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1 **Original Article**

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

4

5 **Authors**

6 Kosuke Ebina, MD, PhD ^{a*}, Masafumi Kashii, MD, PhD ^a, Makoto Hirao, MD, PhD ^a,

7 Jun Hashimoto, MD, PhD ^b, Takaaki Noguchi, MD ^a, Kota Koizumi, MD, PhD ^c,

8 Kazuma Kitaguchi, MD ^a, Hozo Matsuoka, MD ^a, Toru Iwahashi, MD ^a, Yasunori

9 Tsukamoto, MD ^d, and Hideki Yoshikawa, MD, PhD ^a

10

11 **Affiliations**

12 ^aDepartment of Orthopaedic Surgery, Osaka University, Graduate School of Medicine,

13 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

14 ^bDepartment of Rheumatology, National Hospital Organization, Osaka Minami Medical

15 Center, 2-1 Kidohigashi, Kawachinagano, Osaka 586-8521, Japan

16 ^cDepartment of Orthopaedic Surgery, Japan Community Health Care Organization,

17 Osaka Hospital, 4-2-78 Fukushima ward, Osaka 586-8521, Japan

18 ^dDepartment of Orthopaedic Surgery, North Osaka Police Hospital, 1-2-2 Muroyama,

19 Ibaraki, Osaka 567-0052, Japan

20

21 ***Corresponding author**

22 Tel: +81 6 6879 3552; Fax: +81 6 6879 3559

23 E-mail: k-ebina@umin.ac.jp (K. Ebina)

24

25 **Abstract**

26 *Purpose*

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

31 *Methods*

32 Patients (n=127; mean age 75.6 [58-93] years; treatment-naïve [n=28]; oral
33 bisphosphonate-treated [n=59]; daily teriparatide-treated [n=40]) were allocated to
34 either the (1) “DMAb + native VD” group (n=60; cholecalciferol 10 µg + calcium 610
35 mg/day; treatment naïve [n=13]; oral bisphosphonate-treated [n=28]; daily
36 teriparatide-treated [n=19]) or (2) “DMAb + active VD” group (n=67; alfacalcidol
37 0.8±0.0 µg + calcium 99.2±8.5 mg/day; treatment-naïve [n=15]; oral
38 bisphosphonate-treated [n=31]; daily teriparatide-treated [n=21]) based on each
39 physician’s decision. Changes in bone mineral density (BMD), serum bone turnover
40 markers, and fracture incidence were monitored every 6 months.

41 *Results*

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

43 levels, and prior treatments ratio between the two groups. After 12 months, compared
44 with the DMAb + native VD group, the DMAb + active VD group showed similar
45 increases in BMDs of the lumbar spine (6.4% vs 6.5%) and total hip (3.3% vs 3.4%),
46 although it showed significantly greater increases in the BMDs of the femoral neck
47 (1.0% vs 4.9%; $P < 0.001$) and distal forearm (1/3 radius) (-0.8 vs 3.9%; $P < 0.01$).
48 These tendencies were similar regardless of the differences in the prior treatments. The
49 decrease rates of bone turnover markers were similar for TRACP-5b (-49.0% vs
50 -49.0%), PINP (-45.9% vs -49.3%), and ucOC (-56.0% vs -66.5%), while serum
51 intact-PTH levels were significantly lower in the DMAb + active VD group (47.6 vs
52 30.4 pg/ml; $P < 0.001$). The rate of hypocalcemia was 1.7% in the DMAb + native VD
53 group and 1.5% in the DMAb + active VD group, and the rate of clinical fracture
54 incidence was 8.3% in the DMAb + native VD group and 4.5% in the DMAb + active
55 VD group, with no significant difference between the groups.

56 *Conclusions*

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

60

61 **Keywords**

62 Postmenopausal osteoporosis, Denosumab, Cholecalciferol, Alfacalcidol

63

64

65 **Introduction**

66 Receptor activator of NF- κ B ligand (RANKL) is mainly produced by osteoblasts and
67 osteocytes, which play critical roles in osteoclast differentiation and bone resorption
68 [1-3]. Denosumab (DMAb), a fully human monoclonal antibody to receptor activator of
69 NF- κ B ligand (RANKL), has shown a greater increase in bone mineral density (BMD)
70 and reduction in bone resorption than bisphosphonates (BP) such as alendronate (ALN)
71 [4], ibandronate [5], or risedronate [6], and it also significantly increased BMD and
72 decreased bone turnover markers when switched from ALN compared to continuing
73 ALN therapy in postmenopausal osteoporosis [7]. We have previously demonstrated
74 that switching daily teriparatide (TPTD) to DMAb significantly increased BMD and
75 decreased bone resorption markers compared to switching to oral BP [8]. Thus, DMAb
76 may be effective not only in treatment-naïve patients, but also in patients switched from
77 BP or daily TPTD, which may be the major objects of DMAb treatment in the
78 real-world setting.

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

83 hormone calcitriol, and its favorable effects on calcium absorption, bone mineralization,
84 reduction of serum parathyroid hormone (PTH) levels, improving muscle function, and
85 decreasing risk of falls have been reported [11]. Furthermore, some studies
86 demonstrated that active VD analogs including ALF have advantageous effects by
87 preventing bone loss, osteoporosis-related fractures, and falls compared to native VD
88 [12, 13]. Indeed, combination therapy with ALN and ALF was superior in increasing
89 BMD and decreasing fracture rates than a combination with native (plain) VD [14, 15].
90 However, there are no previous reports that demonstrated the different effects of DMAb
91 when combined with native or active VD, and we hypothesized that its combination
92 with active VD may have advantageous effects compared to native VD.
93 The aim of this 12-month **retrospective** study was to compare the effects of DMAb in
94 combination with active VD to DMAb in combination with native VD in patients with
95 postmenopausal osteoporosis.

96

97 **Materials and methods**

98 *Study design and subjects*

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

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

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

115

116 *BMD assessment*

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

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

124

125 *Biochemical markers of bone turnover*

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

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

140

141 *Radiographs*

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

145

146 *Statistical analysis*

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

153

154 **Results**

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

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

172

173 *Change in BMD*

174 BMD was monitored every 6 months (Fig. 2). The DMAb + active VD group showed
175 significant increases in BMD from baseline to 6 months and 12 months in the LS (4.4%;
176 $P < 0.001$ and 6.5%; $P < 0.001$), TH (3.3%; $P < 0.001$ and 3.4%; $P < 0.001$), and FN
177 (4.5%; $P < 0.001$ and 4.9%; $P < 0.001$), and at 12 months in the DF (3.9%; $P < 0.01$).
178 On the other hand, the DMAb + native VD group showed significant increases in BMD
179 from baseline to 6 months and 12 months in the LS (3.2%; $P < 0.001$ and 6.4%; $P <$
180 0.001) and TH (2.1%; $P < 0.01$ and 3.3%; $P < 0.01$), but no significant increases were
181 observed in the FN and DF.
182 Moreover, the DMAb + active VD group showed a significantly greater BMD increase
183 compared to the DMAb + native VD group in the FN from 6 months (4.5 vs 0.6%; $P <$
184 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%;
185 $P < 0.01$).

186 The difference in percent change of areal BMD by prior treatment and combined VD
187 was also evaluated (Table 2). There was no significant difference in the LS and TH
188 between the groups. However, the DMAb + active VD group showed a significantly
189 greater BMD increase of the FN compared to the DMAb + native VD group in the prior
190 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

209 group. On the other hand, the rate of hypercalcemia (corrected serum calcium level >
210 10.2 mg/dl) was 3.3% (2/60) in the DMAb + native VD group and 3.0% (2/67) in the
211 DMAb + active VD group. There were no significant differences in the rates of
212 hypocalcemia and hypercalcemia between the two groups.

213

214 **Discussion**

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

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

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

227 610 mg/day; $P < 0.001$).

228 Ultraviolet B-radiation of sunlight to the skin converts dehydrocholesterol to
229 cholecalciferol (native VD), which has to be activated by two steps of hydroxylation.

230 First, it becomes 25 hydroxycholecalciferol (25-OH-D3) by 25-hydroxylase in the liver
231 (also expressed in the osteoblasts), and subsequently it becomes 1α ,

232 25-dihydroxy-cholecalciferol [1α , 25-(OH) $_2$ -D3] by 1-alpha-hydroxylase in the kidney

233 [11]. Renal 1α -hydroxylation is strongly restricted by a negative-feedback mechanism

234 with a sufficient VD-hormone level, and also in renal dysfunction with a creatinine

235 clearance of < 65 ml/min [11]. ALF can be directly activated in the liver or locally in

236 osteoblasts to be active VD-hormone without renal feedback. Ringe et al. suggested that

237 active VD (ALF) is especially recommended in patients with renal insufficiency; e.g.

238 creatinine clearance < 60 – 65 ml/min [11]. Taken together, native VD is effective only in

239 patients with VD insufficiency ($25(\text{OH})\text{D} < 30$ ng/ml) and normal renal function, while

240 ALF is also effective in both VD replete and renal insufficient patients [11, 13, 23].

241 PTH plays important roles in determining bone resorption and bone mass [24, 25].

242 Serum PTH levels increase in response to a low serum 25-hydroxyvitamin D

243 [$25(\text{OH})\text{D}$] level [26] and low calcium intake [27], which promotes bone resorption

244 and consequent bone loss [28]. A previous report demonstrated that, in ovariectomized

245 monkeys, DMAb treatment with native VD and calcium supplementation did not alter
246 serum intact-PTH levels [29], which was consistent with the present study. However,
247 other previous reports demonstrated that BP monotherapy increased serum intact-PTH
248 levels, while a combination with BP and active VD decreased serum intact-PTH levels
249 [18, 30, 31], and decreased serum intact-PTH levels were positively correlated with
250 BMD increase [18, 30]. Taken together, ALF may have advantageous effects in
251 decreasing serum intact-PTH levels and increasing BMD, compared to native VD in
252 DMAb treatment.

253 Considering bone modeling, it has been reported that active VD (calcitriol and
254 eldecacitol) [32] induces bone modeling or minimodeling, which is considered to be
255 focal bone formation with the resumption of osteoblastic activity of bone lining cells
256 [33]. Furthermore, ALF increased not only focal bone formation on cancellous surfaces,
257 but also periosteal bone formation [34] and cortical bone BMD of rats [35]. However,
258 cholecalciferol did not alter cortical bone morphology in mice [36]. From these
259 observations, active VD including ALF may have stronger effects, especially on cortical
260 bone compared to cholecalciferol, which is consistent with the present study.

261 Since this study was based on a real-world setting, only 22.0% of patients were
262 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

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

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

289

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293

294 **Conflicts of interest**

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

298

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414 **Figure legends**

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

420

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

426

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

430 propeptide; ucOC, undercarboxylated osteocalcin.

431

432 Fig. 4 Mean \pm standard error (SE) absolute values of bone turnover markers TRAP-5b

433 (Panel a), PINP (Panel b), ucOC (Panel c), and intact PTH (Panel d). TRAP-5b, isoform

434 5b of tartrate-resistant acid phosphatase; PINP, type I collagen N-terminal propeptide;

435 ucOC, undercarboxylated osteocalcin; PTH, parathyroid hormone. *** $P < 0.001$ change

436 from baseline within each treatment group. ### $P < 0.001$ DMAb + native VD group

437 versus DMAb + active VD group.

438

1 Table 1. Baseline clinical characteristics

Variable	DMAb + native VD (n=60)	DMAb + active VD (n=67)	<i>P</i> 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.

2 Data are expressed as means ± standard error (SE), unless otherwise noted.
3 BP, Bisphosphonate; DMAB, Denosumab; ALN, Alendronate; RIS, Risedronate; MIN, Minodronate; sc,
4 subcutaneous; TPTD, daily teriparatide; N.S., not significant; n/N (%) = number of patients with
5 measurements / total number of patients (%). Ca, calcium; Bone mineral density; BMD, PTH, parathyroid
6 hormone; PINP, Type I collagen N-terminal propeptide; TRAP-5b, Isoform 5b of tartrate-resistant acid
7 phosphatase; ucOC, Undercarboxylated osteocalcin; eGFR, Estimated glomerular filtration rate;
8 Differences between the groups were determined by the Mann-Whitney U-test or chi-squared test.

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10

11 Table 2. Difference in the percent change of bone mineral density (BMD) of the
 12 lumbar spine (LS), total hip (TH), femoral neck (FN), and distal forearm (DF; **1/3**
 13 **radius**) by prior treatment and combined vitamin D (VD) in denosumab treatment.
 14

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**

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

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

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

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Figure 1

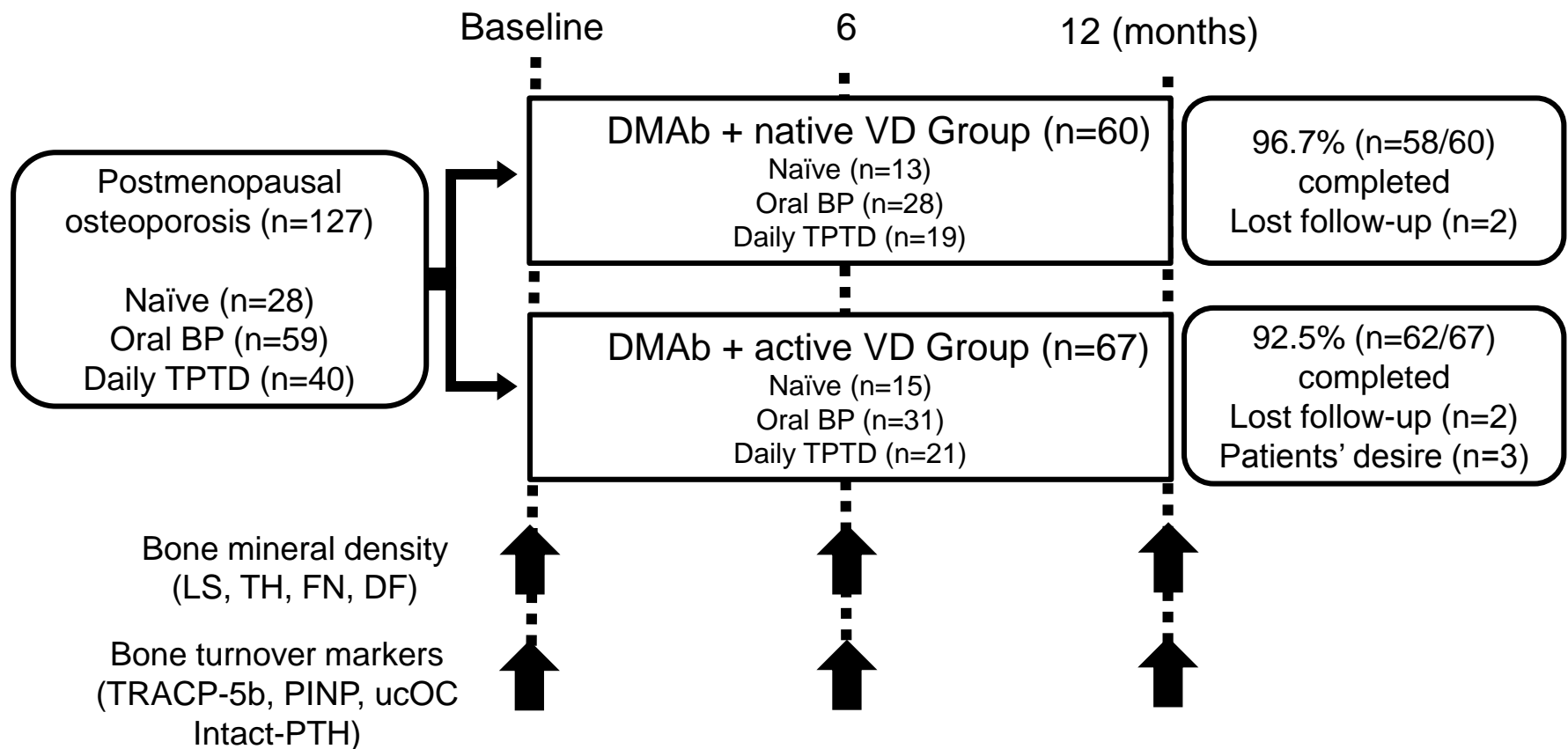


Figure 2

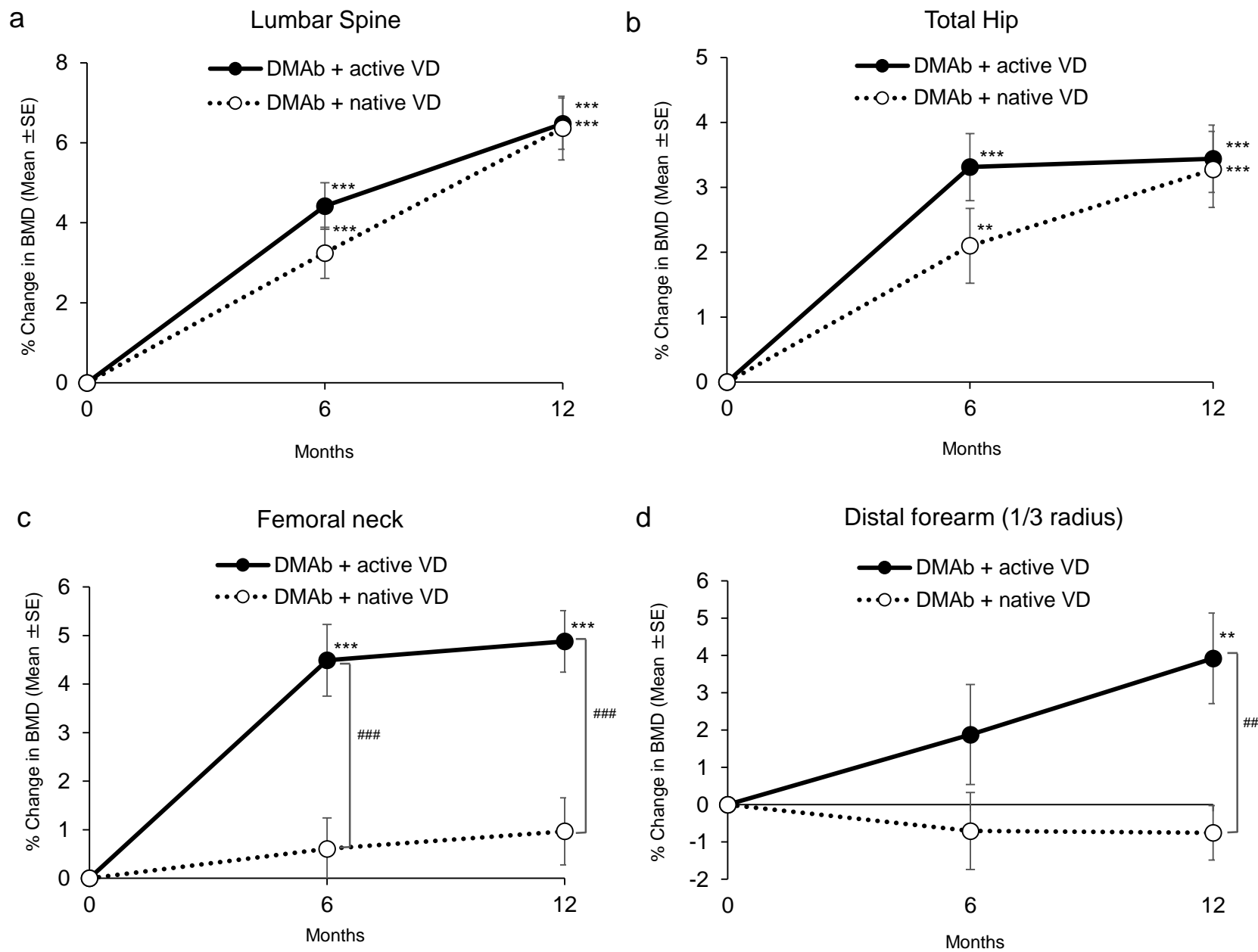


Figure 3

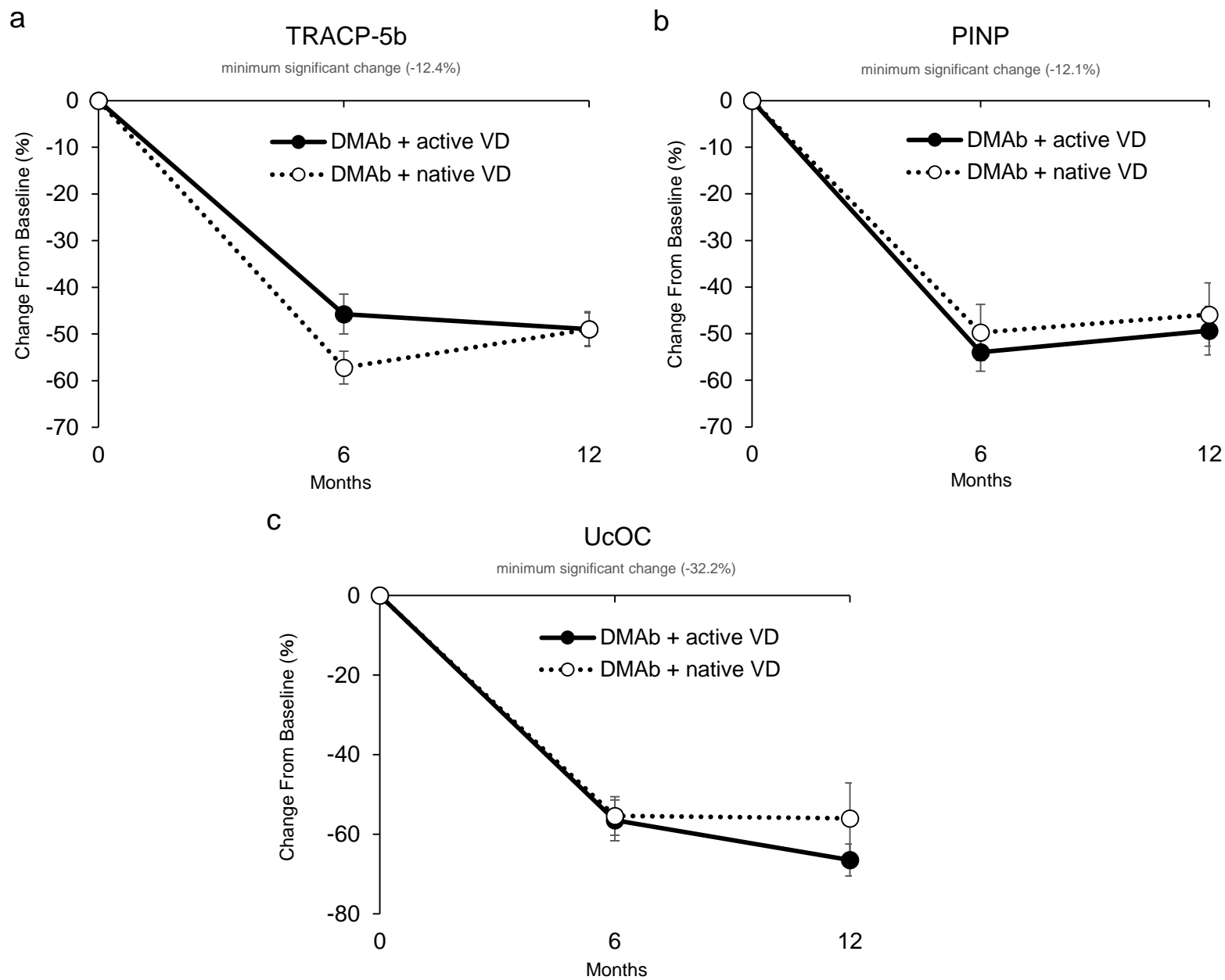


Figure 4

