ORIGINAL ARTICLE

JBMR

Effects of Denosumab on Bone Turnover Markers in Postmenopausal Osteoporosis

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ABSTRACT

Denosumab, a fully human monoclonal antibody to RANKL, decreases bone remodeling, increases bone density, and reduces fracture risk. This study evaluates the time course and determinants of bone turnover marker (BTM) response during denosumab treatment, the percentage of denosumab-treated women with BTMs below the premenopausal reference interval, and the correlations between changes in BTMs and bone mineral density (BMD). The BTM substudy of the Fracture REduction Evaulation of Denosumab in Osteoporosis every 6 Months (FREEDOM) Trial included 160 women randomized to subcutaneous denosumab (60 mg) or placebo injections every 6 months for 3 years. Biochemical markers of bone resorption (serum C-telopeptide of type I collagen [CTX] and tartrate-resistant acid phosphatise [TRACP-5b]) and bone formation (serum procollagen type I N-terminal propeptide [PINP] and bone alkaline phosphatase [BALP]) were measured at baseline and at 1, 6, 12, 24, and 36 months. Decreases in CTX were more rapid and greater than decreases in PINP and BALP. One month after injection, CTX levels in all denosumab-treated subjects decreased to levels below the premenopausal reference interval. CTX values at the end of the dosing period were influenced by baseline CTX values and the dosing interval. The percentage of subjects with CTX below the premenopausal reference interval before each subsequent injection decreased from 79% to 51% during the study. CTX and PINP remained below the premenopausal reference interval at all time points in 46% and 31% denosumab-treated subjects, respectively. With denosumab, but not placebo, there were significant correlations between CTX reduction and BMD increase ($r = -0.24$ to -0.44). The BTM response pattern with denosumab is unique and should be appreciated by physicians to monitor this treatment effectively. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: BONE TURNOVER MARKERS; OSTEOPOROSIS; C-TELOPEPTIDE OF TYPE I COLLAGEN (CTX); PROCOLLAGEN TYPE I N-TERMINAL PROPEPTIDE (PINP); BONE ALKALINE PHOSPHTASE (BALP); TARTRATE-RESISTANT ACID PHOSPHATASE (TRACP 5b); DENOSUMAB

Introduction

Denosumab (Prolia) is a fully human monoclonal antibody to RANKL that blocks its binding to RANK, inhibiting the development and activity of osteoclasts, decreasing bone resorption, and increasing bone density. In the Fracture REduction Evaulation of Denosumab in Osteoporosis every 6 Months (FREEDOM) Trial, denosumab treatment for 3 years

Received in original form March 24, 2010; revised form August 18, 2010; accepted August 31, 2010. Published online September 13, 2010.
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This work was presented in part as an abstract and poster at the 31st Annual Meeting of the American Society for Bone and Mineral Research, Denver, CO, USA, September 11 to 15, 2009.

Journal of Bone and Mineral Research, Vol. 26, No. 3, March 2011, pp 530–537
DOI: 10.1002/jbmr.251

significantly reduced the risk of new vertebral (68%, $p < .001$), hip (40%, $p = .04$), and nonvertebral (20%, $p = .01$) fractures.[1]

Denosumab has an antiresorptive effect on bone and results in a rapid decrease in bone resorption and subsequently bone-formation markers. Such changes have been reported for the bone-resorption markers cross-linked *C*-telopeptide of type I collagen (CTX) and cross-linked *N*-telopeptide of type I collagen (NTX) and the bone-formation markers serum procollagen *N*-propeptide of type I collagen (PINP) and bone alkaline phosphatase (BALP).[2–4] The baseline bone turnover level is related to the bone mineral density (BMD) increase with denosumab (as for alendronate).[5] The effect of denosumab on bone resorption is earlier and of greater magnitude than that of the oral bisphosphonate alendronate, as is the increase in BMD.[6]

The changes in bone-resorption markers with denosumab do not remain steady over the dosing interval; there is suppression within 12 hours of administration of the drug, with median decreases in bone-resorption markers of 80% or more.[2–4,6] There is subsequently a resolution of effect before the next injection, and this cycle of marked reduction followed by a release in bone turnover markers (BTMs) occurs after each injection. After stopping treatment, BTM levels return to baseline, and there follows a transient increase in BTMs above the initial baseline that lasts for up to a year, and this is associated with loss of BMD gained during denosumab therapy.[4]

The decrease in BTMs has been associated with an improvement in fracture risk resulting from anticatabolic treatment; thus larger decreases in BTMs are associated with greater fracture efficacy.[7–10]

The International Osteoporosis Foundation recommends that bone turnover be used for monitoring the treatment of individual patients with osteoporosis.[11] It is therefore important that we have a complete description of the change in BTMs, especially when the drug belongs to a new class and results in a distinct BTM profile compared with existing therapies.

The aims of this study were (1) to describe in detail the time course of BTM response to denosumab, (2) to identify the determinants of BTM response during treatment with denosumab, (3) to estimate the percentage of women with BTMs below the reference interval for premenopausal women while taking denosumab, and (4) to relate the changes in BTMs to those in BMD.

Methods

Study design and patients

The design of the FREEDOM Trial, which has been reported previously,[1] is summarized briefly here. FREEDOM was an international randomized, placebo-controlled trial. A total of 7808 participants were randomly assigned to 1-mL subcutaneous injections of denosumab 60 mg or placebo administered at study sites at baseline and every 6 months for 3 years. Randomization was stratified by 5-year age groups from 60 to 75 years and older.

Women between 60 and 90 years of age with a BMD *T*-score of less than −2.5 at the lumbar spine and/or total hip were eligible for inclusion. Women were excluded if they had a BMD *T*-score of less than −4.0 or conditions that influence bone metabolism or had taken oral bisphosphonates for more than 3 years. If they had taken bisphosphonates for fewer than 3 years, they were eligible after 12 months without treatment before study entry. Women also were excluded if they had used intravenous bisphosphonates, fluoride, or strontium for osteoporosis within 5 years or parathyroid hormone or its derivatives, steroids, systemic hormone-replacement therapy, selective estrogen receptor modulators, tibolone, calcitonin, or calcitriol within 6 weeks of study enrollment. Women enrolled at 18 study sites were given the option of participating in the BTM substudy; 160 women chose to participate.

Efficacy measurements

Concentrations of markers of bone turnover, namely, CTX by ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark), PINP by radioimmunoassay (Orion Diagnostica Oy, Espoo, Finland), TRACP 5b by ELISA (IDS, Scottsdale, AZ, USA), and BALP by immunoassay (Access Bioassay, Beckman Coulter, Brea, CA, USA), were measured in 160 women from fasting serum samples collected before the injection on day 1, at 1 month following the day 1 injection, before injections at 6, 12, and 24 months, and at the 36-month visit. There was a 1-month time window before and after each scheduled visit for injection; thus, for the 6-month visit, women could attend 5 to 7 months after the first injection.

The reference interval applied in this study for CTX, PINP, and BALP was established previously in a study of 145 healthy, young premenopausal women ages 35 to 45 years.[12] The interval for serum CTX was 0.20 to 0.90 ng/mL, for serum PINP was 17.4 to 61.6 ng/mL, and for serum BALP was 5.2 to 17.5 ng/mL. The reference interval for serum TRACP 5b was 1.8 to 4.6 U/L and was based on 20 healthy, young women ages 19 to 39 years (unpublished data).

BMD by dual-energy X-ray absorptiometry (DXA; Hologic, Bedford, MA, USA and GE Lunar densitometers, Madison, WI, USA) was measured at baseline and then annually at the hip and after 36 months at the lumbar spine. Quality assurance of the BMD was provided centrally (Synarc, Portland, OR, USA and Herlev, Denmark).

Statistical analysis

All randomized subjects who participated in the BTM substudy in the FREEDOM Trial and received at least 1 dose of study drug were included for analysis. Missing bone markers at either baseline or postbaseline were not imputed. Any values below the lower limit of quantification (LLOQ) were set to the LLOQ for analysis (0.049 ng/mL for CTX, 10 ng/mL for PINP, and 1.3 U/L for TRACP 5b) and considered observed data, except for BALP, where values below the LLOQ of 9.5 ng/mL were extrapolated in this analysis. Serum samples taken more than 9 months after the previous dose of study drug were excluded. The Wilcoxon rank-sum test was used to assess the significance of the treatment difference at each time point. A Tobit-style model[13] was used to handle the censoring of the CTX values below the quantification limit and to evaluate the relationship between the change in CTX

at month 6 and days since the first injection, adjusting for the baseline CTX value. Spearman correlations were used to assess the relationship between BMD change from baseline at month 36 and change from baseline in BTMs at month 6.

Role of funding source and data access

Study design, data collection, and data analysis were done by Amgen, Inc. All authors, some of whom are employed by the study sponsor, were involved in interpretation of the data. The San Francisco Coordinating Center had access to raw data as requested by the authors, and the authors had access to all relevant summary study data. All requested data and analyses were provided to the authors. The authors were responsible for the decision to submit the article.

Results

Baseline characteristics

The BTM substudy population had similar baseline characteristics to those of the overall population (Table 1). All baseline characteristics also were similar between denosumab and placebo groups except for PINP, which was higher in the placebo group. We had planned to have equal numbers of subjects in each treatment arm, but by chance, more subjects were randomized to denosumab than to placebo in this BTM substudy.

Effects of treatment on bone turnover markers

The changes in BTMs over 36 months are shown as a percentage change from baseline (Fig. 1). This shows the early and large change in the bone-resorption markers CTX and TRACP 5b compared with the bone-formation markers PINP and BALP. The levels of BTMs in absolute units are also shown and compared with the respective reference intervals (Fig. 2). The CTX levels 1 month after denosumab treatment show that these markers were decreased below all marker levels in the placebo group (ie, all denosumab-treated subjects appeared to respond). At 1 month, the levels were below the lower limit of the reference interval in 100% of denosumab-treated subjects and below the lower limit of quantification of the assay (0.049 ng/mL) in 76% subjects. The median CTX levels increased before the next dose at 6 months. The predose CTX levels were higher at the later time points (Fig. 2). The other BTMs showed a similar pattern. The pharmacokinetics of denosumab did not change over the 3 years of the study (data not shown).

The determinants of bone turnover during treatment with denosumab

Since the study protocol allowed for a 28-day time window before or after a scheduled follow-up visit, the "6-month" sample could be collected as early as 5 and as late as 7 months after the first injection [mean (SD) = 179 (16) days]. The level of bone-resorption markers at "6 months" related to the baseline bone-resorption markers and the time since administration of the first dose (Fig. 3). Higher baseline levels of CTX were predictive of higher bone turnover prior to the second injection ($p < .0001$) for both treatment groups (treatment by baseline CTX interaction $p = .8306$). In addition, the longer the time since the initial dose, the higher was the level of bone turnover at 6 ± 1 months ($p < .0001$) for the denosumab group (treatment by time since injection interaction $p < .0001$).

Percentage of women with BTMs below the premenopausal reference interval at the end of the dosing interval during treatment with denosumab

We examined the percentage of subjects with BTMs below the premenopausal reference interval at the time before each injection (Table 2). For CTX, this percentage was as high as 79% at month 6, with lower percentages observed at the later time points. Similar trends in percentages were observed for the markers PINP and TRACP 5b, although these percentages were numerically lower for TRACP 5b. This suggests that more women

Table 1. Baseline Characteristics of Bone Marker Substudy Compared With the Whole FREEDOM Trial

	Bone marker substudy			Whole study
Variable	Placebo ($n = 64$)	Denosumab ($n = 96$)	p Value[a]	Placebo and denosumab ($n = 7808$)
Age, years	72.9 (5.7)	72.3 (5.0)	0.48	72.3 (5.2)
YSM, years	24.1 (8.2)	24.6 (6.4)	0.72	24.2 (7.5)
Height, cm	156.6 (7.0)	156.6 (5.8)	0.99	156.8 (6.7)
Weight, kg	61.2 (7.9)	63.8 (9.9)	0.089	63.8 (10.4)
BMI, kg/m^2	25.0 (3.5)	26.0 (3.9)	0.11	26.0 (4.2)
Prevalent vertebral fractures, %	15.6	24.0	0.20	23.6
Lumbar spine BMD *T*-score	−2.96 (0.59)	−2.88 (0.89)	0.51	−2.83 (0.69)
Total-hip BMD *T*-score	−1.92 (0.76)	−1.93 (0.85)	0.93	−1.90 (0.81)
Serum CTX, ng/mL (median, IQ range)	0.55 (0.42, 0.72)	0.50 (0.35, 0.69)	0.23	0.54 (0.38, 0.72)
Serum PINP, ng/mL (median, IQ range)	51.8 (40.0, 65.6)	44.0 (33.0, 56.3)	0.022	n/a
Serum TRACP 5b, U/L (median, IQ range)	4.4 (3.6, 5.0)	4.1 (3.2, 4.9)	0.42	n/a
Serum BALP, ng/mL (median, IQ range)	14.2 (11.4, 19.1)	13.5 (10.8, 16.4)	0.15	n/a

n = number of randomized subjects; values are mean (SD); YSM = years since menopausal; BMI = body mass index.

[a]Based on *t* test for continuous variables, chi-square test for prevalent vertebral fractures, and Wilcoxon rank-sum test for serum CTX, PINP, TRACP 5b, and BALP.

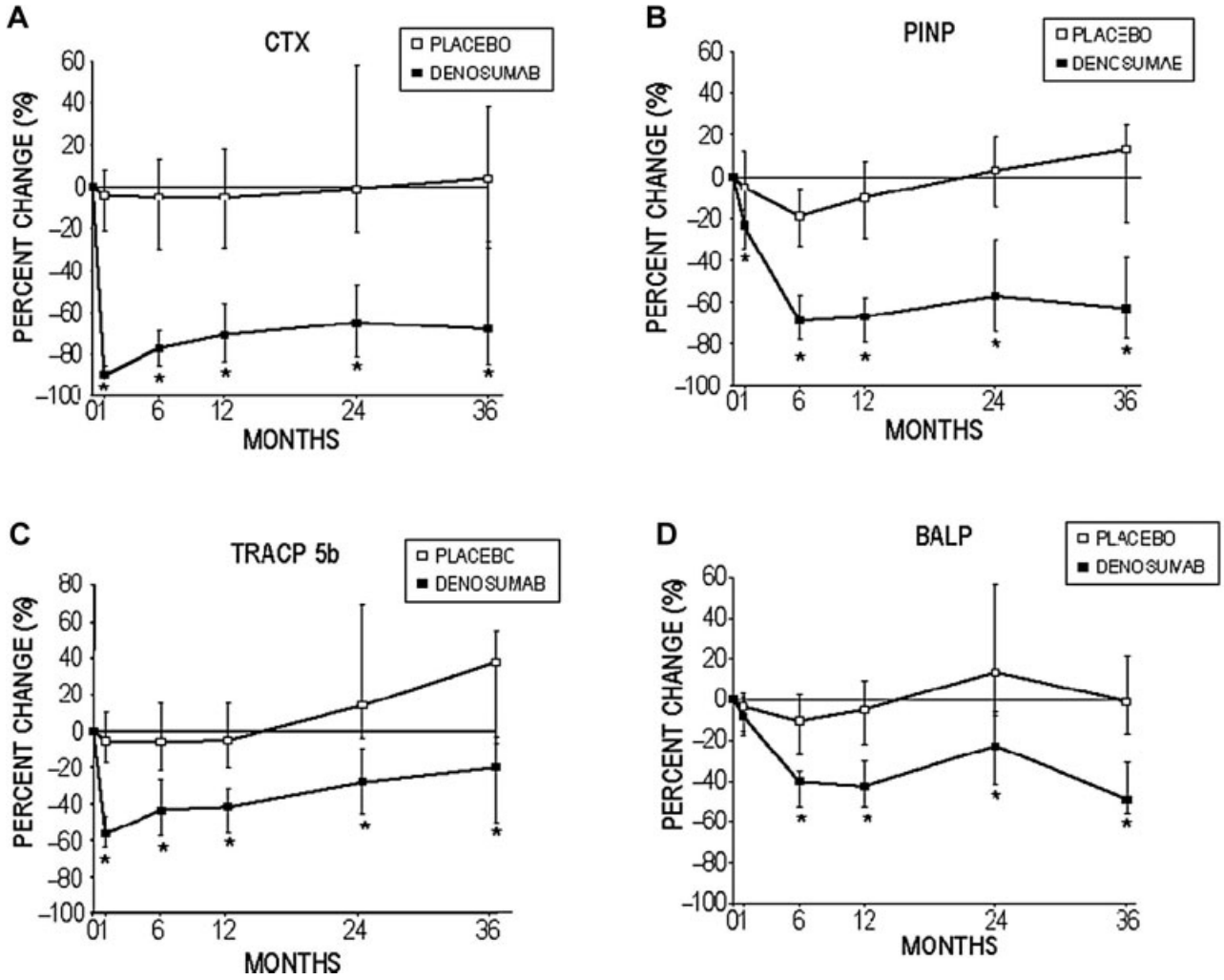

Fig. 1. Percent change from baseline in serum: (*A*) CTX, (*B*) PINP, (*C*) TRACP 5b, and (*D*) BALP over 36 months in the FREEDOM Trial. The horizontal line shows the line of no change, and each point and error bar represent the median percent change and interquartile range, respectively. Samples were measured prior to the next injection, except for the month 1 and month 36 time points, at which a dose was not administered. $^{*}p < .0001$.

in the denosumab group have BTM levels above the lower limit of the premenopausal reference interval at the later time points before dosing in the study (this trend was significant by Mantel-Haenszel at $p < .001$ for CTX, PINP, and TRACP 5b).

Among all denosumab-treated subjects with observed BTM levels at all time points, 46% and 31% of them had BTM levels below the premenopausal reference interval for CTX and PINP, respectively, at all measurements throughout the 36 months (from 6 months onward); no subject had TRACP 5b or BALP below the premenopausal reference interval throughout. Few subjects in the placebo group had values below the lower limit of the premenopausal reference interval at any time point (Table 2), and none in the placebo group had values below the premenopausal reference range at all time points.

Relationship between changes in BTMs and changes in BMD

We examined the relationship between change in BTMs (at 6 months) and BMD changes (at 36 months; Table 3). In general, correlations with markers were stronger for the denosumab group than for the placebo group.

Discussion

There was an initial rapid and very large decrease in CTX such that the CTX values in all denosumab-treated subjects were lower than those in the placebo group, indicating that all subjects responded to treatment. It is notable that the other marker of bone resorption, TRACP 5b, did not decrease to the same extent. TRACP 5b is believed to reflect the number of osteoclasts, whereas CTX is believed to reflect their activity. The clinical significance of an immediate and large decrease in bone resorption (in contrast to the decrease over 3 to 6 months with treatments such as oral bisphosphonates) is not clear. At the level of the bone surface, it appears to result in shallower resorption pits and less bone surface covered by resorption pits.[14]

There was a subsequent increase of CTX into the reference interval in about 20% of women at 6 months before the following

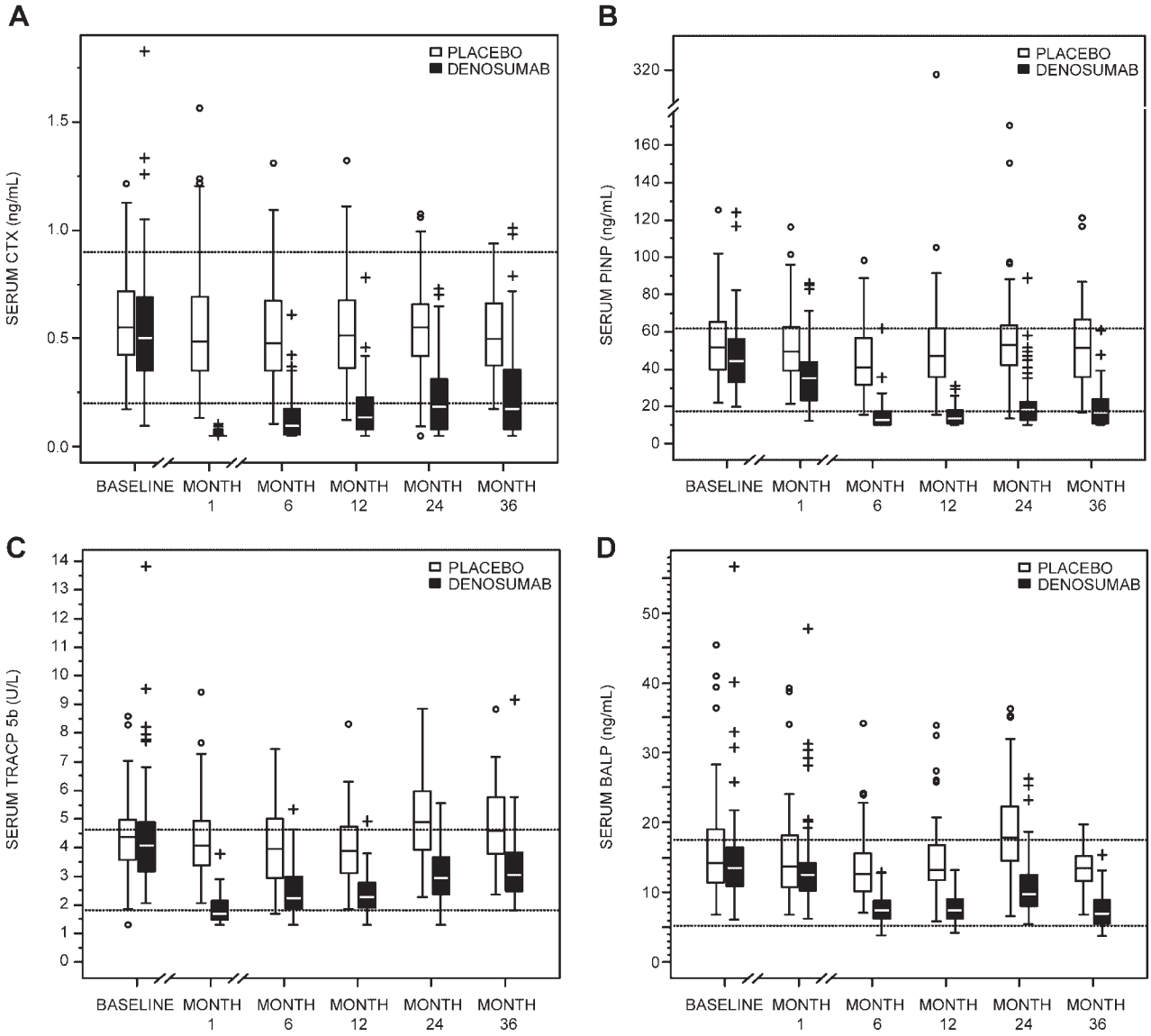

Fig. 2. Absolute values of serum: (*A*) CTX, (*B*) PINP, (*C*) TRACP 5b, and (*D*) BALP over 36 months in the FREEDOM Trial. For each box-and-whisker plot, the whiskers extend to the 10th and 90th percentiles, the boxes extend to the 25th and 75th percentiles, and the middle line represents the median. Horizontal lines represent the premenopausal reference interval[12]: 0.20 to 0.90 ng/mL for CTX, 17.4 to 61.6 ng/mL for PINP, 1.8 to 4.6 U/L for TRACP 5b, and 5.2 to 17.5 ng/mL for BALP. Samples were measured prior to the next injection, except for the month 1 and month 36 time points, at which a dose was not administered.

dose, and this percent increased to about 40% at 3 years. This change in CTX is similar to BTM responses to denosumab reported previously.[2–4] The mechanism for the apparent greater release over time is not fully understood but does not appear to be related to a change in the pharmacokinetics of denosumab over time. It could relate to greater endogenous RANKL synthesis over time to try to "overcome" RANKL inhibition.

Baseline BTM levels vary quite broadly among individual subjects, and the determinants of the variance are not well understood. BTM levels within the premenopausal reference interval at the end of the dosing interval were more likely in an individual if bone turnover was higher at baseline or the next injection was delayed beyond 6 months. The effect of the delay in the timing of the preceding injection on end-of-dosing-interval BTM levels is probably explained by the nature of the relationship between circulating levels of denosumab and its effect on bone turnover.[15] Denosumab levels will have already declined greatly by 5 months, and there would be a further decline by 7 months and hence a release of bone turnover.

The percentage of subjects whose BTM values returned to within or above the reference interval appeared to increase over time (from 21% to 44% for CTX). Between one-third and one-half of subjects remained below the premenopausal reference interval throughout the study. The 6-month change in BTMs was related to the 36-month change in BMD; thus subjects with the largest percent decreases in BTMs at 6 months had the largest increases in BMD at 36 months. The increase in BMD with

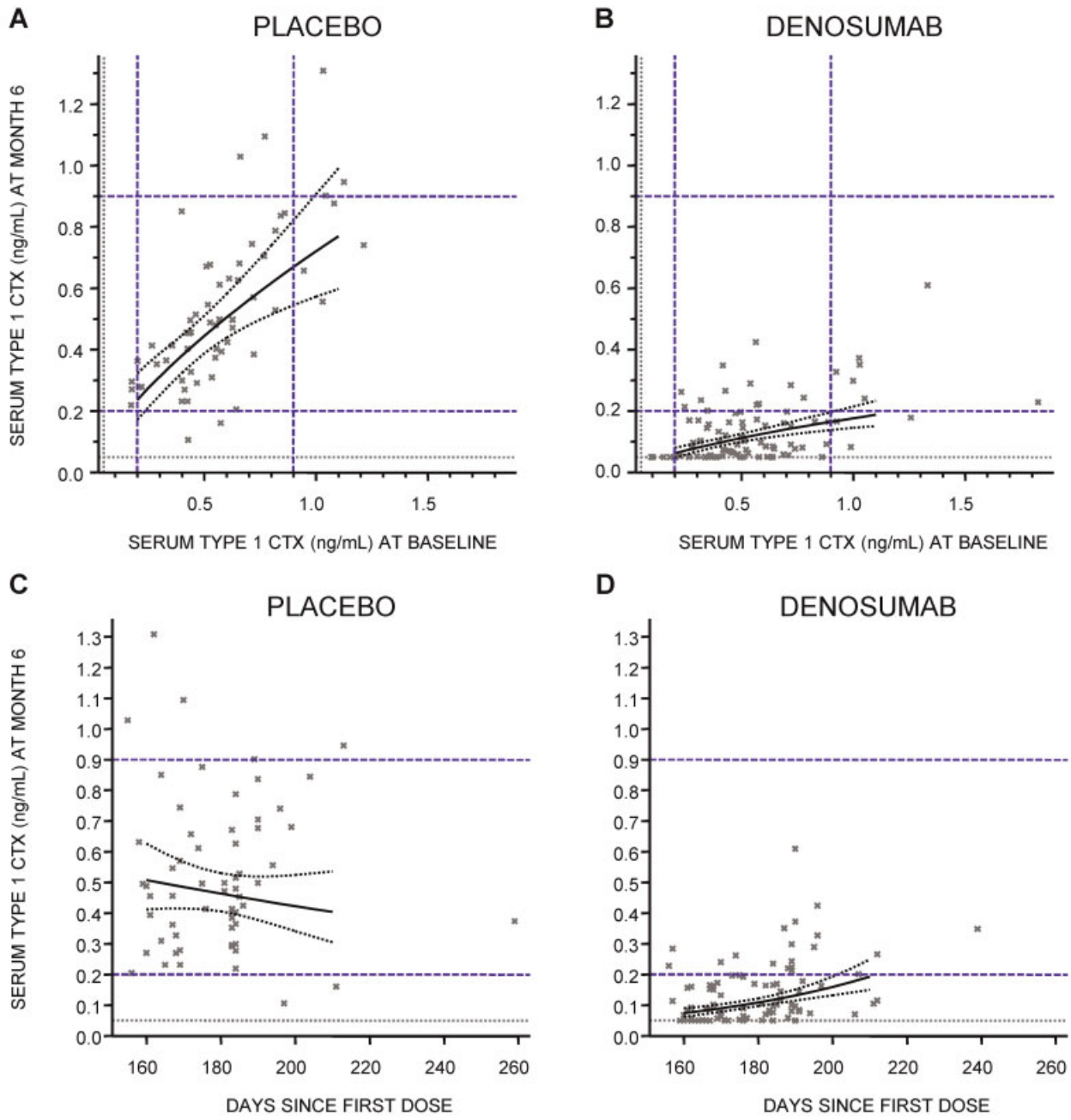

Fig. 3. Relationship between CTX at month 6 and CTX at baseline (*A*, *B*) and time to injection (*C*, *D*). Black lines represent the fit from a Tobit-style model and 95% bands; dashed lines represent the premenopausal reference interval[12]; the lowest dashed lines represent the lower limit of quantification. Both baseline CTX level and time to injection were significant factors for predicting the CTX level at month 6 ($p < .0001$).

antiresorptive therapy is thought to be due to two actions, the closing of the remodeling space and the increase in bone tissue mineralization.[16]

A premenopausal reference interval was used in this study to provide a point of reference, as has been proposed by others.[12,17–19] There appears to be a paradox: The BTMs in the placebo group are mostly within the premenopausal reference interval (only 10% are above this), and yet these patients experience fracture and bone loss. This same paradox was observed in the Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly (HORIZON) study of zoledronic acid.[8] This observation may be explained by the higher median level of BTMs in the placebo group (compared with premenopausal women) and remodeling imbalance with bone formation relatively lower than bone resorption. It is likely that these levels of bone turnover over time are associated with significant deterioration of bone mass and architecture in the postmenopausal woman.

There are some practical clinical consequences of the observations from this study. It will be common to find BTM values below the premenopausal reference interval in women treated with denosumab for osteoporosis. Is this a concern? We do not know for sure, but no adverse skeletal effects were observed over the first 3 years of the FREEDOM Trial.[1] This study has been extended to 10 years of treatment with denosumab so that theoretical concern about bone safety with long-term

Table 2. Percentage of Subjects in the Placebo and Denosumab Groups With BTM Values Below the Premenopausal Reference Interval at the End of the Dosing Interval

	CTX				PINP			
	Placebo ($N = 64$)		Denosumab ($N = 94$)		Placebo ($N = 64$)		Denosumab ($N = 94$)	
	n	%	*n*	%	*n*	%	*n*	%
Month 6	61	3%	92	79%	61	2%	92	78%
Month 12	58	7%	83	69%	58	2%	83	74%
Month 24	49	4%	76	51%	48	2%	76	45%
Month 36	38	5%	59	56%	38	3%	59	56%
	TRACP 5b				BALP			
	Placebo ($N = 64$)		Denosumab ($N = 94$)		Placebo ($N = 64$)		Denosumab ($N = 94$)	
	n	%	*n*	%	*n*	%	*n*	%
Month 6	60	5%	91	23%	59	0%	89	9%
Month 12	58	0%	83	19%	57	0%	82	11%
Month 24	47	0%	74	11%	47	0%	72	0%
Month 36	35	0%	56	0%	21	0%	36	22%

n = number of subjects with an observed value at the time point; % = percentage of subjects with BTMs below reference range (CTX values < 0.20 ng/mL; PINP values < 17.4 ng/mL; TRACP 5b values < 1.8 U/L; BALP values < 5.2 ng/mL).

treatment can be addressed. In the FREEDOM Trial, the pattern of bone turnover observed in response to denosumab was associated with significant reductions in vertebral, nonvertebral, and hip fractures. Data are also available about the association of reduced BTMs and fracture risk from other studies. In the HORIZON study of zoledronic acid given for postmenopausal osteoporosis, about 19% of women had PINP levels below the premenopausal reference interval throughout the 3-year study. The risk of nonvertebral fracture was just as low, if not lower, than in the women with PINP within the premenopausal reference interval.[8] BTM levels within the premenopausal reference interval at the end of the dosing interval with denosumab simply may indicate that the baseline BTMs may have been quite high or that the interval since the last treatment exceeded 6 months.

Table 3. Spearman Correlation Between BMD Change From Baseline at Month 36 and Change in Serum CTX, PINP, TRACP 5b, and BALP at Month 6

	Lumbar spine		Total hip	
	r (*n*)	*r* (*n*)	*r* (*n*)	*r* (*n*)
	Placebo	Denosumab	Placebo	Denosumab
CTX	−0.08 (51)	−0.24 (76)*	−0.21 (51)	−0.44 (74)**
PINP	−0.12 (51)	−0.42 (76)**	−0.11 (51)	−0.47 (74)**
TRACP 5b	0.02 (51)	−0.14 (75)	0.08 (51)	−0.07 (73)
BALP	−0.05 (50)	−0.26 (73)*	0.01 (50)	−0.06 (71)

r = Spearman correlation; *n* = number of subjects with an observed BTM change at month 6 and an observed BMD change at month 36.
*$p < .05$.
**$p < .001$.

Thus denosumab therapy results in an early and large change in the bone-resorption markers CTX and TRACP 5b compared with the bone-formation markers PINP and BALP, followed by a release in BTMs occurring at the end of the dosing interval. This pattern is repeated after each dose.[3,6] Denosumab is a potent inhibitor of bone turnover with a pattern of response that differs, by virtue of its mode of action and pharmacokinetics, from other agents used for the treatment of osteoporosis. This effect on bone remodeling has been associated with significant increases in BMD, and in the FREEDOM Trial of denosumab, significant reductions in the risk of vertebral, nonvertebral, and hip fractures were observed.

Disclosures

RE serves as a consultant, has received honoraria for speaking, and has received grant support from Amgen, AstraZeneca, California Pacific Medical Center, GlaxoSmithKline, Hologic, Kyphon, Inc., Lilly Industries, Maxygen, Nastech Pharmaceuticals, Nestle Research Center, New Zealand Milk, Ltd., Novartis, Novo Nordisk, ONO-Pharma, Organon Laboratories, Osteologix, Pfizer, Procter & Gamble Pharmaceuticals, Roche Diagnostics, Sanofi-Aventis, Servier, Shire, Tethys, TransPharma Medical, Ltd., Unilever, and Unipath. RE contributed to data collection and interpretation, wrote the statistical analysis plan, and wrote the first draft of manuscript. CC is the chairman of Nordic Bioscience A/S and of CCBR/Synarc. He also has received consulting fees from Roche, Wyeth-Ayerst, Eli Lilly, Novartis, Novo Nordisk, Procter & Gamble, Groupe Fournier, Besins EscoVesco, MSD, Chiesi, Boehringer Mannheim, Pfizer, and Amgen. CC contributed to data collection, data interpretation, and critical revision of the manuscript. AG, CL, and AW are employees of Amgen and own stock or stock options in Amgen. AG and CL contributed to study supervision, data collection and interpretation, and critical revision of the manuscript. AW contributed to data analysis and interpretation and critical revision of the manuscript. SK reports receiving consulting fees or participating on paid advisory boards for Amgen and Zentiva; participating as a clinical trial investigator for Amgen, Servier, and Novartis; and providing expert opinions and consultations for Zentiva and Servier. SK contributed to data collection, data interpretation, and critical revision of the manuscript. MRM reports receiving consulting fees or participating on paid advisory boards for Amgen, Eli Lilly, Merck, and Novartis and receiving lecture fees for speaking for Eli Lilly, Novartis, and Sanofi-Aventis. He reports receiving grant support from Amgen, GlaxoSmithKline, Eli Lilly, Merck, Novartis, Procter & Gamble, and Roche. MRM contributed to data collection, data interpretation, and critical revision of the manuscript. IRR reports receiving consulting fees from Amgen, Merck, and Novartis. He receives grant support from Amgen, Novartis, Merck, and Procter & Gamble. IRR contributed to data collection, data interpretation, and critical revision of the manuscript. HR reports no conflicts of interest. HR contributed to data collection, data interpretation, and critical revision of the manuscript. ES reports receiving consulting fees from Amgen, Novartis, and Eli Lilly and has received lecture fees for speaking

for Amgen, Eli Lilly, Novartis, and Sanofi-Aventis. ES contributed to data interpretation and critical revision of the manuscript. DU reports no conflicts of interest. DU contributed to data collection, data interpretation, and critical revision of the manuscript. GW has served as an investigator for Amgen. GW contributed to data collection, data interpretation, and critical revision of the manuscript. SRC reports receiving consulting fees from Amgen, Eli Lilly, and Novartis. SRC contributed to data collection, data interpretation, and critical revision of the manuscript.

Acknowledgments

We thank the patients who participated in the BTM substudy of the FREEDOM trial. Amy K Foreman-Wykert, PhD, of Amgen, Inc., provided editorial and formatting assistance. Matthew Austin, MS, of Amgen, Inc., created the figures. David Shields provided graphics support on behalf of Amgen, Inc. This study was supported by Amgen, Inc.

Clinical trial registration: NCT00089791; registered on August 13, 2004.

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