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Name of Sponsor: Amgen Inc., Thousand Oaks, CA

Name of Finished Product: Denosumab (AMG 162)

Name of Active Ingredient: Fully human monoclonal antibody to RANK ligand

Title of Study: A Multicenter, Randomized Placebo Controlled Pilot MicroCT Study to Estimate the Effect of Treatment with Denosumab (AMG 162) and Alendronate Sodium in Postmenopausal Women with Low Bone Mineral Density

Investigators and Study Centers: This was a multicenter study conducted at 9 centers: 3 in the United States, 2 each in Canada and France, and 1 each in Argentina and Australia.

Publications: Majumdar S, Boutroy S, Boyd SK, et al. Dissection of radial DXA BMD measurements using high resolution-peripheral quantitative computed tomography (HR-pQCT) Parameters. Presented at ISCD 2008 Annual Meeting, 12-15 March 2008, San Francisco, CA, USA. Abstract and Poster 104.

Delmas PD, Hanley DA, Sellmeyer D, et al. High resolution-peripheral quantitative computed tomography (HR-pQCT) facilitates accurate dissection of DXA radial BMD. Presented at ECCEO 8, 9–12 April 2008, Istanbul, Turkey. Abstract and Poster P10.

Study Period: 25 May 2006 (first subject enrolled) to 21 April 2008 (last subject's end-of-study date)

Development Phase: 2

Introduction and Objectives:
Denosumab is a fully human monoclonal antibody with high affinity and specificity for the RANK ligand (RANKL). Denosumab binds to, and neutralizes the activity of, human RANKL. In phase 2 and 3 studies in postmenopausal women with low bone mineral density (BMD), denosumab administration for up to 5 years increased mean BMD at the lumbar spine and other anatomic sites. Also, preclinical studies suggest possible actions of denosumab on other components of bone geometry that contribute to bone strength (eg, cortical thickness and bone microarchitecture), as well as effects on both trabecular and cortical compartments within a skeletal site. The characterization of the effect of denosumab on bone geometry variables and within each skeletal compartment will support a better understanding of the potential effect of denosumab on bone strength.

The primary objective of the study was to estimate the effect of denosumab, compared with placebo, on cortical thickness at the distal radius, as determined by in vivo high-resolution peripheral quantitative computed tomography (HR-pQCT) (XtremeCT®) at 12 months. Secondary objectives were reflective of the secondary efficacy endpoints (described below) and involved evaluation (at month 6, 12, or both) of the effect of denosumab, compared with placebo or alendronate, on other XtremeCT® assessments of the distal radius and distal tibia; on quantitative computer tomography (QCT) assessments of the radius, spine, and hip; dual energy x-ray absorptiometry (DXA) assessments of the forearm and hip; and assessments of bone turnover markers and biochemical parameters.

Methodology:
This multicenter, randomized, double-blind, double-dummy, placebo-controlled, pilot study enrolled postmenopausal women with lumbar spine or total hip BMD T-scores from -2.0 to -3.0 (inclusive). Subjects were randomized (1:1:1) to receive denosumab (60 mg subcutaneously [SC] every 6 months [Q6M] [at day 1 and month 6] plus placebo for alendronate orally once weekly [QW]); alendronate (70 mg orally QW plus placebo for denosumab SC Q6M); or placebo

(placebo for denosumab SC Q6M plus placebo for alendronate orally QW), for a study duration of 12 months. Randomization was stratified by age (≤ 60 years, > 60 years). All subjects received daily calcium (≥ 500 mg elemental calcium) and vitamin D (≥ 400 IU) supplements. Subject safety was monitored on an ongoing basis by an external data monitoring committee.

Number of Subjects Planned: 240 (80 in each treatment group)

Number of Subjects Enrolled:
A total of 247 subjects were enrolled in the study and were randomized (1:1:1) to receive denosumab (83 subjects), alendronate (82 subjects), or placebo (82 subjects). Age was ≤ 60 years for 116 subjects and > 60 years for 131 subjects.

Sex: 100% women

Mean (Standard Deviation [SD]) Age: 60.6 (5.4) years

Ethnicity (Race): 96% white or Caucasian, 2% Asian, < 1% Hispanic or Latino, < 1% Japanese, < 1% Native Hawaiian or other Pacific Islander

Diagnosis and Main Criteria for Eligibility:
Eligible subjects were postmenopausal women, between 50 and 70 years of age, with lumbar spine or total hip BMD corresponding to T scores from -2.0 to -3.0 (inclusive). Subjects were ambulatory, were not receiving medication that affected bone metabolism (other than calcium and vitamin D), were free from any underlying condition that affected their ability to take alendronate or receive denosumab, and had no history of fragility fractures after age 50 or severe or moderate vertebral deformities as evidenced by vertebral fracture assessment (VFA) of a DXA scan.

Investigational Product, Dose and Mode of Administration, Manufacturing Batch Numbers:
Denosumab was provided as a sterile, clear, colorless, preservative-free liquid in glass vials containing 60 mg denosumab per mL of 10 mM sodium acetate and 5% sorbitol in water for injection, with a pH of 5.2. One mL of blinded denosumab was administered SC every 6 months (on day 1 and month 6).

Subjects who received active denosumab (and also subjects in the placebo group) also received placebo for alendronate. Placebo for alendronate was provided as tablets in packages of 15 tablets per pack and contained the same ingredients, microcrystalline cellulose and magnesium stearate, as the commercially available 70-mg tablet of alendronate sodium. The placebo also contained pregelatanized starch, colloidal silicon dioxide, and crospovidone in place of anhydrous lactose and croscarmellose sodium. Also, the active ingredient (alendronate monosodium salt trihydrate) was omitted. Tablets and packages were identical in appearance to alendronate tablets and packages (see below). One tablet of blinded investigational product was taken orally QW.

Reference Therapy, Dose and Mode of Administration, Manufacturing Batch Numbers:
Alendronate (Fosamax®, Merck Sharp & Dohme) was provided as white, crystalline, nonhygroscopic, oval tablets in packages of 15 tablets per pack and contained 91.37 mg of alendronate monosodium salt trihydrate (molar equivalent to 70 mg of free acid) and the following inactive ingredients: microcrystalline cellulose, anhydrous lactose, croscarmellose sodium, and magnesium stearate. One tablet of blinded investigational product was taken orally QW.

Subjects who received active alendronate (and also subjects in the placebo group) also received placebo for denosumab. Placebo for denosumab was provided in vials identical to those provided for denosumab. The placebo formulation was identical to the denosumab formulation with the exception of the protein content. One mL of blinded placebo for denosumab was administered SC at day 1 and at month 6.

Duration of Treatment: 12 months (with administration of denosumab at day 1 and month 6)

Study Endpoints (as specified in the Statistical Analysis Plan)

Primary Efficacy Endpoint:

The primary endpoint was the percent change in cortical thickness (mm) at the distal radius as determined by XtremeCT® from baseline to 12 months.

Secondary Efficacy Endpoints:

Secondary endpoints included various parameters (eg, total BMD) at the distal radius and distal tibia assessed by XtremeCT® at months 6 and 12; parameters at the forearm (distal, ultradistal, and proximal radius) assessed by QCT at months 6 and 12; parameters at the hip (femoral neck, trochanter, and femur) and lumbar spine assessed by QCT at 12 months; parameters at the forearm (distal 1/3, ultradistal, and total radius) assessed by DXA at months 6 and 12; and parameters at the hip (femoral neck, trochanter, and total hip) and lumbar spine assessed by DXA at month 12. Additional secondary endpoints included biochemical markers (serum insulin-like growth factors [IGFs] and intact parathyroid hormone [iPTH]) and bone turnover markers (serum type 1 C-telopeptide [CTX1], procollagen type 1 N-telopeptide [P1NP], osteocalcin, bone-specific alkaline phosphatase [BALP], tartrate-resistant acid phosphatase 5b [TRAP 5b], and N-telopeptide [NTX]) assessed at week 1 and months 1, 3, and 6, month 6 + 1 week, and months 7, 9, and 12.

Safety Endpoints:

- adverse event incidence
- changes in safety laboratory analytes (urine, serum chemistry, and hematology)
- changes from baseline in vital signs and body weight
- subject incidence of anti-denosumab antibody formation

Statistical Methods:
Formal statistical hypothesis testing was not performed for this study; only estimation of treatment effects was performed, and no p-values for the differences between treatments were calculated.

Descriptive statistics were provided for selected baseline characteristics and demographic, efficacy, and safety data. Continuous variables were summarized descriptively using mean, median, standard deviation (SD), minimum, maximum, 25th percentile, 75th percentile, and the number of non-missing observations (denoted as "n"). Frequencies and percentages were presented for nominal categorical variables.

Bone volumetric and geometric parameters derived by XtremeCT®, QCT, and DXA at each assessment time point were summarized by actual treatment group. Primary analyses of these parameters included all randomized subjects who received ≥ 1 dose of investigational product and had a non-missing baseline and a non-missing postbaseline evaluation. Least-squares mean estimates of percent changes from baseline for each treatment group and 95% 2-sided confidence intervals (CI) for the treatment differences of (denosumab – placebo) and (denosumab – alendronate) using an analysis of covariance (ANCOVA) model with last observation carried forward imputation were reported.

The safety analysis set included all randomized subjects who received ≥ 1 dose of investigational product. Safety was characterized by tabulating adverse event incidence by system organ class and preferred term (coded using Medical Dictionary for Regulatory Authorities [MedDRA] version 10.0); changes in safety laboratory analytes (serum chemistry and hematology) at each visit and shifts between baseline and the worst on-study value; changes in vital signs; and subject incidence of anti-denosumab antibody appearance (negative/positive).

Summary of Results:

Subject Disposition:
A total of 247 subjects were enrolled and randomized in the study; 83 subjects were randomized to denosumab, 82 subjects were randomized to alendronate, and 82 subjects were randomized to placebo. All subjects randomized to denosumab received at least 1 dose of active investigational product (denosumab). One subject randomized to the alendronate group received her SC injection (placebo for denosumab), but did not take any oral investigational product (alendronate). Since efficacy and safety analyses were performed according to the actual treatment received, this subject was analyzed in the placebo group for these analyses. Eighty-nine percent of subjects in the denosumab group, 84% of subjects in the alendronate group, and 90% of subjects in the placebo group completed the study.

Efficacy Results:
Note that formal statistical hypothesis testing was not performed for this pilot study; only estimation of treatment effects was performed, and no p-values for the differences between treatments were calculated. Also, note that "mean percent change" in the text below refers to least-squares mean percent change, and "mean treatment difference" refers to least-squares mean difference of the percent change between treatments.

The primary analysis of the primary efficacy endpoint (ie, the percent change in cortical thickness at the distal radius as determined by XtremeCT® from baseline to 12 months) demonstrated that denosumab treatment increased cortical thickness at the distal radius relative to placebo (Table Error! **No text of specified style in document.-1**). The effect of denosumab on cortical thickness at the distal radius was observed at the first assessment time point at month 6. Denosumab also increased cortical thickness relative to placebo at the distal tibia as assessed by XtremeCT® at months 6 and 12. The results of analyses of QCT data corroborated the results of analyses of XtremeCT® data, also demonstrating that denosumab increased cortical thickness at the distal and proximal radius relative to placebo at months 6 and 12. The mean percent increases relative to placebo in cortical thickness at the distal radius and tibia (assessed by XtremeCT® and QCT) in the denosumab group were of larger magnitudes than those observed in the alendronate group at both month 6 and 12.

Table Error! No text of specified style in document.**-1. Percent Change from Baseline in Cortical Thickness at Month 12 (ANCOVA Model, Efficacy Data Set, LOCF)**

	% Change From Baseline [a]							
	XtremeCT®				QCT			
	Distal Radius		Distal Tibia		Distal Radius		Proximal Radius	
	n	LS Mean (95% CI)	n	LS Mean (95% CI)	n	LS Mean (95% CI)	n	LS Mean (95% CI)
Placebo	79	-0.8 (-1.8, 0.3)	79	1.4 (0.6, 2.3)	66	-0.7 (-1.4, 0.1)	66	-1.1 (-1.5, -0.8)
Alendronate 70 mg QW	73	2.4 (1.2, 3.5)	73	4.9 (4.0, 5.8)	71	0.5 (-0.2, 1.2)	71	0.2 (-0.1, 0.6)
Denosumab 60 mg Q6M	75	3.4 (2.2, 4.5)	78	5.8 (5.0, 6.7)	66	1.2 (0.5, 2.0)	66	0.5 (0.1, 0.9)

LS = Least squares, CI = confidence interval
[a] Based on an ANCOVA model adjusting for the baseline value of the variable, age group, and treatment
Source: Table 14-4.7.1, Table 14-4.8.1, Table 14-4.29.5, and Table 14-4.30.3

As shown in Table Error! **No text of specified style in document.-2**, XtremeCT®, QCT, and DXA assessments of BMD consistently demonstrated that denosumab also increased total BMD

at the radius at month 12. This effect of denosumab was observed as early as the first assessment time point at month 6. In addition, XtremeCT® and QCT assessments demonstrated that denosumab consistently increased cortical and trabecular BMD relative to placebo at all radial anatomic sites assessed at months 6 and 12, and XtremeCT® assessments demonstrated that denosumab also increased cortical and trabecular BMD relative to placebo at the distal tibia at these time points. The mean percent increases relative to placebo in total, cortical, and trabecular BMD parameters in the denosumab group were of larger or similar magnitudes to those in the alendronate group.

Table Error! No text of specified style in document.**-2. Percent Change from Baseline in Total BMD at the Radius at Month 12 (ANCOVA Model, Efficacy Data Set, LOCF)**

	% Change From Baseline [a]					
	XtremeCT® [b]		QCT [b]		DXA [b]	
	n	LS Mean (95% CI[a])	n	LS Mean (95% CI[a])	n	LS Mean (95% CI[a])
Placebo	79	-2.1 (-2.7, -1.4)	66	-0.8 (-1.6, 0.1)	79	-0.5 (-1.2, 0.2)
Alendronate 70 mg QW	73	0.0 (-0.6, 0.7)	71	1.3 (0.4, 2.1)	77	1.1 (0.4, 1.8)
Denosumab 60 mg Q6M	75	1.1 (0.5, 1.8)	66	2.8 (2.0, 3.7)	78	2.3 (1.6, 3.0)

LS = Least squares
[a] Based on an ANCOVA model adjusting for the baseline value of the variable, age group, and treatment
[b] XtremeCT assessments performed at the distal radius, DXA and QCT assessments performed at the ultradistal radius
Source: Table 14-4.7.3, Table 14-4.28.2 and Table 14-4.36.3

The effects of denosumab on cortical thickness and BMD at the radius were reflected in increases the polar moment of inertia, a parameter associated with bone strength, as assessed by QCT at all radial sites assessed at months 6 and 12. The mean percent increases in polar moment of inertia in the denosumab group were generally of larger magnitudes than those in the alendronate group.

QCT assessments also demonstrated that denosumab increased total, cortical, and trabecular BMD relative to placebo at the hip (femoral neck, trochanter, and femur) and trabecular BMD (the only BMD variable assessed) at the lumbar spine at month 12 (the only assessment time point for these skeletal sites). At these skeletal sites, the mean percent changes in total, cortical, and trabecular BMD relative to placebo in the denosumab group were generally either similar to or larger in magnitude than those in the alendronate group. The results of analyses of DXA BMD data at the hip and lumbar spine were consistent with those of QCT data.

Results of analyses of QCT assessments of BMC and cortical volume at all radial sites and hip regions assessed were generally consistent with those observed for QCT assessments of BMD. As expected on the basis of the mechanism of action of an anti-resorptive therapy like denosumab, which has its primary site of activity at the endocortical surface of the bone, no notable differences were observed between treatment groups in mean percent changes in total volume at the radial sites and hip regions, nor in circumference at the radial sites. The QCT methodology does not allow evaluation of effects of treatment on trabecular bone volume because the region of interest measured with this technique represents a fixed derived sampling volume and not the actual volume of trabecular bone. DXA assessments of BMC and (projected) area at the hip and spine were generally consistent with DXA assessments of BMD at these skeletal sites and, at the hip, were consistent with QCT assessments of BMC.

Results of analyses of XtremeCT® assessments of the interdependent variables, trabecular number, thickness, separation, and SD of 1/TbN, at the distal radius and distal tibia demonstrated no interpretable differences between treatment groups for these parameters at these anatomic sites.

Denosumab treatment resulted in median percent decreases in bone turnover marker concentrations (CTX1, P1NP, osteocalcin, BALP, TRAP 5b, and NTX) relative to placebo during the study, consistent with the known mechanism of action of denosumab to inhibit osteoclast formation, activation, and survival and to maintain coupling of bone resorption and formation. The median percent decreases relative to placebo in bone turnover concentrations in the denosumab group were generally of larger or similar magnitudes to those in the alendronate group. In addition, denosumab dosing resulted in rapid, transient median percent increases in iPTH concentration relative to placebo, which were of larger magnitudes than those in the alendronate group. No notable differences were observed between the 3 treatment groups in median percent changes from baseline in serum IGF-1, IGF-2, or IGFB3 concentrations during the study.

Safety Results:
Safety analyses were performed for all subjects who received ≥ 1 dose of investigational product (ie, the Safety Analysis Set [N = 247]). The overall incidence of adverse events was similar between treatment groups (91.6% denosumab, 95.1% alendronate, 94.0% placebo); most adverse events were mild to moderate in severity. The most common adverse events (reported for ≥ 10% of subjects in any treatment group) were (denosumab, alendronate, placebo) constipation (18.1%, 16.0%, 14.5%), influenza (16.9%, 12.3%, 18.1%), extremity pain (12.0%, 12.3%, 12.0%), nasopharyngitis (12.0%, 9.9%, 16.9%), arthralgia (12.0%, 9.9%, 9.6%), back pain (12.0%, 7.4%, 12.0%), bronchitis (10.8%, 13.6%, 13.3%), headache (7.2%, 14.8%, 10.8%), upper abdominal pain (6.0%, 12.3%, 9.6%), dyspepsia (6.0%, 11.1%, 8.4%), diarrhea (3.6%, 12.3%, 10.8%), and abdominal pain (2.4%, 11.1%, 3.6%). Treatment-related (investigator attributed) events were reported at a higher incidence in the alendronate group (44.4%) than in the denosumab (31.3%) or placebo (38.6%) groups. Few subjects in all treatment groups discontinued investigational product (6.0% denosumab, 3.7% alendronate, 2.4% placebo) or the study (1.2% denosumab, 3.7% alendronate, 1.2% placebo) because of an adverse event. Serious adverse events were reported for 2 subjects [2.4%] in the denosumab group, 5 subjects [6.2%] in the alendronate group, and 5 subjects [6.0%] in the placebo group. Note that 1 additional serious adverse event was reported in the ARISg safety database for a subject in the denosumab group, which is not included in the clinical trials database; the event was reported after the protocol-specified reporting period for serious adverse events had ended for this subject. No subject died during the study.

No events of hypocalcemia were reported as adverse events. A similar proportion of subjects in each treatment group had adverse events within the infections and infestations system organ class (54.2% denosumab, 55.6% alendronate, 55.4% placebo). One serious adverse event of infection was reported during the study (pneumonia in the placebo group). Adverse events within the neoplasms system organ class were reported in 1.2% of subjects in the denosumab group, 3.7% of subjects in the alendronate group, and 2.4% of subjects in the placebo group.

No trends in serum chemistry or hematology assessments were noted other than expected decreases in albumin-adjusted serum calcium, phosphorus, and total alkaline phosphatase. No subjects in any treatment group had decreases in serum calcium of ≥ grade 2 severity (defined as 1.75 to < 2 mmol/L as per CTCAE v3.0) at any time point.

No subject in the denosumab and placebo groups tested positive for anti-denosumab antibodies (the alendronate group was not tested).