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Denosumab effects on bone density and turnover in postmenopausal women with low bone mass with or without previous treatment

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ABSTRACT

Purpose: Prior osteoporosis therapies may affect the skeletal response to denosumab. We compared the effect of denosumab (60 mg every 6 months for 12 months) on bone mineral density and bone metabolism parameters in postmenopausal women with low bone mass who were either treatment-naïve ($n = 30$), or previously treated either with zoledronic acid ($n = 30$), or teriparatide ($n = 22$).

Methods: We assessed lumbar spine bone mineral density (BMD) and measured serum concentrations of the bone turnover markers pro-collagen type 1 *N*-terminal propeptide (PINP) and C-terminal-cross-linking telopeptide of type 1 collagen (CTX), as well as sclerostin, dickkopf-1 (Dkk-1), and myostatin.

Results: Lumbar spine BMD increased equivalently in all three groups after 12 months of denosumab compared to baseline ($p < 0.001$). Serum PINP and CTX decreased significantly with denosumab in pre-treated women reaching the same nadir levels as in treatment-naïve patients ($p < 0.001$). Women pre-treated with teriparatide displayed lower baseline myostatin concentrations as compared to the other two groups ($p < 0.001$). Changes in lumbar spine BMD in teriparatide pre-treated women correlated with changes in bone turnover markers and myostatin.

Conclusions: Denosumab induced similar increases in lumbar spine BMD in treatment-naïve and pre-treated patients and suppressed serum PINP and CTX to the same levels regardless of prior treatments. In teriparatide pre-treated patients the magnitude of change in bone turnover markers is associated with BMD response.

1. Introduction

Denosumab, a monoclonal antibody against the receptor activator of nuclear factor κB ligand (RANKL), is a potent antiresorptive agent, which profoundly suppresses bone turnover markers (BTMs), increases bone mineral density (BMD), and reduces fracture risk [1]. The effects on BTMs and BMD are completely reversible after denosumab discontinuation, including a rise of BTMs, a rapid reduction of BMD, and a potential risk of multiple vertebral fractures (VFx) [2,3]. Zoledronic acid (ZOL) is a potent bisphosphonate that exerts antiresorptive activity, while teriparatide (TPTD) is an osteoanabolic agent. Both ZOL and TPTD significantly improve BMD and decrease vertebral and non-vertebral fractures [4,5], although they exert opposite effects on BTMs. Upon discontinuation, ZOL displays a sustained effect on bone metabolism [6], while TPTD gradually loses its effects on BMD and BTMs [7].

During the last years, BTMs have gained importance as surrogate markers to monitor both anti-resorptive and anabolic treatment, and may be used in conjunction with BMD measurements in clinical practice [8]. The two most widely utilized BTMs are pro-collagen type 1 *N*-terminal propeptide (PINP), which reflects osteoblast activity, and C-terminal-cross-linking telopeptide of type 1 collagen (CTX), which corresponds to the osteoclastic function [8]. While prior treatment with bisphosphonates results in smaller BMD and BTM changes in patients transitioning to denosumab as compared to treatment-naïve patients beginning denosumab therapy [9,10], it has also been proposed to blunt enhanced bone turnover and/or attenuate BMD loss after denosumab discontinuation [11]. On the contrary, postmenopausal women

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switching from TPTD to denosumab depicted a continued increase in BMD, with bone resorption maximally suppressed after 1-month of denosumab and a delayed suppression of bone formation with maximal effects after 12–24 months of denosumab treatment [12].

ZOL, denosumab, and TPTD are characterized by the coupling effect in their action, the former two agents decreasing both bone resorption and formation, and the latter stimulating both formation and resorption. Interactions between these agents and the (Wnt)/β-catenin signaling pathway, which constitutes a major promoter of bone formation, have been documented [13]. Serum concentrations of Wnt-antagonists sclerostin and dickkopf-1 (Dkk-1) have been reported in both pre-treated and treatment-naïve patients with postmenopausal osteoporosis, and are believed to reflect their expression in the bone microenvironment [14,15]. Myostatin is a member of the transforming growth factor beta (TGF-beta) superfamily, with major effects on muscle, fat, and bone homeostasis [16]. Next to the pivotal finding that myostatin deficiency is associated with increased muscle mass [17], animal studies report that inhibition of the myostatin pathway is associated with enhanced bone turnover and bone mass accrual [18,19]. In a placebo-controlled multiple dose human study, inhibition of myostatin led to a significant increase of muscle mass, while also causing an up-regulation of bone specific alkaline phosphatase and down-regulation of CTX [20], implying uncoupling of bone remodeling.

In this study, we compared the effects of denosumab following previous treatment with ZOL, TPTD, or no treatment, on spinal BMD and serum concentrations of PINP, CTX, sclerostin, Dkk-1, and myostatin in postmenopausal women with low bone mass.

2. Patients and methods

2.1. Patients

This was a 12-month, observational, monocentric, longitudinal study of 82 postmenopausal women with low bone mass. All data was obtained from patients who were treated and regularly monitored at the outpatient clinics for Metabolic Bone Diseases of 424 General Military Hospital, Thessaloniki, Greece. At the time of the first s.c. injection of 60 mg denosumab, patients were either treatment-naïve ($n = 30$), or had received 1–2 yearly i.v. infusions of 5 mg ZOL ($n = 30$), or had completed 24 months of daily s.c. injections of 20 μg TPTD ($n = 22$).

There was no gap between TPTD or ZOL treatment and denosumab e.g. patients under TPTD received the first denosumab injection no more than two weeks after the last TPTD injection, and patients under ZOL received the first denosumab injection in 1 year ± 2 weeks after the last ZOL infusion. Regarding previous treatment with ZOL, twenty of 30 patients had previously received 1 infusion and 10/30 patients had received 2 infusions of ZOL. The main reason for switching from ZOL to denosumab was the difficulty in prescribing and administering ZOL in Greece due to regulatory restrictions. Other reasons were physicians' and patients' preference due to the convenience of a subcutaneous injection versus an intravenous infusion, as well as the avoidance of an acute phase reaction with ZOL treatment.

Women in all groups were supplemented with calcium 1000 mg/d and vitamin D 800 IU/d throughout the study. Exclusion criteria were: (1) history or presence of bone disease other than primary osteoporosis (e.g., hyperparathyroidism, Paget disease of bone, osteogenesis imperfecta), (2) medication known to affect bone metabolism (e.g., glucocorticoids) within the last 3 years, with the exception of previous antiosteoporotic treatment, (3) any type of cancer, (4) renal and/or liver failure, (5) diabetes mellitus, (6) uncontrolled thyroid disease, and (7) history of primary ovarian failure. The study was approved by the local Ethics Committee and was in accordance with the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice. Informed consent was obtained from all patients.

3. Methods

Baseline assessment was defined as the day of the first s.c. injection of 60 mg denosumab, and comprised of history (including history of previous VFx), physical examination, blood sampling, measurement of bone mineral density at the lumbar spine (lumbar spine BMD), and lateral spine radiographs for the presence of VFx. Areal BMD was measured by dual energy X-ray absorptiometry (DXA) at the lumbar spine (LS) (L1–L4) using a Lunar Prodigy densitometer (Lunar Corporation, Madison, WI, USA) at baseline and 12 months after the first denosumab injection. Morning (08:00–09:00 am) fasting blood samples were obtained from all patients at baseline and 3, and 6 months. The first denosumab injection was administered directly after blood drawing in a standardized manner. Samples were centrifuged immediately, and serum was separated and stored at −80 °C until analyzed at the Technische Universität Dresden Medical Center in one batch per measured parameter. Serum PINP concentrations were measured on the IDS-iSYS Multi-Discipline Automated Analyser using a chemiluminescence assay [Immunodiagnostic Systems Limited, Frankfurt am Main, Germany; intra-assay coefficient of variation (CV) ≤ 3%, inter-assay CV ≤ 5.3%]. Serum CTX concentrations were measured by an immunoassay on the Cobas e 601 Automated Analyser (Roche Diagnostics, Mannheim, Germany; intra-assay CV ≤ 4.7%, inter-assay CV ≤ 5.7%). Other parameters measured by ELISA included sclerostin (Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria; intra-assay CV ≤ 7%, inter-assay CV ≤ 10%), Dkk-1 (Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria; intra-assay CV ≤ 3%, inter-assay CV ≤ 3%) and myostatin (Immundiagnostik AG, Bensheim, Germany; intra-assay CV ≤ 10.4%, inter-assay CV ≤ 14%).

3.1. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Normality of the continuous variables was tested with the Kolmogorov-Smirnov test. Within group comparisons were performed with the paired *t*-test or Wilcoxon signed-rank test, when the repeated measures were two, or repeated measures analysis of variance (ANOVA) or Friedman test, when the repeated measures were more than two. In case of statistically significant difference in repeated measured ANOVA or Friedman test, Bonferroni post hoc adjustment was used for multiple pairwise comparisons. Between group comparisons were performed with independent *t*-test or Mann-Whitney test (in case of two groups), or one-way ANOVA or Kruskal-Wallis test (in case of more than two groups); again Bonferroni post hoc adjustment was used for multiple pairwise comparisons. Spearman's coefficient was used for correlation performed between absolute changes of BMD and absolute changes of indicated serum parameters. A two-sided p value of < 0.05 was considered statistically significant in all the above tests. Statistical analysis was performed with Graphpad Prism version 7 (La Jolla, CA, USA).

4. Results

Baseline anthropometric and lumbar spine BMD data, as well as VFx history is summarized in Table 1. Baseline characteristics were similar between treatment-naïve patients ($n = 30$), and patients pre-treated with ZOL [After ZOL] ($n = 30$), or TPTD [After TPTD] ($n = 22$).

In treatment-naïve patients BMD at the lumbar spine increased by 4.4% ± 0.6% ($p < 0.001$) at 12 months of denosumab treatment. The respective change for patients pre-treated with ZOL increased by 4.1% ± 0.8% ($p < 0.001$), and for patients pre-treated with TPTD by 4.6% ± 0.8% ($p < 0.001$) (Fig. 1). Lumbar spine BMD at 12 months did not differ among groups.

At baseline women pre-treated with ZOL had lower PINP and CTX concentrations compared to treatment-naïve patients ($p < 0.05$ and $p < 0.001$, respectively), and women pre-treated with TPTD significantly higher levels of PINP and CTX compared to treatment-naïve

Table 1
Baseline characteristics of the patients in the 3 groups.

Variable	Treatment naive	After ZOL	After TPTD	*p* value
Age, y	68.9 ± 1.3	67.1 ± 1.4	69.3 ± 1.5	0.488
Age at menopause, y	48.1 ± 0.8	48.7 ± 0.7	48.1 ± 1.1	0.833
Weight, kg	71.3 ± 1.8	69.1 ± 2.2	66.3 ± 1.9	0.185
Height, cm	155 ± 1	155 ± 1.5	155 ± 1.5	0.983
BMI, kg/m^2	29.9 ± 0.8	28.7 ± 0.9	27.6 ± 0.8	0.183
Lumbar spine BMD, g/cm^2	0.84 ± 0.02	0.83 ± 0.03	0.85 ± 0.02	0.189
T-score_LS	−2.35 ± 0.11	−2.04 ± 0.2	−2.4 ± 0.16	0.221
Previous VFx [N (%)]	9 (30%)	10 (33%)	9 (41%)	0.734

Data is presented as means ± SEM.
Abbreviations: BMI, Bone Mass Index; Lumbar spine BMD, Bone Mineral Density at Lumbar Spine; TPTD, teriparatide; VFx, vertebral fractures; ZOL, zoledronic acid.

patients ($p < 0.001$ and $p < 0.05$, respectively), as expected (Table 2). There were no differences between groups in PINP or CTX concentrations at 3 or 6 months of denosumab treatment. At month 6, there was a non-significant trend towards lower sclerostin concentrations in women pre-treated with ZOL as compared to treatment-naïve patients ($p = 0.07$). Sclerostin and Dkk-1 concentrations were not statistically different between groups at all time points. On the contrary, women pre-treated with TPTD displayed lower baseline myostatin concentrations as compared to women pre-treated with ZOL and treatment-naïve women ($p < 0.001$) which remained unaffected by denosumab treatment in all groups (Table 2).

Regarding within group comparisons, PINP and CTX concentrations were significantly lower at months 3 and 6 compared to baseline in all groups of patients (all $p < 0.001$). Concentrations of sclerostin and Dkk-1 remained essentially unchanged in all groups (Table 2).

Table 3 summarizes the correlation coefficients between the absolute change in lumbar spine BMD within 12 months following denosumab initiation and the absolute change of measured bone parameters within 3 or 6 months following denosumab initiation. In patients pre-treated with TPTD, there were negative correlations between changes in lumbar spine BMD at 12 months and changes in PINP at 3 ($r = -0.566$, $p < 0.01$) and 6 months ($r = -0.626$, $p < 0.01$), and with CTX ($r = -0.481$, $p < 0.05$), and myostatin ($r = -0.455$, $p < 0.05$) at 6 months. No other correlations were identified. When we performed correlations between the presence of prevalent VFx and serum parameters at baseline separately for each group, TPTD pre-treated patients with prevalent VFx had lower PINP concentrations at baseline compared to patients without VFx (46.95 vs. 77.57, $p < 0.05$). No other differences in measured parameters at baseline were identified when comparing patients with or without VFx.

5. Discussion

In this study lumbar spine BMD increased equivalently in the three groups of patients, seemingly not being affected by previous anti-osteoporotic treatment or absence of treatment. These findings were in line with previous studies highlighting the potent BMD increase achieved with denosumab in treatment-naïve [21], or TPTD pre-treated patients [12]. Conversely, a course of bisphosphonate pre-treatment has been shown to diminish denosumab's effect on BMD in most [22–24], but not all studies [25].

In agreement with the changes in lumbar spine BMD, serum concentrations of PINP and CTX decreased significantly 3 and 6 months after the first injection of denosumab in all groups, irrespective of previous treatment (antiresorptive, osteoanabolic). The changes in BTMs are accordance with previous studies evaluating transition from bisphosphonates to denosumab [25–27]. Similarly, the changes in BTM concentrations in our group of women transitioning from TPTD to denosumab are in line with those recently published in the DATA-Switch study [12]. The difference in PINP and CTX concentrations between the three groups at baseline is expected mainly due to the distinct mechanisms of action of ZOL and TPTD [4,5]. The steep reduction of CTX and the more gradual reduction of PINP concentrations at month 3, with a sustained suppression of both BTMs at month 6 after the first denosumab injection, were also expected based on the established mechanism of action of denosumab [21]. Another interesting observation in our study was the negative correlation between changes in PINP and CTX and changes in lumbar spine BMD in TPTD pre-treated patients, which may imply that higher short-term differences in BTMs were associated with greater BMD increases, which is consistant with current literature [28,29]. Of note, this correlation was already apparent at 3 months for PINP in TPTD pre-treated patients, which might suggest that at least for PINP, the suppression seen at 3 months could predict the lumbar spine BMD increase at month 12.

Sclerostin levels did not differ at baseline and did not significantly change with denosumab treatment in any group, despite a trend for lower sclerostin concentrations in women pre-treated with ZOL as compared to treatment-naïve patients at 6 months. Admittedly, since we have no data on sclerostin concentrations at the time of initiation of either ZOL or TPTD, it is difficult to draw a conclusion about their change (or lack thereof) during each of the two treatments. Although we have previously reported that ZOL treatment decreases serum sclerostin levels [30], in the current study we did not identify a similar

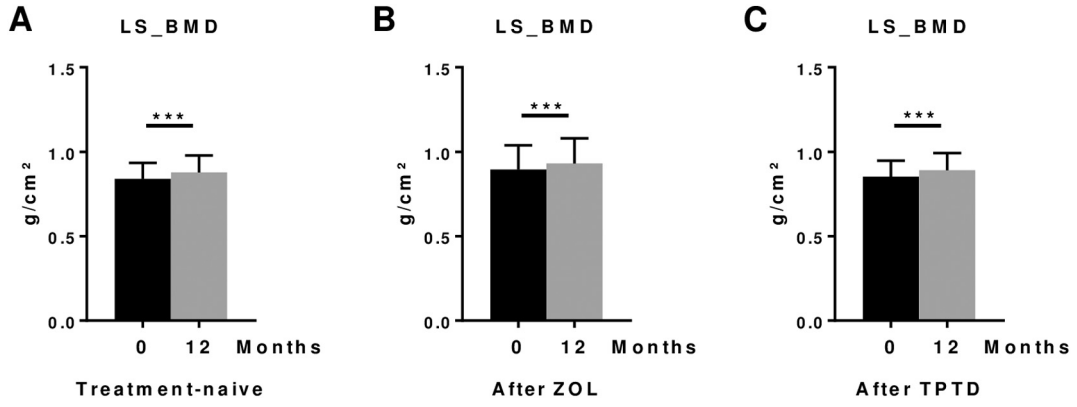

Fig. 1. Changes in lumbar spine bone mineral density at baseline and 12 months. Increases in lumbar spine BMD after 12 months of denosumab therapy in treatment-naïve patients (A), patients pre-treated with ZOL (B), and patients pre-treated with TPTD (C).
Abbreviations: Lumbar spine BMD, Bone Mineral Density at Lumbar Spine; TPTD, teriparatide; ZOL, zoledronic acid.
***$p < 0.001$.

Table 2
Baseline, 3-month and 6-month data of the measured serum parameters in the 3 groups.

Variable and group	Baseline	Month 3	Month 6	*p* value (within groups)*
PINP, ng/mL				
Treatment naïve	45.9 ± 3.1	11.76 ± 0.7[a]	15.9 ± 0.9[b]	< 0.001
After ZOL	23.1 ± 1.5[d]	10.9 ± 0.8[a]	14.2 ± 0.9[b]	< 0.001
After TPTD	76.8 ± 10.7[e,f]	12.8 ± 1.2[a]	16.9 ± 2[b]	< 0.001
p value (between groups)**	< 0.001	0.37	0.28	
CTX, pg/mL				
Treatment naïve	373.7 ± 25.8	47.4 ± 3.6[a]	148.8 ± 15.4[b,c]	< 0.001
After ZOL	157.9 ± 12[d]	44.5 ± 5[a]	127.4 ± 12.6[c]	< 0.001
After TPTD	528.8 ± 65[e,f]	42.5 ± 7.7[a]	136.2 ± 22.8[b]	< 0.001
p value (between groups)**	< 0.001	0.82	0.63	
Sclerostin, pmol/L				
Treatment naïve	30.3 ± 1.2	33.1 ± 1.4	31.6 ± 1.3	0.35
After ZOL	26.1 ± 2.4	26.6 ± 1.9	25.4 ± 1.8	0.92
After TPTD	28.3 ± 2.6	29.3 ± 3.3	27.2 ± 3	0.88
p value (between groups)**	0.32	0.09	0.06	
Dkk-1, pmol/L				
Treatment naïve	36.7 ± 2.4	39.2 ± 3	36.7 ± 2.4	0.74
After ZOL	32.6 ± 3.2	32.9 ± 3.3	29.5 ± 3.6	0.73
After TPTD	40.3 ± 3.2	36.3 ± 3.3	38.4 ± 3.3	0.68
p value (between groups)**	0.21	0.35	0.11	
Myostatin, ng/mL				
Treatment naïve	56.7 ± 1.4	56.6 ± 2.8	54.4 ± 1.5	0.66
After ZOL	53.4 ± 2.3	53.4 ± 3.2	48.7 ± 1.3[d]	0.31
After TPTD	36.8 ± 0.5[e,f]	34.7 ± 1.9[e,f]	33.9 ± 1.6[e,f]	0.38
p value (between groups)**	< 0.001	< 0.001	< 0.001	

Data is presented as means ± SEM.
Abbreviations: CTX, C-terminal-cross-linking telopeptide of type 1 collagen; Dkk-1, Dickkopf-1; PINP, pro-collagen type 1 *N*-terminal propeptide; TPTD, teriparatide; ZOL, zoledronic acid.
* Repeated measures ANOVA or Friedman test.
** One-way ANOVA or Kruskal-Wallis test.
[a] $p < 0.001$ for month 3 compared to baseline.
[b] $p < 0.001$ for month 6 compared to baseline.
[c] $p < 0.001$ for month 3 compared to month 6.
[d] $p < 0.05$ for treatment naïve compared to after ZOL.
[e] $p < 0.05$ for treatment naïve compared to after TPTD.
[f] $p < 0.001$ for after ZOL compared to after TPTD.

trend between treatment-naïve patients and those pre-treated with ZOL at baseline, which might be due to the different study populations. Several groups have also looked into changes of sclerostin levels after denosumab treatment. In treatment-naïve postmenopausal women, denosumab therapy has been shown to increase sclerostin concentrations [30,31], possibly as a feedback loop to overcome inhibition of RANKL through denosumab [32]. The trend for lower sclerostin concentrations in women pre-treated with ZOL as compared to treatment-naïve patients could imply that previous treatment with ZOL blunts serum sclerostin response because of the established down-regulation of bone turnover [33], but this remains to be explicitly shown.

We found no differences in Dkk-1 concentrations at baseline between treatment-naïve patients and patients pre-treated with ZOL or TPTD. In previous clinical studies, increased serum concentrations of Dkk-1 after TPTD treatment have been reported [34,35], and have been proposed to represent a break to the osteoanabolic effect of TPTD [36]. Regarding Dkk-1 levels in postmenopausal women treated with ZOL, we have reported decreased concentrations in a previous study [33], while another group found a transient increase [37].

Another interesting finding was that myostatin concentrations were lower at women pre-treated with TPTD as compared to treatment-naïve and patients pre-treated with ZOL at all time points. To the best of our knowledge, this is the first study to report myostatin responses to different treatments in the setting of postmenopausal osteoporosis. Unfortunately it is not possible to report on myostatin measurements before TPTD (or before ZOL) initiation since patients were recruited after having concluded the respective treatments. As a result, we cannot prove that the differences observed in myostatin concentrations at baseline are the direct result of TPTD pre-treatment per se. Serum myostatin levels have been recently found to negatively correlate with BMD in Chinese elderly women [38], and myostatin is highly expressed in the synovial tissues of patients with rheumatoid arthritis [39], while there are no large-scale evaluations of myostatin concentrations in the setting of osteoporosis to date. Clearly more large-scale studies are warranted to investigate the effects of osteoporosis treatments on myostatin and the clinical consequences of potential changes.

This study has some limitations. Since it is an observational study, randomization was not performed. However, baseline data (Table 1) was similar between groups, which partly balances the lack of randomization. Furthermore, the study reflects everyday clinical practice, in that TPTD is administered in women with more severe osteoporosis, in which randomization may bear ethical considerations. In addition, the sample size was relatively small; however, it was sufficient to show within group differences in lumbar spine BMD in all groups. Moreover, serum measurements were performed at different time-points than BMD assessment; however, our aim was to investigate whether early changes in bone turnover markers were associated with a later BMD response. Since it is not possible to report on myostatin measurements before TPTD initiation (as patients were recruited after having concluded treatment with TPTD), the differences seen in myostatin concentrations at baseline could have been caused by factors other than TPTD pre-treatment. Finally, BMD was measured only in the lumbar spine; however, given the short duration of the study and the predominantly trabecular composition of the spine, the lumbar spine was the measurement site which was expected to reveal the more prominent BMD changes at 12 months.

In conclusion, denosumab increased BMD and suppressed bone turnover in women with postmenopausal osteoporosis irrespective of

Table 3
Correlation between absolute change in lumbar spine BMD at month 12 and absolute change in study parameters at month 3 and month 6 in the 3 groups of patients.

Parameter	Patient group	Correlation lumbar spine BMD change (12 months) and parameter change (3 months)	Correlation lumbar spine BMD change (12 months) and parameter change (6 months)
PINP	Treatment naive	−0.284	−0.282
	After ZOL	−0.131	−0.193
	After TPTD	−0.556**	−0.626**
CTX	Treatment naive	−0.142	−0.157
	After ZOL	0.017	−0.231
	After TPTD	−0.336	−0.481*
Sclerostin	Treatment naive	0.118	−0.011
	After ZOL	−0.227	−0.049
	After TPTD	−0.036	−0.249
Dkk-1	Treatment naive	−0.032	0.173
	After ZOL	−0.224	0.147
	After TPTD	−0.106	−0.269
Myostatin	Treatment naive	0.013	−0.084
	After ZOL	0.098	−0.147
	After TPTD	−0.279	−0.455*

Abbreviations: CTX, C-terminal-cross-linking telopeptide of type 1 collagen; Dkk-1, Dickkopf-1; Lumbar spine BMD, Bone Mineral Density at Lumbar Spine; PINP, pro-collagen type 1 *N*-terminal propeptide; TPTD, teriparatide; ZOL, zoledronic acid.
*$p < 0.05$, **$p < 0.01$.

prior treatments, findings with certain clinical implications. Larger, prospective studies are needed to confirm these observations.

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