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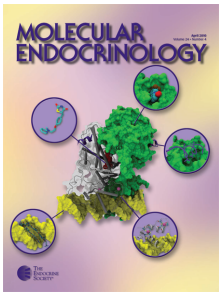

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Effect of Denosumab on Bone Mineral Density and Biochemical Markers of Bone Turnover: Six-Year Results of a Phase 2 Clinical Trial

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Context: This is a study extension to evaluate the efficacy and safety of long-term treatment with denosumab in postmenopausal women with low bone mass.

Objective: Our objective was to describe changes in bone mineral density (BMD) and bone turnover markers as well as safety with 6 yr of denosumab treatment.

Design: We conducted an ongoing 4-yr, open-label, single-arm, extension study of a dose-ranging phase 2 trial. This paper reports a 2-yr interim analysis representing up to 6 yr of continuous denosumab treatment.

Setting: This multicenter study was conducted at 23 U.S. centers.

Patients: Of the 262 subjects who completed the parent study, 200 enrolled in the study extension and 178 (89%) completed the first 2 yr.

Intervention: All subjects received denosumab 60 mg sc every 6 months.

Main Outcome Measures: We evaluated BMD at the lumbar spine, total hip, femoral neck, and one third radius; biochemical markers of bone turnover; and safety, reported as adverse events.

Results: Over a period of 6 yr, continuous treatment with denosumab resulted in progressive gains in BMD in postmenopausal women with low bone mass. Reduction in bone resorption was sustained over the course of continuous treatment. Independent of past treatment and discontinuation period, subjects demonstrated responsiveness to denosumab therapy as measured by BMD and bone turnover markers. The safety profile of denosumab did not change over time.

Conclusions: In this study, denosumab was well tolerated and effective through 6 yr of continuous treatment in postmenopausal women with low bone mass. (***J Clin Endocrinol Metab*** **96: 394–402, 2011)**

At menopause, with reduction in estrogen levels, substantial bone loss commences with accelerated bone turnover. Resultant microarchitectural deterioration, including perforation and loss of trabeculae in cancellous bone, cortical thinning, and increased porosity, leads to an increase in bone fragility and susceptibility to fracture (1, 2). Based on this pathophysiology and the burden of disease, the aim of pharmacological intervention in the treat-

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Abbreviations: BMD, Bone mineral density; BSAP, bone-specific alkaline phosphatase; CTX, C-telopeptide; Q6M, every 6 months; RANKL, receptor activator of nuclear factor-κB ligand.

ment of osteoporosis is to decrease fracture risk. Therapeutics within several classes have been approved for the treatment of osteoporosis and may be categorized by their ability to either decrease bone resorption (antiresorptive therapies) or increase bone formation (anabolic therapies). Evaluation of antifracture efficacy with antiresorptive therapies in randomized, controlled trials and metaanalyses has found associations between the degree of reduction in bone turnover or the increase in bone mineral density (BMD) with the resultant decrease in fracture risk (3–9). Despite the availability of current osteoporosis agents, unmet needs in the treatment of osteoporosis include poor compliance and persistence with therapy, intolerance to therapy, and complexities of administration.

Denosumab (Prolia) is a fully human monoclonal antibody to receptor activator of nuclear factor-κB ligand (RANKL), an osteoblast-derived glycoprotein. Denosumab is an IgG_2 with high affinity ($K_d = 3 \times 10^{-12}$ M) for RANKL (10). Denosumab binds RANKL, preventing the activation of osteoclast-receptor RANK, and inhibiting the formation, activation, and survival of osteoclasts. This results in a reduction in bone resorption and an increase in cortical and trabecular bone mass, volume, and strength (11). Denosumab is a highly specific molecule because it does not bind to other members of the TNF family, including TNFα, TNFβ, TNF-related apoptosis-inducing ligand (TRAIL), or CD40 ligand. As a result of its unique and specific mechanism of action, denosumab is available for treatment of osteoporosis and has been approved in some countries as a therapy for bone loss associated with hormone ablation therapy. In clinical trials, denosumab has been shown to decrease the risk for vertebral, nonvertebral, and hip fractures in postmenopausal women with osteoporosis (12) and risk for new vertebral fractures in men with nonmetastatic prostate cancer receiving androgen-deprivation therapy, with an adverse event profile that was similar to that of placebo (13).

Administration of a product for chronic diseases, such as osteoporosis, warrants characterization of long-term efficacy and safety outcomes. We previously reported results from a phase 2, dose-ranging trial to assess the effects of continuous denosumab administration as compared with placebo or alendronate through 4 yr of treatment (14). This study was extended for an additional 4 yr to permit continued evaluation of efficacy and safety for up to 8 yr of continuous denosumab exposure. We report here interim 2-yr analyses from that extension study, representing up to 6 yr of exposure to denosumab, its longest evaluation to date.

Subjects and Methods

The methods and entry criteria for eligibility in the 4-yr parent phase 2 study have been published elsewhere (14–16). The following summarizes study methodology for the parent study and that for the study extension.

Study design

This open-label, single-arm study extension was conducted in 23 study centers in the United States. An institutional review board reviewed and approved the study protocol at each study center, and the study was performed in compliance with the World Medical Association Declaration of Helsinki principles. All study subjects provided written informed consent.

Subjects

Postmenopausal women aged 80 yr or younger with low bone mass were eligible for the parent study if they had a BMD T-score between −1.8 and −4.0 for the lumbar spine or between −1.8 and −3.5 for either the total hip or femoral neck. For eligibility in the extension study, subjects were required to have successfully completed the parent study, including the end-of-study visit at month 48. Subjects were excluded if they had experienced any of the following during the parent study: severe and/or serious adverse events, including abnormal laboratory results, thought to be related to denosumab; discontinued investigational product due to protocol-specified BMD decrease during the study; missed two or more scheduled administrations of investigational product during yr 3 or 4; used any bone-active drugs; or developed a disease known to affect bone metabolism.

Study procedures

The baseline visit (d 1) of the study extension occurred on the same day as that of the end-of-study visit of the parent study (yr 4 ± 14 d). Subsequent study visits were conducted at the first month of enrollment and every 6 months (Q6M) through yr 5 and 6. Dual-energy x-ray absorptiometry (instrument from GE Lunar, Madison, WI, or Hologic, Waltham, MA) was used to measure BMD at the lumbar spine (L1–L4), total hip, femoral neck, and one third radius at study entry and yr 5 and 6. The same machine was used for the parent and extension studies, and the same side of the body that was measured for the total hip, femoral neck, and one third radius was used whenever possible. Quality control and scan analysis were conducted at Bio-Imaging Technologies Inc. (Newtown, PA).

Serum samples for measuring levels of the bone turnover markers for C-telopeptide (CTX) and bone-specific alkaline phosphatase (BSAP) were drawn after an overnight fast and before the next denosumab dose, and an additional draw for CTX was performed at 1 month after dose in yr 1 and yr 5 of the parent and extension studies. The assay for CTX was Crosslaps Nordic Biosciences (Herlev, Denmark), with coefficient of variation of 4–13%; the assay for BSAP was Tandem-R Ostase (Hybritech Inc., San Diego, CA) or Access Ostase assay (Beckman Coulter, Inc. Fullerton, CA) with coefficient of variation of 3.6–6.4%. In yr 1–4, CTX was processed at Amgen, Division of Pharmacokinetics and Drug Metabolism (Thousand Oaks, CA), where the premenopausal reference range was defined as 200–900 pg/ml. In yr 5–6, CTX was processed by PPD (Richmond, VA), where the premenopausal reference range was defined as 68–661 pg/ml. For the parent and extension studies, BSAP was processed at

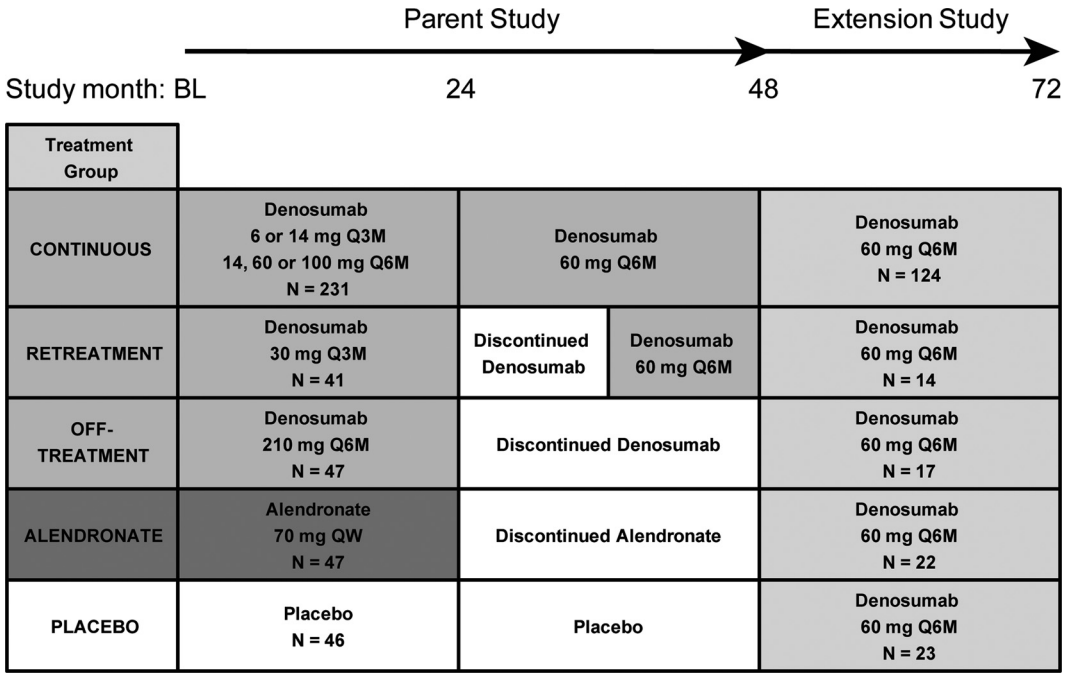

FIG. 1. Study design of the 4-yr parent dose-ranging study showing the initial treatment regimens and changes in treatment that occurred at months 24, 36, and 48 and the 2-yr extension study in which all subjects received denosumab 60 mg Q6M. QW, Every week.

Covance Laboratories (Indianapolis, IN), where the premenopausal reference range was defined as 3.2–20.9 μg/liter. Hematology and safety serum chemistries were performed at study entry, first month of enrollment, and Q6M in yr 5 and 6. Serum denosumab concentrations and anti-denosumab-binding antibody titers were drawn at the time of serum chemistries, except at month 18 in the study extension. Antibody evaluation used a validated electrochemiluminescent immunoassay, and a cell-based assay was used to screen positive samples, as previously described (14–16).

Reports of adverse events were collected at every visit. Information about new fractures was recorded including date, anatomical site, degree of trauma involved, and treatment.

Treatment

Treatment groups in the parent and the extension studies are depicted in Fig. 1. Subjects were instructed to take daily oral supplemental calcium (≥500 mg) and vitamin D (≥400 IU). Although all subjects in the extension study received denosumab 60 mg sc Q6M, they were grouped according to the treatment regimens received during the parent study.

Continuous treatment

Subjects received denosumab for 4 yr. During the first 2 yr, the doses were 6 mg Q3M, 14 mg Q3M, 14 mg Q6M, 60 mg Q6M, or 100 mg Q6M. During yr 3 and 4, all subjects received 60 mg Q6M.

Retreatment

Subjects received denosumab 30 mg Q3M for yr 1 and 2, placebo Q6M for yr 3, and denosumab 60 mg Q6M for yr 4.

Off-treatment

Subjects received denosumab 210 mg Q6M for yr 1 and 2 and placebo Q6M for yr 3 and 4.

Alendronate

Subjects received alendronate 70 mg once weekly for yr 1 and 2 and no treatment for yr 3 and 4.

Placebo

Subjects received placebo for 4 yr.

Statistical analyses

No hypothesis testing was performed because the primary objective was collection of safety information; efficacy endpoints were considered exploratory.

Descriptive statistics were used to summarize extension study subject demographics and baseline characteristics (mean and SD for continuous variables and frequency and percentage for categorical variables). Because this study did not have a control group, data from the first 4 yr of the parent study (14) served as the comparator. We report percent change in BMD at the lumbar spine, total hip, femoral neck, and one third radius from the parent and extension study baseline values as well as actual values of bone turnover markers from the extension study and parent study baseline values. Percent changes in BMD were summarized using analysis of covariance with the treatment groups used singly or pooled as the main effect and geographical location and the parent and extension study baseline BMD values as covariates. The least-squares means and 95% confidence intervals of percent changes from extension and parent study baselines in BMD over the 2- and 6-yr study courses, respectively, were examined. Due to the skewed nature of bone turnover marker data, median and interquartile range (first quartile to third quartile) for actual values were examined over the 6 yr by treatment group.

Safety analysis included subjects who received at least one dose of denosumab. Because this study did not have a control group, subjects were compared with subjects who had received denosumab or placebo in yr 1–4.

TABLE 1. Subject demographics and characteristics at baseline in parent and extension studies

	Parent study, yr 1–4[a]		Extension study, yr 5–6
	All subjects (n = 412)	Denosumab (n = 319)	Denosumab (n = 200)
Age (yr)	62.5 (8.1)	62.3 (8.0)	66.1 (7.7)
Time since menopause (yr)	16.2 (9.9)	16.5 (9.8)	19.3 (9.1)
Lumbar spine BMD T-score	−2.14 (0.78)	−2.14 (0.77)	−1.55 (0.96)
Total hip BMD T-score	−1.44 (0.71)	−1.42 (0.69)	−1.21 (0.73)
Femoral neck BMD T-score	−1.87 (0.67)	−1.86 (0.68)	−1.68 (0.70)
One third radius BMD T-score	−1.48 (1.21)	−1.48 (1.18)	−1.35 (1.19)
Subjects who completed [n (%)]	262 (64)	203 (64)	178 (89)

Values are mean (SD) unless indicated otherwise.

[a] Values from parent study (14–16).

Results

Patients

This 2-yr interim report provided information from May 23, 2006 (first subject enrolled), to May 19, 2009 (last subject completed yr-6 study visit). Of the 200 subjects who entered the extension study, which represented yr 5, 178 (89%) completed the 6-yr assessment (Fig. 1). Baseline demographics for the cohort have been reported elsewhere (14–16). Review of demographics at study extension entry revealed an older cohort, reflecting 4 yr that had passed since enrollment in the parent study (Table 1). Also of note, BMD T-scores at the lumbar spine, total hip, femoral neck, and one third radius were higher compared with their baseline demographics in the parent study, reflecting treatment interventions in the antecedent 4 yr (Table 1).

Efficacy assessments

Continuous treatment cohort

In the continuous treatment group, 2 additional years of denosumab treatment led to further gains in BMD. From the extension study baseline, mean BMD increased at the lumbar spine by 2.9% (Fig. 2A), total hip by 1.1% (Fig. 2B), one third radius by 1.0% (Fig. 2C), and femoral neck by 1.2% (data not shown). Six years of continuous treatment was associated with mean BMD changes from parent study baseline of 13.3, 6.1, and 1.9% for the lumbar spine, total hip, and one third radius, respectively (Fig. 2), and 5.6% for femoral neck (data not shown). At yr 6, serum CTX remained below parent study baseline with a median reduction of 54.8% compared with baseline (Fig. 3). The level of reduction in CTX was similar through all measured time points in the study extension when CTX was measured at the end of the dose interval. To characterize the effects of short- and long-term denosumab administration on the magnitude of reduction in CTX, we compared CTX values 1 month after dose in yr 1 and 5, which showed median reductions of 89.3 and 91.2%, respectively, compared with parent study baseline (Fig. 4). Median reductions in CTX 6 months after dose for these intervals were 72.1% in yr 1 and 47.5% in yr 5 (Fig. 4).

Retreatment, off-treatment, alendronate, and placebo cohorts

These cohorts had exposure or reexposure to denosumab for 2 yr in the study extension. Regardless of previous pharmacological exposure, these four treatment groups similarly showed gains in BMD at the lumbar spine, total hip, one third radius (Fig. 2), and femoral neck (data not shown). All subjects responded to denosumab with reductions in CTX and BSAP, independent of previous treatment assignments (Fig. 5). Both bone turnover markers remained within the premenopausal reference range when measured in the study extension.

Safety evaluations (Table 2)

All 200 subjects in the study extension 2-yr interim analysis received at least one dose of denosumab, and 176 subjects (88%) received all four doses of denosumab. Because the study did not have a control group, incidence rates of adverse events were compared with those of subjects who received denosumab or placebo in the first 4 yr of the study.

One hundred sixty-six subjects (83.0%) reported at least one adverse event. The three most frequent adverse events were upper respiratory infection (13.5%), arthralgia (11.5%), and back pain (9.0%), findings that were consistent with what has been reported during the previous 4 yr of treatment (Table 2). No other adverse events occurred with an incidence of at least 10% in subjects enrolled in the extension study. To provide comparative incidence, adverse events that were reported as at least 10% in yr 1–4 are provided in Table 2 along with rates reported during 2 yr of the study extension. Five subjects (2.5%) reported adverse events of eczema (four subjects with contact dermatitis and one with eczema). Two subjects (1.0%) had skin infection (one case of skin bacterial

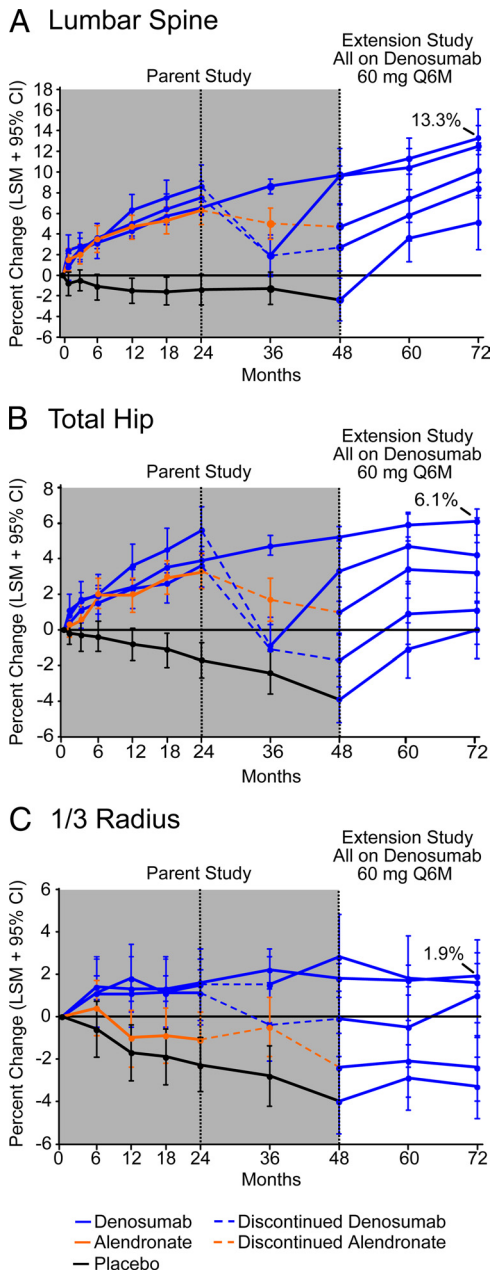

FIG. 2. Effect of 6 yr of denosumab treatment on BMD at the lumbar spine (A), total hip (B), and one third radius (C). BMD values are shown as percent change from parent study baseline [least square mean (LSM) + 95% confidence interval (CI)]. The *vertical dashed lines* at months 24 and 48 indicate changes in dosing regimens. *Gray boxes* indicate the original 4-yr parent study.

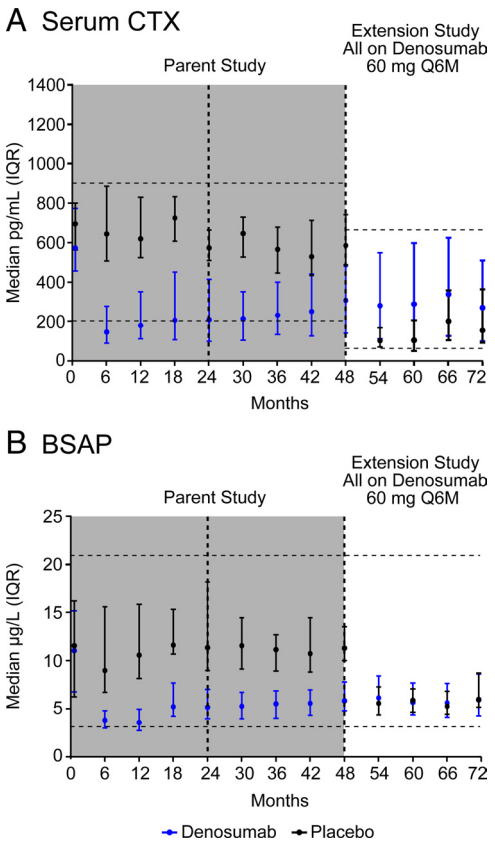

FIG. 3. Effect of 6 yr of continuous denosumab treatment on levels of CTX (A) and BSAP (B). Bone turnover markers are shown as actual values [median with Q1–Q3 interquartile range (IQR)]. The *horizontal dashed lines* represent the premenopausal ranges, and the *vertical dashed lines* at months 24 and 48 indicate changes in dosing regimens. *Gray boxes* indicate the original 4-yr parent study.

infection and staphylococcal infection and one case of skin infection).

Twenty-six subjects (13.0%) experienced an adverse event that was categorized as serious (Table 2). Neoplasms were reported in 3.5% of subjects in the extension study: one subject was diagnosed with liver, bone, and lung metastatic disease (unknown primary), one with breast cancer *in situ*, one with breast cancer, one with benign gastrointestinal neoplasm, two with lung neoplasms, and one with colon cancer. Three infections associated with hospitalization were reported: one case of pneumonia, one case of endocarditis and staphylococcal bacteremia, and one case of diverticulitis (Table 2). Three subjects died during the extension study: one from an unknown cause, 1 from a

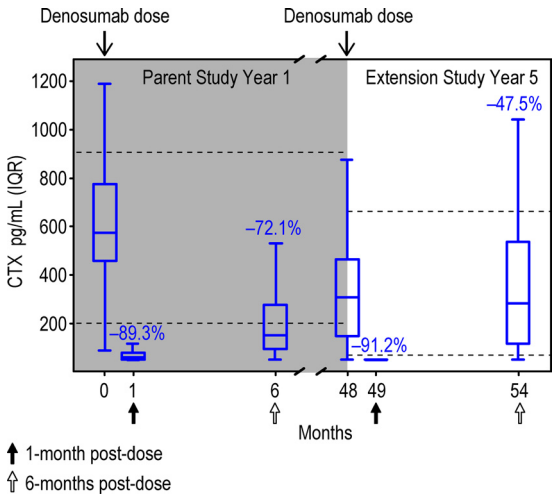

FIG. 4. Comparison of equivalent dosing intervals at 1 and 6 months after dose for serum CTX in yr 1 and 5. Box plots of CTX actual values are shown. The *horizontal dashed lines* represent the premenopausal ranges.

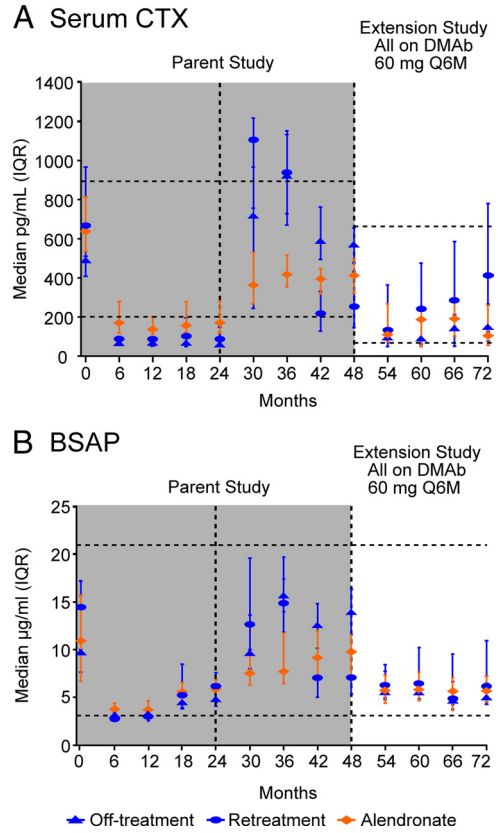

FIG. 5. Effect of bone turnover marker level of CTX (A) and BSAP (B) in the antecedent off-treatment, retreatment, and alendronate treatment groups over 6 yr. Bone turnover markers are shown as actual values [median with Q1–Q3 interquartile range (IQR)]. The *horizontal dashed lines* represent the premenopausal ranges, and the *vertical dashed lines* at months 24 and 48 indicate changes in dosing regimens. *Gray boxes* indicate the original 4-yr parent study.

hepatic malignant neoplasm, and 1 from chronic obstructive pulmonary disease. No case of osteonecrosis of the jaw was reported.

Nine subjects (4.5%) sustained at least one fracture during the extension study; the fractures included fibula, foot, rib, humerus, hand, radius, thoracic vertebra, and tibia (Table 2). There were no reports of delayed fracture healing or fracture nonunion.

No clinically relevant changes in blood chemistries were observed. Although three subjects experienced adverse events considered potential clinical manifestations of hypocalcemia, including paraesthesia (1) and hypoaesthesia (2), no symptoms of hypocalcemia were reported by the investigators. Mean calcium change from parent study baseline was less than or equal to 0.1 mmol/liter for all treatment groups. No subject developed antibodies to denosumab during the extension study.

Discussion

This ongoing study, which was initiated in 2002 and began as a 12-month, phase 2 dose-ranging trial, represents 6 yr of treatment with denosumab, the longest reported clinical experience to date. Phase 2 studies do not require large sample sizes because they are designed to identify optimal dose of investigational product to evaluate in future populations. Nevertheless, important efficacy and safety information may be gleaned from these study subjects (17–20).

In this study extension, gains in BMD were observed for all treated cohorts. For subjects in the continuous treatment cohort, 2 additional years of denosumab treatment led to further gains in BMD. In the other treatment cohorts, subjects with longer duration of prior treatment had smaller gains compared with those who had had shorter prior treatment. Continued BMD accrual over 6 yr, without evidence of plateau, raises the question as to the mechanism by which this finding occurs. One hypothesis is that denosumab, like alendronate, closes the remodeling space and prolongs remodeling with increased mineralization over time (18). Another hypothesis is that denosumab, when compared with alendronate, causes greater reduction in bone resorption and longer remodeling time (21), independent of bone surface available for remodeling, leading to fewer new bone modeling units and concurrent filling in of preexisting resorption cavities (22). A third hypothesis is that the increase in CTX observed at the end of the dosing interval with denosumab permits some degree of remodeling, and the newly formed bone subsequently mineralizes after the next denosumab dose. Our comparison of short- and long-term effects of continuous denosumab administration showed consistent postdose CTX reduction with quantitative CTX increases at the end of the dosing interval. This active profile of serum CTX with denosumab differs from that of alendronate and other bisphosphonate therapies, where a steady state of reduction in bone resorption persists with continued administration (23, 24).

The mechanism for the attenuation in CTX reduction at the end of the dosing interval over time is not fully understood. The bone mechanostat concept suggests that skeletons, even those with low bone mass, appear to possess a bone remodeling set point that might be genetically determined (25, 26). The bone mechanostat concept hypothesizes that each individual has a preset level of bone remodeling and density that is influenced by a variety of genetic and biomechanical stressors on the skeleton. The gradual rise in CTX over time with continuous denosumab exposure may reflect discrete changes in the degree of RANKL expression as a consequence of the mechanostat. Further investigation is required to test such a hypothesis. Development of tolerance to denosumab is unlikely for two reasons: 1) significant reductions in

TABLE 2. Adverse event summary

	Parent study, yr 1–4[a]		Extension study, yr 5–6
	Placebo (N = 46)	Denosumab (N = 314)	Denosumab (N = 200)
Event [% (n)]			
Any adverse event	93.5 (43)	93.3 (293)	83.0 (166)
Infections	67.4 (31)	66.2 (208)	40.0 (80)
Fractures	10.9 (5)	10.5 (33)	4.5 (9)
Serious adverse events	10.9 (5)	17.8 (56)	13.0 (26)
Hospitalized infections	0.0 (0)	3.2 (10)	1.5 (3)
Neoplasms	4.3 (2)	4.8 (15)	3.5 (7)
Treatment-related adverse events	21.7 (10)	20.4 (64)	10.0 (20)
Serious treatment-related adverse events	0.0 (0)	1.3 (4)	1.0 (2)
Withdrawals due to adverse event	4.3 (2)	4.5 (14)	4.0 (8)
Deaths	0.0 (0)	1.3 (4)	1.5 (3)
Adverse events occurring in ≥10 of subjects in any treatment group [% (n)]			
Upper respiratory infection	23.9 (11)	28.0 (88)	13.5 (27)
Arthralgia	30.4 (14)	23.6 (74)	11.5 (23)
Back pain	13.0 (6)	20.1 (63)	9.0 (18)
Nasopharyngitis	15.2 (7)	19.1 (60)	3.0 (6)
Pain in extremity	17.4 (8)	17.5 (55)	5.0 (10)
Hypertension	4.3 (2)	15.3 (48)	6.5 (13)
Influenza-like illness	10.9 (5)	13.1 (41)	1.0 (2)
Urinary tract infection	4.3 (2)	13.1 (41)	5.5 (11)
Gastroesophageal reflux disease	4.3 (2)	12.7 (40)	3.5 (7)
Dyspepsia	6.5 (3)	12.4 (39)	2.5 (5)
Headache	17.4 (8)	12.1 (38)	3.0 (6)
Nausea	4.3 (2)	12.1 (38)	1.5 (3)
Sinusitis	19.6 (9)	11.8 (37)	7.0 (14)
Muscle spasms	15.2 (7)	10.2 (32)	5.5 (11)
Musculoskeletal pain	15.2 (7)	9.6 (30)	4.5 (9)
Diarrhea	13.0 (6)	8.9 (28)	3.0 (6)
Bronchitis	10.9 (5)	8.3 (26)	3.0 (6)
Peripheral edema	10.9 (5)	4.8 (15)	3.0 (6)

N = all subjects who received at least one dose of study drug; n = number of subjects reporting at least one event.

[a] Values from parent study (14–16).

CTX at 1 month after dose were consistent in yr 1 and 5 and 2) CTX levels at the end of the dosing interval were consistently reduced with long-term administration, as evidenced in yr 4–6. These observations suggest a durable response to denosumab with long-term administration.

This study also provides insights into the safety of long-term exposure, where subjects received up to 16 doses of denosumab over 6 yr. No subjects developed neutralizing antibodies to denosumab, demonstrating that there was no evidence of long-term immunogenicity. Reported adverse events were generally mild to moderate in severity and consistent with past reports in yr 1–4 of exposure in this study. There was no discernible pattern of the temporal relationship of the events to investigational product administration, and there was no evidence of increased frequency of a specific event over time. Fracture incidence over time was constant, and there was no evidence of delayed healing. Review of infections during the 2-yr extension was similar to what was observed during the first 4 yr of the study, and there were no cases of opportunistic infections or deaths related to infection. Despite the relatively small sample size in this study, these data provide early information about the safety of denosumab for up to 6 yr of exposure. These data will be expanded upon through a 7-yr study extension to the denosumab pivotal fracture trial, which will provide 10 yr of data regarding long-term efficacy and safety in a larger population of postmenopausal women with osteoporosis.

In conclusion, continuous treatment with denosumab resulted in sustained reduction in bone turnover markers and further gains in BMD over a period of up to 6 yr in postmenopausal women with low bone mass. Independent of past treatment and discontinuation period, subjects demonstrated responsiveness when treated with denosumab therapy, as measured by BMD and bone turnover markers. The overall safety profile in this ongoing study extension did not change over time; denosumab was well tolerated and effective through 6 yr of continuous treatment.

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