

Title	Effects of follow-on therapy after denosumab discontinuation in patients with postmenopausal osteoporosis
Author(s)	Ebina, Kosuke; Hashimoto, Jun; Kashii, Masafumi et al.
Citation	Modern Rheumatology. 2021, 31(2), p. 485-492
Version Type	AM
URL	https://hdl.handle.net/11094/93250
rights	This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.
Note	

Osaka University Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

Osaka University

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 **Original Article**

2 Effects of follow-on therapy after denosumab discontinuation in patients with postmenopausal
3 osteoporosis

5 **Authors**

6 Kosuke Ebina, MD, PhD ^{1)*}, Jun Hashimoto, MD, PhD ²⁾, Masafumi Kashii, MD, PhD ³⁾,
7 Makoto Hirao, MD, PhD ⁴⁾, Akira Miyama, MD ⁴⁾, Hiroyuki Nakaya, MD, PhD ⁵⁾, Shigeyoshi
8 Tsuji, MD, PhD ⁶⁾, Koichiro Takahi, MD, PhD ⁵⁾, Hideki Tsuboi, MD, PhD ⁷⁾, Gensuke
9 Okamura, MD ⁴⁾, Yuki Etani, MD ⁴⁾, Kenji Takami, MD ⁴⁾, and Hideki Yoshikawa, MD, PhD ³⁾

11 **Affiliations**

12 ¹⁾ Department of Musculoskeletal Regenerative Medicine, Osaka University, Graduate School
13 of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
14 ²⁾ Department of Rheumatology, National Hospital Organization Osaka Minami Medical Center,
15 2-1 Kidohigashi, Kawachinagano, Osaka 586-8521, Japan

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

16 ³⁾ Department of Orthopaedic Surgery, Toyonaka Municipal Hospital, 4-14-1 Shibahara-cho,
17 Toyonaka, Osaka 560-8565, Japan

18 ⁴⁾ Department of Orthopaedic Surgery, Osaka University Graduate School of Medicine, 2-2
19 Yamada-oka, Suita, Osaka 565-0871, Japan

20 ⁵⁾ Department of Orthopaedic Surgery, Osaka Toneyama Medical Center, 5-1-1 Toneyama,
21 Toyonaka, Osaka 560-8552, Japan

22 ⁶⁾ Department of Orthopaedic Surgery, National Hospital Organization Osaka Minami Medical
23 Center, 2-1 Kidohigashi, Kawachinagano, Osaka 586-8521, Japan

24 ⁷⁾ Department of Orthopaedic Surgery, Osaka Rosai Hospital, 1179-3 Nagasone-cho, Sakai
25 591-8025, Japan

27 ***Corresponding author:**

28 Phone: +81-6-6879-3552; Fax: +81-6-6879-3559

29 E-mail: k-ebina@umin.ac.jp ORCID: 0000-0002-2426-1024

31 **Funding**

1
2 32 None
3
4
5
6 33
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 34 **Abstract**
3

4
5
6 35 Objectives
7

8
9
10 36 To clarify the effects of follow-on therapy after denosumab (DMAb) discontinuation.
11

12
13 37 Methods
14

15
16
17 38 In this retrospective, multicenter study, postmenopausal patients with osteoporosis who were
18

19
20 39 previously treated by oral bisphosphonates (BP) (n=26) or teriparatide (TPTD) (n=27) were
21

22
23 40 switched to DMAb (administered 2.6 times), and then discontinued. Patients (73.1 years,
24

25
26 41 T-scores of the lumbar spine [LS] -2.7 and femoral neck [FN] -2.2) were switched to either (1)
27

28
29 42 raloxifene (RAL) (n=13) or BP [(2) weekly or monthly BP (wmBP) (n=29) or (3) zoledronate
30

31
32 43 (ZOL) (n=11)], based on each physician's decision (mean interval after final DMAb
33

34
35 44 administration was 7.2 months). Bone mineral density (BMD) at final DMAb administration
36

37
38 45 were set as baseline.
39

40
41
42 46 Results
43

44
45
46 47 Changes in LS BMD at 1.5 years after final DMAb administration were -2.7% in the RAL,
47

48
49 48 0.7% in the wmBP, and 1.9% in the ZOL (P=0.31 between groups), and in FN BMD were
50

51
52 49 -3.8%, -0.8%, and 1.8%, respectively (P=0.02 between the RAL and ZOL; P=0.048 between the
53

54
55 50 RAL and BP). Clinical vertebral fracture incidence during 1.5 years after final DMAb
56

1
2 51 administration was 23.1% in the RAL, 3.4% in the wmBP, and 0.0% in the ZOL (P=0.048
3
4
5 52 between the RAL and ZOL; P=0.015 between the RAL and BP). No significant differences
6
7
8 53 were observed in these parameters between the wmBP and ZOL.
9

10 11 12 54 Conclusions

13
14
15
16 55 These results may contribute to the selection of adequate follow-on therapy after DMAb
17
18
19 56 discontinuation, although further investigations are required.
20
21

22
23 57

24 25 26 27 58 **Keywords**

28
29
30
31 59 Bisphosphonate; denosumab; discontinuation; follow-on treatment; postmenopausal
32
33
34 60 osteoporosis
35
36

37
38 61

39 40 41 42 62 **Introduction**

43
44
45 63 Denosumab (DMAb) is a monoclonal anti-RANKL antibody that acts on bone as a potent
46
47
48
49 64 antiresorptive agent and is associated with reduced vertebral and non-vertebral fracture risk of
50
51
52 65 patients with osteoporosis [1]. However, discontinuation of DMAb is associated with a
53
54
55 66 substantial increase in bone turnover markers above pretreatment levels [2], as well as bone
56
57
58 67 mineral density (BMD) loss and increased vertebral fracture risk [3, 4].
59
60
61
62
63
64
65

1
2 68 To protect patients from the rapid effects that may occur after discontinuation of DMAb,
3
4
5 69 follow-on treatments with bisphosphonates (BP) have been investigated. Previous reports
6
7
8 70 demonstrated some positive effects of treatment with alendronate (ALN) [5] or zoledronate
9
10
11 71 (ZOL) [6]. However, another case report showed that treatment with raloxifene (RAL) was
12
13
14 72 associated with multiple vertebral fractures [7], and treatment with teriparatide (TPTD) was
15
16
17 73 associated with transient loss of BMD [8]. In addition, a previous study showed that serum
18
19
20
21 74 collagen type 1 cross-linked C-telopeptide (CTX) levels of patients with prior exposure to BP
22
23
24 75 remained in the postmenopausal range after DMAb discontinuation [9], suggesting the
25
26
27 76 importance of prior treatment before DMAb. Collectively, most of these previous studies were
28
29
30
31 77 relatively small case series, and the ideal prior and follow-on treatments of DMAb are still
32
33
34 78 unknown.

35
36
37 79 Taken together, the aim of this retrospective study was to clarify the effects of follow-on
38
39
40
41 80 therapy after DMAb discontinuation on bone resorption, BMD, and clinical fracture risk.

42
43
44
45 81

46 47 48 82 **Materials and methods**

49 50 51 52 83 *Study design and subjects*

53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 84 This non-randomized, retrospective study was conducted in 6 centers according to the Japanese
3
4
5 85 Guidelines for Prevention and Treatment of Osteoporosis 2011 [10]. A total of 129
6
7
8 86 postmenopausal patients with osteoporosis who were treated with and discontinued DMAb were
9
10
11 87 enrolled (Fig. 1). Among them, patients who were lost to follow-up 1.5 years after final DMAb
12
13
14 88 administration, who did not receive follow-on treatment or were treated by TPTD, who did not
15
16
17 89 undergo dual-energy x-ray absorptiometry (DXA) or spinal radiograph, or without bone
18
19
20
21 90 resorption marker data were excluded. To minimize the patients' variance, only patients who
22
23
24 91 were treated with oral BP or TPTD before DMAb, and followed by ALN, RIS, or IBN as BP
25
26
27 92 were included. Finally, 53 patients were included, whose physicians chose to treat them with
28
29
30 93 RAL (60 mg/day; n=13) or BP (n=40) [weekly or monthly BP (wmBP; ALN, RIS, or IBN)
31
32
33 94 (dose varies by agent used; n=29) or ZOL (5 mg/year IV; n=11)].
34
35
36
37
38
39
40

41 96 *Ethical statement*
42
43
44

45 97 This study was conducted in accordance with the ethical standards of the Declaration of
46
47
48 98 Helsinki and was approved by the institutional ethical review board of Osaka University
49
50
51 99 Graduate School of Medicine (approval number 18258; Osaka University, Graduate School of
52
53
54 100 Medicine) and each institute. The board waived the requirement for patients' informed consent
55
56
57 101 because of the anonymous nature of the data.
58
59
60
61
62
63
64
65

1
2 102
3
4
5
6 103 *BMD assessment*
7
8
9
10 104 Areal BMD in the lumbar spine (LS; L2-L4) and femoral neck (FN) were assessed by DXA
11
12
13 105 (Discovery, Hologic, Inc., Waltham, MA, USA) at baseline (ie, final DMAb administration) and
14
15
16 106 1.5 years after final DMAb administration. Regions of severe sclerosis, vertebral fracture, and
17
18
19 107 surgical sites were excluded from BMD measurements, as previously described [11].
20
21
22
23 108
24
25
26
27 109 *Biochemical markers of bone resorption*
28
29
30
31 110 The bone resorption marker, isoform 5b of tartrate-resistant acid phosphatase (TRACP-5b)
32
33
34 111 (inter-assay coefficient of variation, 5.0-9.0%) (Immunodiagnostic Systems Ltd., Boldon, UK)
35
36
37 112 was measured by enzyme-linked immunosorbent assay in the morning after overnight fasting, as
38
39
40 113 previously described [12]. A previous report demonstrated that TRACP-5b levels are a useful
41
42
43 114 marker that show higher clinical sensitivity and signal-to-noise ratio compared to serum CTX
44
45
46 115 levels [13]. Serum 25-hydroxycholecalciferol (25(OH)D) levels were measured by
47
48
49
50 116 electrochemiluminescence using the Elecsys system (Roche Diagnostics, Basel, Switzerland).
51
52
53
54 117
55
56
57 118 *Radiographs*
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

119 Spinal radiographs were obtained at final DMAb administration and at unscheduled times if
120 subjects had symptoms suggestive of clinical vertebral fractures during the 1.5-years follow-up.
121 For incidental non-vertebral fractures, radiographs were assessed by the investigator if subjects
122 had symptoms.

123

124 *Statistical analysis*

125 The differences between study groups were tested using the Mann-Whitney U test (for 2
126 groups) or by non-parametric Kruskal-Wallis test (for 3 groups) for continuous variables, and
127 Pearson's chi-squared test (for 2 groups) or Fisher's exact test (for 3 groups) for categorical
128 variables, and multi-way analysis of variance. Changes in BMD and serum TRACP-5b levels
129 from baseline to specified time points within each study group were compared using the
130 non-parametric Wilcoxon signed-rank test. Multivariate logistic regression analysis with a
131 forward stepwise procedure was performed to identify significant indicators of LS or FN BMD
132 change. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical
133 University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical
134 Computing, Vienna, Austria) [14]. A *P* value < 0.05 was considered significant.

135

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

136 **Results**

137 Patients' clinical backgrounds before DMAb discontinuation are shown in Table 1. Of the 53
138 study patients, 49.1% (n=26) were previously treated with an oral BP, and 50.9% (n=27; 19
139 daily and 6 weekly) were previously treated by TPTD before DMAb administration. There were
140 no significant differences between groups in prior therapy duration before DMAb (mean, 18.9
141 months), serum TRACP-5b levels before DMAb administration (347.2 mU/dl), and the number
142 of times that DMAb was administered (2.6 times) between groups. Reasons for discontinuation
143 of DMAb, as evaluated by each physician, were as follows: patients' preference, 20.8%; toxic
144 reasons (malignancy, eruption, itching, swelling of gums, renal failure, and hypocalcemia),
145 13.2%; ineffectiveness, 13.2%; need for dental care, 7.5%; adequate BMD achieved (mostly LS
146 BMD T-score > -2.5), 7.5%; and other nontoxic reasons, 35.8%. Results of these backgrounds
147 separated by the RAL group (n=13) and the BP group (n=40) are shown in supplemental Table
148 1. No significant differences were observed between the groups.

149 Table 2 shows patients' clinical backgrounds at switching from DMAb to other therapy and
150 BMD at final DMAb administration. There were no significant differences between groups in
151 interval between final DMAb administration and start of follow-on treatment (7.2 months),
152 combined active vitamin D (92.5%) and calcium (11.3%) rate, age (73.1 years), body mass
153 index (20.5 kg/m²), estimated glomerular filtration rate (eGFR) (71.9 ml/min/1.73 m²), serum

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

154 25(OH)D (13.8 ng/ml) or serum TRACP-5b levels (192.8 mU/dl), LS T-score (-2.7), FN
155 T-score (-2.2), and prior vertebral (50.9%) and non-vertebral (26.4%) fracture rate. Results of
156 these backgrounds separated by the RAL group (n=13) and the BP group (n=40) are shown in
157 supplemental Table 2. The RAL group showed lower eGFR (P=0.04) and higher rate of prior
158 non-vertebral fracture (P=0.010) compared to the BP group.

159

160 *Bone resorption marker*

161 Percent changes in serum TRACP-5b levels from baseline (before DMAB administration) to
162 each time point are shown in Figure 2a. All groups showed similar and significant reductions in
163 TRACP-5b levels at final DMAB administration (RAL, -38.5%; wmBP, -35.3%; and ZOL, -
164 31.2%) (P=0.32 between groups). However, the RAL group tended to show marked increases
165 (52.9%), whereas the wmBP (14.2%) and ZOL (9.1%) groups showed a similar restoration to
166 pre-DMAB levels at 1.5 years after final DMAB administration (P=0.50 between groups).
167 Results of these parameters separated by the RAL group (n=13) and the BP group (n=40) are
168 shown in supplemental Figure 1a.

169

170 *Changes in BMD*

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

171 Changes in LS BMD from final DMAB administration (baseline) to 1.5 years after final DMAB
172 administration are shown in Figure 2b. The wmBP (+0.7%) and ZOL (+1.9%) groups
173 maintained levels, whereas the RAL group (-2.7%) tended to show a decrease in levels 1.5
174 years after DMAB discontinuation (P=0.31 between groups).

175 Changes in FN BMD from final DMAB administration (baseline) to 1.5 years after final DMAB
176 administration are shown in Figure 2c. The wmBP (-0.8%) and ZOL (+1.8%) groups
177 maintained levels, whereas the RAL group (-3.8%) showed a significant decrease from baseline
178 (P=0.02) and a significant decrease compared to the ZOL group (+1.8%) (P=0.02).

179 No significant differences were observed in these parameters between the wmBP and ZOL, and
180 also between wmBP (ALN, RIS, and IBN; data not shown). Results of these parameters
181 separated by the RAL group (n=13) and the BP group (n=40) are shown in supplemental Figure
182 1b and 1c, respectively. In FN BMD changes, the BP group maintained significantly higher
183 levels (-0.1%) compared to that of the RAL group (-3.8%) (P=0.048).

184

185 *Effects of prior treatment before DMAB and follow-on treatment after DMAB discontinuation on*
186 *bone resorption and BMD changes*

1
2 187 Multi-way analysis of variance was conducted to evaluate the effects of prior treatment before
3
4
5 188 DMAb and follow-on treatment after DMAb discontinuation on serum TRACP-5b levels and
6
7
8 189 BMD changes after DMAb discontinuation (Figure 3a-3c). Patients previously treated by TPTD
9
10
11 190 tended to be protected by bone resorption increases and BMD decreases compared to those
12
13
14 191 previously treated by a BP, especially in the RAL group, although there were no statistically
15
16
17 192 significant differences. Finally, the difference of follow-on treatment after DMAb
18
19
20 193 discontinuation remained a significant factor for FN BMD changes after adjusting for the
21
22
23 194 difference of prior treatment (P=0.043). Results of these parameters separated by the RAL
24
25
26 195 group (n=13) and the BP group (n=40) are shown in supplemental Figure 2a-2c, respectively.
27
28
29 196 The difference of follow-on treatment (RAL or BP) after DMAb discontinuation remained a
30
31
32 197 significant factor for FN BMD changes after adjusting for the difference of prior treatment
33
34
35 198 (P=0.033).

36
37
38
39
40
41 199
42
43
44
45 200 *Effects of number of DMAb treatment and follow-on treatment after DMAb discontinuation on*
46
47
48 201 *bone resorption and BMD changes*
49

50
51 202 Multi-way analysis of variance was conducted to evaluate the effects of number of DMAb
52
53
54 203 treatments and follow-on treatment after DMAb discontinuation on serum TRACP-5b levels
55
56
57 204 (Figure 4a) and FN BMD changes (Figure 4b) after DMAb discontinuation. **There were no**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

205 significant differences between patients who were treated 1 to 2 times with DMAb (n=31) and
206 those previously treated more than 3 times with DMAb (n=22) in the change of serum
207 TRACP-5b levels (F-value=0.59, P=0.45) and FN BMD (F-value=0.0022, P=0.96). Results of
208 these parameters separated by the RAL group (n=13) and the BP group (n=40) are shown in
209 supplemental Figure 3a and 3b, respectively. Patients who were treated by BP tended to be
210 protected by FN BMD decrease compared to that of RAL (F-value=3.65, P=0.063).

211

212 *Significant predictor variables of the change in LS or FN BMD*

213 The possible clinical backgrounds [including baseline age, body mass index, prior therapy
214 before DMAb, number of DMAb administration, interval after final DMAb administration,
215 baseline BMD (LS or FN T-score), the difference of follow-on therapy after DMAb
216 {categorized as RAL (1), wmBP (2), and ZOL (3)}, and the change of TRACP-5b (%) at 1.5
217 years after final DMAb administration] were subjected to stepwise multivariable linear
218 regression analysis to investigate significant predictors of BMD changes at 1.5 years after final
219 DMAb administration. As for LS BMD change, the only significant predictor was the difference
220 of follow-on therapy after DMAb (partial regression coefficient=+3.72, P=0.022). As for FN
221 BMD change, the significant predictors were the difference of follow-on therapy after DMAb
222 (partial regression coefficient=+3.66, P=0.0035), the change of TRACP-5b (%) (partial

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

223 regression coefficient=-0.027, P=0.0032), and the baseline FN T-score (partial regression
224 coefficient=-2.58, P=0.013).

225

226 *Rate of clinical fragility fracture*

227 Figure 4 shows clinical vertebral (Fig. 4c) and non-vertebral (Fig. 4d) fracture rates during the
228 1.5 years period after final DMAb administration. RAL showed the highest rate of clinical
229 vertebral fractures (23.1%) compared to wmBP (3.4%) or ZOL (0.0%) (P=0.048; RAL vs.
230 ZOL), as well as that of non-vertebral clinical fractures (7.7%) compared to wmBP (3.4%) or
231 ZOL (0.0%) (P=0.71 between groups), although differences were not statistically significant.

232 Results of these parameters when separated by the RAL group (n=13) and the BP group (n=40)
233 are shown in supplemental Figure 3c and 3d, respectively. The RAL group showed higher rate
234 of clinical vertebral fractures (23.1%) compared to that of the BP group (2.5%) (P=0.015).

235 The grades of vertebral fracture, evaluated by a semiquantitative method, were grade 2 (n=1)
236 and grade 3 (n=2) in the RAL group, and grade 3 vertebral fracture (n=1) in the wmBP group
237 [15]. There were no patients who had multiple vertebral fractures after DMAb discontinuation.

238 Seventy-five percent of patients who suffered clinical vertebral fracture were treated by oral BP
239 before DMAb (n=3/4).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

240

241 **Discussion**

242 Previous studies concerning follow-on therapy after DMAb discontinuation revealed several
243 factors affecting treatment effectiveness.

244 First, in terms of the protective effects of prior exposure to BP, Uebelhart et al. reported that
245 serum CTX levels of patients with prior exposure to BP remained in the postmenopausal range
246 after DMAb discontinuation [9]. In this study, 49.1% of patients were treated by oral BP, and
247 50.9% were treated by TPTD before DMAb administration. Patients previously treated by
248 TPTD tended to be protected by bone resorption increase and BMD decreases compared to
249 those previously treated by BP, especially in the RAL group. There are no previous reports
250 evaluating the effect of prior TPTD treatment on DMAb discontinuation, although some
251 positive effects may be expected, as we previously reported that prior TPTD treatment followed
252 by DMAb treatment showed beneficial results for continuous increases in BMD [16].

253 Second, patients with a low number of DMAb treatments (especially a single treatment) were
254 also protected from bone resorption increase [9]. **In this study, there were no significant**
255 **differences in the change of bone resorption marker and FN BMD between patients who were**
256 **treated 1 to 2 times with DMAb (n=31) and those previously treated more than 3 times with**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

257 **DMAb (n=22)**. This finding may be due to the relatively small number of patients and the small
258 number of DMAb treatments (mean, 2.6 times).
259 Third, in terms of the strength of bone-resorption inhibition of follow-on treatment, a previous
260 report demonstrated that ZOL was more effective in improving BMD and reducing bone
261 turnover compared to weekly oral ALN [17], and RIS tended to show lower BMD preservation
262 compared to ZOL after DMAb discontinuation [6]. However, no significant differences were
263 observed in the change of BMD and TRACP-5b levels between the wmBP and ZOL, and also
264 between wmBP (ALN, RIS, and IBN; data not shown) in this study. In addition, Freemantle et
265 al. reported that switching DMAb to ALN maintained BMD in DAPS study [5], although
266 another case report demonstrated that ALN was not effective in preventing multiple vertebral
267 fractures after DMAb discontinuation [18]. Taken together, the follow-on effect due to the
268 difference of BP remains controversial. On the other hand, a case report showed that follow-on
269 RAL treatment was associated with bone resorption increase after DMAb discontinuation [7]. In
270 this study, the increase in the TRACP-5b level and decrease in the FN BMD were more
271 apparent in the RAL group compared to the BP group, which suggests that RAL may have little
272 effect on inhibiting bone resorption increase and preserving FN BMD.
273 Fourth, in terms of the timing of follow-on treatment, Horne et al. reported that most of the
274 BMD gain obtained with DMAb was preserved with delayed administration of ZOL (7 to 8

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

275 months after last DMAb administration) [6]. This may be partially due to the fact that BP uptake
276 into the bone is expected to increase as a result of increased bone turnover. In this study, we
277 conducted multi-way analysis of variance to clarify whether the treatment interval after DMAb
278 [within 6 months (n=37) vs. more than 7 months (n=16)] may influence the change in bone
279 resorption marker or BMD. Finally, no significant differences were observed between groups
280 (data not shown).

281 Fifth, the difference of combined vitamin D should be considered. In this study, most patients
282 were treated by active vitamin D, which may be different from a previous study [6]. Previous
283 studies demonstrated that alfacalcidol (active vitamin D) in combination with ALN [19] or
284 DMAb [20] showed a higher increase in BMD compared to that of combination with native
285 vitamin D. However, we should note that RAL in combination with active vitamin D failed to
286 protect against bone turnover increase and FN BMD loss after DMAb discontinuation.

287 There are several limitations to this study. Because of the small number of patients, the
288 statistical power of the results (especially for the fracture incidence) may be attenuated. As
289 spinal X-ray was not routinely performed at 1.5 years after final DMAb administration,
290 subclinical vertebral fractures could not be monitored. There was no control group of patients
291 without follow-on treatment, and we could not monitor the early change of serum TRACP-5b
292 levels and bone formation marker after DMAb discontinuation. When switching DMAb to other

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

293 agents, the RAL group tended to show higher age, lower eGFR, lower serum TRACP-5b levels,
294 and higher LS T-score compared to other groups. These backgrounds may potentially affect
295 physicians' treatment selection and following effects. Larger, randomized studies with longer
296 follow-up periods should be conducted in the future.

297 In conclusion, in this short-term follow-up of postmenopausal patients with osteoporosis who
298 discontinued DMAb, switching to BP showed better FN BMD preservation, as well as
299 prevention of clinical vertebral fractures compared to switching to RAL. No significant
300 differences were observed in these parameters between the wmBP and ZOL. These results may
301 contribute to the selection of adequate follow-on therapy after DMAb discontinuation, although
302 further investigations are required.

303

304 **Acknowledgments**

305 The authors thank Keiko Uchishiba for her excellent cooperation in conducting the study.

306

307 **Conflicts of interest**

308 K. Ebina is affiliated with the Department of Musculoskeletal Regenerative Medicine, Osaka
309 University, Graduate School of Medicine, which is supported by Taisho. K. Ebina, M. Hirao,

1
2 310 and H. Yoshikawa have received research grants from Asahi-Kasei, Astellas, Chugai, Daiichi
3
4
5 311 Sankyo, Eisai, and Ono. K. Ebina has received payments for lectures from Asahi-Kasei,
6
7
8 312 Astellas, Chugai, Daiichi Sankyo, Eisai, Eli Lilly, and Ono. J. Hashimoto has received research
9
10
11 313 grants from Chugai, Teijin Pharma, and Pfizer, and has received payments for lectures from
12
13
14 314 Chugai. M. Kashii has received payments for lectures from Asahi-Kasei and Astellas. S. Tsuji
15
16
17 315 has received a research grant from Eli Lilly. S. Tsuji has received payments for lectures from
18
19
20
21 316 Eisai and Eli Lilly. H. Tsuboi has received a research grant from Chugai, and has received
22
23
24 317 payments for lectures from Asahi-Kasei, Astellas, Chugai, Eisai, Eli Lilly, and Pfizer. A.
25
26
27 318 Miyama, H. Nakaya, K. Takahi, G. Okamura, Y. Etani, and K. Takami declare that they have no
28
29
30 319 conflicts of interest. The funders had no role in the study design, data collection and analysis,
31
32
33 320 decision to publish, or preparation of the manuscript.
34
35
36
37
38
39
40
41
42

322 **Figure legends**

323 **Figure 1. Study design, schedule, and patient flow.**

324 Treatment of patients was changed based on each physician's discretion to the DMAB to RAL
325 group (n=13) or the DMAB to BP group (n=40) [weekly or monthly BP group (n=29) or the
326 DMAB to yearly ZOL group (n=11)]. Bone mineral density, TRACP-5b levels, and clinical
327 fracture incidence were evaluated at each time point. TPTD, teriparatide; DXA, dual-energy

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

328 x-ray absorptiometry; BP, bisphosphonate; DMAb, denosumab; ALN, alendronate; RIS,
329 risedronate; IBN, ibandronate; RAL, raloxifene; ZOL, zoledronate; LS, lumbar spine; FN,
330 femoral neck; TRAP-5b, isoform 5b of tartrate-resistant acid phosphatase.

331

332 **Figure 2. Mean changes of serum TRAP-5b levels (a), changes of BMD in the lumbar spine**
333 **(b) and femoral neck (c).** TRACP-5b, isoform 5b of tartrate-resistant acid phosphatase;
334 DMAb, denosumab; RAL, raloxifene; BP, bisphosphonate; ZOL, zoledronate; BMD, bone
335 mineral density. Bars indicate standard errors (SE). #P < 0.05 change from final DMAb
336 administration within each treatment group. *P < 0.05 RAL group versus ZOL group.

337

338 **Figure 3. Multi-way analysis of variance for prior treatment before DMAb and follow-on**
339 **treatment after DMAb discontinuation on serum TRACP-5b levels (a), lumbar spine BMD**
340 **(b), and femoral neck BMD (c) changes.**

341 TRACP-5b, isoform 5b of tartrate-resistant acid phosphatase; DMAb, denosumab; RAL,
342 raloxifene; wmBP, weekly or monthly bisphosphonate; ZOL, zoledronate. Bars indicate
343 standard deviations (SD).

344

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

345 **Figure 4. Multi-way analysis of variance of number of DMAB treatments and follow-on**
346 **treatment after DMAB discontinuation on serum TRACP-5b levels (a) and femoral neck**
347 **BMD (b) changes. Incidence rate of clinical vertebral fracture (c) and non-vertebral**
348 **fracture (d) from final DMAB administration to 1.5 years after final DMAB**
349 **administration.**

350 TRACP-5b, isoform 5b of tartrate-resistant acid phosphatase; DMAB, denosumab; RAL,
351 raloxifene; wmBP, weekly or monthly bisphosphonate; ZOL, zoledronate; BMD, bone mineral
352 density. Bars indicate standard deviations (SD). *P < 0.05 RAL group versus yearly ZOL group.

353

354 **References**

355 1. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for
356 prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med.* 2009; 361(8):
357 756-65.

358 2. Bone HG, Bolognese MA, Yuen CK, Kendler DL, Miller PD, Yang YC, et al. Effects of
359 denosumab treatment and discontinuation on bone mineral density and bone turnover markers in
360 postmenopausal women with low bone mass. *J Clin Endocrinol Metab.* 2011; 96(4): 972-80.

361 3. Anastasilakis AD, Polyzos SA, Makras P, Aubry-Rozier B, Kaouri S, Lamy O. Clinical Features
362 of 24 Patients With Rebound-Associated Vertebral Fractures After Denosumab Discontinuation:
363 Systematic Review and Additional Cases. *J Bone Miner Res.* 2017; 32(6): 1291-6.

364 4. Cummings SR, Ferrari S, Eastell R, Gilchrist N, Jensen JB, McClung M, et al. Vertebral Fractures
365 After Discontinuation of Denosumab: A Post Hoc Analysis of the Randomized Placebo-Controlled
366 FREEDOM Trial and Its Extension. *J Bone Miner Res.* 2018; 33(2): 190-8.

1 367 5. Freemantle N, Satram-Hoang S, Tang ET, Kaur P, Macarios D, Siddhanti S, et al. Final results of
2 368 the DAPS (Denosumab Adherence Preference Satisfaction) study: a 24-month, randomized, crossover
3 369 comparison with alendronate in postmenopausal women. *Osteoporos Int.* 2012; 23(1): 317-26.
4
5
6
7 370 6. Horne AM, Mihov B, Reid IR. Bone Loss After Romosozumab/Denosumab: Effects of
8 371 Bisphosphonates. *Calcif Tissue Int.* 2018; 103(1): 55-61.
9
10
11 372 7. Gonzalez-Rodriguez E, Stoll D, Lamy O. Raloxifene Has No Efficacy in Reducing the High Bone
12 373 Turnover and the Risk of Spontaneous Vertebral Fractures after Denosumab Discontinuation. *Case Rep*
13 374 *Rheumatol.* 2018; 2018: 5432751.
14
15
16
17 375 8. Leder BZ, Tsai JN, Uihlein AV, Wallace PM, Lee H, Neer RM, et al. Denosumab and teriparatide
18 376 transitions in postmenopausal osteoporosis (the DATA-Switch study): extension of a randomised
19 377 controlled trial. *Lancet.* 2015; 386(9999): 1147-55.
20
21
22
23 378 9. Uebelhart B, Rizzoli R, Ferrari SL. Retrospective evaluation of serum CTX levels after denosumab
24 379 discontinuation in patients with or without prior exposure to bisphosphonates. *Osteoporos Int.* 2017;
25 380 28(9): 2701-5.
26
27
28
29 381 10. Orimo H, Nakamura T, Hosoi T, Iki M, Uenishi K, Endo N, et al. Japanese 2011 guidelines for
30 382 prevention and treatment of osteoporosis--executive summary. *Arch Osteoporos.* 2012; 7: 3-20.
31
32
33 383 11. Ebina K, Hirao M, Hashimoto J, Matsuoka H, Iwahashi T, Chijimatsu R, et al. Impact of switching
34 384 oral bisphosphonates to denosumab or daily teriparatide on the progression of radiographic joint
35 385 destruction in patients with biologic-naive rheumatoid arthritis. *Osteoporos Int.* 2018; 29(7): 1627-36.
36
37
38
39 386 12. Ebina K, Hashimoto J, Shi K, Kashii M, Hirao M, Yoshikawa H. Comparison of the effect of
40 387 18-month daily teriparatide administration on patients with rheumatoid arthritis and postmenopausal
41 388 osteoporosis patients. *Osteoporos Int.* 2014; 25(12): 2755-65.
42
43
44
45 389 13. Nenonen A, Cheng S, Ivaska KK, Alatalo SL, Lehtimäki T, Schmidt-Gayk H, et al. Serum TRACP
46 390 5b is a useful marker for monitoring alendronate treatment: comparison with other markers of bone
47 391 turnover. *J Bone Miner Res.* 2005; 20(10): 1804-12.
48
49
50
51 392 14. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics.
52 393 *Bone Marrow Transplant.* 2013; 48(3): 452-8.
53
54
55 394 15. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a
56 395 semiquantitative technique. *J Bone Miner Res.* 1993; 8(9): 1137-48.
57
58
59
60
61
62
63
64
65

1 396 16. Ebina K, Hashimoto J, Kashii M, Hirao M, Kaneshiro S, Noguchi T, et al. The effects of switching
2 397 daily teriparatide to oral bisphosphonates or denosumab in patients with primary osteoporosis. *J Bone*
3 398 *Miner Metab.* 2017; 35(1): 91-8.
4
5
6
7 399 17. Tan W, Sun J, Zhou L, Li Y, Wu X. Randomized trial comparing efficacies of zoledronate and
8 400 alendronate for improving bone mineral density and inhibiting bone remodelling in women with
9 401 post-menopausal osteoporosis. *J Clin Pharm Ther.* 2016; 41(5): 519-23.
10
11
12 402 18. Lamy O, Fernandez-Fernandez E, Monjo-Henry I, Stoll D, Aubry-Rozier B, Benavent-Nunez D, et
13 403 al. Alendronate after denosumab discontinuation in women previously exposed to bisphosphonates was
14 404 not effective in preventing the risk of spontaneous multiple vertebral fractures: two case reports.
15 405 *Osteoporos Int.* 2019; 30(5): 1111-5.
16
17
18 406 19. Ringe JD, Farahmand P, Schacht E, Rozehnal A. Superiority of a combined treatment of
19 407 Alendronate and Alfacalcidol compared to the combination of Alendronate and plain vitamin D or
20 408 Alfacalcidol alone in established postmenopausal or male osteoporosis (AAC-Trial). *Rheumatol Int.*
21 409 2007; 27(5): 425-34.
22
23
24 410 20. Ebina K, Kashii M, Hirao M, Hashimoto J, Noguchi T, Koizumi K, et al. Comparison of the
25 411 effects of denosumab between a native vitamin D combination and an active vitamin D combination in
26 412 patients with postmenopausal osteoporosis. *J Bone Miner Metab.* 2017; 35(5): 571-80.
27
28
29
30
31
32
33 413
34
35
36
37 414
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 **Table 1. Patients' clinical backgrounds before discontinuation of DMAb**

Variable	RAL group (n=13)	Weekly or monthly BP group (n=29)	ZOL group (n=11)	P-value
Prior therapy before DMAb	Weekly or monthly oral BP (n=6) [ALN 35 mg/week (n=4)]	Weekly or monthly oral BP (n=14) [ALN 35 mg/week (n=7)] RIS 17.5 mg/week (n=4)	Weekly or monthly oral BP (n=6) [ALN 35 mg/week (n=3)] RIS 17.5 mg/week (n=1)	1.00
Prior therapy duration before DMAb (months)	MIN 50 mg/month (n=2)] TPTD (n=7) [daily (n=4) weekly (n=3)]	MIN 50 mg/month (n=3)] TPTD (n=15) [daily (n=13) weekly (n=2)]	IBN 100 mg/month (n=2)] TPTD (n=5) [daily (n=4) weekly (n=1)]	0.84
TRACP-5b level before DMAb (mU/dl)	17.1±12.1	19.0±15.6	21.0±21.9	0.88
DMAb administration (no. of times)	2.5±1.1	2.4±1.5	3.3±2.3	0.50
Reasons for discontinuation of DMAb	Adequate BMD achieved (n=1) Patient's preference	Adequate BMD achieved (n=2) Patient's preference	Adequate BMD achieved (n=1) Patient's preference	N.A.

(n=2)	(n=8)	(n=1)
Dental care (n=3)	Dental care (n=2)	
Other nontoxic reasons (n=3)	Other nontoxic reasons (n=10)	Other nontoxic reasons (n=6)
Ineffectiveness (n=1)	Ineffectiveness (n=3)	Ineffectiveness (n=3)
Toxic reasons (itching, swelling of gum, renal failure) (n=3)	Toxic reasons (malignancy, eruption, renal failure, hypocalcemia) (n=4)	

-
- 2 Mean \pm standard deviation; N.A. = not applicable.
 - 3 DMAB, denosumab; RAL, raloxifene; BP, bisphosphonate; ZOL, zoledronate; ALN, alendronate; RIS, risedronate; IBN, ibandronate; MIN, minodronate; TPTD, teriparatide; TRACP-5b, isoform 5b of tartrate-resistant acid phosphatase; BMD, bone mineral density.
 - 5 Differences between the groups were determined by Kruskal-Wallis test or Fisher's exact test.

1 **Table 2. Patients' clinical background at time of switch from DMAb to other treatment**

Variable	RAL group (n=13)	Weekly or monthly BP group (n=29)	ZOL group (n=11)	P-value
Interval after final DMAb administration (months)	7.0±1.7 (range, 6-11)	7.4±3.7 (range, 5-16)	6.8±2.4 (range, 5-14)	0.85
		ALN 35 mg/week PO (n=11)		
		ALN 900 ug/month IV (n=3)		
Switched therapy from DMAb	RAL 60 mg/day PO (n=13)	RIS 17.5 mg/week PO (n=9)	ZOL 5 mg/year IV (n=11)	N.A.
		IBN 100 mg/month PO (n=4)		
		IBN 1 mg/month IV (n=2)		
Combined active vitamin D	Total (92.3%; n=12) ALF (n=7) ELD (n=5)	Total (89.7%; n=26) ALF (n=23) ELD (n=3)	Total (100.0%; n=11) ALF (n=11)	0.80
Combined Ca, n/N (%)	15.4% (n=2)	10.3% (n=3)	9.1% (n=1)	0.47
Age (years)	77.1±7.9	71.6±11.5	72.8±8.5	0.23
Body mass index	19.7±2.5	20.7±2.7	21.2±1.2	0.19

(kg/m²)

eGFR (ml/min/1.73 m ²)	60.5±22.6	75.3±22.5	76.8±13.3	0.10
Corrected serum Ca (mg/dl)	9.3±0.6	9.3±0.5	9.2±0.4	0.64
Serum 25(OH)D levels (ng/ml)	7.9±3.3	14.4±4.1	16.5±0.4	0.13
TRACP-5b (mU/dl)	160.9±108.1	186.3±141.6	239.1±81.8	0.18
Lumbar spine BMD (T-score)	-2.3±0.9	-2.7±1.4	-2.8±1.8	0.46
Femoral neck BMD (T-score)	-2.2±0.8	-2.2±0.8	-2.3±1.1	0.93
Prior vertebral fracture	46.2% (n=6)	51.7% (n=15)	54.5% (n=6)	0.98
Prior non-vertebral fracture	53.8% (n=7)	17.2% (n=5)	18.2% (n=2)	0.10

2 Mean ± standard deviation; N.A. = not applicable.

3 DMAb, denosumab; RAL, raloxifene; BP, bisphosphonate; ZOL, zoledronate; PO, oral; IV, intravenous;

4 ALN, alendronate; RIS, risedronate; IBN, ibandronate; ALF, alfacalcidol; ELD, eldecacitol; Ca, calcium;

5 eGFR, estimated glomerular filtration rate; 25(OH)D, 25-hydroxy vitamin D; TRACP-5b, isoform 5b of

6 tartrate-resistant acid phosphatase; BMD, bone mineral density.

7 Differences between the groups were determined by Kruskal-Wallis test or Fisher's exact test.

8

Figure 1

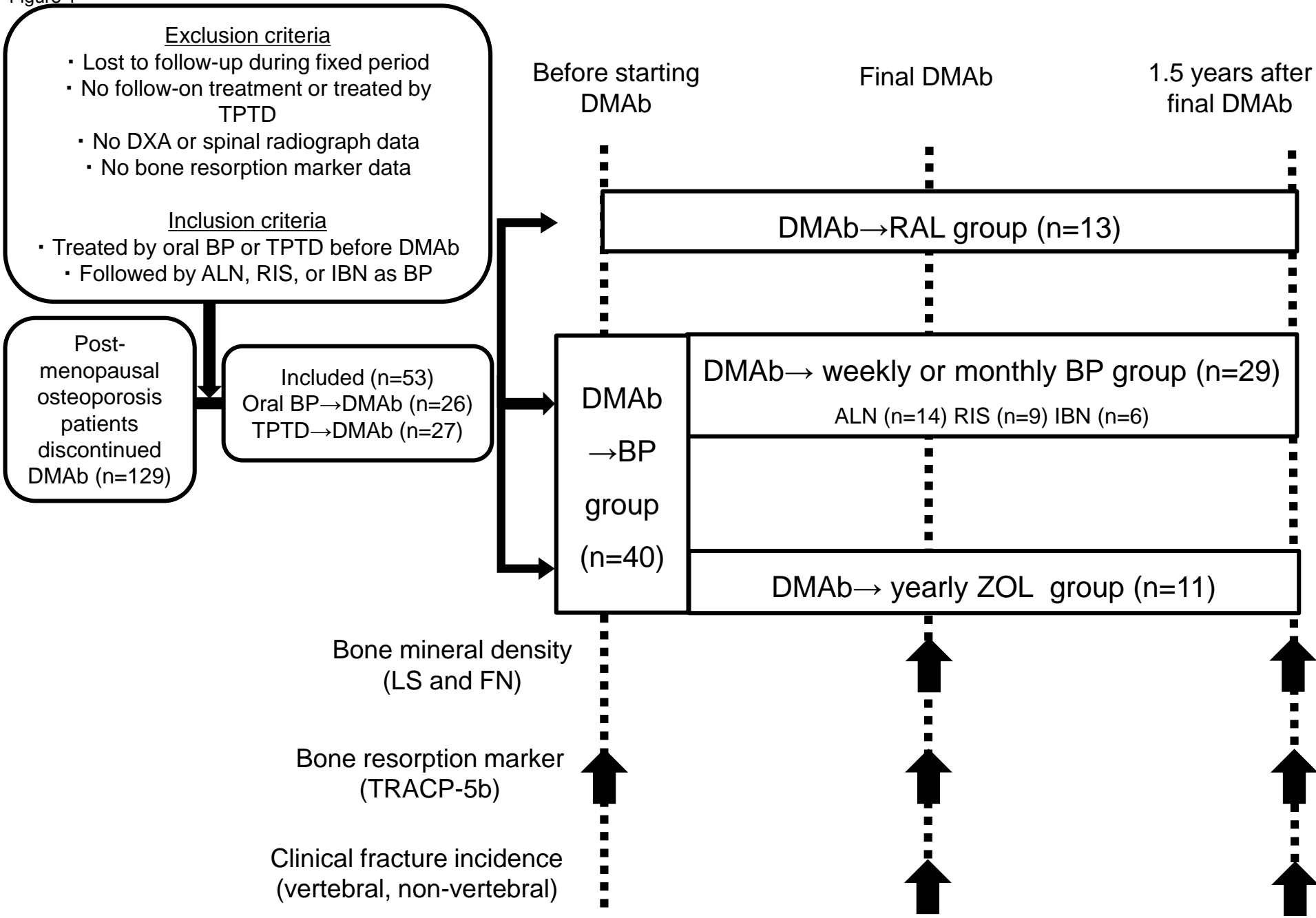


Figure 2

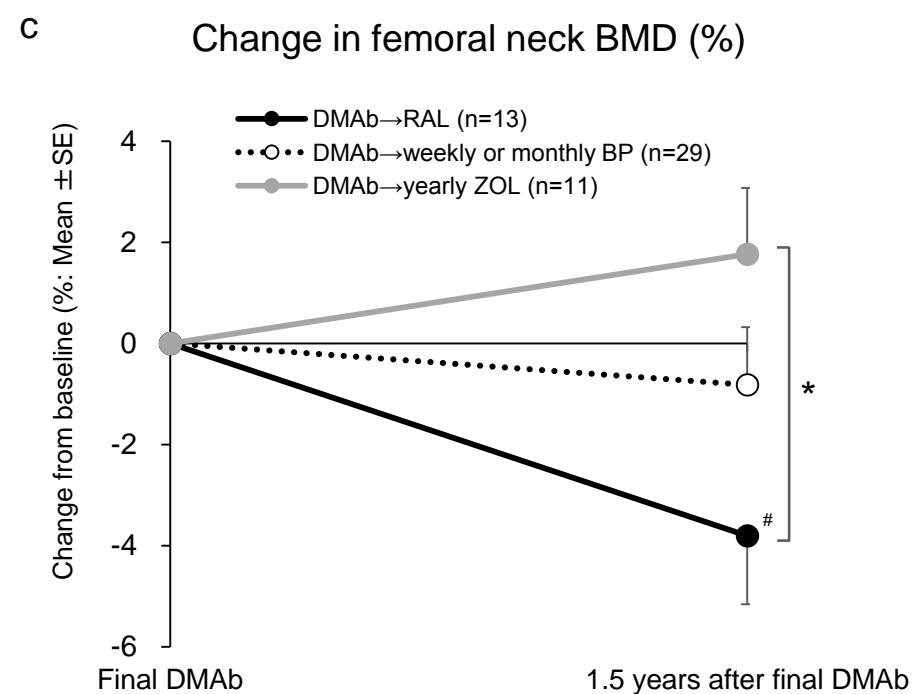
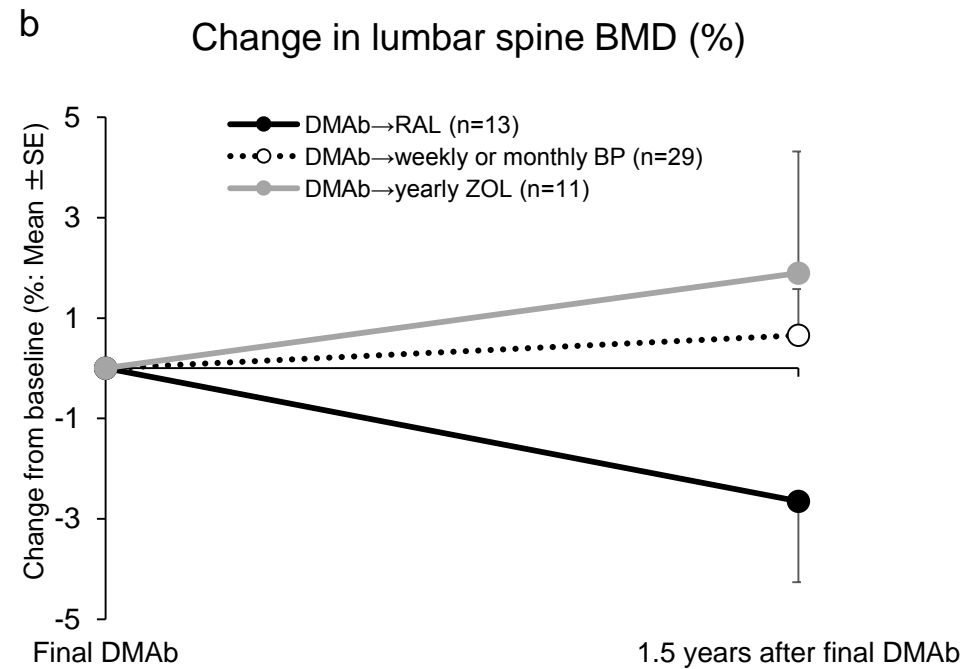
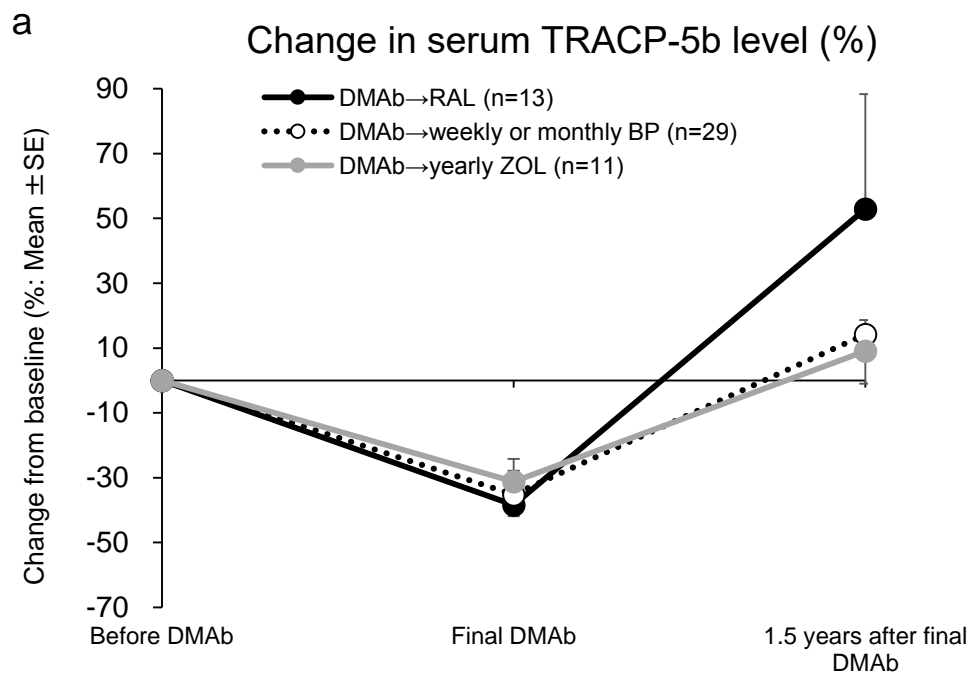
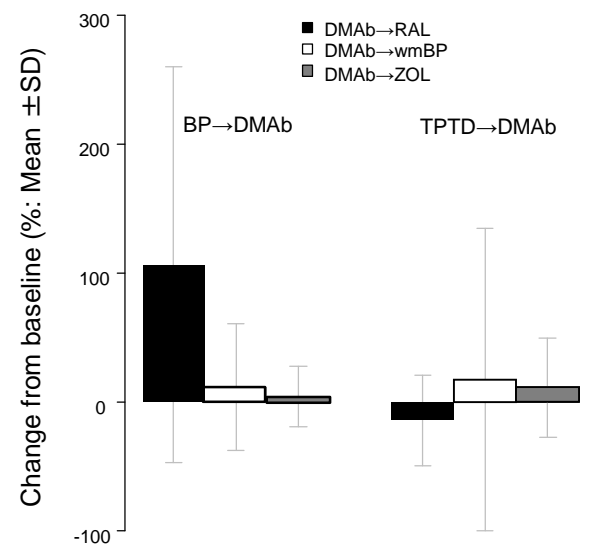


Figure 3

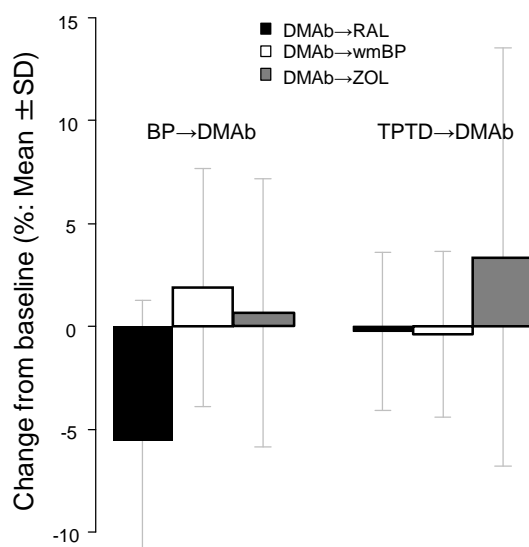
a

Change in serum TRACP-5b levels
from baseline (%)



b

Change in lumbar spine BMD
from baseline (%)



c

Change in femoral neck BMD
from baseline (%)

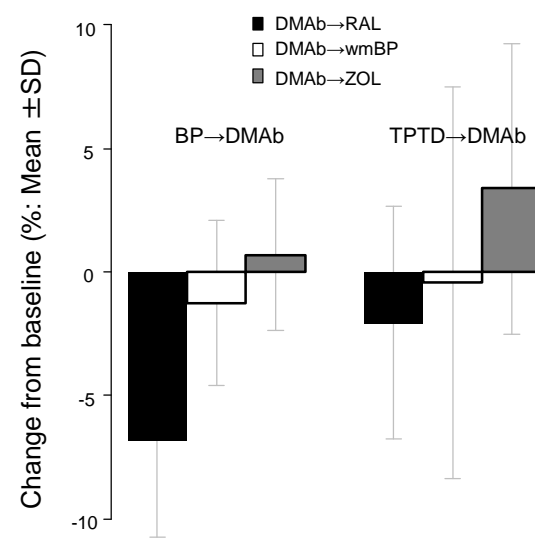
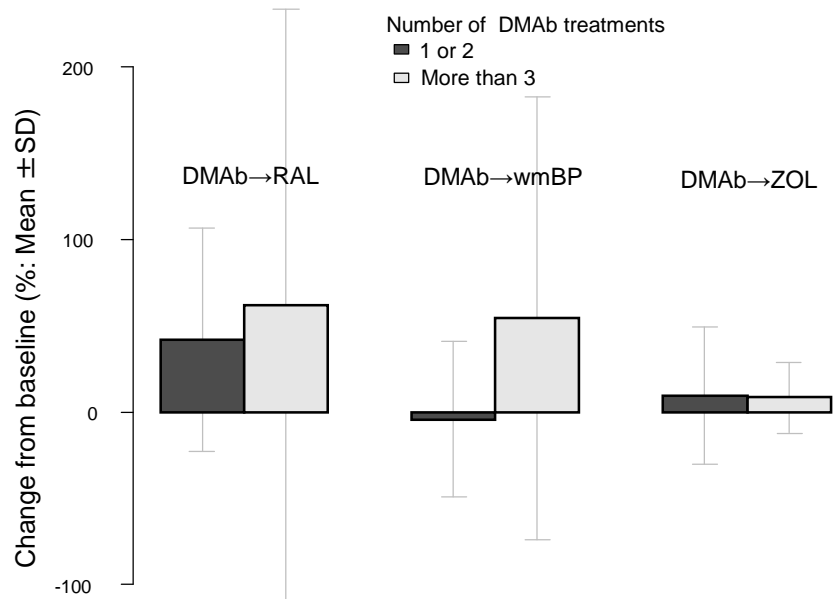
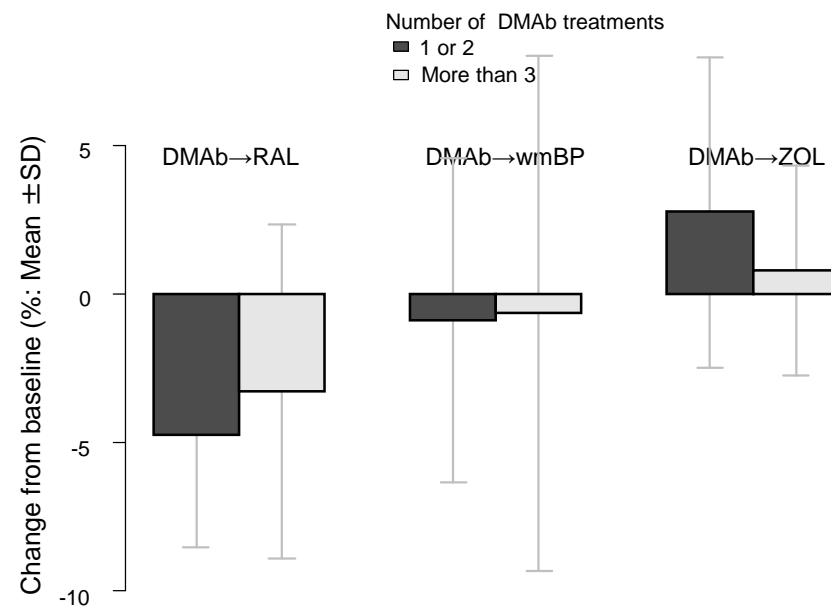


Figure 4

a Change in serum TRACP-5b levels from baseline (%)

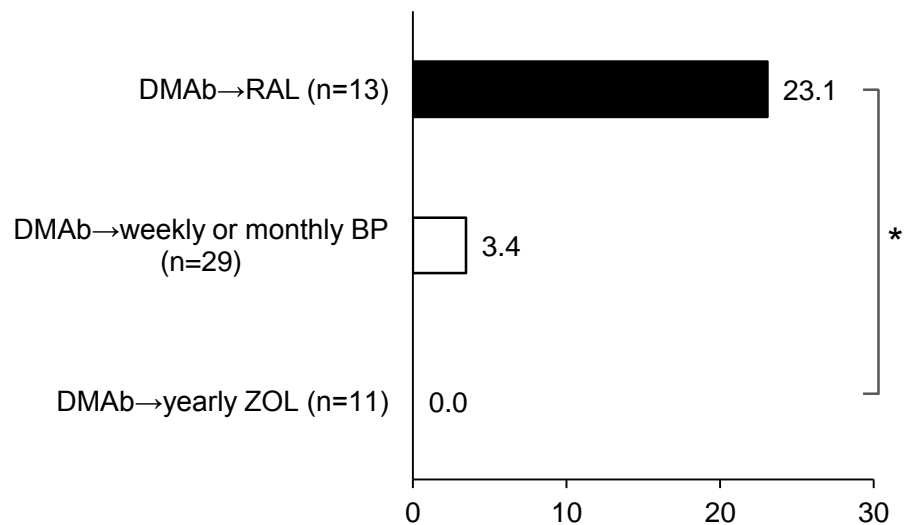


b Change in femoral neck BMD from baseline (%)



c

Clinical vertebral fracture rate (%)



d

Clinical non-vertebral fracture rate (%)

