2. SYNOPSIS

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

Name of Finished Product:
Denosumab (AMG 162)

Name of Active Ingredient:
Fully human monoclonal antibody to receptor activator for nuclear factor-κB ligand

Title of Study:
A Randomized Double-Blind Study to Evaluate Denosumab in the Prevention of Postmenopausal Osteoporosis (DEnosumab FortifiEs BoNe Density – The DEFEND Trial)

Investigators and Study Centers:
This was a multicenter study conducted at 21 centers in North America (16 in the United States and 5 in Canada). Centers and principal investigators are listed in Appendix 4.

Publications: None as of the date of this report.

Study Period:
27 August 2004 (first subject enrolled) to 20 February 2007 (last subject's end of on-treatment period)

Development Phase: 3

Introduction and Objectives:
Denosumab is a fully human monoclonal antibody with high affinity and specificity for the receptor activator of nuclear factor-κB ligand (RANKL). Denosumab binds to, and neutralizes the activity of, human RANKL. In a phase 2 study in postmenopausal women with low bone mineral density (BMD, assessed by dual energy x-ray absorptiometry [DXA]) (-4.0 ≤ T-score ≤ -1.8 for the spine or -3.5 ≤ T-score ≤ -1.8 for the total hip or femoral neck), denosumab administration for up to 3 years increased mean BMD at the lumbar spine, total hip, femoral neck, trochanter, distal 1/3 radius, and total body (without head).

The primary objective of this study was to determine whether denosumab treatment can prevent lumbar spine bone loss (as measured by percent change from baseline in the lumbar spine BMD [by DXA] at 24 months of treatment) in both early and late postmenopausal women with lumbar spine BMD T-score between –1.0 and –2.5.

The primary safety objective was to characterize the safety and tolerability profile of denosumab in this population based on adverse event incidences, changes in laboratory profiles, electrocardiograms (ECGs), and immunogenicity to denosumab.

The secondary objectives were to assess the effect of denosumab on (a) BMD measured by DXA at the hip (total hip, femoral neck, and trochanter), distal radius, and total body (without head), and (b) trabecular, cortical, and total volumetric BMD measured by quantitative computerized tomography (QCT) at the distal radius in both early and late postmenopausal women with lumbar spine BMD T-score between –1.0 and –2.5.

Exploratory objectives are outlined in Section 6.3.

Methodology:
This multicenter, randomized, double-blind, 2 period, parallel-group study enrolled postmenopausal women with lumbar spine BMD T score between 1.0 and 2.5. Subjects were randomized (1:1) to receive either denosumab or placebo; randomization was stratified by time since onset of menopause (≤ 5 years or > 5 years). During the on-treatment period (baseline to month 24), subjects received blinded investigational product Q6M SC (last dose at month 18); this document reports results from the on-treatment period. During the off-treatment period (months 25 to 48), administration of investigational product was discontinued; the off-treatment is still ongoing. Daily supplementation throughout the 48 months of the study was ≥ 1 g elemental calcium and either ≥ 400 IU vitamin D (if screening 25[OH] vitamin D was > 20 ng/mL) or ≥ 800 IU vitamin D (if screening 25[OH] vitamin D was 12 to 20 ng/mL).

Subject safety was monitored on an ongoing basis throughout the study by an external data monitoring committee (DMC).

Final analyses of the primary and key secondary endpoints are reported in this document, along with safety and pharmacokinetic data from the on-treatment period. Results from the off-treatment period will be reported when data become available.

Number of Subjects Planned: 300

Number of Subjects Enrolled:
A total of 332 subjects were enrolled in the study and were randomized (1:1) to receive denosumab (166 subjects) or placebo (166 subjects). Onset of menopause was ≤ 5 years for 170 subjects and > 5 years for 162 subjects.

Sex: 100% women

Mean (SD) Age: 59.4 (7.5) years

Ethnicity (Race): 83% white or Caucasian, 7% Hispanic or Latino, 4% black or African American, 4% Asian, 1% Japanese, 1% other, and < 1% American Indian

Diagnosis and Main Criteria for Eligibility:
Eligible subjects were postmenopausal women with lumbar spine BMD T-score between –1.0 and –2.5 (ie, –2.5 < T-score < –1.0) who were ambulatory, were not receiving medication that affected bone metabolism (other than calcium and vitamin D), were free from any underlying condition (other than low lumbar spine BMD) that might have resulted in abnormal bone metabolism, and had no history of a fracture after the age of 25 years.

Investigational Product, Dose and Mode of Administration, Manufacturing Batch Number:
Denosumab was provided as a sterile, clear, colorless, preservative-free liquid in glass vials containing 60 mg denosumab per mL of [redacted] mM sodium acetate and [redacted]% sorbitol in Water for Injection, with a pH of [redacted]. One mL of blinded investigational product was administered SC at day 1 and at months 6, 12, and 18. Lot numbers for denosumab used in this study were [redacted], [redacted], [redacted], and [redacted].

Reference Therapy, Dose and Mode of Administration, Manufacturing Batch Number:
Placebo was provided in containers 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 investigational product was administered SC at day 1 and at months 6, 12, and 18. Lot numbers for placebo used in this study were [redacted] and [redacted].

Duration of Treatment:
The duration of treatment was 24 months (on-treatment period). Including the screening period (up to 56 days before randomization) and the off-treatment period (24 months), the maximum time for a subject's participation in the study was approximately 50 months.

Study Endpoints

Primary Efficacy Endpoint:

- Percent change from baseline in the lumbar spine BMD (by DXA) at 24 months of treatment.

Secondary Efficacy Endpoints:

- Percent change from baseline in BMD (by DXA) of the total hip, femoral neck, trochanter, distal 1/3 radius, and total body (without head) at 24 months

- Percent change from baseline in trabecular, cortical, and total volumetric BMD of the distal radius as measured by QCT at 24 months.

Exploratory efficacy endpoints are detailed in Section 7.10.3.1.

Safety Endpoints:

Safety was characterized by tabulating adverse event incidence by system organ class, high-level group term and preferred term; changes in safety laboratory analytes (serum chemistry and hematology) at each visit and shifts between baseline and the worst on-study value; changes in the QTc interval and incidence of abnormal ECG; and subject incidence of anti-denosumab antibody appearance (Negative/Positive). Safety endpoints are detailed in Section 7.10.3.2.

Statistical Methods:
Descriptive statistics were provided for selected baseline characteristics and demographic, efficacy, pharmacokinetic, and safety data. Continuous variables were summarized descriptively using mean, standard deviation (SD), minimum, maximum, and the number of non-missing observations, n. Median and other selected percentiles were substituted for mean and SD for parameters exhibiting a lack of normality (continuous or ordinal categorical). Frequencies and percentages were presented for nominal categorical variables.

The analysis of the percent change from baseline in lumbar spine BMD to months 1, 6, 12, and 24 separately within each postmenopausal stratum employed an analysis of covariance (ANCOVA) model with treatment (denosumab or placebo), baseline BMD value, machine type, baseline value-by-machine type interaction as fixed effects. The primary results were based on the point estimate for the least-squares mean and the 2-sided 97.5% confidence interval (CI) for the treatment difference (denosumab – placebo) within each postmenopausal stratum at the 24-month time point. Multiplicity adjustment was applied to primary and secondary efficacy endpoints to maintain the overall type I error at ≤ 0.025 within each strata. The analysis of QCT and BMD data of the additional body sites was conducted in the same manner.

Safety was characterized by tabulating adverse event incidence by system organ class, high-level group term and preferred term (coded using Medical Dictionary for Regulatory Authorities [MedDRA] version 9.0); changes in safety laboratory analytes (serum chemistry and hematology) at each visit and shifts between baseline and the worst on-study value; changes in the QTc interval and incidence of abnormal ECG; and subject incidence of anti-denosumab antibody appearance (negative/positive).

Summary of Results:

Subject Disposition:
A total of 332 subjects were randomized into this study (166 subjects in each treatment group). Randomization was stratified by time since menopause; 162 subjects were in the ≤ 5 years-since-menopause stratum (81 denosumab, 81 placebo) and 170 subjects were in the > 5 years-since-menopause stratum (85 denosumab, 85 placebo). Of the 332 subjects enrolled into the study, 329 subjects (164 denosumab, 165 placebo) received at least 1 dose of investigational product.

Eighty-six percent of subjects in the denosumab group and 87% of subjects in the placebo group completed the 24-month on-treatment period.

Efficacy Results:
Denosumab statistically significantly increased BMD (assessed by DXA) at the lumbar spine, total hip, femoral neck, trochanter, distal 1/3 radius, and total body at month 24 ($p < 0.0001$ after multiplicity adjustment) for both early and late postmenopausal women and for both strata combined (Figure 2-1). Significant increases in BMD were observed both in primarily trabecular bone sites and in primarily cortical bone sites; the magnitude of percent increases in BMD was greatest at the lumbar spine and trochanter. The effect of denosumab was rapid; mean percent changes from baseline in BMD at all anatomic sites were greater in the denosumab group than in the placebo group at each postbaseline assessment, including the 1-month assessment. Results of sensitivity analyses of the BMD efficacy endpoints demonstrated that the results of the primary analyses were robust. In addition, higher proportions of subjects treated with denosumab, as compared with placebo, experienced changes in BMD > 0% and > 3% at the lumbar spine, femoral neck, trochanter, and total hip at most postbaseline assessments.

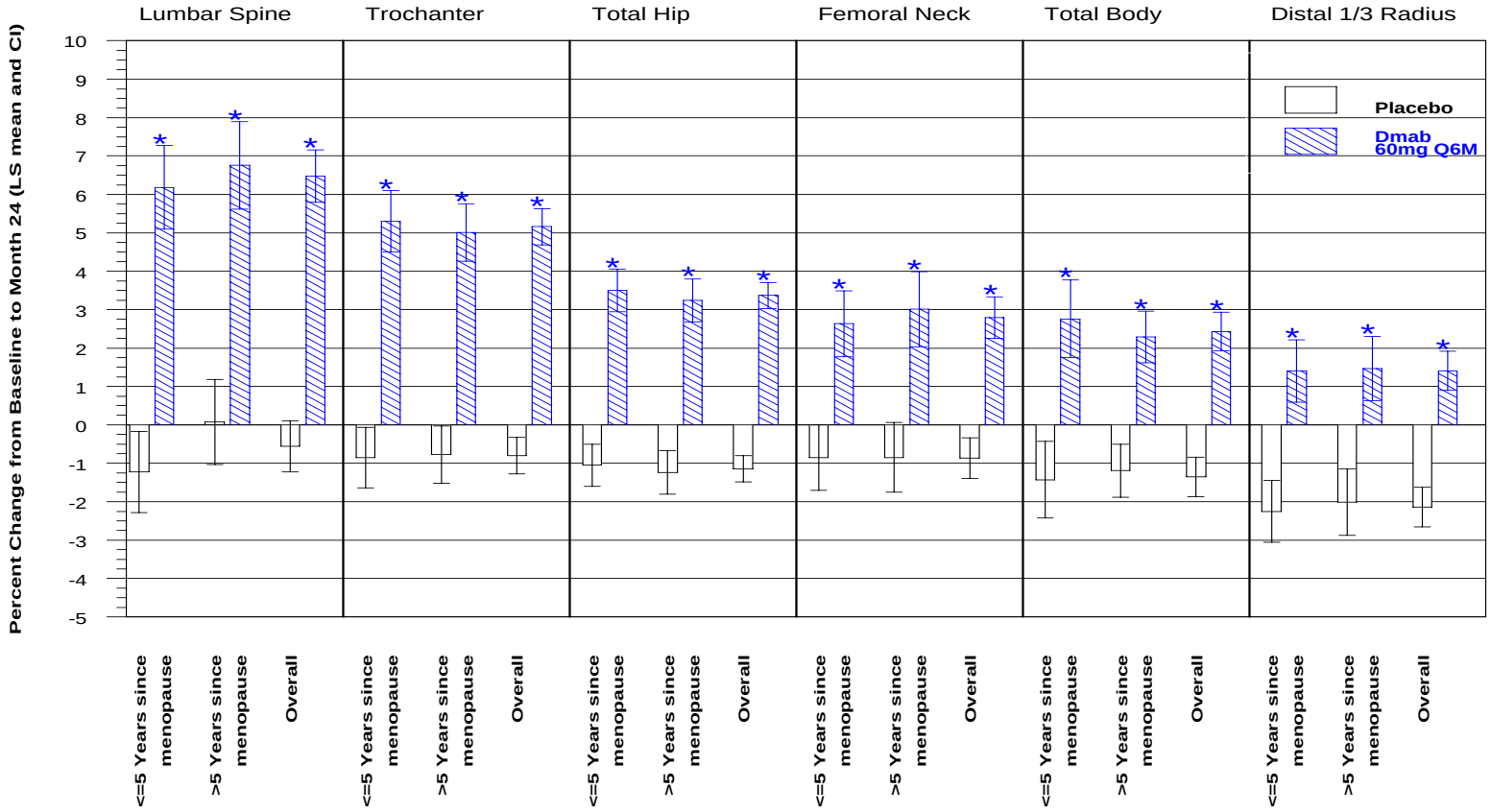
As assessed by QCT, denosumab, compared with placebo, increased cortical, trabecular, and total volumetric BMDs at the distal radius, a predominantly cortical site. Denosumab increased mean section modulus (a measure of resistance to bending loads) and decreased mean buckling ratio (a measure of susceptibility to axial compressive loads) at all 3 sites assessed by HSA (narrow neck, intertrochanter, and shaft).

Denosumab, compared with placebo, decreased serum concentrations of type 1 C-telopeptide (CTX1), intact N-terminal propeptide of type 1 procollagen (P1NP), and tartrate-resistant acid phosphatase (TRAP) 5b at each postbaseline assessment ($p < 0.0001$) (except the day 1, hour 6 assessment for TRAP 5b). No changes in mean serum concentrations of intact parathyroid hormone (iPTH), osteoprotegerin (OPG), and RANKL were observed in either treatment group.

Clinical fractures were reported and confirmed by the imaging vendor for 2 subjects (1%) in the denosumab group and 7 subjects (4%) in the placebo group.

Pharmacokinetics Results:
The mean (SD) serum denosumab concentration approximately 1 month after the first dose was 5640 (2190) ng/mL. Mean serum denosumab trough concentrations for months 6 through 24 were similar, suggesting that denosumab PK did not change with time and that there was no accumulation.

Figure 2-1. BMD by DXA Percent Change from Baseline to Month 24
(Least-squares Means and CIs From ANCOVA Model, LOCF Imputation, Primary Efficacy Subset)



Least squares means with 97.5% CIs for each stratum and 95% CIs for overall assessments based on ANCOVA models (for each stratum) that adjust for treatment, baseline value, machine type, and baseline value-by-machine type interaction; the models (for overall assessment) also adjust for strata
* Indicates significance after multiplicity adjustments at 2.5% level for each stratum and at 5% level for the overall assessment

Program: /stat/amg162/osteo/20040132/analysis/mon24/adhoc/program/g_lsmci_dxa_pchg.sas
Output: g14-01_001_014_lsmci_dxa_pchg.cgm (Date Generated: 23AUG2007: 8:23:32)
Source Data: adam.abmdxa, adam.abmqct

Safety Results:
A total of 329 subjects (164 randomized to denosumab, 165 randomized to placebo) received investigational product and were evaluable for safety. No subjects died during the study. The proportions of subjects who withdrew because of adverse events from either the investigational product (denosumab 3.0%, placebo 3.6%) or from the study (denosumab 0.6%, placebo 1.2%) were similar in the treatment groups. Serious adverse events were reported for 18 subjects (11.0%) in the denosumab group and 9 subjects (5.5%) in the placebo group. The increased incidence of serious adverse events in the denosumab group was primarily due to a greater incidence of serious adverse events of infection reported in subjects who received denosumab; the incidences of other SAEs were similar in the treatment groups.

Ninety-five percent of subjects in both treatment groups reported at least 1 adverse event, most of which were mild to moderate in severity. The most common adverse events (≥ 10% in either treatment group) were (denosumab, placebo) arthralgia (25%, 26%), nasopharyngitis (22%, 19%), back pain (20%, 20%), headache (16%, 12%), pain in extremity (15%, 12%), upper respiratory tract infection (12%, 13%), constipation (11%, 5%), urinary tract infection (11%, 10%), shoulder pain (10%, 6%), influenza (9%, 11%), and sinusitis (6%, 10%). The incidences of treatment-related events were 15% in the denosumab group and 12% in the placebo group. The most common treatment-related adverse events (≥ 2% in either treatment group) were nausea (4%, 2%) and rash (2%, 0%). No adverse events of hypocalcemia were reported, and the incidences of adverse events considered potential clinical manifestations of hypocalcemia (hypoesthesia and paresthesia) were similar in the treatment groups.

The overall infection rate was balanced between the treatment groups; incidences of adverse events in the Infections and Infestations system organ class were 60% in the denosumab group as compared with 61% in the placebo group. More subjects receiving denosumab who developed an infection were hospitalized compared with subjects receiving placebo (8 [4.9%] vs 1 [0.6%]). No unusual pathogens or those typically associated with opportunistic infections (eg, tuberculosis) were reported. Hospitalizations were characterized by uncomplicated courses and successful treatment with standard antibiotics. No deaths due to infection occurred. The nature of these cases of infections in the context of the small sample size, the overall balanced adverse events of infections, and the lack of pattern in body systems or pathogens suggest that the data from this study are insufficient to conclude that the observed imbalance represents an increased risk of infection.

No trends in serum chemistry or hematology parameters were noted other than modest, expected decreases in serum calcium, phosphorus, and total alkaline phosphatase. Two subjects in the denosumab group experienced grade 2 hypocalcemia at month 1 and normalized at subsequent visits; no clinical sequelae resulting from decreases in calcium were noted. Expected changes in albumin-adjusted serum calcium associated with the pharmacologic action of denosumab were observed.

Two subjects (1%) in the denosumab group and 3 subjects (2%) in the placebo group developed non-neutralizing, binding antibodies to denosumab. For these subjects, there was no evidence of an effect of these antibodies on the subjects' safety profiles or on the pharmacokinetics or efficacy of denosumab.

Conclusions:
In conclusion, denosumab is a novel therapeutic modality that rapidly increases BMD throughout the skeleton. This study demonstrates that denosumab effectively increases both trabecular and cortical bone mineral density, and density-derived structural parameters, in postmenopausal women with low bone mass, independent of time since menopause.