## 2. SYNOPSIS

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

**Name of Finished Product:** Denosumab

**Name of Active Ingredient:**

Human monoclonal IgG2 antibody to Receptor Activator of Nuclear Factor-κB Ligand (RANKL)

**Title of Study:** A Randomized, Double-blind, Placebo-controlled, Multi-dose Phase 2 Study to Determine the Efficacy, Safety and Tolerability of Denosumab in the Treatment of Rheumatoid Arthritis

**Investigator(s) and Study Center(s):** This study was conducted at 39 sites in the United States and Canada. A complete list of investigators and sites is included in Appendix 4.

**Publication(s):** None

**Study Period:** The first subject was enrolled on 11 August 2004 and the last subject, last visit occurred on 29 April 2007.

**Development Phase:** 2

---

**Introduction and Objectives:**

Osteoclasts are recognized as being a key mediator of bone destruction in experimental animal models of arthritis and RA (Romas et al, 2002; Gravallese E, 2002). Several studies have shown that Receptor Activator of Nuclear Factor-κB Ligand (RANKL) is an essential factor for osteoclast differentiation, and that its receptor RANK is expressed on cells of the RA synovial membrane (Simonet W, 1997; Yasuda H, 1998; Gravallese et al, 2000; Takayanagi H, 2000; Haynes D, 2001). Administration of osteoprotegerin (OPG), a naturally occurring inhibitor of RANKL (Simonet W, 1997), leads to protection from bone destruction in experimental arthritis (Kong Y, 1999**a**; Redlich K, 2002**a**).

Denosumab is a human monoclonal antibody (IgG2) to RANKL which has demonstrated prolonged mean residence time (MRT) in cynomologous monkeys such that pharmacologically active serum concentrations were maintained for 35-days for a subcutaneous (SC) dose of 1 mg/kg. Similar to OPG, denosumab is a potent inhibitor of RANKL and osteoclastogenesis, preventing terminal differentiation and activation of osteoclasts (Lacey et al, 1998; Yasuda et al, 1998; Burgess et al, 1999) and resulting in decreased bone resorption and increased bone density (Kong et al, 1999b).

Denosumab has been well tolerated in clinical studies conducted to date. The incidence of adverse events, including serious adverse events, was similar between subjects who received the active comparator or placebo treatment.

The purpose of this 24 month, multi-dose study was to demonstrate that denosumab was effective in reducing joint destruction in patients with rheumatoid arthritis (RA) on methotrexate (MTX) therapy. Efficacy based on the RA-MRI erosion score through 6 months will be used to help select an appropriate dose for future studies in RA and radiographic data collected through 12 months will be used to validate this assessment and reinforce subsequent Phase 3 data since radiographic data is currently considered to be the gold standard with regard to claims regarding joint destruction in RA. Subjects were followed through 24 months for long term safety.

**Methodology:**

This was a phase 2, multicenter, randomized, double-blind placebo-controlled parallel-group study of denosumab in patients with RA being treated with methotrexate (MTX).

Patients were screened for study participation within 28 days before randomization. Approximately 210 eligible subjects were planned to be stratified by current use of steroids and prior use of biologic agents (ie, etanercept, infliximab, adalimumab, or anakinra), and then randomized in a blinded fashion to one of the 3 treatment cohorts. Patients were randomized in a 1:1:1 ratio to receive one of two dose levels of denosumab (60 mg or 180 mg) or placebo subcutaneously at baseline and at month 6, with follow-up through month 24.

All subjects were to receive daily supplemental calcium plus vitamin D.

**Number of Subjects Planned:** Approximately 210 patients were planned; 70 subjects in each of the three treatment groups; 60 mg denosumab, 180 mg denosumab or placebo.

**Number of Subjects Enrolled:**

**Sex:** 166/227 (73%) female; 61/227 (27%) male

**Mean (SD) Age:** 57.4 (11.2) years

**Ethnicity (Race):** 82% White/Caucasian; Hispanic (9%); Black (3%); Asian (2%); American Indian/Alaskan Native (2%); Japanese (1%); Other (1%).

**Diagnosis and Main Criteria for Eligibility:**

Subjects ≥ 18 years of age at the time of screening with active RA (disease duration ≥ 24 weeks) and the presence of erosive disease defined as ≥ 3 erosions of the hands and feet or a C-reactive protein (CRP) ≥ 2.0 mg/dL and a positive test for cyclic citrullinated peptide (CCP) antibodies were eligible for the study after giving written informed consent. Subjects were required to be taking a stable dose of MTX for 8 weeks prior to randomization.

Subjects were excluded if they had received any biologic agent (eg, etanercept, infliximab, adalimumab, anakinra) or leflunomide within 8 weeks before randomization; past use of these agents was allowed. Other major criteria which excluded subjects from participation included, but were not limited to, glucocorticoid use > 15 mg/day (prednisone or equivalent), potential or scheduled surgery of the hands /wrists or feet, Felty's syndrome, any uncontrolled clinically significant systemic disease, a malignancy within 5 years, positive test for Hepatitis B surface antigen, Hepatitis C virus, or HIV, others.

Refer to Section 7.5 for a complete list of Inclusion/Exclusion Criteria for this study.

**Investigational Product, Dose and Mode of Administration, Manufacturing Batch Number:** Denosumab, human monoclonal IgG2 antibody to RANKL, 60 mg or 180 mg was administered subcutaneously at baseline and at Month 6. The denosumab manufacturing lot numbers used in this study were [redacted].

**Duration of Treatment:** Subjects were screened during the 28 day period prior to receiving study drug. Subjects received one of three treatments at baseline and at Month 6, with long term follow up through Month 24.

**Reference Therapy, Dose and Mode of Administration, Manufacturing Batch Number:** Placebo for this study was administered subcutaneously at baseline and at Month 6. Placebo consisted of study drug vehicle (minus drug protein content). Placebo batch numbers used in this study were [redacted].

**Study Endpoints**

As defined in the statistical analysis plan (version 3.0), the key endpoints of the study were:

**Primary Efficacy Endpoint:** the change in score in the RA-MRI erosion score (ES) from baseline to 6 months.

**Secondary Efficacy Endpoints:**

- Change in radiographic total modified Sharp score (TSS) from baseline at month 12
- Change in radiographic total modified Sharp score (TSS) and its components, radiographic erosion score (ES) and radiographic joint space narrowing score (JSN) from baseline at months 6 and radiographic erosion score (ES) and radiographic joint space narrowing score (JSN) from baseline at month 12
- Percent change in bone mineral density (BMD) from baseline in lumbar spine, total hip, femoral neck, and trochanter at months 1, 6, and 12
- Percent change from baseline in bone turnover and cartilage markers (including serum Type 1 CTX, P1NP, urine Type II CTX/creatinine, intact parathyroid hormone [iPTH], osteoprotegerin [OPG], receptor activator nuclear KappaB ligand [RANKL]) at months 3, 6, and 12

**Safety Endpoints:**

- Adverse event incidence including all treatment-emergent adverse events, serious adverse events (including death), non-serious severe adverse events, adverse events leading to study withdrawal, adverse events leading to discontinuation of investigational product, investigational product related adverse events, and investigational product related serious adverse events
- Safety laboratory analytes and changes in safety laboratory analytes
- Subject incidence of anti-denosumab antibody

**Pharmacokinetic Endpoint:**

- Denosumab serum concentration at month 1, 6, and 12

**Statistical Methods:**

The primary objective of this study was to evaluate whether denosumab was effective and safe in decreasing the progression of periarticular bone erosions in subjects with rheumatoid arthritis (RA) on methotrexate (MTX) treatment. The study was also designed to determine an optimal dose of denosumab for use in future studies.

The study consisted of a 12-month, double-blind, placebo-controlled period to assess efficacy and safety, and an additional 12-month untreated period to assess long-term safety. Two database snapshots and one final database lock occurred during the study; month 6, month 12, and at the end of the study.

The general analytical approach for efficacy and safety focused on treatment comparisons of each denosumab dose group (60 mg and 180 mg) with placebo.

Analysis of the primary endpoint was based on the change in RA-MRI erosion score at month 6 and was carried out when the data through Month 6 were finalized. The final inferential analysis was performed on radiographic endpoints at month 12. The radiographic endpoints at month 12 evaluated the effect of each denosumab group relative to placebo in reducing joint destruction and in healing of periarticular structural damage associated with RA. BMD changes at key sites of the body during the 12-month treatment period were also evaluated. Analysis of the accumulated safety data and efficacy data (except RA-MRI) through month 12 were also summarized.

The primary analysis compared the changes from baseline at month 6 in the RA-MRI erosion score (ES) between each denosumab dose group and placebo. The key secondary efficacy endpoint was the change in radiographic total modified Sharp score (TSS) from baseline to month12. Each of these analyses were carried out on the primary efficacy subset and included all randomized subjects who received at least 1 dose of investigational product with a non-missing baseline and at least 1 non-missing postbaseline measurement. With the exception of BMD analyses, all efficacy endpoints (including bone turnover and cartilage markers) were analyzed using van Elteren stratified rank test adjusting for baseline use of steroid and previous use of biologic agents.

Analyses of BMD by dual X-ray absorptiometry (DXA) of the lumbar spine, total hip, femoral neck, and trochanter included all randomized subjects who received at least 1 dose of investigational product with a non-missing baseline and at least 1 non-missing postbaseline measurement of BMD. Treatment comparisons for percent changes in lumbar spine, total hip, and femoral neck BMD were performed using a repeated measures model. An ANCOVA model was used for sensitivity analyses with both last observation carried forward (LOCF) and mean of the other group (MOTH) as the methods of imputation.

Pharmacokinetic analyses included all randomized subjects who received at least 1 dose of investigational product and had at least 1 postbaseline serum concentration level. Summary statistics were calculated on the steady-state concentration of each denosumab dose group at each scheduled visit.

Safety and tolerability were assessed by the incidence of adverse events, laboratory analytes, the presence of antibodies to denosumab, and vital signs through month 24. Safety data were summarized by actual treatment received and included all randomized subjects who received at least 1 dose of investigational product without any imputation. There was no statistical testing for the safety analyses.

The overall significance level for the analysis of the primary efficacy endpoint was 0.05. Both comparisons within the primary efficacy endpoint were tested simultaneously and the Hochberg's method for multiple comparisons was employed. The two comparisons within the key secondary efficacy endpoint were only to be tested in the case that both comparisons within the primary efficacy endpoint were significant at the two-sided 0.05 level. Also, both comparisons within the key secondary efficacy endpoint were tested simultaneously and the Hochberg's method (Hochberg, 1988) was employed in order to control the overall type I error at 0.05. All statistical tests were 2-sided. All efficacy and safety analyses were summarized by actual treatment received. Continuous parameters were summarized using descriptive statistics, which included the mean, median, standard deviation or standard error, minimum, maximum, and the number of non-missing observations (n). Nominal categorical parameters were summarized using frequencies and percentages. Ordinal categorical parameters were summarized either with frequencies and percentages, and/or selected percentiles of the data. The off-treatment effect of denosumab was characterized by examining spine and total hip BMD and bone markers at months 18 and 24, and radiographs of the hands and feet at month 24.

Additional details of the statistical analyses can be found in the Statisical Analysis Plan (version 3.0) in Appendix 2.

**Summary of Results:**

**Subject Disposition:**

A total of 227 subjects were enrolled and randomized equally to one of three treatment groups: Denosumab 60 mg, Denosumab 180 mg, or placebo. Of these, 218 (96%) subjects received at least one dose of investigational product, 204 (90%) completed their dosing regimen, and 203 (89%) completed the 12-month treatment period. A total of 14 (6%) subjects discontinued investigational product. Approximately 50% of subjects in each group completed the entire 24-month study period; the distribution was similar across each of the three treatment groups.

**Efficacy Results:**

The primary study endpoint was the change from baseline at month 6 in the MRI erosion score. There was a statistically significant difference in the MRI erosion score at month 6 between the 180 mg denosumab group and the placebo group (p=0.018). Progression of erosion in the 60 mg denosumab group was numerically lower than that in the placebo group; however, this difference was not significant.

Radiographic (x-ray) erosion scores at Month 6 were consistent with the Month 6 RA-MRI erosion analysis. A significant difference in the radiographic erosion score at Month 6 was observed between subjects who received 180 mg denosumab Q6M and placebo (p=0.019). The 12-month comparisons of radiographic data demonstrate significant differences between each of the denosumab groups (60 mg and 180 mg) and placebo.

Treatment effects of denosumab on lumbar spine BMD were evident at Months 6 and 12. The greatest change from baseline in the least squares (LS) mean (SE) BMD of the lumbar spine at Month 12 was observed for subjects who received 180 mg denosumab Q6M [4.09% (0.40); p<0.001]. The LS mean (SE) increase from baseline for the 60 mg denosumab group was slightly lower [3.00% (0.39); p<0.001] than that of the 180 mg denosumab group, but substantially greater than that observed among placebo subjects, ie, 0.80% (0.40).

The percent change from baseline at the femoral neck, trochanter, total hip and hands (in a substudy) also demonstrate significant changes from baseline at Month 12 in BMD compared with placebo at each of these anatomic sites. Subjects who received 180 mg denosumab Q6M had the greatest change from baseline in BMD at each site. Subjects who received 60 mg doses of denosumab Q6M also had marked increases from baseline at Month 12 compared to placebo in BMD at all sites. These increases were slightly lower than those observed in the 180 mg group. Among placebo subjects, there was a mean loss in BMD from baseline at Month 12 at each of the anatomic sites.

Treatment with denosumab also resulted in substantial suppression of CTX-I and P1NP (bone turnover markers) as well as CTX-II and CTX-II/creatinine ratio (cartilage markers). CTX-II levels decreased at Month 3, but recovered to near baseline levels by Month 6. Denosumab-treatment did not substantially affect levels of OPG, iPTH or RANKL at any timepoint. The levels of these markers generally remained consistent with the levels at baseline.

In an ad hoc analysis, a strong correlation was shown between BMD at baseline and following discontinuation of denosumab at month 24, confirming the reversibility of effects, as evidenced by an increase in bone turnover markers and consequent decrease in BMD with denosumab discontinuation.

**Safety Results:**

Safety endpoints in the study included the incidence of all adverse events (AEs), changes in laboratory analytes and the subject incidence of anti-denosumab antibodies. The primary focus

of the safety analyses was the 12-month treatment period (ie, baseline to 12); however, adverse events during the off-treatment period (Months 12 to 24) are also reported.

The adverse event profile during the treatment phase of the study was comparable between treatment groups. During the 12-month treatment period, 68 (91%) placebo subjects and 113 (79%) denosumab-treated subjects reported at least 1 adverse event. Seven (9%) placebo subjects and 18 (13%) denosumab-treated subjects reported AEs that were considered at least possibly related to treatment by the investigator. Treatment-related AEs (as determined by the attribution of the clinical investigator) that were reported by 2 or more denosumab-treated subjects included upper respiratory tract infection (3 subjects), headache (2), herpes zoster (2), and muscle spasms (2). Upper respiratory tract infection and muscle spasms were also each reported by 1 placebo subject. Serious adverse events (SAEs) were reported by 7 (9%) placebo and 9 (6%) denosumab-treated subjects. One placebo subject and one subject who received 180 mg denosumab experienced an AE of breast cancer that led to study withdrawal. These events were not considered related to investigational product.

During the off-treatment period (Months 12 to 24), 41 (68%) placebo and 75 (60%) denosumab-treated subjects experienced at least one AE. Only three subjects (one in each treatment group) experienced an AE that was considered treatment-related by the investigator. These events included 2 reports of sinusitis (one in each denosumab group) and 1 report of rheumatoid arthritis in a placebo subject. SAEs were reported in similar proportions of subjects during the treatment and off-treatment periods, and were similar among treatment groups. During the off-treatment period, 6 (10%) placebo and 10 (8%) denosumab-treated subjects experienced SAEs.

No serious adverse events reported throughout the 24 month study period were considered related to treatment. Two subjects (one in the placebo group and one in the 180 mg denosumab group) were withdrawn from the study due to adverse events. In each case, these subjects were diagnosed with breast cancer. No deaths occurred during the study. No neutralizing antibodies to denosumab were detected throughout the study.

**Other Analyses:**

Pharmacokinetic evaluations included the determination of denosumab concentrations in serum samples collected from patients at Months 1, 3, 6 and 12. Serum denosumab concentrations were dose dependent with the highest values occurring in samples collected at Month 1. Mean month 1 concentrations in the 180 mg group were 3.9-fold that in the 60 mg group. Trough concentrations did not increase after the second dose, which indicate drug accumulation did not occur.

---

**Conclusions:**

This was a phase 2, multicenter, randomized, double-blind placebo-controlled parallel-group study evaluating two different doses of denosumab Q6M versus placebo in patients with RA being treated with methotrexate (MTX). Treatment with denosumab demonstrated marked decreases in the progression of erosion by MRI, increases in BMD at all anatomic sites measured, and decreases in the levels of bone turnover and cartilage markers.

There were no safety events of concern noted for subjects treated with denosumab. Overall, the incidence of adverse events during the treatment period in each of the two denosumab-treated groups was similar to that in the placebo group. There were no treatment-related serious adverse events and no deaths in the study.

This study demonstrated that marked and statistically significant reductions from baseline in joint erosion at Month 6 are possible for subjects with RA administered 180 mg denosumab once every 6 months. This benefit appeared to persist through 12 months of follow-up. This dose also resulted in substantial increases in BMD of the lumbar spine, total hip, femoral neck, and

trochanter, and was also accompanied by increases in BMD of the hand. Bone turnover markers, CTX-I and P1NP, were suppressed 3 and 6 months following both doses of denosumab. Although CTX-II and CTX-II/creatinine levels were decreased at month 3 they recovered to near baseline by Month 6, suggesting that more frequent dosing may be necessary with regard to cartilage preservation.