

Effects of Denosumab on Bone Mineral Density and Bone Turnover in Postmenopausal Women

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Context: Denosumab is an investigational fully human monoclonal antibody against receptor activator of nuclear factor- κ B ligand, a mediator of osteoclastogenesis and osteoclast survival.

Objective: This study evaluated the ability of denosumab to increase bone mineral density (BMD) and decrease bone turnover markers (BTMs) in early and later postmenopausal women with low BMD.

Design and Setting: This 2-yr randomized, double-blind, placebo-controlled study was conducted in North America.

Participants: Subjects included 332 postmenopausal women with lumbar spine BMD T-scores between -1.0 and -2.5 .

Interventions: Subjects were randomly assigned to receive denosumab sc, 60 mg every 6 months, or placebo. Randomization was stratified by time since onset of menopause (≤ 5 yr or > 5 yr).

Main Outcome Measures: The primary end point was the percent change in lumbar spine BMD by dual-energy x-ray absorptiometry at 24 months. Additional end points were percent change in volumetric BMD of the distal radius by quantitative computed tomography; percent change in BMD by dual-energy x-ray absorptiometry for the total hip, one-third radius, and total body; hip structural analysis; percent change in BTMs; and safety.

Results: Denosumab significantly increased lumbar spine BMD, compared with placebo at 24 months (6.5 vs. -0.6% ; $P < 0.0001$) with similar results for both strata. Denosumab also produced significant increases in BMD at the total hip, one-third radius, and total body ($P < 0.0001$ vs. placebo); increased distal radius volumetric BMD ($P < 0.01$); improved hip structural analysis parameters; and significantly suppressed serum C-telopeptide, tartrate-resistant acid phosphatase-5b, and intact N-terminal propeptide of type 1 procollagen. The overall incidence of adverse events was similar between both study groups.

Conclusions: Twice-yearly denosumab increased BMD and decreased BTMs in early and later postmenopausal women. (*J Clin Endocrinol Metab* 93: 2149–2157, 2008)

Osteoporosis is a skeletal disease characterized by low bone mass and deterioration of microarchitecture, leading to compromised bone strength and susceptibility to fracture (1, 2). Bone turnover increases at menopause, with osteoclast-mediated

bone resorption exceeding osteoblast-mediated bone formation, resulting in bone loss. Bone turnover is especially high in the period just after menopause (3). Decreased levels of estrogen result in increased numbers and activity of osteoclasts through a

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Abbreviations: AE, Adverse event; ANCOVA, analysis of covariance; BMD, bone mineral density; CTX-I, C-telopeptide I; DXA, dual-energy x-ray absorptiometry; HSA, hip structural analysis; iPTH, intact PTH; 25-(OH)D, 25-hydroxyvitamin D; P1NP, N-terminal propeptide of type 1 procollagen; QCT, quantitative computed tomography; RANK, receptor activator of nuclear-factor- κ B; RANKL, RANK ligand; TRAP, tartrate-resistant acid phosphatase.

mechanism driven by receptor activator of nuclear-factor- κ B ligand (RANKL) (4, 5).

RANKL is an essential mediator of osteoclast formation, function, and survival (6–9). RANKL acts by binding to its cognate receptor, receptor activator of nuclear-factor- κ B (RANK), on the surface of osteoclasts and their precursors (7, 10, 11). The natural inhibitor of RANKL is osteoprotegerin, a soluble receptor that binds to RANKL, preventing its interaction with RANK (8, 12). Preclinical studies demonstrated that inhibition of RANKL increases trabecular and cortical bone mass and strength (12–16).

Inhibition of activation of RANK in humans may be achieved therapeutically through denosumab, a fully human monoclonal antibody that specifically targets RANKL to reduce bone resorption. In a phase 2 placebo-controlled, dose-finding study, denosumab treatment for 1 yr significantly increased bone mineral density (BMD) at all measured skeletal sites in postmenopausal women with low bone mass (17). Data from this study suggested that a regimen of denosumab, administered sc 60 mg every 6 months, was suitable for further evaluation of its ability to offset postmenopausal bone loss.

Postmenopausal osteoporosis is defined by a BMD measurement at a clinically important anatomical site that is at least 2.5 SD below the mean for young, adult Caucasian women (T-score ≤ -2.5) (18). However, more than half of osteoporotic fractures occur in women with BMD T-scores above this threshold (19, 20), suggesting the importance of evaluating new therapies for the treatment of bone loss in this population. In this article, we report the results of the principal phase 3 study conducted to evaluate the effect of denosumab, 60 mg administered sc once every 6 months, on BMD and biochemical markers of bone turnover in early and later postmenopausal women with low bone mass.

Subjects and Methods

Study population

Subjects were postmenopausal women with lumbar spine BMD T-scores between -1.0 and -2.5 who were: 1) ambulatory, 2) not receiving medication that affected bone metabolism (other than calcium and vitamin D supplements), 3) free from any underlying condition (other than low BMD) that might have resulted in abnormal bone metabolism, and 4) had no history of a fracture after the age of 25 yr. Women were excluded if they had received oral bisphosphonates for 3 or more yr, cumulatively; fluoride, or strontium ranelate within 5 yr of study enrollment; or PTH or PTH derivatives, steroids, hormone replacement therapy, selective estrogen receptor modulators, tibolone, calcitonin, or calcitriol within 6 wk of study enrollment. Women who had taken oral bisphosphonates for less than 3 months were eligible. Those who had taken oral bisphosphonates for longer than 3 months but less than 3 yr cumulatively were eligible after a 12-month washout period.

Study design

This trial was a randomized, double-blind, placebo-controlled, phase 3 study conducted at 21 centers in the United States and Canada. An extension phase is ongoing. Subjects were randomly assigned (1:1) to receive either sc injections of denosumab, 60 mg every 6 months, or placebo for 2 yr; randomization was stratified by time since onset of menopause (≤ 5 yr or > 5 yr). All subjects were instructed to take supplements totaling at least 1000 mg calcium daily. Daily vitamin D supplementation was determined according to baseline serum 25-hydroxyvitamin D [25-(OH)D] levels. The dosage of vitamin D was at least 400 IU daily if screening 25-(OH)D was more than 20 ng/ml or at least 800 IU daily if screening 25-(OH)D was 12–20 ng/ml. Women with screening 25-(OH)D levels less than 12 ng/ml were excluded or could undergo vitamin D repletion with ergocalciferol for 2 wk. If eligible after vitamin D repletion, women received supplementation according to the vitamin D levels described above. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines, and an independent ethics committee or institutional review board for each study site approved the study protocol. All subjects provided

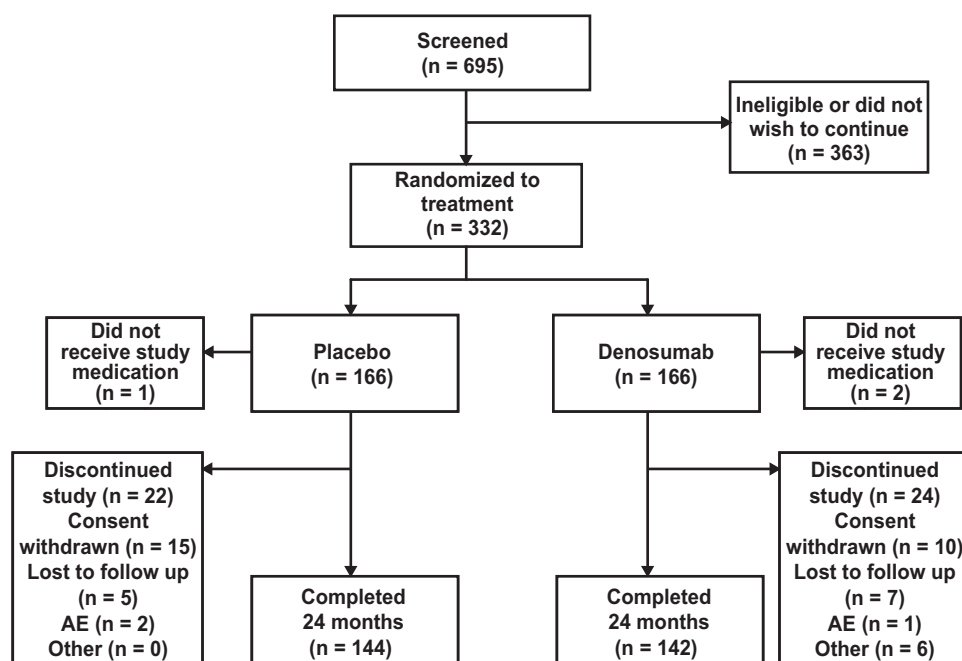


FIG. 1. Disposition of all subjects. In the denosumab group, 142 women completed the 24-month study; in the placebo group, 144 women completed the 24-month study. AE, Adverse events.

TABLE 1. Subject demographics and baseline characteristics [mean (SD)]

Characteristic	Placebo (n = 166)	Denosumab (n = 166)	All (n = 332)
Age (yr)	59.8 (7.5)	59.8 (7.4)	59.4 (7.5)
≤ 5 yr since menopause	53.8 (3.9)	55.2 (3.6)	54.5 (3.8)
> 5 yr since menopause	63.8 (6.9)	64.2 (7.5)	64.0 (7.2)
Years since menopause	9.4 (8.4)	10.5 (9.3)	10.0 (8.9)
≤ 5 yr since menopause	3.4 (2.2)	3.9 (3.2)	3.6 (2.8)
> 5 yr since menopause	15.2 (8.0)	16.9 (8.8)	16.0 (8.4)
Body mass index (kg/m ²)	26.2 (4.8)	26.6 (4.8)	26.4 (4.8)
≤ 5 yr since menopause	25.9 (4.0)	26.7 (5.2)	26.3 (4.6)
> 5 yr since menopause	26.6 (5.5)	26.5 (4.5)	26.5 (5.0)
Lumbar spine BMD T-score	−1.66 (0.44)	−1.55 (0.41)	−1.61 (0.42)
≤ 5 yr since menopause	−1.62 (0.44)	−1.52 (0.39)	−1.57 (0.42)
> 5 yr since menopause	−1.70 (0.43)	−1.58 (0.42)	−1.64 (0.43)
Serum CTX (ng/ml)	0.55 (0.25)	0.53 (0.24)	0.54 (0.25)
≤ 5 yr since menopause	0.58 (0.24)	0.58 (0.27)	0.58 (0.26)
> 5 yr since menopause	0.53 (0.26)	0.48 (0.21)	0.50 (0.24)
Serum P1NP (μg/liter)	59.3 (29.4)	56.9 (25.6)	58.1 (27.5)
≤ 5 yr since menopause	63.5 (30.3)	63.1 (27.2)	63.3 (28.7)
> 5 yr since menopause	55.2 (28.1)	51.1 (22.7)	53.2 (25.5)
Serum TRAP-5b (U/liter)	4.19 (1.28)	4.21 (1.38)	4.20 (1.33)
≤ 5 yr since menopause	4.25 (1.25)	4.39 (1.43)	4.32 (1.34)
> 5 yr since menopause	4.13 (1.32)	4.03 (1.31)	4.08 (1.31)

written informed consent before participating in any study-related procedures. An independent data monitoring committee reviewed all safety and efficacy data at least twice per year.

Assessment of outcomes

Study visits occurred at baseline and months 1, 6, 12, 18, and 24. Denosumab 60 mg (in 1.0 ml, 10 mM sodium acetate, and 5% sorbitol in water for injection) or placebo was administered sc at baseline (d 1) and at months 6, 12, and 18. BMD measurements of the lumbar spine, proximal femur (total hip), one-third radius, and total body were performed by dual-energy x-ray absorptiometry (DXA) using Hologic (Bedford, MA) or Lunar (GE Healthcare, Piscataway, NJ) densitometers. The same DXA instrument was used for all measurements on each subject. Lumbar spine and total hip BMD were measured at baseline and at months 1, 6, 12, and 24. BMD of the one-third radius and total body were measured at baseline and at months 12 and 24.

Trabecular, cortical, and total volumetric BMD were assessed by quantitative computed tomography (QCT) of the forearm at baseline and at 1, 6, 12, and 24 months using standard whole-body spiral computer tomography scanners. The left arm was used unless it was not evaluable, and the same arm was scanned throughout the study. QCT scans were analyzed at a central laboratory (Synarc, San Francisco, CA) using Geanie software (COMMIT, Espoo, Finland). Detailed analyses of the QCT assessments will be presented separately. Here we present the results of total volumetric BMD of the distal forearm. Hip structural analysis (HSA) based on DXA of the total hip was performed on Hologic scans using software developed by Beck *et al.* (21).

Fasting specimens for standard safety chemistries were collected at baseline and at every study visit. Hematology assessments occurred at baseline and months 6, 12, 18, and 24. Fasting samples for determination of serum levels of the bone turnover markers C-telopeptide I (CTX-I), tartrate-resistant acid phosphatase (TRAP)-5b, and intact N-terminal propeptide of type 1 procollagen (P1NP) were collected at baseline and at months 1, 6, 10, 12, 14, 18, and 24. Serum concentrations of CTX-I were assayed at Amgen using Serum CrossLaps ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark); serum concentrations of TRAP-5b and P1NP were assayed at Covance Laboratories using the BoneTRAP Assay ELISA (Medac Diagnostika GmbH, Wedel, Germany) and UniQ P1NP RIA (Orion Diagnostika GmbH, Wedel, Germany),

respectively. Levels of intact PTH (iPTH) in serum were measured at baseline and month 24 by Quintiles Laboratories, Ltd. (N-tact PTH SP ELISA; DiaSorin, Saluggia, Italy). Serum levels of denosumab were determined at all study visits. Antidenosumab antibody assessments were conducted at baseline and at months 1, 6, 12, 18, and 24 using screening methods as described previously (17).

Clinical fractures were defined as new vertebral or nonvertebral fractures for which a subject reported symptoms indicative of a fracture and which were confirmed radiographically (excluding fractures of the skull, facial bones, mandible, metacarpals, and phalanges of fingers or toes). Fractures were also excluded if they were the result of severe trauma as determined by prespecified criteria.

Safety was monitored by recording all adverse events and by evaluating serum chemistry and hematology values. Site investigators classified adverse events as treatment related if they considered the event to be possibly or probably related to the study treatment, without unblinding of the treatment assignment.

End points

The primary efficacy end point was the percent change from baseline in lumbar spine BMD as assessed by DXA at 24 months, compared with placebo. Secondary end points included the percent change from baseline in BMD at the total hip, femoral neck, one-third radius, and total body at 24 months and the percent change from baseline in trabecular, cortical, and total volumetric BMD as measured by QCT at the distal radius at 24 months. Additional end points were the percent change from baseline in bone turnover markers (serum CTX-I, TRAP-5b, and P1NP) at 24 months; the proportion of subjects with BMD gains greater than 0% at the spine and total hip; and parameters of HSA.

Statistical analysis

The primary and secondary efficacy analyses included all subjects with a baseline measurement and at least one postbaseline measurement at or before the month 24 time point [last observation carried forward as the primary imputation method]. Safety analyses included all subjects who received at least one dose of study medication (n = 329; 164 denosumab, 165 placebo).

For each end point analyzed, the primary analysis consisted of an inferential test for treatment differences between the denosumab and placebo groups within the early (≤5 yr) and later (>5 yr) postmeno-

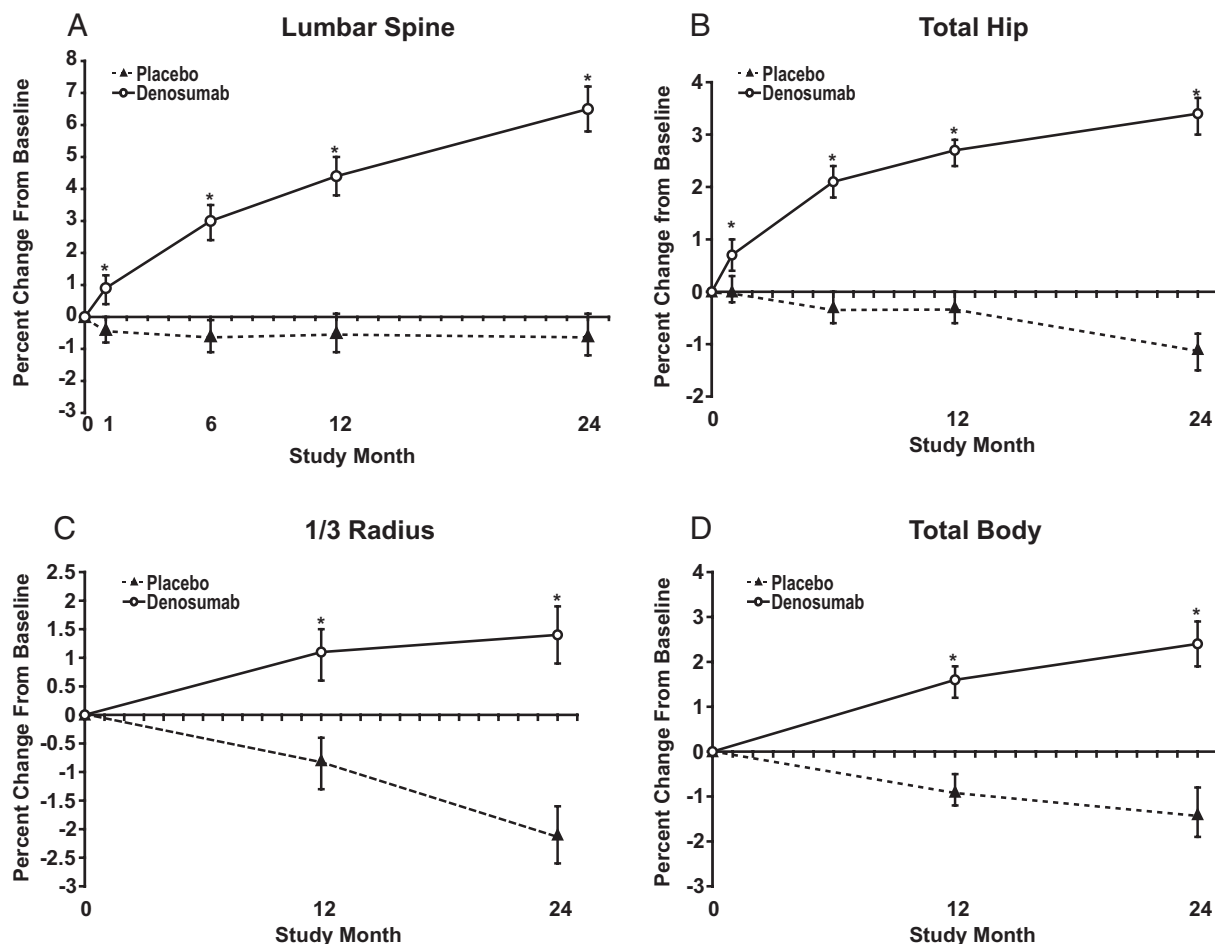


FIG. 2. Percent change from baseline in BMD of the lumbar spine (A), total hip (B), one-third radius (C), and total body (excluding the head) (D) for the overall treatment groups. For one-third radius and total body, BMD was measured at months 12 and 24 only. Results are presented as least-squares means and 95% confidence intervals based on an ANCOVA model adjusting for treatment, machine type, baseline value, baseline value-by-machine type interaction, and strata. *, $P < 0.05$.

pausal strata, respectively. The analysis of the percent change from baseline in lumbar spine BMD for each postmenopausal stratum was performed by using an analysis of covariance (ANCOVA) model with treatment, baseline BMD value, instrument manufacturer, and the interaction of baseline BMD and instrument manufacturer as fixed effects. Multiplicity adjustments were applied to primary and secondary efficacy end points to maintain the overall type I error at 0.025 or less within each stratum. The primary results were based on the point estimate for the least-squares mean and the two-sided 97.5% confidence interval for the treatment difference within each postmenopausal stratum at the 24-month time point. The analyses of QCT and BMD data of the additional body sites were conducted in the same manner. To determine whether there was a difference in treatment effect between strata (the interaction of treatment and strata), an analysis was performed using an ANCOVA model with treatment, baseline BMD value, instrument manufacturer, the interaction of baseline BMD and instrument manufacturer, strata, and the interaction of treatment and strata as fixed effects. If the interaction of treatment and stratum was not significant at the 0.05 level, the treatment effects were presented for the overall results from the ANCOVA models stated above, excluding the interaction of treatment and strata.

Because percent changes in levels of bone turnover markers were skewed, they are reported as medians. Differences in percent change in bone turnover markers between treatment groups for subjects overall and for each postmenopausal stratum were analyzed using the van Elteren stratified rank test (adjusting for time-since-menopause strata) and Wilcoxon rank-sum test, respectively. Comparisons between the denosumab and placebo groups

with regard to safety are considered descriptive and unadjusted for multiple comparisons; P values are based on Fisher's exact tests.

Results

A total of 332 subjects (166 denosumab, 166 placebo) were enrolled in the study. Time since onset of menopause was 5 yr or less in 162 subjects and more than 5 yr in 170 subjects. Eighty-six percent of subjects completed 24 months of treatment. Withdrawal of consent was the most common reason for early discontinuation (6% denosumab, 9% placebo; Fig. 1). The reasons for which subjects withdrew consent were typical for clinical trials and did not display an imbalance between study groups. The most common reasons involved relocation or inconvenience associated with study visits; other reasons ranged from intolerance to the calcium supplement to advice from an herbalist.

Demographics and baseline characteristics of the study subjects are shown in Table 1 and were generally balanced between treatment groups and time-since-menopause strata. Most women (83%) were white. As expected, subjects in the later postmenopausal stratum were older than subjects in the early postmenopausal stratum.

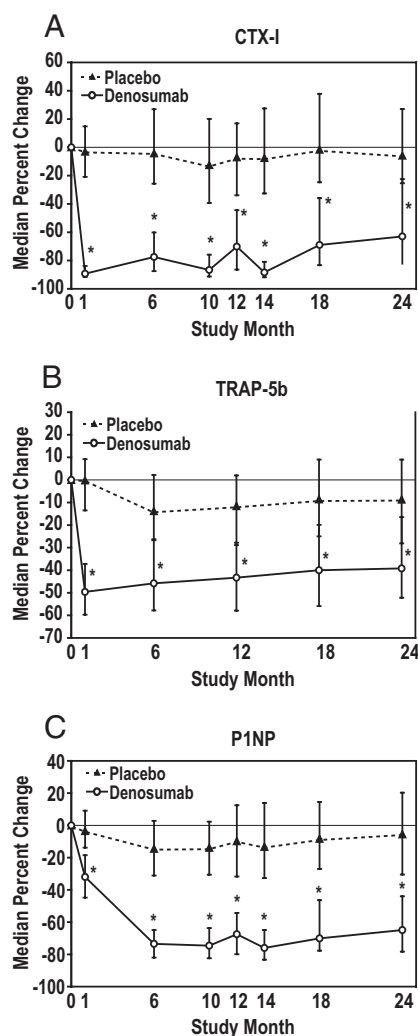


FIG. 3. Percent change from baseline in bone turnover markers: serum CTX-I (A), serum TRAP-5b (B), and serum P1NP (C) for the overall treatment groups. Results are medians. Error bars are interquartile ranges. *, $P < 0.05$, based on the van Elteren stratified rank test. The 10-month data points represent samples collected 16–21 wk after the month 6 dose of the study drug; the 14-month data points represent samples collected 6–15 wk after the month 12 dose of the study drug.

BMD

The lumbar spine BMD increase for the denosumab group overall was 6.5% at month 24, compared with -0.6% for placebo. BMD increases with denosumab were rapid, with significant increases, compared with placebo, observed as early as month 1 and at all measurement intervals thereafter (Fig. 2A). The effect of denosumab treatment was similar between the early and later postmenopausal strata (Table 2).

At month 24, denosumab also significantly ($P < 0.0001$) increased BMD of the total hip, femoral neck, trochanter, one-third radius, and total body regions, compared with placebo for both strata and the strata combined (Table 2). The BMD increases at month 24 for the denosumab group overall were 3.4% at the total hip, 1.4% at the one-third radius, and 2.4% for the total body, compared with changes of -1.1 , -2.1 , and -1.4% , respectively, in the placebo group overall (Fig. 2, B–D).

QCT analysis of the distal forearm showed that denosumab significantly ($P < 0.01$) increased total volumetric BMD at the distal

forearm for both strata and the strata combined, compared with placebo at 24 months (Table 2).

The proportion of women who gained BMD at the lumbar spine (BMD change from baseline $> 0\%$) was 96% in the overall denosumab group and 39% in the overall placebo group ($P < 0.0001$) at 24 months. Similarly, at the total hip, 96% of denosumab subjects experienced a greater than 0% increase in BMD, compared with 31% of subjects in the placebo group, and at the one-third radius, 71 and 22% of denosumab and placebo subjects, respectively, experienced at least a nominal BMD gain. Time since menopause did not influence the BMD response to denosumab.

Hip structural analysis

Cross-sections of the hip at the narrow neck, intertrochanteric, and shaft regions were assessed by HSA using DXA-derived BMD data. Denosumab treatment significantly increased BMD, cross-sectional area, cross-sectional moment of inertia, section modulus, and average cortical thickness relative to placebo at all three cross-sections (Table 3) but had no significant effect on outer diameter. Results were similar for each stratum, except that no significant differences were noted between treatment groups in the early postmenopausal stratum for cross-sectional moment of inertia and section modulus at the shaft.

Bone turnover markers

Markers of bone resorption were rapidly reduced by denosumab treatment. Levels of CTX-I reached a nadir at month 1, with a median reduction of 89% from baseline for the denosumab group overall, compared with a 3% decrease in the placebo group overall ($P < 0.0001$) (Fig. 3A). Continued suppression of CTX-I was maintained thereafter on denosumab treatment with reductions from baseline of 63–88% observed at the remaining study visits. Reductions in serum TRAP-5b showed a similar pattern, with significant reductions from baseline of 40–50% observed throughout the study, beginning at month 1, compared with reductions of 0–14% in the placebo group (Fig. 3B). Denosumab treatment also reduced levels of the bone formation marker P1NP, which declined more gradually than CTX-I or TRAP-5b levels. Significant reductions from baseline in P1NP of 32% were observed with denosumab at month 1, and levels continued to decrease with reductions of 65–76% maintained from month 6 through month 24 (Fig. 3C). P1NP was reduced in the placebo group by median changes of 4–15% during the study. Results for the different time-since-menopause strata were similar.

Fractures

Clinical fractures occurred in seven subjects (4%) in the placebo group and two subjects (1%) in the denosumab group. All the clinical fractures were nonvertebral fractures. One new radiographic vertebral fracture was reported during the study and occurred in the placebo group (1%).

Safety

The overall incidence of adverse events over 24 months was similar between the denosumab and placebo groups. The most common adverse events in both treatment groups were arthralgia, nasopharyngitis, and back pain (Table 4). Sore throat was

TABLE 2. Percent change in BMD (by DXA) of the lumbar spine, total hip, femoral neck, trochanter, one-third radius, and total body and percent change in volumetric BMD of the distal radius by QCT

Site	Stratum	Treatment group	n	Difference from baseline ^a		Difference from placebo ^a		
				LS mean	(CI) ^b	LS mean	(CI) ^b	P value ^c
Lumbar spine	≤ 5 yr since menopause	Placebo	80	-1.2	(-2.3, -0.2)	7.4	(6.1, 8.7)	< 0.0001
		Denosumab	79	6.2	(5.1, 7.3)			
	> 5 yr since menopause	Placebo	83	0.1	(-1.0, 1.2)			
		Denosumab	84	6.8	(5.6, 7.9)			
Overall	Placebo	163	-0.6	(-1.2, 0.1)				
	Denosumab	163	6.5	(5.8, 7.2)				
Total hip	≤ 5 yr since menopause	Placebo	80	-1.0	(-1.6, -0.5)	4.6	(3.8, 5.3)	< 0.0001
		Denosumab	79	3.5	(3.0, 4.1)			
	> 5 yr since menopause	Placebo	83	-1.2	(-1.8, -0.7)			
		Denosumab	84	3.2	(2.7, 3.8)			
Overall	Placebo	163	-1.1	(-1.5, -0.8)				
	Denosumab	163	3.4	(3.0, 3.7)				
Femoral neck	≤ 5 yr since menopause	Placebo	80	-0.9	(-1.7, -0.0)	3.5	(2.3, 4.7)	< 0.0001
		Denosumab	79	2.6	(1.8, 3.5)			
	> 5 yr since menopause	Placebo	83	-0.8	(-1.8, 0.1)			
		Denosumab	84	3.0	(2.0, 4.0)			
Overall	Placebo	163	-0.9	(-1.4, -0.3)				
	Denosumab	163	2.8	(2.3, 3.3)				
Trochanter	≤ 5 yr since menopause	Placebo	80	-0.9	(-1.6, -0.1)	6.2	(5.0, 7.3)	< 0.0001
		Denosumab	79	5.3	(4.5, 6.1)			
	> 5 yr since menopause	Placebo	83	-0.8	(-1.5, -0.0)			
		Denosumab	84	5.0	(4.3, 5.8)			
Overall	Placebo	163	-0.8	(-1.3, -0.3)				
	Denosumab	163	5.2	(4.7, 5.6)				
One-third radius	≤ 5 yr since menopause	Placebo	77	-2.3	(-3.1, -1.4)	3.7	(2.5, 4.8)	< 0.0001
		Denosumab	75	1.4	(0.6, 2.2)			
	> 5 yr since menopause	Placebo	79	-2.0	(-2.9, -1.1)			
		Denosumab	81	1.5	(0.6, 2.3)			
Overall	Placebo	156	-2.1	(-2.6, -1.6)				
	Denosumab	156	1.4	(0.9, 1.9)				
Total body (without head)	≤ 5 yr since menopause	Placebo	77	-1.4	(-2.4, -0.4)	4.2	(2.8, 5.5)	< 0.0001
		Denosumab	75	2.8	(1.7, 3.8)			
	> 5 yr since menopause	Placebo	77	-1.2	(-1.9, -0.5)			
		Denosumab	81	2.3	(1.6, 3.0)			
Overall	Placebo	154	-1.4	(-1.9, -0.8)				
	Denosumab	156	2.4	(1.9, 2.9)				
Volumetric BMD by QCT One-third radius	≤ 5 yr since menopause	Placebo	77	-1.4	(-2.7, -0.1)	2.4	(0.5, 4.2)	0.009
		Denosumab	77	1.0	(-0.3, 2.3)			
	> 5 yr since menopause	Placebo	76	-2.3	(-3.6, -1.0)			
		Denosumab	79	0.6	(-0.6, 1.9)			
Overall	Placebo	153	-1.9	(-2.6, -1.1)				
	Denosumab	156	0.8	(0.0, 1.6)				

^a Based on ANCOVA models that adjust for treatment, baseline value, instrument type, and baseline value-by-instrument type interaction; the models (for overall assessment) also adjust for strata.

^b The 97.5% CI for each stratum and 95% CI for the overall assessment.

^c P values are adjusted for multiple comparisons using both hierarchical testing and Hochberg's procedures.

CI, Confidence interval.

reported more frequently in the denosumab treatment arm than the placebo arm. However, there was no difference in the reported incidence of streptococcal infection (one denosumab, one placebo). The incidence of rashes of all types was greater in the denosumab group than the placebo group. However, the patterns observed were generally not suggestive of drug reactions; there was no consistent pattern in the location or onset of rash in either group, and most of the rashes reported were localized.

Serious adverse events were reported for 18 subjects in the denosumab group (11%) and nine subjects in the placebo group

(5.5%) ($P = 0.074$; Table 4). The higher incidence of serious adverse events in the denosumab group was primarily due to a greater number of subjects who had infections treated as hospital inpatients (eight denosumab, one placebo). The overall incidence of infections reported as adverse events was balanced between the two groups (60% denosumab, 61% placebo). The types of infections reported in the hospitalized subjects were common infections for this subject population: pneumonia, diverticulitis, appendicitis, sepsis, pyelonephritis, urinary tract infection, and cellulitis in denosumab subjects and lobar pneumonia in the placebo subject. No opportunistic

TABLE 3. Summary of hip structural analysis parameters (denosumab difference from placebo in percent change from baseline for combined strata)

Parameter	Narrow neck		Intertrochanter		Shaft	
	Mean (95% CI) ^a	P value	Mean (95% CI) ^a	P value	Mean (95% CI) ^a	P value
BMD	4.9 (3.7, 6.0)	< 0.0001	4.5 (3.6, 5.4)	< 0.0001	2.4 (1.5, 3.4)	< 0.0001
Cross-sectional area	4.9 (3.8, 6.1)	< 0.0001	4.3 (3.3, 5.3)	< 0.0001	2.4 (1.5, 3.3)	< 0.0001
Cross-sectional moment of inertia	4.0 (2.3, 5.7)	< 0.0001	3.9 (2.5, 5.3)	< 0.0001	1.6 (0.5, 2.8)	0.007
Outer diameter	0.1 (−0.5, 0.7)	0.765	−0.2 (−0.7, 0.4)	0.507	0.0 (−0.4, 0.3)	0.783
Section modulus	4.4 (2.8, 6.0)	< 0.0001	4.8 (3.6, 6.1)	< 0.0001	1.7 (0.5, 2.8)	0.004
Endosteal diameter	−0.5 (−1.3, 0.3)	0.227	−1.4 (−3.8, 1.0)	0.256	−1.7 (−2.7, −0.7)	0.001
Average cortical thickness	5.2 (3.9, 6.4)	< 0.0001	4.6 (3.5, 5.8)	< 0.0001	3.1 (1.8, 4.3)	< 0.0001
Average buckling ratio	−5.4 (−6.9, −3.8)	< 0.0001	−5.2 (−6.5, −4.0)	< 0.0001	−3.1 (−4.5, −1.6)	< 0.0001

CI, Confidence interval.

^a Least-squares mean difference from placebo. Based on an ANCOVA model (for overall assessment) that adjusts for strata, treatment, and baseline value. Denosumab group, n = 146; placebo group, n = 143.

infections were reported. Hospitalizations were characterized by uncomplicated courses of 1–6 d and successful treatment with standard antibiotics. None of these infections were considered by the site investigators to be possibly or probably related to the study drug. Neoplasms were reported in four subjects in the denosumab group (breast cancer *in situ*, mycosis fungoides, ovarian cancer, and uterine cancer) and one subject in the placebo group (B cell lymphoma) ($P = 0.215$). There was no specific pattern in the onset of the neoplasms with regard to timing of dose of study drug, and none

were considered by the site investigators to be related to the study drug. Withdrawals due to adverse events were balanced between treatment groups, and no deaths occurred during the study.

As expected, a transient decrease from baseline in mean albumin-adjusted serum calcium levels occurred in the denosumab group at month 1, with levels returning toward baseline and remaining stable thereafter. No subjects experienced symptomatic or clinical adverse events of hypocalcemia; serum calcium transiently fell less than 8.0 mg/dl in 2 subjects. Serum phosphorus levels fol-

TABLE 4. Summary of adverse events

	Placebo (n = 165) n (%)	Denosumab (n = 164) n (%)	P value ^a
Any adverse event	157 (95.2)	156 (95.1)	1.000
Serious adverse events	9 (5.5)	18 (11.0)	0.074
Infection	1 (0.6)	8 (4.9)	0.020
Neoplasm	1 (0.6)	4 (2.4)	0.215
Musculoskeletal or connective tissue disorder	2 (1.2)	3 (1.8)	0.685
Gastrointestinal disorder	0 (0.0)	2 (1.2)	0.248
Injury, poisoning, or procedural complication	1 (0.6)	2 (1.2)	0.623
Reproductive system or breast disorder	1 (0.6)	1 (0.6)	1.000
Hepatobiliary disorder	1 (0.6)	0 (0.0)	1.000
Nervous system disorder	1 (0.6)	0 (0.0)	1.000
Psychiatric disorder	1 (0.6)	0 (0.0)	1.000
Treatment-related adverse events	20 (12.1)	24 (14.6)	0.522
Serious treatment-related adverse events	0 (0.0)	0 (0.0)	
Withdrawals due to adverse events	2 (1.2)	1 (0.6)	1.000
Deaths	0 (0.0)	0 (0.0)	
Adverse events occurring in greater than 10% of subjects in either treatment group or with significant differences between groups			
Arthralgia	42 (25.5)	41 (25.0)	1.000
Nasopharyngitis	31 (18.8)	36 (22.0)	0.496
Back pain	33 (20.0)	33 (20.1)	1.000
Headache	19 (11.5)	26 (15.9)	0.266
Pain in extremity	20 (12.1)	24 (14.6)	0.522
Upper respiratory tract infection	22 (13.3)	19 (11.6)	0.739
Constipation	8 (4.8)	18 (11.0)	0.043
Urinary tract infection	17 (10.3)	18 (11.0)	0.860
Shoulder pain	10 (6.1)	17 (10.4)	0.166
Influenza	18 (10.9)	15 (9.1)	0.714
Sinusitis	17 (10.3)	10 (6.1)	0.228
Pharyngolaryngeal pain (sore throat)	5 (3.0)	15 (9.1)	0.022
Rash	5 (3.0)	14 (8.5)	0.035

^a Based on Fisher's exact test.

lowed the same pattern as serum calcium concentrations with an early decrease from baseline in the denosumab group observed at month 1. Serum iPTH levels measured at baseline and month 24 were similar between treatment groups. No other clinically meaningful differences in blood chemistry measurements and no trends in hematology assessments were observed between treatment groups or over time.

Two subjects (1%) in the denosumab group and three subjects (2%) in the placebo group tested positive for denosumab-binding antibodies; all were negative for neutralizing activity against denosumab. There was no evidence of an effect of these antibodies on safety, pharmacokinetics, or efficacy of denosumab in those subjects receiving denosumab.

Discussion

This phase 3 study evaluated the efficacy of denosumab for increasing BMD and decreasing bone turnover in early and later postmenopausal women with low bone mass. Denosumab, 60 mg administered sc every 6 months, significantly increased BMD at all measured skeletal sites and significantly reduced markers of bone turnover, compared with placebo, with reductions sustained throughout the 24-month treatment period in early and later postmenopausal women. These results confirm the findings of the phase 2 dose-ranging study with denosumab in postmenopausal women (17, 22) and support the ongoing investigation of the denosumab 60 mg, 6-month dosing regimen in phase 3 trials.

The period just after menopause is associated with rapid bone turnover and accelerated bone loss (3). Denosumab treatment produced significant gains in BMD and rapid suppression of bone turnover markers in women within 5 yr of menopause in this study as well as those who were more than 5 yr since menopause.

The effect of denosumab on one-third radius BMD suggests denosumab has a positive and distinctive effect on predominantly cortical bone sites. BMD of the one-third radius typically declines over time in clinical trials with bisphosphonates, although to a lesser degree than seen with placebo (17, 23). The indication of a cortical effect is reinforced by the BMD increases observed with denosumab at the total body and hip regions and by the results of HSA. The HSA data, especially for the femoral shaft region, are consistent with a favorable effect of denosumab on calculated bone structural indices. Thus, it appears that modulation of bone remodeling by monoclonal antibody binding of RANKL results in a pattern of effects on cortical bone that may be beneficial. Whether this observation will translate to a greater reduction in fracture risk remains to be determined and is under study in postmenopausal women with osteoporosis.

A transient decrease in mean serum calcium levels occurred after the first dose of denosumab, as has been observed previously (17). Although serum iPTH levels were not measured at the corresponding time point in this study, a previous study showed the expected transitory increase in iPTH (17). The accompanying decrease in serum phosphorus levels is consistent with such a compensatory increase in iPTH.

Overall rates of infection were similar between the denosumab and placebo groups, with an increased incidence of hospitalized

infections observed in denosumab-treated subjects. The reasons for this finding are unclear. The subjects who developed these common infections did not have unusual clinical courses, and they improved with standard antibiotic treatment. A similar finding of balanced overall infection rates and an increased incidence of hospitalizations associated with infections was observed in another study with denosumab (22). However, in two similarly sized studies with denosumab in patient populations at high risk for infections (women with breast cancer and rheumatoid arthritis patients), the incidence of hospitalizations due to infections and the incidence of overall infections were balanced between the denosumab and placebo groups (24, 25). The overall incidence of rashes of various kinds was also greater in the denosumab group than the placebo group, although the pattern and location of the rashes were not suggestive of a drug reaction. In this study, no differences between the denosumab and placebo groups were noted with regard to mean total white blood cell or differential cell counts. A similar lack of effect on immune cell counts was observed in other studies with denosumab in postmenopausal women (17, 26). In animal models, RANKL inhibition did not affect measures of cell-mediated immunity nor did it alter innate immunity or response to influenza challenge (27, 28). Infection-related adverse events are being closely monitored in ongoing clinical trials with denosumab.

The women in our study had low bone mass but did not meet the diagnostic criteria for osteoporosis. The best treatment strategy for such individuals continues to be debated. Organizations such as the National Osteoporosis Foundation and the American Association of Clinical Endocrinologists recommend consideration of pharmacological treatment for certain women with low BMD (29, 30). The new World Health Organization FRAX tool can be used to estimate fracture risk based on BMD and other factors (31). This may lead to treatment of more patients with BMD in this range when additional risk factors are present. Although their fracture incidence rate is lower than that of osteoporotic women, the majority of fractures occur in this group because they make up a much larger proportion of the population (32). Whereas younger women with low bone mass may have lower near-term fracture risk than older women with similar BMD, the younger women's total lifetime risk may be greater, in view of their longer life expectancy. Factors that should be taken into account in the consideration of preventive therapy for osteoporosis include efficacy, safety, convenience, and simplicity of administration. Denosumab, as a twice-yearly sc injection, is being investigated for its potential to meet these criteria.

In conclusion, twice-yearly treatment with sc 60 mg denosumab in this phase 3 study increased BMD and provided sustained reductions of bone turnover in both early and later postmenopausal women with low bone mass, with an overall incidence of adverse events that was similar to placebo.

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