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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

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25 Abstract

26 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.

31 Methods

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

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.

# 61 Keywords

62 Postmenopausal osteoporosis, Denosumab, Cholecalciferol, Alfacalcidol

63

#### 65 Introduction

66 Receptor activator of NF-KB ligand (RANKL) is mainly produced by osteoblasts and osteocytes, which play critical roles in osteoclast differentiation and bone resorption 67 [1-3]. Denosumab (DMAb), a fully human monoclonal antibody to receptor activator of 68 NF- $\kappa$ B ligand (RANKL), has shown a greater increase in bone mineral density (BMD) 69 and reduction in bone resorption than bisphosphonates (BP) such as alendronate (ALN) 70[4], ibandronate [5], or risedronate [6], and it also significantly increased BMD and 71decreased bone turnover markers when switched from ALN compared to continuing 7273 ALN therapy in postmenopausal osteoporosis [7]. We have previously demonstrated 74that switching daily teriparatide (TPTD) to DMAb significantly increased BMD and decreased bone resorption markers compared to switching to oral BP [8]. Thus, DMAb 75may be effective not only in treatment-naïve patients, but also in patients switched from 76BP or daily TPTD, which may be the major objects of DMAb treatment in the 77real-world setting. 78

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

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), consisting of patients who were treated with DMAb (60 mg sc every 6 months) in 104 combination with oral cholecalciferol 10 µg and calcium 610 mg/day (Denotas<sup>®</sup>; 105106 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  $0.8 \pm 0.0$  (0.25-1.0) µg and calcium formulation 99.2  $\pm$  8.5 (0-260) mg/day, depending on each physician's decision (Table 1). 109 110This 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.

115

116	BMD	assessment
116	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

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

Bone turnover markers were measured in serum obtained from each patient at 126127approximately the same time in the morning after overnight fasting. The bone formation 128marker, 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 129130 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 measured by ELISA as previously described [17, 19, 20]. Levels of undercarboxylated 132osteocalcin (ucOC) were measured by a solid-phase enzyme immunoassay kit 133 134(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 135136released 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	

## **Results**

155Baseline characteristics are shown in Table 1. There were no significant differences in 156the percentage of prior osteoporosis treatment and combined VD, baseline age, body mass index, rate of prior vertebral fracture, areal BMD, or bone turnover markers 157158between 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 159160 months; P < 0.001), lesser VD dose (10.0 vs 0.8 µg/day; P < 0.001), and a lesser 161combined calcium dose (610.0 vs 99.2 mg/day; P < 0.001) and use frequency (100.0%) vs 88.1%; *P* < 0.01). 162

Overall, 96.7% (58/60) of the DMAb + native VD group (2 patients were lost to 163 164follow-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 165166 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 168suffered 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 169fractures (2 vertebral and 1 humerus fractures). No significant difference was observed 170in the fracture rate between the two groups. 171

#### 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	<i>P</i> < 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

Percent changes in bone turnover markers from baseline are shown in Fig. 3. No 195196significant differences were observed between the two groups with regard to the changes in serum TRACP-5b, PINP, and ucOC levels from 6 months to 12 months. The 197198absolute values of bone turnover markers are shown in Fig. 4. There were no significant differences in absolute TRACP-5b, PINP, and ucOC levels from baseline to 6 and 12 199 200months between the groups; all values were within the reference values. However, serum intact-PTH levels were significantly decreased in the DMAb + active VD group 201202from baseline to 6 months (38.2 vs 32.8 pg/mL; P < 0.001) and 12 months (38.2 vs 30.4 203pg/mL; P < 0.001), while no significant change was observed in the DMAb + native VD group. Moreover, serum intact-PTH levels were significantly lower in the DMAb + 204active VD group compared to the DMAb + native VD group from 6 months (32.8 vs 20520651.1 pg/mL; P < 0.001) to 12 months (30.4 vs 47.6 pg/mL; P < 0.001). During this period, the rate of hypocalcemia (corrected serum calcium level < 8.6 mg/dl) was 1.7% 207(1/60) in the DMAb + native VD group and 1.5% (1/67) in the DMAb + active VD 208

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**

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].

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

groups. These results suggest that the difference in the VD may not significantly affect

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

- 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

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 $1\alpha$ ,
232	25-dihydroxy-cholecalciferol $[1\alpha, 25-(OH)2-D3]$ by 1-alpha-hydroxylase in the kidney
233	[11]. Renal 1a-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(OH)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(OH)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

245monkeys, DMAb treatment with native VD and calcium supplementation did not alter 246serum intact-PTH levels [29], which was consistent with the present study. However, 247other previous reports demonstrated that BP monotherapy increased serum intact-PTH 248levels, 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 249BMD increase [18, 30]. Taken together, ALF may have advantageous effects in 250decreasing serum intact-PTH levels and increasing BMD, compared to native VD in 251252DMAb treatment.

Considering bone modeling, it has been reported that active VD (calcitriol and 253254eldecalcitol) [32] induces bone modeling or minimodeling, which is considered to be focal bone formation with the resumption of osteoblastic activity of bone lining cells 255[33]. Furthermore, ALF increased not only focal bone formation on cancellous surfaces, 256but also periosteal bone formation [34] and cortical bone BMD of rats [35]. However, 257cholecalciferol did not alter cortical bone morphology in mice [36]. From these 258observations, active VD including ALF may have stronger effects, especially on cortical 259260bone 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

280 monitored. Whether a greater change in BMD induced by DMAb + active VD than that

279

spinal x-ray is not routinely conducted, subclinical vertebral fracture couldn't be

281of DMAb + native VD may reduce fracture risk should be assessed in a larger cohort. In 282addition, unknown baseline oral calcium intake and baseline VD-hormone levels may 283have affected the results, and possible subclinical hypercalciuria which may promote renal dysfunction couldn't be monitored in some part of the patients. 284In conclusion, DMAb with active VD combination significantly increased BMDs of the 285FN and DF, where cortical bone is relatively abundant and also in maintaining serum 286intact-PTH at lower levels compared to native VD combination, suggesting combining 287active VD may be an effective option of DMAb treatment. 288

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.

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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,

- 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.
- 420

Fig. 2 Mean  $\pm$  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.

426

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

430 propeptide; ucOC, undercarboxylated osteocalcin.

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. $^{\#\#\#}P < 0.001 \text{ DMAb} + \text{native VD}$ group
437	versus DMAb + active VD group.

Variable	DMAb + native VD	DMAb + active VD	P value
variable	(n=60)	(n=67)	
	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.
D	Weekly ALN (n=16)	Weekly ALN (n=4)	
Prior osteoporosis treatment	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.
	Naïve (n=32; 53.3%)	Naïve (n=26; 38.8%)	N.S.
Prior combined VD	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	15 4 1 5	21.0.07	0.001
(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 <sup>2</sup> )	21.6±0.4	20.7±0.4	N.S.
Prior vertebral fracture(s),	42/(0 (71 70/)		NC
n/N(%)	43/60 (71.7%)	40/07 (08.7%)	N.S.
Lumbar spine BMD (g/cm <sup>2</sup> )	0.730±0.016	0.762±0.017	N.S.
Lumbar spine BMD (T-score)	-3.0±0.1	$-2.8\pm0.1$	N.S.
Total hip BMD (g/cm <sup>2</sup> )	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 <sup>2</sup> )	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)	0.246.0.014	0.201 . 0.014	NG
$(g/cm^2)$	0.346±0.014	0.381±0.014	N.S.
Distal forearm (1/3 radius)	47.02	4.4+0.2	NC
(T-score)	-4./±0.2	-4.4±0.2	IN. <b>.</b> .
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.

## 1 Table 1. Baseline clinical characteristics

TRACP-5b (mU/dl)	439.5±30.9	422.2±32.8	N.S.
ucOC (ng/ml)	7.4±1.3	$6.4{\pm}1.1$	N.S.
eGFR (ml/min/1.73m <sup>2</sup> )	67.9±2.2	67.5±2.4	N.S.

2 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.

Areal BMD	Prior treatment	Combined VD	BMD change (%)	
			6 months	12 months
LS naïve BP TPTD total	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 BP TPTD total	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	$1.7{\pm}1.0$
	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

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.

	active	$0.1{\pm}1.7$	4.9±2.2
total	native	-0.7±1.0	-0.8±0.7
	active	$1.9{\pm}0.8$	3.9±0.8**

\*P<0.05, \*\*P<0.01, \*\*\*P<0.001. Data are expressed as means  $\pm$  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.  $\mathbf{27}$ 







