Original Article

Comparison of the effects of denosumab between native vitamin D combination and active vitamin D combination in patients with postmenopausal osteoporosis

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Abstract

Purpose

The aim of this 12-month, retrospective study was to compare the effects of denosumab (DMAb; 60 mg sc every 6 months) with native vitamin D (VD) (cholecalciferol) combination to DMAb with an active VD analog (alfacalcidol) combination in patients with postmenopausal osteoporosis.

Methods

Patients (n=127; mean age 75.6 [58-93] years; treatment-naïve [n=28]; oral bisphosphonate-treated [n=59]; daily teriparatide-treated [n=40]) were allocated to either the (1) "DMAb + native VD" group (n=60; cholecalciferol 10 μg + calcium 610 mg/day; treatment naïve [n=13]; oral bisphosphonate-treated [n=28]; daily teriparatide-treated [n=19]) or (2) "DMAb + active VD" group (n=67; alfacalcidol 0.8±0.0 μg + calcium 99.2±8.5 mg/day; treatment-naïve [n=15]; oral bisphosphonate-treated [n=31]; daily teriparatide-treated [n=21]) based on each physician's decision. Changes in bone mineral density (BMD), serum bone turnover markers, and fracture incidence were monitored every 6 months.

Results

There were no significant differences in baseline age, BMD, bone turnover marker

levels, and prior treatments ratio between the two groups. After 12 months, compared with the DMAb + native VD group, the DMAb + active VD group showed similar increases in BMDs of the lumbar spine (6.4% vs 6.5%) and total hip (3.3% vs 3.4%), although it showed significantly greater increases in the BMDs of the femoral neck (1.0% vs 4.9%; $P < 0.001$) and distal forearm (1/3 radius) (-0.8 vs 3.9%; $P < 0.01$). These tendencies were similar regardless of the differences in the prior treatments. The decrease rates of bone turnover markers were similar for TRACP-5b (-49.0% vs -49.0%), PINP (-45.9% vs -49.3%), and ucOC (-56.0% vs -66.5%), while serum intact-PTH levels were significantly lower in the DMAb + active VD group (47.6 vs 30.4 pg/ml; $P < 0.001$). The rate of hypocalcemia was 1.7% in the DMAb + native VD group and 1.5% in the DMAb + active VD group, and the rate of clinical fracture incidence was 8.3% in the DMAb + native VD group and 4.5% in the DMAb + active VD group, with no significant difference between the groups.

Conclusions

DMAb with active VD combination may be a more effective treatment option than DMAb with native VD combination in terms of increasing BMDs of the femoral neck and distal forearm and also maintaining serum intact-PTH at lower levels.

Keywords

Postmenopausal osteoporosis, Denosumab, Cholecalciferol, Alfacalcidol

Introduction

Receptor activator of NF-κB ligand (RANKL) is mainly produced by osteoblasts and osteocytes, which play critical roles in osteoclast differentiation and bone resorption [1-3]. Denosumab (DMAb), a fully human monoclonal antibody to receptor activator of NF-κB ligand (RANKL), has shown a greater increase in bone mineral density (BMD) and reduction in bone resorption than bisphosphonates (BP) such as alendronate (ALN) [4], ibandronate [5], or risedronate [6], and it also significantly increased BMD and decreased bone turnover markers when switched from ALN compared to continuing ALN therapy in postmenopausal osteoporosis [7]. We have previously demonstrated that switching daily teriparatide (TPTD) to DMAb significantly increased BMD and decreased bone resorption markers compared to switching to oral BP [8]. Thus, DMAb may be effective not only in treatment-naïve patients, but also in patients switched from BP or daily TPTD, which may be the major objects of DMAb treatment in the real-world setting.

Previous clinical studies of DMAb were mostly conducted using a combination of native vitamin D (VD) and calcium [4, 7, 9, 10]. However, both native VD and active VD analog (alfacalcidol) can be used in combination with DMAb in Japan, and still lack reliable evidence for the proper use. Alfacalcidol (ALF) is a pro-drug of active VD

hormone calcitriol, and its favorable effects on calcium absorption, bone mineralization, reduction of serum parathyroid hormone (PTH) levels, improving muscle function, and decreasing risk of falls have been reported [11]. Furthermore, some studies demonstrated that active VD analogs including ALF have advantageous effects by preventing bone loss, osteoporosis-related fractures, and falls compared to native VD [12, 13]. Indeed, combination therapy with ALN and ALF was superior in increasing BMD and decreasing fracture rates than a combination with native (plain) VD [14, 15]. However, there are no previous reports that demonstrated the different effects of DMAb when combined with native or active VD, and we hypothesized that its combination with active VD may have advantageous effects compared to native VD.

The aim of this 12-month retrospective study was to compare the effects of DMAb in combination with active VD to DMAb in combination with native VD in patients with postmenopausal osteoporosis.

Materials and methods

Study design and subjects

This 12-month retrospective study was carried out at 3 centers. A total of 127 patients (treatment naïve n=28, prior treatment with oral BP n=59, prior treatment with daily

TPTD n=40) with postmenopausal osteoporosis who met the criteria of the Japanese guidelines for prevention and treatment of osteoporosis 2011 [16] were enrolled in the study (Fig. 1). Patients were allocated to either the "DMAb + native VD" group (n=60), consisting of patients who were treated with DMAb (60 mg sc every 6 months) in combination with oral cholecalciferol 10 μg and calcium 610 mg/day (Denotas®; Daiichi Sankyo Company, Limited, Tokyo, Japan)**,** or the "DMAb + active VD" group (n=67), consisting of patients who were treated with DMAb in combination with oral ALF 0.8 ± 0.0 (0.25-1.0) μg and calcium formulation 99.2 ± 8.5 (0-260) mg/day, depending on each physician's decision (Table 1).

This observational study was conducted in accordance with the ethical standards of the Declaration of Helsinki and approved by ethical review boards at each clinical center (approval number 13231-2; Osaka University, Graduate School of Medicine) and posted on the hospital homepage, with informed consent obtained from individual patients included in the study.

BMD assessment

Areal BMDs of the lumbar spine (LS, L2–L4), total hip (TH), femoral neck (FN), and distal forearm (DF; 1/3 radius) were assessed by dual-energy x-ray absorptiometry

(Discovery A, Hologic, Inc., Waltham, MA) at baseline and after 6 and 12 months of treatment. Regions of severe scoliosis, previous vertebral fractures, and postoperative sites were excluded from BMD measurements, and at least 2 of the lumbar vertebrae L2–L4 had to be evaluable for BMD. Subjects were excluded from the BMD analyses if the area was fractured or operated on during the study, as previously described [17-19].

Biochemical markers of bone turnover

Bone turnover markers were measured in serum obtained from each patient at approximately the same time in the morning after overnight fasting. The bone formation marker, N-terminal type I procollagen propeptide (PINP) (inter-assay coefficient of variation [CV] 3.2%−5.2%, Intact UniQ assay, Orion Diagnostica, Espoo, Finland) and bone resorption marker, isoform 5b of tartrate-resistant acid phosphatase (TRACP-5b) (inter-assay CV 5.0%−9.0%, Immunodiagnostic Systems Ltd., Boldon, UK) were measured by ELISA as previously described [17, 19, 20]. Levels of undercarboxylated osteocalcin (ucOC) were measured by a solid-phase enzyme immunoassay kit (inter-assay CV 5.2%–8.3%, Takara Bio, Shiga, Japan) with a sensitivity of 0.25 ng/mL. UcOC reflects not only vitamin K deficiency, but also total bone turnover, since it is released from both osteoblasts and absorbed bone extracellular matrix by osteoclasts as

previously described [19, 21]. Intact parathyroid hormone (PTH) was measured using a two-site immunoradiometric assay (inter-assay CV 8.4%, Nichols Institute Diagnostics, Valencia, California).

Radiographs

Spinal radiographs were obtained at baseline and also at unscheduled times if subjects had symptoms suggestive of clinical vertebral fractures. For incidental non-vertebral fractures, radiographs were assessed by the investigator if subjects had symptoms.

Statistical analysis

Differences between each study group were tested using the Mann-Whitney U test or the chi-squared test. Changes in BMD and ranked bone turnover marker data from baseline to specified time points within each study group were compared using the nonparametric Wilcoxon signed-rank test. Results are expressed as means ± standard error. A P value < 0.05 indicated significance. All tests were performed using IBM SPSS Statistics version 22 software (IBM, Armonk, NY).

Results

Baseline characteristics are shown in Table 1. There were no significant differences in the percentage of prior osteoporosis treatment and combined VD, baseline age, body mass index, rate of prior vertebral fracture, areal BMD, or bone turnover markers between the two groups. However, compared with the DMAb + native VD group, the DMAb + active VD group had longer prior TPTD treatment duration (15.4 vs 21.8 months; $P < 0.001$), lesser VD dose (10.0 vs 0.8 μg/day; $P < 0.001$), and a lesser combined calcium dose (610.0 vs 99.2 mg/day; $P < 0.001$) and use frequency (100.0% vs 88.1%; $P < 0.01$).

Overall, 96.7% (58/60) of the DMAb + native VD group (2 patients were lost to follow-up) and 92.5% (62/67) of the DMAb + active VD group (2 patients were lost to follow-up and 3 patients desired to change the medication) completed 12 months of therapy, with no significant differences in dropout rates between the groups (Fig. 1). During the 12-month period, in the DMAb + native VD group, 8.3% (5/60) patients suffered from clinical fractures (2 vertebral, 1 femoral trochanter, 1 humerus, and 1 toe fractures). In the DMAb + active VD group, 4.5% (3/67) patients suffered from clinical fractures (2 vertebral and 1 humerus fractures). No significant difference was observed in the fracture rate between the two groups.

Change in BMD

BMD was monitored every 6 months (Fig. 2). The DMAb + active VD group showed significant increases in BMD from baseline to 6 months and 12 months in the LS (4.4%; $P < 0.001$ and 6.5%; $P < 0.001$), TH (3.3%; $P < 0.001$ and 3.4%; $P < 0.001$), and FN (4.5%; $P < 0.001$ and 4.9%; $P < 0.001$), and at 12 months in the DF (3.9%; $P < 0.01$). On the other hand, the DMAb + native VD group showed significant increases in BMD from baseline to 6 months and 12 months in the LS (3.2%; P < 0.001 and 6.4%; $P < 0.001$) and TH (2.1%; P < 0.01 and 3.3%; $P < 0.01$), but no significant increases were observed in the FN and DF.

Moreover, the DMAb + active VD group showed a significantly greater BMD increase compared to the DMAb + native VD group in the FN from 6 months (4.5 vs 0.6%; $P < 0.001$) to 12 months (4.9 vs 1.0%; $P < 0.001$) and in the DF at 12 months (3.9 vs -0.8%; $P < 0.01$).

The difference in percent change of areal BMD by prior treatment and combined VD was also evaluated (Table 2). There was no significant difference in the LS and TH between the groups. However, the DMAb + active VD group showed a significantly greater BMD increase of the FN compared to the DMAb + native VD group in the prior treatment-naïve (4.9 vs 0.8%; $P < 0.05$) and TPTD-treated groups (6.4 vs 0.7%; $P <$

191 0.01), and of the DF in the prior BP-treated group (3.0 vs -1.6%; $P < 0.05$) at 12
192 months.
193
194 *Bone turnover markers*
195 Percent changes in bone turnover markers from baseline are shown in Fig. 3. No
196 significant differences were observed between the two groups with regard to the
197 changes in serum TRACP-5b, PINP, and ucOC levels from 6 months to 12 months. The
198 absolute values of bone turnover markers are shown in Fig. 4. There were no significant
199 differences in absolute TRACP-5b, PINP, and ucOC levels from baseline to 6 and 12
200 months between the groups; all values were within the reference values. However,
201 serum intact-PTH levels were significantly decreased in the DMAb + active VD group
202 from baseline to 6 months (38.2 vs 32.8 pg/mL; $P < 0.001$) and 12 months (38.2 vs 30.4
203 pg/mL; $P < 0.001$), while no significant change was observed in the DMAb + native VD
204 group. Moreover, serum intact-PTH levels were significantly lower in the DMAb +
205 active VD group compared to the DMAb + native VD group from 6 months (32.8 vs
206 51.1 pg/mL; $P < 0.001$) to 12 months (30.4 vs 47.6 pg/mL; $P < 0.001$). During this
207 period, the rate of hypocalcemia (corrected serum calcium level < 8.6 mg/dl) was 1.7%
208 (1/60) in the DMAb + native VD group and 1.5% (1/67) in the DMAb + active VD

group. On the other hand, the rate of hypercalcemia (corrected serum calcium level > 10.2 mg/dl) was 3.3% (2/60) in the DMAb + native VD group and 3.0% (2/67) in the DMAb + active VD group. There were no significant differences in the rates of hypocalcemia and hypercalcemia between the two groups.

Discussion

A previous report demonstrated that increased BMD may be obtained by a combination of 3 elements: (1) remodeling closure (inhibition of bone resorption); (2) secondary mineralization (related to calcium and vitamin D metabolism); and (3) bone modeling without bone resorption [22].

Considering remodeling closure, the decrease rate and the absolute value of bone turnover markers such as TRACP-5b, PINP, and ucOC were all similar between the groups. These results suggest that the difference in the VD may not significantly affect total bone turnover during DMAb treatment.

Considering secondary mineralization related to VD and calcium metabolism, the DMAb + active VD group showed a significantly greater decrease and lower serum intact-PTH levels compared to the DMAb + native VD group, although it had a lower calcium formulation combination rate (88.1% vs 100.0%; P <0.01) and dose (99.2 vs

610 mg/day; P <0.001).

Ultraviolet B-radiation of sunlight to the skin converts dehydrocholesterol to cholecalciferol (native VD), which has to be activated by two steps of hydroxylation. First, it becomes 25 hydroxycholecalciferol (25-OH-D3) by 25-hydroxylase in the liver (also expressed in the osteoblasts), and subsequently it becomes 1α, 25-dihydroxy-cholecalciferol［1α, 25-(OH)2-D3］by 1-alpha-hydroxylase in the kidney [11]. Renal 1α-hydroxylation is strongly restricted by a negative-feedback mechanism with a sufficient VD-hormone level, and also in renal dysfunction with a creatinine clearance of < 65 ml/min [11]. ALF can be directly activated in the liver or locally in osteoblasts to be active VD-hormone without renal feedback. Ringe et al. suggested that active VD (ALF) is especially recommended in patients with renal insufficiency; e.g. creatinine clearance <60–65 ml/min [11]. Taken together, native VD is effective only in patients with VD insufficiency (25(OH)D < 30 ng/ml) and normal renal function, while ALF is also effective in both VD replete and renal insufficient patients [11, 13, 23].

PTH plays important roles in determining bone resorption and bone mass [24, 25]. Serum PTH levels increase in response to a low serum 25-hydroxyvitamin D［25(OH)D］level [26] and low calcium intake [27], which promotes bone resorption and consequent bone loss [28]. A previous report demonstrated that, in ovariectomized

monkeys, DMAb treatment with native VD and calcium supplementation did not alter serum intact-PTH levels [29], which was consistent with the present study. However, other previous reports demonstrated that BP monotherapy increased serum intact-PTH levels, while a combination with BP and active VD decreased serum intact-PTH levels [18, 30, 31], and decreased serum intact-PTH levels were positively correlated with BMD increase [18, 30]. Taken together, ALF may have advantageous effects in decreasing serum intact-PTH levels and increasing BMD, compared to native VD in DMAb treatment.

Considering bone modeling, it has been reported that active VD (calcitriol and eldecalcitol) [32] induces bone modeling or minimodeling, which is considered to be focal bone formation with the resumption of osteoblastic activity of bone lining cells [33]. Furthermore, ALF increased not only focal bone formation on cancellous surfaces, but also periosteal bone formation [34] and cortical bone BMD of rats [35]. However, cholecalciferol did not alter cortical bone morphology in mice [36]. From these observations, active VD including ALF may have stronger effects, especially on cortical bone compared to cholecalciferol, which is consistent with the present study.

Since this study was based on a real-world setting, only 22.0% of patients were treatment-naïve, and 46.5% of patients were switched from BP treatment. Previous

263 clinical studies, which were mostly conducted in osteoporosis treatment-naïve or
264 treatment washed-out patients with native VD, demonstrated that 12 months
265 administration of DMAb increased BMD by approximately 5.3-6.5% in the LS, 3.5% in
266 the TH, 2.4-2.7% in the FN, and 0.2% in the DF [4, 9, 10, 37]. On the other hand, a
267 previous study showed that switching alendronate to DMAb in combination with native
268 VD increased BMD by 3.0% in the LS, 1.9% in the TH, 1.4% in the FN, and 0.9% in
269 the 1/3 radius at 12 months, which were relatively small compared to previous studies
270 of treatment-naïve or treatment washed-out patients [7]. These tendencies were similar
271 in both groups of the present study, although active VD group achieved higher BMD
272 increase compared to native VD group under such conditions.
273 There are several limitations to this study. Due to the small number of subjects,
274 statistical power of the results may be attenuated, although significant differences were
275 observed between native and active VD groups even if distributed by the difference of
276 prior treatment. As based on real-world setting, patients and treatment selection were
277 not randomized and depended on each physician's decision, while general patients'
278 background and the ratio of prior treatment were similar between the two groups. As
279 spinal x-ray is not routinely conducted, subclinical vertebral fracture couldn't be
280 monitored. Whether a greater change in BMD induced by DMAb + active VD than that

of DMAb + native VD may reduce fracture risk should be assessed in a larger cohort. In addition, unknown baseline oral calcium intake and baseline VD-hormone levels may have affected the results, and possible subclinical hypercalciuria which may promote renal dysfunction couldn't be monitored in some part of the patients.

In conclusion, DMAb with active VD combination significantly increased BMDs of the FN and DF, where cortical bone is relatively abundant and also in maintaining serum intact-PTH at lower levels compared to native VD combination, suggesting combining active VD may be an effective option of DMAb treatment.

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Conflicts of interest

K Ebina has received payments for lectures from Daiichi Sankyo. M Kashii, M Hirao, J Hashimoto, T Noguchi, K Koizumi, K Kitaguchi, H Matsuoka, T Iwahashi, Y Tsukamoto, and H Yoshikawa declare that they have no conflicts of interest.

References

1. Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. Nature 423:337-342

2. Honma M, Ikebuchi Y, Kariya Y, Hayashi M, Hayashi N, Aoki S, Suzuki H (2013) RANKL subcellular trafficking and regulatory mechanisms in osteocytes. J Bone Miner Res 28:1936-1949

3. Setsu N, Kobayashi E, Asano N, Yasui N, Kawamoto H, Kawai A, Horiuchi K (2016) Severe hypercalcemia following denosumab treatment in a juvenile patient. J Bone Miner Metab 34:118-122

4. Brown JP, Prince RL, Deal C, et al. (2009) Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. J Bone Miner Res 24:153-161

5. Recknor C, Czerwinski E, Bone HG, et al. (2013) Denosumab compared with ibandronate in postmenopausal women previously treated with bisphosphonate therapy: a randomized open-label trial. Obstet Gynecol 121:1291-1299

6. Roux C, Hofbauer LC, Ho PR, et al. (2014) Denosumab compared with risedronate in postmenopausal women suboptimally adherent to alendronate therapy: efficacy and safety results from a randomized open-label study. Bone 58:48-54

7. Kendler DL, Roux C, Benhamou CL, Brown JP, Lillestol M, Siddhanti S, Man HS, San Martin J, Bone HG (2010) Effects of denosumab on bone mineral density and bone turnover in postmenopausal women transitioning from alendronate therapy. J Bone Miner Res 25:72-81

8. Ebina K, Hashimoto J, Kashii M, Hirao M, Kaneshiro S, Noguchi T, Tsukamoto Y, Yoshikawa H (2016) The effects of switching daily teriparatide to oral bisphosphonates or denosumab in patients with primary osteoporosis. J Bone Miner Metab

9. Nakamura T, Matsumoto T, Sugimoto T, et al. (2014) Clinical Trials Express: fracture risk reduction with denosumab in Japanese postmenopausal women and men with osteoporosis: denosumab fracture intervention randomized placebo controlled trial (DIRECT). J Clin Endocrinol Metab 99:2599-2607

10. Sugimoto T, Matsumoto T, Hosoi T, et al. (2015) Three-year denosumab treatment in postmenopausal Japanese women and men with osteoporosis: results from a 1-year open-label extension of the Denosumab Fracture Intervention Randomized Placebo Controlled Trial (DIRECT). Osteoporos Int 26:765-774

11. Ringe JD, Schacht E (2009) Potential of alfacalcidol for reducing increased risk of falls and fractures. Rheumatol Int 29:1177-1185

12. Richy F, Dukas L, Schacht E (2008) Differential effects of D-hormone analogs and

native vitamin D on the risk of falls: a comparative meta-analysis. Calcif Tissue Int 82:102-107

13. Richy F, Schacht E, Bruyere O, Ethgen O, Gourlay M, Reginster JY (2005) Vitamin D analogs versus native vitamin D in preventing bone loss and osteoporosis-related fractures: a comparative meta-analysis. Calcif Tissue Int 76:176-186

14. Ringe JD, Farahmand P, Schacht E, Rozehnal A (2007) Superiority of a combined treatment of Alendronate and Alfacalcidol compared to the combination of Alendronate and plain vitamin D or Alfacalcidol alone in established postmenopausal or male osteoporosis (AAC-Trial). Rheumatol Int 27:425-434

15. Ringe JD, Schacht E (2007) Improving the outcome of established therapies for osteoporosis by adding the active D-hormone analog alfacalcidol. Rheumatol Int 28:103-111

16. Orimo H, Nakamura T, Hosoi T, et al. (2012) Japanese 2011 guidelines for prevention and treatment of osteoporosis--executive summary. Arch Osteoporos 7:3-20

17. Ebina K, Hashimoto J, Shi K, Kashii M, Hirao M, Yoshikawa H (2014) Comparison of the effect of 18-month daily teriparatide administration on patients with rheumatoid arthritis and postmenopausal osteoporosis patients. Osteoporos Int 25:2755-2765

18. Ebina K, Noguchi T, Hirao M, Kaneshiro S, Tsukamoto Y, Yoshikawa H (2015) Comparison of the effects of 12 months of monthly minodronate monotherapy and monthly minodronate combination therapy with vitamin K or eldecalcitol in patients with primary osteoporosis. J Bone Miner Metab

19. Ebina K, Shi K, Hirao M, Kaneshiro S, Morimoto T, Koizumi K, Yoshikawa H, Hashimoto J (2013) Vitamin K2 administration is associated with decreased disease activity in patients with rheumatoid arthritis. Mod Rheumatol 23:1001-1007

20. Ebina K, Hashimoto J, Shi K, Kashii M, Hirao M, Yoshikawa H (2014) Undercarboxylated osteocalcin may be an attractive marker of teriparatide treatment in RA patients: response to Mokuda. Osteoporos Int

21. Booth SL, Centi A, Smith SR, Gundberg C (2013) The role of osteocalcin in human glucose metabolism: marker or mediator? Nat Rev Endocrinol 9:43-55

22. Ominsky MS, Libanati C, Niu QT, Boyce RW, Kostenuik PJ, Wagman RB, Baron R, Dempster DW (2015) Sustained Modeling-Based Bone Formation During Adulthood in Cynomolgus Monkeys May Contribute to Continuous BMD Gains with Denosumab. J Bone Miner Res

23. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, Staehelin HB, Bazemore MG, Zee RY, Wong JB (2004) Effect of Vitamin D on falls: a meta-analysis. JAMA 291:1999-2006

24. Arabi A, Baddoura R, El-Rassi R, El-Hajj Fuleihan G (2012) PTH level but not 25 (OH) vitamin D level predicts bone loss rates in the elderly. Osteoporos Int 23:971-980

25. Neer RM, Arnaud CD, Zanchetta JR, et al. (2001) Effect of parathyroid hormone (1-34)

on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 344:1434-1441

26. Dawson-Hughes B, Harris SS, Dallal GE (1997) Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. Am J Clin Nutr 65:67-71

27. Aloia J, Bojadzievski T, Yusupov E, Shahzad G, Pollack S, Mikhail M, Yeh J (2010) The relative influence of calcium intake and vitamin D status on serum parathyroid hormone and bone turnover biomarkers in a double-blind, placebo-controlled parallel group, longitudinal factorial design. J Clin Endocrinol Metab 95:3216-3224

28. Chaitou A, Boutroy S, Vilayphiou N, et al. (2011) Association of bone microarchitecture with parathyroid hormone concentration and calcium intake in men: the STRAMBO study. Eur J Endocrinol 165:151-159

29. Kostenuik PJ, Smith SY, Samadfam R, Jolette J, Zhou L, Ominsky MS (2015) Effects of denosumab, alendronate, or denosumab following alendronate on bone turnover, calcium homeostasis, bone mass and bone strength in ovariectomized cynomolgus monkeys. J Bone Miner Res 30:657-669

30. Barone A, Giusti A, Pioli G, Girasole G, Razzano M, Pizzonia M, Palummeri E, Bianchi G (2007) Secondary hyperparathyroidism due to hypovitaminosis D affects bone mineral density response to alendronate in elderly women with osteoporosis: a randomized controlled trial. J Am Geriatr Soc 55:752-757

31. Shiraki M, Kushida K, Fukunaga M, et al. (1998) A placebo-controlled, single-blind study to determine the appropriate alendronate dosage in postmenopausal Japanese patients with osteoporosis. The Alendronate Research Group. Endocr J 45:191-201

32. Saito H, Takeda S, Amizuka N (2013) Eldecalcitol and calcitriol stimulates 'bone minimodeling,' focal bone formation without prior bone resorption, in rat trabecular bone. J Steroid Biochem Mol Biol 136:178-182

33. Dobnig H, Turner RT (1995) Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. Endocrinology 136:3632-3638

34. Chen H, Tian X, Liu X, Setterberg RB, Li M, Jee WS (2008) Alfacalcidol-stimulated focal bone formation on the cancellous surface and increased bone formation on the periosteal surface of the lumbar vertebrae of adult female rats. Calcif Tissue Int 82:127-136

35. Liu XQ, Chen HY, Tian XY, Setterberg RB, Li M, Jee WS (2008) Alfacalcidol treatment increases bone mass from anticatabolic and anabolic effects on cancellous and cortical bone in intact female rats. J Bone Miner Metab 26:425-435

36. Mallya SM, Corrado KR, Saria EA, Yuan FF, Tran HQ, Saucier K, Atti E, Tetradis S, Arnold A (2016) Modeling vitamin D insufficiency and moderate deficiency in adult mice via

dietary cholecalciferol restriction. Endocr Res 1-10

37. Eastell R, Christiansen C, Grauer A, et al. (2011) Effects of denosumab on bone turnover markers in postmenopausal osteoporosis. J Bone Miner Res 26:530-537

Figure legends

Fig. 1 Study design and schedule. Patients (n=127) were allocated to either the (1) DMAb + native VD group (n=60; treatment-naïve, n=13; oral BP, n=28; or daily TPTD, n=19) or the (2) DMAb + active VD group (n=67; treatment-naïve, n=15; oral BP, n=31; or daily TPTD, n=21) based on each physician's decision. Bone mineral density and bone turnover markers were evaluated every 6 months in all patients.

Fig. 2 Mean ± standard error (SE) change from baseline bone mineral density (BMD) in the lumbar spine (Panel a), total hip (Panel b), femoral neck (Panel c), and distal forearm (1/3 radius) (Panel d); $^{**}P < 0.01$, $^{***}P < 0.001$ change from baseline within each treatment group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ DMAb + native VD group versus DMAb + active VD group.

Fig. 3 Mean ± standard error (SE) change from baseline serum concentration of bone turnover markers TRAP-5b (Panel a), PINP (Panel b), and ucOC (Panel c). TRAP-5b, isoform 5b of tartrate-resistant acid phosphatase; PINP, type I collagen N-terminal

propeptide; ucOC, undercarboxylated osteocalcin.

Fig. 4 Mean ± standard error (SE) absolute values of bone turnover markers TRAP-5b (Panel a), PINP (Panel b), ucOC (Panel c), and intact PTH (Panel d). TRAP-5b, isoform 5b of tartrate-resistant acid phosphatase; PINP, type I collagen N-terminal propeptide; ucOC, undercarboxylated osteocalcin; PTH, parathyroid hormone. ***$P < 0.001$ change from baseline within each treatment group. $^{###}P < 0.001$ DMAb + native VD group versus DMAb + active VD group.

Table 1. Baseline clinical characteristics

Variable	DMAb + native VD (n=60)	DMAb + active VD (n=67)	*P* value
Prior osteoporosis treatment	Naïve (n=13; 21.7%)	Naïve (n=15; 22.4%)	N.S.
	Oral BP (n=28; 46.7%)	Oral BP (n=31; 46.3%)	N.S.
	Weekly ALN (n=16)	Weekly ALN (n=4)	
	Weekly RIS (n=16)	Weekly RIS (n=8)	
	Monthly MIN (n=6)	Monthly MIN (n=19)	
	Daily TPTD (n=19; 31.7%)	Daily TPTD (n=21; 31.3%)	N.S.
Prior combined VD	Naïve (n=32; 53.3%)	Naïve (n=26; 38.8%)	N.S.
	Alfacalcidol (n=22; 36.7%)	Alfacalcidol (n=26; 38.8%)	N.S.
	Eldecalcitol (n=6; 10.0%)	Eldecalcitol (n=15; 22.4%)	N.S.
Prior BP duration (months)	38.7±4.0	26.9±4.0	N.S.
Prior TPTD duration (months)	15.4±1.5	21.8±0.7	< 0.001
Combined VD	Cholecalciferol (n=60/60)	Alfacalcidol (n=67/67)	
Combined VD, μg/day	10±0.0	0.8±0.0	< 0.001
Combined Ca, n/N (%)	60/60 (100.0%)	59/67 (88.1%)	< 0.01
Combined Ca, mg/day	610±0.0	99.2±8.5	< 0.001
Age, (years)	75.3±1.0	75.9±1.0	N.S.
Body mass index (kg/m^2)	21.6±0.4	20.7±0.4	N.S.
Prior vertebral fracture(s), n/N(%)	43/60 (71.7%)	46/67 (68.7%)	N.S.
Lumbar spine BMD (g/cm^2)	0.730±0.016	0.762±0.017	N.S.
Lumbar spine BMD (T-score)	-3.0±0.1	-2.8±0.1	N.S.
Total hip BMD (g/cm^2)	0.607±0.012	0.625±0.010	N.S.
Total hip BMD (T-score)	-2.5±0.1	-2.6±0.1	N.S.
Femoral neck BMD (g/cm^2)	0.561±0.012	0.565±0.013	N.S.
Femoral neck BMD (T-score)	-2.5±0.1	-2.6±0.1	N.S.
Distal forearm (1/3 radius) (g/cm^2)	0.346±0.014	0.381±0.014	N.S.
Distal forearm (1/3 radius) (T-score)	-4.7±0.2	-4.4±0.2	N.S.
Corrected Ca (mg/dl)	9.3±0.1	9.3±0.1	N.S.
Intact-PTH (pg/ml)	45.1±2.5	38.2±2.7	N.S.
PINP (μg/l)	67.8±9.2	61.3±6.7	N.S.

TRACP-5b (mU/dl)	439.5±30.9	422.2±32.8	N.S.
ucOC (ng/ml)	7.4±1.3	6.4±1.1	N.S.
eGFR (ml/min/1.73m^2)	67.9±2.2	67.5±2.4	N.S.

Data are expressed as means ± standard error (SE), unless otherwise noted.

BP, Bisphosphonate; DMAb, Denosumab; ALN, Alendronate; RIS, Risedronate; MIN, Minodronate; sc, subcutaneous; TPTD, daily teriparatide; N.S., not significant; n/N (%) = number of patients with measurements / total number of patients (%). Ca, calcium; Bone mineral density; BMD, PTH, parathyroid hormone; PINP, Type I collagen N-terminal propeptide; TRAP-5b, Isoform 5b of tartrate-resistant acid phosphatase; ucOC, Undercarboxylated osteocalcin; eGFR, Estimated glomerular filtration rate; Differences between the groups were determined by the Mann-Whitney U-test or chi-squared test.

Table 2. Difference in the percent change of bone mineral density (BMD) of the lumbar spine (LS), total hip (TH), femoral neck (FN), and distal forearm (DF; 1/3 radius) by prior treatment and combined vitamin D (VD) in denosumab treatment.

Areal BMD	Prior treatment	Combined VD	BMD change (%)	
			6 months	12 months
LS	naïve	native	5.5±1.2	8.5±1.4
		active	3.8±1.1	6.9±1.5
	BP	native	1.7±1.1	4.4±1.5
		active	4.5±1.1	6.4±1.5
	TPTD	native	3.8±1.2	7.2±1.6
		active	4.7±1.1	6.3±1.0
	total	native	3.2±0.6	6.4±0.8
		active	4.4±0.6	6.5±0.6
TH	naïve	native	3.5±1.4	2.8±1.6
		active	5.2±1.3	5.6±1.1
	BP	native	1.2±0.9	3.3±1.0
		active	2.5±0.8	2.0±1.0
	TPTD	native	2.2±1.1	3.5±1.0
		active	3.1±1.1	3.5±1.1
	total	native	2.1±0.6	3.3±0.6
		active	3.3±0.5	3.4±0.5
FN	naïve	native	1.1±1.4	0.8±1.5
		active	4.5±0.8*	4.9±0.8*
	BP	native	0.8±1.1	1.3±1.3
		active	2.3±1.5	1.7±1.0
	TPTD	native	0.1±1.2	0.7±1.2
		active	6.1±1.2***	6.4±1.1**
	total	native	0.6±0.6	1.0±0.7
		active	4.5±0.7***	4.9±0.6***
DF	naïve	native	0.6±2.7	-0.2±1.3
		active	6.8±2.2	7.2±0.6
	BP	native	-0.7±1.5	-1.6±1.1
		active	1.7±1.5	3.0±1.4*
	TPTD	native	-1.4±1.2	0.6±1.2

		active	0.1±1.7	4.9±2.2
	total	native	-0.7±1.0	-0.8±0.7
		active	1.9±0.8	3.9±0.8**

*P<0.05, **P<0.01, ***P<0.001.

Data are expressed as means ± standard error (SE), unless otherwise noted.

VD, vitamin D; BP, Bisphosphonate; TPTD, daily teriparatide; BMD, Bone mineral density; Differences between the groups were determined by the Mann-Whitney U-test.

Figure 1

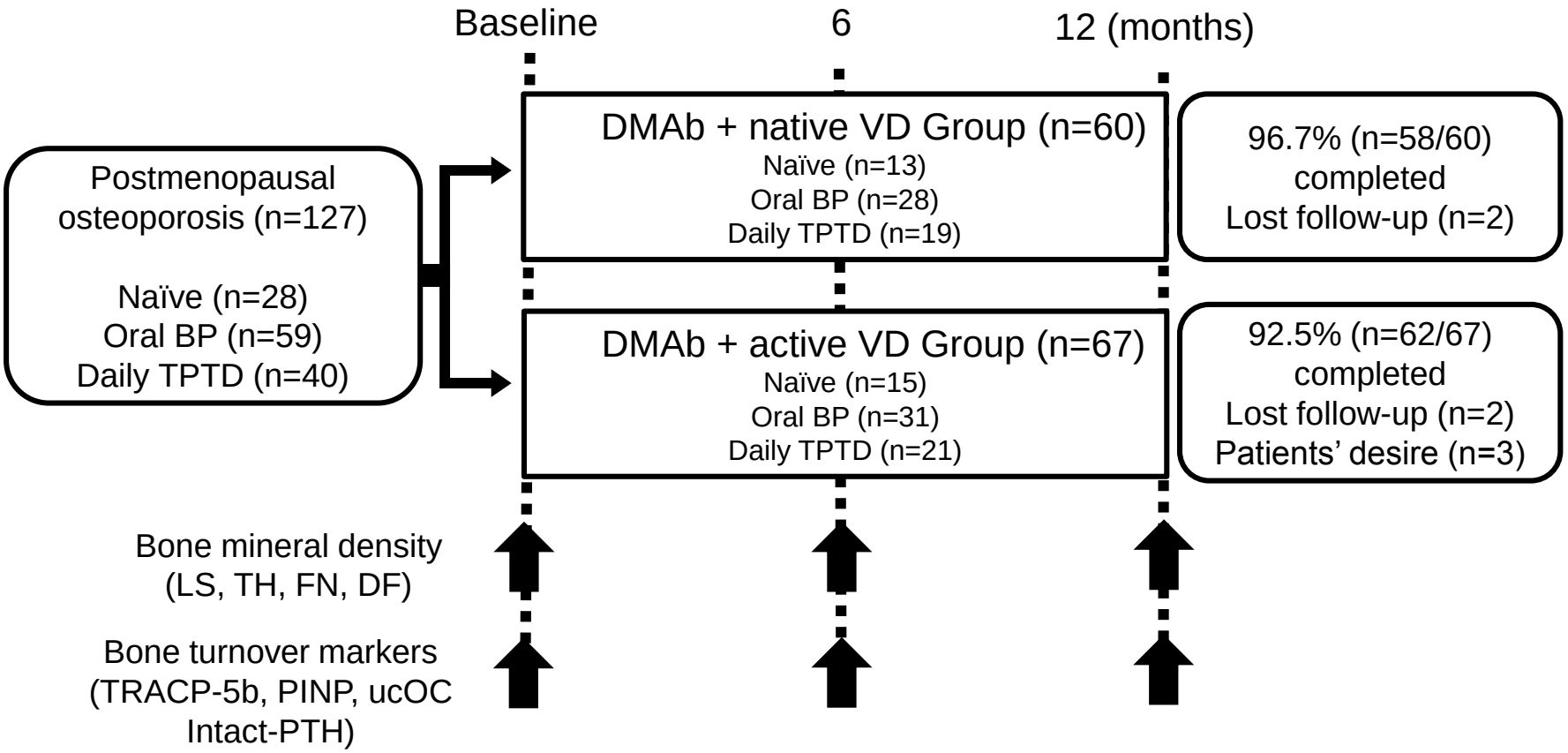

Baseline
6
12 (months)
Postmenopausal osteoporosis (n=127)
Naïve (n=28)
Oral BP (n=59)
Daily TPTD (n=40)
DMAb + native VD Group (n=60)
Naïve (n=13)
Oral BP (n=28)
Daily TPTD (n=19)
96.7% (n=58/60) completed
Lost follow-up (n=2)
DMAb + active VD Group (n=67)
Naïve (n=15)
Oral BP (n=31)
Daily TPTD (n=21)
92.5% (n=62/67) completed
Lost follow-up (n=2)
Patients' desire (n=3)
Bone mineral density (LS, TH, FN, DF)
Bone turnover markers (TRACP-5b, PINP, ucOC Intact-PTH)

Figure 2

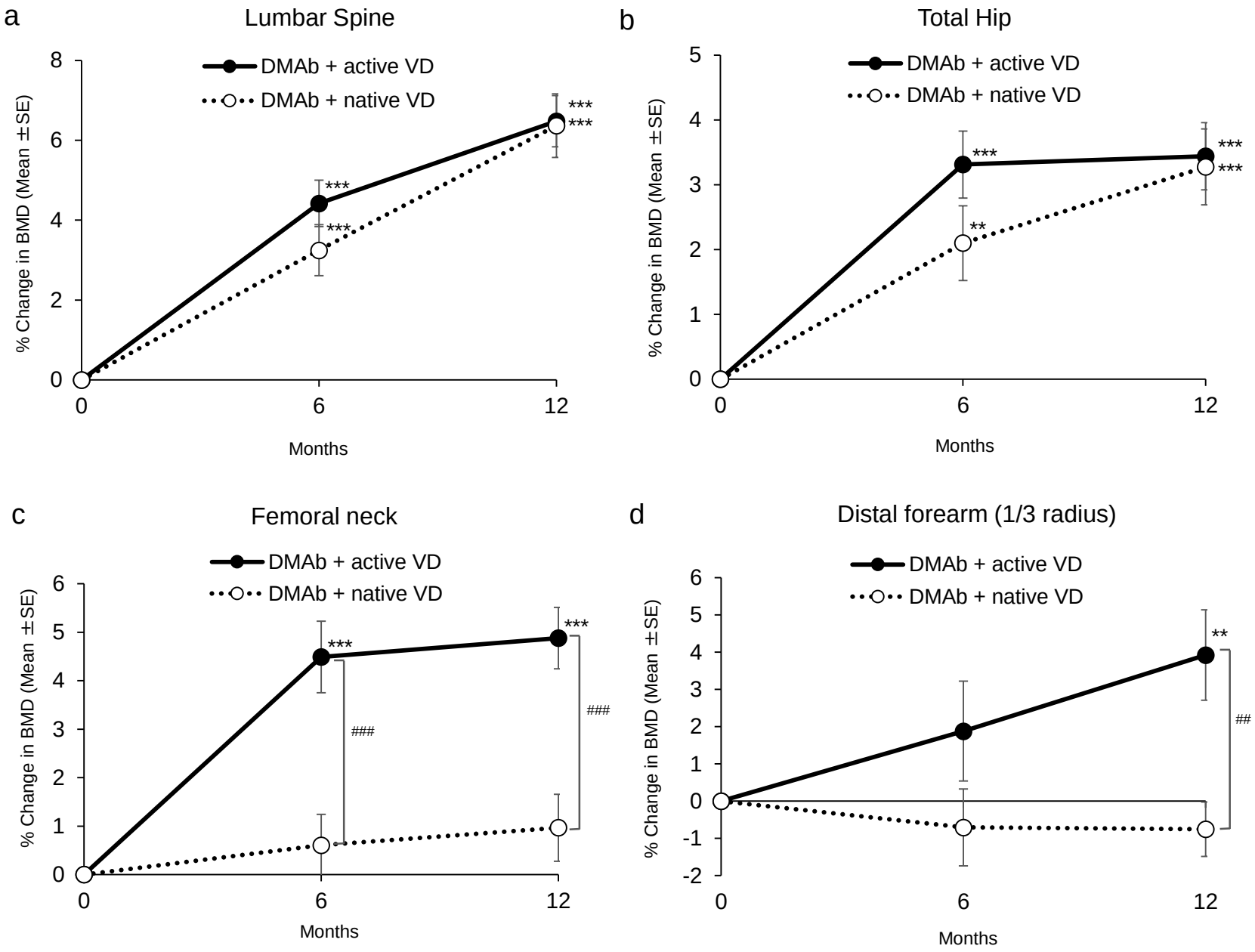

a
Lumbar Spine
DMAb + active VD
DMAb + native VD
% Change in BMD (Mean ±SE)
Months
***
***
***
b
Total Hip
DMAb + active VD
DMAb + native VD
% Change in BMD (Mean ±SE)
Months
***
**
***
***
c
Femoral neck
DMAb + active VD
DMAb + native VD
% Change in BMD (Mean ±SE)
Months
***
###
###
d
Distal forearm (1/3 radius)
DMAb + active VD
DMAb + native VD
% Change in BMD (Mean ±SE)
Months
**
##

Figure 3

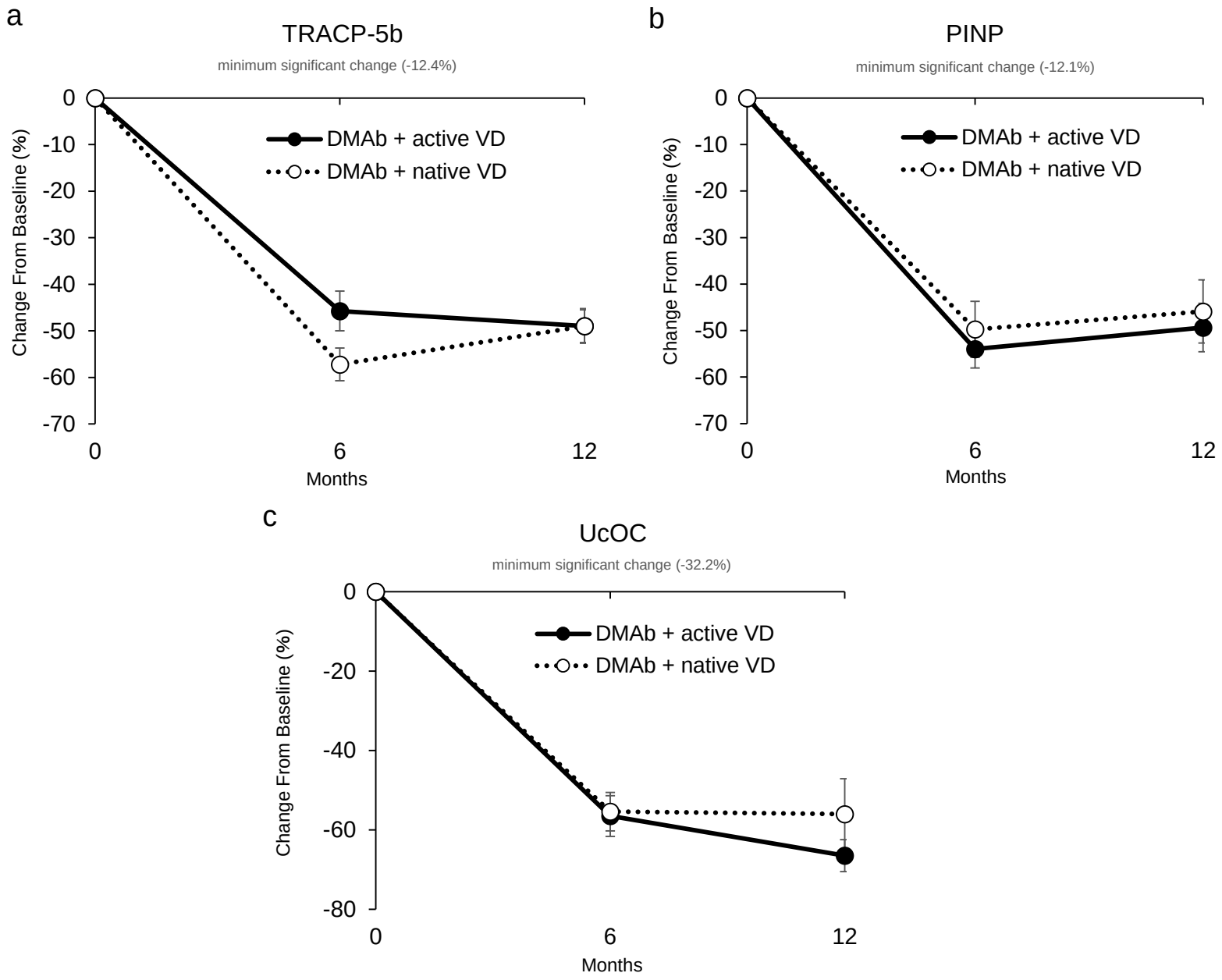
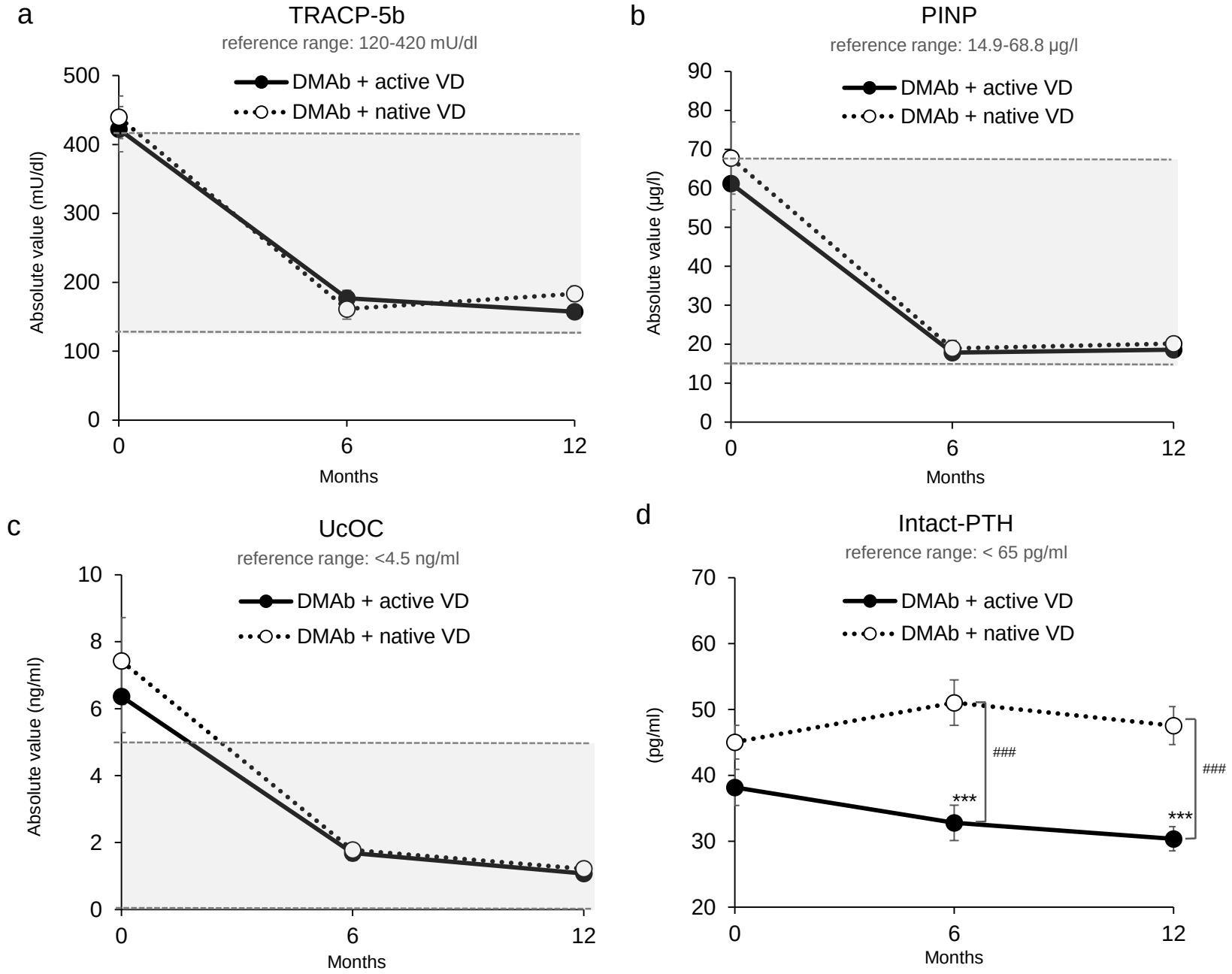

a
TRACP-5b
minimum significant change (-12.4%)
Change From Baseline (%)
0
-10
-20
-30
-40
-50
-60
-70
DMAb + active VD
DMAb + native VD
0
6
12
Months
b
PINP
minimum significant change (-12.1%)
Change From Baseline (%)
0
-10
-20
-30
-40
-50
-60
-70
DMAb + active VD
DMAb + native VD
0
6
12
Months
c
UcOC
minimum significant change (-32.2%)
Change From Baseline (%)
0
-20
-40
-60
-80
DMAb + active VD
DMAb + native VD
0
6
12
Months

Figure 4


a
TRACP-5b
reference range: 120-420 mU/dl
DMAb + active VD
DMAb + native VD
Absolute value (mU/dl)
500
400
300
200
100
0
0
6
12
Months
b
PINP
reference range: 14.9-68.8 µg/l
DMAb + active VD
DMAb + native VD
Absolute value (µg/l)
90
80
70
60
50
40
30
20
10
0
0
6
12
Months
c
UcOC
reference range: <4.5 ng/ml
DMAb + active VD
DMAb + native VD
Absolute value (ng/ml)
10
8
6
4
2
0
0
6
12
Months
d
Intact-PTH
reference range: < 65 pg/ml
DMAb + active VD
DMAb + native VD
(pg/ml)
70
60
50
40
30
20
###
###
***
***
0
6
12
Months