papanicolaou-stained smears according to the WHO guidelines (WHO, 1999) due to the fact that no disorders were observed. A quantitative assessment of testicular cell populations was also made from testicular samples of these animals by flow cytometric analysis.

Gross pathology: All animals (includes early decedents). The scheduled necropsies were performed after an overnight period without food. Three animals of each sex/group were necropsied after 25 weeks of treatment, three animals of each sex/group were necropsied after 53 weeks of treatment, and 2 animals of each sex/group were necropsied after 53 weeks of treatment plus a 3-month recovery period.

Text Table 4: Terminal Procedures

(* - Tissues preserved) († - Organs weighed) (§ - Tissues examined)

Ref no	Tissue / Organ	(*)	(†)	(§)
1	Skin/Animal identification	•		§
2	Mammary glands	•		§
3	Spleen	•	†	§
4	Pancreas	•		§
5	Stomach	•		§
6	Duodenum	•		§
7	Jejunum	•		§
8	Mesenteric lymph nodes	•		§
9	Ileum	•		§
10	Cecum	•		§
11	Colon	•		§
12	Rectum	•		§
13	Adrenals	•	†	§
14	Kidneys	•	†	§
15	Liver	•	†	§
16	Gall bladder	•		§
17	Ovaries	•	†	§
18	Uterus	•		§
19	Vagina	•		
20	Urinary bladder	•		§
21	Testes	• ^c	†	§
22	Epididymides	• ^c	†	§
23	Seminal vesicles*	•	†	§
24	Prostate*	•	†	§
25	Sciatic nerve	•		§
26	Sternum with bone marrow	•		§
27	Femur with bone marrow and articular surface	•		§
28	Skeletal muscle	•		§
29	Mandibular lymph nodes	•		§
30	Salivary glands, mandibular	•		§
31	Salivary glands, lingual			
32	Parotids			
33	Thyroid + parathyroids	•	†	§
34	Tongue	•		§
35	Trachea	•		§
36	Esophagus	•		§
37	Thymus	•		§
38	Rib			
39	Heart	•	†	§
40	Lungs (with mainstem bronchi)	•		§
41	Aorta (arch and anterior abdominal)	•		§
42	Eyes + optic nerves	• ^b		§
43	Lacrimal gland	•		
44	Spinal cord cervical	•		§
45	Spinal cord thoracic			
46	Spinal cord lumbar			
47	Brain (cerebral cortex, thalamus, midbrain, medulla, cerebellum)	•	†	§
48	Pituitary	•	†	§
49	Injection site(s)	•		§
50	Application site			
51	Bone marrow smear (sternum)	• ^{ad}		§
52	Blood sample			
53	Additional tissue			
54	Both femurs	• ^e		
55	Both tibiae	• ^e		
56	Lumbar spine (L1-L6)	• ^e		
	Gross lesions	•		§

Fixative: 10% Neutral Buffered Formalin except where stated as:
a - Methanol
b - Davidson's
c - Bouin's
d - see clinical pathology section of protocol
e - see biomechanics section of protocol

Paired organs were weighed separately
Bone tissue designated for histopathological examination was decalcified using Kristenson's fluid.
* - Organs were weighed from all surviving male animals at necropsy (after Week 53 and at the end of the recovery period).

Organ weights: All animals (includes early decedents). Organs that were weighed are indicated in the table above by a †.

Histopathology: All animals (includes early decedents). Tissues examined are indicated in the table above by an §.

Adequate Battery: yes (x), no ()

Peer review: yes (x), no ()

Toxicokinetics: Serum levels of denosumab were measured by the sponsor. Samples were collected at the times indicated below.

Sampling Scheme: Study Days 1, 85, 169 and 337 prior to dosing and at 12, 24, 48 and 96 hours after dosing; Once in the morning on Study Days 8, 15, 22, 344, 351, 358, 372, 374, 393, 421, 449, 463, and before dosing on Study Days 29, 57, 113, 141, 197, 225, 281, 309 and 365.

Results:

Mortality: Two HD males died intercurrently. One (20855M/407) was found dead during study week 11, while another (20859M/408) was sacrificed moribund during study week 42. According to the Sponsor no cause of death could be determined in either case.

20855M/407: Diarrhea was noted for 2 weeks prior to being found dead. Skeletal muscle was noted to be dehydrated at necropsy. Gross and histopathology findings were consistent with protozoan infection of the GI tract. Other histopathology findings included: hypertrophy of adrenal cortex (slight), depletion of mandibular and mesenteric lymph node follicular centers (minimal), lymphoid atrophy of the spleen (marked), and serous atrophy of femur & tibia bone marrow (minimal). These findings are consistent with prolonged stress and malnutrition. Minimal multifocal acute myocarditis and slight focal acute pericarditis was also noted. According to the Sponsor the presentation of the cardiac pathology was inconsistent with drug-induced cardiac toxicity, in that drug-induced lesions would be expected to be more diffuse, rather than being multifocal in limited areas. Elevated platelets ($\uparrow 69\%$), banded neutrophils ($\uparrow 15x$), BUN ($\uparrow 7.9x$) and creatinine ($6.3x$) were observed, as were serum electrolyte perturbations: Na $\downarrow 7\%$, Cl $\downarrow 15\%$, Ca $\downarrow 22\%$, inorganic P $\uparrow 6.6x$.

20859M/408: Clinical observations in the 30 days prior to moribund sacrifice included diarrhea for 25/30 days, dehydration on 8 occasions, including the last 3 days before sacrifice, and overall bad physical condition. Weight loss was frequently noted, and the animal was noted as being thin (severe) at necropsy. Skeletal muscle was also noted to be dehydrated at necropsy. Gross and histopathology findings indicated GI protozoan infection. Other histopathology findings included: hypertrophy of adrenal cortex (slight), lymphoid atrophy of the lymph nodes (moderate-marked), depletion of mesenteric lymph node (moderate), lymphoid atrophy of the spleen (moderate), serous atrophy of femur & tibia bone marrow (moderate-marked). These findings are consistent with prolonged stress and malnutrition. Elevated platelets ($\uparrow 2.9x$), banded neutrophils ($\uparrow 11x$), BUN ($\uparrow 3.4x$) and creatinine ($\uparrow 14\%$) were also seen, as were perturbations of serum electrolyte levels: Na/Cl $\downarrow 15\%$, Ca $\downarrow 37\%$, inorg. P $\uparrow 82\%$.

From the preceding, it seems likely that the morbid state of both early decedents was caused by acute renal failure (prerenal) as a consequence of hypovolemia secondary to fluid losses from diarrhea. The Sponsor concludes that these deaths were not related to treatment with denosumab, however, in this reviewer's opinion, a potential role for denosumab-induced decrease in immune competence cannot be ruled out as a contributing factor.

Clinical signs: Commencing at about study day 50, diarrhea was frequently observed in animals across all groups, including controls. Feces samples collected on study day 79/74 (males/females) were found to be contaminated with giardia lambia and/or cryptosporidium in the majority of animals (47 of 64). The incidence of diarrhea was reportedly higher in this study than the typical incidence at this testing facility. The explanation for the higher incidence is not known. Individual results of the fecal test

were not provided in the study report. However, the diarrhea was not considered by the Sponsor to be related to denosumab exposure, since there were similar incidences in control animals and there was no indication of a relationship to dose. All surviving animals were treated with fenbendazole (Panacur; broad spectrum anti-parasitic) on days 80-82 (males) and 75-77 (females).

Clinical signs that showed an increased incidence in denosumab-treated animals included abscesses of the teeth and jaw: 2 of 8 females in both the MD and HD groups (animal #358 and #457), beginning at day 260 (37 weeks) in HD animal and at day 401 in MD animal (57 weeks, recovery), as compared to 0/8 females in the control and LD groups. The Sponsor does not address this finding, but it is suggestive of impaired immune response at denosumab doses above 10 mg/kg/day. Dose-related shedding or peeling skin was noted only in denosumab-treated animals, usually occurring in combination with a finding of rough haircoat. The finding of shedding/peeling skin was transient, and did not progress to more severe dermal disturbances. The finding of enteritis was confined to denosumab-treated animals: 2 LD males, 3 HD males, 1 LD female and 1 MD female.

SUMMARY INCIDENCE OF CLINICAL OBSERVATIONS

Weeks 1-13

Male

Female

SEX	FEMALE			
DOSE GROUP	1	2	3	4
# EXAMINED	8	8	8	8

	SEX	MALE			
	DOSE GROUP	1	2	3	4
# EXAMINED		8	8	8	8

NORMAL					
NO SIGNIFICANT CLINICAL OBSERVATIONS		7	8	5	7

DEAD					
FOUND DEAD		0	0	0	1

APPEARANCE					
INJURY TO (PART OF) TAIL		4	2	2	2
BLOODY (PART OF) TAIL		0	1	1	0

HOUSING					
LOW FOOD CONSUMPTION		4	2	4	4
TREATMENT WITH VITAMIN B KOMPLEX		0	0	0	1
ADDITIONAL FOOD SUPPLEMENT		1	0	0	1
TREATMENT WITH NAOL 0.54		2	0	0	0
TREATMENT WITH GLUCOSE		2	0	0	0
TREATMENT WITH CATOSAL		1	0	0	0
TREATMENT WITH KAPORCMT		1	0	0	0
TREATMENT WITH FRANG		0	0	0	1
TREATMENT WITH PANACUR		8	8	8	7

NOSE					
SWOLLEN (PART OF) NOSE		0	0	1	0
INJURY TO (PART OF) NOSE		0	0	1	0

SKIN/FUR					
HAIR THINNING PARTLY		5	5	7	5
HAIR LOSS PARTLY		1	2	2	2
HAIR THINNING ON THE WHOLE BODY		1	0	1	1
INJURY TO (PART OF) EXTREMITY/IES		1	0	0	0
ROUGH HAIRCOAT		1	0	2	2
SHEDDING OR PEELING SKIN		0	1	0	1

EXCRETION					
SOFT FECES		5	2	2	5
DIARRHEA		5	2	2	5
NO FECES		2	2	1	1
PROTOZOA IN FECES		4	5	7	6

NORMAL					
NO SIGNIFICANT CLINICAL OBSERVATIONS		6	7	6	5

APPEARANCE					
INJURY TO (PART OF) TAIL		1	2	5	2
THEN		0	1	0	0
NECROTIC (PART OF) TAIL		0	0	0	1
SWOLLEN (PART OF) TAIL		0	0	1	0
PURULENT (PART OF) TAIL		0	1	2	2
BLOODY (PART OF) TAIL		2	1	2	3

ACTIVITY					
BAD PHYSICAL CONDITION		0	1	0	0

OTHER					
INFRA-RED SUPPLEMENT		0	1	0	0

HOUSING					
LOW FOOD CONSUMPTION		4	5	4	5
TREATMENT WITH NAOL 0.54		0	2	0	2
TREATMENT WITH GLUCOSE		0	2	0	2
ADDITIONAL FOOD SUPPLEMENT		0	1	0	1
TREATMENT WITH KAPORCMT		0	1	0	0
TREATMENT WITH DUOPRIM		0	1	0	0
TREATMENT WITH CATOSAL		0	1	0	0
TREATMENT WITH SIOZNO-N		0	2	0	0
TREATMENT WITH VITAMIN B KOMPLEX		0	1	0	1
TREATMENT WITH FRANG		0	1	0	1
TREATMENT WITH PANACUR		8	8	8	8

NOSE					
BLOODY NASAL DISCHARGE		1	0	0	1

SKIN/FUR					
HAIR LOSS ON THE WHOLE BODY		1	0	2	0
HAIR THINNING ON THE WHOLE BODY		2	1	0	5
HAIR THINNING PARTLY		6	8	6	7
HAIR LOSS PARTLY		1	2	5	4
RASH ON (PART OF) EXTREMITY/IES		0	1	0	0
SHEDDING OR PEELING SKIN		0	1	1	2
ROUGH HAIRCOAT		2	6	2	2
SWOLLEN (PART OF) EXTREMITY/IES		0	1	0	0
BURN TO SHOULDER/S		0	1	0	0
INJURY TO (PART OF) EXTREMITY/IES		0	2	0	0
BOIL ON (PART OF) EXTREMITY/IES		0	0	1	0
FISTULAR ON (PART OF) EXTREMITY/IES		0	0	0	1
STICKY HAIRCOAT		1	0	0	0

EXCRETION					
SOFT FECES		2	2	4	6
DIARRHEA		2	2	2	4
NO FECES		2	2	2	1
PROTOZOA IN FECES		6	5	8	6

Weeks 14-26

	SEX	MALE	
	DOSE GROUP	1 2 3 4	
	# EXAMINED	5 5 5 7	
NORMAL			
NO SIGNIFICANT CLINICAL OBSERVATIONS		7 7 4 5	
DEAD			
INTERIM KILL		2 2 2 2	
APPEARANCE			
INJURY TO (PART OF) TAIL		2 2 2 2	
LOSS OF WEIGHT		0 0 0 2	
BODY TEMPERATURE TOO LOW		0 0 0 2	
FURULENT (PART OF) TAIL		1 0 0 0	
BLOODY (PART OF) TAIL		1 0 0 1	
DEHYDRATION		0 0 0 1	
OTHER			
INFRA-RED SUPPLEMENT		0 0 0 1	
ELSTONE SUPPLEMENT		0 0 0 1	
EYE(S)			
HEMATOMA/E ON EYE/S		0 0 0 1	
HOUSING			
LOW FOOD CONSUMPTION		2 2 4 6	
TREATMENT WITH NAOL 0.9%		0 0 0 2	
TREATMENT WITH GLUCOSE		0 0 0 2	
TREATMENT WITH FRANG		0 0 0 1	
ADDITIONAL FOOD SUPPLEMENT		0 0 0 2	
TREATMENT WITH SIOZNO-N		0 0 1 0	
NO FOOD CONSUMPTION		0 1 1 0	
NOSE			
NOSE BLOODY CRUSTED		0 0 0 1	
MOUTH			
EMESIS		0 0 2 0	
EMESIS OF MASH		1 0 1 0	
SKIN/FUR			
HAIR THINNING PARTLY		6 6 8 4	
ROUGH HAIRCOAT		1 0 2 1	
SHEDDING OR PEELING SKIN		0 0 1 1	
HAIR THINNING ON THE WHOLE BODY		2 0 2 1	
REDDISH SKIN PARTLY		0 0 1 0	
HAIR LOSS PARTLY		1 1 2 2	
EXCRETION			
SOFT FECES		4 5 6 5	
DIARRHEA		5 2 3 5	
NO FECES		6 2 2 2	
ANUS SMELARED WITH FECES		0 0 0 1	
ENTERITIS		0 0 0 2	

	SEX	FEMALE	
	DOSE GROUP	1 2 3 4	
	# EXAMINED	5 5 5 5	
NORMAL			
NO SIGNIFICANT CLINICAL OBSERVATIONS		5 5 2 2	
DEAD			
INTERIM KILL		2 2 3 2	
APPEARANCE			
INJURY TO (PART OF) TAIL		2 2 4 2	
TEIN		0 1 1 0	
LOSS OF WEIGHT		0 1 0 0	
BODY TEMPERATURE TOO LOW		0 1 1 0	
BODY TEMPERATURE WITHIN NORMAL LIMIT		0 0 1 0	
FURULENT (PART OF) TAIL		1 0 0 0	
BLOODY (PART OF) TAIL		2 2 2 2	
ACTIVITY			
BAD PHYSICAL CONDITION		0 1 0 0	
OTHER			
ELSTONE SUPPLEMENT		0 1 1 0	
VAGINAL BLEEDING		1 0 0 0	
HOUSING			
LOW FOOD CONSUMPTION		2 2 7 2	
TREATMENT WITH NAOL 0.9%		1 1 1 0	
TREATMENT WITH GLUCOSE		1 1 1 0	
ADDITIONAL FOOD SUPPLEMENT		0 1 1 0	
TREATMENT WITH FRANG		0 1 0 0	
TREATMENT WITH SIOZNO-N		0 1 0 0	
TREATMENT WITH BAYTRIL		0 1 0 0	
TREATMENT WITH ROMARKION		1 0 0 0	
TREATMENT WITH ALBIOTIC		0 0 1 0	
TREATMENT WITH VETALGIN		0 0 1 0	
NO FOOD CONSUMPTION		0 1 0 1	
TREATMENT WITH ROMFUM		0 0 1 0	
TREATMENT WITH KETAVET		0 0 1 0	
NOSE			
BLOODY NASAL DISCHARGE		0 0 1 0	
INJURY TO (PART OF) NOSE		0 0 1 0	
SWOLLEN (PART OF) NOSE		0 0 1 0	
BLOODY (PART OF) NOSE		0 0 1 0	
MOUTH			
FURULENT INFLAMMATION OF THE GUMS AT MOLAR TOOTH/TEETH		0 0 1 0	
EXTRACTION OF MOLAR TOOTH/TEETH		0 0 1 0	
EMESIS		0 1 0 0	
SKIN/FUR			
HAIR THINNING PARTLY		7 7 7 2	
ROUGH HAIRCOAT		2 2 3 2	
HAIR THINNING ON THE WHOLE BODY		2 2 2 2	
SWOLLEN (PART OF) EXTREMITIES		0 1 0 0	
HAIR LOSS ON THE WHOLE BODY		0 0 2 0	
HAIR LOSS PARTLY		2 2 3 3	
BURN TO SHOULDERS/HEAD		0 1 0 0	
SWOLLEN (PART OF) FACE		0 0 1 0	
FATTY HAIRCOAT		0 0 0 1	
STICKY HAIRCOAT		0 0 0 1	
SHEDDING OR PEELING SKIN		0 0 0 1	
EXCRETION			
DIARRHEA		2 2 2 4	
SOFT FECES		5 4 4 5	
NO FECES		4 2 4 0	

Weeks 27-52

	SEX DOSE GROUP # EXAMINED	MALE 1 2 3 4 5 5 5 5
NORMAL		
NO SIGNIFICANT CLINICAL OBSERVATIONS		2 5 4 2
DEAD		
KILLED MORIBUND		0 0 0 1
TERMINAL KILL		2 2 3 2
APPEARANCE		
INJURY TO (PART OF) TAIL		0 2 2 2
THIN		0 0 0 1
LOSS OF WEIGHT		0 0 0 1
DEHYDRATION		0 0 0 1
BODY TEMPERATURE TOO LOW		0 0 0 1
SKIN DISCOLORATION ON FACE		0 0 0 1
BODY TEMPERATURE WITHIN NORMAL LIMIT		0 2 0 1
ACTIVITY		
BAD PHYSICAL CONDITION		0 0 0 1
OTHER		
ELSTONE SUPPLEMENT		0 0 0 1
HOUSING		
LOW FOOD CONSUMPTION		2 2 2 4
TREATMENT WITH NAOL 0.5%		0 0 0 1
TREATMENT WITH GLUCOSE		0 0 0 1
ADDITIONAL FOOD SUPPLEMENT		0 2 0 2
TREATMENT WITH CATOSAL		0 0 0 1
FORCED FEEDING		0 0 0 1
TREATMENT WITH METRONIDAZOL ARTESAN		0 0 0 1
TREATMENT WITH VITAKEMEX I		0 0 0 1
TREATMENT WITH VITAMIN B COMPLEX		0 0 0 1
TREATMENT WITH SANADERM		0 0 0 1
TREATMENT WITH TARDOMYCEL		0 0 0 1
AMPUTATION OF (PART OF) TAIL		0 0 0 1
TREATMENT WITH ROMPUN		0 1 0 1
TREATMENT WITH KETAVET		0 1 0 1
TREATMENT WITH VETALGIN		0 0 0 1
TREATMENT WITH NEBACETIN POWDER		0 0 0 1
TREATMENT WITH RETAISODONA		0 1 0 1
REMOVE THE STITCHES		0 1 0 1
TREATMENT WITH BAYTRIL		0 1 0 0
AMPUTATION OF (PART OF) EXTREMITY/IES		0 1 0 0
MOUTH		
EMESIS		0 0 0 1
SKIN/FUR		
HAIR THINNING PARTLY		4 4 3 4
HAIR THINNING ON THE WHOLE BODY		1 0 2 1
ROUGH HAIRCOAT		0 0 2 0
SWELLING AT INJECTION SITE		0 0 1 0
HAIR LOSS PARTLY		1 0 1 1
INJURY TO (PART OF) EXTREMITY/IES		1 1 0 1
EXCRETION		
DIARRHEA		1 4 4 4
SOFT FECES		2 5 4 2
NO FECES		2 1 0 4
ENTERITIS		0 2 0 2

	SEX DOSE GROUP # EXAMINED	FEMALE 1 2 3 4 5 5 5 5
NORMAL		
NO SIGNIFICANT CLINICAL OBSERVATIONS		2 2 3 2
DEAD		
TERMINAL KILL		2 2 3 2
APPEARANCE		
THIN		0 0 1 0
INJURY TO (PART OF) TAIL		0 0 2 1
LOSS OF WEIGHT		1 0 0 1
BODY TEMPERATURE TOO LOW		1 1 1 1
ACCESS ON NECK		0 0 0 1
BODY TEMPERATURE WITHIN NORMAL LIMIT		0 1 2 2
OTHER		
SEVERE VAGINAL BLEEDING		1 0 0 0
ELSTONE SUPPLEMENT		1 0 1 1
HOUSING		
LOW FOOD CONSUMPTION		5 5 5 4
TREATMENT WITH ROMPUN		0 0 0 1
TREATMENT WITH KETAVET		0 0 0 1
TREATMENT WITH RETAISODONA		0 0 0 1
TREATMENT WITH ALBISTIC		0 0 0 1
ADDITIONAL FOOD SUPPLEMENT		1 1 2 1
OPERATION AT MANDIBULAR		0 0 0 1
NOSE		
SWOLLEN (PART OF) NOSE		0 0 1 0
BLOODY NASAL DISCHARGE		0 0 1 0
MOUTH		
SECRETION OF PUS ON GINGIVA		0 0 0 1
ABSCCESS/ES ON (PART OF) MANDIBULAR		0 0 0 1
LESION/S ON (PART OF) GINGIVA		0 0 0 1
SWOLLEN (PART OF) MANDIBULAR		0 0 0 1
GROWTH OF SWELLING ON MANDIBULAR		0 0 0 1
GROWTH OF SWELLING ON UPPER JAW		0 0 1 0
SKIN/FUR		
HAIR THINNING PARTLY		2 5 4 4
HAIR LOSS PARTLY		2 2 1 1
HAIR THINNING ON THE WHOLE BODY		1 2 1 2
ROUGH HAIRCOAT		1 0 0 0
FATTY HAIRCOAT		0 0 0 1
STICKY HAIRCOAT		0 0 0 1
SQUAMOUS SKIN PARTLY		0 1 0 0
REDDISH SQUAMOUS SKIN PARTLY		0 1 0 0
EXCRETION		
DIARRHEA		2 1 2 2
NO FECES		0 2 2 2
SOFT FECES		2 2 2 4
ENTERITIS		0 1 1 0

Weeks 53-65 (recovery)

	SEX	MALE		
	DOSE GROUP	1	2	3 4
	% EXAMINED	2	2	2 1
NORMAL				
NO SIGNIFICANT CLINICAL OBSERVATIONS		1	2	2 1
DEAD				
RECOVERY KILL		2	2	2 1
APPEARANCE				
INJURY TO (PART OF) TAIL		1	0	1 0
BODY TEMPERATURE WITHIN NORMAL LIMIT		1	0	0 0
HOUSING				
LOW FOOD CONSUMPTION		1	2	2 1
AMPUTATION OF (PART OF) TAIL		1	0	0 0
TREATMENT WITH ROMPUN		1	0	0 0
TREATMENT WITH KETAVET		1	0	0 0
TREATMENT WITH NEBACETIN POWDER		1	0	0 0
TREATMENT WITH METASODORA		1	0	0 0
TREATMENT WITH VETALGIN		1	0	0 0
TREATMENT WITH BAYTRIL		1	0	0 0
REMOVE THE STITCHES		1	0	0 0
SKIN/FUR				
HAIR LOSS PARTLY		1	0	0 1
HAIR THINNING PARTLY		1	2	1 1
EXCRETION				
DIARRHEA		1	2	0 1
SOFT FECES		1	2	1 0
NO FECES		0	1	0 0

	SEX	FEMALE		
	DOSE GROUP	1	2	3 4
	% EXAMINED	2	2	2 2
NORMAL				
NO SIGNIFICANT CLINICAL OBSERVATIONS		0	1	0 1
DEAD				
RECOVERY KILL		2	2	2 2
APPEARANCE				
BODY TEMPERATURE TOO LOW		0	0	1 0
INJURY TO (PART OF) TAIL		1	0	0 0
TEIN		0	0	1 1
BODY TEMPERATURE WITHIN NORMAL LIMIT		0	0	2 1
HYPOTHERMIA		0	0	1 0
ACTIVITY				
TREMORS		0	0	1 0
OTHER				
ELSTONE SUPPLEMENT		0	0	1 0
HOUSING				
LOW FOOD CONSUMPTION		2	2	2 2
TREATMENT WITH RIVANOL		0	0	0 1
TREATMENT WITH ALSIOTIC		0	0	1 1
ADDITIONAL FOOD SUPPLEMENT		0	0	2 0
TREATMENT WITH LEBERTRAN-ZINK OINTMENT		0	0	1 0
NOSE				
SWOLLEN (PART OF) NOSE		0	0	1 0
MOUTH				
SWOLLEN (PART OF) MANDIBULAR		0	0	0 1
SWOLLEN (PART OF) UPPER JAW		0	0	1 0
ABSCESS/ES ON (PART OF) MANDIBULAR		0	0	0 1
LESSIONS ON (PART OF) MANDIBULAR		0	0	0 1
GROWTH OF SWELLING ON UPPER JAW		0	0	1 0
SUSPICION OF ABSCESS ON UPPER JAW		0	0	1 0
ABSCESS/ES ON UPPER JAW		0	0	1 0
SKIN/FUR				
HAIR THINNING PARTLY		2	1	0 2
HAIR THINNING ON THE WHOLE BODY		2	0	1 0
FATTY HAIRCOAT		0	0	0 1
SWOLLEN (PART OF) FACE		0	0	0 1
SWOLLEN (PART OF) NECK		0	0	0 1
BURN TO SHOULDER/S		0	0	1 0
BURN TO (PART OF) FACE		0	0	1 0
EXCRETION				
NO FECES		1	0	2 2
DIARRHEA		1	1	1 1
SOFT FECES		2	1	1 1
ENTERITIS		0	0	1 0

HEMATOLOGY (Group Mean – excludes early decedents)								
Sex	Male				Female			
Dose (mg/kg/day)	0	1	10	50	0	1	10	50
WBC (10⁹ cells/L)								
Pre-dose	13.4	8.7	10.7	9.3	9.4	10.7	9.6	10.0
Week 12	11.7	12.1	15.5	14.3	11.6	13.9	12.0	14.3
Week 24	9.9	9.0	10.9	11.3	11.7	11.7	11.3	12.9
Week 36	9.5	9.5	9.6	14.1	11.1	11.3	9.7	15.8
Week 52	10.0	10.1	11.3	11.6	8.9	11.7	12.5	14.9*
Week 66 (post recovery)	6.7	7.3	6.9	7.8	12.3	13.0	13.2	18.3
Neutrophilic segmented cells (%)								
Pre-dose	50	33	41	38	45	49	53	44
Week 12	53	53	52	58	51	63	55	60
Week 24	51	43	43	51	50	61	56	61
Week 36	51	56	50	60	40	51	40	60
Week 52	50	53	44	59	40	55	51	58
Week 66 (post recovery)	45	49	43	53	49	59	47	49
Lymphocytes (%)								
Pre-dose	47	62	56	56	51	47	44	52
Week 12	44	44	45	39	46	33	41	36
Week 24	46	54	53	47	45	36	41	36
Week 36	47	40	48	36	54	46	57	37
Week 52	47	43	53	38	51	40	45	37
Week 66 (post recovery)	53	47	55	41	50	34	42	43

Bold indicates significantly different from control.

*Significantly different at 95% confidence level.

n.c. = no change.

There were some apparent abnormalities in several hematological parameters in the early decedent HD males. In interpreting the significance of these findings it should be kept in mind that animal 20855M/407 was found dead, and the week 11 blood sample from this animal was drawn at necropsy. Of particular interest are those parameters which were similarly perturbed in both early decedents. These included ↑platelets and ↑banded neutrophils. The large increase in the % of banded neutrophils (immature neutrophils) in the early decedent HD animals suggests that rapid proliferation and differentiation of neutrophils was occurring in these animals prior to their death. Since the number of mature, segmented neutrophils was not comparably increased, this may indicate rapid turnover (death and resynthesis) of neutrophils in these animals, suggesting the presence of an active immune/inflammatory response.

HEMATOLOGY (Early Decedents)				
	Animal No.		Remainder of HD Group (mean \pm SD)	Control Group (mean \pm SD)
	20855M/407	20859M/408		
RBC (10^{12} cells/L)				
Pre-dose	6.56	n.c.	6.90 \pm 0.42	6.75 \pm 0.21
Week 12	8.36 ^a		6.02 \pm 0.35	6.30 \pm 0.35
Week 36	n.a.		6.56 \pm 0.65	6.67 \pm 0.54
Week 52	n.a.		5.72 \pm 0.25	6.02 \pm 0.27
Hemoglobin (mmol/L)				
Pre-dose	8.3	n.c.	8.7 \pm 0.5	8.3 \pm 0.4
Week 12	10.9 ^a		8.0 \pm 0.6	8.1 \pm 0.7
Week 36	n.a.		8.3 \pm 0.8	8.3 \pm 0.8
Week 52	n.a.		7.6 \pm 0.7	7.7 \pm 0.5
Packed Cell Volume (%)				
Pre-dose	43.5	n.c.	45.8 \pm 3.1	44.6 \pm 2.1
Week 12	52.1 ^a		40.1 \pm 2.6	41.5 \pm 2.7
Week 36	n.a.		42.9 \pm 3.6	43.8 \pm 3.8
Week 52	n.a.		36.6 \pm 3.0	37.9 \pm 2.5
Platelet Count (10^9 cells/L)				
Pre-dose	369	373	353 \pm 52	324 \pm 98
Week 12	622 ^a	463	362 \pm 84	344 \pm 82
Week 36	n.a.	622	354 \pm 173	313 \pm 97
Week 52	n.a.	915 ^b	320 \pm 64	317 \pm 68
WBC (10^9 cells/L)				
Pre-dose	7.7	8.7	9.3 \pm 3.2	13.4 \pm 2.7
Week 12	18.9 ^a	10.8	14.3 \pm 5.6	11.7 \pm 3.8
Week 36	n.a.	13.7	14.1 \pm 9.5	9.5 \pm 5.5
Week 52	n.a.	7.5 ^b	11.6 \pm 3.2	10.0 \pm 3.5
Neutrophilic Band Cells (%)				
Pre-dose	0	1	1 \pm 1	1 \pm 1
Week 12	15 ^a	0	1 \pm 1	1 \pm 1
Week 36	n.a.	11	2 \pm 5	0 \pm 0
Week 52	n.a.	11 ^b	1 \pm 1	1 \pm 1
Lymphocytes (%)				
Pre-dose	n.c.	46	56 \pm 13	47 \pm 15
Week 12		51	39 \pm 11	44 \pm 14
Week 36		38	36 \pm 14	47 \pm 16
Week 52		19 ^b	38 \pm 5	47 \pm 11

^a) Found dead on day 76 (week 11). Value is from blood sample drawn at necropsy.

^b) Sacrificed moribund on day 289 (week 42). Value is from blood sample drawn at necropsy.

n.a. = not applicable. n.c. = no change.

Clinical chemistry: No test item related effect on group mean values for bilirubin, creatinine, BUN, AST, ALT, glucose, cholesterol, sodium, potassium, chloride, total protein, albumin, globulin or A:G ratio. Alkaline phosphatase was significantly reduced in MD & HD monkeys (both sexes) in weeks 12 and 24 and in males at week 52. It is likely that this reduction in serum alkaline phosphatase levels was a consequence of diminished bone remodeling. Notably the depressive effect of

treatment on this parameter peaked between weeks 12 and 24. Neutralizing anti-denosumab antibodies may account for the reduction in the suppressive effect of denosumab on alkaline phosphatase (bone turnover) with continued treatment (see immunogenicity below). γ -Glutamyl transferase was non-statistically significantly reduced in weeks 12-52 in HD males, but was unaffected by treatment in females. Inorganic phosphorus levels were dose-dependently reduced in females during the dosing period. This was also seen in males, especially at high dose. Ca was also reduced in HD males, significantly so at week 12. Decreased osteoclast activity likely also contributed to the reductions in inorganic phosphorus and calcium.

CLINICAL CHEMISTRY (Group Mean)								
Sex	Male				Female			
Dose (mg/kg/day)	0	1	10	50	0	1	10	50
Alkaline Phosphatase (U/L)								
Pre-dose	654.06	699.88	608.54	661.88	599.53	592.48	740.24	642.61
Week 12	534.32	637.81	439.88	205.28*	485.69	463.91	284.72*	218.90*
Week 24	657.24	764.66	497.46	265.62*	423.93	509.64	272.96*	186.61*
Week 36	679.09	711.72	411.35	312.92	459.09	504.63	414.90	301.84
Week 52	696.41	660.50	454.94	319.99*	416.89	477.40	359.11	329.99
Week 66 (post recovery)	535.25	608.25	567.99	784.16	418.66	404.94	684.84	629.70
γ-Glutamyl Transferase (U/L)								
Pre-dose	110.85	109.73	111.95	110.54	n.c.			
Week 12	120.07	110.96	123.13	104.44				
Week 24	167.27	174.04	182.74	142.58				
Week 36	173.94	191.60	167.79	129.91				
Week 52	155.66	162.81	172.38	136.43				
Week 66 (post recovery)	125.82	164.94	200.08	182.98				
Inorg. Phosphorus (mmol/L)								
Pre-dose	1.54	1.65	1.71	1.62	1.56	1.63	1.55	1.65
Week 12	1.51	1.49	1.60	1.32	1.79	1.67	1.48	1.39*
Week 24	1.78	1.76	1.59	1.36*	1.70	1.54	1.46*	1.22*
Week 36	1.84	1.71	1.65	1.45	1.93	1.72	1.50*	1.33*
Week 52	1.81	1.58	1.58	1.37*	1.65	1.60	1.43	1.12*
Week 66 (post recovery)	1.65	1.49	1.47	1.47	1.04	1.47	1.29	1.47
Calcium (mmol/L)								
Pre-dose	2.79	2.74	2.81	2.85	2.63	2.63	2.65	2.68
Week 12	2.59	2.59	2.69	2.34*	2.55	2.49	2.37	2.38
Week 24	2.52	2.60	2.54	2.34	2.30	2.36	2.33	2.35
Week 36	2.57	2.57	2.59	2.29	2.57	2.52	2.50	2.35
Week 52	2.68	2.59	2.64	2.48	2.59	2.76	2.50	2.59
Week 66 (post recovery)	2.57	2.57	2.31	2.85	2.64	2.58	2.80	2.62

Bold indicates significantly different from control.

*Significantly different at 95% confidence level.

n.c. = no change.

A number of clinical chemistry parameters were perturbed in the HD male early decedents compared to the remainder of the HD group or to the control group. Those that were perturbed similarly in both early decedents are:

Creatinine: \uparrow 6.3x in #407 and \uparrow 16% in #408

Urea: \uparrow 7.9x in #407 and \uparrow 2.8x in #408

Inorganic P: ↑6.6x in #407 and ↑82% in #408

Ca: ↓22% in #407 and ↓37% in #408

Na: ↓7% in #407 and ↓18% in #408

Cl: ↓15% in both #407 and #408

Together these differences support renal failure as a contributor to the early deaths of these animals.

CLINICAL CHEMISTRY (Early Decedents)				
	Animal No.		Remainder of HD Group	Control Group
	20855M/407	20859M/408	(mean ± SD)	(mean ± SD)
Creatinine (μmol/L)				
Pre-dose	79.38	92.94	91.36 ± 9.63	93.52 ± 9.55
Week 12	523.35 ^a	85.89	82.89 ± 6.36	87.12 ± 12.58
Week 36	n.a.	114.99	99.26 ± 9.56	96.32 ± 15.72
Week 52	n.a.	106.16 ^b	91.17 ± 5.62	95.63 ± 5.70
Urea (mmol/L)				
Pre-dose	10.25	7.48	8.80 ± 1.48	9.03 ± 1.47
Week 12	64.07 ^a	7.46	8.16 ± 0.99	10.10 ± 4.32
Week 36	n.a.	10.68	8.32 ± 1.45	7.63 ± 0.84
Week 52	n.a.	25.15 ^b	8.96 ± 0.70	7.85 ± 0.63
AST (U/L)				
Pre-dose	32.60	46.92	39.57 ± 8.67	37.67 ± 5.13
Week 12	105.35 ^a	40.36	39.28 ± 6.98	42.85 ± 9.18
Week 36	n.a.	23.83	33.81 ± 10.93	36.12 ± 9.14
Week 52	n.a.	35.20 ^b	30.37 ± 13.25	35.32 ± 7.72
ALT (U/L)				
Pre-dose	24.88	38.65	51.97 ± 22.30	47.66 ± 28.11
Week 12	38.46 ^a	30.04	38.40 ± 20.82	41.40 ± 21.19
Week 36	n.a.	22.19	31.38 ± 6.79	36.37 ± 21.11
Week 52	n.a.	17.94 ^b	25.89 ± 5.94	44.25 ± 13.81
Glucose (mmol/L)				
Pre-dose	2.10	2.67	3.34 ± 0.90	3.35 ± 0.63
Week 12	7.03 ^a	3.07	3.18 ± 0.21	3.30 ± 0.31
Week 36	n.a.	2.85	3.02 ± 0.31	2.97 ± 0.63
Week 52	n.a.	1.59 ^b	3.20 ± 0.16	3.26 ± 0.47
Inorg. Phosp. (mmol/L)				
Pre-dose	1.23	1.85	1.62 ± 0.30	1.54 ± 0.29
Week 12	8.77 ^a	1.27	1.32 ± 0.15	1.51 ± 0.21
Week 36	n.a.	2.31	1.45 ± 0.52	1.84 ± 0.19
Week 52	n.a.	2.49 ^b	1.37 ± 0.26	1.81 ± 0.09
Calcium (mmol/L)				
Pre-dose	2.72	2.64	2.85 ± 0.21	2.79 ± 0.23
Week 12	1.82 ^a	2.38	2.34 ± 0.16	2.59 ± 0.09
Week 36	n.a.	2.00	2.29 ± 0.32	2.57 ± 0.11
Week 52	n.a.	1.56 ^b	2.48 ± 0.19	2.68 ± 0.10
Sodium (mmol/L)				
Pre-dose	153.25	152.52	157.41 ± 3.82	156.66 ± 4.82
Week 12	139.55 ^a	150.37	149.72 ± 2.73	148.24 ± 2.36
Week 36	n.a.	139.57	149.75 ± 5.76	150.14 ± 4.93
Week 52	n.a.	123.45 ^b	149.90 ± 2.46	148.29 ± 2.03
Potassium (mmol/L)				
Pre-dose	5.42	5.90	6.00 ± 0.68	5.81 ± 0.66

Week 12	14.69 ^a	5.37	5.35 ± 0.47	5.06 ± 0.48
Week 36	n.a.	5.61	5.43 ± 0.48	5.25 ± 0.33
Week 52	n.a.	3.21 ^b	4.72 ± 0.36	4.95 ± 0.43
Chloride (mmol/L)				
Pre-dose	104.25	103.70	106.53 ± 2.89	107.23 ± 1.63
Week 12	87.29 ^a	103.85	102.40 ± 3.09	103.56 ± 1.92
Week 36	n.a.	102.16	107.80 ± 3.48	108.69 ± 0.83
Week 52	n.a.	93.92 ^b	110.34 ± 0.80	107.65 ± 1.80
Total Protein (g/L)				
Pre-dose	90.01	92.38	90.28 ± 4.78	89.71 ± 5.56
Week 12	96.48 ^a	85.22	84.67 ± 3.39	85.86 ± 5.03
Week 36	n.a.	78.79	87.92 ± 8.72	85.90 ± 6.00
Week 52	n.a.	61.63 ^b	81.76 ± 5.01	80.14 ± 3.07
Albumin (g/L)				
Pre-dose	58.25	58.34	59.20 ± 5.46	54.94 ± 3.65
Week 12	71.26 ^a	62.57	60.68 ± 3.81	57.87 ± 2.83
Week 36	n.a.	36.10	50.84 ± 8.54	53.02 ± 4.15
Week 52	n.a.	27.23 ^b	49.91 ± 1.96	48.77 ± 1.46
A:G Ratio				
Pre-dose	1.83	1.71	1.94 ± 0.43	1.59 ± 0.14
Week 12	2.83 ^a	2.76	2.55 ± 0.33	2.10 ± 0.29
Week 36	n.a.	0.85	1.44 ± 0.44	1.62 ± 0.16
Week 52	n.a.	0.79 ^b	1.60 ± 0.25	1.57 ± 0.18

^{a)} Found dead on day 76 (week 11). Value is from blood sample drawn at necropsy.

^{b)} Sacrificed moribund on day 289 (week 42). Value is from blood sample drawn at necropsy.

n.a. = not applicable.

Immunoglobulin Isotyping: There were no statistically significant differences between control and treated groups in immunoglobulin levels or isotypes. IgM, and possibly IgA, levels appear to be non-statistically significantly reduced in males, but not females. Note that there are no Ig isotype data for the HD male animal 20855M/407, since this animal died before blood was drawn for isotyping, and isotyping was not conducted on the necropsy blood sample. There were no apparent test item-related effects at week 25 on Ig isotype in HD male animal 20859M/408, which was sacrificed moribund during week 42.

IMMUNOGLOBULIN ISOTYPE (Group Means)				
	Sex		Male	
Dose (mg/kg/day)			0	50
IgA				
Week 25			4.95 ± 2.17	4.38 ± 1.73
Week 52			4.38 ± 1.55	4.04 ± 1.81
IgG				
Week 25			12.47 ± 2.53	12.70 ± 2.05
Week 52			10.77 ± 2.03	11.31 ± 2.28
IgM				
Week 25			0.71 ± 0.28	0.47 ± 0.20
Week 52			1.29 ± 0.42	0.87 ± 0.11

Reported as mean value ± SD.

Immunophenotyping: Although there were no statistically significant changes in the immunophenotype of T cells from HD animals, there was an overall trend for slightly elevated levels ($\uparrow 5\text{-}8\%$) of T cells as a percentage of total lymphocytes. The percentage of T cells that were helper T cells (CD4-positive) also showed a trend towards being increased in HD animals, especially at 25 weeks ($\uparrow 4\text{-}5\%$). There was also a corresponding decrease in the percentage of T cells that are cytotoxic (CD8-positive) cells. No consistent (either across time or sexes) effects of treatment were seen in the relative levels of B cells, natural killer (NK) cells or NK T cells, although there was considerable variability in percentage of NKT cells. These cells make up a minor component of total lymphocytes, and the variability is likely a reflection of the difficulty of accurately measuring the size of this population. No immunophenotyping was carried out for animal 407, since immunophenotyping was only scheduled for weeks 25 and 52. There were no apparent deviations in immunophenotype for animal 408 in week 25, but since this animal was not sacrificed moribund until week 42, it cannot be ruled out that immune deviation contributed to morbidity in this animal.

IMMUNOPHENOTYPING OF T CELLS (Group Means)				
Sex	Male		Female	
Dose (mg/kg/day)	0	50	0	50
% lymphocytes that are CD3 ⁺				
Week 25	58.49 \pm 5.73	63.04 \pm 12.30	65.99 \pm 8.58	68.45 \pm 6.84
Week 52	72.50 \pm 9.54	75.79 \pm 12.91	70.68 \pm 4.69	73.55 \pm 14.38
% lymphocytes that are CD3 ⁺				
Week 25	40.69 \pm 5.86	36.36 \pm 12.19	32.72 \pm 7.58	30.90 \pm 6.75
Week 52	26.45 \pm 9.54	23.65 \pm 13.08	28.57 \pm 4.81	25.60 \pm 14.35
% lymphocytes that are CD4 ⁺				
Week 25	30.29 \pm 6.59	34.50 \pm 5.61	36.41 \pm 7.59	41.59 \pm 6.78
Week 52	39.95 \pm 9.56	41.46 \pm 7.00	39.79 \pm 6.73	43.37 \pm 11.44
% CD3 ⁺ lymphocytes that are CD4 ⁺				
Week 25	51.49 \pm 7.88	55.25 \pm 4.89	55.24 \pm 9.03	60.56 \pm 6.30
Week 52	54.86 \pm 9.49	55.11 \pm 6.58	56.05 \pm 7.02	58.81 \pm 7.96
% lymphocytes that are CD8 ⁺				
Week 25	23.01 \pm 3.45	24.09 \pm 7.15	24.54 \pm 6.52	22.30 \pm 3.42
Week 52	27.54 \pm 5.97	30.02 \pm 8.25	25.84 \pm 4.39	25.55 \pm 6.66
% CD3 ⁺ lymphocytes that are CD8 ⁺				
Week 25	39.68 \pm 6.88	37.51 \pm 4.47	37.09 \pm 8.64	32.83 \pm 6.08
Week 52	38.15 \pm 8.34	39.32 \pm 6.53	36.77 \pm 7.58	34.81 \pm 6.91

Reported as mean value \pm SD.

IMMUNOPHENOTYPING OF B & NK CELLS (Group Means)				
Sex	Male		Female	
Dose (mg/kg/day)	0	50	0	50
% lymphocytes that are CD3 ⁺				
Week 25	53.52 ± 15.87	59.89 ± 10.27	60.37 ± 6.22	62.61 ± 5.54
Week 52	66.49 ± 7.42	66.30 ± 15.26	68.96 ± 5.70	67.86 ± 11.64
% lymphocytes that are CD3 ⁻				
Week 25	45.58 ± 15.30	40.04 ± 10.32	38.22 ± 5.83	36.14 ± 5.48
Week 52	33.09 ± 7.40	33.49 ± 15.28	30.75 ± 5.76	31.72 ± 11.41
% lymphocytes that are CD3 ⁺ CD16 ⁺				
Week 25	9.70 ± 5.50	13.44 ± 8.79	15.69 ± 4.02	12.46 ± 4.42
Week 52	10.01 ± 9.66	6.62 ± 3.43	10.92 ± 4.31	12.70 ± 10.25
% lymphocytes that are CD20 ⁺				
Week 25	22.03 ± 9.05	21.97 ± 14.45	14.72 ± 3.86	17.70 ± 4.85
Week 52	18.36 ± 6.59	18.17 ± 13.75	14.76 ± 3.41	14.62 ± 4.51
% lymphocytes that are CD3 ⁺ CD16 ⁺				
Week 25	0.08 ± 0.09	0.39 ± 0.80	0.14 ± 0.17	0.04 ± 0.03
Week 52	0.02 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.02
% CD3 ⁺ lymphocytes that are CD3 ⁺ CD16 ⁺				
Week 25	0.14 ± 0.15	0.64 ± 1.27	0.24 ± 0.26	0.07* ± 0.04
Week 52	0.03 ± 0.03	0.07* ± 0.02	0.06 ± 0.01	0.08 ± 0.03

Reported as mean value ± SD.

Bold indicates statistical significance.

* significantly different from control at 95% confidence level.

Urinalysis: No apparent test item-related effects.

Gross pathology: Overall, there were no overt macroscopic changes that were indicative of organ toxicity.

GROSS PATHOLOGY								
Sex	Male				Female			
Dose (mg/kg)	0	1	10	50	0	1	10	50
Early Decedents								
NUMBER	0	0	0	2	0	0	0	0
Adrenal gland								
Discolored, dark	-	-	-	1	-	-	-	-
Large				1				
Cecum								
Abnormal contents	-	-	-	2	-	-	-	-
Colon								
Abnormal contents	-	-	-	2	-	-	-	-
Jejunum								
Abnormal contents	-	-	-	1	-	-	-	-
Rectum								
Abnormal contents	-	-	-	2	-	-	-	-
Skeletal muscle								
Dehydration	-	-	-	2	-	-	-	-
Thymus								
Small	-	-	-	1	-	-	-	-

Interim Sacrifice								
NUMBER	3	3	3	2	3	3	3	3
Cecum								
Abnormal contents	0	0	0	1	0	0	0	0
Colon								
Abnormal contents	0	0	0	1	0	0	0	0
Ilium								
Abnormal contents	0	0	0	1	0	0	0	0
Mesenteric Lymph Node								
Large	0	0	0	1	0	0	0	0
Skin & Subcutis								
Fur loss	1	1	1	1	1	2	3	3
Thymus								
Involution	0	0	0	1	0	0	0	0
Terminal Sacrifice								
NUMBER	3	3	3	2	3	3	3	3
Bone Marrow								
Decreased	0	0	0	2	0	0	0	0
Cecum								
Abnormal contents	0	1	0	0	0	0	0	0
Red	0	0	0	0	0	0	0	1
Pituitary								
Mottled	0	0	0	1	0	0	0	0
Skin & Subcutis								
Fur loss	1	1	2	2	0	3	1	2
Tail lesion	0	0	1	1	0	0	0	0
Stomach								
Red	0	0	0	1	0	0	0	0
Recovery Sacrifice								
NUMBER	2	2	2	1	2	2	2	2
Cecum								
Abnormal contents	0	0	0	0	0	0	1	0
Gaseous	0	0	0	0	0	0	1	0
Red	1	0	0	0	0	0	0	0
Colon								
Abnormal contents	0	0	0	0	0	0	1	0
Rectum								
Abnormal contents	0	0	0	0	0	0	1	0
Skin & Subcutis								
Fur loss	1	0	0	1	2	1	1	2
Wound	0	0	0	0	0	0	1	0
Swelling	0	0	0	0	0	0	1	1

Organ weights: There were no statistically significant differences in the group mean organ weight values. Spleen weights trended towards being lower in HD animals, especially males ($\downarrow 6\%$ at interim kill and $\downarrow 23\%$ at terminal kill), but showed partial recovery during recovery period ($\downarrow 14\%$ from control). Differences in the weights of the organs of the male reproductive system were most likely due to differences in the stage of sexual maturity of the individual animals, not exposure to test item. One interim kill HD male (20811M/401) was listed as having a pituitary gland weight of

0.630 g. This is ~10x the weight of the average pituitary of the other males. It seems likely that this is a mis-entry, given the absence of any notable histopathology of the pituitary in this animal, and the fact that there is no trend for increased pituitary size in any of the other HD animals.

The only organ weight findings that were common to both early decedent HD male animals were a marked reduction in thyroid/parathyroid weight (\downarrow 37-70%), and a marked increase in adrenal gland weight (\uparrow 2-4x), as compared to the other members of their dose group. The significance of these changes is unclear, but may have resulted from increased stress in these animals from the frequent diarrhea and dehydration.

Histopathology: In animals surviving to scheduled sacrifice, the clearest histopathologic indication of a dose-dependent effect of denosumab is seen in bone (femur, sternum and tibia). Those MD and HD monkeys in which the epiphyseal growth plates were not yet closed exhibited enlargement of the growth plates in the long bones (see photographs below) at both interim and terminal sacrifice, with the effect being more pronounced at terminal sacrifice. The epiphyseal growth plate normally consists of 5 zones: 1) the zone of reserve cartilage, 2) the zone of proliferating cartilage, 3) the zone of hypertrophying cells and lacunae, 4) the zone of calcifying cartilage and 5) the zone of erosion and ossification. The biggest change in the MD and HD animals was to the cartilage calcifying and ossification zones (zones 4 and 5), with some increase in the hypertrophic zone (zone 3) also being noted. The cause of the enlargement was a marked decrease in chondroclasis due to a decrease in chondroclasts and osteoclasts. A decrease in the number of osteoblasts was also observed. In the sternum, reduced chondroclasis and consequent enlargement of the hypertrophic zone of the symphysis sternalis was also noted in MD and HD animals at terminal sacrifice. In all sampled bones the reduction in chondroclasis, and the numbers of osteoclasts and osteoblasts was at least partially reversed by the 3 month treatment-free recovery period. However, in the recovery group, 1 MD female also exhibited an increase in trabeculae in both the femur and tibia.

The relationship of the test item to another trio of findings in the long bones (serous atrophy, edema and marrow depletion) is unclear. These findings were observed in the 2 early decedent HD males, 1 HD male at interim sacrifice and in 1 C female at recovery sacrifice. Serous atrophy is commonly associated with poor nutritional state. Notably, all four animals presenting with these findings had been in poor nutritional shape (frequent bouts of diarrhea, low food consumption, thin, etc.) for several weeks (or even months) prior to sacrifice.

Most other findings lacked a clear and consistent (i.e. dose-dependent and present in both sexes) relationship with the test item, and were noted by the veterinary pathologist as being typical of individuals of this species and age.

In addition to the findings noted above and in the "Mortality" section, the early decedent HD males had renal findings (slight tubular dilation (both) and minimal

focal unilateral papillary mineralization (#408)), and findings in the lung (slight-moderate) perivascular edema (both), pigment (#407), and multifocal slight vascular dilation and epithelial atrophy of the bronchi (#408)).

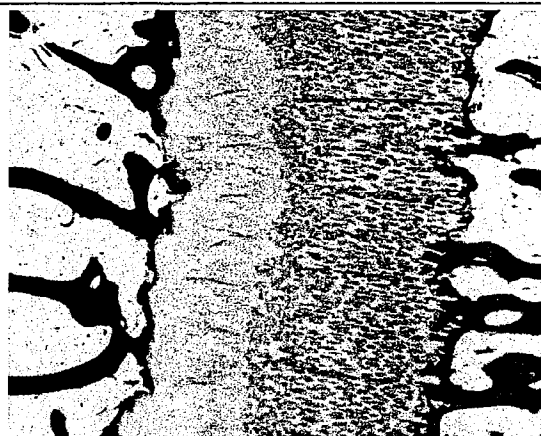


Figure 2a
Animal no. 20811 M, Group 4, left tibia, enlarged epiphyseal growth plate



Figure 2b
Animal no. 20813 M, Group 1 control animal, left tibia, epiphyseal growth plate

HISTOPATHOLOGY (Incidence & Selected Mean Severity Scores)								
Sex	Male				Female			
Dose (mg/kg)	0	1	10	50	0	1	10	50
Early Decedents								
Number	0	0	0	2	0	0	0	0
Adrenal gland								
Cortical hypertrophy	-	-	-	2	-	-	-	-
Congestion	-	-	-	1	-	-	-	-
Nodular hyperplasia	-	-	-	1	-	-	-	-
Cecum								
Acute inflammation	-	-	-	1	-	-	-	-
Erosion	-	-	-	1	-	-	-	-
Crypt microabscesses	-	-	-	2	-	-	-	-
Dilated glands	-	-	-	1	-	-	-	-
Colon								
Acute inflammation	-	-	-	1	-	-	-	-
Crypt microabscesses	-	-	-	1	-	-	-	-
Dilated glands	-	-	-	1	-	-	-	-
Duodenum								
Villous atrophy	-	-	-	1	-	-	-	-
Femur + Marrow								
Serous atrophy	-	-	-	2 (2.0)	-	-	-	-
Edema	-	-	-	2	-	-	-	-
↓ Chondroclasis	-	-	-	2	-	-	-	-
Enlarged epiphyseal growth plate	-	-	-	2	-	-	-	-
↓ Osteoclasts	-	-	-	2	-	-	-	-
↓ Osteoblasts	-	-	-	2	-	-	-	-
Heart								
Myocarditis	-	-	-	1	-	-	-	-

Pericarditis	-	-	-	1	-	-	-	-
Injection site								
Hemorrhage	-	-	-	1	-	-	-	-
Inflammatory cell foci	-	-	-	1	-	-	-	-
Fibrosis	-	-	-	1	-	-	-	-
Ileum								
Mucosal atrophy	-	-	-	1	-	-	-	-
Jejunum								
Autolysis	-	-	-	1	-	-	-	-
Inflammation	-	-	-	1	-	-	-	-
Crypt microabscesses	-	-	-	1	-	-	-	-
Mucosal atrophy	-	-	-	1	-	-	-	-
Hyaline deposits	-	-	-	1	-	-	-	-
Kidney								
Tubular dilation	-	-	-	2	-	-	-	-
Papillary mineralization	-	-	-	1	-	-	-	-
Tibia (left)								
Serous atrophy	-	-	-	2 (2.5)	-	-	-	-
Edema	-	-	-	2	-	-	-	-
↓ Chondroclasis	-	-	-	2	-	-	-	-
Enlarged epiphyseal growth plate	-	-	-	2	-	-	-	-
↓ Osteoclasts	-	-	-	2	-	-	-	-
↓ Osteoblasts	-	-	-	2	-	-	-	-
Lung								
Pigment	-	-	-	1	-	-	-	-
Edema	-	-	-	2	-	-	-	-
Vascular dilation	-	-	-	1	-	-	-	-
Epithelial atrophy	-	-	-	1	-	-	-	-
Mandibular lymph node								
Lymphoid atrophy	-	-	-	1	-	-	-	-
Depleted follicular centers	-	-	-	1	-	-	-	-
Mesenteric lymph node								
Lymphoid atrophy	-	-	-	1	-	-	-	-
Depleted follicular centers	-	-	-	1	-	-	-	-
Depletion	-	-	-	1	-	-	-	-
Sinusoidal dilation	-	-	-	1	-	-	-	-
Necrosis	-	-	-	1	-	-	-	-
↑ tangible body macrophages	-	-	-	1	-	-	-	-
Esophagus								
Epithelial atrophy	-	-	-	1	-	-	-	-
Pancreas								
Atrophy	-	-	-	1	-	-	-	-
Pituitary								
Mineralization	-	-	-	1	-	-	-	-
No sample	-	-	-	1	-	-	-	-
Rectum								
Crypt microabscesses	-	-	-	2	-	-	-	-
Dilated glands	-	-	-	1	-	-	-	-
Sternum + Marrow								
↓ Chondroclasis	-	-	-	1	-	-	-	-
↓ Osteoclasts	-	-	-	2	-	-	-	-
↓ Osteoblasts	-	-	-	2	-	-	-	-
Spleen								

Lymphoid atrophy	-	-	-	2	-	-	-	-
Stomach								
Degeneration	-	-	-	1	-	-	-	-
Erosion	-	-	-	1	-	-	-	-
Mucosal atrophy	-	-	-	1	-	-	-	-
Bacteria in glands	-	-	-	1	-	-	-	-
Skeletal muscle								
Atrophy	-	-	-	1	-	-	-	-
Thymus								
Involution	-	-	-	2	-	-	-	-
Thyroid								
Ectopic thymus	-	-	-	1	-	-	-	-
Urinary bladder								
Inflammation	-	-	-	1	-	-	-	-
Interim Sacrifice								
Number	3	3	3	2	3	3	3	3
Adrenal gland								
Lymphocyte foci	0	0	0	0	0	0	1	0
Cecum								
Hemorrhage	0	0	0	0	0	0	1	0
Inflammation	0	0	0	0	0	0	1	0
Erosion	0	0	0	0	0	0	1	0
Crypt microabscess	0	0	0	0	0	1	0	0
Colon								
Crypt microabscess	0	0	0	0	0	1	0	0
Protozoa	0	0	0	0	0	0	0	1
Epididymis								
Atrophy	0	0	0	1	n.a.			
Oligospermia	1	1	0	1				
Eye								
Lymphocyte foc	0	0	0	0	0	0	0	1
Femur + marrow								
Serous atrophy	0	0	0	1 (3.0)	0	0	0	0
Edema	0	0	0	1	0	0	0	0
Depletion	0	0	0	1	0	0	0	0
↓ Chondroclasis	0	0	3	1	0	0	1	0
No epiphyseal growth plate	0	0	0	0	0	0	0	2
Enlarged epiphyseal growth plate	0	0	3	1	0	0	1	0
↓ Osteoclasts	0	0	2	2	0	0	2	2
↓ Osteoblasts	0	0	2	2	0	0	2	2
Heart								
Inflammatory cell foci	1	1	0	1	1	1	1	2
Degeneration/necrosis	0	0	0	1 (2.0)	0	0	0	0
Injection								
Hemorrhage	0	1	0	1	0	1	0	0
Granuloma	0	0	2	0	0	0	0	0
Kidney								
Tubular Dilation	0	0	0	0	1	0	0	1
Basophilic tubules	0	1	1	0	0	0	0	0
Cortical mineralization	0	0	0	0	0	0	0	1
Papillary mineralization	0	0	0	0	0	1	0	0
Inflammation	0	0	0	1	0	0	0	0
Inflammatory cell foci	3	3	2	0	1	0	0	1

Granuloma	0	0	0	0	0	1	0	0
Glomerulopathy	0	0	0	0	0	0	1	0
Liver								
Hemorrhage	0	0	0	1	0	0	0	0
Inflammatory cell foci	3	1	3	1	3	1	2	2
Inflammatory cell infiltration	0	0	0	0	0	0	0	1
Parasite granuloma	0	0	0	0	0	0	1	0
Tibia (left)								
Serous atrophy	0	0	0	1 (3.0)	0	0	0	0
Edema	0	0	0	1	0	0	0	0
Depletion	0	0	0	1	0	0	0	0
↓ Chondroclasis	0	0	3	2	0	0	2	3
↑ Myelopoiesis	0	0	0	0	0	1	0	0
Enlarged epiphyseal growth plate	0	0	3	2	0	0	2	3
↓ Osteoclasts	0	0	2	2	0	0	2	3
↓ Osteoblasts	0	0	1	2	0	0	2	3
Lung								
Foamy histiocytes	0	0	0	1	0	0	0	0
Mandibular lymph node								
Edema	0	0	0	1	0	0	0	0
Mesenteric lymph node								
Edema	0	0	0	1	0	0	0	0
Sinus histiocytosis	1	0	0	1	0	0	0	1
Depletion	0	0	0	0	0	0	0	1
Ovary								
Mineralization	n.a.				0	0	0	1
Pancreas								
Ectopic spleen	0	0	0	0	0	0	0	1
Pituitary								
Inflammatory cell foci	0	0	1	0	0	0	1	1
Rectum								
Inflammation	0	0	0	1	0	0	0	0
Crypt microabscess	0	0	0	1	0	1	0	1
Salivary gland								
Inflammatory cell foci	0	0	0	0	0	0	0	1
Lymphocyte foci	1	0	1	1	0	0	0	0
Sternum + Marrow								
↓ Chondroclasis	0	0	0	1	0	0	0	0
↓ Osteoclasts	0	0	1	2	0	0	1	2
↓ Osteoblasts	0	0	1	2	0	0	1	2
Spleen								
Lymphoid atrophy	0	0	0	1	0	0	0	0
Glandular dilation	2	0	0	1	0	0	1	1
Thyroid								
Ectopic thymus	0	2	1	1	2	0	0	2
Cyst	0	0	0	0	0	0	1	0
Follicular distension	0	0	0	1	0	0	0	0
C-cell hyperplasia	0	0	0	0	0	0	1	0
Terminal Sacrifice								
Number	3	3	3	2	3	3	3	3
Adrenal								
Lymphocyte foci	0	0	0	1	1	0	0	0
Aorta								

Intimal proliferation	0	0	1	0	0	1	0	0
Brain								
Mineralization, focal Thalamus, vascular wall	0	0	0	0	0	0	0	1 (1.0)
Inflammation	0	1	0	0	0	0	1	0
Inflammatory cell foci Thalamus	0	0	0	2 (1.0)	0	0	0	0
Cecum								
Congestion	0	0	0	1	0	0	0	1
Hemorrhage	0	0	0	1	0	0	0	1
Colon								
Crypt microabscess	0	0	0	0	1	0	0	0
Duodenum								
Pigment	0	0	0	0	0	0	0	1
Epididymis								
Inflammatory cell foci	0	0	1	0	n.a.			
Femur + Marrow								
No epiphyseal growth plate	0	0	0	0	3	3	2	1
↓ Chondroclasis	0	0	1	3	0	0	0	1
Enlarged epiphyseal growth plate	0	0	1	3	0	0	0	1
↓ Osteoclasts	0	0	1	2	0	0	2	3
↓ Osteoblasts	0	0	1	2	0	0	2	3
Heart								
Inflammatory cell foci	0	1 (1.0)	1 (1.0)	2 (1.0)	3 (1.0)	2 (1.0)	1 (1.0)	2 (1.5)
Injection site								
Inflammatory cell foci	0	1	2	0	0	1	1	0
Kidney								
Cortical mineralization	0	0	0	0	0	0	0	1
Papillary mineralization	0	0	0	0	1	0	0	1
Inflammation	0	0	0	0	0	0	1	0
Inflammatory cell foci	2 (1.0)	2 (1.5)	2 (1.0)	1 (1.0)	0	2 (2.0)	1 (1.0)	1 (2.0)
Glomerulopathy	0	0	0	0	0	1	0	0
Liver								
Vacuolation	0	0	1	0	0	0	0	0
Inflammatory cell foci	1 (1.0)	2 (1.0)	3 (1.0)	2 (1.0)	2 (2.0)	3 (1.0)	3 (1.8)	3 (1.0)
Inflammatory cell infiltration	0	0	0	0	1	0	0	1
Tibia (left)								
No epiphyseal growth plate	0	0	0	0	0	1	0	0
↓ Chondroclasis	0	0	1	3	0	0	2	3
Enlarged epiphyseal growth plate	0	0	1	3	0	0	2	3
↓ Osteoclasts	0	0	1	3	0	0	2	3
↓ Osteoblasts	0	0	1	3	0	0	2	3
Lung								
Inflammatory cell foci	0	2	2	0	0	1	0	2
Foamy histiocytes	0	0	2	0	0	0	1	0
Mesenteric lymph node								
Edema	0	0	0	0	0	0	1	1
Sinus histiocytosis	0	2	0	1	0	0	0	0
Ovary								
Follicular cyst	n.a.				0	0	0	1
Pancreas								
Fat infiltration	0	0	0	1	0	0	0	0
Inflammatory cell foci	0	0	1	0	0	0	0	2

Pituitary								
Cyst	0	2	0	1	0	1	0	0
Fat infiltration	0	1	0	0	0	0	0	0
Pituicyte vacuolation	0	0	1	0	0	0	0	0
Inflammatory cell foci	0	1	0	0	0	0	0	2
Parathyroid								
Cyst	0	0	1	0	0	2	0	0
Lymphocyte foci	0	0	1	0	0	2	0	1
Salivary gland								
Lymphocyte foci	0	0	3	1	0	0	1	1
Skin + Subcutis								
Necrosis	0	0	1	0	0	0	0	0
Inflammation	0	0	1	0	0	0	0	0
Abscess	0	0	0	1	0	0	0	0
Sternum + Marrow								
↓ Chondroclasis	0	0	2	3	0	0	1	3
Enlarged symphysis sternalis	0	0	2	3	0	0	1	3
↓ Osteoclasts	0	0	1	2	0	0	2	3
↓ Osteoblasts	0	0	1	2	0	0	2	3
Spleen								
Capsular fibrosis	0	0	0	1	0	0	0	0
Stomach								
Hemorrhage	0	0	0	1	0	1	0	0
Inflammation	0	0	0	1	0	0	0	0
Crypt microabscess	0	0	0	0	1	0	0	0
Glandular dilation	1	0	0	1	2	0	1	1
Skeletal muscle								
Inflammatory cell foci	0	0	0	1	0	0	0	0
Seminal vesicle								
Mineralization	0	0	1	0	n.a.			
Testis								
Edema	0	0	0	1	n.a.			
Thymus								
Cyst	0	0	0	0	2	0	2	1
Involution	0	2	2	2	1	1	2	1
Thyroid								
Ectopic thymus	0	1	1	0	1	1	2	0
Cyst	0	0	1	1	0	0	0	0
Follicular distention	0	0	0	0	0	1	0	0
Fat Infiltration	0	1	0	0	0	0	0	1
Inflammatory cell foci	0	1	0	1	0	0	0	0
Lymphocyte foci	0	0	1	1	0	1	0	0
Uterus								
Squamous metaplasia	n.a.				0	1	0	1
Recovery Sacrifice								
Number	2	2	2	1	2	2	2	2
Adrenal gland								
Pigment	0	0	0	0	0	0	0	1
Brain								
Inflammatory cell foci	0	0	0	0	0	0	1 (1.0)	0
Cecum								
Inflammation	0	0	0	0	0	0	1	0
Crypt microabscess	0	0	0	0	0	0	1	0

Colon								
Acute inflammation	0	0	0	0	0	0	1	0
Crypt microabscess	0	0	0	0	0	0	1	0
Femur + Marrow								
Serous atrophy	0	0	0	0	1 (2.0)	0	0	0
Edema	0	0	0	0	1	0	0	0
Depletion	0	0	0	0	1	0	0	0
No epiphyseal growth plate	1	0	1	0	2	2	1	1
↑ in Trabeculae	0	0	0	0	0	0	1	0
Heart								
Fat Infiltration	0	0	0	1	0	0	0	0
Inflammatory cell foci	0	1	0	0	1	0	1	0
Myocarditis	0	0	0	0	0	0	0	1
Pericarditis	0	0	0	0	1	0	0	0
Degeneration/necrosis	0	0	0	1	1	0	0	0
Kidney								
Tubular dilation	0	0	0	1	0	0	0	0
Basophilic tubules	0	0	0	0	0	0	0	1
Pigment	0	0	0	1	0	0	0	0
Cortical mineralization	0	0	0	0	0	0	1	1
Papillary mineralization	0	0	0	1	0	0	0	0
Inflammation	0	0	0	0	0	0	0	1
Inflammatory cell foci	0	0	1	0	2	1	2	2
Liver								
Inflammatory cell foci	1	1	0	1	2	1	2	2
Lung								
Foamy histiocytes	1	1	0	0	0	0	0	0
Tibia (left)								
Serous atrophy	0	0	0	0	1 (3.0)	0	0	0
Edema	0	0	0	0	1	0	0	0
Depletion	0	0	0	0	1	0	0	0
No epiphyseal growth plate	0	0	0	0	1	1	0	0
Enlarged epiphyseal growth plate	0	0	0	1	0	0	0	0
↑ in Trabeculae	0	0	0	0	0	0	1	0
Mammary gland								
Dilated glands	0	0	0	1	0	0	0	0
Mandibular lymph node								
Sinus histiocytosis	0	0	0	0	0	0	1	0
Mesenteric lymph node								
Edema	0	1	0	0	1	0	0	0
Sinus histiocytosis	0	0	1	0	0	0	0	0
Ovary								
Cyst	n.a.				0	0	1	0
Mineralization					1	0	1	0
Paraovarian cyst					0	0	0	1
Pituitary								
Cyst	0	0	0	0	0	0	0	1
Vacuolation	0	1	0	0	0	0	0	0
Pituicyte vacuolation	0	0	0	0	1	0	0	0
Inflammatory cell foci	1	0	0	0	0	0	0	1
Prostate								
Inflammatory cell foci	0	1	1	1	n.a.			
Parathyroid								

Fat infiltration	0	0	0	0	0	1	0	0
Lymphocyte foci	0	0	0	0	0	0	1	0
Salivary gland								
Lymphocyte foci	0	1	2	0	1	1	0	0
Skin + Subcutis								
Degeneration	0	0	0	1	0	0	0	0
Necrosis	1	0	0	0	0	0	0	0
Inflammation	0	0	0	0	0	0	1	0
Periosteitis	0	0	0	0	0	0	1	0
Abscess	1	0	0	0	0	0	0	1
Hyperkeratosis	0	0	0	0	0	0	1	0
Skeletal muscle								
Inflammatory cell foci	0	0	0	0	1	0	0	0
Thymus								
Cyst	1	0	1	1	1	1	2	0
Involution	0	0	0	1	1	1	1	1
Thyroid								
Ectopic thymus	1	1	2	0	0	1	1	0
Cyst	0	0	2	0	0	0	1	0
Follicular distension	0	1	0	0	0	0	1	0
Lymphocyte foci	0	0	1	0	0	0	1	0
Uterus								
Squamous metaplasia		n.a.			0	2	1	2

n.a. = not applicable.

Light green highlighting: expected pharmacodynamic effect.

Light orange highlighting: possible toxicologic effects

Sperm Motility: No test item-related effect.

Anti-denosumab Antibodies:

Antibody positive: C = 0%, LD = 100%, MD = 50% and HD = 13%.

Positive for bioassay neutralization: C = 0%, LD = 81%, MD = 43% and HD = 13%.

Analysis of the individual serum denosumab concentrations over time (see Toxicokinetics section below) indicates that the dose-dependent reduction in the incidence of anti-denosumab immune response at higher doses is not an artifact of interference of the test item in the serum with the antibody binding assay.

Neither of the early decedent HD male monkeys was positive for anti-denosumab antibodies.

Table 5

Incidence of anti-AMG 162 Binding Antibodies Over Time as Detected by an Electrochemiluminescent Immunoassay in Cynomolgus Monkeys for Study 102090

Group	Dose (mg/kg)	Day 0	Week 12	Week 24	Week 52	Week 66
1	0	4/16	1/16	0/16	0/10	0/4
2	1	5/16	16/16	16/16	10/10	4/4
3	10	7/16	8/16	8/16	6/10	3/4
4	50	4/16	2/15	1/15	2/9	2/3

Table 6

Incidence of anti-AMG 162 Neutralizing Activity Over Time as Detected by AMG 162 NAb in Cynomolgus Monkeys for Study 102090

Group	Dose (mg/kg)	Day 0	Week 12	Week 24	Week 52	Week 66
1	0	0/16	0/16	0/16	0/10	0/4
2	1	0/16	12/16	12/16	7/10	3/4
3	10	0/16	6/16	6/16	6/10	3/4
4	50	0/16	5/15	4/15	2/9	2/3

Table 7

Overall Incidence of Animals that Developed anti-AMG 162 Antibodies at one or more time point through Week 66 as Detected by an Electrochemiluminescent Immunoassay and Neutralizing Antibody Bioassay

Group	Dose (mg/kg)	Total Number of Animals	Number of Animals Positive for anti-AMG 162 Binding Antibodies in Immunoassay	Number of Animals Positive for Neutralizing Antibody in Bioassay*	Percent Incidence of anti-AMG 162 Binding Antibodies in Immunoassay	Percent Incidence of anti-AMG 162 Neutralizing Antibody in Bioassay
1	0	16	1	0	0%	0%
2	1	16	16	13	100.0%	81.3%
3	10	16	8	7	50.0%	43.8%
4	50	15	2	2	13.3%	13.3%
**Overall Incidence in AMG 162 Dosed Animals		47	26	22	55.3%	46.8%

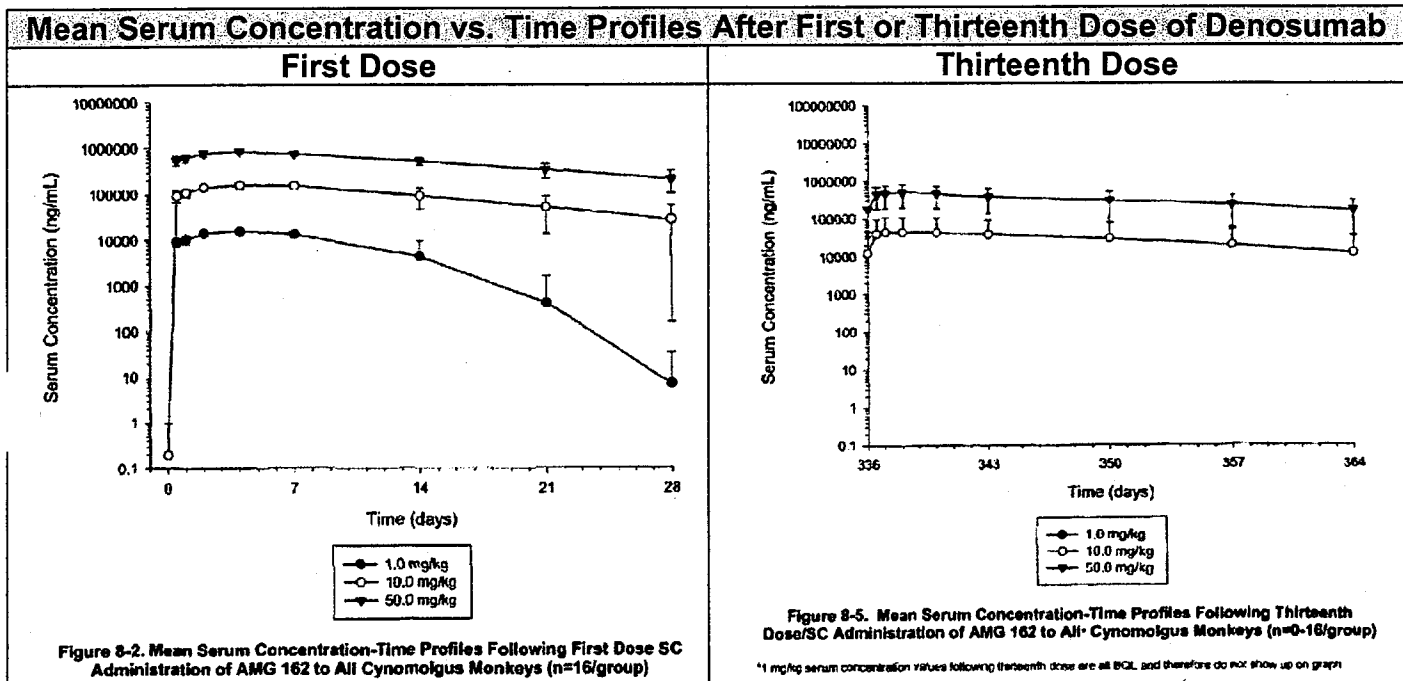
*Animals positive at 1 or more time point for binding antibodies in the immunoassay

** Values do not include Group 1 placebo animals

Toxicokinetics: Denosumab exposure increased hyperproportionally with dose. There was no appreciable accumulation of denosumab over the 12 month dosing period. There were no apparent sex-based differences in exposure. Denosumab exposure was not detected in the control animals. Exposure levels were substantially reduced (2 to 4 logs) following the 13th dose, compared to the 1st dose, in those animals that tested positive for anti-denosumab antibodies. Notably, denosumab could no longer be detected in the serum of any of the LD animals by about 29 days after the first dose, presumably due to increased clearance caused by the circulating anti-denosumab antibodies in these animals.

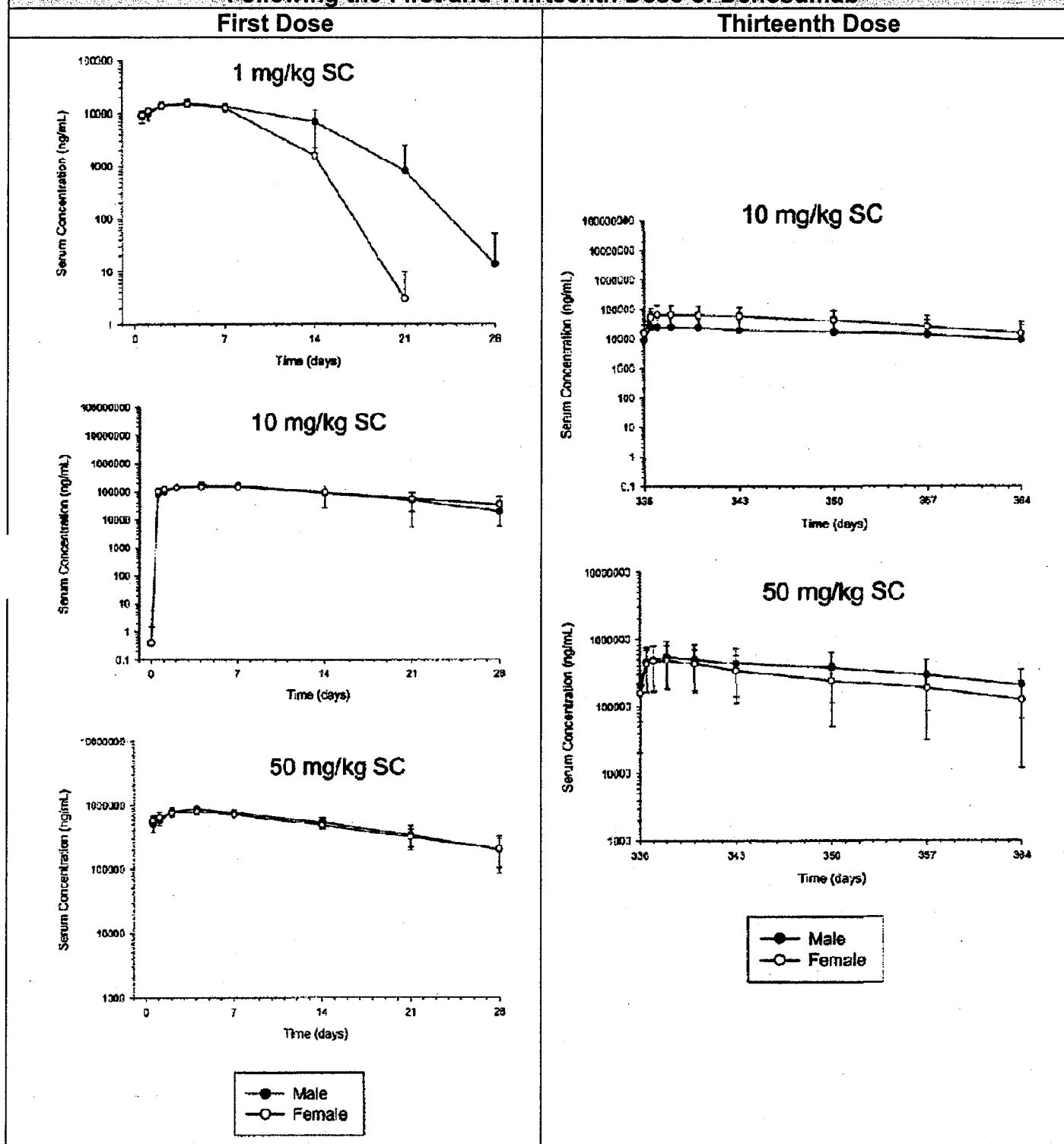
Mean (\pm SD) Non-Compartmental Parameter Estimates						
Group:		2	3	4	3	4
Dose (mg/kg):		1.0	10.0	50.0	10.0	50.0
n ^a		16 ^b	16 ^b	16 ^b	4 ^b	7 ^b
Parameter	Units	First Dose	First Dose	First Dose	Thirteenth Dose	Thirteenth Dose
t _{max} ^c	hr	96 (48-96)	96 (48-336)	96 (48-96)	24 (12-96)	48 (24-96)
C _{max} ^d	μ g/mL	15.8 (1.81)	162 (25.4)	853 (79.3)	115 (37.1)	666 (156)
C _{last} ^e	μ g/mL	0.965 (2.17)	36.0 (39.1)	203 (105)	30.6 (20.8)	209 (101)
AUC _{0-t_{last}} ^f	μ g \cdot hr/mL	4100 (1150)	61500 (16000)	343000 (52200)	48200 (21100)	268000 (90300)
AUC _{0-t_{last}} ^f / Dose ^g	(mg \cdot hr/mL) / (mg/kg)	4.10 (1.15)	6.15 (1.6)	6.86 (1.04)	4.82 (2.11)	5.37 (1.81)

^anumber of animals, ^bantibody negative animals used for calculation, ^ctime at which C_{max} was observed (median and range shown), ^dmaximum observed concentration, ^elast concentration observed for the dosing interval, ^farea under the concentration time curve from time zero to the time of the last sample in the dosing interval, ^gDose Normalized AUC_{0- t_{last}}



The serum concentrations of denosumab were below the lower limit of quantitation, and are not shown for the 13th week.

Comparison of Male and Female Mean Serum Concentration vs. Time Profiles Following the First and Thirteenth Dose of Denosumab



The serum concentrations of denosumab were below the lower limit of quantitation, and are not shown for the 13th week

Individual Serum Concentrations of Denosumab: Effect of anti-denosumab Immune Response**Male****Table 9-3. Individual Serum Concentrations* (ng/mL) of AMG 162 in Male Cynomolgus Monkeys Following up to 14 Monthly SC Injections of 1 mg/kg with a 3 Month Washout Period**

Study Day	Time From First Dose (days)	Animal Number							
		20431	20818	20819	20834	20841	20853	20856	20857
1	0	0	0	0	0	0	0	0	0
1	0.5	15700	7560	7780	9420	9830	2610	10000	7220
2	1	14300	1570	7560	3700	3070	10800	12600	3540
3	2	15800	14900	14900	13800	11200	13600	18000	12700
5	4	12000	14200	18500	16700	16000	14700	20300	12500
8	7	16000	13000	15800	13600	13000	12900	13500	11500
15	14	11400	8900	11200	5220	10100	724	8570	112
22	21	1890	260	118	0	4460	0	0	0
29	28	0	0	233	10000	101	0	0	0
57	56	0	0	0	0	0	0	0	0
85	84	0	0	0	0	0	0	0	0
85	84.5	0	0	0	0	222	0	0	0
86	85	0	0	0	0	231	0	0	0
87	86	0	0	0	0	84.6	0	0	0
89	88	0	0	0	0	0	0	0	0
113	112	0	0	0	0	0	0	0	0
141	140	0	0	0	0	0	0	0	0
169	168	0	0	0	0	0	0	0	0
169	168.5	0	0	0	0	0	0	0	0
170	169	0	0	0	0	0	0	0	0
171	170	0	0	0	0	0	0	0	0
173	172	0	0	0	0	0	0	0	0
197	196	0	0	0	0	0	0	0	0
225	224	0	0	0	0	0	0	0	0
281	280	0	0	0	0	0	0	0	0
309	308	0	0	0	0	0	0	0	0
337	336	0	0	0	0	0	0	0	0
337.6	336.6	0	0	0	0	0	0	0	0
338	337	0	0	0	0	0	0	0	0
339	338	0	0	0	0	0	0	0	0
341	340	0	0	0	0	0	0	0	0
344	343	0	0	0	0	0	0	0	0
351	350	0	0	0	0	0	0	0	0
358	357	0	0	0	0	0	0	0	0
365	364	0	0	0	0	0	0	0	0
372	371	0	0	0	0	0	0	0	0
374	373	0	0	0	0	0	0	0	0
393	392	0	0	0	0	0	0	0	0
421	420	0	0	0	0	0	0	0	0
449	448	0	0	0	0	0	0	0	0
463	462	0	0	0	0	0	0	0	0

*Values have been rounded to 3 significant figures
 *BCL values have been changed to zero
 *Quantity not sufficient
 *No Sample
 Shaded values indicate antibody positive animals

Female**Table 9-4. Individual Serum Concentrations* (ng/mL) of AMG 162 in Female Cynomolgus Monkeys Following up to 14 Monthly SC Injections of 1 mg/kg with a 3 Month Washout Period**

Study Day	Time From First Dose (days)	Animal Number							
		20733	20794	20797	20808	20868	20871	20882	20891
1	0	0	0	0	0	0	0	0	0
1	0.6	16800	3640	7120	9620	8290	11100	1690	2360
2	1	12400	3100	10800	11200	11900	12200	11700	10500
3	2	15200	10800	14400	15400	15400	15000	14000	11100
5	4	12900	1800	16600	18200	18100	18600	15300	13100
8	7	11400	12200	14900	14300	13900	13900	10500	9780
15	14	12.0	187	386	10400	1625	1440	239	735
22	21	0	0	0	18.6	0	4.37	0	0
29	28	0	0	0	0	0	0	0	0
57	56	0	0	0	0	0	0	0	0
85	84	NS	NS	NS	NS	NS	NS	NS	NS
85	84.5	NS	NS	NS	NS	NS	NS	NS	NS
86	85	NS	NS	NS	NS	NS	NS	NS	NS
87	86	0	0	0	0	0	0	0	0
89	88	0	0	0	0	0	0	0	0
113	112	0	0	0	0	0	0	0	0
141	140	0	0	0	0	0	0	0	0
169	168	0	0	0	0	0	104	0	0
169	168.5	0	0	0	0	0	0	0	0
170	169	0	0	0	0	0	0	0	0
171	170	0	0	0	0	0	0	0	0
173	172	0	0	0	0	0	0	0	0
197	196	0	0	0	0	0	0	0	0
225	224	0	0	0	0	0	0	0	0
281	280	0	0	0	0	0	0	0	0
309	308	0	0	0	0	0	0	0	0
337	336	0	0	0	0	0	0	0	0
337.5	336.5	0	0	0	0	0	0	0	0
338	337	0	0	0	0	0	0	0	0
339	338	0	0	0	0	0	0	0	0
341	340	0	0	0	0	0	0	0	0
344	343	0	0	0	0	0	0	0	0
351	350	0	0	0	0	0	0	0	0
358	357	0	0	0	0	0	0	0	0
365	364	0	0	0	0	0	0	0	0
372	371	0	0	0	0	0	0	0	0
374	373	0	0	0	0	0	0	0	0
393	392	0	0	0	0	0	0	0	0
421	420	0	0	0	0	0	0	0	0
449	448	0	0	0	0	0	0	0	0
463	462	0	0	0	0	0	0	0	0

*Values have been rounded to 3 significant figures
 *BCL values have been changed to zero
 *Not Reportable
 *No Sample
 Shaded values indicate antibody positive animals

Table 9-5. Individual Serum Concentrations* (ng/mL) of AMG 162 in Male Cynomolgus Monkeys Following up to 14 Monthly SC Injections of 10 mg/kg with a 3 Month Washout Period

Study Day	Time From First Dose (days)	Animal Number							
		20814	20828	20831	20838	20840	20845	20851	20858
1	0	0	0	0	0	0	0	0	0
1	0.5	93500	85800	77800	81300	104000	83400	47700	70700
2	1	123000	101000	97200	109000	111000	105000	85100	87400
3	2	163000	153000	149000	160000	167000	138000	97500	130000
5	4	173000	168000	158000	177000	227000	157000	128000	160000
7	7	175000	150000	163000	166000	177000	178000	122000	147000
15	14	118000	0	114000	43300	164000	124000	131000	5860
22	21	68700	0	96700	118	39100	81500	55800	0
29	28	38800	0	53300	0	50000	666	9750	0
57	56	45600	0	70000	0	45700	0	7.05	0
85	84	51800	0	74600	0	42700	0	0	0
85	84.5	120000	23.8	167000	4050	143000	22800	41100	0
86	85	132000	14.4	173000	3320	153000	22200	83500	0
87	86	163000	4.32	191000	2830	163000	9190	51000	0
89	88	200000	0	185000	0	168000	4.93	30800	0
113	112	48400	0	81500	0	30200	0	0	0
141	140	43100	0	89300	0	47600	0	0	0
169	168	48400	0	107000	0	58500	0	0	0
169	168.5	106000	0	152000	608	123000	1290	8930	0
170	169	134000	0	197000	1230	142000	1370	12100	0
171	170	172000	0	252000	435	160000	2480	11900	0
173	172	181000	0	208000	0	161000	0	2250	0
197	196	0	0	0	0	42600	0	0	0
225	224	0	0	0	0	5690000	0	0	0
281	280	0	0	0	0	30500	0	0	0
309	308	0	0	0	0	35900	0	0	0
337	336	0	0	0	0	45700	0	0	0
337.5	336.5	0	0	0	0	121000	0	2800	0
338	337	0	0	0	0	119000	0	3390	0
339	338	0	0	0	0	121000	0	2480	0
341	340	0	0	0	0	121000	0	4.18	0
344	343	0	0	0	0	97000	0	0	0
351	350	0	0	0	0	83700	0	0	0
358	357	0	0	0	0	67800	0	0	0
365	364	0	0	0	0	45700	0	0	0
372	371	0	0	0	0	125000	0	NS	NS
374	373	0	0	0	0	0	0	0	0
393	392	0	0	0	0	0	0	0	0
421	420	0	0	0	0	0	0	0	0
449	448	0	0	0	0	0	0	0	0
483	482	0	0	0	0	0	0	0	0

*Values have been rounded to 3 significant figures

*BCL values have been changed to zero

No sample

Shaded values indicate antibody positive animals

Table 9-6. Individual Serum Concentrations* (ng/mL) of AMG 162 in Female Cynomolgus Monkeys Following up to 14 Monthly SC Injections of 10 mg/kg with a 3 Month Washout Period

Study Day	Time From First Dose (days)	Animal Number							
		20718	20727	20728	20731	20740	20886	20887	20902
1	0	0	0	0	0	0	3.13	0	0
1	0.5	126000	108000	104000	136000	48400	120000	78700	105000
2	1	108000	138000	127000	146000	71300	110000	122000	125000
3	2	141000	153000	156000	149000	107000	129000	142000	126000
5	4	139000	168000	178000	164000	111000	155000	131000	140000
8	7	143000	154900	147000	173000	117000	150000	121000	137000
15	14	104000	119000	91200	62800	77500	102000	78700	103000
22	21	64100	97400	18100	155	58800	74900	28000	79500
29	28	42900	62000	368	0	45300	54000	2710	60400
57	56	37100	68600	0	0	32800	63600	155	68800
85	84	NS	NS	NS	NS	NS	NS	NS	NS
85	84.5	NS	NS	NS	NS	NS	NS	NS	NS
86	85	NS	NS	NS	NS	NS	NS	NS	NS
87	86	157000	177000	29800	33.5	118000	180000	128000	186000
89	88	226000	203000	5420	0	133000	141000	144000	142000
113	112	24900	54600	0	0	32000	53100	0	57300
141	140	38700	43400	0	0	57800	38700	0	62900
169	168	37400	47700	0	0	45300	52200	0	37500
169	168.5	120000	83300	9320	4.87	95300	148000	14700	106000
170	169	131000	123000	8560	2.54	118000	152000	16300	123000
171	170	153000	136000	5500	0	131000	177000	13800	119000
173	172	159000	136000	800	0	159000	163000	1010	121000
197	196	0	0	0	0	22800	38300	0	25200
225	224	0	0	0	0	491000	596000	0	551000
281	280	0	0	0	0	9760	58500	0	21400
309	308	0	0	0	0	13000	84300	0	17200
337	336	0	0	0	0	9970	58700	0	11200
337.5	336.5	0	0	0	0	57300	142000	6.78	77400
338	337	0	0	0	0	73100	166000	26.2	85300
339	338	0	0	0	0	85000	164000	222	73200
341	340	0	0	0	0	85400	149000	0	74900
344	343	0	0	0	0	81000	147000	0	64300
351	350	0	0	0	0	53000	117000	0	39500
358	357	0	0	0	0	20000	79100	0	19900
365	364	0	0	0	0	15300	51100	0	10400
372	371	0	0	0	0	80000	137000	0	59200
374	373	0	0	0	0	0	0	NS	NS
393	392	0	0	0	0	0	0	0	12300
421	420	0	0	0	0	0	0	0	330
449	448	0	0	0	0	0	0	0	0
483	482	0	0	0	0	0	0	0	0

*Values have been rounded to 3 significant figures

*BCL values have been changed to zero

No sample

Shaded values indicate antibody positive animals

Table 9-7. Individual Serum Concentrations^a (ng/mL) of AMG 162 in Male Cynomolgus Monkeys Following up to 14 Monthly SC Injections of 50 mg/kg with a 3 Month Washout Period

Study Day	Time From First Dose (days)	Animal Numbers							
		20611	20617	20623	20627	20637	20649	20655	20659
1	0	0 ^b	0	0	0	0	0	0	0
1	0.5	398000	703000	573000	437000	462000	538000	383000	748000
2	1	530000	589000	531000	558000	570000	542000	527000	745000
3	2	710000	929000	892000	753000	783000	855000	752000	881000
5	4	835000	897000	827000	832000	930000	905000	895000	1020000
8	7	618000	910000	783000	735000	762000	887000	738000	832000
15	14	563000	652000	531000	541000	532000	542000	506000	630000
22	21	346000	403000	357000	378000	401000	389000	373000	436000
29	28	153000	278000	198000	242000	209000	270000	202000	327000
57	56	195000	287000	177000	229000	116000	0	175000	407000
85	84	241000	269000	186000	183000	121000	0	0	386000
85	84.5	723000	685000	683000	690000	492000	229000	0	852000
86	85	764000	839000	752000	704000	583000	562000	0	822000
87	86	845000	859000	809000	807000	829000	889000	0	968000
88	88	824000	761000	811000	824000	888000	907000	0	1020000
113	112	220000	314000	242000	181000	277000	0	0	471000
141	140	282000	301000	218000	150000	482000	0	0	520000
169	168	318000	287000	238000	177000	112000	0	0	678000
169	168.5	752000	800000	437000	504000	378000	548000	0	1080000
170	169	777000	679000	497000	528000	517000	547000	0	1040000
171	170	731000	830000	474000	709000	531000	527000	0	1010000
173	172	842000	945000	711000	720000	653000	653000	0	942000
197	196	258000	0	0	218000	197000	0	0	209000
225	224	QNS ^c	0	0	QNS	QNS	0	0	1800000
281	280	243000	0	0	197000	320000	0	0	33100
309	308	225000	0	0	233000	311000	0	0	0
337	336	212000	0	0	278000	332000	0	0	0
337.5	336.5	509000	0	0	619000	681000	28000	0	0
338	337	675000	0	0	555000	779000	32000	0	0
339	338	691000	0	0	653000	902000	32000	0	0
341	340	630000	0	0	618000	738000	29500	0	0
344	343	503000	0	0	579000	650000	0	0	0
351	350	443000	0	0	455000	600000	0	0	0
358	357	370000	0	0	312000	472000	0	0	0
365	364	259000	0	0	249000	329000	0	0	0
372	371	537000	0	0	593000	684000	NS ^d	0	0
374	373	0	0	0	0	0	0	0	0
393	392	0	0	0	0	0	0	0	0
421	420	0	0	0	0	0	0	0	0
449	448	0	0	0	0	0	0	0	0
483	482	0	0	0	0	0	0	0	0

^aValues have been rounded to 3 significant figures

^bBQL values have been changed to zero

^cQuantity not sufficient

^dNo Sample

Shaded values indicate antibody positive animals

Table 9-8. Individual Serum Concentrations^a (ng/mL) of AMG 162 in Female Cynomolgus Monkeys Following up to 14 Monthly SC Injections of 50 mg/kg with a 3 Month Washout Period

Study Day	Time From First Dose (days)	Animal Number							
		20669	20734	20741	20762	20884	20878	20870	20695
1	0	0 ^b	0	0	0	0	0	0	0
1	0.5	621000	672000	425000	514000	505000	540000	779000	645000
2	1	588000	707000	583000	582000	642000	641000	891000	572000
3	2	658000	737000	605000	748000	811000	846000	951000	755000
5	4	789000	775000	735000	788000	821000	741000	972000	782000
8	7	781000	719000	705000	741000	746000	879000	734000	834000
15	14	487000	481000	417000	543000	514000	586000	460000	417000
22	21	338000	421000	184000	307000	374000	399000	389000	153000
29	28	254000	291000	46100	214000	283000	310000	274000	85700
57	56	186000	331000	114000	212000	245000	279000	231000	0
85	84	NS ^d	NS	NS	NS	NS	NS	NS	NS
85	84.5	NS	NS	NS	NS	NS	NS	NS	NS
86	85	NS	NS	NS	NS	NS	NS	NS	NS
87	86	823000	962000	602000	723000	789000	838000	942000	494000
89	88	938000	986000	658000	1020000	940000	856000	943000	170000
113	112	273000	287000	210000	289000	255000	317000	213000	0
141	140	307000	300000	239000	192000	279000	359000	313000	0
169	168	311000	297000	252000	214000	290000	314000	250000	0
169	168.5	606000	732000	658000	522000	805000	864000	885000	157000
170	169	770000	846000	718000	580000	1000000	866000	928000	125000
171	170	812000	923000	761000	706000	936000	1020000	905000	188000
173	172	827000	882000	749300	657000	969000	962000	796000	130000
197	196	0	0	0	212000	229000	264000	124000	0
225	224	QNS	0	0	QNS	2140000	503000	139000	0
281	280	254000	0	0	216000	254000	268000	44900	0
309	308	191000	0	0	191000	226000	309000	65800	0
337	336	231000	183000	328000	231000	183000	328000	39900	0
337.5	336.5	455000	624000	703000	455000	624000	703000	390000	0
338	337	515000	682000	784000	515000	682000	784000	376000	0
339	338	557000	686000	798000	557000	686000	798000	417000	108000
341	340	580000	676000	588000	580000	676000	588000	345000	0
344	343	414000	503000	572000	414000	503000	572000	228000	0
351	350	298000	368000	447000	298000	368000	447000	88600	0
358	357	263000	261000	357000	263000	261000	357000	45700	0
365	364	203000	144000	257000	203000	144000	257000	19500	0
372	371	470000	538000	609000	470000	538000	609000	189000	0
374	373	0	0	0	0	0	0	NS	NS
393	392	0	0	0	0	0	0	21800	0
421	420	0	0	0	0	0	0	120	0
449	448	0	0	0	0	0	0	0	0
483	482	0	0	0	0	0	0	0	0

^aValues have been rounded to 3 significant figures

^bBQL values have been changed to zero

^cNo Sample

Shaded values indicate antibody positive animals

^aValues have been rounded to 3 significant figures

^bBQL values have been changed to zero

^cQuantity not sufficient

^dNo Sample

Shaded values indicate antibody positive animals

Other:

Denosumab Effects on Bone Mineral: As expected, treatment of monkeys with denosumab anti-RANKL antibody has a positive effect on bone mineral density (BMD) and bone mineral content (BMC), as measured by peripheral quantitative computed tomography (pQCT). Only the 10 and 50 mg/kg dose caused statistically significant increases in these parameters. The absence of an effect of a dose of 1 mg/kg is consistent with the observation that antibody development in 100% of the LD animals lowered the serum levels of denosumab to below the lower limit of quantitation after about 30 days on treatment.

Text Table 5: Group Mean Change from Pre-dose to Week 52 [%]

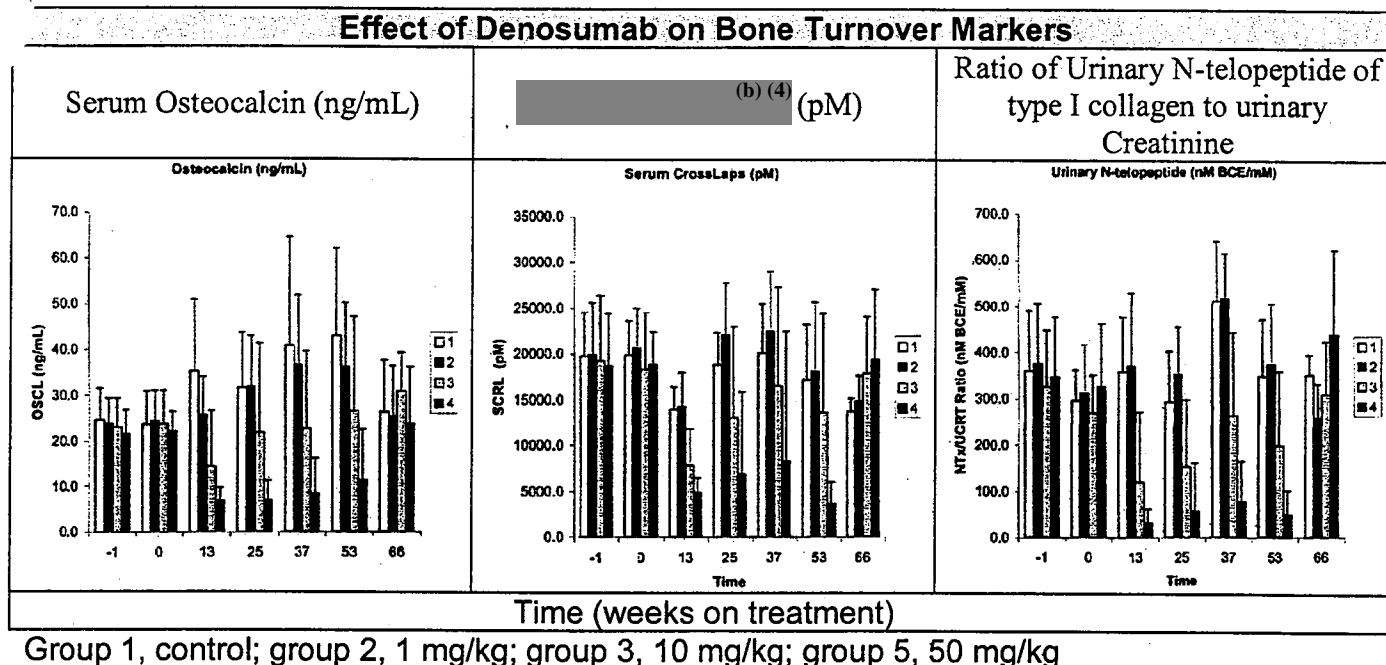
Bone Mineral Density	Males (mg/kg/dose)				Females (mg/kg/dose)			
Parameter	0	1	10	50	0	1	10	50
Radius (cortical)	-0.4	+1.3	+3.5	+3.7	+2.1	+0.9	+3.1	+3.5
Radius (trabecular)	-4.9	+1.8	-2.0	+15.2	-15.3	-17.3	+2.5	+16.5
Radius (total)	-7.6	+3.7	-0.5	+11.8	-3.7	-2.3	+18.9	+21.8
Tibia (cortical)	+1.0	+1.8	+4.1	+6.2	+0.8	+1.8	+3.7	+3.0
Tibia (trabecular)	-13.8	-15.8	-12.1	+2.4	-12.2	-19.5	-3.2	-0.8
Tibia (total)	-11.4	-6.4	-2.1	+30.1	-1.9	-4.2	+7.3	+14.6
Femur (cortical)*	-0.9	+0.1	-0.2	+1.3	+2.2	+1.3	+2.2	+3.2
Femur (trabecular)*	-7.2	-8.7	-8.6	+5.5	-4.4	-12.1	-6.6	+3.0
Femur (total)*	-3.5	+5.1	-10.5	+17.9	+1.3	-4.4	+3.4	+8.2

* group mean change from Week 12 to Week 52

Bone Mineral Content	Males (mg/kg/dose)				Females (mg/kg/dose)			
Parameter	0	1	10	50	0	1	10	50
Radius (total)	-1.0	+7.0	+7.1	+48.6	-4.6	-3.0	+17.5	+39.6
Tibia (total)	-4.1	-3.8	+2.0	+66.0	+2.6	-7.6	+21.0	+30.3
Femur (total)*	-2.2	-2.2	-5.7	+32.9	+1.6	+0.1	+2.0	+16.4

* group mean change from Week 12 to Week 52

Denosumab Effects on Biochemical Markers of Bone Turnover: Serum and overnight urine samples were collected at the times indicated in the figures, and the levels of serum osteocalcin, serum (b) (4), urinary N-telopeptide and urinary creatinine were measured by (b) (4). The results indicate that once monthly s.c. injections of denosumab to monkeys at doses of 10 and 50 mg/kg caused significant and dose-dependent reductions in serum and urinary markers of bone turnover in both male and female monkeys.



2.6.6.4 Genetic toxicology

No genetic toxicology studies were conducted by the Sponsor.

2.6.6.5 Carcinogenicity

No carcinogenicity studies were conducted by the Sponsor. Denosumab is not pharmacologically active in rodent species (mice or rat). While the Sponsor does have a surrogate model of huRANKL KI mice, this model would not serve as an appropriate model for carcinogenicity studies.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

2.6.6.6.1 Study title: Subcutaneous fertility evaluation of AMG 162 in the female cynomolgus monkey

Reviewed by: Kimberly Hatfield, Ph.D.

Key study findings:

- No effects on menstrual cycle length or mating performance.
- No differences in progesterone, estradiol or LH levels from controls.

- NOAEL for fertility and reproductive performance is > 12.5 mg/kg (the highest dose examined; 13 times higher than the recommended human dose of 60 mg based on body weight).

Study no.: 102843

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.3.5.1

Conducting laboratory and location: (b) (4)

Date of study initiation: July 10, 2003

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Denosumab (AMG 162) Lot# A0111300000

Methods

Doses: 0, 2.5, 5, 12.5 mg/kg/d, once weekly

Species/strain: Cynomolgus monkeys

Number/sex/group: 24 females (normally cycling); 6/dose group

Route, formulation, volume, and infusion rate: Subcutaneous (s.c.) (between scapulae in the back); 30 mg/mL concentration; 1.0 mL/kg/d dosing volume

Age and weight: Sexually mature, at least 3 years old; 2.8-5.3 kg

Satellite groups used for toxicokinetics: none

Study design: The following experimental design was utilized:

Group Number	Group Description	Color Code	Dose Level (mg/kg/day)	Number of Females	Application Volume (mL/kg/day)	Concentration (mg/mL)
1	Control	White	0	6	1.0	0
2	Low	Blue	2.5	6	1.0	2.5
3	Intermediate	Green	5	6	1.0	5
4	High	Red	12.5	6	1.0	12.5

Females were maintained on study or treated based on individual menstrual cycle with the following design: 1) two observation cycles without treatment; 2) 2 cycles (or the expected duration of 2 cycles (max 70 days) with treatment; 3) females were mated with fertile males and pregnancy confirmed by ultrasonography; 4) treatment continued until Day 20 post-mating.

Parameters and endpoints evaluated: Mortality and clinical signs (twice daily); body weight (weekly); food consumption (twice daily); menses (daily); menstrual bleeding; toxicokinetics; reproductive hormones (progesterone, 17 β -estradiol, LH); mating performance; menstrual cycle length; pregnancy confirmation.

Results

Mortality: There were no treatment-related deaths. One female (Group 2) was sacrificed moribund during mating due to severe bite wounds from the male.

Clinical signs: No significant treatment-related findings were observed.

Body weight: No significant treatment-related findings were observed.

Food consumption: No significant treatment-related findings were observed.

Necropsy: Necropsy was only conducted on the one animal sacrificed moribund. All other animals were released back to the colony.

Menstrual cycle length: Mean cycle length was comparable between treated and control groups. At week 3, control and Group 4 (high dose) animals had a longer mean cycle length (43 and 49 days, respectively, compared to 28 days the previous cycle), but this was mainly due to one or two outlier animals with very long cycle lengths (>50 days). The same was observed at week 4 in control animals only (53 days), due mainly to one outlier female.

Hormone analysis:

Progesterone: During observation cycle 2, mean progesterone levels were comparable for all groups, and all groups had increased levels starting ~Day 12 and after. During treatment cycles 1 and 2, mean progesterone levels were comparable across groups, and began to elevate ~Day 13-14, though Group 2 was slightly delayed in increased progesterone levels during treatment cycle 1 (Day 15), but not significantly.

17 β -estradiol: During observation cycle 2, mean estradiol levels were comparable for all groups, and all groups had a spike between Days 11-12. During treatment cycles 1 and 2, mean estradiol levels were comparable across groups, and spiked between Days 11-13.

Luteinizing hormone (LH): During observation cycle 2, mean LH levels were comparable for all groups, and all groups had increased levels between Days 12-15. The LH levels for Groups 1 and 3 however began to increase as early as Day 10, but this did not appear to be treatment related. During treatment cycles 1 and 2, mean LH levels were comparable across groups, and increased between Days 12-18 (cycle 1) and Days 12-16 (cycle 2), but were often highly variable, indicating outliers.

Mating performance: Denosumab treatment did not affect female fertility. After 2 cycles of mating, 2 of 6 females in each of Groups 1, 2 and 3 were positive for pregnancy, while 5 of 6 females in Group 4 were positive.

Toxicokinetics: C_{max} and AUC increased in a relatively dose proportional manner following the first administration and prior to second mating, however they increased much more than dose proportional prior to the first mating. Comparison of PK values between first and second matings show comparable C_{max} and AUC between doses. The differences in PK between first s.c. administration and prior to first mating are slight, but in the same relative range, considering variability. Mean T_{max} values are longer after first administration than later on prior to 1st and 2nd mating. During mating, the T_{max} values are within similar ranges for all doses. Accumulation of exposure was noted at the high dose only prior to first mating (~2-fold), and then only at the low and high dose prior to second mating (3-fold). The Sponsor notes that 4/6 low dose, 3/6 mid-dose and 2/6 high dose females showed decreased exposure after

multiple dosing, which is likely attributed to antibody formation. However, antibody analysis was not performed so this cannot be confirmed.

Table 7-4. Mean (±SD) TK Parameters^a Following the First SC Administration of AMG 162 to Female Cynomolgus Monkeys

Parameter	Units	2.5 mg/kg			5 mg/kg			12.5 mg/kg		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
T _{max} ^b	hr	72	30.4	6	72	72-120	6	72	72-168	6
C _{max}	µg/mL	48.8	11.9	6	79.0	16.4	6	186	24.4	6
AUC ₍₀₋₂₄₎	hr•mg/mL	6.77	1.50	6	11.7	2.53	6	26.9	2.70	6

^aValues are reported to 3 significant figures and have been calculated using nonrounded numbers

^bMedian and Range shown for T_{max}

Table 7-5. Mean (±SD) TK Parameters^a Following Administration of AMG 162 to Female Cynomolgus Monkeys Prior to the First Mating

Parameter	Units	2.5 mg/kg			5 mg/kg			12.5 mg/kg		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
T _{max} ^b	hr	24	8-72	4	24	8-24	4	24	8-72	5
C _{max}	µg/mL	28.5	38.7	4	115	81.7	4	476	279	5
AUC ₍₀₋₂₄₎	hr•mg/mL	4.22	5.94	4	16.4	11.4	4	67.8	39.6	5
Dose No.		11	N/A	6	9	N/A	6	10	N/A	6
AR		0.787	1.09	4	1.54	1.08	4	2.59	1.57	5

^aValues are reported to 3 significant figures and have been calculated using nonrounded numbers

^bMedian and Range shown for T_{max}

^cN/A=Not Applicable

Table 7-6. Mean (±SD) TK Parameters^a Following Administration of AMG 162 to Female Cynomolgus Monkeys Prior to the Second Mating

Parameter	Units	2.5 mg/kg			5 mg/kg			12.5 mg/kg		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
T _{max} ^b	hr	64	8-120	2	24	8-72	3	8	8-24	4
C _{max}	µg/mL	121	84.0	2	183	27.2	3	727	148	4
AUC ₍₀₋₂₄₎	hr•mg/mL	17.6	14.8	2	16.9	2.32	3	85.5	20.7	4
Dose No.		18	N/A	5	17	N/A	6	20	N/A	5
AR		3.27	2.64	2	1.61	0.300	3	3.31	1.25	4

^aValues are reported to 3 significant figures and have been calculated using nonrounded numbers

^bMedian and Range shown for T_{max}

^cN/A=Not Applicable

Embryofetal / Prenatal and postnatal development

2.6.6.6.2 Study title: Subcutaneous embryo/fetal development study of AMG 162 in the cynomolgus monkey

Reviewed by: Ronald Wange, Ph.D September 18, 2006.

Key study findings:

- No effect of treatment on the incidence of prenatal loss.
- No treatment-related effect on maternal clinical signs or body weight.
- No evidence of teratogenicity.
- Dose-dependent ↑ in fetal spleen weight (↑28% in HD vs. C). No histopathologic correlate was noted. Sponsor's analysis found no statistical significance; however, my analysis (t-test) shows a significant difference between the C and HD groups (p = 0.021).
- No significant effect of test item exposure on any other measured fetal parameter; however, some parameters showed non-significant trends:
 - 5% ↓ in fetal bodyweight in denosumab-exposed animals.

- Dose-dependent ↓ in fetal adrenal gland weight (↓20% in HD vs. C). This tissue was not microscopically examined.
- Dose-dependent ↓ in fetal heart weight (↓ 9% in HD vs. C). This tissue was not microscopically examined.
- 18% ↑ in fetal ovary weight in denosumab-exposed females. This tissue was not microscopically examined.
- Delayed ossification of sphenoid, hyoid and sella turcia. No dose-dependency.
- Increased incidence of shortened, isolated, rudimentary and/or vestigial cervical ribs. No dose-dependency.
- Anti-denosumab antibodies were detected in 80% of LD, 69% of MD and 50% of HD dams, and 53% of LD, 40% of MD and 6% of HD fetuses. Neutralizing antibodies were detected in 53% of LD, 38% of MD and 13% of HD dams, and 20% of LD, 27% of MD and 0% of HD fetuses.
- For reasons unexplained, the Sponsor did not examine fetal lymph nodes. This is problematic since signaling via RANK has been shown to be required for lymph node development in mice.

Study no.: 102842 (b) (4)

Volume #, and page #: EDR 0000 12/19/08 Section 4.2.3.5.2

Conducting laboratory and location: (b) (4)

Date of study initiation: 05-August-2003

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: Denosumab (AMG 162), lot no. A0111300000, 99.3% pure by SE-HPLC

Methods

Doses: 0, 2.5, 5 and 12.5 mg/kg/week

Species/strain: Cynomolgus monkey

Number/sex/group: 16/g

Route, formulation and volume: Subcutaneous injection (s.c.); Soln. for injection: 30 mg/mL denosumab, 5% Sorbitol, 10 mM sodium acetate, pH 5.1; 1.0 mL/kg

Satellite groups used for toxicokinetics: All animals used for TK

Study design: Control (vehicle) and test item were administered s.c. once weekly from day 20 to 50 of gestation (e.g. on days 21, 28, 35, 42 and 49 of gestation). Fetuses were delivered by cesarean and euthanized on day 100 ± 1 of gestation.

Parameters and endpoints evaluated: maternal bodyweight, maternal food consumption, anti-RANKL antibody titer, pregnancies, abortions, fetal weight, placental weight, distance from coccyx to cranium, distance from nose to occipital, distance from frontal to occipital, width of head, distance between the eyes, fetal organ weights, external examination of fetus, examination of fetal viscera, examination of fetal skeleton, limited histopathology panel (thymus, spleen & Peyer's patches).

ResultsMortality (dams): No deaths.Clinical signs (dams): No test item-related effect.Body weight (dams): No test item-related effect.Food consumption (dams): No test item-related effect.

Toxicokinetics: The single- and multiple-dose exposures to denosumab were determined in pregnant Cynomolgus monkeys over the dose range of 2.5 to 12.5 mg/kg (delivered s.c.). Exposure, based on mean C_{max} and AUC, increased dose-proportionally over the dose range of 2.5 to 12.5 mg/kg. Additionally, there was moderate accumulation (>2-fold) over 5 weekly doses. The average cumulative exposure up to 56 days was estimated at 34.2, 62.0 and 164 mg*hr/mL for the 2.5, 5, and 12.5 mg/kg dose groups respectively. Neutralizing antibodies developed in 34% of drug-treated animals and coincided with a profound decrease in serum concentrations and exposure.

Of note was that control animal 12403 had high denosumab serum levels from GD 38-54 that correlated with the range of concentrations observed following administration of 2.5 or 5 mg/kg.

Table 7-2. Mean (±SD) TK Parameters^a Following the First SC Administration of AMG 162 to Pregnant Cynomolgus Monkeys

Parameter	Units	2.5 mg/kg			5 mg/kg			12.5 mg/kg		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
T _{max} ^b	hr	120	72-120	16	120	72-168	16	120	72-168	16
C _{max}	µg/mL	25.9	8.12	16	56.6	20.1	16	122	39.8	16
AUC _(0-∞)	hr*mg/mL	3.59	1.19	16	7.46	2.95	16	16.7	5.52	16

^aValues are reported to 3 significant figures and have been calculated using nonrounded numbers.

^bMedian and Range shown for T_{max}.

Table 7-3. Mean (±SD) TK Parameters^a Following the Fifth SC Administration of AMG 162 to Pregnant Cynomolgus Monkeys

Parameter	Units	2.5 mg/kg			5 mg/kg			12.5 mg/kg		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
T _{max} ^b	hr	24	8-120	15	24	8-72	16	24	8-72	16
C _{max}	µg/mL	42.7	21.5	15	94.1	61.2	16	291	87.2	16
AUC _(0-∞)	hr*mg/mL	5.78	3.85	15	11.9	8.13	16	41.4	9.97	16
AR		1.71	1.03	15	1.84	1.30	16	2.64	0.770	15

^aValues are reported to 3 significant figures and have been calculated using nonrounded numbers.

^bMedian and Range shown for T_{max}.

Table 7-4. Mean (\pm SD) TK Parameters* Following the Fifth SC Administration of AMG 162 to Neutralizing Antibody Negative Pregnant Cynomolgus Monkeys

Parameter	Units	2.5 mg/kg			5 mg/kg			12.5 mg/kg		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
T_{max}	hr	24	8-120	7	16	8-72	10	24	8-72	14
C_{max}	μ g/mL	58.8	19.8	7	114	52.3	10	282	89.6	14
$AUC_{(0-1)}$	hr \cdot mg/mL	8.80	3.05	7	15.5	6.35	10	41.0	10.6	14
AR		2.46	0.428	7	2.59	0.926	10	2.79	0.696	13

*Values are reported to 3 significant figures and have been calculated using nonrounded numbers

*Median and Range shown for T_{max} **Table 7-5. Mean (\pm SD) TK Parameters* Following the Fifth SC Administration of AMG 162 to Neutralizing Antibody Positive Pregnant Cynomolgus Monkeys**

Parameter	Units	2.5 mg/kg			5 mg/kg			12.5 mg/kg		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
T_{max}	hr	48	8-120	8	24	8-72	6	24	24-24	2
C_{max}	μ g/mL	28.5	10.1	8	61.2	65.3	6	356	8.27	2
$AUC_{(0-1)}$	hr \cdot mg/mL	3.14	2.14	8	5.95	7.56	6	43.9	2.75	2
AR		1.04	0.948	8	0.579	0.681	6	1.65	0.486	2

*Values are reported to 3 significant figures and have been calculated using nonrounded numbers

*Median and Range shown for T_{max} Antibodies:**Table 3. Development of anti-AMG 162 antibodies over time in Dams**

Group	Predose		GD 49		GD 94, 99, 100 or 101	
	Immunoassay	Bioassay	Immunoassay	Bioassay	Immunoassay	Bioassay
1	0% (0/16)	0% (0/16)	7% (1/15)	0% (0/15)	8% (1/13)	0% (0/13)
2	6% (1/16)	0% (0/16)	33% (5/15)	7% (1/15)	80% (12/15)	53% (8/15)
3	0% (0/16)	0% (0/16)	19% (3/16)	6% (1/16)	69% (11/16)	38% (6/16)
4	0% (0/16)	0% (0/16)	0% (0/16)	0% (0/16)	50% (8/16)	13% (2/16)

* The denominator values are based on the number of animals that were tested at that time point in the immunoassay

Table 4. Percent Incidence of Antibodies

Group (Dose)	Anti-AMG 162 Antibodies (Dams)	Anti-AMG 162 Antibodies (Fetus)	Neutralizing Antibodies (Dams)	Neutralizing Antibodies (Fetus)
1 (0 mg/kg)	7% (1/15)	62% (8/13)	0% (0/15)	0% (0/13)
2 (2.5 mg/kg)	80% (12/15)	53% (8/15)	53% (8/15)	20% (3/15)
3 (5.0 mg/kg)	69% (11/16)	40% (6/15)	38% (6/16)	27% (4/15)
4 (12.5 mg/kg)	50% (8/16)	6% (1/13)	13% (2/16)	0% (0/13)
Total of all AMG 162 Dosed Animals	66% (31/47)	35% (15/43)	34% (16/47)	16% (7/43)

* The denominator values are based on the number of animals that were tested at at least 1 post dosing time point in the immunoassay

Dams with neutralizing anti-denosumab antibodies (* indicates that fetus of this dam was also positive for neutralizing antibodies):

C: none

LD: 12295*, 12406*, 12423, 12473*, 12490, 12516, 12532, 12579

MD: 11969*, 12028*, 12426, 12468, 12493*, 12569*

HD: 12425, 12428

Terminal and necroscopic evaluations:

Following C-section, the mothers were returned to the primate colony; therefore no maternal terminal data are available. There was no apparent effect of the test item on the outcome of pregnancy.

Table 1
Pregnancy Outcome Summary

During Gestation		(b) (4)			
Parameter	Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 4 12.5 mg/kg/day (once weekly)	
Number of pregnant females	16	16	16	18	
Number of females with false positive pregnancy test	0	0	0	1	
Number of females with abortion	3	1	0	0	
Number of females with abortion prior to start of treatment	0	0	0	1	
Number of females with early cesarean section	0	0	1	0	
Number of females with cesarean section	13	15	15	16	

Offspring (malformations, variations, etc.): There were no significant differences in any measured parameter.

Fetal Measurements: The group mean body weight was non-statistically significantly ↓5% in fetuses delivered of test item-treated dams; however, there was no dose-dependence.

Gross Pathology: No findings were noted; however, only the following tissues were macroscopically examined at necropsy (stomach, cecum, small and large intestine, testes, epididymides, vas deferens, ovaries, uterus, ureters, kidneys, adrenals, urinary bladder, liver, spleen, gallbladder, diaphragm, thymus, thyroid, heart, aortic arch, cardiac septum, cardiac auricles, lungs, esophagus, trachea, eyes, brain):

Histopathology: No findings were noted; however the tissue panel evaluated was limited to H&E stained sections of the thymus, spleen and Peyer's patches (isolated from the intestine). Notably, the lymph nodes were not examined either in the macroscopic or microscopic examination (see discussion below).

Organ weights: Dose-dependent non-statistically significant increase in spleen weight (↑28% in HD vs. C), although no histopathological correlate was noted. Dose-dependent non-statistically significant decrease in adrenal gland weight (↓ 20% in HD vs. C). Small dose-dependent non-statistically significant decrease in heart weight (↓9% in HD vs. C). Increase in ovary weight (↑13.5% in HD vs. C), not statistically significant or dose-dependent.

External findings: No test item-related effects.

Visceral findings: No test item-related effects.

Skeletal findings: The test item may have contributed to delayed ossification ("incomplete ossification" or "not ossified") at several bone sites, including the sphenoid, hyoid, and sella turcia. There were also increased findings of shortened, isolated, rudimentary and/or vestigial cervical ribs in fetuses from test item-treated females, as well as misaligned vertebrae. However, the effects were typically not dose dependent and were not generalizable to flat or long bone development overall. The Sponsor considers these findings to be incidental.

Table 4
Group Mean Fetal Body Measurements

		(b) (4)			
Parameter		Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 4 12.5 mg/kg/day (once weekly)
Weight of placenta (g)	Mean	57.9	59.6	64.6	56.1
	SD	7.6	10.8	11.1	6.7
	SE	2.1	2.8	2.9	1.7
	N	13	15	15	16
Weight of fetus (g)	Mean	115.5	109.3	109.6	110.0
	SD	12.8	13.2	16.8	7.0
	SE	3.6	3.4	4.3	1.8
	N	13	15	15	16
Distance from coccyx to cranium (cm)	Mean	13.1	12.8	12.8	13.0
	SD	0.7	0.7	0.7	0.6
	SE	0.2	0.2	0.2	0.2
	N	13	15	15	16
Distance from tip of nose to os occipitale (cm)	Mean	5.1	5.0	5.0	5.0
	SD	0.2	0.2	0.2	0.1
	SE	0.0	0.0	0.1	0.0
	N	13	15	15	16
Distance from os frontale to os occipitale (cm)	Mean	4.6	4.4	4.4	4.4
	SD	0.1	0.1	0.2	0.1
	SE	0.0	0.0	0.1	0.0
	N	13	15	15	16
Width of head (cm)	Mean	3.7	3.6	3.6	3.6
	SD	0.2	0.1	0.2	0.2
	SE	0.0	0.0	0.1	0.0
	N	13	15	15	16
Distance between the eyes (cm)	Mean	1.5	1.5	1.5	1.5
	SD	0.1	0.1	0.2	0.1
	SE	0.0	0.0	0.0	0.0
	N	13	15	15	16

Statistical analysis was performed using SAS release 6.12

Table 5
Group Mean Fetal Organ Weights

		(b) (4)			
Parameter		Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 4 12.5 mg/kg/day (once weekly)
Weight of fetus (g)	Mean	115.5	109.3	109.6	110.0
	SD	12.8	13.2	10.8	7.0
	SE	3.6	3.4	4.3	1.8
	N	13	15	15	16
Spleen (mg)	Mean	210.2	214.9	237.8	269.9
	SD	46.5	65.2	66.8	76.8
	SE	12.9	16.8	17.3	19.2
	N	13	15	15	16
Adrenal left (mg)	Mean	34.3	31.9	31.2	29.1
	SD	16.7	9.8	11.3	10.6
	SE	4.6	2.5	2.9	2.6
	N	13	15	15	16
Adrenal right (mg)	Mean	27.5	26.8	25.3	22.3
	SD	12.9	8.0	10.1	7.2
	SE	3.6	2.1	2.6	1.8
	N	13	15	15	16
Kidney left (mg)	Mean	390.5	362.9	381.6	374.8
	SD	81.3	42.2	106.5	64.4
	SE	22.6	10.9	27.5	16.1
	N	13	15	15	16
Kidney right (mg)	Mean	396.6	356.5	377.9	369.3
	SD	85.9	39.8	109.1	63.1
	SE	23.8	10.3	28.2	15.8
	N	13	15	15	16
Liver (g)	Mean	3.9	3.8	3.7	3.9
	SD	0.6	0.6	0.7	0.4
	SE	0.2	0.2	0.2	0.1
	N	13	15	15	16

Statistical analysis was performed using SAS release 6.12

Table Group Mean Fetal Organ Weights

(cont.)

(b) (4)

Parameter		Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 5 12.5 mg/kg/day (once weekly)
Ovary left (mg)	Mean	8.7	8.9	12.0	9.4
	SD	3.7	4.6	3.2	3.8
	SE	1.5	1.8	1.4	1.4
	N	6	7	5	8
Ovary right (mg)	Mean	8.6	9.5	10.9	10.6
	SD	3.8	4.8	3.8	5.1
	SE	1.6	1.8	1.7	1.8
	N	6	7	5	8
Uterus (mg)	Mean	48.3	45.7	43.3	52.8
	SD	13.2	12.5	8.4	6.9
	SE	5.4	4.7	2.9	2.4
	N	6	7	5	8
Testis left (mg)	Mean	14.4	13.6	12.9	13.8
	SD	3.2	2.4	2.1	2.4
	SE	1.2	0.8	0.7	0.9
	N	7	8	10	8
Testis right (mg)	Mean	14.6	14.2	12.9	13.9
	SD	3.1	1.9	1.6	2.7
	SE	1.2	0.7	0.5	1.0
	N	7	8	10	8
Thymus (mg)	Mean	242.9	222.4	244.5	223.3
	SD	45.1	67.4	80.0	51.1
	SE	12.5	17.4	20.7	12.8
	N	13	15	15	16
Heart (mg)	Mean	661.1	621.9	607.3	604.3
	SD	99.0	82.3	108.5	56.6
	SE	27.5	21.2	27.5	14.1
	N	13	15	15	16
Lungs (g)	Mean	2.2	2.3	2.1	2.1
	SD	0.4	0.4	0.6	0.3
	SE	0.1	0.1	0.1	0.1
	N	13	15	15	16

Statistical analysis was performed using SAS release 6.12

Table Group Mean Fetal Organ Weights

(cont.)

(b) (4)

Parameter		Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 5 12.5 mg/kg/day (once weekly)
Eye left (mg)	Mean	473.7	458.0	464.2	467.4
	SD	39.1	42.5	40.1	29.3
	SE	10.9	11.0	10.4	7.3
	N	13	15	15	16
Eye right (mg)	Mean	480.0	456.6	462.3	463.1
	SD	35.9	37.1	43.5	29.3
	SE	10.0	9.6	11.2	7.3
	N	13	15	15	16
Brain (g)	Mean	14.8	14.7	14.7	14.8
	SD	1.7	1.4	1.4	1.3
	SE	0.5	0.4	0.4	0.3
	N	13	15	15	16

Statistical analysis was performed using SAS release 6.12

Table 6
Group Fetal Findings

Parameter	(b) (4)			
	Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 4 12.5 mg/kg/day (once weekly)
Number of fetuses examined	13	15	15	16
<u>External Findings</u>				
Number of fetuses with findings	10	8	12	10
Prepuce not patent	7	5	9	8
Prepuce reddened	0	1	0	0
Small tissue ball at tail end	2	2	3	2
(Slightly) bent tail end	1	0	0	2
Hematoma at right back of the head	1	0	0	0
Genital region slightly enlarged	1	0	0	0
Anterior vulva region slightly reddened	1	0	0	0
Slight hematomae between eyebrows	1	0	0	0
Hematoma at left ear	1	0	0	0
Small incisure at tail end	0	0	1	0
Tip of tail reddened	0	0	1	0
Hematomae on whole body	0	0	1	0
<u>Visceral Findings</u>				
Number of fetuses with findings	12	14	12	13
Right adrenal of soft consistency	1	0	0	0
Light- to dark red foci at mucosa of stomach	0	0	0	1
(Brown/light-/dark-) red foci at cardia	12	12	11	12
Red foci in stomach or fundus of stomach	2	6	1	3
Red foci at thymus	1	2	0	0
Black foci at left lobe of the liver	0	0	1	0
Thymus (partly dark) red patterned	1	0	0	1
Thymus spotted	0	2	0	0

Table

Group Fetal Findings

(cont.)

(b) (4)

Parameter	Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 4 12.5 mg/kg/day (once weekly)
<u>Skeletal findings</u>				
Number of fetuses with findings	13	15	15	16
<u>Skull</u>				
Greater wing of os sphenoidale (unilateral / bilateral) incompletely ossified	10	11	15	15
Lesser wing of os sphenoidale (unilateral / bilateral) incompletely ossified	8	13	14	13
Greater horn of os hyoideum (unilateral / bilateral) incompletely ossified	3	5	2	5
Greater horn of os hyoideum (unilateral / bilateral) not ossified	3	2	4	1
Greater horn of os hyoideum unilateral additional ossification site	0	0	0	1
Lesser horn of os hyoideum (unilateral / bilateral) incompletely ossified	4	2	2	3
Lesser horn of os hyoideum (unilateral / bilateral) not ossified	2	3	1	4
Os hyoideum incompletely ossified	0	1	0	2
Os hyoideum not ossified	3	4	5	4
Os frontale (unilateral / bilateral) incompletely ossified	11	14	10	11
Os occipitale incompletely ossified	8	8	9	8
Os temporale (unilateral / bilateral) incompletely ossified	9	8	4	4
Clivus incompletely ossified	2	6	6	4
Sella turcica incompletely ossified	9	11	14	10
Petrosal bone (unilateral / bilateral) incompletely ossified	7	11	14	11
Lateral part of os occipitale (unilateral / bilateral) incompletely ossified	9	5	12	10
Os parietale bilateral incompletely ossified	2	0	1	0

Table

Group Fetal Findings

(cont.)

(b) (4)

Parameter	Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 4 12.5 mg/kg/day (once weekly)
<u>Skeletal findings</u>				
Number of fetuses with findings	13	15	15	18
<u>Vertebrae</u>				
Additional ossification site prior to 1st vertebral centrum	1	2	2	1
Vertebral centrum/centra incompletely ossified and/or bipartite	1	3	3	2
Vertebral centrum/centra not ossified	0	0	1	0
Additional ossification site on 6th vertebral arches	0	1	0	0
Vertebral centra incompletely ossified	1	0	0	0
Lumbar vertebra and sacral vertebra misaligned	1	0	0	0
Vertebra misaligned	0	0	3	2
Last but one coccygeal vertebra incompletely and misaligned	0	0	1	0
Zygostyle incompletely ossified and/or misaligned	5	3	2	3
Zygostyle asymmetrically ossified	1	1	0	0
Ossification site in the tissue ball	0	0	1	0
<u>Ribs</u>				
Cervical rib(s) shortened	0	2	2	2
Cervical rib isolated and/or rudimentary	0	0	1	2
Cervical rib(s) vestigial	0	0	0	1
Bilateral cervical rib	0	0	0	1
Rib(s) unilateral/bilateral shortened	0	1	2	0
Rib(s) unilateral rudimentary	1	0	0	0
Only 11 ribs on the left side	1	0	0	0
Extra pair of ribs shortened and/or isolated	0	2	1	2
Extra rib	0	0	0	1
Extra pair of ribs	0	0	2	0

Table Group Fetal Findings

(cont.)

(b) (4)

Parameter	Group 1 0 mg/kg/day (once weekly)	Group 2 2.5 mg/kg/day (once weekly)	Group 3 5 mg/kg/day (once weekly)	Group 4 12.5 mg/kg/day (once weekly)
<u>Skeletal findings</u>				
Number of fetuses with findings	13	15	15	18
<u>Sternebrae</u>				
Sternebra(e) incompletely ossified	13	15	15	18
Sternebra(e) not ossified	13	15	14	14
Sternebra(e) asymmetrically ossified	0	1	0	0
Sternebra bipartite	0	0	1	0
<u>Pelvic girdle</u>				
Bilateral insertion of pelvic girdle on last lumbar vertebra	0	1	3	2
Unilateral / Bilateral insertion of pelvic girdle on last lumbar vertebra and 1st sacral vertebra	1	1	0	0
Pubis bilateral incompletely ossified	5	5	6	7
Penial bone incompletely or not ossified	0	1	3	1
<u>Extremities</u>				
Talus bilateral incompletely ossified	3	8	3	5
Talus bilateral not ossified	1	0	2	0
Calcaneus bilateral incompletely ossified	0	1	2	1

2.6.6.7 Local tolerance

No specific local tolerance toxicology studies were conducted by the Sponsor.

2.6.6.8 Special toxicology studies

No special toxicology studies were conducted by the Sponsor.

2.6.6.9 Discussion and Conclusions**General toxicology:**

Two repeat dose toxicology studies in cynomolgus monkeys were conducted to support the general toxicity of denosumab – a 1-month and a 6/12-month study. Toxicity was evaluated as well as effects on bone parameters and potential immunogenicity.

In the 1-month study, male and female monkeys were dosed once per week with 0, 0.1, 1, 10 mg/kg (s.c.) or 10 mg/kg (i.v.) (control, LD, MD, HD). These doses are 0.1-10 times the recommended human dose of 60 mg based on body weight (mg/kg). Major nonclinical findings included moderate urinary occult blood (one HD i.v. male at week 4) and severe urinary occult blood (one LD s.c. female and one HD i.v. female at week 4), and significantly decreased calcium levels in males (LD s.c. and HD s.c. and i.v.) that were not observed in females. After dose recovery, the differences in calcium levels were no longer present. Bone parameters were also assessed in this study, and denosumab was shown to be pharmacologically active in males and females based on observed dose-dependent reductions in serum osteocalcin and N-telopeptide and statistically significant increases in total and cortical BMD in males only at doses ≥ 1 mg/kg. Denosumab did not induce changes in BMD in the female monkeys in any dose group following 4 weeks of dosing. Based on antibody analysis, 28/30 animals tested positive for anti-denosumab antibodies which confounded the true exposure levels to denosumab in this study. Animals testing positive for anti-denosumab antibodies had an approximately 30% reduction in exposure as compared to antibody negative monkeys. In addition, there was an approximate 2-fold increase in accumulation observed between the first and last dose of s.c. administered denosumab.

To evaluate the chronic toxicity of denosumab, the Sponsor submitted a 6/12-month toxicity study in male and female monkeys, dosed once per month s.c. with 0, 1, 10 or 50 mg/kg denosumab (control, LD, MD, or HD). These doses are 1-50 times the recommended human dose of 60 mg based on body weight. Overall, there did not appear to be any overt toxicological findings as a result of treatment, as the primary effects were related to the pharmacodynamics of denosumab. Notably, there was a high amount of immunogenicity in this study, with 100% of LD, 50% of MD and 13% of HD monkeys rapidly developing anti-denosumab antibodies. By day 29, denosumab could not be detected in the serum of LD animals, likely due to accelerated clearance by anti-denosumab antibodies. In MD and HD animals however, circulating denosumab levels were detected despite the formation of antibodies. Neutralizing antibodies were detected in 81%, 43% and 13% of LD, MD and HD animals, respectively. Of the monkeys that developed anti-denosumab antibodies, 81% of the antibodies produced by LD monkeys were neutralizing, as were 86% of MD and 100% of HD antibodies produced in these groups. As a result, antibody and neutralizing antibody formation did affect the exposure of animals to agent, and correspondingly, relatively no effects of treatment were observed in LD animals due predominantly to lack of exposure.

There was a high amount of infection noted by day 79 in both control and treated animals of this study (47 of 64 animals), with an undetermined reason for the high incidence based on facility historical controls. The majority of animals had diarrhea, and fecal samples were found to be contaminated with cryptosporidium and/or giardia lamblia. Potential clinical outcomes of increased infection and the possibility of immune suppression of this drug (as addressed below) could not be evaluated from a nonclinical standpoint because of the high background of infection.

Two HD male deaths were observed while on study – one found dead at week 11 and one sacrificed moribund at week 42. These animals were treated at doses 50 times the recommended human dose of 60 mg based on body weight. The cause of death could not be determined, but it

was likely the result of infection. Both monkeys had been sick in the weeks leading up to their death, with frequent bouts of diarrhea, and both also showed histopathological signs of protozoan infection. Of note, the HD male found deceased at week 11 also had some cardiac findings (minimal multifocal acute myocarditis and slight focal acute pericarditis) that could relate to other cardiac findings in study animals discussed below. Both males also showed increases in immature neutrophils, without comparably increased mature neutrophils prior to death. This could indicate rapid neutrophil turnover, and the potential presence of an active immune/inflammatory response.

As mentioned, there were no overt toxicological findings that were treatment-related in this study. Clinical chemistry findings (\downarrow alkaline phosphatase, \downarrow inorganic phosphorus, \downarrow calcium) and histopathology findings (\downarrow chondroclasis, \downarrow osteoblasts, \downarrow osteoclasts, \uparrow no. of trabeculae), are a consequence of the pharmacodynamic effect of denosumab. In animals where the epiphyseal plates had not fully closed prior to treatment, there were potentially deleterious changes related to denosumab treatment at doses ≥ 10 mg/kg. Growth plates were markedly enlarged with reduced chondroclasis and expanded growth plate zones 4 and 5, which are associated with cartilage calcification (zone 4) and cartilage erosion and calcification (zone 5). This growth plate finding suggests that denosumab should only be used patients in which the epiphyses are closed (adult populations only) until such time as these changes are demonstrated to be benign.

One treatment-related clinical sign of note (that could be related to outcomes observed in the clinical trials with denosumab) is an increased incidence of abscesses of the teeth and jaw. This was observed in 2 of 8 females in both the MD and HD groups (animal #358 and #457; doses 10 and 50 times the recommended human dose of 60 mg based on body weight), beginning at day 260 (37 weeks) in the HD animal and at day 401 in the MD animal (57 weeks, recovery), as compared to 0/8 females in the control and LD groups. This finding is suggestive of impaired immune response at doses ≥ 10 mg/kg.

Pharmacodynamic effects of denosumab were observed in this study as BMC and BMD were dose-dependently elevated in the sampled bone sites in MD and HD animals. Also, serum osteocalcin, serum C-telopeptide, and urine N-telopeptide were all reduced (markers of bone turnover). There was no difference in these parameters between the LD and control groups, likely due to reduced exposure to denosumab due to anti-denosumab antibody formation.

There is a concern that denosumab has the potential to suppress immune system function based on findings from this 6/12-month monkey study (unexplained HD deaths (impairment of the ability to control the infection) and teeth/jaw abscesses), and from published literature indicating: 1) RANK-mediated activation of T cells by dendritic cells;⁹ 2) RANK-mediated activation of CD4⁺ T helper cells in response to viral infection the CD40^{-/-} mouse;¹⁰ and 3) disruption of RANK signaling during pancreatitis potentiates the development of diabetes through

⁹ Green EA and Flavell RA (1999) TRANCE-RANK, a new signal pathway involved in lymphocyte development and T cell activation. *J Exp Med*, 189:1017-1020.

¹⁰ Bachmann MF, Wong BR, Josien R, Steinman RM, Oxenius A and Choi Y (1999) TRANCE, a tumor necrosis factor family member critical for CD40 ligand-independent T helper cell activation. *J Exp Med*, 189:1025-1031.

CD4⁺/CD25⁺ regulatory T cell disruption in the pancreas.¹¹ To address the potential for immunosuppression, Amgen submitted supporting data and articles indicating that 1) there is not a requirement for RANKL/RANK signaling in mounting an effective adaptive phase immune response in the adult mouse; 2) disruption of RANK signaling with osteoprotegerin has no effect on multiple standard measures of immune function (cellular and humoral immunity) or T or B cell proliferation; 3) in the 6/12-month toxicology study, immunoglobulin levels and immunophenotyping of lymphocytes did not show evidence of immunosuppression; and 4) the 16-month bone-quality study did not show any indication of immunosuppressive effects of denosumab or similar findings to the 6/12-month study (no early deaths, infection, or abscesses in teeth/jaws, and no effect of denosumab on immunophenotyping or primary antibody response to a T cell-dependent antigen). Based on the review of this information under IND 9837, it was concluded that no further nonclinical studies were necessary to further investigate the immunosuppressive potential of denosumab. In addition, the Sponsor had an adequate risk management plan for their clinical program, and at the time of review, there was no clear indication of immunosuppression in clinical trials. It was also concluded that based on the available data, disruption of RANKL/RANK signaling in immunologically intact patients would be of negligible clinical significance, but that it was unclear whether disruption in signaling could be of clinical significance in immune-compromised patients as a result of concurrent therapy or age-related immunosenescence. Current clinical findings from submitted trials do indicate that the overall incidence of serious adverse events of infection is slightly higher in denosumab versus placebo-treated subjects. These events include pneumonia, endocarditis, serious skin infections, gastrointestinal infections, urinary tract infections, infective arthritis, and ear infections, along with serious bacterial infections and serious infections due to an unspecified pathogen. A review of adverse events and serious adverse events of infection in older subjects did not identify any unusual trends in regards to infection.

Reproductive toxicity:

The primary treatment populations of denosumab are in postmenopausal women with osteoporosis, or women with bone loss associated with hormone ablative therapy, and as such, reproductive toxicity may not be relevant. However, some women who are perimenopausal or who are of child bearing age being treated for cancer could potentially become pregnant while on treatment. Therefore, effects on reproduction were evaluated in a fertility study and an embryo/fetal study with denosumab.

Fertility and early embryonic development were examined in female cynomolgus monkeys through once weekly dosing with denosumab. The study design consisted of observation through 2 menstrual cycles without treatment, then two cycles with treatment, followed by mating with fertile males and confirmation of pregnancy, and treatment through gestation day (GD) 20. Overall, denosumab produced no overt toxicity to the fertility of the mothers or during the early embryonic period. Menstrual cycle length, mating performance and overall hormone levels (progesterone, estradiol, or luteinizing hormone) were not different from control animals. Fertility was also not compromised, with treated animals having similar or greater instances of positive pregnancy than controls.

¹¹ Green EA, Choi Y and Flavell RA (2002) Pancreatic lymph node-derived CD4⁺CD25⁺ T_{reg} cells: Highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity*, 16:183-191.

In the embryo/fetal study, pregnant cynomolgus monkeys were treated once weekly by s.c. injection with 0, 2.5, 5 and 12.5 mg/kg denosumab (control, LD, MD, HD) from GD 20-50 (period of organogenesis). These doses are 3-13 times the recommended human dose of 60 mg based on body weight. C-sections were performed on GD 100 to assess effects on fetal development, and mothers were returned to the colony. Denosumab had no effect in mothers on mortality, clinical signs, body weight or food consumption. Cmax and AUC were dose proportionally increased over all doses, though some accumulation (2-fold) was noted at all doses at the end of treatment. Antibody formation was noted in 66% of treated mothers and 35% of fetuses, while neutralizing antibodies were found in 34% of treated mothers (8/16 LD, 6/16 MD, 2/16 HD) and 16% of fetuses (3/15 LD, 4/15 MD, 0/15 HD). Antibody formation is similar in frequency (primarily at low dose versus high dose) as in previous chronic studies, but did not appear to interfere with fetal assessment at sacrifice. There was no effect of denosumab on pregnancy outcome as compared to controls, and overall no teratogenicity was observed. In the fetal assessment, there were no histopathology findings of note, however limited tissues were analyzed (H&E stained sections of thymus, spleen and Peyer's patches isolated from the intestine), and most notably, lymph nodes were not examined. Gross pathology did not present any findings in the fetuses, and there were no statistically significant changes in organ weights. No external or visceral findings were observed in fetuses, however skeletal findings in exposed fetuses showed delayed ossification at several bone sites, increased findings of shortened, isolated, rudimentary and/or vestigial cervical ribs, and misaligned vertebrae, though the effects were typically not dose dependent and were not generalized to flat or long bone development overall.

Overall, the embryo/fetal study was adequately designed, but it may not have optimally assessed fetal toxicity to denosumab. While dosing occurred during the period of primate organogenesis (GD 20-50), antibodies do not typically cross the placenta until later in development. Therefore, the study likely only assessed potential secondary effects of denosumab on the fetus resulting from maternal exposure. In addition, only limited organs were evaluated by histopathology, and fetal lymph nodes were not examined. This would have been beneficial since signaling via RANK has been shown to be required for lymph node development in mice. The published literature shows that either RANK or RANKL knockout mice or disruption of RANK signaling in mice is associated with developmental defects such as: 1) absence of mature osteoclasts; 2) severe osteopetrosis with total occlusion of bone marrow space; 3) defective tooth eruption; 4) absence of peripheral lymph nodes; and 5) defective B cell development with a 60% increase in splenic B220+ B cells.¹² RANKL (osteoprotegerin (OPG) ligand) knockout mice also have impaired lymph node formation¹³, and absence of lactation due to inhibition of mammary gland maturation at the stage of development of lobulo-alveolar structures during pregnancy.¹⁴ Earlier stages of mammary development in RANKL knockout mice appear to occur normally. Finally,

¹² Theill LE, Boyle WJ and Penninger JM (2002) RANKL and RANK: T Cells, Bone Loss, and Mammalian Evolution. *Annu. Rev. Immunol.* 20:795-823.

¹³ Kong Y-Y, Yoshida H, Sarosi I, Tan H-L, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ and Penninger JM (1999) OPG is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 397:315-323.

¹⁴ Martin TJ and Gillespie MT (2001) Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL): Another Link Between Breast and Bone. *Trends Endocrinol. Metab.* 12:2-4.

this study did not examine whether denosumab treatment caused the same effects as observed in treatment of neonatal rats with RANKL inhibitors OPG-Fc or RANK-Fc. These animals showed decreased axial skeleton and femur length, along with flared morphometry of the femur, and delayed molar eruption. Also, while this could not have been assessed in this study, the 6/12-month chronic monkey study did indicate potentially deleterious changes in the epiphyseal growth plates of monkeys that had not closed prior to treatment, indicating concern for potential skeletal effects continuing into childhood, or concern for treatment in pediatric populations. As the target population for this study is postmenopausal women, these reproductive outcomes are not a major concern for this product. However, the product labeling should include appropriate language and warnings for use in pregnant and lactating women. If women of childbearing potential are ever chosen as a population for denosumab treatment, an appropriately designed study addressing the developmental issues above should be conducted, as well as a study examining the effect of denosumab on mammary gland development and lactation.

Evaluation of human dose multiples:

NOAELs for the pivotal toxicology and pharmacology studies (102090, 103981 and 102842) were derived and are presented in the table below. Dose multiples were calculated based on body weight (mg/kg), and provide an appropriate safety margin for clinical use. The proposed clinical dose of denosumab is a single s.c. injection of 60 mg every 6 months, which for a 60 kg female, equates to a 1 mg/kg dose every 6 months.

Study	Dose (NOAEL in animals)	Dose Multiple (based on mg/kg)
12-mo monkey (102090)	50 mg/kg (monthly)	50
16-mo monkey (103981)	50 mg/kg (monthly)	50
Embryo/fetal in monkey (102842)	12.5 mg/kg (weekly)	13
PK in human (20010223)	1 mg/kg (Q6M)	

Correlation of nonclinical findings and key clinical issues

Four areas of interest were noted by the clinical team where they inquired about nonclinical support of safety. These included the potentials for ocular toxicity, cardiovascular toxicity, delayed fracture healing and immunosuppression. A nonclinical assessment of these areas and the data provided in this application to support safety are provided below.

Ocular Effects: In nonclinical study 104105 in monkeys, denosumab concentrated in the cornea. In clinical trial 20040138, a signal for cataract (9 cases in placebo vs. 34 cases in denosumab group) was also noted. Due to this finding, and the observations that bisphosphonates and antiresorptive agents are associated with rare adverse events of eye inflammation including uveitis, we looked more closely at the nonclinical study to determine if ocular toxicity could be confirmed in monkeys. In study 104105 in the monkey, ¹²⁵I-radiolabeled denosumab was found in the cornea of the eye for up to 672 hours postdose (1 mg/kg) at levels higher than that in

serum. At 1344 hrs, radioactivity could no longer be detected in the cornea. When compared to amounts in serum at similar timepoints, denosumab levels in the cornea were high, but these higher amounts of radiolabel in the cornea at 672 hours are likely not as much a function of 'drug accumulation in the cornea', but that blood levels at 672 hours were very low and close to the lower limit of quantitation, indicating that radiolabel remains in the cornea longer than in the blood. No remarkable ocular effects were noted in the 1-month or 6/12-month toxicity studies with denosumab treatment in monkeys. Tissue cross reactivity studies with human, monkey, rat and rabbit tissues also did not show binding of denosumab to ocular tissues.

The Sponsor was asked to address visual adverse events in the clinical studies and the nonclinical cornea findings. The Sponsor's response appears to be a valid argument that due to the avascular structure of the cornea, denosumab would likely be found in the tears, causing a subsequent interaction with corneal tissue. In addition, since the human lacrimal glands secrete very low levels of IgG, only relatively little denosumab would be available in tears to bind to the cornea. Furthermore, in mRNA expression studies, RANKL mRNA expression was not found to be present in retina, iris, ciliary bodies, eyecup, lens or cornea. Hence, the argument is that biodegradation of the radiolabeled denosumab protein occurred prior to reaching the eye, and what is observed as preferential distribution of denosumab to the cornea, is actually the presence of free radiolabel in the cornea due to low level secretion of iodine into tears. Since the presence of denosumab in the cornea does not correlate with any remarkable toxicological finding in longer term studies in monkeys with higher doses, this argument appears reasonable.

Cardiovascular Effects: Cardiovascular toxicity was reassessed in the nonclinical studies since the clinical team noted that there is an association between osteoprotegerin (OPG) levels, RANKL and coronary artery disease: OPG/RANKL contributes to osteolysis in bone, and also contributes to arterial calcification. Upon examining the 6/12-month toxicity study in monkeys for potential cardiac toxicity (mainly histopathology findings in the heart), minor findings were noted. Minimal to slight focal myocarditis/pericarditis was observed in one male treated at 50 mg/kg that died while on study (death proposed to be due in part to infection of gastrointestinal tract with secondary dehydration), slight myocardial degeneration/necrosis observed in one denosumab treated male (50 mg/kg) at the interim 6 month sacrifice, and in one denosumab treated male (50 mg/kg) treated for 12 months and sacrificed after a 3 month treatment-free period. Minimal to slight inflammatory cell foci were observed in male and female treated animals at all sacrifices (6 months, 12 months, and recovery), but was also observed in 3 female control animals. In examining the potential for arterial calcification in these animals, no calcification was noted by the investigator; however intimal proliferation in the aorta was noted in 1 of 3 males (10 mg/kg) and 1 of 3 females (1 mg/kg) at the 12 month sacrifice, with no findings in controls. Overall though, the number of animals examined for histopathology in this study was low (n=2-3 per dose and sacrifice; n=1-2 per dose at recovery), and while these findings could be indicative of potential clinical cardiac toxicity, the low number of animals examined at each sacrifice, and the presence of some findings in control animals makes it hard to draw any clear conclusions as to whether these cardiac histopathologies are actually treatment-related. Nonclinical findings in a safety pharmacology study in male monkeys also showed no remarkable differences in blood pressure or heart rate at any dose between the timepoints of predose (-60 minutes) to 168 hours postdose. Dr. Wange also noted in his review of the 6/12-

month monkey study that there were no apparent test item-related effects on blood pressure or heart rate in that study either.

Upon request, the Sponsor submitted historical control data for the monkeys that were used on study. The Sponsor-submitted data included histopathology data, along with two literature articles to support that the findings of note were common in control non-human primates. Chamanza et al. (2006) concluded from a retrospective study of spontaneously arising histopathological findings of the cardiovascular system in purpose-bred laboratory non-human primates (84 studies; 2050 cynomolgus monkeys), that common cardiac findings included focal myocardial inflammation (inflammatory cell infiltration or focal myocarditis) in association with variable degrees of myocardial degeneration or necrosis, edema or fibrin deposition, with other findings including mineralization, endocarditis, pericarditis and myocardial fibrosis.¹⁵ The incidence rate and types of cardiac findings reported in Study 102090 were similar to the reported spontaneous lesions, therefore the lesions could not be definitively classified as drug-related.

Fracture Healing: Fracture healing was also examined further due to clinical concerns based on the mechanism of action of denosumab, and to determine if there were differences in the effect of denosumab on fracture healing in clinical versus nonclinical subjects. Briefly, fracture healing involves the production of a callus of cartilage and woven bone that stabilizes the fracture. The fracture callus gradually mineralizes and is remodeled over time to form lamellar bone and complete the fracture repair. Bone remodeling (bone formation and resorption) is critical in fracture healing, and patients with bone loss are at increased risk and incidence of fracture. While denosumab is primarily an antiresorptive agent, bone resorption and formation are a coupled process, and therefore denosumab suppresses bone formation as well. In clinical studies, information on fracture healing complications was collected for all nonvertebral fractures. In the primary clinical study (20030216), there was a reduction in the number of nonvertebral fractures, and fracture healing complications were actually few and well-balanced between placebo and treated groups. In the nonclinical application, only one study (in human RANKL KI and WT mice) specifically examined fracture healing through treatment with control, denosumab or alendronate, with denosumab doses 10 times higher than the recommended human dose of 60 mg, based on body weight. At either 21 or 42 days post femur fracture, the fracture and contralateral femora were analyzed by microCT, torsion testing and histology. The study concluded that fracture healing was delayed in both denosumab and alendronate treated mice, however, mechanical strength was not affected, and strength and stiffness were actually increased compared to controls. Callus remodeling was also delayed compared to controls, and denosumab treated bones showed decreased woven bone formation.

Immunosuppression: Finally, the clinical team asked if nonclinical findings indicated any potential for immunosuppression and an increase in potential for cancer risk. Following his review of the 6/12-month monkey study, Dr. Wange indicated in his conclusions that denosumab showed possible signs of immune suppression at doses ≥ 10 mg/kg, based on the unscheduled deaths of 2 males (50 mg/kg) due to protozoal infection, and an increased incidence of abscesses of the teeth and jaws in females treated at 10 and 50 mg/kg. In addition, published literature had

¹⁵ Chamanza R, Parry NMA, Rogerson P, Nicol JR and Bradley AE (2006). Spontaneous lesions of the cardiovascular system in purpose-bred laboratory nonhuman primates. *Toxicol. Pathol.*, 34:357-363.

noted: 1) RANK-mediated activation of T cells by dendritic cells; 2) RANK-mediated activation of CD4⁺ T helper cells in response to viral infection in the CD40^{-/-} mouse; and 3) disruption of RANK signaling during pancreatitis potentiates the development of diabetes through CD4⁺/CD25⁺ regulatory T cell disruption in the pancreas. An external comment was submitted, which was subsequently addressed by the Sponsor, and reviewed again by Dr. Wange. Based on the assessment submitted by the Sponsor, he concluded that "...based on presently available data...disruption of RANKL/RANK signaling in immunologically intact patients is likely to be of negligible clinical significance. However, it is presently unclear whether disruption of RANKL/RANK signaling will be of clinical significance in patients that are immune compromised either as a result of concurrent therapy (e.g. corticosteroids, tacrolimus, cyclosporin) or age-related immunosenescence." He recommended that no further nonclinical studies were necessary to investigate the immunosuppressive potential of denosumab, and that clinical monitoring for signs of immunosuppression be continued.

Subsequent to Dr. Wange's assessment, the final report of the 16-month pharmacology (bone quality) study with denosumab was submitted. In the immunology assessment of this study, there were no remarkable findings related to test article in the assay assessing T-cell dependent antibody response using KLH. However, with blood immunophenotyping, the total lymphocyte count was slightly and statistically significantly decreased at pre-necropsy for the high dose. Absolute counts of CD3⁺/CD8⁺ cytotoxic T lymphocytes were also slightly and statistically significantly decreased at the high dose compared to ovariectomized controls at pre-necropsy. The Sponsor's justification for these changes is that "Similar variations were also found during the pretreatment period, thus these changes are considered to be within the normal range of the assay and are not considered biologically significant or related to the test article" and "Denosumab did not appear to have a detrimental effect on immunocompetence in treated monkeys."

Of note, the 6/12-month repeat dose toxicology study and 16-month pharmacology were conducted at two different contract research organization facilities: the 6/12-month study at (b) (4) and the 16-month study at (b) (4). (b) (4) It is highly possible that this difference could account for the discrepancy in number and types of infections observed in one study versus the other.

Overall, I do agree with Dr. Wange's assessment of the potential of denosumab to be immunosuppressive. While the 2 males treated at the high dose of 50 mg/kg in the 6/12-month toxicology study that had unscheduled deaths did have a protozoal infection of the gastrointestinal tract, a number of both control and treated animals (47/64 animals) also had infections, based on frequent diarrhea and presence of giardia lamblia and/or cryptosporidium in the feces. The incidence of diarrhea was also reportedly higher in this study than the typical incidence in this test facility, though the explanation was unknown. So the infections cannot necessarily be considered treatment-related, but it could be concluded that since the 2 deaths occurred in the high dose population, that these animals may have had an impaired ability to control the infection due to treatment with denosumab. Therefore, a healthy population may not experience these effects, but it is unclear whether effects would be seen in an immune-compromised population. The doses where immunosuppressive potential was noted (10 and 50 mg/kg) are 10-50 times the recommended human dose of 60 mg based on body weight.

As for cancer risk, no carcinogenicity studies were performed due to the lack of an appropriate animal model. In the two long term studies (6/12-month toxicity and 16-month pharmacology), there were no incidences of tumor formation indicated with the exception of squamous metaplasia (benign) in the uterus in one low dose (1 mg/kg) and one high dose (50 mg/kg) female in the 6/12-month toxicology study. Standard organ histopathology was not conducted in the 16-month pharmacology study. Due to the lack of an appropriate animal model, formal carcinogenicity studies were not recommended.

2.6.6.10 Tables and Figures

None to be added.

2.6.7 TOXICOLOGY TABULATED SUMMARY

The following table was submitted by the Sponsor (found in Module 2.6.7)

2.6.7 – Toxicology Tabulated Summary

Denosumab (AMG 162) Page 5

Table 3. Overview of Toxicokinetics Data
Test Article: AMG 162

Dose ^c (mg/kg)	Dose Frequency	Mean (SD) Steady-State ^a Exposure in Cynomolgus Monkeys			Exposure Margin ^b Based on:	
		C _{max} (µg/mL)	AUC _{0-24h} (µg·hr/mL)	Corresponding ^d AUC _{0-6 month} (µg·hr/mL)	C _{max}	AUC _{0-6 month}
0.1	weekly ^e	11.3 (31.2)	349 (869)	9074	1.6	0.8
1	weekly ^e	27.5 (14.0)	3410 (2080)	88660	4.0	8.2
2.5	weekly ^f	58.8 (19.8)	8800 (3050)	228800	8.5	21
5	weekly ^f	114 (52.3)	15500 (6350)	403000	16	37
10	weekly ^e	302 (151)	42000 (22700)	1092000	44	100
10 (IV)	weekly ^e	663 (133)	68600 (23000)	1783600	96	170
10	monthly ^g	115 (37.1)	48200 (21100)	289200	17	27
12.5	weekly ^f	282 (89.6)	41000 (10600)	1066000	41	99
50	monthly ^g	666 (156)	268000 (90300)	1608000	96	150

^a At end of dosing. ^b Relative to mean C_{max} and AUC_{0-6 month} values [6.94 µg/mL and 448 µg·day/mL (10752 µg·hr/mL)], respectively; 2nd 60-mg dose for post-menopausal women in study 20010223 (rounded to 2 significant figures). ^c SC dosing except where indicated and compared to clinical exposure of 60 mg Q6M SC. ^d Calculated by multiplying AUC_{0-24h} values for weekly and monthly dosing by 26 and 6, respectively. ^e Study 101447 (dosed for 4 weeks, combined male and female data). ^f Study 102842 (dosed for 5 weeks, gestation days 20 through 50, females only). ^g Study 102090 (dosed for 12 months, combined male and female data). SD = standard deviation; C_{max} = maximum observed serum denosumab concentration; AUC_{0-24h} = area under the concentration-time curve during the dosing interval; AUC_{0-6 month} = area under the concentration-time curve over a 6-month period; IV = intravenous; SC = subcutaneous; Q6M = once every 6 months.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Pharmacology/Toxicology recommends approval for denosumab (60 mg subcutaneous injection once every 6 months) for treatment of postmenopausal osteoporosis, and for prevention of postmenopausal osteoporosis if a positive clinical risk:benefit profile has been demonstrated.

Unresolved toxicology issues (if any): None

Recommendations: None

Suggested labeling: Submitted labeling is acceptable with the following modifications (additions are underlined, deletions are crossed through)

(b) (4)

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA OR SUPPLEMENT

NDA/BLA Number: 125-320 & 125-331 **Applicant:** Amgen

Stamp Date: 12-19-2008

Drug Name: Prolia (denosumab)

NDA/BLA Type: NME

On initial overview of the BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Y		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Y		
3	Is the pharmacology/toxicology section of the NDA legible so that substantive review can begin?	Y		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animal studies*, animal ADME studies, safety pharmacology, etc)?	Y		Tissue cross reactivity studies are provided with human and animal tissues; pivotal studies are in appropriate species (monkey); transgenic models used for pharmacodynamics; antibody formation addressed in studies 101447, 102090, 103948, 103981; CV and respiratory safety pharmacology with single dose study incorporated; PK and PD studies; repeat dose studies over appropriate duration; local tolerance incorporated into repeat dose studies. No carcinogenicity or genotoxicity studies are required since this is a biologic product.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).		N	Clinical and nonclinical studies have used the same formulation of drug product (b) sorbitol, (b) (4) sodium acetate, pH 5.2), but the proposed labeling gives a slightly different formulation content (4.7% sorbitol, 17mM acetate, pH 5.2). The formulation found in the labeling is also different than that noted on page 12 of the Quality overview (which is the same as that noted in clinical and nonclinical studies).
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	Y		The subcutaneous route for clinical trials was used in all pivotal nonclinical studies.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	Y		Located on page 5 of the nonclinical overview.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	Y		See comments below for information requested via meetings and submission comments.

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA OR SUPPLEMENT**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?	Y		Labor and Delivery, and Carcinogenicity and Mutagenicity sections should be added with a statement that they have not been evaluated. The Sponsor uses the terminology "x-fold higher than human exposure to 60mg" – this is appropriate. -At this time, the accuracy of dose multiples has not been evaluated.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	Y		Toxicity studies have not been performed on any impurities. Impurities are listed as product- (HMWS and LMWS) and process- related impurities, and are addressed in the Quality section.
11	Has the applicant addressed any abuse potential issues in the submission?	Y		No indication that drug interacts with receptors associated with drug dependence or neurotropic activity (p 29 of nonclinical overview). Label indicates "no experience with overdosage" and "Doses up to 180mg Q 4wks with no additional AEs".
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? YES

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

At this time, we have no review issues.

Additional notes:

Nonclinical studies submitted to the BLA:

Pharmacology	
Study #	Title
R2004430	Effects of denosumab (AMG 162) on bone mass and bone resorption in human RANK ligand knock-in mice
R2004321	Effects of denosumab (AMG 162) on bone mass and bone resorption in aged human RANK ligand knock-in mice
106564	A 12-mo osteoporosis prevention study of denosumab with and without 6-month alendronate pretreatment in the cynomolgus monkey
R2006351	Denosumab, a fully human monoclonal antibody, has selective effects on human RANK ligand and human osteoclasts
R2006458	Comparison of two anti-resorptive therapies (alendronate vs AMG 162) on murine fracture healing
103981	AMG 162 - a monthly s.c. injection osteoporosis prevention study for 16-months in the cynomolgus monkey

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Safety Pharmacology	
Study #	Title
R20080340	The effects of OPG-Fc, RANK-Fc, or alendronate on tooth eruption, bone density, geometry, and strength in neonatal rats
101606	Final report - A single dose s.c. admin of AMG 162 for cardiovascular and respiratory evaluation in cynomolgus monkeys

Pharmacokinetics	
Study #	Title
101494	PK study of denosumab (AMG 162) in male mice following i.v. or s.c. administration
104192	Absorption, distribution and excretion in cynomolgus monkeys following a single s.c. administration of ¹²⁵ I-AMG 162
106893	A single dose PK study of denosumab (AMG 162) following i.v. administration to male or female FcRn knockout and wild-type mice
106892	PK report for a single dose PK study of denosumab (AMG 162) following i.v. administration to male or female huRANKL knock-in and wild-type mice
101398	A single dose i.v. and s.c. PK and PD study of AMG 162 in cynomolgus monkeys
101002	Pilot PK study of AMG 162 administered s.c. or i.v. in male and female Sprague-Dawley rats
104105	Quantitative whole body autoradiography of cynomolgus monkeys following a single s.c. administration of ¹²⁵ I-AMG 162
103948	PK and PD comparability study for two manufacturing processes of AMG 162 in female cynomolgus monkeys

Toxicology	
Study #	Title
101447	A 1-month study evaluating the effect on bone of AMG 162 administered s.c. or i.v. in cynomolgus monkeys with a 3-month recovery period
102090	A 6-12-month s.c. toxicity study of AMG 162 in the cynomolgus monkey with an interim kill after 6-months and a 3-month recovery period
102843	Subcutaneous fertility evaluation of AMG 162 in the female cynomolgus monkey
102842	Subcutaneous embryo-fetal development study of AMG 162 in the cynomolgus monkey
101758	Cross-reactivity of AMG 162 with normal cynomolgus monkey and human tissues
101348	Cross-reactivity of AMG 162 with normal human tissues
102700	Cross-reactivity of AMG 162 with cynomolgus monkey, rat and rabbit tissue ex vivo

Additional comments:

Notes on pharmacology study design:

Based on the guidelines for preclinical evaluation of agents used in the prevention or treatment of postmenopausal osteoporosis, examination of bone quality in two species is felt to be necessary, with one study conducted in the ovariectomized rat model and the second in a non-rodent model. While the two monkey studies (103982 and 106564) were conducted using ovariectomized female monkeys, the two transgenic knock-in rat studies (2004430 and 2004321) were not conducted using ovariectomized female rats. Study 2004321 did use aged rats however (10 months). The fact that they are knock-in transgenic models of osteoporosis may supersede the necessity of ovariectomy.

It appears that all appropriate investigations of bone turnover markers, BMD measurement, and bone architecture/histology were conducted in these studies.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA OR SUPPLEMENT

Notes concerning the formulation of drug product:

During development, denosumab has been manufactured by two versions of the intended commercial process, CP1 and CP2 (b) (4)

Material from CP1 was used in the pivotal toxicology studies to support clinical development. It appears from meeting minutes that the discussions on this issue were handled by the CMC review team. At an April 20, 2004 meeting, FDA requested additional information to determine if CP1 was comparable to CP2, and stated that if CP2 was used in clinical studies prior to a comparability agreement, a bridging study might be required. Then at a September 21, 2004 meeting (after Amgen had submitted comparability data), FDA stated that open questions remained regarding consistency of amidation and the potential for difference in immunogenicity between CP1 and CP2. In addition, although the modification may not have an effect on toxicity, it may impact exposure levels, and the Agency would consult with colleagues and continue to collaborate to further examine comparability. The Sponsor did conduct a PK-PD study in female cynomolgus monkeys with CP2 material to determine if modifications had an influence on biological activity. To date, this study has not been reviewed, and no further information on whether this study was acceptable or if CP1 and CP2 were considered to be comparable has been located.

- The Sponsor stated in the nonclinical overview that the collective data indicated that changes introduced in CP2 denosumab did not have a meaningful effect on PK or PD in the monkey, and that the drug substance and product planned for commercial use are comparable to the test materials used previously in the pivotal nonclinical studies. Additional information is located in Module 2.5, Section 2 and Module 2.7.1, which are clinical overviews and summaries).

General issues of note:

We noted in Item 5 above that there is a discrepancy in the drug formulation as stated in the clinical and nonclinical reports from what is stated in the current version of labeling. Based on the purification of protein products and changes in concentrations of excipients that can occur, the differences are likely to be minor and unremarkable, but have been noted for reference as the reviews proceed.

In addition, we have found that the Agency had remaining questions regarding the consistency of amidation and the potential for difference in immunogenicity between CP1 and CP2 at a September 2004 meeting where comparability issues were discussed. Because nonclinical studies utilized CP1 only, a PK/PD bridging study with CP2 suggests that the two products are comparable, and nonclinical immunogenicity will likely not correlate with clinical immunogenicity, these issues are not directly related to pharm/tox and are more related to clinical or CMC, but have been noted for reference as the reviews proceed.

Meeting minute and external comment issues:

Meeting 4/20/2004:

- FDA requested justification for the cynomolgus monkey as the appropriate model for "cortical bone remodeling species comparable to humans"
 - This was provided in a response to information request letter from Amgen dated 6/4/2004 (SDN# 75) and Sponsor serial number 0076.
- FDA recommended that Amgen collect blood samples to evaluate total and neutralizing antibodies over the course of the 16-month pharmacodynamic study in monkeys.
 - The final report shows that this advice was followed.
- FDA suggested that histopathologic evaluation of the bone biopsy sites be included in the 16-month study to provide safety data for potential fracture healing in the clinical population.
 - Amgen agreed to include histopathological evaluation of the two biopsy sites at terminal sacrifice.
 - FDA concurred that strength testing at these sites would not be required, only microscopic evaluation of the biopsy sites for evidence of proper healing and/or remodeling.

Amendment dated 10/5/2005:

- The Division of Therapeutic Biological Internal Medicine Products (DTBIMP) asked the Sponsor to address whether AMG 162 impairs fracture repair in the postmenopausal treatment trial
 - The Sponsor proposed, among other clinical trial additions, a nonclinical study of the effects of AMG 162 on tibial fracture healing using a knock-in mouse model.
 - DMEP agreed to the conceptual design and asked Amgen to submit the final protocol for this study to DMEP for formal review and comment.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA OR SUPPLEMENT

- A study was completed and submitted examining femoral fracture healing using a knock-in mouse model.

Study 102090 (external comment to Sponsor)

The previous nonclinical reviewer for IND 9837 in DMEP (Dr. Ron Wange) was concerned about the immunosuppressive potential of AMG 162 after reviewing the 12-month monkey toxicity study. He asked that the Sponsor submit for review their plans for conducting nonclinical trials to address the immunosuppressive potential of denosumab, or provide justification for why such studies are considered to be unnecessary. Amgen subsequently responded to the Agency's request (SDN #273), contending that, "...when taken in aggregate, the available published literature, nonclinical data and human clinical data show that disruption of RANK-L/RANK signaling, and specifically denosumab treatment, is not associated with immunosuppression." This submission was then reviewed by Dr. Wange in DMEP (1-8-2007), and he concluded that, "...based upon presently available data...disruption of RANKL/ RANK signaling in immunologically intact patients is likely to be of negligible clinical significance. However, it is presently unclear whether disruption of RANK-L/RANK signaling will be of clinical significance in patients that are immune compromised either as a result of concurrent therapy (e.g. corticosteroids, tacrolimus, cyclosporin) or age-related immune-senescence. His recommendation was that the Sponsor need not conduct additional nonclinical studies into the immunosuppressive potential of denosumab at this time, but that continued clinical monitoring for signs of immunosuppression is recommended. As a result, no further nonclinical studies specifically addressing the immunogenicity of AMG 162 were submitted, and this is not considered an outstanding item in regards to NDA fileability.

Meeting agreements based on nonclinical:

- 4/20/2004 – Due to species specificity of denosumab, nonclinical studies of long-term bone quality in rodents are unnecessary.
- 4/20/2004 – FDA concurred that the proposed nonclinical study of denosumab in oophorectomized, adult female cynomolgus monkeys (25 and 50mg/kg, monthly dose) will provide information about the effects of long term treatment with denosumab on cortical and callous bone mass. Also, the high dose (50mg/kg) should provide sufficient exposure to overcome any total binding or neutralizing antibody formation.
- 4/20/2004 – FDA asked for a comment as to why there would be no plans to follow treated monkeys for evidence of sustained effect or reversal of BMD following treatment in the 16-month monkey PD study. Amgen provided data demonstrating a linear correlation between time of treatment with AMG 162 and bone strength, and a loss in bone strength after discontinuing treatment, such that benefits of treatment are lost by the end of recovery. As a result, FDA concurred that no additional recovery studies would be required for the 16-month evaluation study in monkeys.