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 RANK ligand

Title of Study:
A Transiliac Crest Bone Histology and Histomorphometry Study in Postmenopausal Women with Low Bone Mass or Osteoporosis Previously Treated with Denosumab

Investigator(s) and Study Center(s):
This study was conducted at 2 sites, 1 in Canada and 1 in Argentina:

[redacted], MD

[redacted], MD

Publication(s):

Brown JP, Dempster DW, Ding B, et al. Effects of denosumab discontinuation on bone histology and histomorphometry [abstract]. Osteoporos Int 2010:21(Suppl1):S134.

Brown JP, Dempster DW, Ding B, et al. Remodeling status in postmenopausal women who discontinued denosumab treatment. [oral presentation 1069] J Bone Miner Res 2010; 25 (suppl 1). Available at http://222.asbr.org/Meetings/AnnualMeeting/AbstractDetail.aspx?aid=51d4e88b-f79d-47e2-a15b-134f0c57b52e. Accessed 26 November 2010.

Wagman RB, Brown JP, Dempster DW, et al. Bone Remodeling in Postmenopausal Women who Discontinued Denosumab Treatment. [oral presentation 2161] Arthritis & Rheumatism 2010; 62(10) Suppl: S 905.

Study Period:
02 June 2009 (first subject enrolled) through 10 June 2010 (last subject's end-of-study visit)

Development Phase: 2

Introduction and Objectives:
Denosumab is a fully human IgG2 monoclonal antibody that binds with high affinity and specificity (Kd 3 x 10^{-12} M) to RANK ligand (RANKL). This binding prevents the activation of RANK and inhibits the formation, function, and survival of osteoclasts. The result is a reduction in the number and function of osteoclasts. The 3-year data from the randomized, double-blind, placebo-controlled, phase 3 trial in women with postmenopausal osteoporosis (20030216) demonstrated that denosumab treatment reduced the incidence of new vertebral fractures, new non-vertebral fractures, and hip fractures when compared with placebo (Cummings et al 2009). Evaluation of transiliac crest bone biopsy samples in this study showed evidence of reduced bone turnover at the tissue level in subjects receiving denosumab, and up to one third of subjects treated with denosumab did not have evidence of tetracycline labeling in trabecular or cortical bone (Reid et al, 2010).

Discontinuation of denosumab therapy, as assessed by biochemical markers of bone turnover and bone mineral density (BMD), has been evaluated in the osteoporosis clinical trial program in two studies: Study 20010223 (phase 2 study of denosumab for the treatment of postmenopausal osteoporosis) and Study 20040132 (phase 3 study of denosumab for the prevention of postmenopausal osteoporosis). These studies demonstrated that the effects of denosumab are reversible. Discontinuation of treatment was associated with increases above baseline levels in biochemical markers of bone turnover, which overall remained within the laboratory reference range and approached pretreatment levels by 18-24 months after therapy cessation. BMD generally returned to pretreatment levels at all measured sites following discontinuation (but remained above levels seen in the placebo group), indicating that the magnitude of the reduction in BMD following discontinuation of denosumab treatment was similar to the level of increase in BMD during treatment. While observed changes in bone density and biochemical markers of bone turnover suggested reversibility of the effect of denosumab, the precise nature of changes in microstructure and mineralization in bone has not been well understood. In a previous study, bone biopsy results in subjects treated with denosumab showed normal bone architecture, lamellar appearance, and mineralization, as well as histormorphometric changes consistent with decreased bone turnover (Reid et al, 2010). The present study investigated the reversibility of the effect of denosumab at the tissue level by evaluating histology and structural and rate-dependent histomorphometric variables.

The primary objective of the study was to characterize the effects of discontinuation of denosumab therapy on variables of bone histology in postmenopausal women with low bone mass or osteoporosis.

The secondary objectives were to characterize the effects of discontinuation of denosumab therapy on:

- variables of bone histomorphometry in postmenopausal women with low bone mass or osteoporosis, and
- level of the biochemical markers of bone turnover C-telopeptide (CTX-1) and procollagen type 1 amino-terminal propeptide (P1NP)

Methodology:
This was a phase 2 cohort study that enrolled subjects who had previously received denosumab and completed one of the following studies: study 20050179, study 20050141, study 20060237, or study 20030216 (without enrolling in the extension study 20060289). Subjects who participated in the off-treatment imaging study for 20080747 also were eligible. The study comprised a screening visit, 2 cycles of tetracycline (or tetracycline derivative) dosing, and a transiliac crest bone biopsy procedure, with a follow-up visit 7 days after the biopsy procedure. No study drug was administered.

Number of Subjects Planned:
15

Diagnosis and Main Criteria for Eligibility:
Subjects were postmenopausal women who had received denosumab and completed study 20050179, completed study 20050141, completed study 20060237, or completed study 20030216 but did not enroll in study 20060289. Subjects who participated in the off-treatment imaging study for 20080747 also were eligible. Subjects had to have completed participation in the eligible studies ≥ 12 and ≤ 36 months prior to screening into this study.

Investigational Product, Dose and Mode of Administration, Manufacturing Batch Number:
No investigational product was administered in this study.

Duration of Treatment:
A transiliac crest bone biopsy was performed following standard labeling procedures with tetracycline or tetracycline derivative. The duration of the study for each subject was expected to be 32 to 41 days.

Reference Therapy, Dose and Mode of Administration, Manufacturing Batch Number:
No reference therapy was administered in this study.

Study Endpoints

Primary Endpoint:

- bone histology as part of the qualitative assessment of bone, with normal bone histology characterized by presence of normal lamellar bone, mineralization, and osteoid, as well as absence of osteomalacia, marrow fibrosis, and woven bone

Secondary Endpoints:

- bone histomorphometry variables including cancellous bone volume, trabecular number, separation and thickness, cortical width, surface density, osteoblast-osteoid interface, osteoid surface, width and volume, wall thickness, eroded surface, osteoclast number, single label and double label surface, mineralizing surface, mineral apposition rate, adjusted apposition rate, bone formation rate, formation period, activation frequency, and mineralization lag time

- biochemical markers of bone turnover, CTX-1 and P1NP

Statistical Methods:
No formal hypothesis was tested in this study. All analyses were descriptive in nature. Frequencies and percentages were presented for all categorical variables. Continuous variables were summarized descriptively using mean, standard deviation (SD), minimum, maximum, median, and other selected percentiles. A descriptive comparison to placebo-treated subjects enrolled in the biopsy substudy for study 20030216 was tabulated. Since the 2.5 and 97.5 percentiles for histomorphometry measurements were either the same or very close to the minimum and maximum values observed in the 20030216 substudy placebo biopsies, the observed range was used as the comparison instead of the 95% confidence interval.

Summary of Results:

Subject Disposition:
A total of 15 subjects were screened and all 15 subjects enrolled. Five of the enrolled subjects had originally participated in study 20050141 and 10 subjects had participated in study 20050179. All 15 subjects had 1 evaluable biopsy and all 15 completed the study. Mean time since denosumab discontinuation, defined as 6 months after last denosumab injection to first tetracycline labeling cycle, was 25.1 months and ranged from 21 to 29 months. All subjects had previously received 1 year of denosumab treatment in the parent studies.

Efficacy Results:
All subjects' biopsies were evaluable for histology and showed normal histology, defined as normal lamellar bone and mineralization, without evidence of osteomalacia, woven bone, or marrow fibrosis. Fourteen of the 15 subjects had biopsies that were evaluable for histomorphometry; one specimen had crush artifact and was not evaluable. Thirteen specimens had double label in trabecular and cortical bone, and 1 specimen had single label in trabecular bone and double label in the cortical compartment. The specimen with crush artifact had evidence of single label in trabecular bone and no label in cortical bone.

Values for static and dynamic histomorphometry variables were generally within the range as compared with the placebo-treated postmenopausal women in Study 20030216.

The biochemical markers of bone turnover CTX-1 and P1NP had returned to levels similar to those seen at baseline in the parent studies 20050141 and 20050179. Approximately 2 years after discontinuing treatment with denosumab and without starting new treatment for osteoporosis, the median value (Q1, Q3) for CTX-1 was 0.646 ng/mL (0.494, 0.789), compared with the parent study baseline of 0.573 ng/mL (0.471, 0.769) and the median (Q1, Q3) for P1NP was 50.70 µg/L (40.80, 58.90) compared with the parent study baseline of 43.08 µg/L (37.60, 50.34).

Safety Results:
All subjects experienced biopsy-related adverse events, with procedural pain (12 subjects, 80%) and post-procedural hematoma (6 subjects, 40%) the most frequent. Other infrequently reported incidence of procedure-related adverse events included dyspepsia, hot flush, myalgia, and vomiting. These adverse events were consistent with what is expected after administration of tetracycline for bone biopsy labeling and after the bone biopsy procedure. No serious adverse events were reported.

Conclusions:
The effects of denosumab on bone remodeling, as assessed by biochemical markers of bone turnover and histomorphometry, are reversible. These data suggest that subjects who discontinue denosumab return to pretreatment levels of bone remodeling. Bone histology does not show evidence of pathology with treatment discontinuation.