## 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, Placebo-controlled, Multi-dose Phase 2 Study to Determine the Efficacy, Safety, and Tolerability of AMG 162 in the Treatment of Postmenopausal Women With Low Bone Mineral Density

**Investigators and Study Centers:**
This was a multicenter study conducted at 29 centers in the United States.

**Publications:**
McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med*. 2006;354:821-831.

Lewiecki EM, McClung MR, Cohen SB, et al. Two-year treatment with denosumab in a randomized phase 2 study of postmenopausal women with low bone mineral density. *J Bone Miner Res.* 2007;22:1832-1841.

Multiple abstracts reporting results of this study also have been presented and/or published.

**Study Period:**
09 May 2002 (first subject enrolled) to 16 April 2007 (last subject last visit)

**Development Phase:**
Phase 2

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**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 1 study (protocol 20010124), a single subcutaneous (SC) dose of denosumab resulted in a dose-dependent, rapid, and sustained decrease in urine N-telopeptide/creatinine (uNTX/Cr).

The primary objective of this study was to determine the effect of denosumab treatment compared with placebo over 12 months on bone mineral density (BMD) of the lumbar spine in postmenopausal women with low BMD.

The secondary objectives were to:

- choose a dose regimen of denosumab for future studies, based on changes from baseline in BMD and bone turnover markers over 12 months;
- evaluate the effect of denosumab relative to placebo on BMD of the total hip, distal radius, and total body, and the safety and tolerability profile (including bone safety profile based on histology and histomorphometry) over 12 months;
- evaluate the effect of denosumab relative to placebo on efficacy (based on BMD and bone turnover marker changes) and safety over 48 months;
- assess whether denosumab treatment has a different efficacy or safety profile compared with alendronate;
- assess the off-treatment effect of denosumab based on BMD and bone turnover marker changes;

- assess the efficacy and safety of retreatment with denosumab; and
- assess the administrative feasibility of subject-reported outcomes assessments, and to determine the reliability and validity of the scales.

Exploratory objectives were to characterize the efficacy and safety of denosumab following investigational product withdrawal and retreatment in a subset of subjects.

**Methodology:**
This was a phase 2, randomized, double-blind, placebo-controlled, multicenter, parallel-group, dose-ranging study in postmenopausal women with low BMD (T-score ≤ 1.8 at either the lumbar spine, femoral neck, or total hip, but not lower than -4.0 at the lumbar spine, or -3.5 at the femoral neck or total hip). The study was designed to determine the effect of denosumab versus placebo (denosumab's effect on lumbar spine BMD at 12 months was the primary endpoint) and to choose a denosumab dose regimen for future studies. The dose regimen from month 24 onwards and for future studies was selected based on changes in BMD and bone turnover markers after the first 12 months of treatment. The study also was designed to evaluate the safety and tolerability of denosumab. The primary analysis was conducted once all subjects completed the month 12 visit. Final analyses of the primary and secondary endpoints are reported for the entire 4-year study duration in this report.

All subjects were instructed to take daily supplements of calcium (≥ 1000 mg) and vitamin D (≥ 400 IU).

Screening Through Month 24
After meeting eligibility criteria and completing screening assessments, subjects were randomized equally to 1 of 9 treatment cohorts, with approximately 40 subjects per cohort. Eight cohorts received double-blind subcutaneous (SC) injections at the following doses and frequencies for months 1 to 21 of the study: placebo; 6, 14, or 30 mg denosumab every 3 months or 14, 60, 100, or 210 mg denosumab every 6 months. The ninth cohort received open-label, oral alendronate 70 mg once weekly.

Month 24 Through Month 48

Beginning at month 24, subjects in 5 of the denosumab dose cohorts (6 and 14 mg every 3 months and 14, 60. and 100 mg every 6 months) received denosumab 60 mg every 6 months, in accordance with the proposed dosing regimen that was selected based on the first 12 months of data in this study. Collectively, these cohorts are the continuous-treatment cohorts.

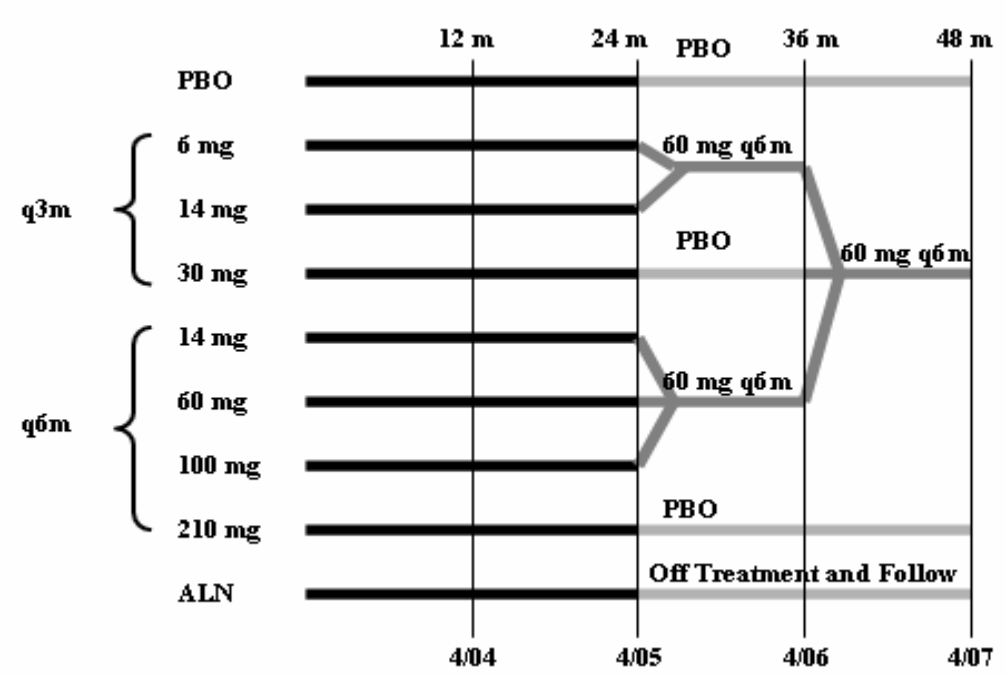
Subjects in the remaining 2 denosumab cohorts were reallocated to placebo treatment to collect data on the off-treatment and retreatment effects of denosumab:

- The denosumab cohort receiving 210 mg every 6 months was reallocated to receive placebo for the remainder of the study. This is the off-treatment cohort.
- The denosumab cohort receiving 30 mg every 3 months was reallocated to receive placebo. At month 36, this cohort was reallocated to receive denosumab 60 mg every 6 months. This is the retreatment cohort.

Subjects in the placebo cohort continued receiving placebo for the remainder of this 48-month study, but were dosed at 6-month intervals.

Subjects in the alendronate cohort discontinued treatment with alendronate, but were followed for the remainder of this 48-month study.

The dosing schema is depicted below.




**Number of Subjects Planned:**
The planned enrollment was 40 subjects in each of 9 treatment cohorts, for a total of 360 subjects.

**Number of Subjects Enrolled:**
A total of 412 subjects were enrolled in the study; 406 received at least one dose of investigational product (denosumab, 314; placebo, 46; alendronate, 46).

**Sex:** 100% women

**Mean (SD) Age:** 62.5 (8.1) years

**Ethnicity (Race):** 86% white, 9% Hispanic, 3% black, 1% Asian, < 1% Japanese, < 1% American Indian

**Diagnosis and Main Criteria for Eligibility:**
Eligible subjects were postmenopausal women with low BMD ($-4.0 \leq$ T-score $\leq -1.8$ for the lumbar spine or $-3.5 \leq$ T-score $\leq -1.8$ for the total hip or femoral neck) who were $\leq 80$ years of age at the time of randomization, not receiving medication that affected bone metabolism, and free from any underlying condition that might have resulted in abnormal bone metabolism.

**Investigational Product, Dose and Mode of Administration, Manufacturing Batch Number:**
Denosumab was provided as a sterile, clear, colorless, preservative-free liquid in glass vials. Three concentrations of denosumab were provided: 30, 60, or 70 mg denosumab per mL of 10 mM sodium acetate and 5% sorbitol in Water for Injection, with a pH of 5.2.

In the first 24 months of the study, denosumab was administered SC to subjects in doses of 6, 14, and 30 mg every 3 months or 14, 60, 100, or 210 mg every 6 months. In the second 24 months of the study, denosumab was administered to subjects SC as a 60 mg dose every 6 months.

**Reference Therapy, Dose and Mode of Administration, Manufacturing Batch Number:**
Placebo
Placebo was provided as a sterile, clear, colorless, preservative-free liquid in glass vials that were identical in appearance to denosumab. The placebo formulation was identical to the denosumab formulation with the exception of the protein content.

In the first 24 months, subjects in the placebo cohort received SC injections of placebo every 3 months. In the last 24 month of the study, subjects in this cohort received placebo every 6 months.

In the first 24 months, subjects in the denosumab cohorts who received denosumab every 6 months also received placebo injections at 3, 9, 15, and 21 months to keep the study blind intact.

Alendronate
One cohort of subjects received open-label alendronate as a reference therapy for the first 24 months of this study. Subjects were to take a 70-mg oral tablet once weekly in accordance with the labeled directions. Study sites provided alendronate (Fosamax®, Merck & Co, Inc) as 70-mg oral tablets to subjects assigned to this cohort.

**Duration of Treatment:**
The treatment period was 48 months.

**Study Endpoints:**

***Primary Efficacy Endpoint:***
- percent change from baseline to month 12 in the BMD of the lumbar spine for the placebo and denosumab treatment arms

***Secondary Efficacy Endpoints:***
- percent change from baseline to month 12 with respect to:
  - uNTX/Cr and serum CTX 1 for all treatment arms (including alendronate)
  - BMD of the lumbar spine for the alendronate treatment arm
- percent change from baseline to months 24, 36, 42, and 48 with respect to:
  - BMD of the lumbar spine for all treatment arms
  - uNTX/Cr and serum CTX 1 for all treatment arms
- percent change from baseline to months 12, 24, 36, 42, and 48 with respect to:
  - BMD of the total hip, distal radius, and total body for all treatment arms
  - Bone-specific alkaline phosphatase (BAP) for all treatment arms

***Pharmacokinetic (PK) Endpoints:***
- maximum observed serum denosumab concentration ($C_{max}$)
- time of $C_{max}$ ($T_{max}$)
- trough serum denosumab concentration ($C_{min}$)
- area under the serum concentration-time curve over the dosing interval ($AUC_{0\text{-}tau}$)
- half-life describing the majority of exposure ($t_{1/2}$, when determinable)

***Subject Reported Outcomes Endpoints:***
- percent completion in subject-reported outcomes
- scale reproducibility
- scale construct validity

***Safety Endpoints:***
- subject incidence of treatment-emergent adverse events
- changes from baseline in laboratory assessments (serum chemistry, hematology, and intact parathyroid hormone [iPTH]) at each visit
- changes from baseline in vital signs at each visit
- clinical fracture incidence
- bone histologic and histomorphometric parameters

- subject incidence of anti-denosumab antibody formation in the denosumab treatment arms
- changes in electrocardiogram (ECG) QTc intervals

**Statistical Methods:**
Descriptive statistics were provided for selected baseline characteristics and demographic, efficacy, pharmacokinetic, subject-reported outcomes, 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 primary analytical approach for all efficacy endpoints was hypothesis testing using results from the first 12 months of randomized treatment; hypothesis testing using results from data collected after 12 months was considered exploratory. Placebo-adjusted denosumab treatment effect estimates were provided. Results from the active control arm (alendronate group) were used as a descriptive comparator.

The primary analysis of the efficacy parameters employed an analysis of covariance (ANCOVA) model with treatment as the main effect and geographic location and baseline value as covariates. The mean percent changes in BMD reported in this report are the model-adjusted (least-squares) means derived from the ANCOVA model.

Pair-wise comparisons between each denosumab dose cohort and the placebo group were performed for each efficacy parameter. Hypotheses were tested across denosumab doses within an endpoint using Hochberg's procedure at a significance level of 0.05 to account for multiple testing. Note that although p-values for post-12 months' results are presented in this report, they should be considered as descriptive statistics because the family-wise type 1 error rate of 0.05 was planned and applied for the 12-month assessment. Any inferences for time points after month 12 were made using 0.001 as the statistical significance level.

Psychometric analyses were used to assess the reliability and validity of the subject-reported outcomes scales. Baseline descriptive statistics were also calculated. In addition, completion rates were calculated to assess administrative feasibility for all health-related quality of life (QOL) instruments at various time points.

Summary statistics, including mean, SD, % coefficient of variation (%CV), and median values, for $C_{min}$ were calculated for the pharmacokinetic analyses. Summary statistics were reported by nominal time points. A value was excluded if the sample for $C_{min}$ was obtained either after dosing or more than 7 days before dosing. Comparisons of mean serum concentrations were made using graphs and tables of data.

Safety was characterized by tabulating adverse event incidence by system organ class 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 corrected QT (QTc) interval and incidence of abnormal ECG; and subject incidence of anti-denosumab antibody appearance (negative/positive). T- and B-cell enumerations and bone biopsy results (substudies) were also summarized.

**Summary of Results:**

**Subject Disposition:**
A total of 412 subjects (319 denosumab, 46 placebo, 47 alendronate) were enrolled at 29 study centers in the United States. The number of subjects enrolled into each of the denosumab dose cohorts ranged from 41 to 54 subjects. Sixty-four percent of all subjects completed the study. Overall, the most common reasons for subjects in the denosumab, placebo, and alendronate groups to discontinue the study were because of withdrawn consent (23%, 24%, and 23%, respectively), adverse events (4%, 4%, and 6%), and loss to follow-up (3%, 7%, and 4%).

A total of 406 subjects (314 denosumab, 46 placebo, 46 alendronate) received at least 1 dose of investigational product and were included in the safety analysis dataset. Sixty-eight percent of all subjects completed dosing with investigational product.

**Efficacy Results:**

Primary Endpoint at 12 Months

At month 12, denosumab increased BMD of the lumbar spine from baseline in all denosumab cohorts (range: 3.0% to 6.7%). The differences between each denosumab cohort and placebo (- 0.8% change from baseline) were statistically significant ($p < 0.001$). Sensitivity analyses supported these findings.

The First 24 Months of the Study

In the first 24 months, increases in BMD of the lumbar spine were observed in all denosumab-treated cohorts (range: 3.9% to 8.8%) compared with a 1.3% decrease for placebo. Bone mineral densities of the total hip, distal 1/3 radius, total body (without head), femoral neck, and trochanter increased steadily at each time point and were greater for all denosumab dose cohorts than placebo through month 24. Overall, the magnitudes of mean percent increases in BMD at each of the anatomic sites were similar between denosumab and alendronate. Across denosumab dose cohorts, the magnitude of the increase in BMD was similar with the exception of the 14 mg every 6 months dose cohort.

Denosumab treatment resulted in rapid and sustained decreases in bone turnover markers serum CTx, uNTX/Cr, and BAP through month 24. In the first 12 months, the magnitude of suppression among denosumab-treated cohorts ranged up to 89% for serum CTx, 73% for uNTX/Cr, and 75% for BAP. At month 24, these decreases were 73% for serum CTx, 50% for uNTX/Cr, and 58% for BAP. Across the denosumab dose cohorts, the maximal suppression of bone turnover markers was similar. In the lower dose cohorts, the bone turnover markers trended towards baseline levels before the next dose.

Dose Selection for Future Phase 3 Trials

The denosumab dose regimen 60 mg every 6 months was selected for future clinical trials. Doses of 6, 14, and 30 mg denosumab administered every 3 months and 14, 60, 100, and 210 mg administered every 6 months reduced bone loss with similar dose-response relationships in the 3- and 6-month dose cohorts. Dosing with denosumab 60 mg every 6 months resulted in robust increases in BMD of all anatomic sites and suppression of bone turnover markers over the entire dosing interval. In addition, no dose-related adverse events were observed. Furthermore, denosumab doses $\geq$ 60 mg administered every 6 months were at least as effective as 70 mg of alendronate administered once a week. Since denosumab was effective when dosed either using a 3- or a 6-month dosing interval, the 6-month dosing interval was selected for reasons of increased convenience and compliance.

The Second 24 Months of Study

*Continuous-treatment Denosumab Cohorts*

Overall, BMD of all anatomic sites continued to increase for subjects in the continuous-treatment denosumab cohort, while BMD of all anatomic sites declined for subjects in the placebo cohort. At month 36, BMD of the lumbar spine increased 9.0% for the continuous-treatment cohort compared with a 1.8% decrease for placebo. At month 48, BMD increased 10.3% for the denosumab cohort compared with a 2.4% decrease for placebo. BMD of the hip followed a similar pattern. At months 36 and 48, BMD increased 4.8% and 5.1%, respectively for the denosumab cohort compared with 2.9% and 3.5% decreases for placebo. Increases in BMD also were observed for other anatomic sites.

Bone turnover markers remained decreased from baseline levels at months 24 through month 48.

*Off-treatment Denosumab Cohort*

BMD of all anatomic sites initially decreased for subjects in the off-treatment cohort in the first year after they were reallocated to placebo, approaching baseline levels at month 36. In the second year off-treatment (up to month 48), BMD remained similar to levels at month 36 in these

subjects. BMD of all anatomic sites was numerically higher than placebo at all time points following denosumab subjects' reallocation to placebo at month 24.

There were transient increases of bone turnover markers above baseline levels following withdrawal of denosumab that returned to baseline at month 48.

In an ad hoc analysis, the relationship of BMD at baseline and following discontinuation of denosumab at months 36 and 48 in the off-treatment dose cohort confirmed the reversibility of effects, as evidenced by an increase in bone turnover markers and consequent decrease in BMD with denosumab discontinuation. The magnitude of the reduction in BMD appeared associated with the subject's pretreatment BMD level.

*Retreatment Denosumab Cohort*
Subjects in the retreatment denosumab cohort stopped receiving denosumab at month 24, then resumed receiving denosumab at month 36. In the year subjects were off denosumab treatment, BMD of all anatomic sites decreased, approaching baseline levels at month 36. Upon resumption of treatment with denosumab at 42 and 48 months (6 and 12 months, respectively, after dosing resumed), the BMD increased to levels similar to those that occurred when denosumab was first administered. For example, mean percent increases in BMD of the lumbar spine at months 6 and 12 were 4.4% and 6.7%, respectively, and mean percent increases at months 42 and 48 were 5.1% and 9.0%, respectively.

There were transient increases of bone turnover markers following withdrawal of denosumab during the 12-month period when subjects received placebo, similar to that noted for the off-treatment cohort. However, retreatment with denosumab decreased turnover marker activity to an extent similar to initial treatment.

Osteoporotic Fractures
In this 4-year study, osteoporotic fractures occurred in 8.7% of placebo subjects, 7.0% of denosumab-treated subjects, and in 4.3% of alendronate subjects. Following withdrawal from denosumab treatment in the off-treatment and retreatment cohorts, there did not appear to be a higher incidence of osteoporotic fractures.

**Pharmacokinetic Results:**
Denosumab displayed non-linear pharmacokinetics with dose across both the 6 to 30 mg every 3 months and 14 to 210 mg every 6 months dose ranges, with exposure based on mean area under the serum denosumab concentration-time curve during the dosing interval ($AUC_{0\text{-}tau}$) values increasing greater than dose-proportionately (~10- and 36-fold for the 5- and 15-fold increases in dose, respectively). However, mean $AUC_{0\text{-}tau}$ values increased approximately dose-proportionately between the 60 and 210 mg every 6 months doses. Additionally, exposure based on mean $C_{max}$ values increased approximately dose-proportionately across both the every 3-months and every 6-months dose ranges. $C_{max}$ values were generally observed in median times of less than 1 week for the every 3 month dose groups and between 2 and 3 weeks for the every 6 months dose groups. No marked (> 2-fold) accumulation was observed for any dosing regimen. For the 60, 100, and 210 mg dose groups, mean $t_{1/2}$ values that described the disposition of denosumab over large proportions of both exposure (based on AUC) and the every 6 month dose intervals were similar, ranging from approximately 25 to 34 days.

For both the every 3 months and every 6 months dose schedules, mean exposures were comparable (< 20% different) between the first and subsequent doses, indicating a lack of change in denosumab pharmacokinetics with time or upon multiple dosing. The latter results are also consistent with similar mean $C_{min}$ values with time, within each cohort, across all dose regimens for up to 48 months.

**Subject Report Outcomes Results:**

Administrative Feasibility

Completion rates of subject-reported outcomes questionnaires were high for all time points between baseline and month 12, ranging from 93.5% to 99.8% for the Medical Outcomes Study Short-Form 36 (SF-36), Quality of Life in Reflux and Dyspepsia (QOLRAD), and Gastroesophageal Reflux Disease Symptom Assessment Scale (GSAS). The completion rate for the experimental Treatment Satisfaction Assessment (TSA) questionnaire ranged from 70% at month 18 to 80% at month 24. At month 48, completion rates for the SF-36, QOLRAD, GSAS, TSA, and EuroQol-5 Dimension (EQ-5D) were satisfactory, ranging from 87.6% to 97.7%. Overall, these results indicate that it is feasible to conduct health-related quality of life (HRQOL) assessments in postmenopausal women with low BMD.

Psychometric Analysis

Baseline descriptive statistics showed that SF-36 scores for the study population were similar to the general population. Additionally, QOLRAD and GSAS scores at baseline showed that study patients had fewer gastrointestinal symptoms with less frequency, severity, and distress than adults with gastroesophageal reflux disease (GERD). For SF-36, QOLRAD, and GSAS at month 48, small but non-significant reductions were observed in mean scores over baseline.

There was evidence for good reliability and validity. Item discrimination for the SF-36 and the QOLRAD were good; however, item discrimination for the GSAS was poor. As hypothesized, higher QOLRAD scores were associated with higher SF-36 scores, and lower GSAS scores were correlated with higher SF-36 scores. Additionally, the QOLRAD and GSAS scales were also responsive to adverse gastrointestinal events. The association between month 12 SF-36 scores and BMD was weak and not significant in most cases, although some data suggested that the distal 1/3 radius measure was weakly and positively associated with the physical scales of the SF-36. A similar association was observed at month 48 between EQ-5D scores and distal 1/3 radius BMD. Furthermore, the EQ-5D and SF-36 were highly correlated, indicating both instruments concordantly measured concepts of general health.

**Safety Results:**

A total of 406 subjects (314 denosumab, 46 placebo, 46 alendronate) received at least 1 dose of investigational product and were included in the safety analysis set. Since the tolerability of denosumab was similar across denosumab dose cohorts, safety results for the denosumab treatment group are generally presented in aggregate for all dose cohorts combined.

Most subjects (93% to 96%) reported at least 1 adverse event, and most events were mild or moderate in severity. The 3 most common adverse events in the denosumab cohorts were upper respiratory tract infection (28%), arthralgia (24%), and back pain (20%). The 3 most common adverse events in the placebo cohort were arthralgia (30%), upper respiratory tract infection (24%), and sinusitis (20%), and the 3 most common adverse events in the alendronate cohort were upper respiratory tract infection (30%), dyspepsia (26%), and nausea (22%).

Overall, treatment-related adverse events occurred in 22%, 20%, and 41% of subjects in the respective treatment groups; the relatively higher incidence in the alendronate group may be due to the open-label design for this group as alendronate has a well-known adverse event profile. Similar percentages of subjects in each treatment cohort withdrew from the study due to adverse events (4% placebo, 4% denosumab, and 7% alendronate).

Serious adverse events occurred in 18% of subjects in the denosumab cohort, 11% of subjects in the placebo cohort, and 17% of subjects in the alendronate cohort. Serious adverse events related to investigational product occurred in 1%, 0%, and 0% of subjects in these cohorts, respectively. Serious adverse events that occurred in ≥ 1% of subjects in the denosumab cohorts were angina pectoris (1.3%) and osteoarthritis (1.0%) and syncope (1.0%). A number of individual serious adverse events were reported in the placebo dose cohort (2.2%). The most frequent serious adverse events reported in the alendronate cohort were osteoarthritis (4.3%) and non-cardiac pain (4.3%).

In this study, there were 4 deaths. Adverse events that contributed to subjects' deaths, each occurring in 1 subject each, were gastric cancer, adenocarcinoma of the lung, brain neoplasm, and a cerebrovascular accident; all who died were randomized to denosumab. None of the deaths was considered related to investigational product by the respective investigator.

Eighteen subjects (5.7%) in the denosumab treatment cohorts, 3 (6.5%) in the placebo cohort, and none (0%) in the alendronate cohort had adverse events considered potential clinical manifestations of hypocalcemia (hypoesthesia and paresthesia). For all but 2 subjects, these adverse events were mild or moderate in severity and not related to investigational product. One subject in the denosumab 14 mg every 6 months cohort experienced hypoesthesia and paresthesia that was moderate in severity and considered by the investigator related to investigational product, and 1 subject in the placebo cohort experienced hypoesthesia that was severe, but not considered by the investigator related to investigational product. None of the subjects who experienced adverse events of potential clinical manifestations of hypocalcemia had albumin-adjusted serum calcium below the normal range at scheduled visits.

The overall infection rate was balanced among the treatment groups; incidences of adverse events in the Infections and Infestations system organ class were 66%, 67%, and 70% in the denosumab, placebo, and alendronate cohorts, respectively. Three percent of subjects receiving denosumab developed an infection and were hospitalized; none of the placebo or alendronate subjects were hospitalized due to infection. Serious infections among subjects in the denosumab cohorts included bronchopneumonia, primary atypical pneumonia, bacteremia, labyrinthitis, pneumonia, appendicitis, and urinary tract infection, and diverticulitis in 2 subjects, and catheter site cellulitis and urinary tract infection, both of which occurred in 1 subject. The onset of serious infections ranged from days 242 to 1340. 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.

In the last 24 months of the study, there did not appear to be any changes in the types and frequencies of adverse events for either the off-treatment or retreatment denosumab cohorts when compared with the denosumab continuous-treatment cohorts and to the first 2 years of the study.

No trends in serum chemistry or hematology parameters were noted other than modest, expected decreases in serum calcium, phosphorus, and total alkaline phosphatase during dosing with denosumab. When denosumab was withdrawn in the off-treatment and retreatment cohorts, some subjects experienced transient increases in alkaline phosphatase, which was likely a result of increases in the bone-specific isoform of alkaline phosphatase, which is known to cross-react with total alkaline phosphatase.

Two subjects developed transient, non-neutralizing, binding antibodies to denosumab at a single time point following dosing with investigational product. No evidence of an effect of the binding antibodies on the pharmacokinetics of denosumab was observed in these subjects. Furthermore, individual safety and efficacy profiles for these subjects did not appear to be different than for subjects who were antibody negative.

In a small substudy conducted in the first 24 months of the study, T- and B-cells (CD3, CD4, CD8, and CD19) and natural killer (NK) cells (CD16/CD56) were counted to assess any potential effect of denosumab on immune cells. The study showed no evidence of a clinically significant effect of denosumab on these cell counts.

Histologic analyses of all evaluable bone biopsy samples from a second substudy demonstrated that subjects had normal bone architecture, lamellar appearance, and mineralization without evidence of pathologic findings, including woven bone and marrow fibrosis. These results indicate that the bone histologic parameters examined were not affected by treatment, regardless of dose level of denosumab. Histomorphometric review of unpaired biopsies showed changes consistent with decreased bone turnover and prolonged remodeling. Denosumab did not impair matrix mineralization.

MicroCT data obtained in the bone biopsy substudy did not show consistent changes from baseline in trabecular and cortical bone parameters after treatment for 12 months. Results of the bone biopsy microCT analysis yielded no significant findings over time or with drug treatment.