CrossMark

Improvement of cancellous bone microstructure in patients on teriparatide following alendronate pretreatment

Astrid Fahrleitner-Pammer [a,*], David Burr [b], Harald Dobnig [a], Jan J. Stepan [c], Helmut Petto [d], Jiliang Li [e], John H. Krege [f], Imre Pavo [d]

[a] Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria
[b] Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA
[c] Institute of Rheumatology and Faculty of Medicine 1, Charles University, Prague, Czech Republic
[d] Lilly Research Centre, Vienna, Austria
[e] Department of Biology, Indiana University Purdue University, Indianapolis, IN, USA
[f] Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, USA

ARTICLE INFO

ABSTRACT

An increase in procollagen type I amino-terminal propeptide (PINP) early after teriparatide initiation was shown to correlate with increased lumbar spine areal BMD and is a good predictor of the anabolic response to teriparatide. Few data exist correlating PINP and bone microstructure, and no data exist in patients on teriparatide following prior potent antiresorptive treatment. This exploratory analysis aimed to investigate the effects of teriparatide on cancellous bone microstructure and correlations of bone markers with microstructure in alendronate-pretreated patients. This was a post hoc analysis of changes in bone markers and three-dimensional indices of bone microstructure in paired iliac crest biopsies from a prospective teriparatide treatment study in postmenopausal women with osteoporosis who were either treatment-naïve (TN, $n = 16$) or alendronate-pretreated (ALN, $n = 29$) at teriparatide initiation. Teriparatide (20 µg/day) was given for 24 months; biopsies were taken at baseline and endpoint, and serum concentrations of PINP and type 1 collagen cross-linked C-telopeptide (βCTX) were measured at intervals up to 24 months. In the TN and ALN groups, respectively, mean (SD) increases in three-dimensional bone volume/tissue volume were 105 (356)% ($P = 0.039$) and 55 (139)% ($P < 0.005$) and trabecular thickness 30.4 (30)% ($P < 0.001$) and 30.8 (53)% ($P < 0.001$). No significant changes were observed in trabecular number or separation. In the ALN patients, 3-month change of neither PINP nor βCTX correlated with indices of cancellous bone microstructure. However, 12-month changes in biochemical bone markers correlated significantly with improvements in bone volume/tissue volume, $r = 0.502$ ($P < 0.01$) and $r = 0.378$ ($P < 0.05$), trabecular number, $r = 0.559$ ($P < 0.01$) and $r = 0.515$ ($P < 0.01$), and reduction of trabecular separation, $r = -0.432$ ($P < 0.05$) and $r = -0.530$ ($P < 0.01$), for PINP and βCTX, respectively. We conclude that cancellous bone microstructure improved with teriparatide therapy irrespective of prior antiresorptive use.

1. Introduction

Teriparatide treatment stimulates bone formation and reduces fracture rates in postmenopausal women with osteoporosis [1,2]. The increase in BMD is associated with various structural improvements resulting in improved biomechanical properties of the bone [3–7]. Teriparatide also rapidly increases biochemical markers of bone formation, followed by an increase of resorption markers, and data suggest that the increases in biochemical markers correlate with indices of

bone formation, such as BMD, quantitative CT-based finite element analysis, or dynamic histomorphometry [7–13]. Among the different markers, serum procollagen type I amino-terminal propeptide (PINP), which reflects collagen formation, appeared to be the most sensitive for predicting BMD change in this setting [9,11,14]. Early increase of PINP after the initiation of teriparatide treatment has been demonstrated to positively correlate with the increase of lumbar spine areal BMD [8,9,15]. Consequently, PINP response has been proposed to be predictive for the individual bone-building capability of teriparatide.

While substantial data exist on these correlations between biochemical markers and X-ray-based bone quality indices, few reports have investigated the relationship between such markers and histomorphometric indices of bone microstructure. Teriparatide

* Corresponding author at: Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Auenbruggerplatz 15, A-8036 Graz, Austria.
E-mail address: astrid.fahrleitner@medunigraz.at (A. Fahrleitner-Pammer).

therapy significantly increased indices of cancellous bone volume and connectivity, improved cancellous bone morphology, and increased cortical bone thickness [16]. Some of these structural improvements were correlated with early increases of biochemical markers measured in the same teriparatide-treated patients [3]. Moreover, teriparatide-induced early increase of PINP was correlated with subsequent increases in indices of dynamic histomorphometry suggesting an anabolic response of teriparatide [10,13].

However, the microstructure-only analysis was performed in postmenopausal women with osteoporosis who were treated for less than the currently recommended full duration of teriparatide treatment (mean 19 months versus 24 months) [3,16] and in those with no meaningful pretreatment with antiresorptives [16]. These may be important limitations, because some observations suggest that pretreatment with potent antiresorptives such as alendronate may blunt the PINP response and the lumbar spine BMD increase in the initial treatment phase, for up to 6 months [5,7,17–20]. Lumbar spine BMD is rich in cancellous bone, characterized by rapid turnover, and may be a more sensitive early marker of changes in bone formation than bone areas containing large amounts of cortical bone, such as the femoral neck. There are currently no data available to indicate whether long-term pretreatment with a potent antiresorptive such as alendronate interacts with teriparatide with respect to its effects on cancellous bone microstructure. It is also not known whether alendronate pretreatment affects the correlations between biochemical markers of turnover and microstructural changes. We have demonstrated in our previous study that increases of dynamic bone formation markers with teriparatide are not meaningfully different between alendronate pretreated and treatment-naïve patients [16], but no data exist on comparisons for microstructural changes. Thus, the aim of this analysis was to investigate the effects of teriparatide on cancellous bone microstructure, either with or without alendronate pretreatment, and to examine the correlations between bone markers and microstructure in patients pretreated with alendronate as a means to predict patient response to treatment.

2. Materials and methods

2.1. Patients and treatments

The present study was a post hoc analysis from a prospective teriparatide treatment study (ClinicalTrials.gov: NCT00191893) carried out in two study centers in Graz, Austria, and Prague, Czech Republic. The study design, patient characteristics and methods have been described previously [10,21], and are summarized briefly here. Prior to study, all patients signed informed consent to the treatment and investigation protocol, which was approved by the Institutional Review Board for Research Involving Human Subjects at the two study centers involved.

The single-arm teriparatide study included 66 ambulatory postmenopausal women with osteoporosis at either the total hip or lumbar spine (T-score ≤ -2.5) and who were aged at least 55 years. Patients were excluded if they had a history of any secondary cause of osteoporosis, malignant neoplasm in the previous 5 years, nephrolithiasis or urolithiasis in the previous 2 years, abnormal thyroid function, active liver disease, impaired renal function, treatment with androgens or other anabolic steroids, treatment with vitamin D > 50.000 IU/week or active vitamin D analogs, or treatment with raloxifene, calcitonin, fluoride, progestins, estrogens, estrogen analogs, agonists or antagonists, selective estrogen receptor modulators, tibolone, systemic corticosteroids, or bisphosphonates other than alendronate in the previous 3 years.

Of the 66 enrolled patients, 38 had been treated previously with alendronate (10 mg/day or 70 mg/week), calcium (1000 mg/day), and vitamin D (800 IU/day) for a minimum duration of 33 months (mean 63.6 months), and 28 were naïve to osteoporosis treatment. During the 24-month study period, all patients self-administered once-daily subcutaneous injections of teriparatide 20 μg; additionally, all patients received daily calcium 1000 mg and vitamin D3 400–1200 IU.

2.2. Study measurements

Blood samples were collected in the morning, after an overnight fast, at baseline and at 1, 3, 6, 12, and 24 months/study endpoint. The samples were sent to a central laboratory where serum concentrations of the bone formation markers, PINP and bone alkaline phosphatase (B-ALP), and the bone resorption marker, type 1 collagen cross-linked C-telopeptide (βCTX), were assessed. Intact PINP was measured by radioimmunoassay (Procollagen Intact PINP, Orion Diagnostica, Finland); the assay was not sensitive to the small molecular weight degradation products of the propeptide. Intra-assay variance was below 5% and inter-assay variance was below 7% at concentrations between 20 and 90 μg/l. βCTX concentration was assessed using electrochemiluminescence-based immunoanalysis (Elecsys 1010 Analyzer®, Roche Diagnostics GmbH, Germany). Detection limit was <10 ng/l; intra- and inter-assay variance was below 5% and below 7%, respectively, for samples with βCTX concentration > 500 ng/l, below 7% and below 9% for samples between 200 and 500 ng/l, and below 10% for samples <200 ng/l. B-ALP concentration was determined using an immunoradiometric assay (Tandem-R Ostase®, Hybritech, Inc., San Diego, CA, USA), with coefficients of variation 4.2–6.8% and 7.4–7.9% for intra- and inter-assay, respectively. Least significant changes were 28%, 20% and 21% for βCTX, PINP and B-ALP, respectively.

BMD was measured with a QDR 4500 A bone densitometer (Hologic, Inc., Waltham, MA, USA), with normative values provided by Hologic, Inc. Short-term precision errors were 0.7% for the lumbar spine (L1–L4) and 1.9% for the femoral neck; long-term precision error determined with the Hologic phantom was 0.3%.

A manual drill with a 7.5 mm trephine (Medical Innovations International, Inc., Rochester, MN, USA) was used to obtain transiliac crest bone biopsies. The two biopsies performed for each patient were taken from opposite sides of the body; the first biopsy was obtained either from the left or the right side, according to a random allocation scheme. Biopsies were taken at baseline, before teriparatide initiation, and at the 24-month endpoint, and were sent in 70% ethanol for assessment at a central laboratory. Biopsies were considered complete and suitable for analysis if they consisted of two well-defined cortical tables from tetracycline double-labeling and did not have any complete fractures between the cortices. High-resolution quantitative CT was performed on specimens in 70% ethanol at room temperature, using a micro-CT scanner (General Electric Enhanced Visions Systems, London, ON, Canada). From scanning of the iliac crest biopsies, a three-dimensional (3D) reconstruction was produced using 22.6 μm isotopic voxels to ensure identical resolution in all three orthogonal directions. Image gray-level values for each specimen were calibrated using a cortical bone mimicking material standard (SB3, Gammex, RMI, Middleton, WI). Images were thresholded into bone and non-bone voxels to measure bone density, area, mineral content, and mineralization.

The biopsy specimens were processed, embedded, and sectioned, as previously described [10]. Two-dimensional (2D) histological assessment was performed on 5-μm thick biopsy sections stained with McNeal's tetrachrome or left unstained. Active bone formation and resorption were evaluated on the cancellous bone surface. Bone-forming activity toward bridging the trabeculae was also assessed.

2.3. Statistics

The initial sample size was estimated to enable detection of a difference in active mineralizing surfaces between the treatment-naïve and alendronate-pretreated groups. The present post hoc study used data only from patients who provided evaluable biopsies and evaluable samples for bone turnover markers at both baseline and the 24-month endpoint of teriparatide treatment. Differences between the treatment-

Table 1
Baseline characteristics of patients and serum markers of bone turnover, by treatment prior to teriparatide initiation.

	Treatment-naïve (n = 16)	Alendronate pretreated (n = 29)
Age (years)	67.6 (7.0)	69.2 (7.3)
Time since menopause (years)	17.8 (6.8)	18.2 (7.8)
Prior alendronate therapy duration (months)	–	64.5 (16.4)
Lumbar spine BMD (g/cm^2)	0.70 (0.06)	0.77 (0.09)*
Femoral neck BMD (g/cm^2)	0.60 (0.12)	0.60 (0.09)
Serum turnover markers		
Procollagen type I amino-terminal propeptide (µg/l)	48.9 (15.7)	26.0 (13.4)**
Type 1 collagen cross-linked C-telopeptide (ng/l)	430.7 (176.1)	221.6 (138.7)**
Bone-specific alkaline phosphatase (µg/l)	15.8 (3.6)	10.6 (4.6)**

Data show mean (SD). Part of the baseline characteristics has been published elsewhere [10].
  * $P < 0.01$ for between-group difference.
 ** $P < 0.001$ for between-group difference.

naïve and alendronate-pretreated groups were examined by Wilcoxon rank-sum tests, with no adjustment for confounders. Within-group changes from baseline during teriparatide treatment were examined using Wilcoxon signed-rank tests. Non-parametric analyses were used due to the small sample size and the possibility of non-normality of the data. Pearson correlation coefficients were determined for evaluation of correlations between bone turnover markers (absolute changes) and bone microarchitecture indices (percentage changes), at baseline, at endpoint, and for changes over time. For all of these exploratory analyses, a P-value < 0.05 was considered statistically significant.

3. Results

The baseline characteristics of the 29 alendronate-pretreated patients and 16 treatment-naïve patients who completed 24 months of teriparatide treatment have been presented previously [10,21] and are briefly summarized in Table 1. Prior to teriparatide treatment, patients pretreated with alendronate, for a minimum of 34 months to a maximum of 93 months, had significantly greater lumbar spine BMD and significantly lower concentrations of the bone turnover markers than the treatment-naïve patients, as expected with long-term bisphosphonate treatment.

Serum concentrations of PINP, βCTX, and B-ALP increased rapidly in both groups after starting teriparatide treatment (Table 2). No significant differences were observed in serum marker concentrations

between the treatment-naïve group and the alendronate-pretreated group at any time after baseline. For PINP, the increase from baseline to 24 months remained significant for both the treatment-naïve and the alendronate-pretreated groups, whereas the changes at both 12 and 24 months for βCTX and B-ALP were significant only in the alendronate-pretreated group. The median change at 12 and 24 months as a percentage of the baseline concentration was greater for PINP than for βCTX and B-ALP.

The median percentage increase in lumbar spine BMD at 12 months was 1.1% in the alendronate-pretreated group, which was not significantly different from baseline ($P = 0.141$). The percentage increase in the treatment-naïve group was 6.2%, which was significant versus baseline ($P < 0.001$) and versus the alendronate-pretreated group ($P = 0.004$). However, the percentage changes at 24 months were significantly different from baseline in both groups ($P < 0.001$ for both), with no significant between-group difference (treatment-naïve 10.2% versus alendronate-pretreated 5.3%, $P = 0.077$).

There were no significant differences observed between the treatment-naïve and alendronate-pretreated groups at baseline or at endpoint for any of the 2D or 3D microstructural parameters assessed (Table 3), and no differences for change from baseline to endpoint except for the change in 2D trabecular number which was greater in the alendronate-pretreated patients ($P = 0.040$). The 3D bone volume and trabecular thickness increased significantly from baseline in both the alendronate-pretreated group and in the treatment-naïve group. The increase of 2D trabecular width reached the level of statistical significance in the alendronate-pretreated group only, while 2D trabecular separation increased in the treatment-naïve group. With this exception, the observed increases in trabecular number and separation were not statistically significant. Micro-CT and isosurface imaging from the 3D reconstructions of a representative pair of biopsies with or without alendronate pre-treatment (Fig. 1) showed a denser structure to the bone following 24 months of teriparatide treatment in both groups. The images showed an increase in active bone formation (Fig. 2), and greater osteoblast recruitment and activity, as indicated by the labeling surface and the double-labeling distance with tetracycline (Fig. 3) in both treatment-naïve and alendronate-pretreated patients. An increase in trabecular connectivity was shown by the bridging between the trabeculae following 24 months of teriparatide treatment (Fig. 4).

Pearson correlations between the bone turnover marker concentrations and the 3D microstructure indices showed no significant correlations at baseline in the alendronate-pretreated group (data not shown). For the treatment-naïve patients at baseline, both PINP ($r = -0.535, P = 0.033$) and βCTX ($r = -0.571, P = 0.021$) concentrations were inversely correlated with trabecular thickness. No significant

Table 2
Median serum concentrations of bone turnover markers by time of treatment with teriparatide in treatment-naïve and alendronate-pretreated patients.

|	PINP (µg/l)		βCTX (ng/l)		B-ALP (µg/l)	
	TN (n = 16)	ALN (n = 29)	TN (n = 16)	ALN (n = 29)	TN (n = 16)	ALN (n = 29)
0 months	48.9	22.6***	427.6	179.7***	16.8	9.8***
3 months	92.5	92.7	536.4	634.8	14.3	13.1
6 months	120.6	150.4	663.3	812.7	16.1	17.7
12 months	142.3	174.1	646.5	753.8	20.0	18.8
24 months	77.3	96.0	487.7	545.9	14.5	16.6
Percentage change 0 to 12	191	529***	70	297***	26	84*
P-value 0 to 12	<0.001	<0.001	0.008	<0.001	0.051	<0.001
Percentage change 0 to 24	108	340***	39	197**	−5	67**
P-value 0 to 24	0.001	<0.001	0.093	<0.001	0.632	<0.001

ALN = alendronate-pretreated; B-ALP = bone alkaline phosphatase; βCTX = type 1 collagen cross-linked C-telopeptide; PINP = procollagen type I amino-terminal propeptide; TN = treatment-naïve. P values are for within-group percentage changes from baseline; between-group differences at specified times in the study are shown as *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Table 3
Two- and three-dimensional histomorphometry indices of cancellous bone microarchitecture at baseline and after 24 months of teriparatide treatment in patients who were previously treatment-naïve or had received alendronate pretreatment.

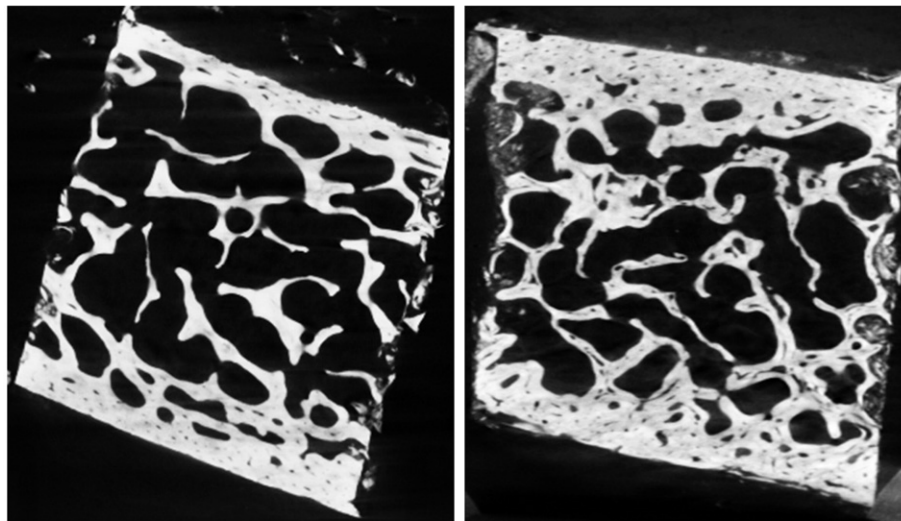|	Treatment-naïve (n = 16)			Alendronate pretreated (n = 29)			All (n = 45)		
	Baseline	24 Months		Baseline	24 Months		Baseline	24 Months	
	Mean (SD)	% Change (SD)	P-value	Mean (SD)	% Change (SD)	P-value	Mean (SD)	% Change (SD)	P-value
Two dimensional (2D)									
Bone area/total area (B·Ar/Tt. Ar, %)	20.1 (5)	−3.6 (30)	0.273	19.0 (6)	33.2 (66)	0.016	19.5 (5)	19.6 (58)	0.138
Trabecular width (Tb·Wi, μm)	0.14 (0.03)	7.0 (24)	0.273	0.14 (0.03)	19.6 (35)	0.033	0.14 (0.03)	14.9 (32)	0.018
Trabecular number (Tb·N, mm⁻¹)	1.4 (0.3)	−9.9 (14)	0.068	1.3 (0.3)	8.0 (28)	0.l63	1.4 (0.3)	1.3 (25)	0.810
Trabecular separation (Tb·Sp, μm)	0.58 (0.12)	15.2 (20)	0.017	0.64 (0.20)	−3.4 (39)	0.126	0.62 (0.17)	3.5 (34)	0.798
Three dimensional (3D)									
Bone volume/tissue volume (BV/TV, %)	17 (6)	105 (356)	0.039	18 (8)	55 (139)	0.005	18 (7)	73 (237)	<0.001
Trabecular thickness (Tb·Th, μm)	0.12 (0.03)	30.4 (30)	<0.001	0.13 (0.03)	30.8 (53)	<0.001	0.12 (0.03)	30.7 (46)	<0.001
Trabecular number (Tb·N, mm⁻¹)	1.47 (0.43)	36.7 (179)	0.298	1.44 (0.55)	12.7 (54)	0.254	1.45 (0.51)	21.2 (114)	0.637
Trabecular separation (Tb·Sp, μm)	0.89 (1.40)	3.1 (36)	0.706	0.71 (0.49)	10.4 (75)	0.237	0.77 (0.91)	7.8 (64)	0.439

A. Treatment-naïve patient
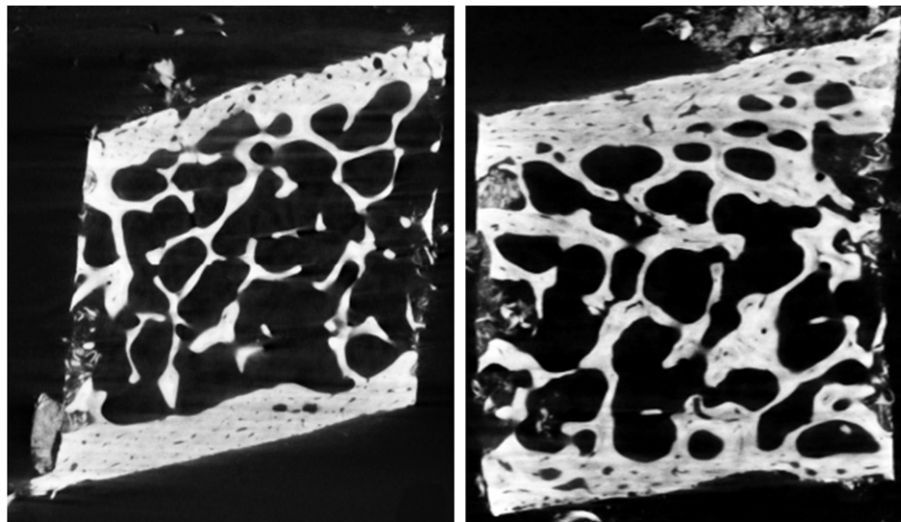
Baseline   24 Months of Teriparatide
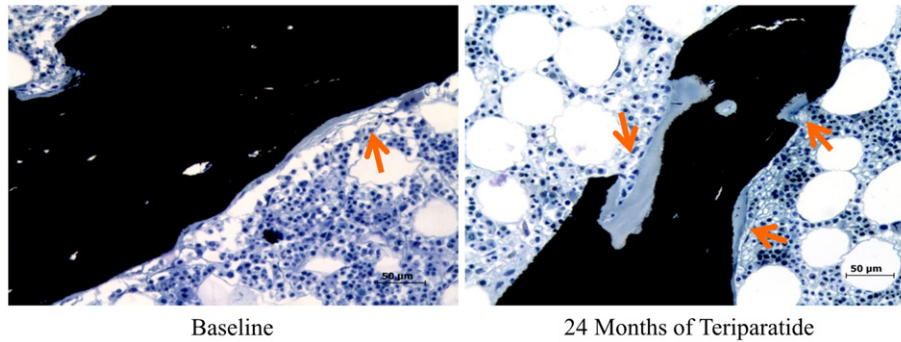
B. Alendronate-pretreated patient

Baseline   24 Months of Teriparatide

Fig. 1. Teriparatide improved trabecular structure in both treatment-naïve and alendronate-pretreated patients. The representative images are from three-dimensional reconstruction of high-resolution quantitative tomography scans of paired iliac crest biopsies from the same patients at baseline and after 24 months of teriparatide treatment. Teriparatide treatment increased bone volume and trabecular thickness.

A. Treatment-naïve patient

Baseline   24 Months of Teriparatide

B. Alendronate-pretreated patient

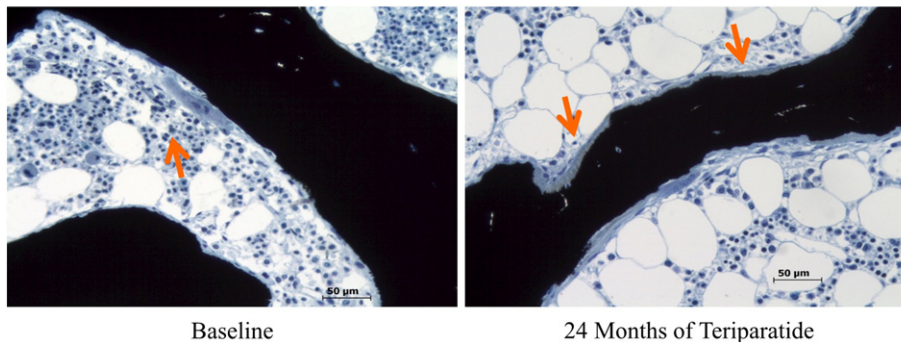Baseline   24 Months of Teriparatide

Fig. 2. Teriparatide stimulated bone anabolic response in both treatment-naïve and alendronate-pretreated patients. The representative images of paired iliac crest biopsies, stained with McNeal's tetrachrome, from the same patients, demonstrate an increase in osteoblast number (arrowed) and the osteoid surface area (arrowed) in both treatment-naïve and alendronate-pretreated patients.

correlations were found for the PINP and βCTX concentrations at 24 months of teriparatide and 24-month trabecular microstructure indices in the alendronate-pretreated group (data not shown). For patients overall, 24-month PINP and βCTX were correlated with 24-month trabecular thickness (PINP $r = 0.340$, $P = 0.026$; βCTX $r = 0.346$, $P = 0.023$), and in the treatment-naïve group 24-month βCTX was correlated with 24-month trabecular thickness ($r = 0.556$, $P = 0.025$); there were no significant correlations with other microstructure indices at 24 months (data not shown).

In the alendronate-pretreated patients, the 3-month change in PINP did not correlate with 24-month microstructural changes, while the 12-month PINP changes were significantly correlated. Scatter plots showing BV/TV changes versus PINP changes at 3 and 12 months are shown in Fig. 5. Similarly to PINP, no meaningful correlations of 3-month βCTX changes could be observed, but 12-month βCTX changes correlated with microstructural improvement in the alendronate-pretreated patient group (Table 4). Respective correlations with B-ALP were not meaningful (data not shown).

4. Discussion

This is the first study to indicate that teriparatide treatment improves cancellous bone microstructure irrespective of antiresorptive pretreatment. The improvement in cancellous bone microstructure was consistent with changes observed in paired biopsies of previously treatment-naïve postmenopausal women treated with either teriparatide 20 or 40 μg for a mean of 19 months [16]. This was also in agreement with earlier studies with parathyroid hormone fragments and various treatment regimes, which indicated an anabolic effect on cancellous bone microarchitecture [22–24]. Other investigations did not find a significant effect on cancellous bone, probably due to insufficient sample size [25,26]. Our data confirm the anabolic effect of teriparatide on cancellous bone microstructure, which extends our

earlier findings concerning signs of increased cortical bone formation in the identical biopsies [21].

We found no significant differences in the anabolic response of cancellous bone between patients naïve to antiresorptive treatment and those pretreated with the potent antiresorptive alendronate. The large variability of the results, however, does not allow us to address this issue with certainty. Some earlier findings suggested that initial bone formation by teriparatide was influenced by the level of bone turnover at the time of initiation. Teriparatide-induced bone turnover and BMD increase were shown to occur earlier and to a greater extent in patients with higher baseline bone turnover who were pretreated with the less potent antiresorptive raloxifene when compared with patients with more reduced baseline bone turnover due to pretreatment with alendronate [20]. Similar findings were reported with regard to risedronate or alendronate pretreatment [27], and no or diverse antiresorptive pretreatment [7]. In agreement with the present microstructural results, dynamic histomorphometric indices on the cancellous bone surface of the investigated biopsies have demonstrated a distinctly suppressed bone turnover in the alendronate-pretreatment group at baseline, with almost no differences between the two treatment groups at the end of 24 months of teriparatide treatment [10]. Thus, longer-term teriparatide treatment could override any initial inhibitory effect of antiresorptive pretreatment. Similar conclusions could be drawn from the respective BMD increase after 24 months of teriparatide treatment [7].

In the present study, the 12-month increases of PINP and βCTX in the alendronate-pretreated group were correlated with improvements of cancellous bone microstructure after 24 months. Our study was not sufficiently powered to investigate properly the correlations in the smaller treatment-naïve patient group. When the two groups were pooled for these correlation analyses, the results were not different from those obtained for the alendronate-pretreated group (data not shown). Correlations between increases in biochemical markers of bone turnover and
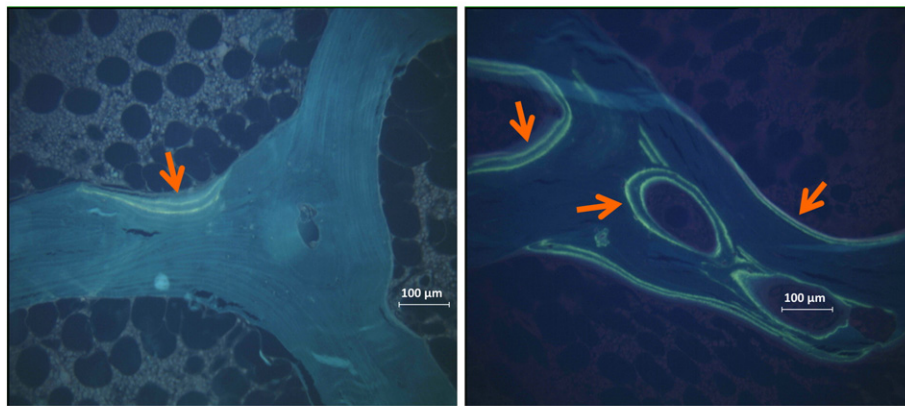
A. Treatment-naïve patient
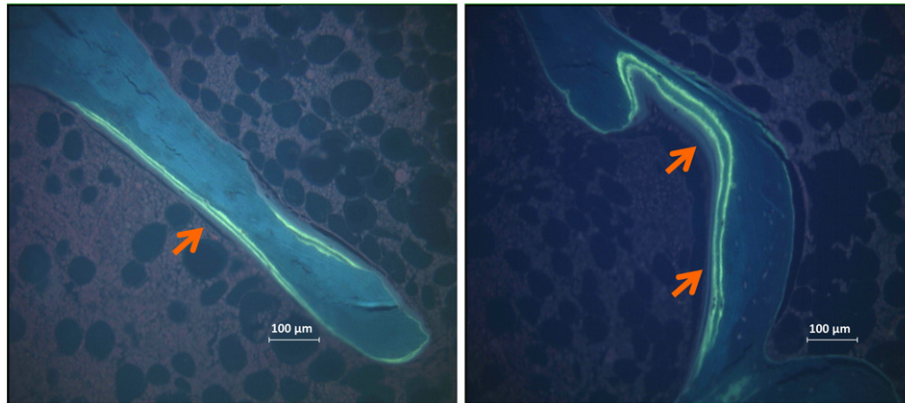
Baseline   24 Months of Teriparatide

B. Alendronate-pretreated patient

Baseline   24 Months of Teriparatide

Fig. 3. Teriparatide stimulated bone mineralization in both treatment-naïve and alendronate-pretreated patients. The representative images of paired iliac crest biopsies, from the same patients at baseline and after 24 months of teriparatide treatment, demonstrate active bone formation, indicated by tetracycline fluorochrome labels (arrowed), on bone surfaces in both treatment-naïve and alendronate-pretreated patients after 24 months of teriparatide treatment in comparison with baseline.

subsequent structural improvements in the skeleton during teriparatide treatment without antiresorptive pretreatment have been extensively studied (reviewed by Krege et al. [9]). Most of the significant correlations were demonstrated between a short-term (1 to 6 months) increase in PINP and an increase in BMD at 1 to 2 years [14,15,17,28–31]. One study showed correlations between bone marker increases (procollagen I C-terminal propeptide and B-ALP) and microstructural improvement in paired biopsies of the iliac crest [3]. Several same-time cross-sectional correlations between biochemical markers of bone formation and dynamic histomorphometric indices such as the mineralizing surface indicate that biochemical markers are reliable surrogates of the level of bone formation on the bone surface of patients treated with teriparatide. For example, PINP concentration correlated with mineralizing surface in iliac bone biopsies after 6 months of teriparatide treatment [31]. Similarly, in the current study, PINP, βCTX, and B-ALP correlated significantly with diverse dynamic histomorphometric markers (osteoid, osteoblast and mineralizing surfaces, and activation frequency) prior to and after 24 months of teriparatide treatment [10]. In line with the earlier findings that BMD improvements are predicted by PINP, the present data indicate that increases in PINP and βCTX are associated with microstructural improvement in patients treated with teriparatide.

In the present study, the increases in turnover markers were already evident after 1 month of teriparatide treatment in the alendronate-

pretreated group, for whom bone turnover had been initially suppressed. Earlier observations in the European Study of Forsteo (EUROFORS) also indicated a sharp increase in these markers in patients receiving teriparatide who were pretreated with antiresorptives. After 1 month on teriparatide, the increase in PINP was significantly more pronounced in the treatment-naïve group than in the alendronate-pretreated group, but no difference was evident after 6 months [14, 32]. Similarly, other studies found only a transitional delay on teriparatide-induced bone turnover increase in patients, as a function of suppressed bone turnover at baseline [19,27]. Overall, the evidence from numerous studies suggests a mild and transitional impact, with limited clinical importance, of antiresorptive pretreatment on bone turnover during subsequent teriparatide therapy.

Changes in PINP and βCTX concentrations at 1 or 3 months did not appear to predict cancellous bone microstructural changes after 24 months of teriparatide treatment. Baseline PINP concentration and changes up to 6 months have previously been reported to correlate with changes in BMD and bone strength [8,9,11,13–15]. The lack of predictive value of 1 and 3-month changes in PINP and βCTX concentration on changes of cancellous bone microstructure indices was different from the significant correlations of bone marker concentrations with dynamic histomorphometric markers found previously in this study [10]. However, the slopes of the correlations of bone turnover markers and dynamic markers were previously noted to increase with duration
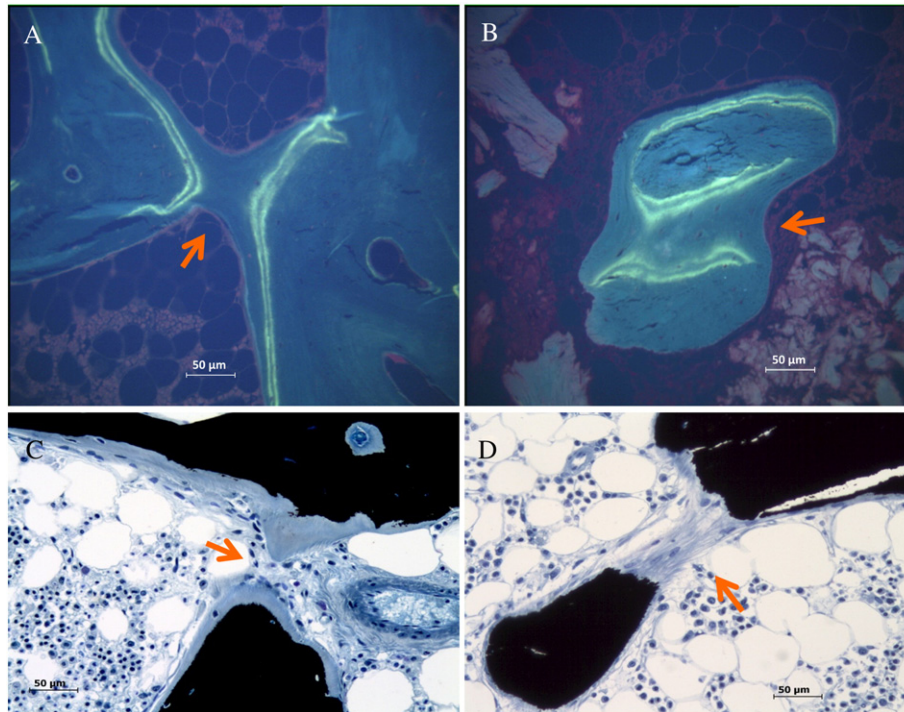
Fig. 4. Teriparatide induced trabecular connectivity in both treatment-naïve and alendronate-pretreated patients. The representative images from iliac crest biopsies after completion of 24 months of teriparatide treatment demonstrate newly formed bone that is going to bridge (A and B, bone sections stained with McNeal's tetrachrome) or has bridged (C and D, unstained bone sections) the adjacent trabeculae (arrowed) in treatment-naïve (A and C) and alendronate-pretreated (B and D) patients.

of teriparatide treatment [10]. It is possible that the high variability in cancellous bone indices and the comparatively small sample size masked any correlations at early times.

No correlation between the 3-month increase in PINP and microstructural improvement was evident in the alendronate-pretreatment group, even though the PINP increase was significant in both patient groups at this time point. Because the increases in bone turnover markers peaked at around 12 months, the changes at 12 months may be the most sensitive to predict improvements in cancellous bone microstructure, especially in patients with long-term antiresorptive pretreatment. Errors in measurement, both of PINP and of microarchitecture, could mask a stronger true relationship between these variables than we have demonstrated. Like all histomorphometry studies, there was a limited sample size, especially in the treatment-naïve group, and significant variability of the individual responses, so the inter-individual differences between changes in microstructural indices was large. Until additional data are available, we suggest interpreting these results with caution.

The strongest correlation between changes in PINP and microstructural indices was observed with BV/TV, which was the primary bone histomorphometry measure that increased the most during teriparatide treatment. A numerically smaller, but significant, increase of trabecular thickness, another primary measure, was also observed with teriparatide treatment. However, the lower magnitude of this change was probably not sufficient to result in significant correlations between trabecular thickness and PINP changes. Trabecular number and trabecular separation are derived from BV/TV and trabecular thickness as secondary measures [33] and had variable levels of correlation with PINP. Therefore, similar to earlier findings, our study was not powered to detect correlations with high certainty between individual cancellous microarchitectural indices and PINP changes on teriparatide [3,16].

This study had several limitations. It was an open-label study in which all patients were assigned to teriparatide treatment. The relatively small sample size, when compared with larger cohorts, limited the conclusions regarding the changes in biochemical markers. Only valid biopsies could be used for analysis and the number of paired biopsies
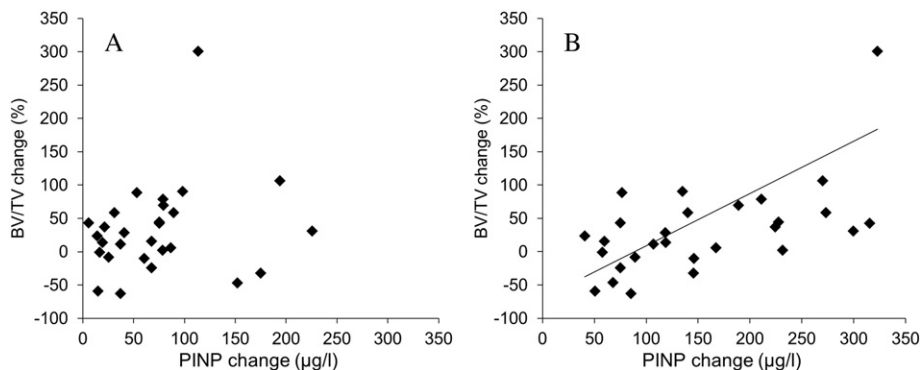

Fig. 5. Scatter plots showing the changes in procollagen type I amino-terminal propeptide (PINP) concentration from baseline to 3 (A; $r = 0.152$, $P = 0.430$) and 12 months (B; $r = 0.502$, $P = 0.006$) and 24-month changes in bone volume/tissue volume (BV/TV) with teriparatide treatment in patients who were alendronate-pretreated. One data-point with BV/TV change of 688% is not shown. The analysis results with or without inclusion of this data-point were not different (data not shown).

Table 4

Correlations (r values) between percentage changes in three-dimensional indices of cancellous bone microstructure after 24 months of teriparatide and changes in PINP and βCTX after 3 and 12 months of teriparatide in patients pretreated with alendronate ($n = 29$).

	Procollagen type I amino-terminal propeptide (PINP)		Type 1 collagen cross-linked C-telopeptide (βCTX)	
	3 months	12 months	3 months	12 months
Bone volume (BV/TV)	0.152	0.502**	0.249	0.378*
Trabecular thickness (Tb·Th)	0.273	0.351	0.284	0.164
Trabecular number (Tb·N)	0.070	0.559**	0.212	0.515**
Trabecular separation (Tb·Sp, μm)	0.074	− 0.432*	− 0.235	− 0.530**

Values show Pearson correlation coefficients.
* $P < 0.05$ for correlation.
** $P < 0.01$ for correlation.

was less in the treatment-naïve than in the alendronate-pretreated group, limiting the statistical power to detect significant changes and correlations, especially differences between treatment-naïve and alendronate-pretreated groups.

5. Conclusions

The present analysis shows that cancellous bone microstructure is significantly improved with teriparatide treatment, whether or not patients receive antiresorptive pretreatment. Additionally, changes in PINP show correlations with cancellous bone microstructure indices at 24 months in treatment-naïve patients and in those who have been pretreated with alendronate. The results warrant further research on the clinical value of PINP changes for predicting subsequent improvements in cancellous bone microstructure in patients receiving teriparatide.

Disclosures

Astrid Fahrleitner-Pammer has received speaker fees/research grants from Amgen, Eli Lilly, Genzyme, GSK, Novartis, Roche, Servier, and Takeda. David Burr served as a consultant for Agnovos and Abt Associates; has received grants from Eli Lilly and Amgen; received royalties from Springer and Elsevier; and received travel/accommodations/meeting expenses unrelated to activities listed as part of the Board of Directors for FASEB (reimbursements only). Harald Dobnig has received speaker fees and travel/accommodations/meeting expenses unrelated to activities listed from Eli Lilly. Jan Stepan served on Amgen Advisory Board, received speaker fees from Eli Lilly and Roche and was supported by the project for conceptual development of research organization 00023728 (Institute of Rheumatology, Prague). Helmut Petto, John Krege and Imre Pavo are full-time employees of Eli Lilly and Company. Jiliang Li has declared no conflict of interest.

Acknowledgments

Authors' roles: All authors were involved in the performance and interpretation of the study, and had full access to all of the data. AF-P, HP, JL and IP take responsibility for the integrity of the data. HP, JL and IP performed the data analysis. AF-P and IP drafted the manuscript. All authors participated in critical revision of the manuscript, and approved the final version for submission.

References

[1] R.M. Neer, C.D. Arnaud, J.R. Zanchetta, R. Prince, G.A. Gaich, J.Y. Reginster, et al., Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis, N. Engl. J. Med. 344 (19) (2001) 1434–1441, http://dx.doi.org/10.1056/NEJM200105103441904.

[2] J.H. Krege, X. Wan, Teriparatide and the risk of nonvertebral fractures in women with postmenopausal osteoporosis, Bone 50 (1) (2012) 161–164, http://dx.doi.org/10.1016/j.bone.2011.10.018.

[3] H. Dobnig, A. Sipos, Y. Jiang, A. Fahrleitner-Pammer, L.S. Ste-Marie, J.C. Gallagher, et al., Early changes in biochemical markers of bone formation correlate with improvements in bone structure during teriparatide therapy, J. Clin. Endocrinol. Metab. 90 (7) (2005) 3970–3977, http://dx.doi.org/10.1210/jc.2003-1703.

[4] K.K. Nishiyama, A. Cohen, P. Young, J. Wang, J.M. Lappe, X.E. Guo, et al., Teriparatide increases strength of the peripheral skeleton in premenopausal women with idiopathic osteoporosis: a pilot HR-pQCT study, J. Clin. Endocrinol. Metab. 99 (7) (2014) 2418–2425, http://dx.doi.org/10.1210/jc.2014-1041.

[5] F. Cosman, T.M. Keaveny, D. Kopperdahl, R.A. Wermers, X. Wan, K.D. Krohn, J.H. Krege, Hip and spine strength effects of adding versus switching to teriparatide in postmenopausal women with osteoporosis treated with prior alendronate or raloxifene, J. Bone Miner. Res. 28 (6) (2013) 1328–1336, http://dx.doi.org/10.1002/jbmr.1853.

[6] C. Graeff, Y. Chevalier, M. Charlebois, P. Varga, D. Pahr, T.N. Nickelsen, et al., Improvements in vertebral body strength under teriparatide treatment assessed in vivo by finite element analysis: results from the EUROFORS study, J. Bone Miner. Res. 24 (10) (2009) 1672–1680, http://dx.doi.org/10.1359/jbmr.090416.

[7] B.M. Obermayer-Pietsch, F. Marin, E.V. McCloskey, P. Hadji, J. Farrerons, S. Boonen, et al., Effects of two years of daily teriparatide treatment on BMD in postmenopausal women with severe osteoporosis with and without prior antiresorptive treatment, J. Bone Miner. Res. 23 (10) (2008) 1591–1600, http://dx.doi.org/10.1359/jbmr.080506.

[8] R. Niimi, T. Kono, A. Nishihara, M. Hasegawa, A. Matsumine, T. Nakamura, et al., An algorithm using the early changes in PINP to predict the future BMD response for patients treated with daily teriparatide, Osteoporos. Int. 25 (1) (2014) 377–384, http://dx.doi.org/10.1007/s00198-013-2426-2.

[9] J.H. Krege, N.E. Lane, J.M. Harris, P.D. Miller, PINP as a biological response marker during teriparatide treatment for osteoporosis, Osteoporos. Int. 25 (9) (Sep 2014) 2159–2171, http://dx.doi.org/10.1007/s00198-014-2646-0.

[10] J.J. Stepan, D.B. Burr, J. Li, Y.L. Ma, H. Petto, A. Sipos, et al., Histomorphometric changes by teriparatide in alendronate-pretreated women with osteoporosis, Osteoporos. Int. 21 (12) (2010) 2027–2036, http://dx.doi.org/10.1007/s00198-009-1168-7.

[11] P. Farahmand, F. Marin, F. Hawkins, R. Möricke, J.D. Ringe, C.C. Glüer, et al., Early changes in biochemical markers of bone formation during teriparatide therapy correlate with improvements in vertebral strength in men with glucocorticoid-induced osteoporosis, Osteoporos. Int. 24 (12) (2013) 2971–2981, http://dx.doi.org/10.1007/s00198-013-2379-5.

[12] Y. Chevalier, E. Quek, B. Borah, G. Gross, J. Stewart, T. Lang, et al., Biomechanical effects of teriparatide in women with osteoporosis treated previously with alendronate and risedronate: results from quantitative computed tomography-based finite element analysis of the vertebral body, Bone 46 (1) (2010) 41–48, http://dx.doi.org/10.1016/j.bone.2009.09.032.

[13] R.R. Recker, F. Marin, S. Ish-Shalom, R. Möricke, F. Hawkins, G. Kapetanos, et al., Comparative effects of teriparatide and strontium ranelate on bone biopsies and biochemical markers of bone turnover in postmenopausal women with osteoporosis, J. Bone Miner. Res. 24 (8) (2009) 1358–1368, http://dx.doi.org/10.1359/jbmr.090315.

[14] A. Blumsohn, F. Marin, T. Nickelsen, K. Brixen, G. Sigurdsson, J. González de la Vera, et al., Early changes in biochemical markers of bone turnover and their relationship with bone mineral density changes after 24 months of treatment with teriparatide, Osteoporos. Int. 22 (6) (2011) 1935–1946, http://dx.doi.org/10.1007/s00198-010-1379-y.

[15] M. Tsujimoto, P. Chen, A. Miyauchi, H. Sowa, J.H. Krege, PINP as an aid for monitoring patients treated with teriparatide, Bone 48 (4) (2011) 798–803, http://dx.doi.org/10.1016/j.bone.2010.12.006.

[16] Y. Jiang, J.J. Zhao, B.H. Mitlak, O. Wang, H.K. Genant, E.F. Eriksen, Recombinant human parathyroid hormone (1-34) [teriparatide] improves both cortical and cancellous bone structure, J. Bone Miner. Res. 18 (11) (2003) 1932–1941, http://dx.doi.org/10.1359/jbmr.2003.18.11.1932.

[17] R. Niimi, T. Kono, A. Nishihara, M. Hasegawa, A. Matsumine, T. Kono, et al., Determinants associated with bone mineral density increase in response to daily teriparatide treatment in patients with osteoporosis, Bone 66 (2014) 26–30, http://dx.doi.org/10.1016/j.bone.2014.05.017.

[18] E.F. Eriksen, T.M. Keaveny, E.R. Gallagher, J.H. Krege, Literature review: the effects of teriparatide therapy at the hip in patients with osteoporosis, Bone 67 (2014) 246–256, http://dx.doi.org/10.1016/j.bone.2014.07.014.

[19] E.T. Middleton, S.A. Steel, S.M. Doherty, The effect of prior bisphosphonate exposure on the treatment response to teriparatide in clinical practice, Calcif. Tissue Int. 81 (5) (2007) 335–340, http://dx.doi.org/10.1007/s00223-007-9066-5.

[20] B. Ettinger, J. San Martin, G. Crans, I. Pavo, Differential effects of teriparatide on BMD after treatment with raloxifene or alendronate, J. Bone Miner. Res. 19 (5) (2004) 745–751, http://dx.doi.org/10.1359/jbmr.040117.

[21] Y.L. Ma, Q.Q. Zeng, A.Y. Chiang, D. Burr, J. Li, H. Dobnig, et al., Effects of teriparatide on cortical histomorphometric variables in postmenopausal women with or without prior alendronate treatment, Bone 59 (Feb 2014) 139–147, http://dx.doi.org/10.1016/j.bone.2013.11.011.

[22] J. Reeve, P.J. Meunier, J.A. Parsons, et al., Anabolic effect of human parathyroid hormone fragment on trabecular bone in involutional osteoporosis: a multicentre trial, Br. Med. J. 280 (6228) (1980) 1340–1344.

[23] J.N. Bradbeer, M.E. Arlot, P.J. Meunier, J. Reeve, Treatment of osteoporosis with parathyroid peptide (hPTH 1-34) and oestrogen: increase in volumetric density of iliac cancellous bone may depend on reduced trabecular spacing as well as increased thickness of packets of newly formed bone, Clin. Endocrinol. 37 (3) (1992) 282–289.

[24] M. Vogel, R.D. Hesch, G. Delling, Morphologic study of iliac crest spongiosa in patients with osteoporosis treated with combination therapy of pulsatile administration of parathyroid hormone (1–38 hPTH) and sequential addition of calcitonin nasal spray, Med. Klin. (Munich) 85 (2) (1990) 82–86 (German).

[25] A.B. Hodsman, M. Kisiel, J.D. Adachi, L.J. Fraher, P.H. Watson, Histomorphometric evidence for increased bone turnover without change in cortical thickness or porosity after 2 years of cyclical hPTH(1–34) therapy in women with severe osteoporosis, Bone 27 (2) (2000) 311–318, http://dx.doi.org/10.11016/S8756-3282(00)00316-1.

[26] D.W. Dempster, F. Cosman, E.S. Kurland, H. Zhou, J. Nieves, L. Woelfert, et al., Effects of daily treatment with parathyroid hormone on bone microarchitecture and turnover in patients with osteoporosis: a paired biopsy study, J. Bone Miner. Res. 16 (10) (Oct 2001) 1846–1853, http://dx.doi.org/10.1359/jbmr.2001.16.10.1846.

[27] P.D. Miller, P.D. Delmas, R. Lindsay, N.B. Watts, M. Luckey, J. Adachi, et al., Early responsiveness of women with osteoporosis to teriparatide after therapy with alendronate or risedronate, J. Clin. Endocrinol. Metab. 93 (10) (2008) 3785–3793, http://dx.doi.org/10.1210/jc.2008-0353.

[28] R. Eastell, J.H. Krege, P. Chen, E.V. Glass, J.Y. Reginster, Development of an algorithm for using PINP to monitor treatment of patients with teriparatide, Curr. Med. Res. Opin. 22 (1) (2006) 61–66, http://dx.doi.org/10.1185/030079905X75096.

[29] P. Chen, J.H. Satterwhite, A.A. Licata, E.M. Lewiecki, A.A. Sipos, D.M. Misurski, et al., Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis, J. Bone Miner. Res. 20 (6) (2005) 962–970, http://dx.doi.org/10.1359/JBMR.050105.

[30] M.R. McClung, J. San Martin, P.D. Miller, R. Civitelli, F. Bandeira, M. Omizo, et al., Opposite bone remodeling effects of teriparatide and alendronate in increasing bone mass, Arch. Intern. Med. 165 (15) (2005) 1762–1768, http://dx.doi.org/10.1001/archinte.165.15.1762.

[31] D.W. Dempster, H. Zhou, R.R. Recker, J.P. Brown, M.A. Bolognese, C.P. Recknor, et al., Skeletal histomorphometry in subjects on teriparatide or zoledronic acid therapy (SHOTZ) study: a randomized controlled trial, J. Clin. Endocrinol. Metab. 97 (8) (2012) 2799–2808, http://dx.doi.org/10.1210/jc.2012-1262.

[32] S. Boonen, F. Marin, B. Obermayer-Pietsch, M.E. Simões, C. Barker, E.V. Glass, et al., Effects of previous antiresorptive therapy on the bone mineral density response to 2 years of teriparatide treatment in postmenopausal women with osteoporosis, J. Clin. Endocrinol. Metab. 93 (3) (2008) 852–860, http://dx.doi.org/10.12/jc.2007-0711.

[33] D.W. Dempster, J.E. Compston, M.K. Drezner, F.H. Glorieux, J.A. Kanis, H. Malluche, et al., Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee, J. Bone Miner. Res. 28 (1) (2013) 2–17, http://dx.doi.org/10.1002/jbmr.1805.